Manzoor Ahmad Mir *Editor*

Cytokine and Chemokine Networks in Cancer

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This book is dedicated to my family
Ayzel Manzoor Mir
Aariz Manzoor Mir
Sumaira Manzoor Mir

Foreword

I am pleased to provide the foreword to Dr. Manzoor Ahmad Mir's valuable book, Cytokine and Chemokine Networks in Cancer.

Cancer is a common illness and comprises over 400 distinct histologic diagnoses. Each diagnosis is associated with specific biologic behaviors and patterns of spread. This variability is reflected in the multitude of diagnosis-specific anticancer regimens. Despite the diversity of cancer, there are striking similarities in the clinical manifestations of patients with progressive disease. Even patients who only have local disease, for example chest wall spread from breast cancer with no evidence of distant metastases, can experience systemic effects, such as anorexia and cachexia, that are synonymous with advanced cancer. These observations suggest that there is a final common pathway underlying deterioration. One feature common to all cancers is uncontrolled growth. Cytokines/chemokines play a prominent role in facilitating not only growth but also metastatic spread.

Chemokines are small proteins that primarily regulate the traffic of leukocytes under homeostatic conditions and during specific immune responses. The chemokine-chemokine receptor system comprises of almost 50 chemokines and approximately 20 chemokine receptors; thus, there is no unique ligand for each receptor, and the binding of different chemokines to the same receptor might have disparate effects. Complicating the system further, these effects depend on the cellular milieu. In cancer, although chemokines are associated primarily with the generation of a protumoral microenvironment and organ-directed metastasis, they also mediate other phenomena related to disease progression, such as angiogenesis and even chemoresistance. Therefore, the chemokine system is becoming a target in cancer therapeutics.

Thus, targeting cytokines/chemokines with their specific inhibitors in cancer is considered to be very useful. In this book, Dr. Mir has shed light on the role of cytokines/chemokines in cancer progression, and their dysregulation in cancer progression and metastasis, their prognostic significance, and the specific inhibitors used to overcome the cancer progression.

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Raid Saleem Al-Baradie

Preface

Introduction to the Book

During the past 40 years, cytokines and chemokines have been extensively investigated as either cancer targets or cancer treatments. A strong preclinical rationale supports therapeutic strategies to enhance the growth inhibitory and immunostimulatory effects of interferons and interleukins, and chemokines including IL-2, IL-7, IL-12, IL-15, and CXCR4, or to inhibit the inflammatory and tumor-promoting actions of cytokines such as TNFs, IL-1β, and IL-6. This rationale is underscored by the discovery of altered and dysregulated cytokine and chemokine expression in all human cancers. These findings prompted clinical trials of several cytokine/chemokine antagonists, revealing relevant biological activity but limited therapeutic efficacy. However, most trials involved patients with advanced-stage disease, which might not be the optimal setting for cytokine/chemokine-based therapy. The advent of more effective immunotherapies and an increased understanding of the tumor microenvironment have presented new approaches to harnessing these cytokine and chemokine networks in the treatment of cancer, which include using cytokine- or chemokine-based therapies to enhance the activity or alleviate the immune-related toxicities of other treatments as well as to target early-stage cancers. Many challenges remain, especially concerning delivery methods, context dependencies, and pleiotropic, redundant, and often conflicting actions of these networks. In this book, we will provide a deep understanding of the mechanism underlying the cytokine and chemokine networks in tumor growth, angiogenesis, and cancer metastasis. Also, we will provide the recent developments and findings in the role of CXCR4-CXCL12 axis in tumor metastasis. Further, we discuss the lessons learnt from the initial trials of single-agent cytokine-based therapies and subsequent efforts to better exploit such agents for the treatment of solid tumors.

Target Audience

The book is designed in such a way so as to target a huge number of audiences including the following ones:

- Graduate and postgraduate students of applied medical field like nursing, oncology, biotechnology, biochemistry, immunology, medical laboratories, biomedical lab technology, and physiotherapy
- Postgraduate and graduate students pursuing normal graduations in life sciences with one of the subjects as cancer biology and medicine
- Researchers and medicos in the field of cancer biology and molecular medicine
- Health professionals related to pharmacy, drug designing, and oncology
- Paramedical staff involved in oncology and chemotherapy
- Staff working in pharma companies related to oncology and cancer research

Importance of the Book

- The book is most important in terms of the significance of cytokines/chemokines being targeted in cancer.
- The book also carries its importance in terms of the cytokines/chemokines and their receptors being targeted by their inhibitors and thus being used as prognostic factors in cancer.
- Last but not least, the book has illustrated the significance of cytokine/chemokine cancer through high-quality visual graphical figures and tables, thus providing a better glimpse of the whole book.

Highlights of the Book

(a) It will be the first book to highlight the role of cytokines and chemokines in tumor development and metastasis.
(b) The book will highlight up-to-date information on the therapeutic implications of cytokines and chemokines in cancer.
(c) It will shed light on various inhibitors targeting cytokine and chemokine axis.
(d) The book will also throw light on the prognostic and diagnostic significance of cytokines and chemokines in cancer.
(e) The book will have many illustrations, flowcharts, and diagrams highlighting the role of cytokines and chemokines in cancer.

Srinagar, Jammu and Kashmir, India Manzoor Ahmad Mir

Acknowledgments

I would like to take this opportunity to pen down my appreciation for a great number of people whose contribution in numerous ways immensely helped me to move toward my destination. It gives me immense pleasure to express my sense of gratitude and respect to my mentor Dr. Javed Naim Agrewala, whose constant support, utmost patience, invaluable advice, and guidance rekindled my interest in science from time to time. A special vote of thanks goes to Prof. Nilofer Khan for her efficient guidance to work hard with sincerity, dedication, and devotion both in professional and personal life. The staff, scholars (especially Miss Shazia, Miss Nusrat, and Miss Hina Qayoom), and students at the Department of Bioresources deserve a special mention for all the help rendered at the hour of need. I am also thankful to my soulmate Miss Sumaira Manzoor and my parents Mohammad Abdullah Mir, Mohd Ashraf Malik, Zona Begum, and Posha Begum for their constant support, love, and care throughout the whole journey. I would like to mention here the affection and love showered by my angels Ayzel Manzoor and Aariz Manzoor throughout the writing process of the book. Finally, I would like to thank and acknowledge the *Jammu and Kashmir Science Technology and Innovation Council (JKST&IC)* for providing the financial support vide *Project No. JKST&IC/SRE/885–87, dated 27-10-2021*, sanctioned to Dr. Manzoor Ahmad Mir (Principal Investigator), and this book is part of the same project entitled "*Targeting Drug Resistant Cancer Stem Cells by Combinational Delivery of Paclitaxel and Quercetin Zerumbone in Breast Cancer.*"

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About the Editor

Manzoor Ahmad Mir, MSc, PhD, PGDHE, JRF-NET, FRSB Dr. Manzoor Ahmad Mir holds a master's degree in zoology with gold medal from HNBG Central University, and after qualifying the prestigious national level CSIR-JRF-NET examination, he worked jointly for his Ph.D. at Jawaharlal Nehru University, New Delhi, and CSIR-Institute of Microbial Technology, Chandigarh, in the field of immunopathology. His basic research interests include molecular immunology and immunobiology of microbes (MTB). His Ph.D. research title was "Role of co-stimulation in the survival of intracellular pathogens." He has published more than 50 high-impact research papers and book chapters, in recognition of which he has received several awards and royalties from international publishing houses. Dr. Manzoor has authored more than 15 books with international publishers like Elsevier, USA; Nova Science Publishers, USA; Bentham Science; IGI Global; and Springer Nature. He is on the editorial board and is a reviewer of some prestigious journals, like DOVE Medical Press, Springer Plus, Cancer Biomarkers, Frontiers, and PLOS, and has been an invited speaker at various scientific meetings/conferences within India and abroad. He is a member of many scientific organizations and societies, like the American Association of Cancer Research, the Royal Society, the International Union of Immunological Societies, the Indian Cancer Society, the Indian Immunology Society, the Indian National Science Academy, and the IMMUNOCON. Dr. Manzoor was awarded Teachers Associate Research Excellence (TARE) Fellowship by the DST, Government

of India. He has been awarded Summer Research Fellowship Programme (SRFP-2019) by the Indian Academy of Sciences and the National Science Academy. He was awarded a research project on combination therapy in breast cancer by the J&K Science Technology and Innovation Council. Dr. Manzoor has developed massive open online courses (MOOCs) in immunology and endocrinology for UG students sanctioned by the UGC-Consortium for Educational Communication (CEC), SWAYAM, Ministry of HRD, Government of India. He has research and teaching experience of 15 years. He currently teaches cancer biology and immunology at the Department of Bioresources, School of Biological Sciences, University of Kashmir. He is presently heading the Department of Bioresources, University of Kashmir. Department of Bioresources, School of Biological Sciences, University of Kashmir, Srinagar, Jammu and Kashmir, India.

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Introduction to Cytokine and Chemokine Networks

1

Manzoor Ahmad Mir, Asma Jan, and Shazia Sofi

Abstract

Cytokines are small-molecular-weight, proteinaceous, immunomodulating molecules that are crucial for controlling the growth and activity of blood and other immune cells. They are peptide molecules that cannot cross the lipid bilayer of a cell to enter the cytoplasm but are important for cell signaling. Cytokines bind to specific cell surface receptors and send intracellular signals. They are involved in autocrine, paracrine, and endocrine signaling. Normally, cytokines affect cell activation, division, apoptosis, or movement, but their biological effect depends on the type of cytokine and the cell involved. These essential chemical mediators function as biomarkers for a wide range of illnesses and control immunological and inflammatory responses through intricate networks. They can be discussed in terms of their pro- and anti-inflammatory activities as well as on the basis of their clinical significance. They take part in the immune response and serve as significant immune system communication network mediators. The development, proliferation, and response of immune cells are dynamically controlled by cytokines, which play a significant role in determining health. Multiple biological activities can result from a single cytokine's ability to act on various cell types and can be released by numerous kinds of cells. Cytokines have two common characteristics, redundancy and promiscuity, which are particularly notable in chemokines that are a specific type of cytokine, and can direct immune cell movement toward their target. There are different types of chemokines, like interferons, interleukins, tumor necrosis factor (TNFs), and growth factors. Chemokines, sometimes also referred to as chemotactic cytokines, are a broad family of small, secreted proteins that communicate by binding to the cell sur-

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faces that have heptahelical chemokine receptors (CKRs). They are mostly well-known for their capacity to encourage the movement of cells, particularly that of leukocytes (white blood cells (WBCs)). Since they are involved in all inflammatory and immunological responses, whether beneficial or harmful, chemokines are essential for homeostasis of the immune system and development. This chapter provides a general review of the cytokine network, chemokines, and chemokine receptor families, highlights the chemokine network's numerous physical interactions, and covers the fundamental principles of chemokine function while providing specific examples.

Keywords

Proinflammatory Cytokines · Anti inflammatory Cytokines · Cancer Therapy · Chemokines · Interferons

1.1 An Overview of Cytokines

The word "cytokine" refers to a class of protein cell regulators also known as monokines, lymphokines, interferons, and interleukins, which are also released by a large number of cells throughout the body. Cytokines are produced by different cell types like mast cells, macrophages, T lymphocytes, B lymphocytes, endothelial cells, and fibroblasts among others. The cytokines produced by monocytes are monokines, lymphocytes are lymphokines, chemotactic activities are chemokines, and leukocytes are interleukins. Cytokines that are secreted by leukocytes, also known as white blood cells (WBCs), and mainly exert effects on other WBCs are termed "interleukins." Cytokines that exhibit a chemoattractant activity are termed "chemokines." Those cytokines that cause differentiation and proliferation of stem cells are termed "colony-stimulating factors." The cytokines that have a role in defense and interfere with viral replication are termed "interferons" (IFNs). Virally infected cells secrete IFNs to create both a barrier around neighboring cells and viral resistance. Interleukin (IL)-2 and IFN-α cytokines and their antagonists are increasingly being used for therapeutic purposes for the treatment of melanoma (Nicholas and Lesinski 2011). Cytokines bind to specific cell surface receptors and send intracellular signals. Although most cytokine receptors exist in the soluble form, some may be membrane-anchored, making the differentiation between the cytokine and receptor roles extremely difficult to define. They have therapeutic effects, are essential for the pathophysiology of several disorders, and have a significant role in numerous physiological processes, as shown in Fig. 1.1.

These diverse groups of proteins share the following traits:

- Cytokines may function in a paracrine, autocrine, or endocrine manner, that is, they may act on the cell that produces them (autocrine action), on the nearby cells (paracrine action), or on distant cells (endocrine action).

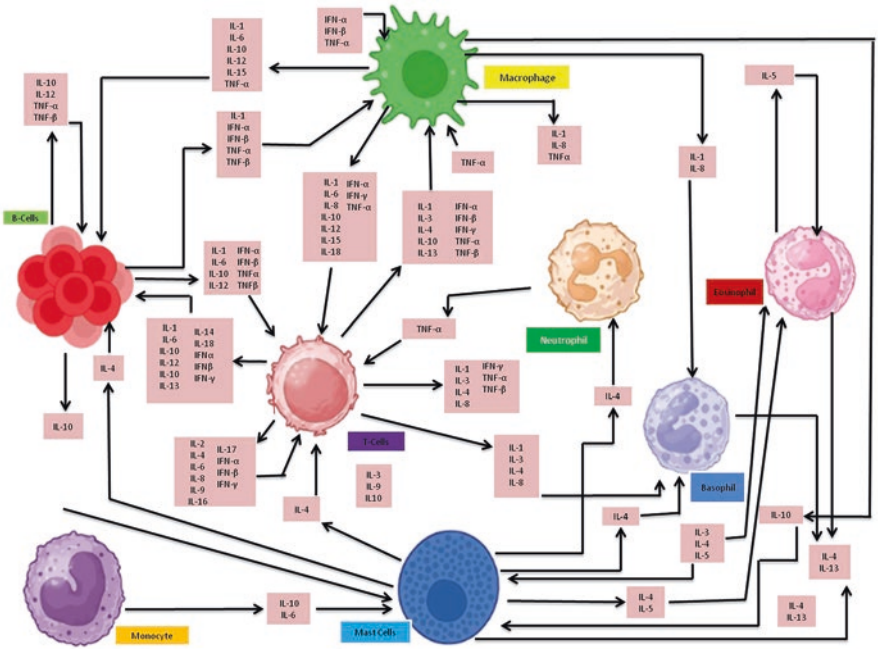

Fig. 1.1 The cytokine network. Adapted from eBioscience (ref: https://in.pinterest.com/pin/83387030580587632/)

- Cytokines are pleiotropic in nature, meaning that different cell types may secrete the same cytokine or a single cytokine can act on different cell types.
- Cytokines are redundant in nature, meaning that different cytokines can stimulate similar functions.
- They are mostly produced in cascades, as one cytokine causes the release of other cytokines.
- Cytokines can work both synergistically and antagonistically.
- Every cytokine has a matching cell receptor, and it can either downregulate or upregulate the activity of genes.
- They control the duration and intensity of responses in immune and inflammatory processes.
- Cytokines have a high potency and often act at picomolar levels.
- They often work in an autocrine or paracrine manner, and they are typically released momentarily and locally, as shown in Fig. 1.2.
- Each cytokine or set of cytokines has a unique high-affinity cell surface receptor with which they engage. The difficulty in elucidating their structure and sequence is due to their lower degree of expression (10–10,000 per cell). Five receptors for cytokines, such as those for IL-1α and IL-1β, interferon-gamma (IFN-γ),

interleukin 2 (IL-2), IL-6, and M-CSF (macrophage colony-stimulating factor) (Smith 1988) are now known to exist. IL-l and IL-6 receptors are associated with integrin receptors and belong to the immunoglobulin (Ig) gene superfamily. The proto-oncogene c-fms encodes the M-CSF receptor.

- As a result of their cell surface binding, cells behave differently and their pattern of RNA and synthesis of protein is altered.
- Each cytokine has numerous overlapping cell regulation functions. The way that a cell reacts to a specific cytokine depends on the kind of cell, local concentration of the cytokine, and additional regulators of the cell to which it is concurrently exposed. The induction of similar proteins (Beresini et al. 1988), which may have shared response regions in their DNA (Reid et al. 1989), can at least partially account for the overlapping regulatory activities of these physically different proteins when they connect to diverse cell surface receptors.
- Cytokines interact with one another and with cell function through three different mechanisms: initially, by inducing one another; second, through transmodulation of cell surface receptors for cytokines; and, finally, by interactions that are synergistic, additive, or antagonistic.

Cytokines may also refer to growth factors, including transforming growth factor-α (TGF-α), platelet-derived growth factor (PDGF), and epidermal growth

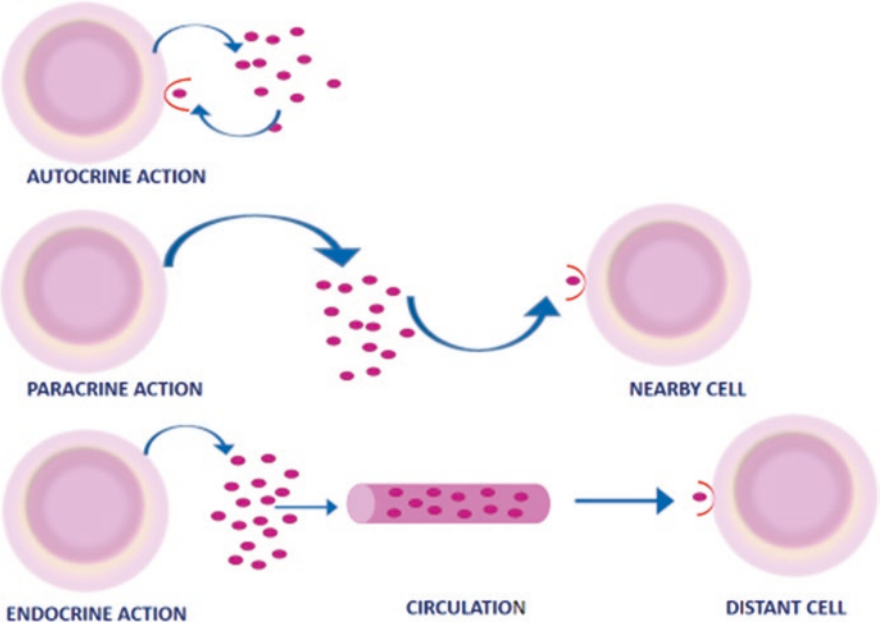

Fig. 1.2 The mechanism of action of cytokines

factor (EGF). Links between these molecules and other peptide-regulating compounds are being discovered. PDGF and M-CSF receptors, for instance, are tandemly coupled and have a similar organizational structure and amino acid sequence (Roberts et al. 1988).

1.2 Why Do Cytokines Have Such a Diverse Range of Functions?

Cytokines are critical controllers of cells, and hence tissues, development, differentiation, growth, and migration. The cytokine family includes growth factors such as epidermal and hepatocyte growth factors, inflammatory cytokines such as interleukins and interferons, and chemokines such as the macrophage inflammatory proteins MIP-1α and MIP-1β. They have important roles in the control of cell replication and apoptosis, in chemically induced tissue damage repair, and in cancer development and progression in the modulation of immune reactions such as sensitization. It is challenging to comprehend how complicated the cytokine network (Fig. 1.3) is given our existing knowledge. Cytokines are secreted products of cells, which act locally and cause activation of cells, especially immune cells, as well as toxic effects in high doses. Factors specifically activating or increasing lymphocyte growth in culture are called lymphokines. Numerous cross talks (interactions) between substances have been described as "cytokine networks," and these are complicated and challenging as well. The distinction between pro- and anti-inflammatory cytokine effects is not always entirely clear, as the pathway interactions and the combination of several cytokines can contribute to upregulation or downregulation of other cytokines that leads to various and differing physiological effects.

Numerous experiments are performed in basic media or with additions like calf fetal serum (CSF) or fetal bovine serum (FBS), to which a specific cytokine is added. When a cell receives numerous diverse and sometimes conflicting regulatory messages, it is challenging to determine whether such actions would be observed in the in vivo environment at that moment. A cellular response's range and length may be constrained by the complexity of simultaneous signals that can be received by any single cell. This is because there may be numerous checks and balances in place. Individual regulatory proteins have been compared by Sporn and Roberts (1988) to an alphabet or a code, a sophisticated cell signaling language, in which a variety of messages received simultaneously on the surface determine the cell's final reaction. The fact that various induction signals cause the creation of unique cytokines with the same array of functions is another reason for the complexity of cytokine functioning and quantity. Table 1.1 demonstrates the various cytokines linked to specific biological processes.

The production of several kinds of cells or subsets may also result in the production of a unique spectrum of cytokines. When it comes to activating T

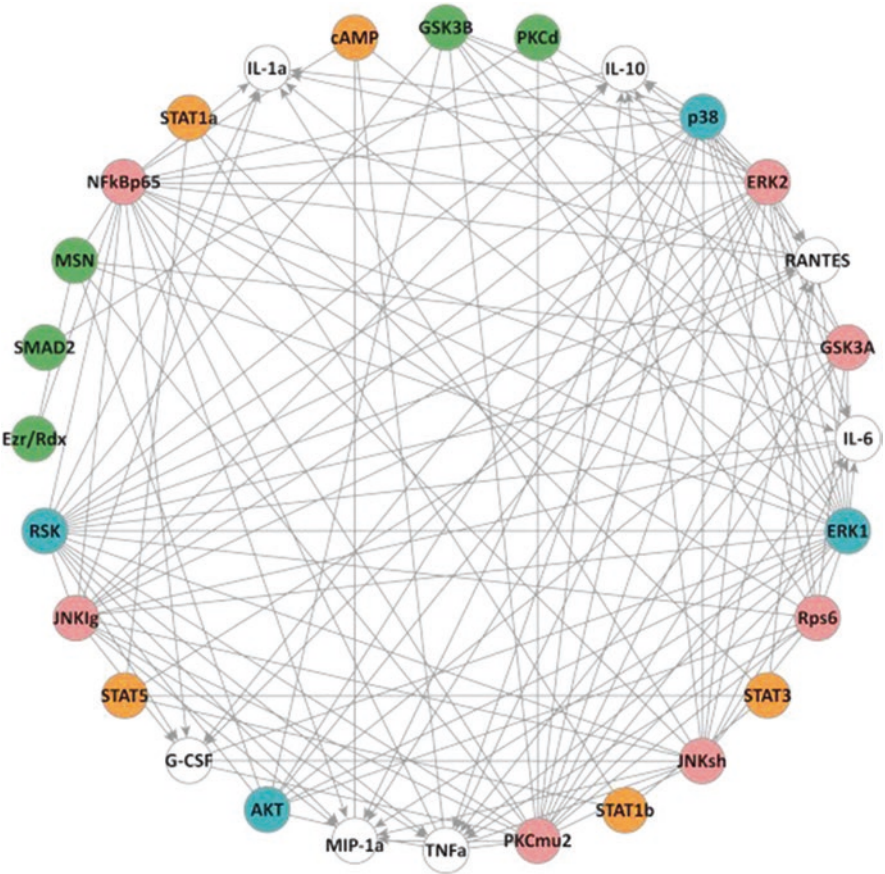

Fig. 1.3 Interconnecting cytokine networks. These events are intracellular. The reconstructed phosphoprotein–phosphoprotein/cytokine network from combining networks from a non-toll dataset (orange) and a toll dataset (pink). The white nodes represent the cytokines, the blue nodes are involved in cytokine regulation from both datasets, and the green nodes are not directly involved in cytokine regulation (adapted from source: Farhangmehr F, Maurya MR, Tartakovsky DM, Subramaniam S. Information theoretic approach to complex biological network reconstruction: application to cytokine release in RAW 264.7 macrophages. *BMC Syst Biol* 2014;8:77)

cells, for instance, IFN-γ and IL-4 are similar or synergistic with one another, yet they have distinct or antagonistic effects when it comes to immunoglobulin isotype selection or major histocompatibility class-II (MHC class II) expression (Snapper and Paul 1987). T_H1 cells in clones of murine T cells generate IL-2, IL-3, and IFN-γ, whereas T_H2 cells generate IL-4 and IL-5 (Mosmann et al. 1986). When clones of human T cells are examined, the situation is not entirely obvious. However, in situ hybridization has recently been used to investigate the generation of these lymphokines in human T cells that have undergone polyclonal activation (Lewis et al. 1988). In all, 60% of $CD4^+$ (cluster of

Table 1.1 Various cytokines linked to specific biological processes

Diseases	Related cytokines	References
Depression	IL-1, IL-2, IL-6, IFN-γ, TNF-α	Schiepers et al. (2005)
Cancer	TRAIL (tumor necrosis factor-related apoptosis-inducing ligand), TNF-α, IL-6, IL-10, IL-12, IL-17, IL-23	Kabel (2014)
Atherosclerosis	IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-17, IL-18, IL-20, IL-33, IL-37, TGF-β, IFN-γ, TNF-α	Ait-Oufella et al. (2011)
Cardiovascular disorders	IL-1, IL-6, IL-10, IL-17, IL-18, TGF-β, TNF-α	Kofler et al. (2005)
Autoimmune diseases	IFN-γ, IFN-α, TNF-α, IL-1, IL-2, IL-6, IL-12, IL-15, IL-16, IL-17, IL-18, IL-23	Trudeau et al. (2003)
Alzheimer's disease	IL-1, IL-4, IL-6, IL-10, TNF-α, TGF-β	Rubio-Perez and Morillas-Ruiz (2012)
Allergy	IL-1, IL-4, IL-5, IL-9, IL-10, IL-13	Romagnani (2002)
Sepsis	IL-1, IL-6, IL-4, IL-10, IL-12, MIF, IFN-γ, TNF-α, TGF-β	Schulte et al. (2013)
Aging	IFN-γ, TNF-α, IL-6, IL-8, IL-10, IL-13	Koelman et al. (2019)
Gastrointestinal diseases	IL-1, IL-4, IL-6, IL-8, IL-10, IFN-γ, TGF-β, TNF-α	Przemioslo and Ciclitira (1996)

differentiation 4^+) cells have IFN-γ and IL-2 messenger RNA (mRNA) and less than 5% of $CD4^+$ T cells can be found to contain IL-4 mRNA. One mechanism for limiting the potentially pleiotropic effects of lymphokines to the correct responder cells may be such selective regulation.

The regulation of the cytokine network may be further clarified by more research using in situ hybridization methods to analyze cytokine message expression, particularly in tissues mounting immunological and inflammatory responses.

1.3 Characteristics of the Cytokine Network

Although Fig. 1.1 is mostly for reference, it allows us to draw some broad generalizations about the cytokine network:

- Although every cytokine has growth factor activity, only lymphotoxin (LT), tumor necrosis factor (TNF), IFNs, TGF-β, and IL-1s have the capacity to directly impede cell growth or kill cells.
- To our knowledge, colony-stimulating factors and IL-5 in human (but not murine) cells are the only cytokines that do not act on B and T cells at a certain point in their responses (Sofi et al. 2023; Mir and Mir 2022). As two or more cytokines are frequently needed to elicit a response, these activities on B- and T-cell responses now overlap and are unclear. IFN-γ appears to regulate IgG2a, IL-4 controls IgE

and IgGl, and IL-5 and TGF-β control IgA, suggesting specificity in the regulation of immunoglobulin isotypes.

- Cytokine regulation of MHC expression seems to be more constrained, with IFNs and TNF/LT reportedly upregulating MHC class I and IFN-γ being the main regulator of MHC class II (although IL-4 upregulates this in B cells).
- At the present time, it also seems that cytokines have a more constrained effect on eosinophils, granulocytes, macrophages, and natural killer (NK) cells. Only IL-2, and maybe IFN-γ, can cause cells to activate the lymphokine-activated killer (LAK) pathway.
- Human IL-5 seems to be the sole cytokine that currently exhibits an extremely limited action.
- Differences across species are of some relevance. For instance, human IL-5 primarily affects eosinophils, whereas mouse IL-5 can also affect B-cell responses. In murine cells, where it can be inhibitory, IL-4 is a strong activator of LAK activity but not in human cells.
- The stimulation of osteoclastic bone resorption, activation of the vascular endothelium, induction of chemotactic migration, and development of a cellular antiviral state are other cytokine effects that seem to be unique to a small number of the currently recognized cytokines.
- The majority of the activities described here are upregulatory, stimulating growth and improving functional activity, and, in turn, increasing immune response and inflammation. IFN-β and TGF-β, at least in in vitro models, can, nevertheless, have a downregulatory effect. TGF-β is unusual from other protein cell regulators discussed here, in that, despite its message increasing occasionally, for instance, during activation of T cells, it is always present in a variety of body cells. However, this regulator is released from cells as an inactive precursor that must be cleaved, most likely by a protease, in order to bind to its cell surface receptor. The activity of several cytokines is controlled by TGF-β in a reciprocal manner.

1.4 A Classification of Cytokines and an Analysis of Their Clinical Importance

Interferons (IFNs), monokines, tumor necrosis factors (TNFs), transforming growth factors (TGFs), interleukins (ILs), colony-stimulating factors (CSFs), and lymphokines are among the different kinds of cytokines that can be categorized. Two groups of cytokines are distinguished by the cells from which they are produced; $CD4^+$ (cluster of differentiation 4^+) T-helper-1 (Th1) cells release type 1 cytokines, such as IL-2, IL-12, TNF-β, and IFN-γ, and $CD4^+$ Th2 cells release type 2 cytokines, such as IL-4, IL-5, IL-6, IL-10, and IL-13 (Sprague and Khalil 2009).

Cytokines can also be categorized as either pro- or anti-inflammatory depending on their function (Sprague and Khalil 2009). IL-1β, IL-6, IL-8, IL-12, TNF-α, and other pro-inflammatory cytokines promote inflammatory responses and have a

tendency to excite immune-competent cells. Anti-inflammatory cytokines, on the other hand, like IL-4, IL-6, IL-10, IL-11, IL-13, TGF-β, and IL-1RA (IL-1 receptor antagonist), restrain immune cells and reduce inflammation (Boshtam et al. 2017). Certain cytokines, including IL-6, have the ability to simultaneously promote and inhibit inflammation. These cytokine classifications, particularly the pro- and anti-inflammatory cytokine families, provide a wide range of views for comprehending the pathways set off by the host response.

Depending on the situation, just one cytokine with pro- and anti-inflammatory properties that is released by various cells, leads to a range of immunological reactions (Monastero and Pentyala 2017; Mir and Gul 2022). As a result, by mediating and controlling inflammation, the equilibrium between cytokines that are pro- and anti-inflammatory has a substantial impact on the immunological system of the host. Anti-inflammatory cytokines make it easier for inflammation to subside for the body to recover from the acute phases of autoimmune diseases, whereas pro-inflammatory cytokines aid in the development and spread of autoimmune inflammation (Moudgil and Choubey 2011).

This part discusses the clinical and biological importance of pro- and anti-inflammatory cytokines, providing readers with a thorough and unbiased explanation of their functions in the inflammatory response that is so important to human health. Table 1.2 compares the roles of pro- and anti-inflammatory cytokines while summarizing the properties of various cytokines and cell sources.

1.4.1 Pro-Inflammatory Cytokines

Cytokines are crucial for regulating the inflammatory response, causing an acute-phase response to defend the body from infections, tissue damage, and irritations. TNF-α, IL-6, IL-8, IL-12, IFN-γ, and IL-1β are pro-inflammatory cytokines, which are released from the distinct or the same cell to initiate this reaction. The main function of these cytokines is to alert the tissues nearby to the presence of an injury or an infection. Additionally, these cytokines have the ability to circulate throughout the body, activating immune cells and significantly altering host physiology by causing symptoms like an acute-phase reaction and fever (Boshtam et al. 2017).

Pro-inflammatory cytokines have immunological qualities, which can help the host fight off invasions from bacteria and various microbes in the immediate environment as well as the gastrointestinal tract and endogenous flora of the skin (Dinarello 2000). In order to fight off infections, macrophages generate pro-inflammatory cytokines (Cheng et al. 2014). Macrophages perform crucial functions in the beginning of adaptive immunological responses, serving as the initial line of defense for the host against bacterial infection. TNF-α, IFN-α/γ, IL-6, IL-8, IL-12, IL-18, and IL-1 are among the pro-inflammatory cytokines that are released when they are triggered by bacterial products. IFNs regulate host defense against viral infection, and direct cytostatic and cytocidal actions; IL-6 is regarded as a key modulator of inflammatory and immunological responses, and IL-12 improves T-cell responsiveness. Consequently, such cytokines cause macrophages to become

Table 1.2 Properties of several cytokines and their cell source

Type of cytokines	Cytokine	Source of cells	Biological role	Half life
Pro-inflammatory	IL-1β	Monocytes/ macrophages	The primary mediator of IL-1's systemic effects; IL-1 has an impact on IL-6-induced gene expression	21 min
Pro- and anti-inflammatory	IL-6	Some tumor cells, monocytes, endothelial cells, B and T cells, and fibroblasts	Acute-phase response as well as a particular cellular and humoral immune response are both induced. The macrophages have the ability to reduce the production of IL-1 and TNF	15.5 h
Pro-inflammatory	IL-8	Chondrocytes, hepatocytes, tumor cells, epithelial cells, endothelial cells, and monocytes/ macrophages	Leukocyte recruitment to inflammatory areas is coordinated by pro-inflammatory mediators	24 min
Pro-inflammatory	IL-12	Dendritic cells and microglial and phagocytic cells	Differentiation of Th cells and production of IFN-γ and TNF-α	–
Pro-inflammatory	TNF-α	Vascular smooth muscle cells (VSMCs), NK cells, B and T cells, mast cells, and macrophages	Resorption of the bone, tumor necrosis, anticoagulant, pro-inflammatory, neutrophil stimulation, activation of adhesion molecules, and stimulation and increase in permeability	18.2 min
Pro-inflammatory	IFN-γ	B cells, Tc cells, Th1 cells, natural killer (NK) cells, VSMCs, and macrophages	Release of Th1-associated cytokines and Th1 immune responses are encouraged; pro-inflammatory and smooth muscle cell (SMC) MHC-I expression restrict the production of the extracellular matrix (ECM)	–
Anti-inflammatory	IL-1RA	Dendritic cells and macrophages/ monocytes	Blocks the IL-1α- and IL-1β-mediated activation of cells at the level of the IL-1 receptor in cells	4–6 h
Anti-inflammatory	IL-4	B cells, Th2 (T cells), stromal cells, and mast cells	Encourages the growth of Th2 lymphocytes and prevents lipopolysaccharide (LPS) from causing pro-inflammatory cytokine formation	20 min

(continued)

Table 1.2 (continued)

Type of cytokines	Cytokine	Source of cells	Biological role	Half life
Anti-inflammatory	IL-10	Macrophages/ monocytes, B cells, and Th2 (T cells)	Neutrophil and monocyte/ macrophage cytokine production is reduced, and Th1-type lymphocyte responses are also reduced	–
Anti-inflammatory	IL-11	Stromal cells and fibroblasts	Th2 lymphocyte response is stimulated, and the responses of pro-inflammatory cytokines by monocytes and macrophages are inhibited	–
Anti-inflammatory	IL-13	Th2 (T cells)	It is homologous to IL-4 and has a common IL-4 receptor; reduction in the activity of macrophages and monocytes	–

inflammatory in a direct manner. These cytokines are connected to one another because they control the immunological reaction to defend the host and are coordinately released from active macrophages (Muñoz-Carrillo et al. 2018; Mir and Mehraj 2019).

It is essential to keep in mind that an extremely high pro-inflammatory response can cause chronic inflammation and disrupt the biological homeostasis-maintaining pathways, which can have negative effects on one's health and contribute to conditions like diabetes (Pickup et al. 2000), gastrointestinal disease (Mendes et al. 2019), aging as well as age-related diseases (Rea et al. 2018), Parkinson's disease (Lin et al. 2019), cardiovascular disease (Kofler et al. 2005), and cancer (Lin and Karin 2007; Mehraj et al. 2022a, b).The crucial functions of cytokines in these numerous medical diseases have been succinctly outlined in several outstanding studies (Rodney et al. 2018). In an autoimmune disease (Miossec 1997), the immune system incorrectly assaults the healthy tissues, leading to illnesses like inflammatory bowel disease, rheumatoid arthritis, multiple sclerosis, and type 1 diabetes. Santamaria (2003) explains how various autoimmune disorders are affected by specific cytokines and chemokines. Autoimmune illnesses emerge as a result of pro-inflammatory interferons, which are crucial. Whether cancer is present or absent, evidence on the involvement of IFN-γ in the etiology of autoimmune illness and its effect on the comorbidities and the adverse consequences of therapeutic measures are available (Hodge et al. 2014; Mir et al. 2020a, b). In this regard, it has been demonstrated that continuous IFN-γ expression leads to the gradual development of a low-to-moderately active autoimmune disease caused by IFN in a preclinical autoimmune mouse model (Hodge et al. 2014). With the help of these models,

researchers may examine how inflammation and autoimmune illness progress at various IFN-γ–protein threshold levels that, when crossed, result in considerably more severe immunopathology. According to a recent study by Bae et al. (2020), integrated multi-omics data using a pathway-based approach can offer cellular and systemic insights regarding how IFN-γ-induced chronic inflammation leads to the emergence of autoimmune disorders with particular etiopathological characteristics (Bae et al. 2020).

Studies have shown that pro-inflammatory cytokines, including TNF-α, IFN-γ, and IL-1, produce neutrophil-attracting chemokines, which are essential for the production of carcinogenesis and reactive oxygen species, during the formation and spread of malignancies (Dinarello 2000). High pro-inflammatory cytokine levels (TNF-α, IL-1β,and IL-6) are seen in animal models of Parkinson's disease, which is pertinent to our research (Shen et al. 2020). Pro-inflammatory cytokines also cause metalloproteinases and adhesion molecules to open up certain pathways for tumor invasion. These highly pro-inflammatory reactions must be controlled and regulated as a whole in order to prevent the development of pathological conditions linked to the immunological mediators that are expressed incorrectly.

1.4.2 Anti-Inflammatory Cytokines

A group of immunoregulatory molecules called anti-inflammatory cytokines, including the IL-1 receptor antagonist, TGF-β, IL-4, IL-10, IL-11, and IL-13, prevent pro-inflammatory cytokines from causing an excessive amount of inflammation (Opal and DePalo 2000). For example, the secretion of various pro-inflammatory cytokines is inhibited by the powerful anti-inflammatory cytokine IL-10, which also has immunoregulatory characteristics. A vital component for the management and regulation of allergies and asthma is IL-10, and it has anti-inflammatory properties on mast cells, basophils, and eosinophils (Al-Dabbagh et al. 2013).

Anti-inflammatory cytokines have been found to possess certain physiological traits (Opal and DePalo 2000; Mir 2015a, b, c, d) that regulate the possible harmful consequences of prolonged or excessive production of pro-inflammatory responses under physiological circumstances. Such anti-inflammatory cytokines have previously demonstrated their worth in a number of clinical situations involving excessive inflammation and regulation of immune responses. Anti-inflammatory cytokines, for instance, are medications that can be utilized to treat disorders linked to inflammation. Contrary to anti-inflammatory biologics like neutralizing antibodies, cytokine treatment has a number of drawbacks as well (Rider et al. 2016; Mir et al. 2022a, b, c, d). For instance, certain anti-inflammatory cytokines may successfully prevent arthritis by influencing the innate immune cells or preventing the B cells or T cells from activating (Qayoom et al. 2023a; Chen et al. 2019; Mir et al. 2022a, b, c, d). It lessens the severity of collagen-induced arthritis because an anti-inflammatory cytokine, IL-35, controls the activity of T cells and pathogenic cells like T_h1 and T_h17 cells are suppressed (Niu and Chen 2014). On the other hand, these anti-inflammatory mediators inhibit the immunological response and may

overcompensate under pathological conditions, leaving the host vulnerable to a systemic infection (Opal and DePalo 2000). Even the evidence suggests that endogenous IL-10 protects against acute sepsis by lowering the synthesis of TNF, and excessive IL-10 production results in extreme TNF downregulation, which may be harmful since it impairs the antibacterial activity that is supplied by TNF (Marchant et al. 1995).

1.5 Chemokines

Cytokines that exhibit a chemoattractant activity are called chemokines. Leukocytes, also known as white blood cells, eliminate the necrotic tissue and pathogens via proteolytic destruction and phagocytosis and accumulate in wounded or infected tissues, which is the characteristic of an inflammatory response. The identification of chemokine receptors (CKRs) and chemokines marked a significant advancement in our knowledge of the molecular processes underpinning leukocyte movement (trafficking) (Mehraj et al. 2022b; Moser et al. 2004). During immune surveillance, small proteins called chemokines are produced in tissues that are normal or in order to respond to an infection or injury. They then attach to and stimulate chemokine receptors, such as G protein-coupled receptors (GPCRs), embedded in WBC membranes, causing adherence of WBCs to the vessel wall, alterations in morphology, extravasation into the inflammatory tissue, and chemotaxis to the location of an infection or injury along the gradient of chemokines (Moser et al. 2004).

Chemokine receptor stimulation can result in a range of other responses from tissues and cells, such as activation, proliferation, extracellular matrix remodeling, angiogenesis, differentiation, and tumor metastasis, beyond their functions in leukocyte trafficking (Luther and Cyster 2001). In order to penetrate host cells, two important diseases (malarial parasite *Plasmodium vivax* and human immunodeficiency virus type 1 (HIV-1)) have developed strategies to use chemokine receptors (Choe et al. 2005).

Other parasites or viruses release proteins that block chemokines and their receptors in order to suppress the host immune response. Several chemokine receptors (and to a smaller degree, chemokines) have been discovered as possible therapeutic targets in a variety of inflammatory illnesses because to their prominent activities in inflammation (Proudfoot 2002).

Given the significance of interactions between chemokines and receptors in response to the dangers posed by the environment as well as the possible hazards of high recruitment of leukocytes, it is perhaps not shocking that a variety of mechanisms (Fig. 1.4) have been developed to control the functions of chemokine receptors and chemokines. These processes could affect the levels of such proteins in particular tissues and changes in their interactions or their molecular structures, all of which would have an impact on leukocyte trafficking.

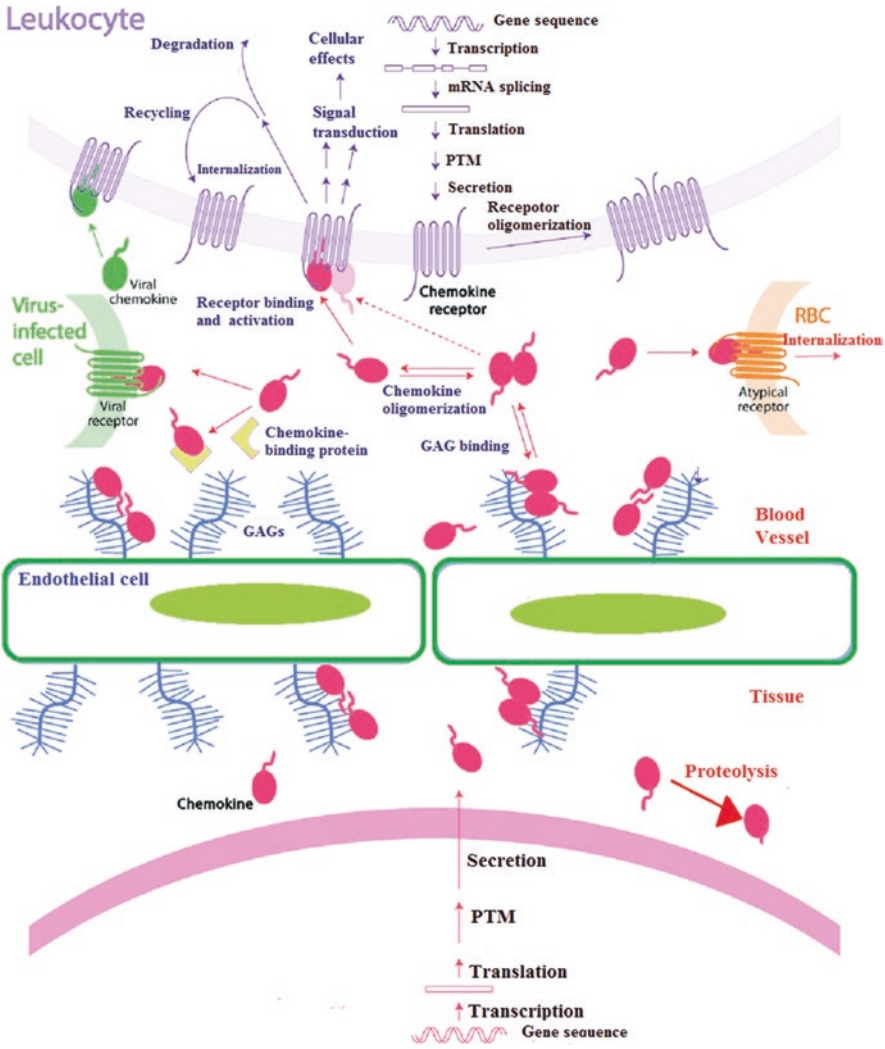

Fig. 1.4 An outline of the chemokine receptor network's regulatory processes in a diagrammatic form

1.5.1 Chemokines: A Unique Instance of Complexity

Cytokines that exhibit a chemoattractant activity are called chemokines. About 8–15-kDa tiny, structurally similar cytokines make up the wide superfamily known as chemokines. They control cell trafficking of different leukocyte subtypes to damage sites and carry out numerous functions in both homeostatic and inflammatory mechanisms (Springer 1994; Mir 2015a, b, c, d). There are currently 18 known chemokine receptors (CKRs) and 42 known chemokines to exist among humans,

although this figure does not really compensate for a number of documented variations that may enhance their actual complexity. Based on the structural cysteine motif, which is located close to the NH2-terminus, the four subfamilies of chemokines are C, CC, CXC, and CX3C chemokines (Zlotnik and Yoshie 2000; Mir 2015a, b, c, d). In all, 20–95% of the identity of the sequence of amino acids is shared by chemokines. Even when the overall sequence identity is low, they all fold in a similar manner. The chemokine scaffold is made up of three β-sheets at the core and an α-helix at its C-terminus, followed by a region at the N-terminus and related to it by cysteine bonds. Chemokines attach to GPCRs (G protein-coupled receptors) with seven-transmembrane domains that are seen in several endothelial, leukocytic, and other cell types (Murphy 1994; Mir 2015a, b, c, d). These receptors let chemokines carry out their biological tasks.

Chemokines are often categorized as either constitutive (homeostatic) or inducible (inflammatory), depending on where and under what conditions they are expressed. Typically, induced chemokines are produced as a result of inflammatory events. The expression of constitutive chemokines, in contrast, occurs in the absence of an infection or injury and regulates a variety of processes, including cell movement in the fetus and embryo and homing of homeostatic leukocytes for immunological monitoring. The immune system has a number of cluster genes with notable allelic diversity, with the genes of the MHC serving as the human genome's really extreme instance. Diversification through the formation of many alleles is quite prevalent in evolution.

The chemokine system is a less well-known but incredibly instructive example of how an intricate gene network evolved to perform a wide range of interrelated tasks. In the next session, we will examine the relationships that already exist between the genomic organization of the various chemokine groups as mini-clusters and clusters and their associated role that will aid in a deeper understanding of the somewhat perplexing chemokine system.

1.5.2 Genomic Organization of Chemokines and Their Functional Complexity

Here, in this section, we will try to understand how the chemokine network uses redundancy and promiscuity to balance precise control, dependability, and robustness with phylogenic flexibility and great variability. A change in any one of the constituent parts has minimal effect on the chemokine network in order to maintain the overall function. Despite its wide range of variabilities, the chemokine superfamily has actually managed to maintain an increased level of robustness, largely due to the promiscuity and redundancy of its ligands and receptors in terms of binding (Mantovani 1999). The chemokine superfamily exhibits the maximum level of this complexity, which is a characteristic of all cytokines. There is no specific leukocyte population in which a chemokine is only active, and most WBC populations have receptors for a variety of chemokines. Additionally, the consequences of chemokines on the cell targets are redundant, and their interactions with receptors are

likewise highly promiscuous (this means that several ligands interact with more than one receptor, and the majority of the known receptors interact with several ligands).

There is a significant amount of functional overlap among several chemokines, even though redundancy among chemokines is not always present (it holds true for CKRs as well). Due to the robustness of the chemokine/CKR system, the primary biological activities may typically be maintained in the presence of an alternate set of CKRs or chemokines when one specific chemokine or receptor is deficient. The CXCL12/CXCR4 combination stands out as an obvious exception to this rule. The embryonic mortality of CXCR4 and CXCL12 lacking mice (Mir et al. 2023; Ma et al. 1998) is a striking illustration of the broad variety of biological consequences of chemokines, which play a role in angiogenesis, development of organs, angiostasis, and immunological control in addition to attracting leukocytes.

The secretion of particular sets of chemokines as well as the chemokine production is under temporal and spatial control, and they finely regulate the chemokines' function in homeostatic and inflammatory processes. This is true even though one chemokine may act on multiple cells, and the opposite is also true—one cell may respond to many chemokines (as well as the receptor expression of specific leukocyte subtypes is controlled). Comparing the characteristics of each of the several ligands for a single receptor raises the possibility that there are other methods to reduce redundancy (Devalaraja and Richmond 1999). There may be considerable differences between a pair of ligands that bind to the same receptor in their affinities to the receptor, their half-lives, their protease modification sensitivity, or in the distribution regions (e.g., the endothelium, extracellular matrix, etc.), their ability to cause receptor desensitization, and binding affinity to glycosaminoglycans (GAGs). Chemokines interact with both GAGs fixed on the extracellular matrix and surface of cells and soluble GAGs in addition to interactions with chemokine G protein-coupled receptors. The creation of haptotactic chemokine gradients is facilitated by chemokine immobilization via the GAG interaction, which also increases their concentration at the site of generation (Tanaka et al. 1993). It is interesting to note that a recent in vivo research using mutations of recombinant chemokines has suggested that for biological responses, selective binding of oligomeric chemokines to distinct types of GAGs is essential (Proudfoot et al. 2003), Interactions with GAGs may regulate the movement of cells and potentially offer an additional degree of specificity, beyond that specified by the involvement of the receptor.

Because chemokines can serve as antagonists of receptors as well, a new level of chemokine control has been revealed. Chemokines can exhibit dual action, which means that they can stimulate or attract a cellular population with a single receptor while inhibiting the stimulation and recruitment of a distinct population of cells with a separate receptor (Petkovic et al. 2004).

Decoy receptors, which are characterized as chemokine-specific proteins that bind ligands with a strong affinity on the cell surface, play an essential role in controlling the chemokine activity by regulating the levels of chemokines in the body (Comerford and Nibbs 2005). Three so-called "silent" receptors like CCX-CKR, D6, and DARC have been discovered, and each of them interacts with a particular

group of chemokines. Because successful inflammatory cell recruitment needs low blood chemokine levels and high tissue chemokine levels, this scavenging of chemokines is crucial for preserving chemokine homeostasis.

In conclusion, all of these processes appear to function together to improve the cell recruitment level of selection and, in a broader sense, to offer methods to exert fine control over the diversity, promiscuity, and redundancy of the chemokine network.

1.5.3 Chemokine Receptors

Conventional chemokine receptors (cCKRs) and atypical chemokine receptors (ACKRs) are two categories of molecules with a heptahelical surface, which interact with chemokines as shown in Fig. 1.5. Let us discuss the chemokine receptors in detail.

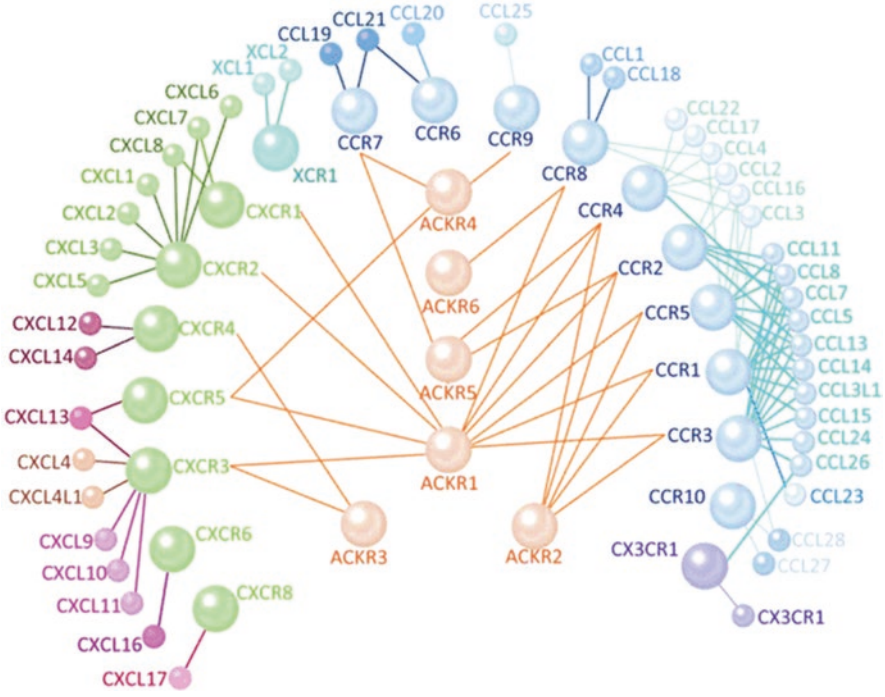

Fig. 1.5 The chemokine signaling network of humans (ref: https://www.researchgate.net/figure/Human-chemokine-signaling-networks-are-highly-promiscuous-There-are-25-receptors-and_fig1_339490694)

1.5.3.1 Conventional Chemokine Receptors

The movement of cells, their adherence, and/or a range of other biological reactions are eventually triggered by chemokine-bound cCKRs, which normally transfer impulses via $G\alpha_i$ G proteins and β-arrestins, which are pertussis toxin-sensitive. The N-terminus of the receptor and the extracellular loops are assumed to be the first points of contact between chemokines and their associated cCKRs. Addition of sulfated tyrosine residues, polysialylation, and glycosylation can increase the negative charge on these cCKR domains. N-terminal sulfated tyrosines improve chemokine binding to CCR2 (Tan et al. 2013), CCR3 (Millard et al. 2014), CCR5 (Farzan et al. 1999), CCR8 (Gutiérrez et al. 2004), CXCR3 (Colvin et al. 2006), CXCR4 (Farzan et al. 2002), and CX3CR1(Fong et al. 2002) and facilitate binding of HIV gp120 to CCR5 (Farzan et al. 1999). CCL21 appears to be released from an autoinhibited conformation by polysialylation of CCR7, which is necessary for CCL21 to activate CCR7 (Kiermaier et al. 2016).

After a chemokine binds to a cCKR, the cCKR's heptahelical bundle receives its unstructured N-terminus to cause a structural shift, which is converted into intracellular signals (Kufareva et al. 2015). Physical and allosteric connections between the two purportedly distinct ligand-binding sites are possible, and recent studies have suggested that complete receptor stimulation is probably achieved by subsequent chemokine–receptor interactions (Kleist et al. 2016). As a result, the traditional chemokine/receptor interaction with a two-site model is likely oversimplistic. The complicated signaling networks that are connected to chemokine receptors include, among others, the Janus kinase–signal transducers and activators of transcription (JAK–STAT) pathways, β-arrestins, and heterotrimeric G proteins (Kehrl 2006).

There are now 18 cCKRs that are identified by the kind of chemokine that they bind most frequently (XC, CX3C, CXC, or CC); the sequence of the discovery is denoted by a numeral after indicating the "receptor" by the letter R. For instance CX_3CR and XCR, 6 CXCRs, and 10 CCRs. Since the original CXCR7 was renamed ACKR3 (Bachelerie et al. 2014), GPR35, which has been recently discovered as a CXCL17 receptor and given the name CXCR8 (Maravillas-Montero et al. 2015). The CXCR3, CXCR4, CCR2, and CCR9 variants have been described with their signaling capabilities or ligand binding (Ehlert et al. 2004), indicating that cCKR transcripts are vulnerable to alternative splicing. The crystal structures of CXCR4, US28, CCR2, CCR5, and CCR9 have been resolved, and a chemokine receptor encoded by the cytomegalovirus as well as other biophysical approaches, significant detailed insights into the structure of the cCKR, chemokine binding, and antagonism mechanisms have been gained (Kufareva et al. 2017).

Several chemokines bind to various cCKRs, and certain cCKRs have a large number of ligands, making receptor specificity complicated. This is common among cCKRs and chemokines that are engaged in inflammation, whereas the ones that are predominantly engaged in movement of homeostatic cells have only one or two ligands that are devoted to a specific cCKR. The affinity of different chemokines to a given cCKR varies, and functional selectivity, or biased signaling, is developing as a significant characteristic of cCKRs. As a result, the specific pathways that a cCKR activates relies on the ligand it attaches to as well as the cellular environment

in which it does so (Zweemer et al. 2014). Currently, biased signaling in six cCKRs has been observed (Zweemer et al. 2014). Additionally, a few cCKRs can also be triggered by substances other than chemokines. For example, β-defensins can activate CCR6 (Yang et al. 1999). One of the most important CXCR4 ligands is the "alarmin" HMGB1 (high mobility group box 1 protein) (Tirone et al. 2018; Schiraldi et al. 2012), and, in response to the macrophage migration inhibitory factor (MIF), cells that express CXCR2 or CXCR4 move (Bernhagen et al. 2007).

CCKRs can be found as homodimers, just like other GPCRs. Higher-order oligomers can also form when they group together and form heterodimers with different functions along with GPCRs that do not bind chemokines (like opioid receptors), ACKRs, other membrane proteins, and other cCKRs (Hayasaka et al. 2015). Several chemokines have the ability to naturally block the cCKR (Xanthou et al. 2003). It has been reported that the non-GPCR phosphatidylinositol transfer protein (PITPMN3), which has six transmembrane domains, functions as a CCL18 receptor with relation to the invasion of tumor cells (Chen et al. 2011). However, CCL18 more commonly binds to the cCKR, CCR8, and controls leukocyte migration through this receptor (Islam et al. 2013).

Most likely, the chemokine network exhibits promiscuous receptor/ligand interaction developed to enhance infection-related WBC responses in order to prevent microbial subversion; heptahelical receptor, chemokines, and chemokine-binding protein genes are found in a large number of viral genomes that can interfere with certain aspects of the chemokine system in the host. They also have genes for chemokine receptors and chemokines, which either trigger or are triggered by chemokines of the host or cCKRs (Alcami and Lira 2010). Blood-sucking tick saliva is considered to contain chemokine-binding proteins that reduce inflammation at the bite site (Agrawal et al. 2004; Déruaz et al. 2008; Bonvin et al. 2016). HIV (human immunodeficiency virus) most commonly uses cCKRs: A crucial co-receptor that mediates HIV entrance into cells is CXCR4, CCR5 in particular, and, after binding to $CD4^+$ T cells, it can adhere to the HIV gp120 envelope protein (Wu et al. 1996). The CXCL12, CCR5, and CCR5 ligands prevent HIV from entering cells through cCKR downregulation or steric hindrance, and genetic diversity in these genes has a significant effect on HIV infection susceptibility and on the rate at which acquired immunodeficiency syndrome (AIDS) develops (Liu et al. 1996). In particular, having a $\triangle$32-CCR5 allele homozygote, which is inactive, offers significant protection from HIV infection, although heterozygosity for this allele is linked to a slower onset of AIDS in the majority of cohorts infected with HIV (Dean et al. 1996). Along with other medications, the CCR5 antagonist Maraviroc (Woollard and Kanmogne 2015), which is now used clinically, could prevent HIV-positive people from developing AIDS, and, transplanting stem cells from the homozygotes of the $\triangle$32-CCR5 gene that are matched for HLA proved to be highly successful in treating HIV-positive individuals (Hütter et al. 2009), as evidenced by the significant media attention gained. Moreover, additional care is needed because, following West Nile virus infection, $\triangle$32-CCR5 homozygosity enhances the risk of experiencing encephalitic symptoms and passing away from the illness (Glass et al. 2006),

most likely as a result of abnormalities in the trafficking of defensive WBCs into the brain (Glass et al. 2005).

Chemokine receptors are a target for other infections. Certain malarial parasites penetrate erythrocytes via ACKR1; *Toxoplasma gondii*'s cyclophilin-18 binds to and signals via CCR5 (Aliberti et al. 2003). Through CX_3CR1, the respiratory syncytial virus (RSV) infects a subpopulation of B lymphocytes using its glycoprotein G (Zhivaki et al. 2017); meanwhile, γ-hemolysin AB toxin (HlgAB) and leukotoxin ED (LukED) from *Staphylococcus aureus* target ACKR1 as well as different cCKRs, respectively, for lysis of red blood cells (RBCs) and destroy WBCs (Reyes-Robles et al. 2013). The virulence of these microbes is significantly impacted by these interactions.

1.5.3.2 Atypical Chemokine Receptors

The four ACKRs, which have a structural relationship with cCKRs, do not link to several, if any, of the signal transduction pathways that cCKRs activate (Bachelerie et al. 2014; Mir et al. 2020a, b). Numerous studies that detail signaling and related biological reactions mediated by ACKR3, regarding the ability of ACKR1, 2, and 4 to transmit signals following chemokine binding, are still debatable and/or ambiguous (Nibbs and Graham 2013). The cCKRs' second intracellular loop, which contains the canonical DRYLAIV motif and other signaling patterns that are normally present on the ACKRs' intracellular surface, may contribute to this in part (Nibbs and Graham 2013). However, ACKRs have a strong affinity for (Nibbs and Graham 2013) and like cCKRs, they can bind chemokines more effectively by using sulphated tyrosine residues (Choe et al. 2005).

All ACKRs seem to have a role in chemokine positioning, quantity, and distribution, which indirectly controls how chemokines interact with cCKRs (Nibbs and Graham 2013). For instance, on erythrocytes, ACKR1 buffers the blood's chemokine abundance (Mei et al. 2010), by delivering chemokines through endothelial cells in order to be presented to blood-borne leukocytes (Pruenster et al. 2009; Lee et al. 2003). This likely inhibits the desensitization of cCKRs on circulating WBCs resulting from chemokine overexposure. Surprisingly, ACKR1 can regulate the phenotype as well as the amount and phenotype of neutrophils in the blood, and it controls progenitor cells and hemopoietic stem cells in the bone marrow (BM); however, the fundamental molecular processes are yet unknown (Reich et al. 2009). The malarial parasites *Plasmodium knowlesi* and *Plasmodium vivax* hijack ACKR1 by interacting with it and allowing access into erythrocytes with the help of their Duffy binding protein (DBP) (Wertheimer and Barnwell 1989). In sub-Saharan African populations, total deletion of ACKR1 from erythrocytes is fairly prevalent, and the genetic variation in ACKR1 has a significant effect on sensitivity to these parasites' infections. A lack of ACKR1 in erythrocytes is also believed to contribute to benign ethnic neutropenia, and it may affect HIV infection by impairing CCR5 ligand regulation or impairing the expression of HIV through ACKR1 (Kulkarni et al. 2009; He et al. 2008); however, other investigations (Horne et al. 2009; Julg et al. 2009; Walley et al. 2009; Winkler et al. 2009) did not support this.

The chemokine scavenger ACKR2 is well-known. In addition to internalizing any chemokines it comes into contact with while being in the extracellular environment, it can move between cell surfaces on its own without signals induced by chemokine. Chemokines that have been internalized are released from ACKR2 and destroyed (Weber et al. 2004). The key regulatory roles for ACKR2 are played by placental trophoblasts, innate-like B cells, and lymphatic endothelial cells (LECs) (Wilson et al. 2017). By either heterodimerizing with CXCR4 and modulating its activity or scavenging CXCL12, ACKR3 plays a significant role in modulating the CXCL12–CXCR4 axis (Valentin et al. 2007). Adaptive immunological responses and CCR7-dependent dendritic cell movement can be regulated by scavenging of either CCL19 or CCL21 through ACKR4 (Comerford et al. 2010), and the thymus has been found to have a function for the ACKR (Lucas et al. 2015). Although less effectively than ACKRs, cCKRs may also eliminate extracellular chemokines, and chemokine/cCKR surface complex internalization is a crucial component for the regulation of cCKR. As a result, animals with a cCKR gene deletion may exhibit higher concentrations of the chemokine(s), which typically bind with that cCKR (Cardona et al. 2008). As a result, ACKRs are not the only receptors that can regulate chemokines by receptor-mediated internalization.

1.5.4 The Role of the Chemokine Network

Cellular movement, especially that of WBCs, is the chemokine network's activity that has received the most importance in terms of research. The activation of leukocyte cCKRs can cause a broad range of additional biological events, such as multiplication, viability, differentiation, cytokine generation, degranulation, and respiratory burst, as recently discussed elsewhere (López-Cotarelo et al. 2017) (Fig. 1.5). The direct antibacterial activity of certain chemokines is another feature (Wolf and Moser 2012). Additionally, a range of non-leukocytic cell types, such as endothelial cells, epithelial cells, mesenchymal cells, astrocytes, and neurons, are able to express cCKRs and react to chemokines in numerous diverse methods (Chamberlain et al. 2008). For instance, numerous chemokines directly control angiogenesis, with different subsets exhibiting either positive or negative angiogenic activity (Dimberg 2010; Mehrad et al. 2007; Qayoom et al. 2023b). It is noteworthy that non-leukocytic cancerous cells are able to develop cCKR expression and chemokine response, which can promote local invasion, distributed to lymph nodes that drain, and seeding of distant tissues by metastatic disease (Zlotnik et al. 2011). Generally considered the classical chemokine-driven mode of movement, chemotaxis as well as transcellular migration, haptotaxis, haptokinesis, and chemokinesis have all been shown to be regulated by chemokines. The primary biological event controlled by chemokine receptors and chemokines is cell movement. Chemokines have been found to either induce cell adhesion, which stops cell movement, or to control the collective migration of groups of cells (Bussmann and Raz 2015) (Fig. 1.6).

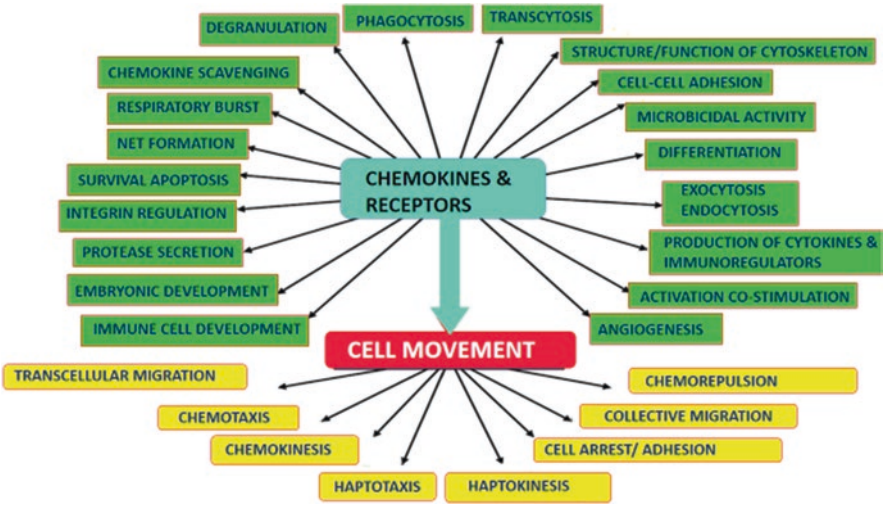

Fig. 1.6 The role of chemokines and chemokine receptors

Immunologically speaking, leukocyte movement is crucial. In order for leukocytes' immunological actions to be properly localized and directed, they must be present at the appropriate time and location. Leukocytes must continuously move from the bone marrow (BM) to other body tissues and back again in order to maintain immune monitoring. Rapid innate immune cell recruitment is crucial for eliminating pathogens, stopping the spread of bacteria, triggering inflammation, and aiding in tissue healing when tissues are damaged or contaminated. Leukocyte migratory pathways must be carefully orchestrated in order for an adaptive immune response to be developed, which is controlled, and for immunological memory to form afterward. Leukocytes are driven into and out of lymphatic vessels and blood as well as moved and positioned in the interstitial space by chemokines, which are fundamental to all of these activities. Immunological tolerance dissipates, protective immunological responses are impaired, and immunosurveillance fails in the absence of chemokine-directed leukocyte migration. However, autoimmune diseases, chronic inflammatory diseases, allergies, cancers, atherosclerosis, and many other illnesses that have an immunological or inflammatory component are also impacted by chemokine-directed leukocyte migration. Here, blocking chemokine-directed leukocyte migration may have therapeutic benefits (Mir et al. 2022a, b, c, d).

Recruitment of leukocytes can be adapted to the immunological requirements of tissues owing to the size of the cCKR families and chemokines. While numerous chemicals are needed for cells to exit the bloodstream and move about inside the tissues (Nourshargh and Alon 2014), in most cases, the expression of a specific cCKR allows a leukocyte to move when its ligands bind to that receptor. Genes for chemokine receptors are expressed in a range of mouse stromal cells and leukocytes. This information is largely preserved in humans and agrees with the

information on protein expression. While others, most notably Cxcr4, exhibit more consistent expression, other cCKR genes, including Cxcr1, Cxcr2, and Ccr3 obviously exhibit highly restricted patterns of expression. Despite the fact that erythrocyte progenitors express Ackr1, ACKRs are mostly found in stromal cells (Duchene et al. 2017) and certain subsets of B cells (Sierro et al. 2007).

In order to link alterations in migratory potential and immune function, leukocyte activation can substantially alter the expression patterns of the cCKR protein. This makes sense if the newly acquired role calls for the cell to become localized to various tissues or microanatomical niches. Within the context of $CD4^+$ T cells, this is described further below. Similar to how different tissues or tissue domains exhibit varied chemokine expression patterns under homeostatic settings, when the tissue's immunological demands vary due to an injury or infection, it varies. For instance, the small intestine of mice expresses CCL25 in a constitutive manner (Kunkel et al. 2000), and CXCL14, CCL8, and CCL27 are produced in significant amounts by the mouse skin (Islam et al. 2011). However, in case of damage, inflammation, or infection to any of these or even other tissues, a huge proportion of chemokines that are inflammatory are generated to control the quick recruitment of cells of innate immunity and the effector T cells' subsequent homing.

1.6 Cytokines and Chemokines for Cancer Therapy

During the past few decades, cytokine receptors and cytokines have been extensively studied and investigated as either cancer treatments or cancer targets. Strong preclinical rationale supports therapeutic strategies to enhance the growth inhibitory and immunostimulatory effects of interleukins and interferons, including IL-7, IL-12, IL-2, and IL-15, or to inhibit the inflammatory and tumor-promoting actions of cytokines like IL-6, TNF-β, and IL-1β. It is now well-established that in all human cancers there is altered and dysregulated cytokine expression and that exploring the use of these cytokines is the right way forward (Mehraj et al. 2021a, b; Qayoom et al. 2021). These findings have prompted clinical trials of several cytokines or cytokine antagonists, revealing relevant biological activity but limited therapeutic efficacy. An increased understanding of the tumor microenvironment and the advent of more effective immunotherapies have presented new methods to harness cytokine networks in the treatment of cancer, which include alleviating the immune-related toxicities of other treatments, using cytokine-based therapies to enhance the activity, and targeting early-stage cancers (Mehraj et al. 2021a, b; Mir et al. 2022a, b, c, d). As we know, most cancers express a complex array of chemokines that influence the local microenvironment by stimulating angiogenesis and recruiting stromal cells. The discovery of chemokine receptors on tumor cells has led to the assumption that the chemokine network system may be involved in cancer cell growth and survival and also the possible development of site-specific spread of cancer.

Therefore, understanding the chemokine networks and their receptors in tumors will enable manipulation of this system. Both chemokines and their receptors

represent targets for therapeutic intervention either with small molecule antagonists or antibodies. However, due to the complexity of the system, and the number of chemokines and chemokine receptors that are expressed by normal cells, issues still remain regarding whether systemic or local drug delivery is preferable and if one chemokine or chemokine receptor is targeted, then whether the redundancy of the system will compensate. Furthermore, challenges like the pleiotropic, redundant, and often conflicting actions of many cytokines and delivery methods remain. However, the lessons learnt from the initial trials of single-agent cytokine-based therapies and subsequent efforts to better utilize such agents for the treatment of solid tumors are going to play a vital role in this regard.

1.7 Conclusions

Pure recombinant cytokines or their inhibitors have shown some effectiveness when used clinically to treat cancers, infections, and autoimmune diseases, and numerous trials are still ongoing (Mehraj et al. 2021a, b; Qayoom et al. 2021). It should be possible to develop more effective therapeutic approaches with a deeper understanding of the cytokine network and how it is abnormally regulated in autoimmune illness and cancer (Mehraj et al. 2021a, b; Mir et al. 2022a, b, c, d). The body produces cytokines in tiny amounts (in the picometer range), they have dynamic secretion processes, and they have short half-lives, all of which make accurate detection of them difficult. The immune system is modulated by cytokines, which are organized into intricate networks. Depending on the cytokine, a biological activity may be influenced by an antagonistic, additive, or synergistic effect from other cytokines. It is difficult to measure cytokines in real time as they react to the microcellular environment because of how complicated the network is. The tremendously intricate chemokine network is made up of numerous interdependent ligands, receptors, and regulatory proteins involved in a wide range of overlapping and distinct physiological functions. The primary biological goal of this process is to induce migration, especially of leukocytes, although its effects go far beyond this. The chemokine network makes a significant physiological contribution by performing critical functions in growth, homeostasis, immunological surveillance, infection prevention, inflammation, innate and adaptive immunity, and tissue repair. Chemokines and their receptors have a role in almost all diseases. Despite the slow progress of clinical translation, medicines that target cCKRs have reached the clinic. Also, there is still a lot to learn, and chemokines and their receptors will probably continue to be discussed often in research papers for many more years to come.

Further Reading

For further reading, some textbooks mentioned below also provide further understanding of the cytokine and chemokine network.

The Cytokines of the Immune System: The Role of Cytokines in Disease Related to Immune Response by Zlatko Dembic

The Cytokine Network and Immune Functions by Jacques Thèze

Chemokines and Viral Infection by T.E. Lane

Cytokines and Chemokines in Infectious Diseases by M Kotb and T Calandra

The below links for video lectures may also be helpful:

https://youtu.be/TAqO0Mq19JQ

https://youtu.be/6wEnYfvoBqk

https://youtu.be/A9fRRCUlMms

https://youtu.be/zIWvmCsKr34

For more insights into the topic, we would suggest detailed findings from the books of Mir et al. (2022a, b, c, d) https://doi.org/10.1016/C2021-0-02565-7 andhttps://doi.org/10.1016/C2022-0-00074-X, Mir (2021) https://doi.org/10.52305/WXJL6770, Mir (2015a, b, c, d) https://doi.org/10.1016/C2014-0-02898-5,and from the cancer.net website on the following mentioned links:

https://www.cancer.net/cancer-types/breast-cancer/types-treatment

https://discovery.ucl.ac.uk/id/eprint/1472740/

https://www.jmedsciences.com/doi/JMEDS/pdf/10.5005/jp-journals-10045-00138

For diagrammatic illustrations and descriptive tables, see Lazzeroni (2012): http://www.eurekaselect.com/article/49928

See video links on the overall status of cancers, various types of cancers, and the current possible new treatment options available at

https://www.sciencedirect.com/science/article/pii/S205970292032278X

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Cytokines and Chemokines in Tumor Growth and Progression

2

Manzoor Ahmad Mir, Mina Rashid, and Nusrat Jan

Abstract

The significance of cytokines as signaling molecules, which has long been acknowledged, is expanding as a result of their connection to malignancies and the control of the immune system's antitumor activity. They significantly affect cellular activities concerning viruses and other diseases as well as inflammation. They are a vast family of tiny associated proteins that, upon connecting to the appropriate receptors, play crucial roles in immunological responses, proper physiology, and the evolution of new organs. These chemicals contribute to the regulation of immune cell signaling pathways as well as to the coordination and recruitment of immune cells to and from the organs. A form of this chemokine/chemokine receptor axis, in addition to the commonly understood antimicrobial immunity, also has a tumor-promoting role in numerous different cancers and is implicated in the development of repressive and protecting immunity. Consequently, they could be used as predictive indicators of different solid and hematopoietic malignancies. In addition, chemokines and their receptors play a significant role in practically all inflammatory disorders, including cancers, in one way or another. The use of novel medications for clinical assessment in the treatment of associated disorders relies on modifying the transcription of chemokines and their receptors on immune or malignant cells. The development and metastasis of tumors depend heavily on chemokines. Cancers, carcinomas, tumors, melanoma, pulmonary cancer, stomach carcinoma, acute lymphoblastic leukemia, colon cancer, non-small cell lung cancer, and non-Hodgkin's lymphoma (NHL) are only a few of the malignancies to which cytokines are linked. Numerous cancers have altered expression of several cytokines and their recep-

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tors, which results in abnormal receptor signaling. This chapter focuses on the functions of tumor necrosis factors (TNFs), interferons (INFs), and interleukins (ILs), which encourage growth, metastases, epithelial–mesenchymal transition (EMT), immune evasion, and vasculature of tumors.

Keywords

Interleukins · Interferons · Cytokines · Chemokines · Metastasis · Hematopoiesis · Angiogenesis

2.1 Introduction

Cytokines, a broad category of peptides or glycoprotein signaling molecules, are secreted by particular immune system cells. This class of signaling molecules mediate and control hematopoiesis, inflammation, and immunity. The Greek words "cyto" and "kinos" mean “cell” and “movement,” respectively. Cytokines are known to promote cell interactions during immune responses and stimulate cell migration to wound, infection, and inflammation spots. Throughout the body, cells with various embryological ancestries create cytokines. Other names are defined based on their alleged function, secretory cell, or intended target of action; “cytokine” is a broad term. For instance, the word “lymphokines” is another name for cytokines produced by lymphocytes. Since many lymphokines can influence leukocytic cellular responses in addition to being secreted by leukocytes, they are often referred to as interleukins (ILs) or cytokines. Monokines are cytokines that are released by monocytes or macrophages. Cytokines with chemotactic properties are known as chemokines. The following characteristics of cytokines allow them to coordinately and interactively regulate cellular activity. Pleiotropy is the ability of one cytokine to exhibit many functionalities. Different cytokines can regulate the same or related activities, which is known as redundancy. When two cytokines work together to enhance cellular activity, their combined effect is greater than the average of their individual effects, and this is known as synergism. Antagonism occurs when one cytokine's actions limit or wipe out the functions of another cytokine. Various cytokines are discussed and described in this chapter. IFNs (IFN-α, IFN-β, and IFN-γ), ILs (IL-1, IL-2, and others), TNFs (TNF-α and TNF-β), chemokines, and others will be grouped according to their kind, followed by a presentation of their function and a brief overview. The actions of several cytokines, including their pro- and anti-inflammatory effects, cellular immunological responses, function in hematopoiesis, and their role in cancer growth and progression, will also be briefly explained. Cells emit cytokines, which influence the actions of many adjacent cells and occasionally produce cells on their own. Interleukins, interferons, lymphokines, tumor necrosis factors, and chemokines are examples of cytokines; hormones and growth factors are often not cytokines (Mehraj et al. 2022; Watford et al. 2003). A wide variety of cells, such as immune cells like macrophages, T lymphocytes, B lymphocytes, and mast cells, together with endothelial cells, fibroblasts, and diverse stromal cells,

release cytokines; a particular cytokine may be released by more than a form of the cell (Mir 2022). Cytokines influence the ratio between adaptive immunity and cell-based immunogenicity as well as the development, expansion, and activity of specific cell types. They operate via receptors and are crucial for immune function. Some cytokines work in intricate ways to either increase or decrease the activity of certain other cytokines (Prashant et al. 2013; Mir 2015a, b). Cytokines are viable therapeutic targets in many diseases (Kierszenbaum and Sztein 1994). Chemokines are cytokines that actively stimulate certain cell types (Groves and Jiang 1995; Mir 2015a, b). Cells express these chemokines one at a time. Various immune cell groups are switched on and off by some of these cytokines. A wide range of interleukins, interferons, and growth factors are among them. They have a crucial role in both wellness and illness, particularly in the host's reactions to infections, swelling, trauma, sepsis, malignancy, and reproduction. T cells are produced by the immune system as a result of interleukin-2 (IL-2). The immune-stimulating abilities of IL-2 have long been used to detect injuries or disease and alert additional immune cells to the area to aid in healing or fending off the invader. Chemokines are significant players in the inflammatory process and present a prospective target for novel medications that aim to control immune reactions (Van der Meide and Schellekens 1996). Additionally, cytokines function as cell-signaling protein complexes that are heavily utilized in signaling pathways. Proteins, peptides, and glycoproteins can all be used to categorize cytokines. The term "cytokines" refers to a broad and varied family of proteins made up of cells with various origins throughout the body (Mousa and Bakhiet 2013). Every mediator has a specific cell receptor corresponding to it (Fig. 2.1).

Stimulation triggers intracellular signaling cascades that change how a network performs. The generation of additional cytokines, an increase in the number of receptor proteins for several other molecules, or a reduction by their action through feedback inhibition can all occur due to the up- or downregulation of a variety of genes and associated transcription factors (Shizuo and Takeda 2004; Mir et al. 2022a, b; 2023). Tumors, Alzheimer's disease, serious depression, and other illnesses are all related to the negative effects of cytokines that are either high or altered. The existence of, or even the prognostic value of, inflammatory conditions implicated in autoimmune disorders can be determined by measuring the levels of several cytokines in plasma (Miller et al. 2009). A subgroup of cytokines termed “lymphokines” are released by lymphocytes, a class of immune cells. They are peptide intermediates that T cells generally create to control the immune system’s reaction by communicating among its cells. Lymphokines play a variety of roles, such as attracting macrophages and certain other immune cells to an infected area and activating them there to get ready to initiate an immune reaction. When lymphokines are present, moving lymphocytes can recognize them at extremely low concentrations and travel up the gradient to the site where an immune reaction is needed. In both regular and disordered physiological processes as well as immune resistance, cytokines carry out a range of regulatory functions (Mehraj et al. 2021a, b). They may be generated by a range of cell types and act on a range of cells. Their

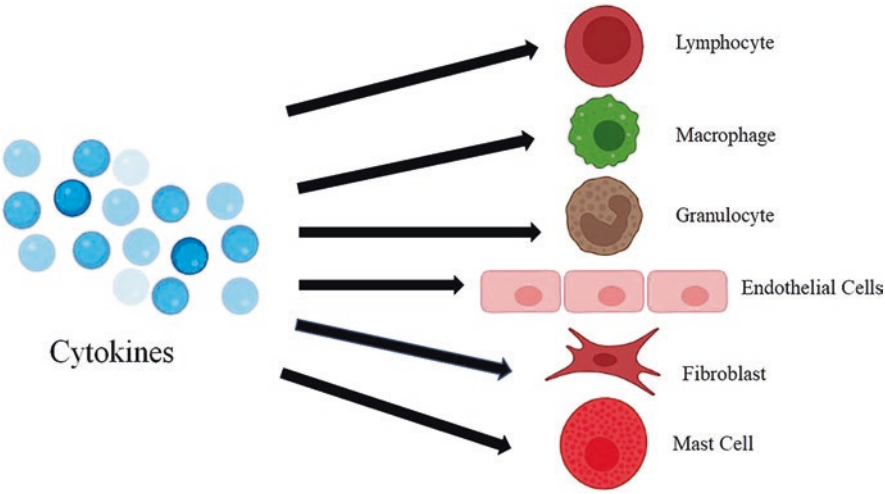

Fig. 2.1 Secretion of cytokines by various immune cells

impacts on cell growth, maturation, stimulation, and migration might be either repressive or encouraging. Furthermore, whether indirectly or via stimulating cells with cytodestructive potential, cytokines have a cytodestructive impact on cancer cells or infectious organisms. Every cytokine has a wide range of potential physiological consequences. Typical signal transduction processes like protein phosphorylation are engaged in the method of action of many cytokines, but the mechanisms by which one activity is preferable to the other when a particular cytokine is associated with a particular biochemical process are still poorly understood. However, understanding their function in other pathological conditions has helped us understand autoimmune diseases, allergies, and other widespread ailments. Cytokines are employed in a variety of medicinal contexts for both neoplastic and infectious disorders due to their extensive spectrum of activities. Cytokines are essential players not only in tissue homeostasis but also in the pathophysiology of numerous infectious diseases, according to several sources of data (Brombacher 2000). A careful balancing act between the anabolic and catabolic processes is necessary for the preservation of tissue homeostasis, a process in which cytokines play critical roles. There is a specific cell receptor for each cytokine. Then, the following downstream signaling pathways modify cell functioning. The creation of additional cytokines, a rise in the number of receptor proteins for other chemicals, or the inhibition of their action by feedback inhibition may come from the up- or downregulation of a variety of genes and related transcription factors. A cytokine's ability to affect a specific cell relies on several factors, including the cytokine itself, its cell surface abundance, the existence and enormous amount of the comparable receptor on the cell surface, and the downstream signals that are stimulated by receptor affinity (Funes et al. 2018). These last two aspects can differ depending on the cell type. Since multiple

cytokines seem to play similar roles, they are characterized by significant "redundancy." It appears to be a contradiction that those antibodies that cytokines attach to have a higher immune response than do the cytokines themselves. Reduced medical dosages could result from this. As mediators of both innate immunity (inflammation, chemotaxis, stimulation of macrophages, and natural killer (NK) cells) and acquired immunity (humoral and cell-mediated), cytokines can control cellular proliferation, division, and initiation. In response to inflammation and infections, cytokines are produced and released in a large part by the innate immune system. Innate immune cells are groups of white blood cells such as tissue-resident mast cells, macrophages, circulating dendritic cells (DCs), monocytes, NK cells, neutrophils, eosinophils, and basophils (Bryant and Monie 2012). These cells regulate the aggressive penetration of several pathogens, including bacteria, fungi, viruses, and parasites (Lacy and Stow 2011). Antibodies or complement receptor-mediated signaling as well as infections via a wide range of cellular receptors, such as recognition receptors like toll-like receptors (TLRs), can directly elicit the production of cytokines (McGettrick and O'Neill 2007). Lipopolysaccharide (LPS), a subunit of Gram-negative bacteria's outer coating and the primary cause of toxic shock syndrome and sepsis, is an extremely effective TLR4 cytokine production trigger. The majority of cytokines use the so-called transport apparatus, which is composed of membrane-bound and cytoplasmic protein substrates, to facilitate their passage through the membrane (Stenmark 2009). Cells actively produce cytokines either in the bloodstream or in tissues. These cytokines find specific leukocytes and connect with their receptors. Target cells respond in a certain way as an outcome of the interaction (McInnes et al. 1997). The transmembrane junction complexes termed "SNAREs" (Soluble N-ethylmaleimide-sensitive factor activating protein receptor) are some of the transport mechanism proteins in cytokine production that have received the most attention (Jahn and Scheller 2006). Vesicle-associated membrane proteins (VAMPs) and syntaxins, two subfamilies of the SNAREs, are classified based on the amino acid composition of their core SNARE domains as R-SNAREs and Q-SNAREs, respectively. The SNAREs contain subfamilies that are categorized. The four-helix spiral loop formed by Q- and R-SNAREs on both target and vesicle surfaces often pulls tethered membranes with each other for merging (Jahn and Scheller 2006). Numerous cytokines work through common signal transduction processes such as protein phosphorylation. The function in various pathological conditions has shed light on some systemic ailments, including autoimmune and allergy disorders. Cytokines are employed in a range of therapeutic situations for both neoplasia and infectious disorders due to their broad range of activities (Cohen and Cohen 1996). Immunohistological analysis of developed peripheral blood neutrophils reveals that peroxidase-negative compartments are where TGF, TNF, IL-6, IL-12, and CXCL2/IL-8 are kept (Beil et al. 1995; Mir et al. 2022a, b) .The SNAREs contain categorized subfamilies. Numerous cytokines and chemokines are produced by neutrophils, but their exact intracellular sites are unknown (Mollinedo et al. 2003). Additional granulocytes that contribute to the production of cytokines,

especially IL-4 in allergic inflammation, are known as basophils (Phythian-Adams et al. 2010). Mast cells, which are crucial tissue-resident innate immune cells in allergic and inflammatory disorders, release a large number of cytokines and chemokines, many of which are retained in their secretory granules as preforming agents (Phythian-Adams et al. 2010). It is clear that cytokines are created on traditional degranulation because of the rapid discharge of IL-4 and TNF following receptor-mediated exocytosis by bridging the cell surface compounds of immunoglobulin (Ig)E and IgG (Gordon and Tem 2011). Mast cell granules also include the CCR3 and CXCR2 subfamilies of chemokine receptors (Lippert et al. 1998). Eosinophils, which may produce more than 35 distinct cytokines, chemokines, and growth factors, are key effector cells in allergies and asthma (Hogan et al. 2008). It has been discovered that eosinophils transport TNF, IL-6, and IL-10 as well as T-helper (Th)1 (IFN, IL-12), Th2 (IL-4, IL-13), and other cytokines through a tubulovesicular system and tiny secretory vesicles that sprout from crystalloid granules (Melo et al. 2005). The tubulovesicular system and tiny secretory vesicles are used to transfer IL-4, a strong chemokine that elicits cellular responses in eosinophils (Spencer et al. 2006). In their intracellular granules, other innate immune cells also produce cytokine receptors. Neutrophils, in general, display the IL-10 receptor along with their unique granules and release IL-10 (Piskin et al. 2005). NK cells are cytotoxic lymphocytes that can kill tumor-causing or virally infected tissues. Similar to mast cells and eosinophils, NK cells mediate cytokine production without the use of lytic granules (Stinchcombe and Griffiths 2007). TNF is a strong pro-inflammatory cytokine that is released by a variety of innate immune cells, especially neutrophils, mast cells, eosinophils, dendritic cells, and NK cells (Beil et al. 1993). Activated macrophages are the main source of TNF. IL-6, IL-10, IL-12, and a variety of chemokines are among the additional pro-inflammatory and anti-inflammatory cytokines that macrophages produce and excrete. These cytokines are transported and released via constitutive exocytosis and occasionally move through the endoplasmic reticulum (ER) and Golgi complex all at the same (Shurety et al. 2000). One of the most potential mediators of inflammation and host responses to infection is the pro-inflammatory cytokine IL-1 (Relkin et al. 2005) (Fig. 2.2).

2.2 Cytokine Receptors

The transmembrane proteins that make up the cytokine receptors are made up of many components. They play a part in internalizing the stimulus. Typically, cytokines have a localized effect on the cells (autocrine in behavior) and on the cells nearby (paracrine action). Usually, they affect cells and tissues that are not at the same location as their source (endocrine nature). Unfortunately, some cytokines, particularly those that cause inflammation, like IL-1 and TNF, only work once they have traveled via the blood to far-off target tissues. Cytokines come in many different varieties, some of which have even been cloned. For instance, in humans and

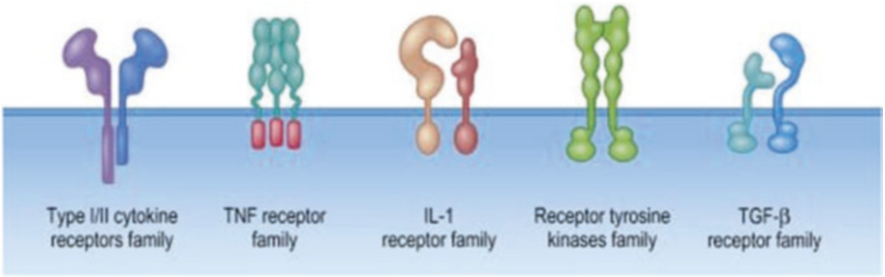

Fig. 2.2 Different receptors for different cytokine molecules

mice, 36 cytokines have been cloned, whereas only 19 are known from the porcine species, as only 19 pro-inflammatory cytokines and chemokines are known to exist in the swine species. Pro-inflammatory cytokines and chemokines such as TNF-α, IL-1, IL-6, IL-8, and others foster the emergence of illness. Along with immunomodulatory cells, cancer cells, tumor-associated macrophages, and stromal cells also generate these cytokines and chemoattractants. Chemokine family receptors connect with G proteins and have membrane-spanning helices. IL-8, macrophage inflammatory protein-1 (MIP-1), and RANTES (regulated upon activation, normal T cell expressed and presumably secreted) receptors are members of this family. The human immunodeficiency virus (HIV) typically enters macrophages or T cells using the chemokine receptors CCR5 and CXCR4 (Sprague and Khalil 2009). Two monomers make up hematopoietic cytokine receptors: a single carrier subunit and a cytokine-specific subunit (Pernis et al. 1995). In the granulocyte macrophage colony-stimulating factor (GM-CSF) subfamily, for instance, a distinct subunit has a weak specificity for binding GM-CSF, IL-3, or IL-5, but a common subunit signal transducer improves the cytokine's binding interactions. The subunits are encouraged to dimerize by cytokine binding, and once they do, they join forces with cytoplasmic tyrosine kinases to phosphorylate proteins that trigger messenger RNA (mRNA) synthesis. GM-CSF, as well as IL-3, influence hematopoietic stem and progenitor cells and stimulate monocytes. Along with IL-5, they promote basophil degranulation and eosinophil growth. The identical protein in the cytoplasm is phosphorylated by all three receptors. Competition for scarce supplies of the subunit can be used to explain antagonistic GM-CSF and IL-3 actions (Sprague and Khalil 2009). The signal-transducing chain is shared by the IL-2R subfamilies of IL-2, IL-4, IL-7, IL-9, and IL-15 receptors. They each have a different cytokine-specific chain. As trimers, IL-2 and IL-15 share an IL-2R chain. For IL-2, monomeric IL-2R has a low affinity, dimeric IL-2R has an intermediate affinity, and trimeric IL-2R has a high affinity. Activated T cells express the IL-2R chain (Tac) but dormant T cells do not. Low levels of IL-2R are constitutively expressed by resting T cells and NK cells (Sprague and Khalil 2009). High-affinity T-cell production is stimulated by antigen activation. IL-2R trimers and IL-2 release allow for the antigen-specific autocrine activation of T-cell proliferation. The closeness of antigen-presenting B cells and macrophages with their T-helper cells ensures that cytokines are produced

in the direction of and near the membrane of the target cell, maintaining the antigen specificity of the immune response. A flaw in the IL-2R family chain, which results in the lack of activity from this family of cytokines, is the root cause of X-linked severe combination immunodeficiency (X-SCID) (Pernis et al. 1995). Antagonists, or substances that attach to cytokines or their receptors, can prevent cytokine action (Ryffel et al. 1997). A particular antagonist for IL-1 prevents IL-1 from attaching to their receptors. Membrane receptor fragments may be lost during immune reactions when they compete for cytokine binding. Cytokine activity is also influenced by microbes. For instance, the Epstein–Barr virus (infectious mononucleosis) encodes a protein similar to IL-10 that inhibits immunological function in the host, whereas the vaccinia virus (smallpox and cowpox) generates soluble molecules that bind IFNs (Phillips 2002). Effector T cells have cell surface ligands called CD40L and FasL that the TNF receptor family members CD40 and Fas bind. The plasma membranes of B cells and macrophages express CD40 (Elgueta et al. 2009). B-cell proliferation and isotype switching are stimulated by T-cell CD40L binding to B-cell CD40. T-cell CD40L interaction with macrophage CD40 induces TNF secretion and increases IFN sensitivity in macrophages. When T-cell FasL binds to Fas, caspase proteases are activated, initiating the membrane-expressing cell's death. Activated lymphocytes that produce Fas can regulate the immune response by eradicating other stimulated cells. An immune deficiency condition associated with the development of a mutant Fas is characterized by excessive lymphocytic development (Yolcu et al. 2008) (Fig. 2.3).

2.3 Types of Cytokines

In response to immune cells, cytokines are also expressed by a range of other cells, including endothelial cells and fibroblasts. Cytokines are most commonly produced by monocytes and T lymphocytes. They formerly went by a range of names based on their derivation (lymphokines, which are generated by lymphocytes, their function (chemokines, interleukins, and interferons), or both. Interleukins and interferons are two examples of immunomodulating substances that have been referred to as cytokines (Lucey et al. 1996) (Table 2.1).

2.4 Interferons

A family of cytokines with a high level of expression is the interferon class. Types I, II, III IFNs are its three primary types. The 2 primary type I IFNs are IFN-α and IFN-β, which are also divided into 13 distinct subgroups, including IFN-1 to IFN-21 and IFN-β. These cytokines interfere with viral replication, and this is where the term “interferon” originated (Fig. 2.4). Natural killer (NK) cells’ ability to lyse viruses and the presentation of the major histocompatibility complex (MHC) class I on virus-damaged cells are both increased by type I IFNs, which also block viral replication and promote the growth of Th1 cells. This kind of interferon increases in

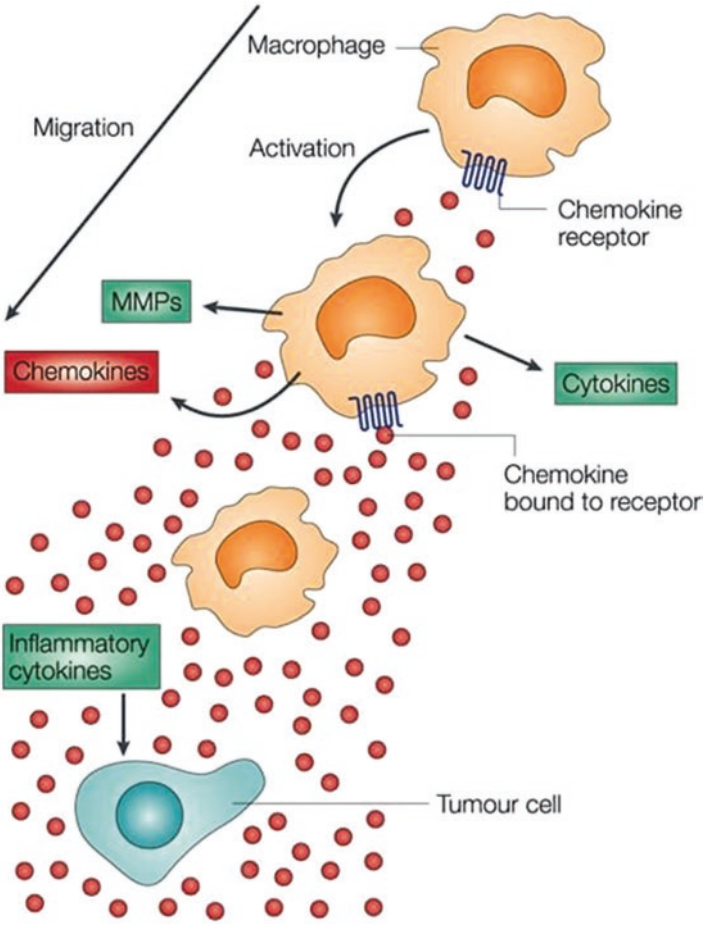

Fig. 2.3 Cytokines bind to their specific receptors and thus initiate the migration of tumor cells

Table 2.1 Specific mode of action by different cytokines

Cytokines	Actions
Interferon family	Antiviral protein
Chemokine family	Direct cell migration adhesion and activation
Tumor necrosis factor family	Regulate inflammatory and immune responses
Interleukin family	Variety of actions dependent upon interleukin and cell type
Hematopoietins	Promote cell proliferation and differentiation
Transforming growth factor-β family	Regulation of immune cells

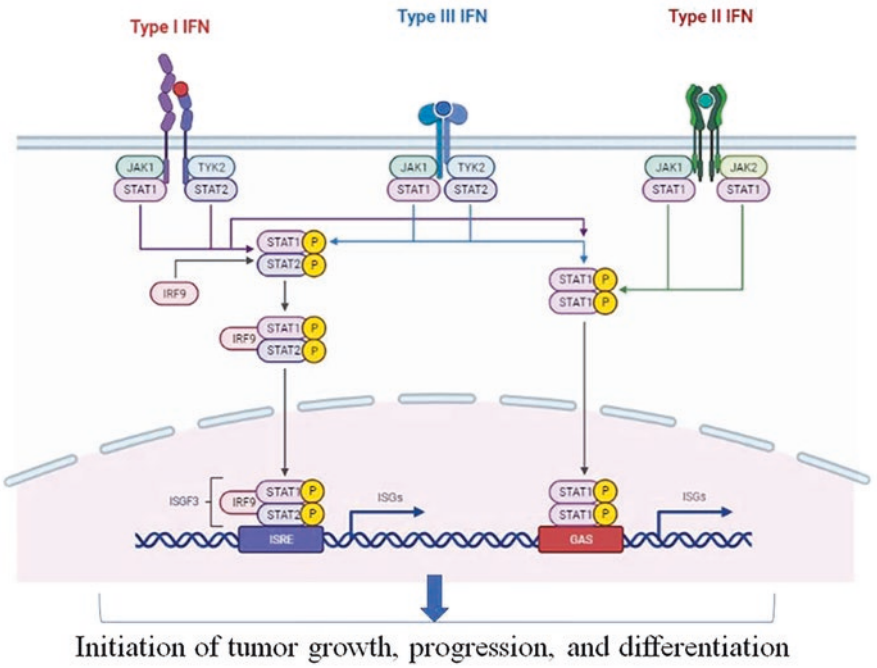

Fig. 2.4 Types I, II, and III interferons activate the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathways and initiate tumor growth, progression, and differentiation

abundance and is immediately found in the blood in an acute infection. IFNγ-, however, are the main proponents of type II IFNs. In both chronic and intrinsic defense responses, this cytokine is crucial for activating macrophages. Identical to type I IFNs, type III IFNs, also known as IL-28/29, play a significant role in the recipient's defense against viral infections. The very first component of the group of protein molecules is commonly referred to as an interferon. They are now widely recognized for the role they play in the innate immune system in modulating reactions toward viral infections (De Andrea et al. 2002). This role of the IFNs was initially recognised by Hoskins' experiments, which showed that rabbits with earlier herpes simplex virus infections were protected from acquiring the exact same strain in the future. Just several years following Hoskins' study, in 1937, Findlay and MacCallum demonstrated that virus-contaminated animals similarly exhibited resistance to impurities brought on by various viruses, thus supporting and enhancing the preexisting relevant data on IFN actions at the time. However, Isaacs and Lindenmann's 1957 study on cell cultures, which showed that virus-infected cells could create a peptide that could render cells susceptible to other viruses, supported their observations. Glasgow postulated in 1966 that interferon formation was not restricted to the initial viral infection and that this cytokine may even be important after a secondary

infection. As a result, by the end of the 1960s, the idea of "immune induction" of interferons was well-recognized. In the early 1970s, through two landmark investigations, it was established that the immune-induced interferons (now known as type II IFNs) and the traditional virus-induced interferons (currently known as type I IFNs) exist in two distinct groups of interferons that differ both physicochemically and physiologically. The designations IFN-α and IFN-β emerged in 1980 to denote the "classical interferons," which had been produced in homogeneous, unadulterated states. Even though the "immune-induced interferon" had not yet been isolated in its purest state, it was understood that this component stood apart from IFN-α and IFN-β, leading to its designation as IFN-γ. IFN-γ was first included in the IFN class due to its capacity to 'interact' with viral infections, which characterized the original meaning of IFNs, but the cytokine's obvious differences from IFN-α and IFN-β. In the past 10 years, the IFN-λ, a third form of IFN (type III IFNs), has been identified. Interleukins IL-28A and IL-28B (IFN-λ2 and IFN-λ3, respectively) and IL-29 (IFN-λ1) are other names for this kind (Platanias 2005; Fitzgerald-Bocarsly and Feng 2007; Billiau and Matthys 2009; Ferreira et al. 2018).

2.4.1 Interferons and Cancer

2.4.1.1 Type I IFN Signaling and Cancer

Numerous research studies have demonstrated a relationship between type I IFNs and cancer physiology (Parker et al. 2016; Ahmed and Cassol 2017). Moreover, due to the intricacy of regulation by numerous cellular external and internal signals, the pathway involved in this altered metabolism is incompletely defined and not well-researched (Hollingshead et al. 2007). The metabolic pathway is controlled by signaling pathways such as Janus kinase/signal transducer and activator of transcription (JAK/STAT), extracellular signal-regulated kinase/mitogen-activated protein (ERK/MAP), p38, and phosphoinositide-3/Akt (PI3/Akt) (Leonidas 2005). Furthermore, it has been demonstrated that interferon regulatory factor (IRF) has a significant impact on controlling cancer biology (Zhao et al. 2015). The JAK/STAT signaling system is crucial for controlling mortality, immunological response, and growth. It controls how initial response genes are expressed (Darnell 2012). The genes responsible for glucose regulation, hepatic glucose production, the Krebs cycle, and mitochondrial oxidative phosphorylation are affected by STAT1 and STAT3 (oxidative phosphorylation (OXPHOS)). In addition to this physiological route, STAT1 and STAT3 are crucial for controlling how lipid metabolism is regulated in cancer (Camporeale et al. 2014). Additionally, it has been demonstrated that they affect mitochondrial function and cellular respiration. Inhibition of peroxisome proliferator-activated receptor-gamma (PPARG) coactivator-1 (PGC-1), a master promoter of mitochondrial biogenesis, reduces the activity of the mitochondria (Sisler et al. 2015). Instead, STAT3 localizes in the mitochondria and engages in interactions with complexes I and II of the electron transport chain (ETC), enhancing the oxidation reaction (Zuckerkandl and Pauling 1965). Most critically, even though these adjustments to metabolic activities are required to generate effective

immunogenicity, alterations related to STAT activation may trigger pathogenic processes when IFNs are activated. Particularly, it has been demonstrated that STAT1-mediated signaling mediates carcinogenesis and chemotherapeutic and ionizing radiation by upregulating the expression of the genes involved in glucose metabolism, the Krebs cycle, and OXPHOS. On the other hand, STAT3-driven changes in mitochondrial metabolism cause medication tolerance in cancer patients by regulating the development of the mitochondrial transition pore (Qayoom et al. 2022; Jacinto et al. 2004; Poli and Camporeale 2015). To fully comprehend how these STAT-mediated processes support both functional and nonfunctional type I IFN signals, more research is necessary. Akt/mammalian target of rapamycin (Akt/mTOR) signaling has been demonstrated to be crucial for the control of type I IFN effector function in addition to the STAT signaling pathway. Different impacts of the two mTOR complexes (mTORC1 and mTORC2) are seen in type I IFN responses (Kai et al. 2011). Interferon-stimulated gene translation is significantly aided by mTORC1 (Kaur et al. 2007), whereas mTORC2 uses interferon-stimulated response elements to carry out IFN-dependent expression of genes (Kaur et al. 2007). Likewise, mTOR systemically organizes metabolic activity regarding endogenous and external inputs (Saxton and Sabatini 2017). Additionally, it has been linked to pyrimidine synthesis, ribosome assembly, adipogenesis, lipid synthesis, and adipocyte signaling (Buel et al. 2013). Type I interferons and mTOR signaling, OXPHOS, fatty acid oxidation (FAO), and glycolysis have all been linked in past studies. IRFs, which mainly control immune cell growth and effector function, are regulators of interferon responses (Man et al. 2013). The most well-known IRF, i.e., IRF4, regulates the production of the key molecules necessary for aerobic glycolysis as well as lipid metabolism-related genes in lipogenesis and lipolysis stimulation (Routy et al. 2016). It also suppresses the transcription of these genes. Inflammatory macrophages are said to induce the glycolytic process when IRF5 stimulates the gluconeogenic gene and Akt. IRF expression anomalies and their connection to metabolic disorders such insulin deficiency, atherosclerosis, and hepatic steatosis have been the subject of numerous research studies (Sofi et al. 2022).

2.4.2 Type I IFNs

The latest research findings indicate that metabolites (succinate and citrate) and the enzyme pyruvate kinase M2 may play an important role in regulating immune function and inflammatory responses (Tannahill et al. 2013). These molecules operate as transcription factors and signaling molecules. Upregulated glucose metabolism is a key trait of type I IFNs in cancer metabolism (Le Bourgeois et al. 2018). The metabolic switch is crucial for the cell to produce adenosine triphosphate (ATP) as soon as possible to meet its energy needs. Type I IFN-associated shift and enhanced glucose absorption in fibroblasts are both influenced by PI3/Akt signaling (Fruman et al. 2017). Alternately, in human squamous carcinoma, STAT1 mediates aerobic glycolysis (Avalle et al. 2012). Additionally, many cancers have been found to display 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3) with

elevated levels (Yi et al. 2019). Additionally, the Warburg hypothesis, tumor growth, and development are influenced by the metabolic switch from OXPHOS to glycolysis (Lu et al. 2015a, b). The glycolytic shift in immune cells is associated with a reduced rate of mitochondrial OXPHOS in tumor cells (Pearce et al. 2013). The mouse L929 cell stimulated with type I IFNs had indications of decreased OXPHOS and ATP generation, which is similar to our results. Additionally, in contrast to normal people, $CD4^+$ T cells isolated from patients with multiple sclerosis who received IFN- had dose-dependent OXPHOS impairment. Patients' lower intracellular ATP production levels and altered therapeutic response to IFN- were found to be related to a one nucleotide variation (single nucleotide polymorphism (SNP)) in the mitochondrial biogenesis gene PGC-1 (Nishimura et al. 2011). However, another research indicates that cancer may experience high metabolic reprogramming. Cancers driven by IFNs may be context- and cell-type-dependent (Lewis et al. 1996). IFN-stimulated mouse plasmacytoid DCs (pDCs) are associated with elevated glycogen synthesis genes, which boost glycolysis, OXPHOS, and FAO to satisfy the cells' need for nutrition (Reyes et al. 2020). OXPHOS and FAO are upregulated by mTOR stimulation, which is crucial for establishing an immunological response. In contrast to functional T-cell activation, which has not been proven to change OXPHOS function, $CD8^+$ memory T-cell stimulation by IFN- is linked to increased OXPHOS in T cells. Moreover, tumor cells cause cancer-associated fibroblasts (CAFs), which are prevalent in the tumor stroma, to engage in aerobic glycolysis in the opposite Warburg effect. Pyruvate, lactate, and ketone bodies produced by CAFs enter the tricarboxylic acid cycle (TCA) cycle in tumor cells to produce mitochondrial OXPHOS. In actuality, these stromal cells are linked to tumors, such as tumor-related macrophages; therefore, tumor cells impact each other in terms of not only growth factors or cytokines, such as IFNs, but also dependency on metabolic pathways. Tumor-associated macrophages (TAMs) derive their ATP from OXPHOS rather than aerobic glycolysis (Ganeshan and Chawla 2014).

2.4.3 IFN-γ and Cancers

Although metabolic reprogramming of macrophages in malignancies has received much study, there is currently scientific concern about its relation to inflammatory cellular activity. Scientists have relied on 2-deoxy-D-glucose (2-DG), a selective antagonist of glucose, in the first step of the reaction, to understand the function of the Warburg phenomenon (aerobic glycolysis) in M1 macrophages. The induction of 2-DG was found to have a significant, dose-dependent impact on cell survival and ATP levels while downregulating aerobic glycolysis and mitochondrial OXPHOS. As a solution, they took advantage of galactose, which is converted into glucose-6-phosphate at an extremely slow pace and greatly lowers the throughput of the glycolytic process. Furthermore, it was found that the extracellular acidification rate (ECAR) levels decreased with little impact on the oxygen consumption rates (OCRs), allowing for a more focused assessment, thereby facilitating a more exclusive evaluation of the importance of glycolysis in M1 macrophages (Kursunel and

Esendagli 2016). IFNs altered macrophages into M1-type phenotypes regardless of the abovementioned circumstances, depending on the activation of surface markers and cytokines, including IL-6 and TNF-α Galactose, on the other hand, significantly suppressed IL-1 and hypoxia-inducible factor-1 (HIF-1) levels as well as nitric oxide (NO) synthesis and generation. This shows that aerobic glycolysis in tumors is highly specific and performs a substantial function for the HIF-1 and STAT1 gene transcription pathways in IFN-stimulated macrophages, which is as per current observations. According to this, IFN-triggered STAT1 phosphorylation in M1 macrophages was raised via the JAK/STAT1 pathway in the malignancy, and this activation was suppressed by employing 2-DG as a competitive inhibitor. Additionally, TAMs displayed an elevation in glycolysis, and the functional phenotype of the malignancy was reversed by inhibiting glycolysis with the competitive inhibitor 2-DG. Galactose strongly suppressed STAT1 phosphorylation, thus demonstrating the significance of the glycolytic pathway in the JAK/STAT1 system. Glucose by itself could not activate the JAK/STAT1 pathway in the absence of IFN-γ. These results emphasize the significance of the IFN-triggered upregulation in M1 macrophages in cancer metabolic alteration (Shen et al. 2018).

2.5 Tumor Necrosis Factors

The term "tumor necrosis factor" (TNF) refers to a cytokine that was first identified as a tumor necrosis-inducing molecule in 1975. Soon after, it was shown that immune system cells encouraged TNF expression. These discoveries were crucial for the later characterization of the TNF group and for more than 40 members of TNF and their receptor subfamilies and include the much more notable TNF-α and TNF-β as well as cellular proteins and cytokines with homologous sequences and a homotrimeric pyramidal configuration (e.g., the CD40 ligand, Fas ligand, OX40 ligand, glucocorticoid-induced TNF receptor-related protein (GITR) ligand, and other several proteins). Particularly, inflammatory responses are brought on by this class of cytokines interacting with their specific receptors. A type II transmembrane protein called TNF-α plays a crucial role in the formation of Peyer's patches and lymph nodes as well as in the upkeep of lymphoid organs. Lymphocytes are primarily responsible for stimulating TNF-α production. Although numerous receptors have been discovered over the years, TNF receptor (TNFR)1 (55 kDa) and TNFR2 (75 kDa) are the two that are the most well-known. These receptors are cellular membrane clippers, and while TNFR2 is mostly generated by immune system cells, TNFR1 is produced by the majority of human cells. It is crucial to note that TNFR2 has a greater affinity to TNF (Chu 2013; Mir et al. 2023). They are related to provocative reactions because cytokines trigger the mobilization of molecules that are necessary for the response when they bind to receptors. Numerous immune system cells, such as T cells, NK cells, monocytes, and macrophages, generate this cytokine. One of the mechanisms that lead to the generation of TNF is the lipopolysaccharide (LPS) that attaches to pathogens and other stimuli. IFN-γ is highlighted as the trigger for TNF, along with other cytokines and toll-like receptors (TLRs) (Beutler 1999) (Fig. 2.5).

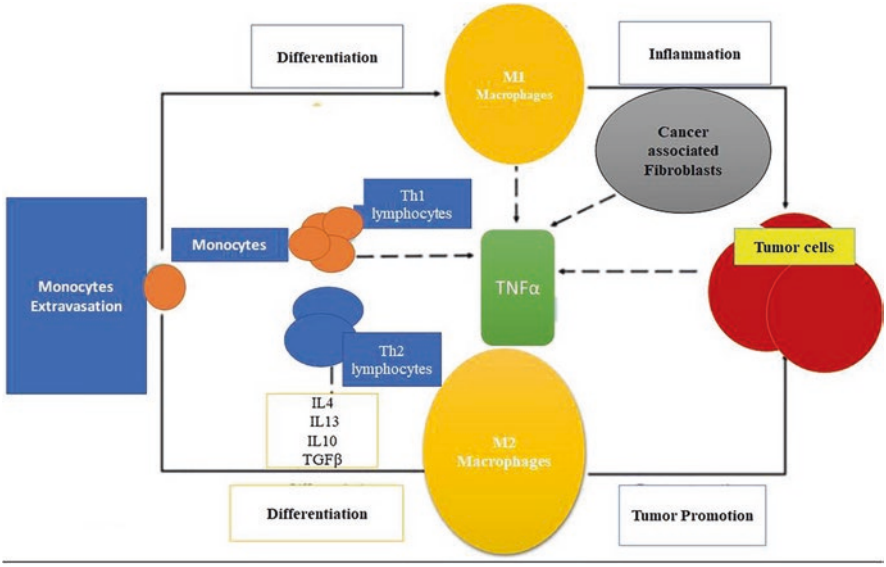

Fig. 2.5 TNF-α secreted by lymphocytes and by endocrine activity promotes inflammation and differentiation of cancer-associated cells

TNF is generated as a type II membrane protein that is arranged as it appears and is not glycosylated. A multi-subunit soluble cytokine is released from the TNF bilayer by metalloprotease TNF-converting enzymes by proteolytic degradation (a polypeptide that measures approximately 17 kDa and has a triangular shape). This is the expanded version that was found in blood plasma and provides the body with a strong capacity to dislocate, which is how it plays the endocrine's role. It is not clearly outlined, but it is conceivable to combine three of these circulating TNFs to create a 51-kDa peptide, which makes it easier for the cytokine to attach to three locations at once. TNF performs a wide range of physiological functions, involving immunological and inflammatory functions as well as the existence and demise of various cell types. Cytokines' primary job is to draw immune cells to the infected sites, trigger them, and then destroy microorganisms like bacteria and viruses. In this situation, TNF stimulates the expression of adhesion molecules by vascular endothelial cells, such as selectins and ligands for leukocyte integrins, which enables immune system cells to attach to the blood vessel wall. TNF also stimulates the creation of chemokines, which enhance leukocyte affinities to their ligands, the expression of IL-1, and the activation of immune system cells' microbicidal capabilities, which operate as a balance to the inflammatory response. If just small amounts of TNF are available in the area, despite its significance in the inflammatory process, then the localization of the disease may be compromised (Camussi et al. 1991). It is understood that TNFs contribute to the well-established inflammatory reactions of several autoimmune disorders, like rheumatoid arthritis and inflammatory bowel disease. Numerous autoimmune, neoplastic, and other disorders are caused by abnormalities in this manufacturing. In these circumstances, pharmacological medicines that attack TNF are used to address these disorders, with the goal

of either decreasing the range of TNF molecules present or blocking their receptors and also inducing apoptotic cell death, which can be cytolytic, which aids in the necrosis of tumor cells. TNFR1 stimulates the migration of expiry signaling proteins such as Fas-associated protein with death domain, TNFR-associated factor (TRAF)-1, and TNFR-associated death domain protein (TRADD), which, in turn, activates the apoptosis pathway. Additional proteins, such as pro-caspase-8, which when triggered activates caspase-3/6/7 and other cytosolic substrates, is produced by these intracellular proteins. Through their interactions with latent DNAse, these proteins cause DNA fragmentation and cell death. Evidence also indicates that TNF can maintain tumors and drive tumorigenesis, which is the opposite of what has previously been described by genetic abnormalities and its process of healing (i.e., genotoxic potential). This is conceivable as a result of nuclear factor-kappa B (NF-kB) activation in tumor cells and increased secretion of the tumor-promoting cytokine IL-6, which both help cancer cells spread and bypass the immune system's defense. TNF also triggers further cellular processes and functions. Due to decreased vascular muscle tone and myocardial contractility, this cytokine may induce a severe increase or a reduction in blood pressure when generated in large quantities, as in the case of a serious disease. TNF can also lower blood sugar levels and induce intravascular thrombosis in excessive quantities (by decreasing the anticoagulant capabilities of the endothelium). TNF is also referred to as an endogenous pyrogen since it causes fever by inducing the production of prostaglandins by hypothalamus cells (Willrich et al. 2015).

2.6 Interleukins

A category of associated proteins with a variety of forms and roles is known as interleukins (ILs). These proteins interact with receptors and let leukocytes communicate with one another. They have a close connection to cell growth, immune response stimulation, and inhibition. Monocytes, macrophages, $CD4^+$ T-helper lymphocytes, and endothelial cells produce the majority of the interleukins “ILs” with a number is the term given to interleukins. Historically, the same IL has been referred to by other names. For instance, IL-1 was also known as T-cell replacement factor III, mitogenic protein, and lymphocyte-activating factor. The name interleukin was first used to standardize the terminology in 1979 at the second International Lymphokine Workshop. Since then, interleukins began receiving sequential names based on the time of their identification. There are currently 40 recognized interleukins, some of which are further subdivided into subgroups (such as IL-1 and IL-12). Based on commonalities in the receptor chains and sequences, as well as other physiological factors, these ILs are categorized into groups. In all, 11 cytokines make up the interleukin-1 family, including 7 agonistic ligands (IL-1α, IL-1β, IL-18, IL-33, IL-36α, IL-36β, and IL-36γ), 3 antagonistic receptors (IL-1Ra, IL-36Ra, and IL-38), and 1 anti-inflammatory cytokine (IL-37) (Kaiser et al. 2004). There were only two members of the interleukin-1 family at first: IL-1 α and IL-1β. More ILs with comparable structures and/or behaviors have been found over time and included

in the family. Each agonist in this family exhibits pro-inflammatory behavior. These cytokines have equivalent intron placement and have a common C-terminal three-dimensional structure with a characteristic trefoil fold made up of 12 strands joined by 11 loops. As such, it is reasonable to conclude that they most likely resulted from the doubling of a single-origin gene (Akdis et al. 2011).

Except for IL-18 and IL-33, all of the group components have genes located on the second chromosome in a 400-kb area in humans. In addition to IL-1Ra, none of the cytokines are released by the endoplasmic reticulum–Golgi pathway, even though they are all extracellular. The remaining family members' secretion mechanisms are currently unknown. These cytokines connect to receptors that are strongly linked to one another, and most of the genes that encode them are gathered in a small area of chromosome 2. Extracellular immunoglobulin domains and a toll/IL-1 receptor (TIR) domain are present in the cytoplasm of the receptors. Both IL-1 and IL-1 fix to the ligand-binding chain type I to remain active (IL-1R1). The accessory protein (IL-1RAcP), a co-receptor, is then drawn in, and the two combine to form a tetrameric complex. The enrollment of the connector protein MyD88 to the toll/IL-1 receptor (TIR), which is accompanied by removing the phosphate of kinases, the transmission of nuclear factor-kappa B (NF-kB) to the nucleus, and the appearance of inflammatory genes, initiates the signaling that will result in a variety of inflammatory events. They also have progenitor forms: IL-α and IL-β. The progenitor of IL-1 is found in the epithelial layers of the digestive system, in respiratory, hepatic, renal, and endothelial cells and in astrocytes. It can interact with IL-1R1 and start the signal transduction, which occurs basically after induction of apoptosis via necrosis (e.g., myocardial infarction and stroke). The IL-1 progenitor, on the other hand, is not functional and cannot attach to the receptor. For it to take on the active form, there must be a split (Sabat et al. 2010). Autoimmune, viral, progressive, and, notably, auto-inflammatory disorders are all closely associated with IL-1. Inherited flaws in the intrinsic inflammatory processes are a significant contributor to auto-inflammatory illnesses and typically manifest at a young age. TNF receptor 1-associated periodic syndrome (TRAPS) was the initial illness to be labeled auto-inflammatory. Other cases include Still's disease in children and adults as well as familial Mediterranean fever. With few exceptions, these categories of disorders react quickly to IL-1 blocking therapy. Many auto-inflammatory disorders have a condition of elevated IL-1 secretion. The activity of caspase-1 causes the progenitor to transform into an activated state. The deactivated variant of this enzyme is also present in tissue macrophages and dendritic cells; it must be converted by autocatalysis to remain functional (Akdis et al. 2011). Moreover, macrophages that are moving in human blood contain it in the activated state. Blood monocytes' strictly controlled production of IL-1 in healthy persons takes many hours. However, relative to healthy people, persons with auto-inflammatory diseases produce more mature IL-1, which exacerbates inflammation. A receptor antagonist, IL-1Ra, the same cells that create IL-1 and IL-1, also synthesizes it (monocytes, macrophages, dendritic cells, and others). Since there is no morphological change during IL-1Ra's interaction with the receptor, IL-1RAcP is not attracted. IL-1Ra controls how much

IL-1 is active. However,, it must be present in quantities that are roughly 100 times stronger than the agonist cytokine to effectively suppress the IL-1 action.

Similar to IL-1, IL-18 is generated as an inactive progenitor and must be processed by caspase-1 to become effective. Both IL-1 and its ancestor are found in practically all human body cells. When there is an imbalance between the quantity of IL-18 and IL-18 receptor protein complex, which is in charge of regulating the degree of IL-18 activity, disorders associated with IL-18 typically manifest. Like IL-1, this cytokine is typically secreted by apoptotic cells. Since it was shown to be an activator of IFN production, IL-18 was initially referred to as an "IFN-inducing factor." Therefore, IL-18 by itself does not significantly increase IFN- synthesis. It has to function in concert with IL-12 for it to occur. Along with inducing IL-2 and IL-13 synthesis in T cells and NK cells, IL-18 also stimulates TH1 and Th2 cell reactions. Encouraging the expression of the Fas ligand in NK cells, it also increases NK toxicity (Fasoulakis et al. 2018). Numerous autoimmune illnesses, myocardial infarction, metabolic disorders, and other conditions are impacted by IL-18. Cytokine IL-33 is immediately generated in response to cellular injury. It mostly affects inflammation and type 2 immunity. Keratinocytes, epithelial and endothelial cells, and monocytes all produce this cytokine. As a progenitor, IL-33 is created. The progenitor is functional and can be degraded by further proteolytic enzymes into more powerful forms. IL-36Ra is a receptor antagonist that prevents the stimulation of the receptor and interacts with IL-36, serving as a modulator. IL-36α, IL-36β, and IL-36γ are receptor agonists. Owing to their similarity to the first interleukin-1 relatives, these cytokines are classified as family members. Their IL-Ra and IL-1 similarity ranges from 20 to 52%. Additionally, the 12-fold, trefoil-fold basic structure of IL-36β and IL-36γ as well as the absence of a coding sequence are distinctive characteristics of the IL-1 family. To reach their activated state, all of these cytokines require an N-terminal treatment, although the enzyme that catalyzes this activity is still unknown. Because IL-36 cytokines are mostly present in skin cells, they are linked to many skin conditions, including psoriasis. After binding to the receptor (IL-36R and IL-1RAcP co-receptor), DCs are triggered and contribute to the splitting of T-helper reactions. In contrast to the other family members, IL-37 is a pro-inflammatory cytokine that lowers both acquired immune responses and innate inflammation. The epidermis, pharynx, esophagus, placental membrane, mammary gland, prostrate, and colon have all already been found to contain it. The human body expresses IL-37 in five distinct subtypes at various sites: IL-37a, IL-37b, IL-37c, IL-37d, and IL-37e. The majority of investigations have so far focused on IL-37b, with a 12β-strand motif characteristic of the IL-1 family and looks to be the most physiologically active. In addition to inhibiting dendritic cellular proliferation, IL-37 reduces the release of pro-inflammatory mediators like IL-1A, IL-6, CC chemokine ligand (CCL12), colony-stimulating factors (CSF-1 and CSF-2), chemokine ligand-13 (CXCL13), IL-1, IL-23A, and IL-1Ra. Since its discovery in 2001, IL-38 has been the most current component of the interleukin-1 family (Garlanda et al. 2013). It interacts with the IL-36R receptor, which is shared

by the IL-36 cytokines. Identical to IL-36Ra, it functions as an antagonist, though. As a result, IL-38 reduces chronic inflammation. IL-38 and IL-1Ra and IL-36Ra have 41% and 43% similarity, respectively. The epidermis, pharynx, thyroid, liver, fetal organs, and mucous membranes all contain this cytokine. Research on the characteristics and biological functions of IL-38 is ongoing. The very first component of the typical IL-2Rα-chain group, also referred to as the T-cell growth factor, is IL-2. Although DCs and natural killer (NK) cells can also generate this cytokine, $CD4^+$ and $CD8^+$ T cells are the largest producers. IL-2 is widely employed as a treatment for cancer.

Th2 cells, basophils, eosinophils, and mastocytes all release IL-4. It has two type II receptors, which fixes to both IL-4 and IL-13 and ends up with IL-4R and IL-13R1, respectively. Type I of the IL-4R only binds to IL-4 and is made up of CD124 (IL-4R), and type II of the receptor is made up of CD132. The human body is covered by these receptors. IL-4 is recognized to perform a variety of functions, directing aversion reactions and triggering the immune system's defense against extracellular parasites (B-cell class substituting to IgE). It is the main cytokine that promotes the growth of Th2 cells. Homeostatic cytokines include IL-7. T cells, B-cell precursors, and bone marrow macrophages contain it. Like its further relatives, its receptor (IL-7R) is made up of two units: the IL-7R and the general -chain fraction (CD127). Thymocyte success and advancement, as well as the maturation of T cells, NK cells, recollection, and naive B and T cells, depend on IL-7 (Kumari et al. 2016). Immunodeficiency, autoimmune conditions, and leukemia are caused by IL-7 abnormalities. Phagocytes and mast cells from asthmatic clients also generate IL-9 but to a lesser extent than do Th2 cells. The CD132 and IL-9R subunits make up its binding site, the IL-9R. As an incredible growth factor for mastocytes and T cells, IL-9 also inhibits the generation of IgE, cytokines by Th1 cells, and mucus generation by the respiratory epithelium. Th9, a newly found subgroup of functional T cells, is believed to be closely associated with the generation of IL-9. IL-9 has been linked to the prevention of helminthic diseases and allergy disorders. Hodgkin's lymphoma has increased levels of this cytokine, later IL-9 inhibitors are being researched as a probable action for this condition. IL-15 and IL-2 share a relatively similar makeup. The family-specific CD132 subunit, along with both IL-15Rα and IL-2Rβ, make up the receptor IL-15R. Keratinocytes and skeletal muscle cells both produce IL-15 in addition to cues that stimulate innate immunity; the epithelial tissues, skeletal muscle cells, monocytes, and activated $CD4^+$ T cells generate IL-15. IL-15 stimulates NK cell growth and acts similarly to IL-2 in terms of activating T cells, but it also affects $CD8^+$ memory cells, NK cells, and NKT cell homeostasis. IL-15 concentrations are higher in autoimmune diseases like celiac disease, psoriasis, and rheumatoid arthritis. T cells, NKT cells, and Th17 cells all generate IL-21. The IL-21 receptors, which comprise CD132 and IL-21R, are found at many locations across the human body. This mediator affects how B cells work and promotes the growth of $CD8^+$ T cells, NK cells, and NKT cells. Cancer treatment using IL-21 is actively being researched (Tanaka and Kishimoto 2014).

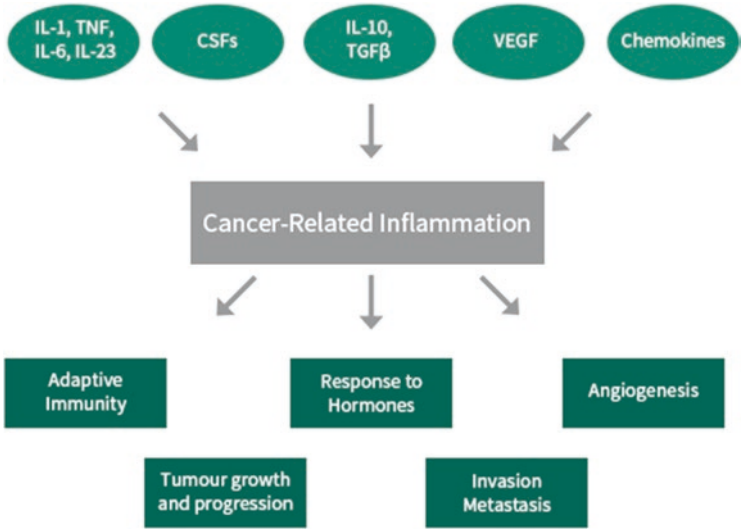

Fig. 2.6 Cytokines related to cancer-related inflammation, invasion, metastasis, angiogenesis, growth, and development

2.7 Cytokines as the Key Components in Cancer-Related Inflammation

Cancer risk is increased in various tissues by inflammatory situations. A complex conversation between cytokines and chemokines promotes vascularization, spread, adaptive immunity, and altered sensitivity to hormones and drugs. As a target for novel therapeutic and diagnostic approaches, cytokines related to cancer-related inflammation will pose a challenge to researchers and physicians in the future (Mir et al. 2020; Germano et al. 2008) (Fig. 2.6).

2.8 Cytokines and Cancer: TNF and Interleukin-1

Research on TNF that showed an improvement in cancer cell penetration capacity provides the first evidence for the antitumor role of inflammatory cytokines. TNF can directly affect tumor cells when found in low concentrations, interact with the chemokine system by inducing CXCR4, or promote the epithelial–mesenchymal transition (EMT), among other mechanisms. These results have provided the additional context required for the creation of experimental regimens using TNF antagonists as a cancer treatment. A result that at the time was connected to the initiation of adhesion molecules in target organs showed that IL-1 increased malignancy (Haabeth et al. 2016). The pro-inflammatory cytokine IL-1 induces a first wave of myc-driven vascularization in a pancreatic islet tumor model. Unexpectedly, it was subsequently discovered that IL-1 also plays a crucial role in the etiology of liver

cancer. Recent research has shown an unpredicted connection between steroid hormones and tumors. Selective androgen receptor modulators (SARMs) control the susceptibility to hormonal changes in prostate carcinoma, an androgen-dependent malignancy. The inflammatory cytokine IL-1 formed by macrophages in the tumor microenvironment (TME) alters SARMs from blockers to stimulators (Nicolini et al. 2006).

2.8.1 Cytokines and Cancer: IL-6

Large concentrations of IL-6 are produced by human colon cancer sufferers. The most prevalent form of hepatocellular carcinoma (HCC) frequently develops after years of ongoing activation brought on by either long-term infection (hepatitis C virus (HCV), hepatitis B virus (HBV)) or heavy alcohol use. These people, who are at a risk for HCC, typically have increased plasma levels of IL-6. Since high levels of IL-6 and certain polymorphisms in the IL-6 promoter area are hereditary risk factors for Breast Cancer (BC) and worse outcomes, IL-6 also plays a significant role in this disease (Kumari et al. 2016).

2.8.2 Cytokines and Cancer: IL-10, TGF-β, and IL-23

The restrictive cytokines IL-10 and TGF-β are typically seen in high concentrations in the TME's lower doses of IL-12, and poor stimulation of traditional Th1 responses is typical in late neoplasia. Genomic data suggest that cytokine IL-23, which is linked to IL-12, encourages the development of skin cancer. Through orchestrating neutrophil-initiated resistance to extracellular pathogens and inflammation, IL-23 encourages the development of Th17 cells. TGF-β is a tumor suppressor that is frequently engaged in human tumors and linked to metastasis while also operating as a diagnostic marker in certain circumstances. Lack of the type II TGF-β receptor is linked to metastasis and the activation of myeloid-derived suppressor cells (MDSCs) in a breast cancer model. MDSC activation is mediated by the chemokines CXCL5 and CXCL12, which, accordingly, act on the chemokine receptor CXCR2. Through their action as metalloproteinases, MDSCs promote metastasis. It is interesting to note that the elevated levels of TGF-1 were linked to chemokine-mediated mobilization in malignancies lacking TGF-RII (da Silva Alves et al. 2020).

2.9 Chemokines

Chemokines, chemotaxis cytokines, are a complex family of tiny cellular proteins that play a critical role in immune response homeostasis, in functions in the body, and in the formation of human organs. G protein-coupled receptors (GPCRs), commonly known as the traditional transmembrane domain proteins, connect to all chemokines, which are 8–12-kDa peptides that control cellular chemoattractant

movement and attachment, movement, and contacts among cells. Anomalous chemokine receptors (ACKRs), which are non-G protein-coupled, seven-transmembrane channels that do not support guided cell growth, may also bind chemokines extremely strongly. ACKRs primarily trigger arrestin-mediated mechanisms that restrict the accessibility of chemokines and modify the production of many other conventional receptors or a subsequent signaling cascade, thus playing an important role in chemokine bioavailability regulation in the immune response (Bonecchi and Graham 2016). Chemokines have a wide range of traits and purposes. Some chemokines, known as homeostatic chemokines, are released without even being stimulated and control immune cell migration during immunological observation, like the recruitment of lymphocytes to the lymph nodes. By engaging with antigen-presenting cells in tissues, homeostatic chemokines can regulate pathogenic invasion. Other chemokines, which play an important role during development, promote the growth of new blood vessels (angiogenesis), make it easier for cells to enter tissues, and transmit particular instructions for cell differentiation. Additionally, certain cells can generate chemokines in reaction to bacterial or viral infections; these chemokines can also be generated in reaction to noninfectious stimulation, such as the existence of kidney stones and silica inhalation, and they have significant biological effects. The majority of the chemokine class is made up of such a category of inflammatory chemokines, which are secreted by numerous various cell types. Inflammatory chemokines initially function as chemoattractive molecules for immune cells (such as neutrophils and monocytes), drawing them from the bloodstream to the area of the damage or physical injury and stimulating cells to elicit an immune reaction or accelerate tissue repair. They are regarded as essential for cellular penetration and subsequent expression of inflammatory reactions because of their cellular durability and binding accuracy, which helps the host's innate and adaptive immune response. Because of the range and continuous instruction of the chemokine ligand and its receptor synthesis by tumor cells, mast cells, and immune cells, the function of pro-inflammatory cytokines in tumor resistance is complicated. Cancer cells, tissue-resident cells (such as fibroblasts and endothelial cells), and invading immune cells that produce several chemokines and their receptors all make up the TME (Qayoom et al. 2023; Palomino and Marti 2015). Chemokines control the proliferation, invasiveness, and stem cell qualities of tumor cells in the TME; in turn, tumor cells produce chemokines that influence the microenvironment by encouraging leukocyte infiltration, regulating neurogenesis and fibrogenesis, and inducing vascularization. However, only recently have researchers begun to recognize how chemokines play an important role in controlling immune cell stimulation, localization, mobilization, morphological development, and activity. The fact that both protumor and anticancer immune reactions are influenced by the same chemokine system further complicates matters. The equilibrium among the various activities may be influenced by the disease's stage of development, immune cells' level of activity, and the existence of CXCL receptor sites on target tissues that are both restrictive and operational. A deeper awareness of cancer biology will result from additional research on the chemokine systems seen in cancerous tumors, but even more crucially, therapeutic strategies and approaches for cancer immunotherapy

will be suggested. Asthma, type 2 diabetes, atherosclerosis, rheumatoid arthritis, multiple sclerosis, and numerous inflammatory disorders other than malignancies are all influenced by chemokines, and their receptors modulate the immune system (Mir et al. 2022c; Raman et al. 2011).

2.9.1 The Structure of Chemokines

Approximately 20 GPCRs, 50 chemokine mediators, and 4 ACKRs make up the chemokine system, which is important for the body's immunological balance, inflammatory processes, infectious agents, and cancer growth. The majority of physiological investigations that have been recently published have demonstrates that the chemokine ligand axis adheres to the conventional GPCR initiation paradigm. After the peptide is triggered, the G protein separates from the associated binding site and starts a sequence of signaling processes that can finally result in a variety of reactions, including cell growth, viability, penetration, movement, and transcriptional regulation. All chemokines are tiny, and they share 20–50% of their sequences with other pro-inflammatory cytokines, indicating that their gene and peptide configurations are similar. Common chemokine molecules are created as peptide precursors, and, when they are secreted from lymphocytes, a signal sequence of 20–30 amino acids is separated from the protein's active portion (Kufareva et al. 2015). To construct their tri or tertiary structure, all chemokines have unique sequences of amino acids, typically containing four cysteines, which combine to create a Greek key shape. The first couple of cysteines are adjacent to the N-terminus, the third cysteine is in the center, and the fourth cysteine is next to the C-terminus of the mature protein. The N-ring, which is made up of roughly 10 amino acids, comes after the first 2 cysteines. G protein-bound chemokine receptors relay cellular signals by attaching to them. Chemokines start the separation of G protein components, and, when they attach to the seven-transmembrane GPCRs, which then activates phospholipase-C (PLC) that breaks the phosphatidylinositol 4,5-bisphosphate (PIP2) molecule into two subsequent messengers, namely, inositol triphosphate (IP3) and diacylglycerol (DAG), they subsequently trigger protein kinase C (PKC), the calcium ions to be released from within the cell, thus promoting cell polarization, attachment, and movement by IP3. Several intracellular signaling cascades, such as PI3 kinase (PI3K)/Akt and JAK/STAT signaling, are also set off by these interactions. These pathways drive active signaling components into the nucleus where they begin regulatory activities (Salanga and Handel 2011; Sofi et al. 2023).

2.9.2 Chemokine Types and Their Receptors

Chemokines are divided into four subgroups on the basis of quantity and position of their preserved N-terminal cysteines: CC, CXC, CX3C, and XC. The terminology of the receptors is primarily the same as that of the correlating chemokines, i.e., the ligand (CCL) binds to the (CCR) receptor and CX3C to (CX3CR). Chemokines are

also grouped into pro-inflammatory, homeostatic, or chemokines with both roles, based on how they behave in the body. Homeostatic chemokines, such as CCL17/14/15, are constitutively released in lymphocytes or other areas in suitable physiological circumstances and are vital for immunological management as they largely regulate the homeostatic movement and recruitment of diverse immune cells. Damage, sickness, and other pro-inflammatory stimuli cause the production of pro-inflammatory chemokines. Their primary job is to quickly draw leukocytes to the area of infection or lesion to work as acute agents (Lazennec and Richmond 2010). Depending on the various cells they affect, chemokines are indeed categorized into six main categories. Monocyte/macrophage chemokines, such as CCL2, CCL3, CCL5, CCL7, CCL8, CCL13, CCL17, and CCL22, work as important chemokines to draw macrophages to areas of inflammation. CCL1, CCL2, CCL17, and CCL22 are four chemokines related to the enrollment of T cells in inflammatory areas. Additionally, interferon (IFN)-stimulated chemokines CXCL9–11 and CXCR3 expression are elevated by stimulated T cells at inflammatory sites. Mast cell chemokines exhibit CCR1–5, CXCR2, and CXCR4 among other co-receptors on their membrane. Lung mast cells are attracted to and activated by the ligands for these receptors, CCL2 and CCL5. Various chemokines from the CC subclass, including CCL3, CCL5, CCL7, CCL11, CCL13, CCL24, and CCL26, are primarily responsible for directing eosinophil movement to various tissues. Eosinophils are generally the primary immune cells to be drawn into the lesion, where they are activated by the chemokines CCL5 and CCL11 when they attach to the CCR3 receptor on their membrane. CXC kinds of chemokines make up the majority of neutrophil chemokines. One such chemotaxis mediator for neutrophils in the TME is CXCL8 (IL-8), which induces neutrophils into the TME and activates their processing and downregulation (Griffith et al. 2014).

2.9.3 The Roles of the CCL2/CCR2 Signaling Axis in Tumor Progression

It has been established that CCL2 and its receptor CCR2 have a role in the initiation and spreading of some cancers, including prostate, breast, hepatic, lung, renal, pancreatic, and nasopharynx cancers. The CCL2/CCR2 signaling axis contributes to various phases of oncogenic progression, such as preserving the emergence and stemness of tumor cells at the spot of the main tumor and, when malignant tumors metastasize, encouraging the attack of cancer cells on nearby cells and the bloodstream and moving downward a particular chemotaxis staircase to the site of the metastatic disease. The remaining circulation tumor cells effectively colonize and develop after entering a new tissue or organ owing to connections with multiple TME elements. The proliferation of tumor cells may be aided by CCL2, which has been hypothesized to function as an autocrine or paracrine chemokine, and may be substantially inhibited by CCR2 blockers or PI3K blockers. By preventing autophagy, CCL2 can increase antibiotic confrontation in gastrointestinal cancer cells, and either CCL2 reduction or autophagy activation is effective in reversing drug

resistance in tumor cells (Xu et al. 2021). Similar to this, in vitro research revealed that upregulating CCL2 protected A549 cells against docetaxel (DTX)-induced cytotoxicity, whereas downregulating CCL2 reduced the survival of the cells and increased DTX-induced cytotoxicity. The stress response of cells that produce CCL2 may be a mediator of the cell survival that exists within lung cancer cells, making CCL2 a potential target for improving the therapeutic effectiveness of DTX on lung cancer. Additionally, CCL2 draws various immune cells together to create an immunosuppressive milieu that encourages the establishment of malignant cells associated with the microvasculature and fosters the spread of tumor cells. Downregulation of CCL2 by small-interfering RNA (siRNA) or antibody neutralization substantially suppressed DC recruitment, lowered $CD68^+$ macrophage infiltration, and decreased tumor development and metastasis in mouse melanoma and hepatocellular carcinoma models. Additionally, radiation significantly increases the migration of CCL2 and $Ly6C^+CCR2^+$ monocytes, which speeds up tumor growth and vasculature in pancreatic ductal adenocarcinoma (PDAC). When combined with radiotherapy, anti-CCL2 antibodies specifically prevent treatment-dependent monocyte/macrophage infiltration and slow tumor growth. However, different outcomes have been drawn from various studies' conflicting findings. Fader and others investigated the association between CCL2 production in tumor samples and clinical responsiveness to treatment and survival outcomes in 37 patients with primary carcinoma. According to their findings, higher levels of CCL2 production in ovarian tumors were linked to greater treatment response and better survival rates. The standard chemotherapy medicines paclitaxel and cisplatin were also shown to be more effective against ovarian cancer cells with increased CCL2 production in in vitro tests. It is worth noting that more CCL2 has been linked to better progression-free survival (PFS) and overall survival (OS) in individuals with squamous lung cancer; nevertheless, individuals with lung cancer cells with elevated CCL2 expression had shorter OS and PFS than did those with a less expression level (Mir et al. 2022b). Additionally, in vitro, results from one study showed that CCL2 might trigger neutrophils and facilitate the death of BC cells, whereas in mouse BC models, intranasal delivery of the CCL2 protein was effective in inhibiting tumor growth and was capable of encouraging tumor progression and dispersion by growing the penetration of $CD4^+$ T cells within the lungs (Li et al. 2013).

2.9.4 The Role of the CCL5/CCR5 Axis in Cancer Progression

It has been widely known that many tumor cells, including those in BC, acute lymphocytic leukemia, multiple myeloma (MM), Hodgkin's lymphoma, and rectal cancer, overexpress CCL5/CCR5. Normal ductal epithelium and benign breast tumor aggregates rarely exhibit CCL5, although tumor cell transition can result in its production. Furthermore, breast tissues from persons who have moderate breast illness or have undergone a significant breast reduction exhibit an overexpression of CCL5, but those from individuals with advanced triple-negative breast cancer do not (Velasco-Velázquez et al. 2014). Studies conducted in living organisms show that

BC cells induce mesenchymal stem cells (MSCs) to release CCL5, which then has an indirect effect on tumor cells (Qayoom et al. 2021). Additionally, they discovered that giving mouse BC cells and MSCs boosted lung metastasis and tumor cell colonization, indicating that CCL5 aids in tumor cells' capacity to spread across the body. Hematological cancers are strongly affected by the CCL5/CCR5 axis as well. For example, serum concentrations of CCL5 were higher in those with myeloid leukemia who might have a monocytic character or alterations in the inner triple amplification of FMS-like protein kinase 3. Furthermore, CCL5 expression is elevated when CD40 is cocultured with typical Hodgkin's lymphoma cells or with MSCs made from Hodgkin's lymph nodes (Aldinucci et al. 2020).

2.9.5 The Roles of CCL19/CCL21/CCR7 in Cancer Progression

It has been hypothesized that raising the contents of CCL19/21 among tumors could improve their treatment by improving the immune reactions to malignancies due to the crucial involvement of the CCL19/CCL21/CCR7 signaling axis in both the recruitment and initiation of T-cell-facilitated reactions. The very first of these is to intensify the immune reaction to the tumor by raising the CCL19/21 levels inside the TME. For example, intratumoral infusion of the CCL19 protein immediately elevated DCs along with $CD4^+/CD8^+$ T cells in the TME and promoted advanced production of inflammatory cytokine decreases due to anti-inflammatory substances such as interferons as well as chemokines CXCL9, CXCL10, IL-12, and granulocyte macrophage colony-stimulating factor (GM-CSF). According to this, intratumoral treatment of CCL21 raised the number of T cells, NK cells, and DCs within the tumor, decreased tumor growth, and enhanced the recovery duration of cancerous mice in a mouse neo-model of BC. Another strategy to increase CCL19/CCL21 protein amounts uses Polymerase Chain Reaction (pCR) product techniques to introduce exogenous genes into tumor cells (for example, lentiviral transfection). For instance, when mouse melanoma and mouse ovarian cancer cells were transfected to generate mouse CCL19 and then injected into C57BL/6 mice, the tumor growth was much slower than in the group lacking CCL19 production (Hillinger et al. 2006; Lu et al. 2015a, b).

2.9.6 The Role of CCL20/CCR6 in Cancer Progression

The development requires the CCL20/CCR6 axis, notably in mediating cancer metastasis and growth inside the TME. According to Ding et al., tissue activation of CCL20 was connected to tumor volume, heterogeneity, recurrence, and vascular invasion in tissue samples of liver carcinoma (HCC), and strong CCL20 transcription was linked to lower PFS and OS in individuals. Matrix metalloproteinase (MMP)2 and MMP9 secretion was increased by CCL20, which significantly amplified the bulk of triple-negative BC cell lines to invade; in contrast, intraperitoneal injection of an anti-CCL20 antibody prevented the expansion of skeletal metastases

in a mouse BC model (Ding et al. 2012). According to research, serum concentrations of CCL20 and IL-17A were more in Colorectal Cancer (CRC) individuals than in normal people, and the mixture of CCL20 and IL-17A characteristic pattern examination was able to successfully distinguish volunteers with benign CRC from those with malignant CRC (Lee et al. 2017). In the research data on resistance to the folinic acid, fluorouracil and oxaliplatin (FOLFOX) regimen, tumor cells' ability to release CCL20 enabled regulatory T cells (Tregs) to be drawn into the TME, which increased chemoresistance and was directly associated with lower mortality chances. Furthermore, epithelial–mesenchymal transition (EMT) is induced in practice by the CCL20/CCR6 axis, which enhances hepatocellular cancer growth and metastatic disease (Wang et al. 2019). M2-type macrophages activated with IL-4 exhibit CCL20 significantly (Liu et al. 2016). Additionally, it was discovered that CCL20 and CCR6 molecules were substantially produced in kidney cells, the lungs, cervix, and gastrointestinal, endometrial, and cervical cancer tissues, enabling tumor development and movement through autocrine or paracrine signals (Zhang et al. 2017).

2.9.7 Chemokines in EMT

During an epithelial–mesenchymal transition (EMT), a biological process, an epithelial cell establishes a mesenchymal cell phenotype with enhanced capacity for movement and penetration (Kalluri and Weinberg 2009). To proliferate, migrate, and invade, tumor cells acquire the mesenchymal phenotype. The chemokine CXCL8 is activated in cells that undergo TGF-driven EMT. The ligand CXCL8 and its target CXCR1 have restricted expression, which is associated with EMT, as seen by the greatly elevated levels of CXCR1 during EMT (Bates et al. 2004). TGF-1 treatment makes rat hepatoma cell populations more likely to exhibit the EMT phenotype. TGF- promotes CXCR4 transcription, and, phenotypically, mesenchymal cells have an advanced range of CXCR4. Cell movement is nevertheless restricted by CXCR4 reduction in such cells or treatment with a CXCR4 inhibitor. This research revealed that CXCR4 stimulation following TGF- treatment may be crucial for initiating EMT and hence promoting cell movement (Bertran et al. 2009). Another analysis confirms the importance of CXCL8/CXCR1 in initiating EMT. In human tumor cells that exhibit the transcription factor brachyury, upregulation of several chemokines, including IL-8, CCL5, and CXCL1, is associated with the activation of EMT. Additionally, the control of the CXCL8/CXCR1 axis is crucial for EMT. The researchers claim that hindering CXCL8 signaling may successfully prevent EMT by focusing on cells with a mesenchymal and competitive nature. A current investigation has found that the CXCR7 inhibitor CCX733 dramatically reduced bladder cancer's EMT, demonstrating the importance of CXCR7 in regulating EMT and in urethral tumor growth (Hao et al. 2012). The fact that the transcription factor SNAIL, which is essential for modifiable EMT, also regulates the synthesis of the chemokine CXCL8 in human colorectal carcinoma cells provides additional proof of the role of CXCL8 or IL-8 in the procedure of EMT in malignancies. These results validate the status of receptor molecule transmission in EMT, an

important stage before cancer cells metastatically transform (Hwang et al. 2011; Qayoom et al. 2023a) (Fig. 2.7).

2.9.8 Chemokines in Tumor Growth

Chemokines have an important role in the progress of malignancies. The MAPK/ERK signaling pathway is stimulated by chemokines that encourage the growth of tumor cells, gliomas, BC, ovarian cancer, chronic B-cell leukemia, and acute lymphoblastic leukemia. Small cell lung cancer, non-Hodgkin's lymphoma, and colon cancer are just a few of the cancers that the chemokine CXCL12 is increasingly connected to, according to various researchers (Hall and Korach 2003). Additionally, it has been demonstrated that the chemokines CXCL1, CXCL2, and CXCL3 contribute to the development of hepatic malignancy, melanoma, lung cancer, adenoma, and stomach carcinoma. The chemokine receptor CXCR4 is exclusively transcribed and aids in the growth of numerous cancer types. These cancers include melanoma, breast, endometrial, prostate, pulmonary, glioma, renal, and B-cell chronic lymphocytic cancers. Ovarian cancer, hepatocellular carcinoma, and chronic lymphocytic leukemia cells exhibit apoptosis when the chemokine receptor CXCR4 is inhibited, highlighting the significance of such a receptor for cell growth and survival (Messmer et al. 2011). Through growth-regulated oncogene (GRO)α and GROβ, another chemokine receptor, CXCR2, is essential for the development of esophageal cancer cells (Wang et al. 2006). Additionally, human gastric carcinoma cells exhibit the CXCR1 and CXCR2 chemokine receptors for CXCL8, which are important for the growth of cancer cells (Kitadai et al. 2000). The relevance of these

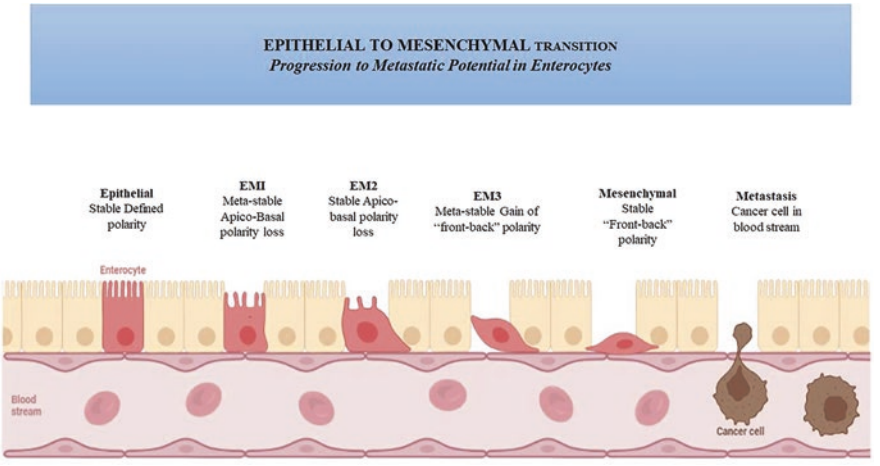

Fig. 2.7 Blood vessel containing monocytes, granulocytes, Tregs, and T-helper cells all binding to their specific membrane receptors recruit MDSCs and $CD8^+$ cells in the TME and lead to the metastasis, and angiogenesis, of tumor cells

receptors in the development and evolution of melanoma tumors was demonstrated by the oversynthesis of both CXCR1 and CXCR2 in human tumor cells, which accelerated cell expansion and attack in vitro and markedly improved tumor formation in situ. Colorectal cancer cells are stimulated by the chemokine ligand CCL20 to proliferate because the chemokine receptor CCR6 is present in this disease. Additionally, the prognosis of prostate cancer patients who expressed CXCR6 and its ligand CXCL16 was associated, and CXCL16 encouraged the proliferation of CXCR6-expressing tumors. In colorectal cancer, the chemokine receptor CCR6 is present, and activation by the chemokine ligand CCL20 encourages colorectal malignancy cells to grow. In colon cancer, the associated protein CCR6 is present, and activation by the ligand molecule CCL20 encourages tumor cell proliferation. Moreover, either CXCR6 or its ligand CXCL6 was linked to a better prognosis in prostate cancer, as was CXCL16's ability to accelerate the tumor growth that expresses CXCR6 (Fu et al. 2017). The chemokine receptor CCR10 is activated in tumor cells. The ligand CCL27 stimulates Akt and phosphatidylinositol-3-kinase in melanoma cells and stops apoptosis. CCL27/CCR10 thereby encourages the growth of melanoma cells by stimulating the PI3K/Akt pathway and inhibiting the host's antitumor action. The chemokines CCL19 and CCL21 are secreted by squamous carcinomas of the cervical cells, and, by activating CCR7, they promote the growth and spread of the tumor. Some receptors, on the other hand, prevent the growth of malignant cells. For instance, the chemokine receptor can block human liver cancerous cells from proliferating (Fig. 2.8).

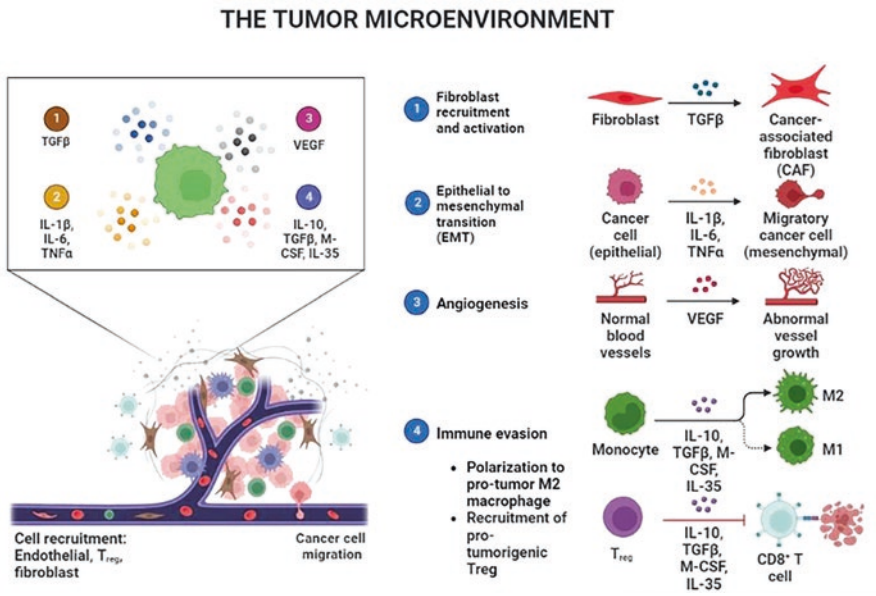

Fig. 2.8 The network in the TME consists of T cells, Tregs, and MDSCs, which downregulate the signaling pathway and contribute to the survival of the tumor cell

2.9.9 Chemokines in Angiogenesis

Angiogenesis, the method by which new blood vessels are created from preexisting capillaries, is essential for the development of tumors. Angiogenesis is significantly influenced by various chemokines and their receptors. Chemokines can attach to the surface receptors on the surface of epithelial cells or trigger the release of proangiogenic molecules or the opposite. The ELR motif found in CXC chemokines is crucial for fostering angiogenesis (Strieter et al. 1995). While chemokines without the ELR sequence encourage angiostasis, CXC chemokines with the ELR sequence are powerful angiogenic agents (Strieter et al. 1995). The angiostatic chemokines include CXCL4, CXCL9, CXCL10, CXCL11, and CXCL14, whereas the angiogenic chemokines are CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL8, CXCL11, CXCL 12, and CCL16 (Kiefer and Siekmann 2011). Melanoma tumors with lower ratios of the chemokines CXCL1, CXCL2, and CXCL3 experience less tumor development, signifying the relevance of these chemokines in angiogenesis (Luan et al. 1997). Endothelial cells display the chemokine receptors CXCR1, CXCR2, and CXCR4. Human microvascular endothelial cells with CXCR1 and CXCR2 knockdown saw decreased recruitment, penetration, and needle pattern generation, highlighting the significance of these receptors in angiogenesis (Singh et al. 2011) (Fig. 2.9). Additionally, the endothelial cells' CXCR4 chemokine receptor is activated by the chemokine CXCL12, which encourages endothelial cell growth and movement in ovarian carcinogenic cells. Endothelial cells also show the surface receptor CCR2. According to a new analysis, the CCR2–CCL2 axis is also important for angiogenesis (Weber et al. 1999; Izhak et al. 2012).

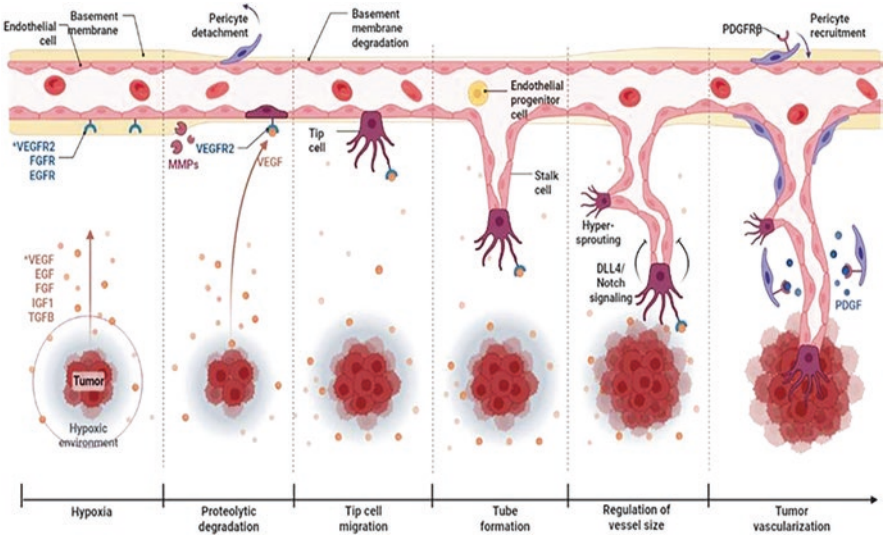

Fig. 2.9 New blood vessel formation (angiogenesis) in a tumor niche by endothelial cells, which line the inside wall of the blood vessels to nourish tumor cells and supply oxygen

2.9.10 Chemokines in Metastasis

Malignant tumor cells depart from the initial site of the tumor and spread to other tissues or locations throughout the organism by a procedure termed "metastasis." The path of metastasis is sequential, nonrandom, and organ-specific. The liver, lungs, central nervous system, lymphatic system, and stem cells are among the regions that frequently experience metastasis, whereas the kidneys, pancreas, and epidermis are less frequently affected (Zlotnik et al. 2011). One of the important characteristics that separate malignant tumors from benign tumors is the potential to metastasize, which is the primary cause of mortality for most solid tumors. Research has shown that whereas metastasis is a complicated method that involves numerous variables and small molecule regulators, chemokines and their receptors play an important role. Homeostatic chemokines control leukocyte movement throughout normal physiological events by luring particular lymphoid cell groups to particular tissues during innate or induced immune function (Homey et al. 2002). For example, in the bone marrow, CXCL12 attracts Hematopoietic Stem Cells (HSCs) that express the receptor CXCR4 and the ligand CCL27 stimulates the recruitment of leukocyte CLA^+ antigen. T lymphocytes express the receptor CCR10 on the epidermis. While immune cells' movement is generally regulated by chemokines, other types of cells can use these chemokine "pathways" by carrying the right receptor. Current findings suggest that to spread to distant areas, malignant cells simply co-opt these chemokine systems. Tumor cells must enter the hemoglobin, pass through vascular barriers, and then move throughout the body before extravagating at distant, nonrandom, organ-specific places, much like leukocytes do naturally. Since cytokine receptors and their ligands control leukocyte transportation, chemokines may also be important for triggering and controlling tumor cell movement. One of the most extensively researched chemokine receptor axes, the CXCR4/CXCL12 axis, is essential for a malignancy. Only the CXCR4 receptor is present in invasive BC, and they migrate to areas with high concentrations of the CXCL12 protein, also called Stromal cell-derived factor-1 (SDF-1) (Miller et al. 2009). The CXCR4 receptor is persistently elevated in advanced BC cell lines, lymph gland tumors, and hepatocellular metastasis, but the ratio patterns are unknown in normal epithelial cells. The lungs, brain, lymphatic system, kidneys, and marrow, which are the furthermost common regions of breast cancer metastasis, on the other hand, are the primary areas where its ligand CXCL12 is generated. In addition, inhibiting CXCR4–CXCL12 relations in vivo suggestively diminishes the invasion of lymph glands and lungs by breast cancer cells. Links between CXCR4 and CXCL12 also trigger migrating reactions that allow cancer cells to invade. Breast cancer CXCR4–CXCL12 receptor–ligand activities have resulted in actin polymerization, which enables tumor cells to efficiently move to nearby cells and spread (metastasize), promote spatial growth of breast tumor cells to drive proboscis establishment, and more. When CXCL12–CXCR4 linkages are reduced, anti-CXCR4 or anti-CXCL12 immunoglobulins' importance diminishes these relocation activities by approximately 63–76% and 60–62%, respectively (Miller et al. 2009; Bruce et al. 1970; Verschueren et al. 1994; Qayoom et al. 2023a).

Numerous investigations have demonstrated that organ-specific CXCR4 de novo production is enough to promote tumor penetration and distribution. The murine B16 melanoma cell line shows a considerably higher rate of lung metastases when treated with CXCR4. Additionally, the small molecule inhibitor T22 prevents the metastasis of CXCR4-B16 cells (Murakami et al. 2002). Murkami et al. approve the status of CXCR4–CXCL12 in facilitating cancer and reveal that CXCR4 activation is crucial for spreading Moreover, CXCR4 is active in a significant number of human-invasive lobular tumors. In prostate cancer, CXCR4 production increases the tumor cells' capacity to attack and spread in the context of the CXCL12 ligand, whereas CXCR4 suppression reduces this capacity (Arya et al. 2004). Contrarily, the existence of CXCL12 did not influence the capacity of healthy prostate epithelial cells to migrate. Integrins 5 and 3 also help CXCR4 promote prostate tumor cell attachment (Engl et al. 2006). Elevated concentrations of CXCR4 activity in human prostate tumors are linked to more regular tumor recurrence and malignant transformation, indicating that CXCR4 may be used as a prognostic indicator in addition to being a possible therapeutic target (Jung et al. 2011). It is interesting to note that prostate cancer stem cells (CSCs) that are $CD44^{+}/CD133^{+}$ also exhibit high levels of CXCR4. The CXCR4–CXCL12 pathway may inadvertently increase cancer by preserving and enhancing CSC stemness, as CSCs have the power to initiate spread and aid in the formation of new or recurring malignancies following primary treatment (Xiao et al. 2017). According to research, enhanced CXCR4 and CXCL12 expression encourages $CD133^{+}/CD44^{+}$ prostate CSCs' adherence to the extracellular protein fibronectin, which is crucial for the establishment of proximal regions and for the development of secondary malignancies. Moreover, CXCL12 stimulates the PI3K pathway and encourages the growth of $CXCR4^{+}$ prostatic CSCs. Therefore, via controlling cell contact, multiplication, pluripotency, and metastatic potential of prostate CSCs, the active CXCR4–CXCL12 pathway may indirectly enhance metastasis (Dubrovska et al. 2012; Qayoom et al. 2023b). Studies have shown that CXCR4 is crucial for the tumor progression of a variety of cancers, including breast, respiratory, colon, gastrointestinal, endometrial, prostate, pancreatic, melanoma, oropharyngeal, head and neck, urethra, acute lymphoblastic leukemia, bone, myeloma, and glioblastoma. CXCR4 promotes carcinoma growth of BCs in the pulmonary, hepatic, and lymphatic systems. CXCR4 enhances gastric malignancy and expands to the lymphatic system, according to studies. Additionally, research reveals that the presence of CXCR4 in esophageal cancer promotes the spread to the marrow and lymphatic system. In ovarian cancer, the CXCR4/CXCL12 axis may promote both lymph node and peritonitis metastases, according to more studies. CXCR4 may potentially be responsible for the metastasis of neuroblastoma to the bone and bone marrow as well as for that of osteosarcoma to the lung. As a result, a wide range of studies have shown CXCR4's crucial role in spreading. Leukocyte migration mediated by CCR7 is crucial for healthy immunological responses. Excited cell lines and naive T cells are drawn to the lymph nodes by CCR7, where they interact with each other and launch the adaptive response (Jafarzadeh et al. 2019).

2.9.11 CCL19/CCL21

The two chemokine ligands CCL19 and CCL21 bind to the CCR7 receptor; CCL19 stimulates T cells, whereas CCL21 guides naïve T cells moving to the secondary lymphoid organs (Yoshida et al. 1998; Kim et al. 1999). T-cell homing to secondary lymphoid tissues is markedly compromised by CCL21 loss or CCR7 gene suppression (Gunn et al. 1999). Numerous studies indicate that to spread to the lymph nodes, tumor cells co-opt the typical CCR7–CCL21 leukocyte pathway. There is a strong association between CCR7 transcription and lymph system metastasis, according to studies, which demonstrate that the mainstream original BC-invasive cells in the lymphatic system exhibit CCR7. Additionally, decreased duration and a worse prognosis are associated with greater CCR7 expression in breast cancer patients. There exists a link between lymph node metastasis and CCR7 transcription in several cancers, including epithelial cell cancer, glioblastoma, semi-cellular pulmonary, head and throat, gastrointestinal, and cervical cancers. Many human and animal breast carcinoma cells in culture, which ordinarily only metastasize to the lungs when CCR7 is not expressed, are induced to preferentially metastasize to the lymph glands by transcription of CCR7 (Cunningham et al. 2010). In nonmetastatic B16, the cancerous cell line's presence of CCR7 also promotes lymph node migration (Wiley et al. 2001). Intriguingly, overexpression of CXCR4 in murine B16 cells promotes lung metastasis, but overexpression of CCR7 in B16 tumor tissues leads to metastasis of the lymph glands. In vivo, proliferation toward lymphatic endothelial cells is also induced by CCR7 overexpression in the metastatic disease (Emmett et al. 2011). Through the PI3K/Akt signal pathway, high CCR7 transcription in human non-Hodgkin's lymphoma promotes metastatic growth (Yang et al. 2011). In human ductal adenocarcinomas, CCR7 overexpression also promotes lymph dissemination. According to a research study, CCR7 enhances the expression of matrix metalloproteinase-9 (MMP-9) in colon cancer, which encourages metastasis (Li et al. 2011). Further research demonstrates a correlation between coupled CCR7 and CXCR4 transcription and lymph node metastasis in breast cancer, gastric cancer, cervical cancer, and melanoma, among other cancers. Despite the fact that CCR7 is also expressed in healthy mammary epithelial cells, breast tumor cells persistently show high amounts of CCR7 together with CXCR4. Similar to CXCR4, CCR7–CCL21 connections lead to the development of invagination, actin folding, and directed infiltration of breast tumor cells, all of which promote the invasion and metastasis of tumor cells. Given that CCL21 and CXCL12 too are abundantly generated in the surrounding tissue and that receptor–ligand combinations, including both chemokines, enhance angiogenesis, it is likely that now the two ligands collaborate to encourage metastasis of the lymphatic system. CCR7 seems to be particularly significant in leukemia and lymphomas. Several lymphomas and leukemia have high CCR7 expression and frequently metastasize to the lymph nodes because they have lymphoid origins. Various myelomas or acute myeloid leukemia convey low concentrations of CCR7 and minimal-to-medium concentrations of CXCR4, whereas B-cell malignant tumors that encounter little tumor growth at the lymph glands, such as B-cell chronic lymphocytic leukemia (B-CLL), assert elevated

amounts of CCR7, which moderates B-cell entrance into the lymphatic system (López-Giral et al. 2004).

2.9.12 CCR9–CCL25

CCR9 plays a role in lymphocytes and mucosal resistance and translocation while T-cell maturation is in a physiologically normal state (Zabel et al. 1999). The intestinal tract and thymus both generate their ligand, CCL25, also known as TECK (Wurbel et al. 2000). Research shows that CCR9–CCL25 also drives malignant spread to the intestine because migration of CCR9-positive lymphocytes to the intestinal tract depends on the chemotactic effects of CCL25 and the activity of integral heterodimers. The bulk of gastrointestinal tumors, which are uncommon and make up only 2% of all gastrointestinal cancers, are malignant metastases (Gill et al. 2001). CCR9 is extensively shown in melanoma cells and small intestine metastases, according to research on malignant metastases (Letsch et al. 2004), indicating that melanoma cells are operational in the activation of CCR9 and facilitate movement to the intestinal tract (Amersi et al. 2008). CCR9 is stated by numerous BC cell lines (Johnson-Holiday et al. 2011). According to a recent study, CCR9 may facilitate cellular proliferation, increase the production of metalloproteinase-9 (MMP-9) in the matrix via CCL25, and boost tumor cell viability in distant areas, all of which may contribute to metastases in breast cancer (Johnson-Holiday et al. 2011). CCR9 is expressed in some ovarian cancer cell lines and tumors; nevertheless, more investigation is required to clarify CCR9's function in additional malignancies and to identify the method by which it encourages spread.

2.9.13 CCR10–CCL27/CCL28

The ligand CCL27, which is extensively produced in both the healthy and inflamed skin, interacts with the chemokine receptor CCR10 (Homey et al. 2000). Leukocyte protein CLA^+ T lymphocytes that exhibit the chemokine CCR10 receptor are involved in the membrane by CCL27. Malignant melanomas exhibit significant amounts of CCR10 together with CXCR4 and CCR7, according to a research study (Payne and Cornelius 2002). Melanoma and breast cancer both exhibit large levels of CXCR4 and CCR7, and, while they both have a common metastatic pattern, melanoma frequently metastasizes to the skin. CCR10 transcription leads to the spread of carcinoma to the epidermis, considering the function of CCR10–CCL27 in leukocyte relocation to the membrane. Conversely, a different study contends that in addition to encouraging invasion, development, and metastasis, CCR10 activation in melanoma increases lymph node metastasis. The production of CCR10 and its ligand CCL27 is connected with the progress of squamous epithelial cell malignancy and is elevated there as well (Simonetti et al. 2006).

2.9.14 CXCR3–CXCL9, CXCL10, and CXCL11

In various human melanoma cells and in the mouse B16F10 melanoma cell line, the CXCR3 protein is constitutively expressed and is correlated with tumor growth in cutaneous metastatic melanoma (Kawada et al. 2004). CXCR3 may be involved in lymph node metastases because a decrease of CXCR3 concentration in mouse B16F10 melanoma results in a 15% reduction in lymph node metastases. Although CXCR3 expression is constitutively present in lung adenocarcinoma, there is no connection between it and lymph node metastasis (Maekawa et al. 2008). Additionally, some carcinoma produces CXCR3 continuously, and individuals with tumors that do so have a worse future than do those who do not (Cambien et al. 2011). Research suggests that CXCR3 and its ligands cause osteosarcoma to metastasize to the lungs and then promote the growth and extension of the metastases (Pradelli et al. 2009). In two mouse models of osteosarcoma, the CXCR3 inhibitor AMG487 dramatically lowers lung metastasis and tumor growth within the lung (Pradelli et al. 2009). In a breast cancer metastatic mouse model, CXCR3 suppression also lowers lung metastasis. According to this research, CXCR3 may be crucial for initiating lung metastases (Walser et al. 2006).

2.10 Cytokines and Breast Cancer

It is unclear how a tumor develops; however, several hypotheses have emerged in this regard. Recent research has revealed that angiogenesis, movement, and the spread and proliferation of cancers are all influenced by the inflammatory response. To name a few, oncogenic modifications, hypoxia, cytokines, and growth regulators encourage the production of inflammatory cells. Inflammation in the TME is caused by immune cells that enter the tumor and hyperactive fibroblasts that release regulators, inflammatory cytokines, and growth regulators exposed to the tumor. An inflammatory environment brought on by obesity may promote the growth of tumors. Menopause and aging are both related to systemic inflammation. Through substantial tumor death, cancer treatment can affect an inflammatory TME (Esquivel-Velázquez et al. 2015). Several cytokines are responsible for regulating an inflammatory TME. The cytokines interleukin-1, 6, 11, and TGF-β encourage the progress and attack of cancer cells. TGF-β is the breast cancer cytokine that has been studied the most. As a member of the TGF-β subfamily, it plays a crucial role in controlling some processes, such as mobility, tolerance, growth, specialization, and death. Important theories on TGF-β's dual functions in tumor growth have evolved. It functions as a negative regulator and has an anticancer effect in the early stages of carcinogenesis, but later-stage tumor cells escape this effect, and it is unclear whether TGF-β triggers any development that follows. Increased IL-8 production is associated with metastases in breast cancer patients (Omokehinde and Johnson 2020). IL-19 encourages the migration of breast cancer cells like Hs578T and 4T1 by overexpressing many molecules involved in tumor progression and

metastasis, including CXCR4, MMP-2, MMP-9, TGF-β, IL-1b, and IL-6. 67NR cells, which normally have low endogenous IL-19 levels, and Michigan Cancer Foundation (MCF)-7 cells multiply and move more quickly when administered to mice, leading to the development of higher tumors and metastatic micronodules in the respiratory system. In vitro, IL-20 promotes BC migration and proliferation by upregulating the synthesis of MMP-9, MMP-12, cathepsin K, and cathepsin G. Breast cancer-related osteosarcoma releases IL-20 in high amounts. Monocyte chemoattractant protein MCP-1/CCL2, which is created by MSCs, and IL-17B encourage BC cell motility. In addition to encouraging angiogenesis, MSCs are providers of substances like vascular endothelial growth factor (VEGF) and IL-6 that cause BC cells to migrate. VEGF also increases BC cell infiltration by activating the MAPK and PI3K/Akt pathways. Hypoxia, which is characterized by abnormally low levels of oxygen in cells, is present in many significant tumors, including BC. The HIF group mostly controls the results that this disorder orchestrates. The creation of numerous components in cells that are related to stem cell survival, angiogenesis, metabolic activity, invasion, and dissemination is triggered when HIFs penetrate the nucleus in the presence of low levels of oxygen. These components include a variety of cytokines, including TGF-α, insulin-like growth factor (IGF)-2, and IGF-Bp2. In the case of BC, hypoxic settings enable MSCs to release development factors and cytokines, including TGF-β1, TGF-β2, and TGF-β3, which have an impact on the development, movement, and overexpression of BC. Furthermore, hypoxia (via HIF-1a) and TGF-β jointly induce cancerous growths in BC and progressively raise the production of the prometastatic molecules VEGF and CXCR4 by regulating a conserved set of genes (including CTGF (connective tissue growth factor), OPN (osteopontin), MMP-1, IL-6, and IL-8) (Geng et al. 2013). TGF-β improves the tumorigenicity of RAS-transformed MCF-10AneoT (M2) cells and noncancerous epithelial MCF-10A1 (M1) cells in spheroid experiments. Additionally, in human breast carcinoma cells, junctional adhesion molecule-A (JAM-A) production is negatively linked to TGF-β1 and TGF receptor expression and cell invasiveness. TGF downregulation of JAM-A, which comprises the TGF-β/Smad and TGF-β/p54 c-Jun N-terminal kinase (JNK) pathways, promotes BC cell invasion. TGF-β1 promotes an EMT that may also cause human mammary epithelial (HMLE) cells to display CSC-like traits. Human primary normal mammary epithelial cells called HMLER cells, which have been immortalized using human telomerase reverse transcriptase (hTERT), SV40 large T antigen, and H-Ras to make them tumorigenic, may facilitate the early dissemination of breast cancer cells. Breast cancer cells can survive at sites of dispersion and may eventually outgrow them over time. Inflammatory tumors are more likely to metastasize than are noninflammatory breast cancers (Bel'skaya et al. 2022).

2.11 Conclusions

Several studies indicate that chemokines and their receptors not only play a part in the growth of tumors at different stages but also support EMT and angiogenesis, which aids in the growth of tumors. Particular chemokine receptors, when expressed on a cancer cell's surface, promote tumor spread and occurrence of organ-specific metastasis. One of the extensively researched chemokine receptors is CXCR4. The CXCR4 blocker plerixafor was authorized by the US Food and Drug Administration in 2010 for use in individuals with multiple myeloma (MM) and non-Hodgkin's lymphoma (NHL). Additionally, even though some receptors, like CXCR4 and CCR7, have undergone substantial research, more research is required to determine how other chemokines affect the growth and spread of tumors. Scientific investigations should concentrate on figuring out the biological mechanisms and signaling networks that chemokines use to mediate tumor growth and metastasis. Additionally, it will be fascinating to investigate the possibility of chemokine inverse agonist combination treatment with other chemotherapeutic medicines to assess the impact on patient survival. Chemokines and their receptors may ultimately prove to be helpful clinical targets in halting tumor growth and metastasis, hence enhancing survival outcomes.

Further Reading

For further reading, some textbooks mentioned below also provide a further understanding of the cytokine and the chemokine network.

- The Cytokines of the Immune System: The Role of Cytokines in Disease Related to the Immune Response by Zlatko Dembic
- The Cytokine Network and Immune Functions by Jacques Thèze
- Chemokines and Viral Infection by T.E. Lane
- Cytokines and Chemokines in Infectious Diseases by Malak Kotb and Thierry Calandra

The below links for video lectures may also be helpful:
https://youtu.be/TAqO0Mq19JQ
https://youtu.be/6wEnYfvoBqk
https://youtu.be/A9fRRCUlMms
https://youtu.be/zIWvmCsKr34

For more insights about the topic, we would suggest detailed findings from the books of Mir et al. (2022a) https://doi.org/10.1016/C2021-0-02565-7, Mir (2022) https://doi.org/10.1016/C2022-0-00074-X, Mir (2021) https://doi.org/10.52305/WXJL6770, and Mir (2015a, b, c) https://doi.org/10.1016/C2014-0-02898-5 and from the cancer.net website on the following mentioned links:
https://www.cancer.net/cancer-types/breast-cancer/types-treatment
https://discovery.ucl.ac.uk/id/eprint/1472740/
https://www.jmedsciences.com/doi/JMEDS/pdf/10.5005/jp-journals-10045-00138

For diagrammatic illustrations and descriptive tables, see Grunt and Mariani (2013): http://www.eurekaselect.com/article/49928

See video links on the overall status of cancers, various types of cancers, and the current possible new treatment options available at

https://www.sciencedirect.com/science/article/pii/S205970292032278X

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Chemokine and Cytokine Network in Angiogenesis

3

Manzoor Ahmad Mir, Aanisa Ishrat, and Mohd Zahoor Ul Haq Shah

Abstract

Angiogenesis is a hallmark of cancer and has been the target of new therapies since last few years; however, it is a complex process and involves many redundancy pathways that ensure its maintenance. The chemokines and cytokines are two important families of signaling molecules that play an important role in angiogenesis through complex networks of interactions. They regulate angiogenesis by modulating the proliferation, differentiation, and migration of endothelial cells, which are the building blocks of blood vessels. Many cytokines have been shown to regulate angiogenesis, including tumor necrosis factor-alpha (TNF-α), vascular endothelial growth factor (VEGF), and interleukin-1 (IL-1). Some of the chemokines that are known to regulate angiogenesis include CXCL12, CCL2, and CXCL8. One among them CXCL12, also known as stromal cell-derived factor-1 (SDF-1), is a potent angiogenic factor that promotes endothelial cell migration and proliferation. A variety of mechanisms are set in place by tumors to facilitate their growth during carcinogenesis. Due to its role in the blood vessel development within the tumor, angiogenesis is a crucial step for the growth of cancer. The supply of oxygen and nutrients to the tumor is ensured by these recently established blood vessels, which promotes the growth of the tumor. New treatments have focused on angiogenesis in recent years because it is a defining feature of cancer. The maintenance of angiogenesis is ensured by a number of redundant routes, although it is a complex event. The sequential

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events/phases that make up angiogenesis and its traditional regulators will be discussed in the first section of this chapter. Then, we will focus on how the chemokine and cytokine in the tumor microenvironment affect angiogenesis and how they can either promote or inhibit it. The therapeutic options that target cancer angiogenesis will be the final topic of discussion, along with the difficulties they face. So, in nutshell, the role of chemokine and cytokine network in the regulation of angiogenesis and targeting these signaling pathways which represent a promising approach for the treatment of a variety of diseases will be discussed. Here, we will also discuss how the cytokines and chemokines present in the tumor microenvironment can induce or block angiogenesis. Further, we will focus on the therapeutic arsenal targeting angiogenesis in cancer and the challenges they have to overcome.

Keywords

Cytokines · Chemokine · Angiogenesis · Cancer · Therapy · Proliferation · Tumor microenvironment · VEGF · TNF · IL-1 · CXCL12 · CCL2 · CXCL8

3.1 Introduction

Angiogenesis and vascular morphogenesis are the two main procedures that result in formation of blood vessel. Vascular morphogenesis relates to the development of new blood vessels. In a biological process called angiogenesis, which is often referred to as neovascularization, existing blood vessels are used to form new blood vessels (Shibuya 2013; Griffioen and Dudley 2021). Numerous physiological and pathological processes in living things depend on it. Numerous angiogenic growth factors, lipids, enzymes, and carbohydrates, including components of the superfamily-chemokine, influence the angiogenesis process (Griffioen and Dudley 2021). Some associates of the chemokine superfamily can applaud the maturation of new blood vessels and operate as proangiogenic molecules, whereas other members can inhibit these processes, making them angiostatic (Adams and Alitalo 2007; Shibuya 2013) (Fig. 3.1).

Angiogenesis is necessary for physiological functions like embryonic development and the menstrual cycle. The development of cancer is also heavily influenced by this mechanism. The contribution of this procedure in cancer started to gain attention around 1800 (Griffioen and Dudley 2021). German researchers did observe that some tumors had considerable vascularization, implying that new blood vessels formed during these malignancies. Algire later demonstrated in mice in 1948 that blood vessel expansion occurs before melanoma growth (Algire and Legallais 1948). There are consecutive stages that are taken to generate new blood vessels from preexisting vessels. Angiogenesis factors connect to endothelial cells' surface-located receptors in a hypoxic environment, causing the cells to dilate and become activated. Simultaneously some proteases that cause basement

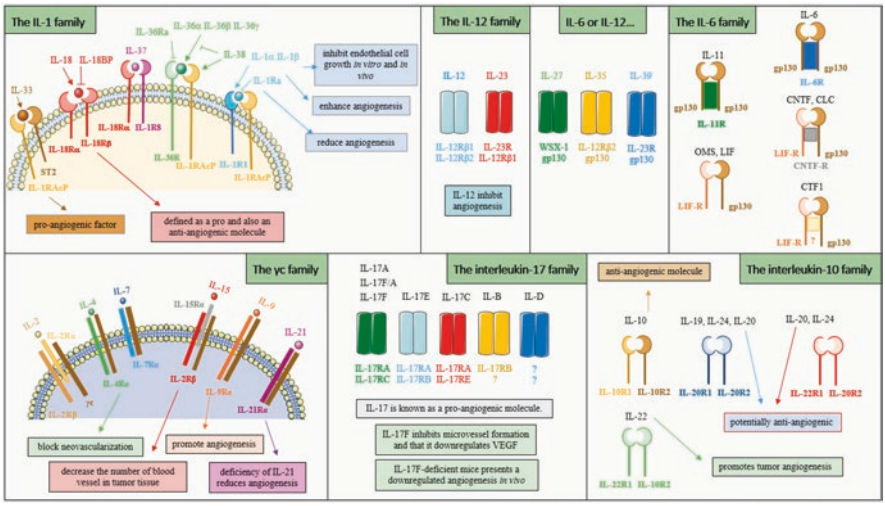

Fig. 3.1 Role of cytokines in angiogenesis

membrane breakdown and pericytes to detach are expressed more frequently when hypoxia is present. In response to the angiogenic stimuli, highly mobile endothelium tip cells migrate. The proliferation of endothelial cells encourages the growth of new blood vessels. The basement membrane is then exposed at a later period. Pericytes are enlisted as well as rehabilitated. The various blood vessels eventually converge, bringing an end to tumor vasculature development (Zanotelli and Reinhart-King 2018).

Hypoxia, which encourages an inequality in favor of proangiogenic factors, is the key cause that triggers this mechanism, which is strictly regulated by a balance between anti and proangiogenic factors. Indeed, hypoxia causes the buildup of hypoxia-inducible factor-1 (HIF-1), which in turn lead to the production of angiogenic-promoting substances like vascular endothelial growth factor (VEGF) (Lv et al. 2017). Briefly, the tumor environment changes once the tumor has reached about 2 mm hypoxic, stimulating angiogenesis and causing an angiogenic switch (Manuelli et al. 2022).

Chemokines and cytokines are soluble proteins that can influence cells and tissues from a distance. They attach to certain high-affinity receptors and then affect the target cells. Chemokines are a broad class of low molecular weight cytokines that have been shown to attract and activate various cell types in a specific manner. Although their primary role as leukocyte attractants is well known, studies have suggested that chemokine's also play a part in tumor-related processes, including angiogenesis, metastasis, and growth (Singh et al. 2007). Cytokines and chemokine's function in controlling angiogenesis in a cancer context (Qayoom et al. 2020). Let us now discuss various chemokine and cytokine regulators of angiogenesis in detail.

3.2 Regulators of Angiogenesis

3.2.1 Family of Vascular Endothelial Growth Factors (VEGF)

The VEGF family is the best significant angiogenesis inducer. The family of VEGF comprises the proteins VEGF-A, VEGF-C, VEGF-D, VEGF-B, and placental growth factor (PlGF). Three receptors control how they perform biologically: the VEGF-2, VEGF-1, VEGF-3, and Neuropilin and Heparins sulfate proteoglycans are two coreceptors for the same function. VEGF-C, VEGF-D, and VEGF-A bind to Vascular EGFR-2, while Vascular EGF-B, Vascular EGF-A, and PlGF bind to vascular EGFR-1, respectively. Both vascular EGF-D and vascular EGF-C bind to vascular EGFR-3 (Melincovici et al. 2018; Shibuya 2013).

Angiogenesis, both healthy and unhealthy, is mostly dependent on the Vascular EGF-A/Vascular EGFR-2 flagging pathway. Because of aberrant vascular development, mice lacking VEGFR-2 or VEGF-A are in viable and die early in the embryo. On endothelial cells, the VEGF family stimulates mitosis, possesses antiapoptotic properties, and both of which involve movement and expansion. These growth factors additionally support blood vessel blood and lymphatic vessel remodeling through permeabilization (Melincovici et al. 2018). HIF-1, the stimulation of oncogenes like growth factors, Ras and cytokines like IL-1, tumor necrosis factor-alpha (TNFa), epidermal growth factor (EGF), and platelet-derived growth factor all help to increase the expression of VEGF. Numerous cells, including smooth muscle cells, keratinocytes, endothelial cells, platelets, neutrophils, and macrophages, generate VEGF-A. It is thought that this chemical is secreted by about 60% of cancers. We opted not to go into detail because there have been evaluations on the VEGF family (Claesson-Welsh and Welsh 2013; Melincovici et al. 2018).

VEGFR-1 and two coreceptors, neuropilin-2 and neuropilin-1, modulate the biological activity of PlGF by promoting tumor survival, proliferation, and angiogenesis by enhancing tumor vascularity, blood vessel growth, and also endothelial cell transfer (Park et al. 1994; Gluzman-Poltorak et al. 2000). HIF-1 overexpression enhances the appearance of placental growth factor (PlGF) in endothelial and vascular cells (Yamakawa et al. 2003). PlGF is not necessary, in contrast to VEGF for the development of embryogenic vessels, but it also supports diseased blood vessel formation. It is true that mice lacking PlGF develop functional blood vessels, but tumor angiogenesis and development are reduced (Carmeliet et al. 2001; Melincovici et al. 2018). However, Yang et al. have shown that tumor growth is slowed in LLC and T241, two tumor mouse replicas that have had their genes changed to overexcite PlGF. Additionally, these tumors have few microvessels and regular blood vessels. They also demonstrated the fascinating finding that T241-VEGF-null cells overexciting PlGF caused mice to grow more quickly, demonstrating that PlGF promotes tumor production in cells that lack VEGF expression (Yang et al. 2013). PlGF is secreted by Th17 cells both in vivo and in vitro, as has recently been demonstrated. PlGF, on the other hand, controls Th17 distinction through a STAT3-dependent pathway and can take the role of IL-6 in the process (Yoo et al. 2019).

3.2.2 Angiopoietin (Ang)–Tie System

The Angiopoietin–Tie pathway system constitutes the second endothelial growth factor receptor signaling pathway, which accomplishes a necessary function to control blood and lymphatic vessel remodeling during mid-gestation, afterward the VEGF–VEGFR-driven phase of active angiogenesis. The endothelial Tie receptors and the angiopoietin growth factors regulate blood and lymphatic vessel development, and vascular permeability, inflammation, angiogenic remodeling, and tumor vascularization in adult tissues (Eklund and Saharinen 2013). Tie receptors are activated by the angiopoietins in unique in trans complexes at endothelial cell-cell and cell-matrix contacts. Angiopoietins control vascular maturation and remodeling and increase angiogenesis. Four members are there in the group: Ang-2 and Ang-1 are well defined, but not much is studied regarding the other two: Ang-3 and Ang-4 (Maisonpierre et al. 1997; Valenzuela et al. 1999; Davis et al. 2015). Tie-2 and Tie-1 receptors, that are mostly indicated in the endothelium but can also be found in some hematopoietic cells, mediate their biological roles (Batard et al. 1996; Hashiyama et al. 1996). Tie-1 is a rare receptor., which means that angiopoietin activates it by interacting with Tie-2 (Saharinen et al. 2005).

To control endothelial cell survival and vascular maturation Angiopoietin/Tie system acts as a vascular specific ligand/receptor system. The family of Angiopoietin includes four ligands (Angiopoietin-1, Angiopoietin-2, and Angiopoietin-3/4) and two (Tie1 and Tie2) tyrosine kinase corresponding receptors. Ang-2 and Ang-1 are specific ligands of Tie2 binding the receptor with similar affinity (Thomas and Augustin 2009; Eklund and Saharinen 2013). Activation of Tie2 promotes maturation and vessel assembly by regulating the recruitment of mural cells and mediating survival signals for endothelial cells. Ang-1 in a paracrine agonistic manner acts and induces Tie2 phosphorylation and subsequent vessel stabilization. On the other hand, endothelial cells produce Ang-2 and acts as an autocrine antagonist of Ang-1-mediated Tie2 activation. Ang-2 thereby primes the vascular endothelium to exogenous cytokines and induces the vascular destabilization at higher concentrations. Expression of Ang-2 is very strongly expressed in vasculature of many tumors and it has been suggested that Ang-2 may act synergistically with other cytokines such as vascular endothelial growth factor to promote tumor progression and tumor-associated angiogenesis (Thomas and Augustin 2009).

In angiogenesis, this mechanism is essential. Indeed, mice lacking in 1-Ang, 1-Tie, or Tie-2 exhibit an aberrant vascular system that causes fetal mortality (Sato et al. 1995; Suri et al. 1996). Developmental angiogenesis is largely intact in mice lacking Ang-2, but the animals' newborns have lymphatic malfunction and occasionally die after birth from chylous ascites (Gale et al. 2002). It is interesting to note that excessive Ang-2 expression kills embryos. Depending on the situation, 2-Ang may work as a 2-Tie agonist or antagonist. Ang-2 causes vascular instability and leakiness when it functions as an antagonist, which causes vascular regression (Benest et al. 2013). Blood vessels remain steady and dormant in a normal environment. Weibel–Palade bodies are where Ang-2 is kept, and its expression is modest (Fiedler et al. 2004). Ang-1 prevails because it suppresses Ang-2 transcription.

Tie-2 is fundamental agonist by Ang-1. The fibroblasts, tumor cells, nonvascular cells, and mural cells all express this molecule (Huang et al. 2010). By affecting the EC-EC joint and the actin cytoskeleton, the Ang-1/Tie-2 signaling alleyway improves vascular stability and reduces vascular permeability. (Saharinen et al. 2017). When there is inflammation, Ang-2 is elevated and take part with combining Ang-1 to bind to Tie-2. Endothelial cells quickly release Ang-2, and cytokines like TNF and VEGF enhance its effects (Oh et al. 1999; Kim et al. 2015). Ang-2 changes to an antagonistic mode during inflammation, and this is accomplished through a mechanism involving the cleavage of the Tie-1 receptor (Kim et al. 2016; Korhonen et al. 2016). Numerous tumor forms, including melanoma, RCC, glioblastoma, breast, and colorectal cancer, have significant levels of Ang-2 expression (Sfiligoi et al. 2003; Helfrich et al. 2009; Goede et al. 2010). Mice lacking Ang-2 have reduced tumor development and metastatic colony formation in the lung (Im et al. 2013).

3.2.3 Hepatocyte Growth Factor (HGF)

Hepatocyte growth factor (HGF) promotes angiogenesis (tissue vascularization), cell motility, and cell differentiation. It is a pleiotropic cytokine composed of an alpha-chain and a beta-chain, and these chains contain four Kringle domains and a serine protease-like structure, respectively. HGF-induced activation of its cell surface receptor, the Met tyrosine kinase, drives morphogenesis and mitogenesis in a wide spectrum of target cell types and embryologic, developmental, and homeostatic contexts. Paracrine HGF/Met signaling in a typical way is regulated by HGF activation at target cell surfaces, HGF binding-induced receptor activation, internalization and degradation. Despite these controls, HGF/Met signaling contributes to tumor angiogenesis, oncogenesis, invasiveness, tumor metastasis in various types of cancer, leading to the rapid growth of pathway-targeted anticancer drug development programs (De Silva et al. 2017). Proteases which are upregulated in tumor cells are primarily responsible for activating HGF, which is produced in the inactive preform (pro-HGF) (Owen et al. 2010; Owusu et al. 2017). As a result of alterations in the HGF promoter region, stromal cells like fibroblasts frequently produce this growth factor together with colorectal and breast cancer cells (Bottaro et al. 1991; Ma et al. 2009; Seneviratne et al. 2015). A bad prognosis for patients is linked to the overexpression of HGF in colorectal cancer stages III and II (Toiyama et al. 2009). The development, survival, and migration of endothelial cells are encouraged by this chemical, which also drives the epithelial mesenchymal transition (EMT) by activating its receptor, the mesenchymal-epithelial transition factor (c-MET) (Bussolino et al. 1992). In many malignancies, including nonsmall cell lung carcinoma (NSCLC), stomach, ovarian, hepatic, thyroid, breast, neck and head, intestinal, and kidney carcinomas, the two molecules c-MET and HGF have an elevated expression (Sierra and Tsao 2011). Additionally, an in vitro investigation has shown that HGF can induce the upregulation of Vascular EGF and IL-8 in esophageal squamous cell carcinoma and accelerate the movement and incursion of cancer cells

(Ren et al. 2005; Mir 2015a, b, c, d). It has been demonstrated that HGF can enhance vascular EGF transcription through SP1 phosphorylation, which in turn increases angiogenesis by increasing vascular EGF expression (Reisinger et al. 2003). The signaling pathway of hepatocyte growth factor (HGF) and its receptor (HGFR, also known as c-Met), which plays important roles in angiogenesis and tumor growth is one promising target. In multiple in vitro and in vivo models, inhibitors of this signaling pathway have been shown to inhibit angiogenesis. The HGF/c-Met signaling pathway is now recognized as a promising target in cancer by inhibiting angiogenesis, tumor growth, invasion, and metastasis.

3.2.4 Fibroblast's Growth Factor (FGF)

Fibroblast Growth Factor (FGF) is a family of growth factors which plays a critical role in many biological processes, including angiogenesis (Klein et al. 1999). In all stages of angiogenesis, FGFs are involved like the initiation, differentiation, proliferation, and migration of endothelial cells (Simons et al. 2005). FGFs bind to their high-affinity tyrosine kinase receptors (FGFRs) on the surface of endothelial cells and initiates the signaling cascade. This signaling due to binding of FGF results in the activation of downstream signaling pathways, like the PI3K–Akt and Ras–MAPK pathways which promote endothelial cell proliferation, tube formation, migration, and finally angiogenesis (Ortega et al. 2015).

FGF are involved in a variety of cellular processes, such as stemness, proliferation, antiapoptosis, drug resistance, and angiogenesis. FGF-1 to FGF-23 are 22 members of the Fibroblast growth factor family, which is further subdivided in seven subfamilies: FGF1/2/5, FGF3/4/6, FGF7/10/22, FGF8/17/18, FGF9/16/20, FGF19/21/2, and FGF 11/12/13/14 (Liu et al. 2021). Their four receptors, FGFR1 to FGFR4, mediate their biological functions. Additionally, FGFR5, a receptor without an intracellular kinase domain, collaborates with FGFR1 as a coreceptor. Endothelial cells express these receptors. The signaling alleyway of FGF/FGFR controls a number of organic activities, comprising angiogenesis, endothelial cell differentiation, survival, propagation, and tube formation (Turner and Grose 2010). The FGF family's role in angiogenesis, however, is debatable. Different models have demonstrated the proangiogenic capabilities of FGF-8, FGF-4, and especially FGF-2 and FGF-1. Vascular endothelial cells express VEGF when exposed to FGF-2, according to in vitro studies. This is accomplished through paracrine and autocrine mechanisms (Seghezzi et al. 1998). In addition, endothelial cells lacking FGF signaling downregulate VEGFR-2 expression by activating Erk1/2 (Murakami et al. 2011). Nonsmall cell lung cancer, head and neck cancer, gastric cancer, urothelial cancer, and breast cancer are only a few of the cancer types that might exhibit FGFR change (Krook et al. 2021).

Many studies have demonstrated that FGFs, especially FGF-2, are crucial for angiogenesis in both pathological and physiological conditions. In vitro studies have shown that FGF-2 promotes the proliferation and migration of endothelial cells, while in vivo studies have demonstrated that FGF-2 induces

neovascularization in various animal models, including mice, rats, and rabbits. Further, FGF-2 has shown to stimulate the production of other angiogenic factors, such as vascular endothelial growth factor (VEGF) and IL-8, which further enhance angiogenesis (Presta et al. 2017; Ortega et al. 2015; Simons et al. 2005).

In nutshell, FGFs plays a very important role in angiogenesis by promoting the proliferation, migration, differentiation, and stabilization of endothelial cells. However, numerous studies have demonstrated the importance of FGFs, particularly FGF-2, in angiogenesis, and their potential as therapeutic targets for various diseases characterized by abnormal angiogenesis.

3.2.5 Platelet-Derived Growth Factor (PDGF)

Platelet degranulation allows the release of a large number of soluble mediators, which is an essential step for angiogenesis. Angiogenesis requires the action of a variety of growth factors that act in an appropriate physiological ratio to assure functional blood vessel restoration. Platelets release main regulators of angiogenesis: vascular endothelial growth factors (VEGFs), basic fibroblast growth factor (FGF-2), and platelet-derived growth factors (PDGFs), among others. The continuous release of these growth factors has been proposed to promote angiogenesis both in vitro and in vivo (Martínez et al. 2016). After the inflammatory phase has been initiated, the wound healing response requires angiogenesis as a process that modulates the activation, proliferation, and migration of endothelial cells to establish new blood vessels from pre-existing vasculature (Oklu et al. 2010).

A key function of the PDGF/PDGFR signaling pathway in angiogenesis is the enrollment of pericytes to vessels, which promotes vascular stability and vasculature cell survival. PDGF comes in a variety of isoforms, including Platelet DGF-CC, Platelet DGF-AB, Platelet DGF-DD, Platelet DGF-AA, and Platelet DGF-BB. These molecules attach to the Platelet DGFR receptors and are generated by endothelial and epithelial cells. With the exempting of Platelet DGF-DD, all Platelet DGF ligands activate PDGFR-α. All PDGF ligands, with the exclusion of PDGF-AA, activate PDGFR-α. PDGFR-β is activated by PDGF-DD and PDGF-BB. Pericytes and Vascular smooth muscle cells express PDGFR, while endothelial cells also express PDGFR. Changes in these molecules are linked to tumor angiogenesis, metastatic disease, and poor survival. Proangiogenic factors including VEGF and FGF are stimulated by PDGF/PDGFR, and endothelial cell generation and endothelial predecessor cell migration to vessels are also induced. Studies conducted in both in vitro and in vivo have revealed that PDGF-D overexpression in HCT116 is linked to tumor aggressiveness while PDGF-D inhibition in SW480 suppresses migration, tumor growth, and angiogenesis (Chen et al. 2016).

Bertrand-Duchesne et al. (2010) investigated how platelet-derived fractions may encourage angiogenesis by analyzing the presence of angiogenic growth factors in PRP samples. Their findings revealed high levels of VEGF, PDGF-BB, EGF, and

basic fibroblast growth factor (bFGF). To examine the effects of each growth factor, PRP supernatants were incubated with blocking antibodies and the response was measured via proliferation assays in Human Umbilical Vein Endothelial Cells (HUVEC). Interestingly, the use of EGF neutralizing antibodies reduced the proliferation of HUVEC, whereas the other antibodies did not affect this response. Mammoto et al. also conducted a mechanistic study to explore the role of angiopoietin-1 in driving the vascular response in mouse PRP and PRF formulations. They found that angiopoietin-1-containing mouse platelet-derived fractions were responsible for increasing the proliferation, migration, and differentiation of human microvascular endothelial cells (Mammoto et al. 2013). Additionally, Li et al. (2014) observed that PRP could stimulate endothelial progenitor cells to form vessel-like structures and promote vascular growth. Recently, Anitua et al. (2015) studied the effect of a platelet concentrate called PRGF (plasma rich in growth factors) (P-PRP low leukocyte content) on angiogenesis. This product was found to increase cell proliferation and reduce apoptosis in primary HUVEC and skeletal myoblasts by forming a fibrin matrix system.

Numerous growth factors play a vital role in angiogenesis, and platelets secrete many of these growth factors. Given the importance of angiogenesis in regulating wound healing and the significant role platelet-derived factors play in activating and stabilizing blood vessels, it is natural to wonder whether platelet-derived formulations used in regenerative medicine can promote angiogenesis (Peterson et al. 2010). In vitro experiments and animal studies have shown promising results, indicating that platelet-derived fractions may have a positive effect on promoting angiogenesis (Fig. 3.2).

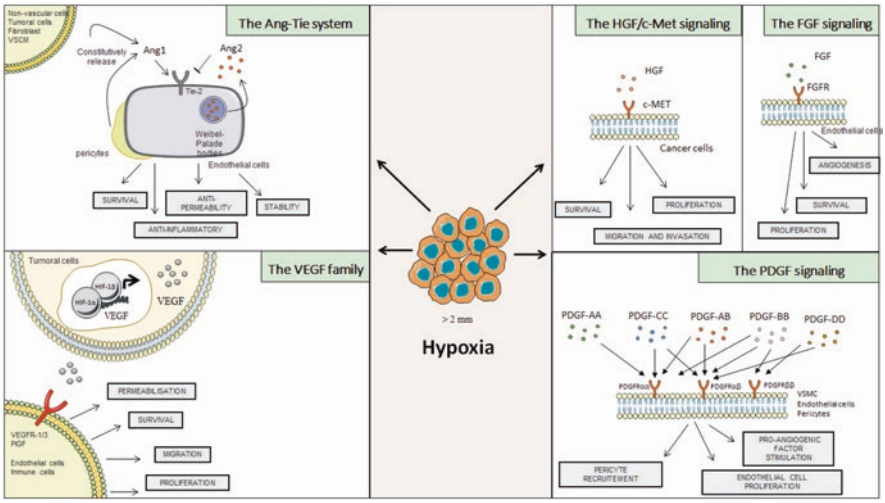

Fig. 3.2 Classical regulators of angiogenesis

3.2.5.1 Interleukins: An Immune System and Angiogenesis Connection

Various components of the immune system play important roles in the process of angiogenesis and in turn the angiogenesis can modulate the immune system as well (Mir et al. 2022a, b, c, d). Angiogenesis has been shown to modify the appearance of proteins on endothelial cells to decrease immune cell invasion. Additionally, angiogenesis creates a tumor microenvironment that is immunosuppressive (Mehraj et al. 2021a, b, c; Mir et al. 2022a, b, c, d). In fact, it motivates the tumor's acquisition of immune-suppressive cells including Treg and MDSC, while decreases cytotoxicity, $CD3^+$ proliferation, and DC maturation. In contrast, angiogenesis can be modulated by certain immune cells (Geindreau et al. 2021).

In recent years, the role of interleukins in angiogenesis, the process by which new blood vessels are formed from existing ones, has been an area of active research. Various cytokines like IL-1, IL-6, IL-8, IL-17, IL-33, and IFNs play important roles in the process of angiogenesis.

IL-1 is a proinflammatory cytokine can stimulate the migration and proliferation of endothelial cells, which are the cells that make up blood vessels. It has also been shown to promote the production of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), which are both important factors in the formation of new blood vessels (Voronov et al. 2003). IL-6 another proinflammatory cytokine produced by a variety of cells, including endothelial cells has been shown to play a role in angiogenesis. It acts by promoting the expression of VEGF and bFGF, as well as by stimulating the proliferation and migration of endothelial cells. IL-8 a chemokine produced by a variety of cells, including endothelial cells has been shown to play a role in angiogenesis by promoting the migration of endothelial cells toward areas where new blood vessels are needed (Zhang et al. 2016). IL-17 also a proinflammatory cytokine has been shown to promote the proliferation and migration of endothelial cells and promote angiogenesis by stimulating the production of VEGF and bFGF. Recently IL-33 has been shown to promote the proliferation and migration of endothelial cells, as well as the formation of new blood vessels (Nascimento et al. 2021).

3.2.5.2 Interferons and Angiogenesis

The cytokine family known as interferon's (IFNs) was discovered over 50 years ago due to their antiviral capabilities. IFNs play a part in antitumor and immunomodulatory responses in addition to having significant antiviral effects. Interferons (IFNs) play a critical role in the immune response against viral infections and cancer. In addition to their well-known antiviral and antitumor effects, IFNs have also been shown to regulate angiogenesis, the process of forming new blood vessels from preexisting ones. In humans, interferon has three subsets: type I, which includes IFN/, type II, which involves IFN, and type III, which contain IFNs. IFNs of type II and type I employ different but related receptor systems. The purpose and signaling pathways of various cytokines and cytokine receptors are now better understood because the studies on these systems.

Several studies have demonstrated the antiangiogenic properties of IFNs. For example, IFN-alpha has been shown to inhibit angiogenesis by downregulating the expression of vascular endothelial growth factor (VEGF), a key regulator of angiogenesis, in various cancer cell lines (Chen et al. 2017; Liu et al. 2017). Similarly, IFN-beta has been reported to suppress tumor angiogenesis by inhibiting the proliferation and migration of endothelial cells. However, recent studies have suggested that IFNs may also have proangiogenic effects under certain conditions. For instance, a study by Kim et al. demonstrated that IFN-gamma can promote angiogenesis by stimulating the expression of proangiogenic factors such as VEGF and angiopoietin-1 in human umbilical vein endothelial cells (Kim et al. 2011). Another study by Ohtani et al. showed that IFN-alpha can enhance the angiogenic potential of endothelial progenitor cells (Ohtani et al. 2010). It is well known that type I IFNs limit angiogenesis (Albini et al. 2000), as they stop the formation of proangiogenic factors such bFGF, VEGF, and IL-8 by tumor cells (Gough et al. 2012). Additionally, IFNα/prevents the growth of endothelial cells and the molecules involved in endothelial cell chemotaxis are secreted (Metzemaekers et al. 2018). IFN specifically prevents tumor cells in human bladder cancer cells from producing bFGF and IL-8 (Izawa et al. 2002). Mice in the B16F10 and MCA205 cancer models, those lacking IFN exhibit a quicker rate of tumor growth. The blood vessels of wild-type mice are more fully developed. IFN-deficient mice display an increased expression of these cells and a rise in neutrophil infiltration (Jablonska et al. 2010). These above-mentioned different findings suggest that the role of IFNs in angiogenesis is complex and context-dependent. The specific effects of IFNs on angiogenesis may vary depending on the type of IFN, the source of IFN production, and the microenvironment in which they are present.

3.2.5.3 Interleukin-1 Family

Interleukin-1 (IL-1) is a proinflammatory cytokine that plays a crucial role in various physiological processes, including angiogenesis. It stimulates angiogenesis process through its effects on endothelial cells, by promoting endothelial cell proliferation, migration, and tube formation, which are all essential steps in the process of angiogenesis. It also enhances the expression of proangiogenic factors such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF), which further promote angiogenesis. IL-1 has been shown to promote the angiogenesis process in various experimental models for instance IL-1β was found to stimulate angiogenesis in a mouse cornea model, and this effect was blocked by a neutralizing antibody against VEGF (Oh et al. 1999). In another study, IL-1β was shown to promote angiogenesis in a rat model of hind limb ischemia (Zbinden et al. 2003). IL-1α has also been shown to promote angiogenesis in a mouse model of skin wound healing (Rezzani et al. 2011). IL-1 in addition to its effects on endothelial as mentioned above also stimulates angiogenesis indirectly by promoting the recruitment of inflammatory cells, such as neutrophils and macrophages, to the site of angiogenesis. These cells release proangiogenic factors, such as VEGF and FGF, which further enhance angiogenesis.

Eleven molecules make up this family. There are agonist ligands like, IL-18, IL-1, IL-33, IL-36, IL-36, and antagonist ligands such as IL-1Ra, IL-36Ra, IL-37, and IL-38. This group has the capacity to either directly or indirectly mediate angiogenesis. Instantly by triggering proangiogenic factors like vascular EGF, it was demonstrated some 30 years ago that IL-1 can stop the development of endothelial cells both in lab and in vivo and can prevent the development of vessels brought on by FGF (Cozzolino et al. 1990; Mir 2015a, b, c, d). Studies conducted in vitro have demonstrated that pancreatic, stomach, and colon cancer cells can secrete IL-1, which promotes angiogenesis (Ma et al. 2008; Matsuo et al. 2009a, b; Mir et al. 2022a, b, c, d). Studies showed that IL-1 can promote angiogenesis in the prostate and stomach tumors is possible (Murakami et al. 2013; Kwon et al. 2014) for melanoma cells to release IL-1 and IL-1, which then upregulate intracellular adhesion molecule-1 (ICAM-1), interleukin (IL)-6, interleukin (IL)-8, and vascular cell adhesion. Expression of molecule-1 (VCAM-1) in endothelial cells (Strozyk et al. 2014). In a Lewis model, IL-1 encourages tumor growth (Bar et al. 2004). IL-1-deficient animals demonstrate a lung cancer model by increasing VEGF and CXCL2. No local tumor or lung metastases in an intravenously injected B16 melanoma model (Voronov et al. 2003).

IL-1 is also involved in pathological angiogenesis, such as that seen in cancer. IL-1β has been shown to promote angiogenesis in various types of cancer, including prostate cancer, breast cancer, and melanoma (Orlotti et al. 2011). Further, IL-1β has been shown to enhance the invasiveness of cancer cells and promote the formation of blood vessels in the tumor microenvironment. In summary, IL-1 plays a crucial role in angiogenesis through its effects on endothelial cells, recruitment of inflammatory cells, and promotion of proangiogenic factors. Targeting IL-1 signaling pathways may offer a potential therapeutic strategy for diseases involving aberrant angiogenesis, such as cancer.

Depending on the tissues and environment, IL-18 is either a pro- or an antiangiogenic molecule in the literature. In vivo research first showed that it negatively influences neovascularization. IL-18 treatment exhibits an anticancer effect and lowers the microvessel density in mice that had received a subcutaneous injection of T241 (Cao et al. 1999). Two decades later, the development of endothelial tubes can be induced by IL-18, according to later in vivo and in vitro research. In cases of rheumatoid arthritis (Park et al. 2001). In a Lewis lung cancer mice model, IL-18 suppresses tumor growth by down-regulating VEGF-A and VEGF-C expression in tumor tissues. In addition, VEGF has been shown to promote IL-18 production, which increases gastric migration of cancer cells (Kim et al. 2007). Recent in vivo research demonstrated that IL-18 produced by macrophage prevents the growth of tumor blood vessels (Xing et al. 2016).

A cytokine with potent angiogenesis properties is IL-33 (Küchler et al. 2008; Choi et al. 2009; Stojkovic et al. 2014). In colorectal cancer cells, stromal cells, and microvessels, its receptor ST2 is significantly expressed (Cui et al. 2015). Human umbilical vein endothelial cells respond to IL-33 in a proangiogenic manner

(HUVECs) via means of the Akt pathway (Choi et al. 2009). Additionally, IL-33 encourages myofibroblasts to create the MMP2 and MMP9, metalloproteinases engaged in the formation of advanced vasculature (Liu et al. 2014; Akimoto et al. 2016; Mertz et al. 2016).

In summary, the IL-1 family plays a complex role in angiogenesis, with some members promoting and others inhibiting the process of angiogenesis. Further research is needed to fully understand the mechanisms by which these cytokines regulate angiogenesis and to explore their potential as therapeutic targets for diseases characterized by abnormal angiogenesis, such as cancer and inflammatory disorders.

3.3 C Family

C family of chemokines which include IL-4, IL-7, IL-2, IL-21, IL-9, and IL-15 make up this group (Leonard et al. 2019) and they play a crucial role in angiogenesis by stimulating the migration, proliferation, and tube formation of endothelial cells, as well as inducing the expression of proangiogenic factors. Hence, targeting these chemokines and their receptors could be a potential strategy for inhibiting angiogenesis in various pathological conditions, such as cancer and inflammatory diseases. Their similar aspect of the receptor cytokine c serves as the foundation for this family. It has been demonstrated that IL-4 can by inhibiting basic fibroblast growth factor, which prevents corneal blood vessel formation, human microvascular cells migrating (Volpert et al. 1998). In a model of spontaneous breast cancer, IL-4 was found to help remodeling of vessel in mice (PyMT) lacking the hormone (Kodama et al. 2021). The NSCLC IL-9 expression is linked to a poor prognosis and encourages angiogenesis by STAT3 (He et al. 2019). IL-15 reduces prostate cancer cell mobility and lowers the number Vivo in mice of blood arteries in tumor tissue (Rohena-Rivera et al. 2017). In mice that spontaneously erupt gastrointestinal tumors, angiogenesis is reduced due to deficiency in IL-21 (De Simone et al. 2015).

Moreover, the CXC chemokines, which belong to the C family, are particularly important in angiogenesis. They bind to their specific receptors on endothelial cells and stimulate a range of cellular processes that promote angiogenesis, such as cell proliferation, migration, and tube formation. For example, CXCL8 (also known as interleukin-8 or IL-8) is a potent angiogenic chemokine that is expressed in response to hypoxia (low oxygen levels) in the tissues. CXCL8 promotes angiogenesis by recruiting and activating endothelial cells, as well as inducing the expression of proangiogenic factors such as vascular endothelial growth factor (VEGF). Other CXC chemokines that have been shown to play a role in angiogenesis include CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL12 (also known as stromal cell-derived factor-1 or SDF-1), and CXCL16. These chemokines are expressed by various cell types, including endothelial cells, immune cells, and stromal cells, and they can act synergistically to promote angiogenesis.

3.4 Interleukin 6 Family

The interleukin-6 (IL-6) family of cytokines has been implicated in a wide range of physiological processes, including inflammation, immune regulation, and angiogenesis. The family include IL-6, IL-11, oncostatin M (OSM), and leukemia inhibitory factor (LIF), have been shown to have proangiogenic effects in various experimental models. These cytokines act on endothelial cells, which are the cells that line the inner surface of blood vessels, to promote their proliferation, migration, and tube formation. IL-6, in particular, has been shown to play a role in both physiological and pathological angiogenesis. IL-6 can induce the production of vascular endothelial growth factor (VEGF), which is a key regulator of angiogenesis, and can also stimulate the migration and proliferation of endothelial cells. IL-11 has also been shown to promote angiogenesis by upregulating VEGF expression. OSM and LIF have been shown to induce angiogenesis in vitro and in vivo by promoting endothelial cell proliferation and migration. OSM has also been shown to enhance VEGF-induced angiogenesis by promoting the recruitment of immune cells to the site of angiogenesis. Moreover, in addition to promoting angiogenesis, the IL-6 family of cytokines can also regulate angiogenesis by modulating the expression of angiogenic inhibitors such as thrombospondin-1 and angiostatin. These findings suggest that targeting IL-6 family cytokines may be a promising strategy for the treatment of diseases characterized by abnormal angiogenesis, such as cancer (Fig. 3.3).

Numerous members of this family are present, including IL-6, IL-11, ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), oncostatin M (OSM), cardiotrophin 1 (CTF1), CCTF-1, or cardiotrophin-like cytokine factor (CLCF1). Recent research has found that 27-IL, 35-IL, and 39-IL are newly included in the interleukin-6 group (Kang et al. 2020). IL-6 can induce VEGF mRNA expression in

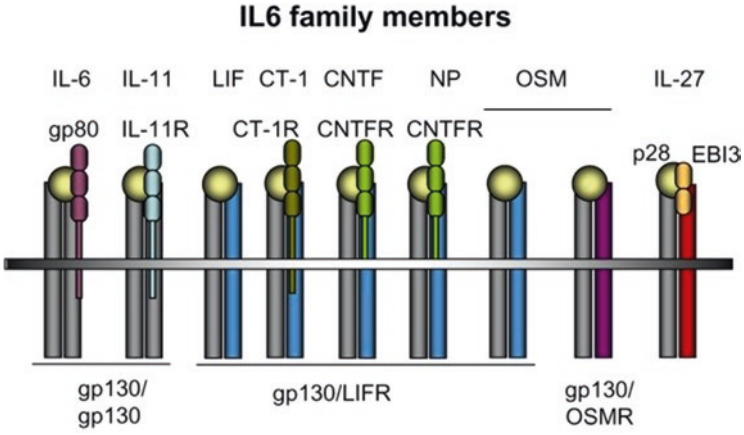

Fig. 3.3 The IL-6 cytokine family members and their receptors (schematic)

A431 cells, a human cell line of epidermoid carcinoma, and also in a rat glioma cell line C6 as in cancer (Porta et al. 2013). EGF-dependent angiogenesis mechanisms are used by IL-6 to increase tumor growth in human cervical carcinoma C33A (Wei et al. 2003). A needful reaction to sunitinib and bevacizumab, a tyrosine kinase prohibit that targets the VEGF/VEGFR, is related with elevated levels of circulating IL-6 in glioblastoma, colorectal cancer, renal cell carcinoma, and hepatocellular carcinoma. Respectively a route and an anti-VEGF antibody (Porta et al. 2013). A549 cells produce less proangiogenic factors when exposed to IL-27; indeed, A549 In cells exposed to IL-27, VEGF, IL-8/CXCL8, and CXCL5 expression are reduced. In contrast to cells that were not treated. It is interesting to note that adding siRNA against STAT1 indicates that IL-27 suppresses the production of these pro-angiogenic molecules by raising their levels. Shows that IL 27 reduces VEGF and angiogenic factor production in human NSCLC via the STAT1 pathway (Kachroo et al. 2011, 2013; Qayoom et al. 2023a). Human diseases such large B cell lymphoma, melanoma, and nasopharyngeal carcinoma, produce IL-35. This interleukin accelerates the maturation of tumors by new vessel formation in tumors (Wang et al. 2013). Prostate cancer development is accelerated by IL-35 via angiogenesis in tumors (Zhu et al. 2020).

3.5 Family of Interleukin-17

Interleukin-17 (IL-17) produced by a variety of immune cells, like T-helper 17 (Th17) cells, gamma-delta (γδ) T cells, natural killer T (NKT) cells, and innate lymphoid cells (ILCs) is a proinflammatory cytokine. The IL-17 cytokine family includes six members (IL-17A to IL-17F) that bind to different receptors and have diverse biological functions. IL-17 family members play an important role in the process of angiogenesis (Wu et al. 2018; Numasaki et al. 2003).

IL-17A, the prototypical member of the IL-17 family, has been shown to promote angiogenesis in several models of inflammation and cancer. IL-17A promotes angiogenesis indirectly by recruiting immune cells to the site of angiogenesis, which can release proangiogenic factors. IL-17A stimulates the proliferation and migration of endothelial cells, which are essential for angiogenesis. IL-17A also induces the production of proangiogenic factors, such as VEGF, by endothelial cells and other cell types. In addition, IL-17A can activate other cytokines and growth factors that contribute to angiogenesis, such as IL-6 and TGF-β. IL-17E (also known as IL-25), a member of the IL-17 family, has been shown to have both pro- and antiangiogenic effects. It can induce the production of VEGF and other proangiogenic factors by endothelial cells, but it can also inhibit angiogenesis by inducing the production of antiangiogenic factors, such as thrombospondin-1 (TSP-1) and soluble endoglin (sEng), by endothelial cells and other cell types. IL-17B, IL-17C, and IL-17D have not been extensively studied in the context of angiogenesis, but they have been shown to have other biological functions, such as promoting inflammation and immunity.

IL-17F, another member of the IL-17 family, can induce the production of VEGF by endothelial cells and enhance angiogenesis in vitro and in vivo. IL-17F can also

induce the production of other proangiogenic factors, such as IL-8 and matrix metalloproteinase-9 (MMP-9), in endothelial cells and other cell types. Further, IL-17F has a defending role in the growth of colon tumors since mice lacking IL-17F exhibit accelerated tumor growth, particularly with a reduced angiogenesis in vivo. An in vivo investigation consistently, demonstrates that IL-17F inhibits the formation of tumors in mice that are carrying the hepatocarcinoma cell line SMMC-7721. The same study demonstrates that IL-17F suppresses the impression of VEGF, IL-6, and IL-8 in hepatocellular carcinoma and prevents the development of microvessels (Wu et al. 2018; Tong et al. 2012; Numasaki et al. 2003) (Fig. 3.4).

The IL-17RA, RB, RC, RD, and RE are ligands for these molecules (Gu et al. 2013). Patients with colorectal cancer who have high IL-17 assertion, which is linked to increased microvessel frequency in colorectal cancer tissues fragment, have a bad prognosis (Zhang et al. 2009). IL-17R is expressed by colorectal cancer cell lines, and they can also release VEGF and IL-6. It's interesting to note that IL-17 stimulates the growth of angiogenic molecules including VEGF and IL-6 in colorectal cancer cells (Liu et al. 2011). Similar to this, patients with hepatocellular carcinoma who exhibit an overgrowth of Th17 tumor cell have a bad prognosis. The amount of Ang-2 and Vascular EGF transcripts rises in the CMS-G4 fibrosarcoma cell line culture when IL-17 is added. Additionally, an osteosarcoma cell line's production of VEGF is influenced by IL-17 (Wakita et al. 2010). According to in vivo research, CD31, MMP9, and VEGF production was lowered in tumor tissues when IL-17A was inhibited at the tumor locations (Hayata et al. 2013). The promotion of VEGF inhibition treatment resistance is another effect of IL-17 (Chung et al. 2013).

In conclusion, we can say that the role of the IL-17 family in angiogenesis is context-dependent and is very much complex. While some members of the family, like IL-17A and IL-17F, promote angiogenesis, others, like IL-17E, can have both pro- and antiangiogenic effects. Further studies are required to fully understand the

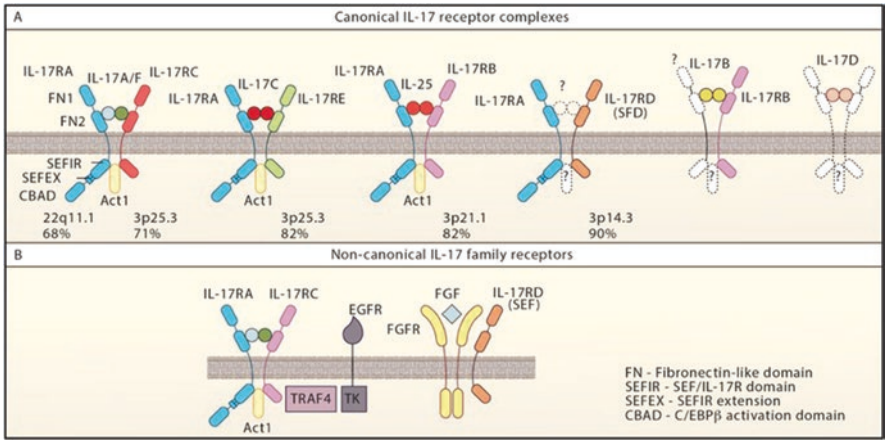

Fig. 3.4 IL-17 cytokine members and their receptors

mechanisms by which IL-17 family members regulate angiogenesis and to explore their potential as targets for antiangiogenic therapies in cancer and other diseases.

3.6 Interleukin 12 Family

As its first identified member, IL-12 named a whole family of cytokines, which includes IL-12, IL-23, IL-27, and IL-35 (Fig. 3.5), and has been found to plan an important role in angiogenesis process (Gabrilovich and Nagaraj 2009). IL-12, a heterodimeric cytokine composed of p35 and p40 subunits, has been shown to have both pro- and antiangiogenic effects.

IL-12 can inhibit angiogenesis by suppressing the expression of vascular endothelial growth factor (VEGF) and other angiogenic factors, as well as inducing the apoptosis of endothelial cells. On the other hand, IL-12 can also promote angiogenesis by stimulating the production of angiogenic factors, such as VEGF and basic fibroblast growth factor (bFGF) and enhancing the proliferation and migration of endothelial cells. IL-12 has shown to suppress angiogenesis in vivo investigations. In vivo angiogenesis-inhibiting properties of IL-12 are reduced by NK cell neutralization, according to one study, indicating that NK cells are involved in mediating this effect (Yao et al. 1999). The proangiogenic task of human primary lung adenocarcinoma cells is inhibited by IL-12 by reducing angiogenic genes like CCL2, HIF-1, VEGF-C, VEGF-D, and IL-6 (Airoldi et al. 2009). IL-12 also reduces VEGF and MMP9 assertion in a mouse breast cancer model (Dias et al. 1998). IL-23, a heterodimeric cytokine composed of p19 and p40 subunits, has been shown to promote angiogenesis in several experimental models. IL-23 can stimulate the production of angiogenic factors, such as VEGF and bFGF, and enhance the migration and tube formation of endothelial cells. IL-23 also promotes the recruitment of inflammatory cells, such as macrophages and neutrophils, which can further promote angiogenesis (Carmi et al. 2013; Gabrilovich and Nagaraj 2009; Qayoom et al. 2023b). IL-27, a heterodimeric cytokine composed of p28 and Epstein–Barr virus-induced gene 3 (EBI3) subunits, has been shown to have both pro- and

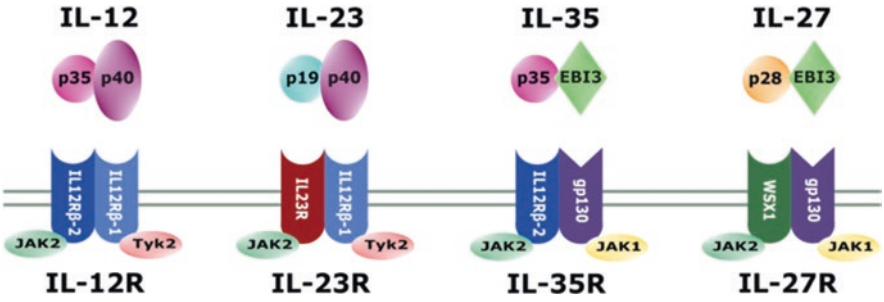

Fig. 3.5 The IL-12 family. Schematic representation of IL-12 family members, their associated receptors, and corresponding subunits

antiangiogenic effects. IL-27 can inhibit angiogenesis by suppressing the expression of VEGF and other angiogenic factors, as well as inducing the apoptosis of endothelial cells. On the other hand, IL-27 can also promote angiogenesis by stimulating the production of angiogenic factors, such as VEGF and bFGF, and enhancing the proliferation and migration of endothelial cells. IL-35, a heterodimeric cytokine composed of p35 and EBI3 subunits, has been shown to inhibit angiogenesis in several experimental models. IL-35 can suppress the production of angiogenic factors, such as VEGF and bFGF, and induce the apoptosis of endothelial cells. IL-35 can also suppress the recruitment of inflammatory cells, such as macrophages and neutrophils, which can further inhibit angiogenesis.

Despite occasionally being categorized as components of the IL-6 family, IL-27, IL-35, and IL-39 are also identified as belonging to this group (Mirlekar and Pylayeva-Gupta 2021). Most activated NK and T cells express the IL-12R receptor (Mir and Mir 2022).

Taken together, IL-12 cytokine is positioned at an important nexus between the innate and adaptive immune response and displays various crucial functions in health and disease that are translationally relevant. But despite being known for more than 30 years now, we are still far away from completely understanding the involvement of IL-12 in physiological and pathological processes. In conclusion, the IL-12 family of cytokines has complex and often opposing roles in angiogenesis. The precise mechanisms by which these cytokines regulate angiogenesis are still being elucidated and may depend on the specific context of the angiogenic stimulus and the cell types involved.

3.7 Family of Interleukin-10

The IL-10 family of cytokines include the interleukins like IL-10, IL-19, IL-20, IL-22, IL-24, and IL-26 (Wei et al. 2019). These cytokines have been shown to play various roles in inflammation, immunity, and tissue repair. Some members of this family have also been implicated in angiogenesis. IL-10, produced usually by helper T type-2 cells (Th2 cells), regulatory T cells (Tregs), and macrophages, has been shown to have antiangiogenic properties (Fig. 3.6). While angiogenesis is primarily regulated by proangiogenic factors, such as vascular endothelial growth factor (VEGF), IL-10 has also been implicated in the regulation of angiogenesis. IL-10 inhibits angiogenesis by suppressing the expression of proangiogenic factors like VEGF and basic fibroblast growth factor (bFGF). In addition, IL-10 inhibits the proliferation and migration of endothelial cells, which are crucial steps in angiogenesis. Studies have also shown that IL-10 inhibits the activation of nuclear factor-kappa B (NF-κB) signaling pathway, which is involved in the regulation of angiogenesis (Mir et al. 2023; Wei et al. 2019). IL-10 a negative regulatory cytokine is an inhibitor of angiogenesis. In fact, tumor formation was inhibited in SCID mice subcutaneously injected with IL-10 transfected B-cell lymphoma DG75 cells as compared to normal cells. In vitro, IL-10 has been revealed to limit the proliferation of microvascular endothelial cells restoring by VEGF and FGF2, and the authors

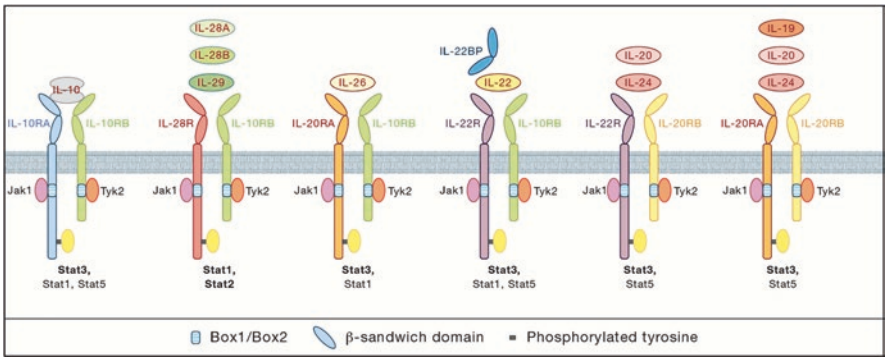

Fig. 3.6 IL-10 family of cytokines

demonstrated that these cells stop angiogenesis (Cervenak et al. 2000). When mice are treated with a strain of ovarian cancer cells that produces VEGF, IL-10 also prevents angiogenesis (Kohno et al. 2003). It was hypothesized that IL-10 generated by tumor cells prevents the production of angiogenic molecules in macrophages (Mir and Mehraj 2019), because it suppresses the production of COX-2 and VEGF (Huang et al. 1996) in NSCLC. IL-19 cytokine has been found to promote angiogenesis in vitro and in vivo through the upregulation of vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs).

Similarly, IL-20 has been shown to induce angiogenesis in a VEGF-dependent manner. In addition, IL-20 promotes the recruitment and activation of endothelial cells, which further enhances angiogenesis (Zhu et al. 2020). IL-20 may have antiangiogenic effects by enhancing endothelial cell survival, proliferation and migration, IL-22 encourages tumor angiogenesis (Heuzé-Vourc'h et al. 2005; Baird et al. 2011). IL-22 has been found to have both pro- and antiangiogenic effects depending on the context. In some cases, IL-22 has been shown to promote angiogenesis by upregulating VEGF and inducing the proliferation and migration of endothelial cells (Gao et al. 2009). In other cases, IL-22 has been found to inhibit angiogenesis by downregulating VEGF and inhibiting the proliferation and migration of endothelial cells (Andoh et al. 2005). Additionally, angiogenesis, tumor development, and microvascular density are all inhibited by the management of IL-22 neutralizing antibodies. In nude mice, the xenografted cervical carcinoma HeLa exhibits a reduction in tumor growth when combined with the drug cisplatin (Protopsaltis et al. 2019). Additionally, through reducing the expression of VEGF, VEGFC, and PDGF-B, this combination also prevents angiogenesis (Wang et al. 2015). In a recent study IL-22, expressed by TH17 cells, has been shown to directly acts on endothelial cells to promote tumor angiogenesis. IL-22 induces endothelial cell proliferation, survival, and chemotaxis in vitro and neovascularization in an ex vivo mouse choroid explant model. Blockade of IL-22, with a neutralizing antibody, significantly inhibits tumor growth associated with reduced microvascular density. No synergistic effect of IL-22 with VEGF was observed. These results

identify IL-22 as a potential therapeutic target for blocking tumor angiogenesis (Protopsaltis et al. 2019). Besides the roles played by the members of Il-10 family of cytokines in the process of angiogenesis the other members like IL-24 have been found to inhibit angiogenesis by downregulating VEGF and MMPs and IL-26 has also been shown to have antiangiogenic effects by inhibiting the proliferation and migration of endothelial cells.

In conclusion the various members of the IL-10 family of cytokines appears to play a complex role in angiogenesis, with some members inhibiting it while as other members promoting it. The exact mechanisms by which the cytokines of IL-10 family regulate angiogenesis are still being investigated, but the involvement of MMPs and VEGF proposes that these may act in part by modulating the extracellular matrix and the signaling pathways that regulate endothelial cell proliferation and migration.

3.8 Chemokines: Role in Angiogenesis

Chemokines are a broad class of low molecular weight cytokines that have been shown to captivate and operate various cell types in a specific manner (Mehraj et al. 2022b). Although their primary role as leukocyte attractants is well known, current research suggests that chemokines play also a part in various tumor-related operations, including new vessel formation, development, and metastasis. Chemokine's stimulate cells through G-protein-coupled receptors and seven trans membranes on the cell surface (GPCR). Chemokines and their receptors play a complex function in the pathophysiology of tumors since some of them promote tumor development and spread while others may strengthen antitumor immunity (Mir 2015a, b, c, d). Chemokine's serve a variety of functions that make them essential mediators between tumor cells and their microenvironment. They also play a significant role in angiogenesis and tumor progression.

Leukocyte trafficking to inflammatory areas is regulated by a sizable variety of less molecular weight chemotactic proteins that belong to the chemokine superfamily (Zlotnik and Yoshie 2000; Locati et al. 2002). Four conserved cysteine amino acid residues, connected by disulfide bonds, make up the majority of the 8–11 kDa chemokines (Singh et al. 2007). Chemokines (CXC, CC, C, and CX3C) are structurally divided into four subfamilies. There are more than 50 chemokines, most of which are members of the CXC and CC subfamilies, which are the most prevalent (Murphy et al. 2000). Chemokine ligands and receptors are now referred to as "L" or "R," respectively, in a new classification. Due to the fact that each receptor binds to a different subfamily of chemokines, receptors are further divided into four subfamilies (Zlotnik and Yoshie 2000; Mehraj et al. 2022a, b).

On the basis of configuration of the two cysteine residues that are closest to the N-terminal of chemokines, they are categorized into four families: C, C-C, C-X-C, and C-X3-C. There is only one N-terminal cysteine on the C chemokines. These cysteines are next to each other in the C-C chemokines, an amino acid separates them in the C-X-C chemokines, and three amino acids separate them in the C-X3-C

chemokines. These compounds are mostly known to promote leukocyte migration, but many investigations have shown that they also contribute to tumor angiogenesis.

3.9 C-X-C Chemokines

On the basis of ELR (Glu-Leu-Arg) design, this family of chemokines exhibits either pro- or antiangiogenic effects (Mir 2015a, b, c, d). This shape stimulates angiogenesis and inhibits it when it is absent. The ELR^+ chemokines are predominantly neutrophil chemotactic, making those strong angiogenesis promoters. However, ELR members primarily target T cells and B cells, which are powerful angiogenesis inhibitors. Parts of the CXC chemokine family that are angiogenic include CXCL1–3 and CXCL5–8, whereas those that are angiostatic include CXCL4 (12) and CXCL9–11. Even though the ELR chemokine CXCL12 was first identified as a pre-B-cell growth-promoting factor, it has now been demonstrated to have angiogenic action. Additionally, it has been displayed that ELR CXC chemokines prevent neovascularization brought on by traditional angiogenic agents involving basic fibroblast growth factor and vascular endothelial cell growth factor (VEGF). In contrast to CXCL4, CXCL10, and CXCL14, which are ELR-negative and limit angiogenesis, CXCL1, CXCL6, and CXCL8, as well as CXCLR5, are ELR-positive and promote angiogenesis. CXCL12, on the other hand, stimulates angiogenesis despite being ELR-negative. Intriguingly, CXCL14 transgenic animals injected with LLC or B16 melanoma cells displayed slower tumor growth than WT mice, and they also displayed fewer and smaller visible blood vessel diameters in tumors. Furthermore, WT mice had larger tumor CD31-positive cell percentages (Izukuri et al. 2010; Sofi et al. 2023). CXCL5 overexposed is positively linked with CD31 expression. Through CXCR2, this chemokine is able to encourage HUVEC tube formation, migration, and proliferation in addition to inducing the production of VEGF-A in HUVEC (Chen et al. 2019). In colorectal cancer, CXCL1 is also able to encourage angiogenesis. Intriguingly, CXCL1 accelerates tumor growth and increases microvessel density, whereas CXCR2—the receptor for CXCL1—is raised in CRC tissue (Wang et al. 2006). CXCL10 can reduce blood vessel growth by preventing endothelial cells from migrating. By attaching to CXCR3 that is expressed on newly formed vessels, CXCL10 prevents angiogenesis (Yates-Binder et al. 2012).

3.10 CXCL8: Role in Angiogenesis

Angiogenesis was demonstrated to be induced by CXCL8, also known as IL-8 (Végran et al. 2011). CXCL8 is one of the most investigated chemokines as a powerful angiogenesis mediator. Rat mesenteric window, rat and rabbit corneal, and a subcutaneous sponge model assays were used to confirm the proangiogenic action of CXCL8 in vivo (Hu et al. 1993). When impregnated in the rat cornea, human recombinant CXCL8 was found to be angiogenic and to stimulate the growth and

chemotaxis of human umbilical vein endothelial cells (Koch et al. 1992). Further, antisense oligonucleotides inhibited the angiogenic effects of conditioned media from activated monocytes and macrophages (Mehraj et al. 2021a, b, c). Since CXCL8 stimulates angiogenesis in the absence of inflammatory cells, many researchers have hypothesized that its angiogenic action is distinct from its chemotactic and proinflammatory activities (Hu et al. 1993). It has been noted that in nude xenograft models of human cancer cells, there is a direct association between high levels of CXCL8 and tumor angiogenesis, development, and metastasis (Luca et al. 1997; Xie 2001). The expression of CXCL8 was directly linked with neovascularization and weak survival in an experimental model of ovarian cancer (Yoneda et al. 1998). Given that blood levels of CXCL8 are higher in individuals with prostate and breast cancer and correlate with disease stage (Veltri et al. 1999; Benoy et al. 2004), CXCL8 may also play a significant role in angiogenesis in these cancers. The expression of its receptor by endothelial cells determines whether CXCL8 can cause angiogenic activity. Endothelial cells have been found to express CXCL8 and its receptors, CXCR1 and CXCR2, and it has been illustrated that these molecules contribute to endothelial cell proliferation (Koch et al. 1992; Salcedo et al. 2000; Uehara et al. 2005). Recent research shows that human microvascular endothelial cells (HMEC) express CXCR1 and CXCR2 at high and moderate levels, respectively, while human umbilical vein endothelial cells (HUVEC) express CXCR1 and CXCR2 at low levels. Nullifying antibodies to CXCR1 and CXCR2 prevented endothelial cells from migrating in response to CXCL8, demonstrating that these two receptors are essential for the angiogenic response to CXCL8. The role of CXCR2 in mediating chemokine-induced angiogenesis was shown to be crucial to CXCL8-induced neovascularization (Addison et al. 2000). CXCR2 is one of these two high affinity receptors for CXCL8. Other tumor systems have supported the function of CXCR2 in enhancing tumor-associated angiogenesis.

In addition to CXCL8, other chemokine family members have been demonstrated to be crucial in angiogenesis. The vascularity of nonsmall cell lung cancer (NSCLC) was related with upraised levels of CXCL5 and CXCL8 (Numasaki et al. 2005). Depletion of CXCL5 attenuated tumor development, angiogenesis, and spontaneous metastasis in an acute combination immune-deficient (SCID) animal model system (Arenberg et al. 1998). CXCR2 was discovered to be expressed on endothelial cells within tumor biopsies in renal cell carcinoma, and higher levels of CXCL1, CXCL3, CXCL5, and CXCL8 were revealed to be expressed in the tumor tissue and found in the plasma. Thus, the several studies that were discussed before show how crucial a function ELR^+ CXC chemokines play in tumor angiogenesis.

In spite of a chemokine CXC that is not an ELR, CXCL12 is highly relevant to tumor angiogenesis. CXCL12 causes endothelial cell migration, proliferation, tube formation, and increases in Vascular EGF release by endothelial cells, according to a number of lines of evidence (Kanda et al. 2003). By inhibiting angiogenesis in a VEGF-independent way, CXCL12/CXCR4 blockade reduces tumor growth in vivo (Guleng et al. 2005). It is likely that tumor cells and specialized stromal cells are the source of the CXCL12 that fuels angiogenesis. The capability of breast carcinoma-associated fibroblasts to stimulate angiogenesis has been hypothesized to be

partially attributed to CXCL12 in a recent study. While CXCL12 neutralization inhibited metastasis in a model of NSCLC, it did not result in a decrease in tumor angiogenesis, indicating that the effects of CXCL12 on angiogenesis cannot be extended to all tumor systems (Phillips et al. 2003). These findings indicate that CXCL12 may have a tumor-specific effect or may work in concert with other angiogenic proteins.

3.11 C-C Chemokine

The two cysteine residues that are nearest to the N terminal adjacent are found in C-C chemokines. With CCL9 and CCL10 being the same chemokine, this family consists of 27 chemokines from CCL1 to CCL28. These molecules attach CCR1 to CCR10, a group of 10 distinct chemokine receptors (Korbecki et al. 2020). One of the key chemoattractants macrophages (Mir et al. 2022a, b, c, d), CCL2 also persuades the release of proangiogenic molecules like VEGF. Bevacizumab can cause bevacizumab resistance in some glioblastoma multiforme (GBM) patients, and tumor-associated macrophages are a key factor in this mechanism (Mehraj et al. 2021a, b, c). Recent research has demonstrated that mNOX-E38 decreases macrophage recruitment to GBM cells that produce CCL2. Additionally, they showed that compared to CCL2-expressing cells alone, angiogenesis was increased in cocultures of macrophages and CCL2-expressing cells. Inhibiting CCL2 may improve the effectiveness of antiangiogenic therapies in GBM, as evidenced by the fact that using this hindrance in connection with bevacizumab enhances mice survival when contrast to using bevacizumab only (Cho et al. 2019). Compared to healthy persons, endometrial cancer (EC) patients have higher levels of CCL4 and VEGF-A expression in EC tissues, and there is a positive association between these expressions. Studies carried out both in vitro and in vivo highlight that CCL4 stimulates tumor development by increasing the production of VEGF-A, which in turn affects the STAT3 signaling pathway in EC cells (Hua et al. 2017).

3.12 Chemokine CX3C

Between the two first cysteines, there are nonconserved three amino acids in the CX3C chemokine (-chemokine's). One chemokine from this group has so far only been depicted. This substance, CX3CL1, which is often referred to as fractalkine (FKN), connects to CX3CR1. Angiogenesis is regulated by this chemokine. In fact, ex vivo and *in vivo* experiments shown that FKN mimic angiogenesis, and *in vitro* research demonstrated that these chemical increases human umbilical vein endothelial cell proliferation, migration, and tube formation. This work demonstrated that the signaling pathways Raf-1/MEK/ERK and PI3K/Akt/eNos are activated by CX3CL1 to promote angiogenesis (Lee et al. 2006).

3.13 Cancer Therapies That Focus on Angiogenesis

3.13.1 VEGF Family: The Targeting Therapies

The extremely researched and applied cancer treatments; target the VEGF signaling pathway. Bevacizumab, aflibercept, and ramucirumab are the three recombinant proteins that have been allowed for use in the treatment of cancer. Two humanized monoclonal antibodies that target all VEGF-A isoforms and VEGFR-2, respectively, are bevacizumab and ramucirumab. Aflibercept is a protein that consists of the Vascular EGFR-2 and Vascular EGFR-1 recognition domains binding to the Fc region of a human IgG1. The ability of aflibercept to bind to PlGF, VEGF-A, and VEGF-B. Tyrosine kinase inhibitors (TKI) like tegorafenib, sorafenib, and sunitinib have also been allowed for the medication of cancer. We made the decision not to go into great detail because there are variety of reviews of the anti-VEGF-based medicines. Although it is a promising method, focusing on the VEGF signaling pathway as a single therapy seems to be futile due to several resistances. The angiogenic signaling pathways are redundant; when the VEGF signaling route is stopped, other pathways take over to keep angiogenesis going (Qayoom et al. 2021). Targeting multiple angiogenic elements at once is therefore desirable to overcome this resistance.

3.14 Therapies for Targeting Angiopoietin

Recently, treatments for cancer patients that target the Ang–Tie signaling pathway have been available (Saharinen et al. 2017). They are Ang-2-specific monoclonal antibodies like MEDI3617, Nesvacumab (REGN910), and LY3127804. In numerous xenograft tumor models, including colorectal cancer (LoVo and Colo205), renal cell carcinoma (786-0), ovarian carcinoma (HeyA8), and hepatocellular carcinoma (PLCPRF/5). MEDI3617 dramatically slows tumor growth (Khan et al. 2016). Its safety in advanced solid tumors has been determined by Phase I (NCT01248949), and patients with ineradicably Stage III or Stage IV melanoma are still the subject of another study (NCT02141542). Different xenograft tumor models, such as colorectal cancer (Colo205), prostate cancer (PC3), and epidermoid carcinoma, are inhibited by REGN910 in terms of tumor growth and tumor vascularity (A431). REGN910 has also been demonstrated to enhance the effects of aflibercept (Papadopoulos et al. 2016). Another method is the ABTAA protein, which not only inhibits Ang-2 but also stimulates TIE-2 to improve vascular normalization and hence boost medication delivery. In a subcutaneous LLC tumor model, this chemical slows tumor growth (Park et al. 2016). Recombinant proteins are also available that target Ang-2 as well as the interconnection of Ang1/Ang2 with Tie-2. Another one of these compounds is trebananib. Although this chemical is still being studied, it improves progression-free survival when used in conjunction with paclitaxel to treat recurrent ovarian cancer (NCT01204749). Bevacizumab + Trebananib was effective and tolerated as first-line therapy for patients with metastatic colorectal

cancer, according to a recent research (Mooi et al. 2021). Vanucizumab is an antibody that specifically targets VEGF-A and Ang-2. However, a newly research found that in patients with metastatic colorectal cancer, the mixture of Vanucizumab/mFOLFLOX-6 did not increase the PFS compared to Bevacizumab/mFOLFLOX-6 biotherapy.

3.15 Therapies Targeting HGF

The HGF/MET pathway is involved in various cancer types, making targeting it a promising therapy. HGF constraint, MET antagonists, MET kinase inhibitors, and HGF stimulating inhibitors are some of the numerous ways that this signaling system might be targeted. Epithelial, endothelial, neuronal, hematopoietic, and hepatocyte cells are among the cell types that express MET. A number of biological reactions, including proliferation, angiogenesis, migration, invasion, metastasis, and survival, are linked to the activation of the HGF/MET axis. Different solid cancers have abnormal activation of the HGF/MET signaling pathway, which is linked to a poor prognosis. Several human cancers, involving renal, lung, gastrointestinal, thyroid, and breast carcinomas, as well as sarcomas and malignancies of the nervous system, such as GBM, are influenced by HGF/MET aberrant activation. A fully humanized monoclonal antibody called rilotumumab targets HGF. A preclinical investigation found that combining rilotumumab with docetaxel or temozolomide reduced the maturation of tumors in nude mice carrying the U-87 MG tumor (Mooi et al. 2021). This chemical had an acceptable profile in clinical investigations in individuals with mRCC, but no impact was found (NCT00422019). Rilotumumab and mFOLFOX6 first-line chemotherapy do not have any advantages in patients with newly gastroesophageal adenocarcinoma (Malka et al. 2019). Bevacizumab alone did not produce a better response in patients with recurrent malignant gliomas than Rilotumumab combined with it did. This compound is not accepted by the FDA at this time. A fully humanized monoclonal antibody called onartuzumab targets the MET extracellular domain. This chemical failed to enhance the therapeutic efficacy of paclitaxel in patients with metastatic triple-negative breast cancer when used alone or in combination with bevacizumab (NCT01186991). Additionally, it did not enhance mFOLFOX6's effectiveness in treating gastric cancer (Shah et al. 2016). The aggregation of Onartuzumab with mFOLFOX-6 and bevacizumab did not enhance the therapeutic usefulness as much in patients with metastatic colorectal cancer. MET tyrosine kinase inhibitors are cleaved into two classes, class I and class II, according to how they bind to the MET structure. The European Medical Agency (EMA) and the Food and Drug Administration (FDA) have both approved crizotinib as a type I TKI for patients with NSCLC under specific circumstances (Kazandjian et al. 2014). In some circumstances, this drug is also licensed for use in people with anaplastic large cell lymphoma. Cabozantinib is a type II TKI that the EMA has approved under specific circumstances for patients with medullary thyroid carcinoma. This drug has also been given FDA approval for use in individuals with differentiated thyroid carcinoma that has spread locally or distantly.

3.16 Therapies for FGF Targeting

Tyrosine kinase inhibitors and monoclonal antibodies directed against FGFR and FGF are two medications being researched as potential FGF/FGFR signaling pathway inhibitors. Numerous monoclonal antibodies that target FGF have shown intriguing findings. Antibodies against FGF2 and FGF8b have been focused to have antitumor and antiangiogenic properties in in vitro and in vivo studies (Chudzian et al. 2018). SMMC-7721, HEP-G2, and SK-HEP-1 are three distinct xenograft mouse models of human HCC cell lines that were used to test GAL-F2 as a tumor suppressor. Numerous monoclonal antibodies, including FPA144, PRO-001, RG7444, and SSR128129E, target FGFR. Antibody–drug associates that target this route are also available. The substance BAY 1187982 is a monoclonal antibody that targets FGFR-IIb and FGFR-IIIc and is coupled to an auristatin that disrupts microtubules. In various cancer models, including those for breast, gastric, and ovarian cancer, this chemical slows tumor growth (Kim et al. 2019). There are various tyrosine obstacles that target FGFR and other receptors including VEGFR, FGFR, and PDGFR, including AZD4547, BAY1163877, BGJ398, and AXL1717, as well as Cediranib and Dovotinib.

3.17 Therapies for PDGF

PDGF restrictor, inhibitors of the connection between PDGF and PDGFR, and TKIs are a few of the treatments that target the PDGF/PDGFR signaling system. Human monoclonal antibodies are also available that specifically target PDGFR. The xenograft lung cancer tumor model Calu-6 and A549 showed less tumor growth in the presence of this chemical (Loizos et al. 2005). In xenografts of glioblastoma (U118) and leiomyosarcoma (SKLMS-1), it also slows tumor growth. Doxorubicin with a PDGFR-targeting antibody increase overall survival in neutral stages Ib and II when compared to doxorubicin alone (Tap et al. 2016) The FDA granted conditional clearance to this chemical in 2016 for the medication of soft tissue sarcoma, and the EMA has granted full authorization. This chemical is ineffective in the therapy of glioma, prostate cancer, or ovarian cancer. The PDGFR signaling pathway is targeted by a variety of TKI that have received clinical approval, including Imatinib, Nilotinib, Dasatinib, Ponatinib, Sunitinib, Axitinib, and Sorafenib (Papadopoulos and Lennartsson 2018) [162].

3.18 Conclusion

The purpose of this paper was to highlight the close relationship among angiogenesis and the microenvironment tumor, in particular the cytokines and chemokines that may be present in tumors. These chemicals are produced by stromal cells and immune cells, who both carefully regulate angiogenesis within the tumor. An important aspect of carcinogenesis and a defining characteristic of cancer, infection is a

potent proangiogenic signal. It is obvious that inflammation and angiogenesis are linked to cancer given that cytokines like IL-1, IL-6, and TNF all have proangiogenic characteristics. It is interesting to note that cytokines processed by traditional protumor immune cells like Tregs, which produce IL-10 and IL-35; Th17 cells, which create IL-17 and IL-22; or Th2 cells, which produce IL-4, are all proangiogenic factors. Further, NK cells, Th1, and cytotoxic CD8 T lymphocytes are connected to antiangiogenic chemicals like IFN and IL-12 with recognized antitumor immune cells. Chemokines function to draw cells in a gradient-dependent way, and their influence on angiogenesis is influenced by the cells they draw as well as by the direct effects they have on angiogenesis. In fact, CCL2 and CCL4 both work to attract macrophages to the tumor and stimulate their formation of VEGF. Additionally, it has been discovered that CXCL1 and tumor cells directly communicate with CXCL1, causing the creation of VEGF by tumor cells. Although some chemokines, like CXCL10, also can prevent angiogenesis by directly influencing recently created blood vessels or by encouraging the development of the antiangiogenic miR206, like CCL19. Immune cell recruitment to the tumor is frequently linked to chemokines that have antiangiogenic actions.

For the treatment of cancer, three recombinant proteins that target the VEGF/VEGFR pathway have been allowed. But because there are so many unnecessary mechanisms that lead to angiogenesis, many patients grow resistant to these treatments. Many of the new medicines that target these redundant pathways are still in research or being tested in clinical phases after significant effort has been put into their creation. Furthermore, it is evident that inhibiting angiogenesis does not produce a strong immune response by itself. It is beginning to show encouraging effects when antiangiogenic therapy is combined with chemotherapies or immunotherapies, and it is anticipated that additional relationships of this kind will emerge in the future.

Further Reading

For further readings, some textbooks mentioned below also give further understanding of cytokine and chemokine network.

- Cytokines and Their Therapeutic Potential by Manzoor Ahmad Mir.
- The Cytokines of the Immune System: The Role of Cytokines in Disease Related to Immune Response by Zlatko Dembic.
- The Cytokine Network and Immune Functions by Jacques Thèze.
- Chemokines and Viral Infection by T.E. Lane.
- Cytokines and Chemokines in Infectious Diseases.

The below links for video lectures may also be helpful:
https://youtu.be/TAqO0Mq19JQ
https://youtu.be/6wEnYfvoBqk
https://youtu.be/A9fRRCUlMms
https://youtu.be/zIWvmCsKr34

For more incites about the topic, we would suggest detailed findings from the books of (Mir et al. 2022a, b, c, d) https://doi.org/10.1016/C2021-0-02565-7, https://doi.org/10.1016/C2022-0-00074-X and https://doi.org/10.52305/WXJL6770 (Mir 2015a, b, c, d) https://doi.org/10.1016/C2014-0-02898-5 and from the cancer.net website on the following mentioned below links

https://www.cancer.net/cancer-types/breast-cancer/types-treatment

https://discovery.ucl.ac.uk/id/eprint/1472740/

https://www.jmedsciences.com/doi/JMEDS/pdf/10.5005/jp-journals-10045-00138

For diagrammatic illustrations and descriptive tables, see Lazzeroni http://www.eurekaselect.com/article/49928

See also video links on overall status of cancer, its various types, and current new treatment possible options available at

https://www.sciencedirect.com/science/article/pii/S205970292032278X.

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Implications of Chemokine Heterogenicity in Cancer Metastasis

4

Manzoor Ahmad Mir, Sumaya, and Nusrat Jan

Abstract

The amazing genetic complexity of malignant tumors has been revealed because of advancements in genomic technology. A phenomenon known as intratumor heterogeneity is emerging evidence that solid tumors may have subpopulations of cells with different genetic changes within the same tumor. Particularly in the era of focused treatment, intratumor heterogeneity is anticipated to have ramifications for cancer treatments and biomarker development, and there is mounting evidence that it may be related to clinical outcomes. The primary factor in cancer-related death is tumor metastasis. The metastatic cascade and metabolic rewiring are closely related and work together to support various stages of cancer metastasis. Cancer cell metabolites have an impact on the metastatic cascade, which includes the epithelial–mesenchymal transition (EMT), the persistence of cells of cancer in the bloodstream, and metastatic colonization at out-of-reach sites. The action of prometastatic metabolites, which are tumor-derived, is mediated by several molecular pathways, involving epigenetic dysregulation, stimulation of matrix metalloproteinases (MMPs), enhancement of cancer stemness, and reduction of oxidative stress. On the other hand, prometastatic metabolites are produced by rate-limiting metabolic enzymes when metastatic signaling affects their expression and activity and the metastasis cascade is reinforced. Knowing how metabolism and metastasis interact intricately may lead to the discovery of new molecular targets, whose treatment may improve cancer therapy.

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Keywords

Heterogenicity · Intratumor · Next-generation sequencing · Therapeutics · Cancer metabolism · Metastasis

4.1 Introduction

For a primary tumor and any metastases it produces, including tumors of the same histopathological subtype, there are subpopulations of cells with unique genotypes and phenotypes that may harbor diverse biological behaviors. This is known as tumor heterogeneity (intra- and inter-tumor, respectively). Deep sequencing techniques have made it possible to better understand the scope and prevalence of intra- and inter-tumor heterogeneity. Although intratumor heterogeneity has characteristics that are part of a standard pathological evaluation, clinical decision-making does not yet include its determination. Tumor -omics in cancer cells vary significantly over space and time. Clinicians use tumor mutation profiling and gene expression to determine the type of cancer a patient has, how to treat it, and how likely they are to survive (Trainer et al. 2010; Jekunen 2014). Researchers have thoroughly investigated the phenomenon of tumor diversity through space by validating contrasting mutations found in either various primary tumor samples or metastases (Campbell et al. 2010; Hardiman et al. 2016). According to Morrissey and colleagues' (2015) analysis of the geographic diverseness of medulloblastoma, high-grade glioma, and renal cell carcinoma (RCC), at least five biopsies are required to detect at least 80% of the somatic variations (Shah et al. 2009).

4.2 The Spectrum of Cancer Heterogenicity

4.2.1 Clinicopathological Heterogeneity and Its Molecular Basis

The treatment of cancer is complex owing to the heterogeneity of tumors, which is connected to unsatisfactory outcomes. The lofty Mutant Allele Tumor Heterogeneity (MATH) scores, a measure of intratumor heterogeneity depending on whole-exome sequencing data, were linked to poor overall survival in the Cancer Genome Atlas for head and neck cancer cohort (Mroz et al. 2015). Similar to this, the clonality of neoantigens correlates with the response to immune checkpoint inhibitors in non-small cell lung cancer (NSCLC) (Mehraj et al. 2021a; McGranahan et al. 2016). For example, first-line therapy with either chemotherapy or tyrosine kinase inhibitors resulted in a mixed response in 21% of cancer patients with non-small lung cells (Dong et al. 2017). In reality, there are limited circumstances in which a mixed response occurs more frequently (Lee et al. 2014). Adaptable n-of-1 clinical trials are considered to be the ideal mode to assess the effects of a patient's tumor heterogeneity in actual time because of the clear-cut evolutionary history of every patient's malignancy (Lillie et al. 2011; Bedard et al. 2013; Catenacci 2015). These studies

could make use of serial liquid biopsies to judge temporal heterogeneity and multiple regional liquid biopsies to evaluate spatial heterogeneity (Joung et al. 2016; Lennon et al. 2016). Since targetable mutations are not automatically clonal, therapies will likely need to focus on truncal mutations or pathways that are shared by subclones to treat patients effectively (Mir et al. 2022a; McGranahan et al. 2012; Lohr et al. 2014). Histopathologists have long been aware of the variety of morphologies within a tumor. Since only the highest grade is reported, pathologists often evaluate various sections of the same tumor (Fig. 4.1). Other illustrations of intratumor heterogeneity are taken into account when grading breast cancer as a nuclear pleomorphism. Additionally, the behavior of tumors greatly varies across people with similar tumor types and in between various sites of tumors within the same patient. The latter is typically revealed by inconsistent or blended responses to therapy. Common clinicopathological findings can be intuitively simplified by intratumor heterogeneity, but studies are just now starting to systematically assess the connection. It may be also possible that additional elements, like as in pharmacodynamics, have a unique role in the unevenness of a pharmacological response.

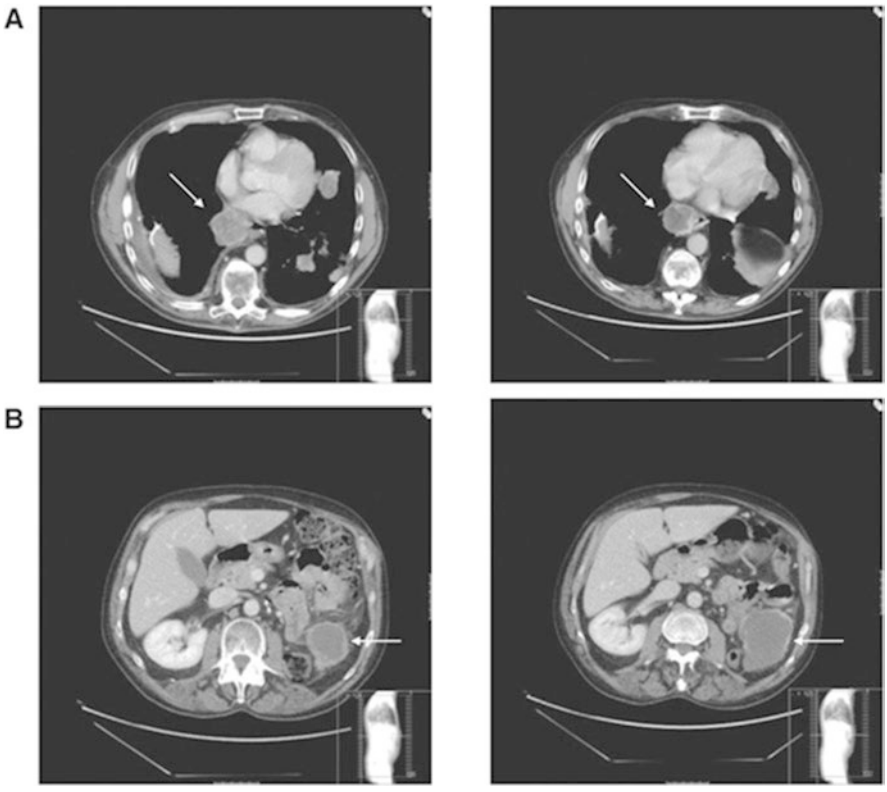

Fig. 4.1 (**a**, **b**) Serial computed tomography

G-banded karyotyping and fluorescence in situ hybridization (FISH) experiments, which revealed unique chromosomal rearrangement patterns and copy number variations of the key genomic loci, provided the first evidence that various subclones with varying genetic statuses existed inside the same tumor (Navin and Hicks 2010). The fulfillment of the human genome project gave researchers a an opportunity to access the all-inclusive somatic coding sequences and noncoding abnormalities on a wide scale, an important step in apprehending the full genetic complexity that drives cancers. This chapter will concentrate on how genetic intratumor heterogeneity affects therapy outcomes and treatment stratification, despite the existence of other kinds of intratumor heterogeneities, like phenotypic heterogeneity.

4.2.2 Clonal Evolution as a Model of Tumor Progression and Heterogeneity

A clonal evolutionary model of cancer development was first proposed by Pater Nowell in 1976 (Nowell 1976) that also explained how Darwinian models of natural selection worked, describing how genetically unstable cells undergo genetic changes and how selection pressures enable the growth and survival of variant subpopulations that have the advantage of biological fitness. This model aggravates structural and numerical chromosomal instability (CIN) in co-occurrence with cancer genomic instability pathways (McGranahan et al. 2012) and might be a cause of the molecular heterogeneity that exists in tumors, which increases not only the number of genetic variants that can be inspected by selection but also the chance that a subclone will have an advantage of growth and/or survival. An important part of this paradigm is the idea of "driver" and "passenger" mutations: somatic mutations, also known as "passenger mutations," are either neutral or cause harmful changes that continue because they are genetically connected to "driver mutations," which improve the cell fitness and drive the cell lineage to form tumors (Sprouffske et al. 2012). We suggest that both kinds of mutations have direct inferences for the therapeutics of cancer because the resistance outgrowth is determined by heterogeneous events and finally patient demise, and even driver mutations are at the core of our recent understanding of the genetic basis of cancer in humans (Mehraj et al. 2021b).

Recent research studies on hematological cancers as well as brain, breast, and pancreatic tumors have proven the role of clonal evolution as a substrate for tumor growth (Sidransky et al. 1992; Yachida et al. 2010; Ding et al. 2012; Mir et al. 2022b). In individuals with transformed myelodysplastic syndromes, matching samples from the initial myelodysplastic stage and the secondary acute myeloid leukemia (AML) stage were also clonal; the transition to AML was associated with the acquisition of several additional mutations in a founding clone to establish a daughter subclone (Walter et al. 2012). The cancer stem cell (CSC) hypothesis, another model for tumor growth, might provide an additional, not compulsorily mutually exclusive, simplification for intratumor heterogeneity (Mir et al. 2022c; Qayoom et al. 2021; Navin and Hicks 2010; Sprouffske et al. 2012).

4.2.3 Tumor Heterogeneity: A Dynamic State

A tumor may be able to adapt to changes in the tumor microenvironment (TME) due to its dimensional and temporal heterogeneity. In multiple myeloma patients, there are interesting clinical examples of diversity in the temporal patterns of tumor genomic architectures, and, in some individuals, a clear pattern of complicated clonal rivalry has been seen (Qayoom et al. 2022; Keats et al. 2012) (Fig. 4.2). Longitudinal copy number analysis was possible in one patient with a cytogenetically high risk because serial samples were available throughout the disease. Two significant clonal progenitors and subsequent divergence were shown in this work. The subdual and repetition of clones at different time points appeared to be linked to the therapy of drug timing. However, the plasma cell leukemic clone 2.2 that caused patient mortality was rarely identifiable at the time of diagnosis, creating real concerns for predictive or prognostic biomarker techniques. In recent times, patients with chronic lymphocytic leukemia have been shown to have the same result (Mir and Mehraj 2019; Schuh et al. 2012). Our explication of the experimental data is that, in the context of systemic medication, two subclones may coexist in

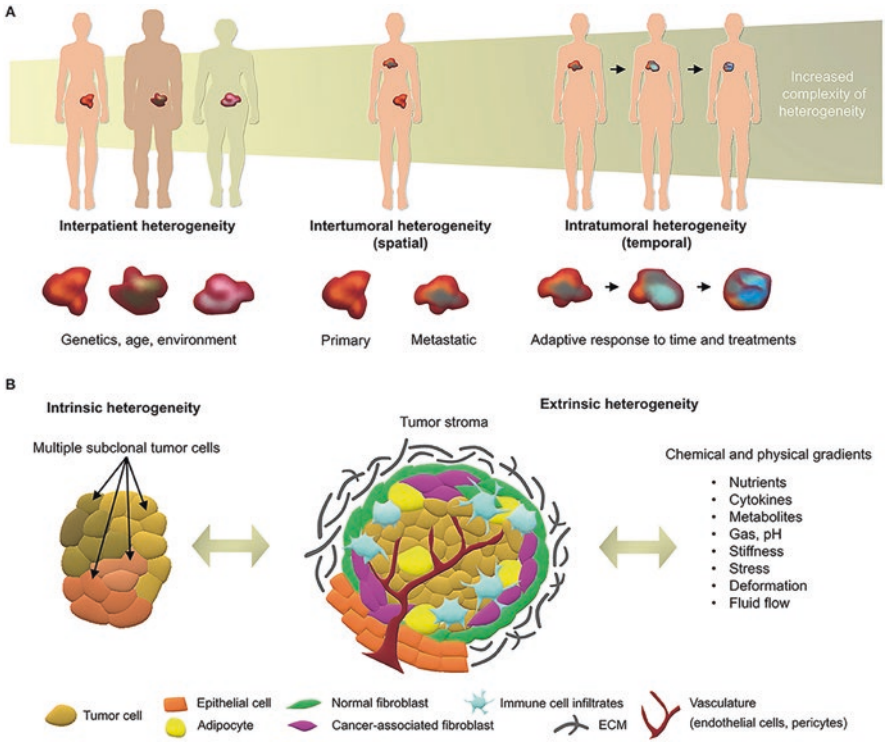

Fig. 4.2 Overview of the cancer heterogeneity: The complexity of tumor microenvironments is largely attributed to the interaction of genetic/epigenetic intrinsic variables, stromal extrinsic factors, and chemical/physical factors

"dynamic equilibrium," continuously contending for dominance. Using whole-genome sequencing, a third study found subclones present at diagnosis, which fluctuated in dominance as the malignancy developed in a patient whose multiple myeloma had led to plasma cell leukemia (Egan et al. 2012; Sofi et al. 2023).

In addition, the cooperation of clones has been documented (Bonavia et al. 2010), and it was revealed that both in vitro and in vivo co-cultures of cells of glioma with wild-type and mutant epidermal growth factor receptors (EGFRs) promote tumor formation. Leukemia inhibitor factor and interleukin (IL)-6 were produced by EGFR-mutant cells through a paracrine pathway, which activated the wild-type EGFR and promoted its growth. This led to the results that were observed.

4.3 Existence of Tumor Heterogeneity at Various -Omics Levels

Tumor -omics differ significantly between cells of cancer across time and space. Clinicians use tumor mutation profiling and expression of genes to show the type of cancer a patient has, how to care for it, and how likely they are to survive (Trainer et al. 2010; Jekunen 2014). The interconnection between immune cells in the TME and tumors is also an aspect of tumor heterogeneity. Cytokines are emitted by immune cells, which are known to increase the number of cancer stem cells (Qayoom et al. 2023; Sansone et al. 2007; Liu et al. 2011). One of the cytokines, namely, transforming growth factor (TGF)-β, is produced by epithelial, immunological, and/or cancerous cells, and stimulating signaling in this pathway can lead to the suppression of cytolytic gene expression, thus weakening cytotoxic T cells and enabling cancer cells to evade the immune system (Mir et al. 2022; Vinay et al. 2015; Thomas and Massagué 2005).

4.3.1 Genomic Heterogeneity

Tumor cells are extremely different both in terms of space and time, according to genetic studies on tumor heterogeneity. Researchers have thoroughly investigated the phenomenon of tumor diversity through space by validating contrasting mutations present in either numerous primary tumor samples or metastases (Campbell et al. 2010; Hardiman et al. 2016). As a venture, the sequencing of the exome of 23 places of a single hepatocellular carcinoma (HCC) tumor showed the availability of 20 different subclones, and envisioning the HCC tumor's whole volume revealed a determined 100 million somatic coding mutations along all subclones. These data demonstrate the substantial geographic heterogeneity of a clinically unexceptional tumor, and it is possible to use different biopsies to capture all clinically relevant alterations and to reliably distinguish between truly clonal mutations and those that are sub-clonal (Ling et al. 2015). Profiling 4–5 regions of primary tumors from 11 patients with HCC was carried out by Lin et al., and the results showed that, on average, 39% of somatostatin mutations varied among the geographic samples collected

from each patient's tumor (Lin et al. 2017). Comparable results were seen in patients with oligodendroglioma (43% in these patients) and in those with esophageal squamous cell carcinoma (ESCC) (36% across 3–4 samplings from each of the 13 patients with ESCC) (Aihara et al. 2017).

When comparing full-exome sequencing of primary formalin-fixed paraffin-embedded (FFPE) samples and 5–12 metastatic locations during a quick autopsy of 4 patients with metastatic breast cancer, Savas et al. demonstrated that metastases can be seeded by polyclonal groupings of cells (Savas et al. 2016). Metastatic cells can probably spread to other places. Studies have used serial circulating tumor DNA (ctDNA) sampling in addition to standard cancer cell collection techniques such as draining ascites fluid to investigate temporal heterogeneity. For example, in a large, parallel study, using ctDNA sequencing, De Mattos-Arruda et al. detected somatic nonsynonymous mutations in their patient's primary tumor samples and liver metastases and thereafter used ipatasertib for treatment (De Mattos-Arruda et al. 2014). Therefore, the development of methods for collecting and analyzing ctDNA from straight blood samples has the potential for improvement in a more approachable and less noninvasive manner and adds an understanding of temporal heterogeneity (Bulfoni et al. 2016; Han et al. 2017).

4.3.2 Heterogenicity of Transcription

In addition to the genetic level, a tumor cell shows heterogeneity based on the transcriptome. We address RNA investigations at the level of an individual cell, which produces surprising insights into the transcriptomes of numerous malignancies, considering that the bulk tumor transcriptome may also contain signals from normal cells (Tellez-Gabriel et al. 2016). Through the development and application of several computational methods, such as spanning-tree progression analysis of density-normalized events (SPADE), data from these single-cell assays can be utilized to categorize cells according to phenotypic characteristics to infer sub-clonal hierarchy (Qiu et al. 2011; Saadatpour et al. 2015). A theory that claims that the cellular expression of specific pathways is more diverse than may be deduced from bulk pathway evaluation is supported by studies of single-cell transcriptomics along a variety of cancers. For instance, single-cell RNA sequencing of more than 400 primary glioblastoma cells from 5 patients revealed that these cells undergo a wide range of changes in the expression of their cell cycle, hypoxia, and immune/complement gene programs (Mir et al. 2022; Patel et al. 2014). In studies of more than 4000 cells from 6 patients with oligodendroglioma, the same variance across the stemness spectrum was seen (Tirosh et al. 2016). Zhang et al. examined the transcriptomic heterogeneity of 75 cells from a primary squamous cell carcinoma bladder tumor and found that the expression of the genes included in the mitogen-activated protein kinase (MAPK) signaling pathway varied significantly between each cell (Zhang et al. 2016).

4.3.3 Proteomic Heterogeneity

As per recent data, proteomic heterogeneity in cancer samples from patients may be less prominent than genetic, epigenetic, and transcriptomic heterogeneities. For example, Turtoi and colleagues (2014) noticed that in colorectal cancer patients, the protein distribution along the metastatic lesions of the liver was consistent from one patient to another (Mir et al. 2023b; Ahmed et al. 2016), by discovering that the site-specific spatial heterogeneity in one pancreatic cancer patient's lung, liver, and metastases was only in the peritoneum. Kim et al. state that proteomic heterogeneity justifies the use of target-specific treatment for each metastatic organ (Kim et al. 2014; Mir et al. 2023a). The study of proteomic heterogeneity has benefited from single-cell research, just as that of genomic and transcriptomic heterogeneities. For example, to assign proteomic heterogeneity, Giesen et al. employed mass cytometry on single cells that had been laser-ablated from 21 FFPE samples of breast cancer (Mir et al. 2023a; Giesen et al. 2014).

4.4 Current Evidence for Intratumor Heterogeneity

The use of next-generation sequencing (NGS) technology is producing fresh proof of the genetic variants present in both common tumors and between them. NGS techniques offer the benefit of being able to simultaneously sequence heterogeneous mixtures of genomes in a given sample, which may consist of tumor, stromal, and immune cells. They comprehensively and methodically determine both the nucleotide sequence and copy number of the genomic loci.

Breast cancer studies using NGS and comparative genomic hybridization both point to considerable genetic intratumor heterogeneity (Navin et al. 2010; Navin and Hicks 2010) (Fig. 4.3). Samples particularly acquired from different regions of the same primary tumor can be distinguished from subclones with different DNA copy number variation patterns by the specific existence of subclones that were created by a common clonal progenitor. Tumors that are polygenic may be physically distinct or mixed in subpopulations of the cells. The heterogenicity of a genome is not related to numerous grades of tumors or immune histochemical patterns of staining.

Like breast cancer, It is also clear that a glioblastoma is heterogeneous (Snuderl et al. 2011; Szerlip et al. 2012). Studies have shown that cell inhabitants were mixed and had amplifications that were mutually exclusive of numerous receptor tyrosine kinases (RTKs), including platelet-derived growth factor receptor alpha (PDGFRA), mesenchymal–epithelial transition factor (MET), and EGFR. Additionally, a subgroup of these cells may exhibit comparable alterations in the CDKN2A and TP53 genes, raising the possibility of a singly shared ancestral precursor (Snuderl et al. 2011). Another study on glioblastomas verified the intratumor heterogeneity of amplification of RTKs and showed that this heterogeneity causes functionally different and decreased susceptibility to targeted treatment (Szerlip et al. 2012). Recurrent tumor samples and even a primary tumor sample from one patient with

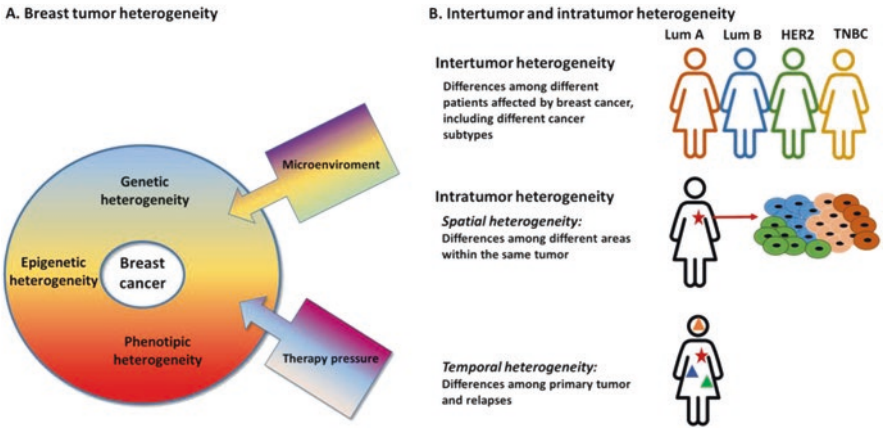

Fig. 4.3 Breast cancer heterogeneity: Intertumoral heterogeneity and intratumoral heterogeneity

glioblastoma were shown using targeted NGS to characterize spatial and temporal heterogeneity. In this investigation, the somatic mutations in EGFR, phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PI3KCA), phosphatase and tensin homolog (PTEN), and tumor protein (TP)53's variant allelic frequency compared to wild-type changed between focal tumor locations in addition to the times of diagnosis between the second and the first recurrences (Nickel et al. 2012).

Recent evidence has shown that spatial genomic heterogeneity in renal cell carcinoma (RCC) raises concerns about the possibility of biased tumor sampling impairing the interpretation of biomarkers (Gerlinger et al. 2012). Various samples of tumors from metastatic and primary lesions with clear cell RCC in two patients were sequenced using exome technology, revealing considerable intratumor heterogeneity that was confirmed by genetic and transcriptomic studies. Multiple tumor suppressor genes had a distinct lack of function of a somatostatin event in spatially distinct areas of the same tumor, indicating evolution (convergent) and possibly predictable paths for the growth of the tumor. Regions collected from various primary and metastasis sites shared about 30–35% of the mutations. Clear cell RCC's single-cell exome sequencing revealed a heterogeneous genomic landscape, with a less gene number. Alterations in a sizable part of cells with a more gene number changes on less frequency, provides a clear evidence for intra-tumor heterogeneity in clear cell RCC (Xu et al. 2012).

4.5 The Genomic Relationship Between Primary and Metastatic Tumors

Emerging data from deep sequencing analyses suggest that understanding clonal heterogeneity and the evolution of tumor sub-clonal architecture from the primary to the metastatic tumor sites and during therapy may provide an important insight

into the metastatic process and the emergence of drug resistance during systemic therapy (Mir et al. 2022a). There is now compelling evidence to support a branched evolutionary pattern of tumor growth across many hematological (Anderson et al. 2011) and solid tumors (Shah et al. 2009; Yachida et al. 2010; Gerlinger et al. 2012; Wu et al. 2012). Studies that reconstruct the evolution of tumors based on phylogenetic analysis revealed that a small minority of primary and metastatic tumor sites may have somatic mutational statuses that change regularly. In a novel "bicompartmental" mouse model of medulloblastoma, complex genomic events were shared across metastatic tumor regions and a limited population of their matched primaries, providing elegant proof of this. This strongly supports the idea that a shared progenitor cell initiates the initial metastatic expansion (Wu et al. 2012). The hypothesis that subsequent evolution and genetic divergence occur in both areas irrespective of tumor dissemination is based on the discovery that some of the genetic abnormalities were present only in primary or metastatic cancers. Additionally, the identification of somatic mutations that are shared only by the primary tumor or sites of metastasis, or that are particular to one tumor region (private mutations) in clear cell RCC, provides further evidence for ongoing independent sub-clonal evolution within distinct and spatially separated tumor regions (Gerlinger et al. 2012).

Xu et al.'s study suggested that approaches of single cells may be necessary to test rarer subclones. It is significant that some earlier studies, depending on Sanger sequencing or low-depth NGS, might have misjudged the degree of genomic heterogeneity because of sequencing depth limitations that stop the identification of tumor rarer subpopulations. In addition, the image of intratumor heterogeneity is anticipated to become more complicated because of aberrations caused by posttranslational changes and changes in epigenesis as well as unpredictable behavioral and stochastic variation of subclones with identities of the same genotypes (Fisher et al. 2013).

4.6 Implications for Targeted Therapeutics

Cancer drug therapy may be significantly impacted by the problem of cancer heterogeneity, involving the relationships between subpopulations within and between tumor lesions. It is known that all molecularly selected patients are not benefited from targeted therapies with advanced diseases, and even when a clinical benefit is seen, it is frequently only for a short duration. Targeted therapy, which tries to utilize a tumor's dependence on a critical proliferation or survival pathway, has significantly improved patient outcomes in a variety of solid tumor types (Sofi et al. 2021; Diaz et al. 2012). These clinical findings may be partially explained by tumor heterogeneity.

It may be possible to identify strong predictive biomarkers that are less prone to the sampling of tumor bias by taking into account an "actionable mutation" within a model of clonal dominance that occurs in early cancer cell progenitors and is present in the tumor's trunk (Yap et al. 2012). Such widespread, clonally dominant driving events would be present in all tumor cells and constitute such actionable "trunk"

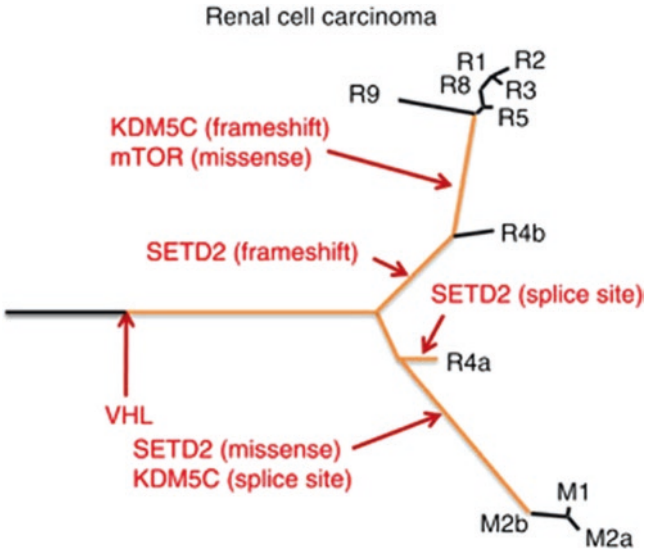

Fig. 4.4 Using a single patient's case of renal cell carcinoma as an example, the trunk–branch model of tumor heterogenicity

mutations (Fig. 4.4). Nevertheless, if a tumor exhibits intratumor heterogeneity through multiple branched events, then even focusing on a driver event may not have a significant impact on treatment outcomes because a population with less frequency may harbor resistance in the tumor branches. For example, the less frequency gatekeeper mutation in EGFR in non-small cell lung cancer (NSCLC) can result in sub-clonal selection and the establishment of treatment resistance (Su et al. 2012; Yap et al. 2012). It is conceivable that clinical approaches may need to change to account for the likelihood that patient outcomes and therapy success are determined by branching, low-frequency somatic events. In fact, by extension, diverse somatic events may make the need for early truncal drivers that depend on tumors unnecessary.

4.6.1 Secondary Somatic Mutation Heterogeneity and Medication Resistance

With certain significant exceptions, such as seminoma, in metastatic solid tumors, nearly all patients succumb to their illness. Drug resistance is undoubtedly the most serious issue facing oncologists. There are numerous instances of medication resistance being brought on by subclones that carry particular somatic gene alterations. Patients with chronic myeloid leukemia have been shown to have imatinib-resistant mutations in the BCR–ABL fusion gene; some of these mutations have also been demonstrated to occur before systemic therapy and to co-occur with subclones carrying various imatinib-resistant variations in treatment-naive patients (Shah et al.

2002). Gastrointestinal stromal tumors (GISTs) that receive imatinib or sunitinib treatment exhibit intratumor heterogeneity of drug resistance mechanisms, with secondary drug-resistant mutations developing in 9 out of 11 patients having mutated oncogenic KIT. They had different mutations in separate metastasis out of six, two or more of them had distinct mutations, and three of them had two secondary KIT variations in the same metastasis (Liegl et al. 2008). Multiple different mutations that appear in separate or identical metastases with a dizzying degree of intricacy shed light on the enormous somatic mutational pool that exists in these tumors.

Patients with clinical resistance to the EGFR–tyrosine kinase inhibitor (TKI) gefitinib as well as untreated patients have been reported to have secondary EGFR mutations that make them insensitive to the drug, which is a common theme in NSCLCs with an EGFR mutation (Inukai et al. 2006; Su et al. 2012). NSCLC samples from patients who had never received treatment as well as samples from patients before and after receiving EGFR–TKIs were examined for one particular EGFR mutation, the T790M mutation. Before treatment, the existence of less frequency T790M variations predicted a shorter progression-free survival. About 25% of patients with lung cancers that have anaplastic lymphoma kinase (ALK) rearranged showed secondary mutations in the ALK tyrosine kinase domain, but these varied greatly in a group of patients. Additionally, non-mutational and potentially reversible epigenetic pathways can contribute to drug resistance in patients, suggesting that multiple resistance mechanisms occurred at the same time (Katayama et al. 2012).

A fatal transplacental melanoma case has been recently presented as a unique illustration of transplacental melanoma and intratumor heterogeneity and its association with therapy failure (Sekulic et al. 2012). The mother's melanoma tumors (both before and after vemurafenib therapy) and the child's tumors (before vemurafenib therapy) were both identified using genomic analysis.

One therapy might not be sufficient to treat a genetically heterogeneous tumor because cancer cell populations that harbor resistance mutations before treatment, even if they are present at a low frequency, might contribute to therapeutic failure and a bad prognosis (Mir et al. 2020; Qayoom et al. 2023a). According to these data, it may be necessary to take into account acceptable combinatorial strategies tailored to the sub-clonal genetic heterogeneous makeup of each patient's tumor to prevent the development of drug resistance.

Furthermore, it is conceivable that an opportunity for therapeutics exists and that driver mutations can be treated without clonal expansion or divergence (Gerlinger and Swanton 2010; Ding et al. 2012). Studies have revealed that interval cytotoxic chemotherapy affected the loss of founding clones and gain of mutations in the genomes of eight patients with recurrent AML, thus highlighting the hypothesis that cytotoxic therapy may cause relapse by causing sub-clonal evolution.

Notably, tumor heterogeneity is not the only factor contributing to cancer treatment resistance; the microenvironment of the tumor also plays a significant role. For example, it was demonstrated that the release growth factor of a hepatocyte and its receptor, in a BRAF-mutant tumor cell line, MET, by stromal cells causes innate

resistance to BRAF inhibition. To overcome resistance, BRAF and MET inhibitors were combined (Mehraj et al. 2022; Lackner et al. 2012; Straussman et al. 2012).

4.6.2 Tumor Heterogeneity and the Validation of Biomarkers for Targeted Therapy

Targeted therapy that is clinically helpful and financially feasible depends on biomarkers that predict treatment response. The identification and validation of prognostic biomarkers may have been negatively impacted by cancer heterogeneity and tumor sampling bias. For instance, there are few genomics methods available in metastatic RCC that allow for the forecasting of therapeutic response or resistance, despite the identification of signaling pathways; it should be highlighted that this might potentially be a result of insufficient translational research and systematic tissue collection in the therapeutic development studies for this illness.

Intratumor heterogeneity and baseline genetic instability may act as biomarkers for a subpar clinical result (Maley et al. 2006; Lee et al. 2011). For instance, clonal diversity in Barrett's esophagus seems to indicate a higher chance of developing into an invasive cancer (Maley et al. 2006), and numerous research studies have demonstrated the connection between CIN and a poor clinical result (McGranahan et al. 2012). An expanding body of proof points to intratumor heterogeneity as a mechanism of treatment resistance, with CIN likely playing a significant role in this process (Gerlinger and Swanton 2010). A growing body of evidence implicates intratumor heterogeneity as a mechanism of therapy resistance, with CIN probably being a key player (Swanton et al. 2009), and colorectal cancer cell lines from CIN seem to have an innate resistance to multiple drugs (Lee et al. 2011). New NGS data may help these theories. Breast cancer patients in the ACOSOG Z1031 trial who received neoadjuvant aromatase inhibitors (AIs) had tumors that were more molecularly diverse at the beginning, based on whole-genome or exome sequencing of biopsy samples taken before and after therapy. Nearly twice as many AI-resistant cancers had background mutations as did AI-sensitive tumors (Ellis et al. 2012).

4.6.3 Utilizing the Therapeutic Potential of Intratumor Heterogeneity

Although it is depressing to consider treating various genetic subpopulations within a tumor, it is possible that therapeutic benefit could come from utilizing intratumor heterogeneity. Gatenby et al. proposed an experimental "evolutionary double-blind therapy" that involves the sequential application of two therapies, each of which causes the tumor cells to undergo particular modifications that leave them open to the second therapy. They contend that a flexible and dynamic approach to cancer treatment is more likely to result in long-lasting tumor control (Cunningham et al. 2011). Although this approach has not yet been used in clinical cancer therapy, it serves as an excellent illustration of how a greater understanding of intratumor

heterogeneity might be extremely important to advancements in the field of medical oncology. Recent research has suggested that patients with breast cancer who have tumors classified as extreme in comparison to those with intermediate CIN have a better prognosis. This shows that carcinogenesis may require a specific level of genomic instability and that too much of it might have a negative impact on the survival of cancer cells (Roylance et al. 2011). It is fascinating to think about how this system of genetic instability, which contributes to intratumor clinical outcomes and heterogeneity, may be therapeutically abused. In solid tumors, the genomic instability of drivers or suppressors, whose activation or inactivation is necessary for initiation of intratumor diversity and heterogeneity, might offer a feasible way to eventually try to stop cancer evolutionary processes.

4.7 Therapeutic Opportunities Targeting the Metastasis–Metabolism Cross Talk in Cancer

Evidence from glycolysis inhibitors is mounting that suggests that glycolysis inhibition may be useful for the treatment of metastatic cancers (Warburg 1924). Hexokinase (HK)-targeting glycolysis inhibitors include 3-bromopyruvate (3-BrPA), lonidamine (LND), and 2-deoxy-D-glucose (2-DG). By inhibiting bioenergetics, inducing reactive oxygen species (ROS), and inactivating Akt/mammalian target of rapamycin (mTOR)/p70S6K signaling, mitochondria-targeted LND displayed an antimetastatic impact **in vitro** and **in vivo**. By suppressing vimentin, Snail, Slug, and Twist and upregulating E-cadherin, glycolysis **blockage with 2-DG or 3-BrPA reduced** nicotinamide adenine dinucleotide phosphate (NAD(P)H): quinone oxidoreductase-1 (NQO1)-induced epithelial–mesenchymal transition (EMT), resulting in less NQO1-induced glycolysis and metastasis in breast cancer cells (Yang et al. 2019). Various metastatic tumors are being studied in phase I–III clinical trials with lonidamine and 2-DG. Alternative tactics focus on phosphofructokinase 1 (PFK1), which catalyzes the second rate-limiting phase of the glycolysis process. Fructose-2,6-bisphosphate, a by-product of 6-phosphofructo-2-kinase/fructose-2,6-biphosphatases (PFKFB1–4), allosterically activates PFK1. Overexpression of the PFKFB3 isoform, which exhibits higher kinase activity than phosphatase activity, increases fructose-2,6-bisphosphate and allosteric activation of PFK1 (Hay 2016; Mir et al. 2023). **A PFKFB3 inhibitor called 3PO** (3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one) prevented glycolysis and reduced head and neck squamous cell carcinoma (HNSCC) cells' migration and invasion (Li et al. 2017). Through encouraging cell quiescence and the normalization of tumor vessels, 3PO treatment of endothelial cells also has antimetastatic effects (Cantelmo et al. 2016; Conradi et al. 2017). Advanced solid malignancies are being tested in phase I clinical trials with ACT-PFK-158 (inhibitor of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatases (PFK-2/FBPase) isoform 3 (PFKFB3) with potential antineoplastic activity), a 3PO derivative. Thus, glycolysis inhibitors show promise in stopping cancer's metastatic cascades.

4.7.1 Isocitrate Dehydrogenase (IDH)1/2 Inhibitors

To reduce the formation of the oncometabolite 2-oncometabolite (2-HG), inhibition of IDH1/2 has been pursued in phase I, II, and III clinical trials. Several mutant IDH1R132H inhibitors have been reported to selectively inhibit mutant $IDH1^{R132H}$ in the nanomolar range (Wong et al. 2017). The progression of metastatic intrahepatic cholangiocarcinoma was stopped by administering AGI-5027 (ML309) to mutant $IDH1^{R132H}$-expressing hepatoblasts. This treatment reduced 2-HG, suppressed liver progenitor cells, and restored cell differentiation Intra-hepatic cholangiocarcinoma (IHCC) (Saha et al. 2014). Additionally, administration of AGI-5198 to human fibrosarcoma HT1080 cells with the heterozygous IDH1R132C ^{R132C} mutation reduced the activity of mTORC1/2, whose oncogenic activation promotes angiogenesis and metastasis (Carbonneau et al. 2016). These proof-of-concept studies show that differentiation therapy to prevent cancer metastasis could potentially target mutant IDH1/IDH2.

4.7.2 Lipid Metabolism Inhibitors

Fatty acid synthase (FASN) and acetyl-CoA carboxylase (ACC) work together to simultaneously activate endogenous fatty acid (FA) biogenesis, which serves as a metastatic stimulus that promotes tumor growth (Luo et al. 2017). The thioesterase domain of FASN was demonstrated to be inhibited by the proton pump inhibitor omeprazole, preventing the formation of the acyl carrier protein and free palmitate. Omeprazole's inhibition of FASN decreased palmitate and, in a dose-dependent manner, prevented breast cancer cells from migrating and metastasizing (Qayoom et al. 2023b; Tripathi et al. 2019).

4.7.3 Cholesterol Metabolism Inhibitors

The rate-limiting step in the synthesis of cholesterol, the conversion of β-hydroxy β-methylglutaryl-coenzyme A (HMG-CoA) to mevalonate, is catalyzed by HMG-CoA reductase. Statins, which are HMG-CoA reductase inhibitors, have been demonstrated to reduce tumor growth, metastasis, and stemness by altering the expression of genes related to sonic hedgehog (Shh) (Li et al. 2016; Yin et al. 2018). By suppressing matrix metalloproteinases (MMPs), pravastatin therapy significantly lowers the frequency and extent of spontaneous lung metastasis, indicating a positive role for statins in reducing tumor growth and metastasis (Taras et al. 2007).

4.7.4 S-Adenosylmethionine (SAM) Cycle Inhibitors

SAM blockers prevent the activity of DNA methyltransferases (DNMTs) and histone methyltransferases (HMTs), which will probably have an impact on metastasis

caused by DNA and histone methylation. S-adenosylhomocysteine (SAH) is hydrolyzed into adenosine and homocysteine by SAH hydrolase. Targeting SAH hydrolase interferes with DNMT/HMT activity because SAH produced by-product inhibition of these enzymes. DZNep, an SAH hydrolase inhibitor, blocks the transcription of SNAIL and polycomb repressive complex 2 (PRC2) and enhancer of zeste homolog 2 (EZH2)-mediated H3K4me3, which reverses EMT (Crea et al. 2011). Another SAH hydrolase inhibitor, adenosine dialdehyde, reduced MMP-9 expression and cancer cell invasion (Kim et al. 2013).

4.7.5 Nucleotide Metabolic Inhibitors

Phosphodiesterase (PDE) targeting can control the second messengers of G protein-coupled receptor signaling linked to the metastatic cascades (Gulati et al. 2019). A PDE5 inhibitor called MY-5445 increased cyclic guanosine monophosphate (cGMP) and protein kinase A (PKA) signaling, which reduced the number of CSCs involved in metastasis (Gulati et al. 2019). Phase I, II, and III trials of sildenafil, another PDE5 inhibitor with US Food and Drug Administration (FDA) approval for pulmonary arterial hypertension treatment, were conducted in advanced solid tumors. By focusing on surgery, sildenafil has been found to reduce postoperative, metastasis-induced myeloid-derived suppressor cells (MDSCs) (Tai et al. 2018). The expression of arginase-1 (Arg1), IL-4Ra, and ROS was decreased by sildenafil to prevent MDSC-mediated suppression of natural killer (NK) and T cells. Metastatic squamous cell carcinoma of the head and neck and esophageal cancer were both studied in phase II trials. Tadalafil, which targets PDE5, also reduced associated metastasis between MDSC and NK cells (Tai et al. 2018). Thus, PDE5 is a possible antimetastatic therapeutic target.

4.7.6 Glycogenolysis and Gluconeogenesis Inhibitors

To control glycogenolysis and gluconeogenesis, inhibition efforts have been directed at the enzyme glucose-6-phosphate translocase (G6PT), which moves G6P from the cytoplasm into the endoplasmic reticulum (ER) lumen. By blocking MMP-2, suppression of G6PT prevents tumor spread, MMP-9, and also angiogenesis. G6PT inhibitors, such as mumbaistatin, AD4-015, and chlorogenic acid, are now being studied as cancer treatments. Phase I, II, and III trials involving chlorogenic acid are being conducted for advanced lung cancer and glioblastoma (Huang et al. 2019).

4.8 Conclusions

Tumor heterogeneity has been identified at various -omics levels. Recurrent tumor cell phenotypes emerge despite spatial and temporal diversity at the genomic, transcriptomic, and proteomic levels. Assessment of interpatient tumor heterogeneity

and cellular phenotypes has the potential to redefine cancer care. Assessment of cellular phenotypes is crucial for targeting cancer cell weaknesses, and development of routine assays that determine these phenotypes is necessary before phenotypic targeting can be fully implemented in the clinic.

Further Reading

For further reading, some textbooks mentioned below also provide further understanding of the cytokine and chemokine network.

- Cytokines and Their Therapeutic Potential by Manzoor Ahmad Mir
- The Cytokines of the Immune System: The Role of Cytokines in Disease Related to Immune Response by Zlatko Dembic
- The Cytokine Network and Immune Functions by Jacques Thèze
- Chemokines and Viral Infection by T.E. Lane
- Cytokines and Chemokines in Infectious Diseases by J. Kerr

The below links for video lectures may also be helpful:
https://youtu.be/TAqO0Mq19JQ
https://youtu.be/6wEnYfvoBqk
https://youtu.be/A9fRRCUlMms
https://youtu.be/zIWvmCsKr34

For more insights into the topic, we would suggest detailed findings from the books of Mir (2022) https://doi.org/10.1016/C2021-0-02565-7 and https://doi.org/10.1016/C2022-0-00074-X, Mir (2021) https://doi.org/10.52305/WXJL6770, and Mir (2015) https://doi.org/10.1016/C2014-0-02898-5, and from the cancer.net website on the following mentioned links:
https://www.cancer.net/cancer-types/breast-cancer/types-treatment
https://discovery.ucl.ac.uk/id/eprint/1472740/
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The Role of Interleukin (IL)-6/IL-6 Receptor Axis in Cancer

5

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Abstract

As a pleiotropic cytokine, interleukin (IL)-6 plays a key role in mediating the host's immune defense. Multiple cell types are involved in IL-6 production, including macrophages, dendritic cells, fibroblasts, mast cells, vascular endothelial cells, monocytes, dendritic cells, T cells, B cells, etc. Furthermore, stromal cells, immune cells that infiltrate tumors, and cancer cells present in the tumor microenvironment are also in charge of producing IL-6. Although the blood levels of IL-6 are barely noticeable at physiological levels (1–5 pg/ml), they have been observed to increase by more than 100,000 times during an immediate inflammatory reaction. Elevated intensities of IL-6 have been identified in almost all human cancers. Overexpression of IL-6 in the tumor microenvironment stimulates carcinogenesis by controlling all the pathways that lead to cancer. There are two categories of interleukin-6 receptors: the first one comprises the cell membrane-bound receptor of IL-6 (IL-6R). Upon binding with IL-6R, IL-6 forms a complex with glycoprotein 130 (gp130) (a signal transducer), and, thus, the classical signaling pathway that exhibits anti-inflammatory activity gets activated. The second category comprises a soluble derivative of the interleukin-6 receptor, i.e., soluble IL-6R (sIL-6R). Upon IL-6 binding to sIL-6R, a membrane-associated signal transducer (gp130) complex is formed and trans-signaling gets activated. Signaling pathways, for instance, Ras/Raf/mitogen-activated protein kinase (MAPK), Janus kinase/signal transducer and activator of transcription (JAK/STAT), phosphatidylinositol-3 kinase (PI3K), or Src/yes-associated protein (Src/YAP), exhibit inflammatory activity, as a result of which they get activated. Almost all human malignancies are related to higher IL-6 levels in the

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blood. In this chapter we have summarized the function of interleukin-6 and its associated signaling pathways that promote cancer progression via various processes such as angiogenesis and increased expression of antiapoptotic genes known to promote cancer. Moreover, we have discussed the potential agents targeting IL-6R/IL-6/JAK/STAT3 that restores the anti-tumor activity by inhibiting IL-6/JAK/STAT3 axis.

Keywords

Interleukin-6 · Glycoprotein 130 · mbIL-6Rα · sIL-6R · IL-6/JAK/STAT3 signaling · ADAM17 · Tumorigenesis · Tumor microenvironment · Angiogenesis · Metastasis · Invasiveness · Chronic inflammation

5.1 Introduction

Interleukins are a type of cytokines, which are cellular messengers with a low molecular weight of about 15–20 kDa, produced by both immune and nonimmune cells in response to pathogens and other antigens that mediate communication between cells as they bind to their receptors and trigger a cellular response in a target cell (Schafer and Brugge 2007; Gandhi et al. 2016; Takeuchi and Akira 2010). In 1979, the term "interleukin" was initially coined and used for several proteins secreted by leukocytes, which primarily act as a mediator between leukocytes, and were thus named "interleukins," meaning "between leukocytes." It is known that IL is also secreted by a variety of body cells (Brocker et al. 2010; Mir 2015a, b). The human genome encodes more than 50 different interleukins; they vary significantly in terms of structure and tend to overlap when it comes to their functions. Interleukins play a part in the differentiation, proliferation, and activation of immune cells (T and B lymphocytes) during inflammatory and immune responses. They exhibit properties of both anti- and pro-inflammatory cytokines (Scheller et al. 2011; Mir 2015a, b, c). Interleukin signaling mainly occurs through autocrine and paracrine mechanisms. Interleukins are not stockpiled within cells; rather, in response to a stimulus, they are rapidly secreted as they move toward their intended cell targets and elicit many cellular responses by binding to high-affinity receptor molecules on the cell surface. This interaction initiates a series of signal transduction pathways within the target cell that eventually change the cellular response (Vaillant and Qurie 2021).

A family of signaling molecules known as the cytokine family of interleukin-6 (IL-6)/glycoprotein 130 (gp130) came about as a consequence of infections and tissue injuries (Tanaka et al. 2014; Rose-John et al. 2015). IL-6 family members exhibit functional pleiotropy as well as redundancy. In their receptor complex, they have a core structure in common (Taga and Kishimoto 1997) and a signal transducer known as gp130 that performs a variety of functions in the body. Moreover, several molecules, such as tyrosine kinases of the JAK family and a STAT family member that communicates directly with the cytoplasmic domains of these receptors, have

been identified. Amongst the members of this family, IL-6 deregulation is responsible for many diseases. Inhibiting IL-6 signaling is extremely effective against diseases like cytokine release syndrome, Castleman disease, and rheumatoid arthritis (RA). The axis of IL-6/IL-6R is known to contribute to the growth of a number of autoimmune disorders and cancer as well (Hirano et al. 1988; Hodge et al. 2005; Hirano et al. 1987; Schafer and Brugge 2007). Undoubtedly, inhibitors of IL-6, IL-6R, or JAK family proteins are useful in the treatment of various immunological conditions. Several cell types are linked to the secretion of the cytokine interleukin-6, including keratinocytes, mesangial cells, fibroblasts, mast cells, macrophages, vascular endothelial cells, monocytes, dendritic cells, B cells, and T cells (Mir 2015a, b, c). Furthermore, stromal cells, tumor-infiltrating immune cells, and the tumor cells located in the tumor niche are also responsible for IL-6 production (Fig. 5.1).

As a pleiotropic cytokine, interleukin-6 has many different names depending on the type of biological activity it carries out. It was discovered nearly 50 years ago in 1973 and was originally identified as a T-cell-derived soluble mediator essential for B cells to produce antibodies (Kishimoto et al. 1978). IL-6 was first identified as a B-cell differentiation factor or a B-cell stimulatory factor 2 (BSF-2) (Howard et al. 1982). It was formerly referred to as interferon-2 (IFN-2) due to its antiviral interferon-like action (Zilberstein et al. 1986). Because of its capacity to encourage the generation of acute-phase proteins like fibrinogen, serum amyloid A (SAA), and C-reactive protein (CRP), interleukin-6 became characterized as a hepatocyte-stimulating factor shortly after (Gauldie et al. 1987). Furthermore, it was reported to

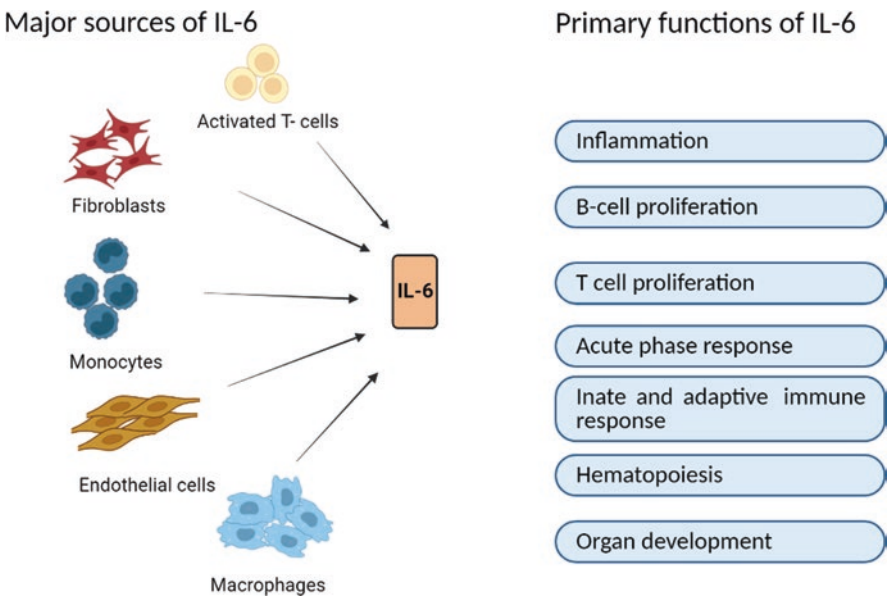

Fig. 5.1 The major cell types linked to the secretion of IL-6

be identical to MGI-2A, also known as myeloid blood cell differentiation-inducing protein, macrophage and granulocyte inducer, type 2A, reflecting the cytokine's myeloid-differentiating capabilities (Shabo et al. 1988). Lastly, in 1988, this multifunctional cytokine was finally named "IL-6" (Hirano 2014).

IL-6 influences various metabolic and immune system functions, such as inflammation, generation of the acute-phase protein (CRP) in hepatocytes, antigen-specific immune responses, apoptosis, hematopoiesis, mitochondrial activity, insulin resistance (for instance, mice develop glucose intolerance and insulin resistance in the absence of IL-6, and, at the same time, liver inflammation is also seen) (Wunderlich et al. 2010), cell differentiation, vascular disease, and lipid metabolism (Ji et al. 2011), and it has also been reported to be associated with the neuroendocrine and neuropsychological systems (Rohleder et al. 2012; Rose-John et al. 2015). Moreover, as mentioned, it contributes to the progression of various disease types such as cancers and autoimmune diseases (Hirano et al. 1987, 1988; Hodge et al. 2005; Schafer and Brugge 2007).

In this chapter, we will discuss the function of interleukin-6 (IL-6) and its signaling, how it helps in regulating tumor progression, and learn exactly how agents targeting the IL-6/IL-6R/JAK/STAT3 or JAK/STAT signaling inhibitors can be therapeutic for various cancers.

5.2 IL-6 and Its Receptor Structure

IL-6 belongs to the hematopoietic cytokine family and is a glycosylated protein with a molecular weight of about 21–26 kDa (Fig. 5.2). In 1986, the IL-6 gene was cloned that encoded IL-6 precursor protein containing 212 amino acids that comprised of a mature segment of 184 amino acids and a signal sequence of 28 amino acids (Hirano et al. 1986). In 1986, the IL-6 gene was cloned that encoded IL-6

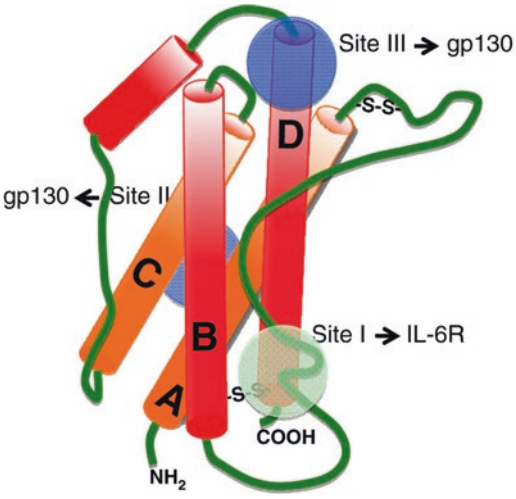

Fig. 5.2 The structure of interleukin-6 (https://www.researchgate.net/figure/Schematic-representation-of-the-domain-structure-of-human-IL-6-notes-The-human-IL-6_fig1_281674578)

precursor protein containing 212 amino acids that comprised of a mature segment of 184 amino acids and a signal sequence of 28 amino acids (Simpson et al. 1997). The mature segment of IL-6 consists of long antiparallel α-helices A, B, C, and D connected through various loops. These helices are structured in such a way that they have an up-up-down-down geometry (Scheller et al. 2011), resulting in the formation of three binding sites for epitopes, namely, sites I, II, and III, at which binding of the receptor occurs (Brakenhoff et al. 1990, 1994; Ehlers et al. 1994).

Helix D and AB loop's C-terminal residues form site I, which serves as a requisite site for a nonsignaling component of the interluekin-6 receptor (Savino et al. 1994a, b; Paonessa et al. 1995). Site II is produced by the dual association of IL-6 and IL-6R with the signal transducer glycoprotein (gp130), which is located on helices A and C and in the middle of domains 2 and 3 (Yawata et al. 1993; Savino et al. 1994a, b; Boulanger et al. 2003). Site III is composed of residues found at the C-terminal of helix D and at the N-terminal of the AB loop. This location is known as domain 1 (D1) because it interacts with immunoglobulin G (IgG) to produce a reliable signaling complex, much like the gp130 (glycoprotein 130) domain does (Yawata et al. 1993).

The IL-6 receptor is a member of the family of cytokine receptors that comprise of IL-6 binding chain domain and a signal transducing chain (gp180) both of these proteins have a Trp-Ser-X-Trp-Ser motif and are cytokine receptors. Two isoforms of the IL-6-binding chain have been found: one is a transmembrane receptor (mbIL-6R) with a molecular weight of 80 kDa and the other is soluble IL-6R (sIL-6R) of molecular weight 50–55 kDa. The interleukin-6 receptor consists of an 80-kDa IL-6-binding protein (α-chain) (CD126), with a cytoplasmic domain containing only 82 amino acids, and gp80, which is present only in limited cell types. IL-6 binds to low-affinity receptors by means of the residues present in the interior of domains 2 and 3, which subsequently recruit the gp130 signal transducer, a β-subunit of the interluekin-6 receptor, to create a high-affinity IL-6R complex that transmits signals and carries out signal transduction. It is worth mentioning that the cytoplasmic and the transmembrane domains of the interluekin-6 receptor are not competent for signaling, and, as such, if both these domains are removed, it will not affect IL-6 signaling (Yamasaki et al. 1988; Taga et al. 1989). Interleukin-6 is not able to transduce signals alone. The IL-6/IL-6R complex is associated with the signal transducer glycoprotein 130 (gp130), also known as CD130, and IL-6Rβ, a glycoprotein of molecular weight of about 130 kDa, has about 277 amino acids in its cytoplasmic domain containing several motifs and sites for phosphorylation. It is universally expressed as the IL-6-type cytokine family's signal transducer, which comprises leukemia inhibitory factor (LIF), OSM (oncostatin M), IL-11 (interleukin-11), ciliary neurotrophic factor (CNTF) , and cardiotrophin-1 (CT-1) (Sprang and Bazan 1993). Since the gp130 subunit exclusively interacts with the complex of IL-6/IL-6R through the generated dual site II engages with gp130's multiple CBDs (cytokine-binding domains) D2 & D3 as well as via site III is present at D1 of gp130, neither IL-6 nor IL-6R can be activated by themselves (Hibi et al. 1990). Meanwhile, functional investigations indicate that two distinctive copies of IL-6, IL-6R, and gp130 combine to form the hexameric structure of the high-affinity IL-6/IL-6R

complex. One molecule of IL-6, one molecule of IL-6R, and two molecules of gp130 are already part of a tetrameric model (Grötzinger et al. 1999; Takeuchi and Akira 2010).

5.3 IL-6 Signaling in Cancer

Malignant cells as well as lymphoid and nonlymphoid cells exhibit the type I cytokine receptor on their cell surfaces, and this is how IL-6 communicates. There are two categories of IL-6 receptors: the first is the cell membrane-bound IL-6 receptor (IL-6R), which has a low affinity and binds to gp130 to activate a classical signaling pathway, and the second is the soluble IL-6 receptor isoform that activates trans-signaling.

By attaching to a soluble variant of the IL-6 receptor (sIL-6R), IL-6 may be able to "trans-signal" in a way that is more convenient (sIL-6R). It first attaches to IL-6 and then to the membrane receptor b-chain (gp130), resulting in an intracellular signal. An IL-6/IL-6R or an IL-6/sIL-6R complex, formed with the membrane-associated signal transducer, triggers signal transduction. The stimulation of particular signaling, including JAK/STAT3 (signal transducer and activator of transcription 3)/IL-6, as well as other signaling pathways involved, leads to the amplification of pro-inflammatory cytokine IL-6. This condition is a hallmark of chronic inflammatory conditions, autoimmune diseases, vascular diseases, and, in a large percentage of patients, several neoplastic disorders, such as hematological malignancies, ovarian cancer, colon cancer, breast cancer, and prostate cancer (Fig. 5.3) (Mir and Mehraj 2019).

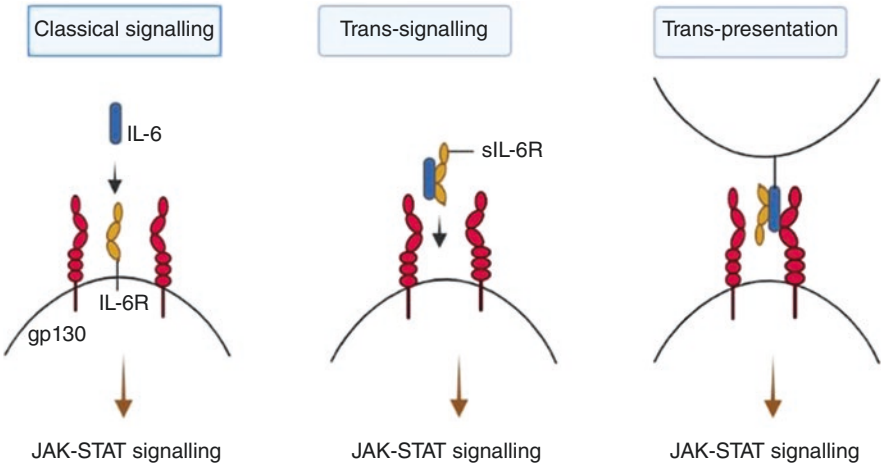

Fig. 5.3 Modes of IL-6 signaling

5.4 Classical Signaling and Trans-Signaling

The two known isoforms of the interleukin-6 receptor, namely, mbIL-6R (a membrane-bound receptor) and sIL-6R (a soluble isoform), are the factors that will determine whether classical signaling or trans-signaling will occur. The proteolysis of the ectodomain of mbIL-6R by a disintegrin and metalloproteases 17 and 10 (ADAM17 and ADAM10, respectively) results in the shedding of the soluble receptors of IL-6, ADAM17, and ADAM10. These are zinc (Zn dependent metalloproteinases. sIL-6R is produced via alternative splicing of the messenger RNA (mRNA) of IL-6 and also that of IL-6R, but the latter occurs less commonly, i.e., about 1–20% (Mülberg et al. 1993; Lust et al. 1992; Riethmueller et al. 2017). Both these isoforms of IL-6R have a similar affinity toward IL-6 binding (Fig. 5.4) (Narazaki et al. 1993).

It is notable that an IL-6/sIL-6R complex can induce IL-6 signaling through autocrine and paracrine mechanisms in any cell type with a gp130 signal transducer, which results in activation of the trans-signaling of sIL-6R. On the other hand, only few epithelial cells, hepatocytes, and few leukocytes express cell membrane-bound

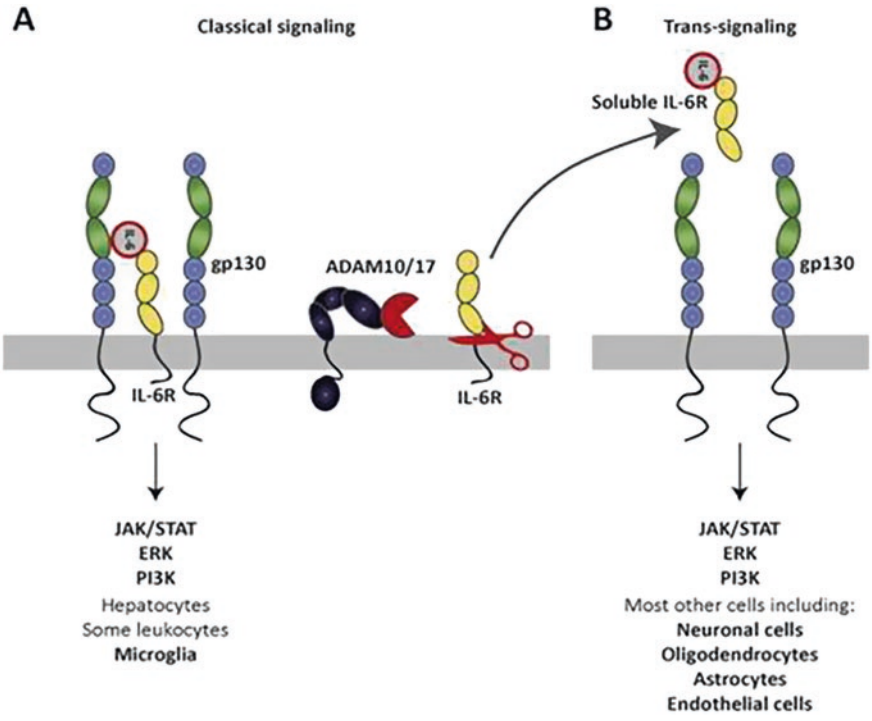

Fig. 5.4 Classical signaling and trans-signaling via ADAM17-mediated cleavage of IL-6R or other events; soluble isoforms of IL-6R are produced

IL-6R (mbIL-6R), and, as such, classical signaling of IL-6 gets activated. Classical signaling is anti-inflammatory, whereas trans-signaling stimulates pro-inflammatory processes. Both times, signaling is brought on by gp130 homodimerization.

The term "IL-6 trans-presentation," which has recently been established, was used to describe the third way of IL-6 signaling. Once dendritic cells (DCs) specifically interact with an antigen, IL-6 binds intracellularly to its receptor and moves to the cell membrane, where the membrane-anchored receptor of IL-6 interacts with the gp130 subunit of T cells when combined with transforming growth factor-beta 2 (TGF-β2) to form prime T-helper (Th)17 cells that lead to chronic inflammation and regulatory T (Treg) cell suppression by IL-27 (Qayoom et al. 2023; Narazaki et al. 1993; Heink et al. 2017). It is still not known whether this novel IL-6 signaling mechanism works in humans because this study was conducted experimentally using a murine model of multiple sclerosis of humans. gp130 dimerization is necessary for IL-6 signaling through the traditional or trans-signaling pathways because it activates the JAK protein that is linked to gp130 (Heink et al. 2017).

Trans-signaling plays an important role in the tumor microenvironment by regulating leukocyte recruitment and inducing the inflammatory activation of stromal cells that are linked to the tumor (Mehraj et al. 2021a, b, c). It is notable that alternative splicing of gp130 mRNA generates four different soluble isoforms of gp130 (sgp130). This adds to the complexity, given that when secreted sgp130 proteins interact with the IL-6/sIL-6R complex, they specifically prevent the trans-signaling facilitated by IL-6 (Baran et al. 2018). As a result of its binding, an inactive ternary complex is formed. The inhibitory activity of trans-signaling occurs at low doses and also depends on the IL-6/sIL-6R ratio because the classical signaling mediated by IL-6/mIL-6R is not affected by it (Nakajima et al. 1993; Jostock et al. 2001). Numerous downstream signaling processes occur after the formation of the IL-6/IL-6R/gp130 complex, enabling IL-6 to exhibit cell differentiation, proliferation, survival, and death, among a wide range of other biological processes. The signaling of IL-6 involves the Ras/Raf/mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K), JAK/STAT, and Src/yes-associated protein (Src/YAP) pathways (Fig. 5.5) (Nakajima et al. 1993; Taniguchi et al. 2015, 2017).

5.5 Signaling Pathway for IL-6/JAK/STAT3

The JAK/STAT3 signaling pathway is essential for mediating IL-6's diverse effects on tumor promotion, i.e., cancer cell survival, proliferation, invasion, metastasis, and immunity-suppressing properties (Kumari et al. 2016; Qayoom et al. 2021). The JAK/STAT (Janus kinase/signal transducer and activator of transcription) signaling pathway is highly conserved. IL-6 signaling can happen via traditional or trans-signaling routes, and the heterohexameric complex formed between IL-6R, IL-6, and gp130 is what initiates intracellular signaling. This complex activates JAKs that are linked to gp130. There are four mammalian JAKs: tyrosine kinase 2 (TYK2), JAK1, JAK2, and JAK3. There are seven members of the STAT family: STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6 (Seif et al. 2017).

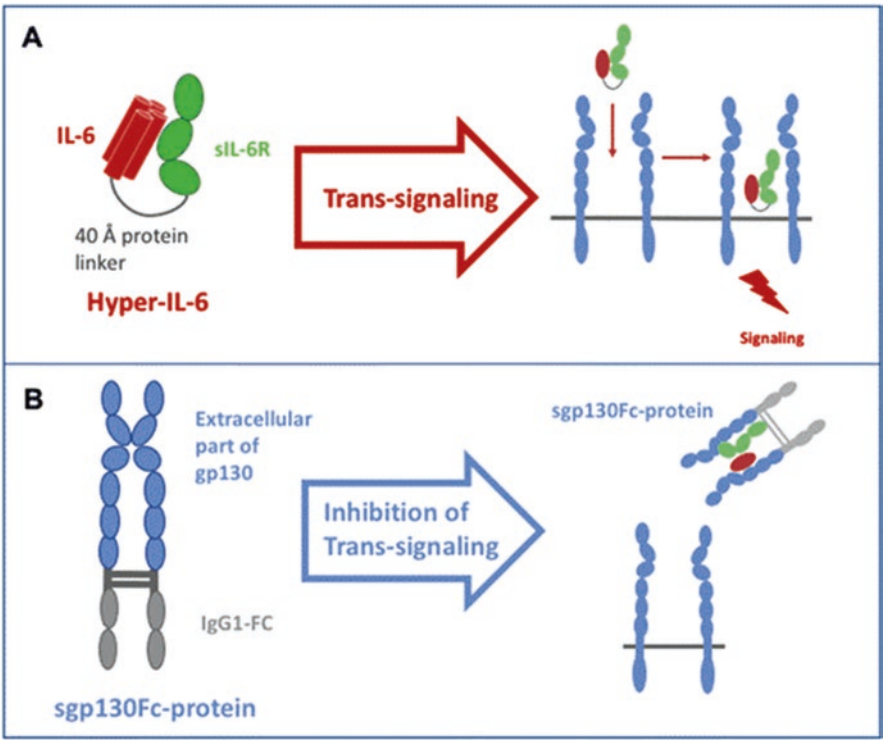

Fig. 5.5 Inhibition of trans-signaling

The JAK Family: This consists of protein tyrosine kinases that are not receptors. JAK tyrosine kinases TYK2, JAK1, JAK2, and JAK3 are stimulated when cytokines connect to their receptors. The other members of JAK3 are uniformly present in all tissues, whereas JAK3 is restricted only to the lymphatic system, bone marrow, vascular smooth muscle cells, and endothelial cells (Rane and Reddy 1994; Takahashi and Shirasaw 1994; Lai et al. 1995; Verbsky et al. 1996). Members of the JAK family consist of seven different JAK homology domains (JH). There is the first JH, JH1, which originates from the carboxyl terminus and has roughly 250 amino acid residues. JH1 is also known as the kinase domain because it creates the kinase structural domain and phosphorylates a substrate by encoding a kinase protein. Although JH2 shares structural similarities with the kinase domain, it lacks kinase activity. It is known as the Pk domain or pseudo kinase domain and takes part in the interaction between JAK and STAT. Its major aim is to regulate the kinase domain's activity, and, by binding to the kinase domain, it can also inhibit Tyr kinase activity. JH3 and one-half of JH4 make up the Src homology 2 (SH2) domain, whereas JH4 and one of each of JH5, JH6, and JH7 make up the FERM (F for four point one protein, E for ezrin, R for radixin and M for moesin) domain. When the IL-6/IL-6R complex homodimerizes gp130, JAK1, JAK2, and/or TYK2 are selectively activated through the SH2 and FERM domains, mostly through interactions

of these enzymes with gp130's Box domains 1 and 2, which are membrane-proximal areas. Due to the disruption of JH2-mediated inhibition of JH1 kinase activity caused by these contacts, reciprocal transphosphorylation that results in complete activation of JAKs takes place. After the JAK enzyme is activated in the cytoplasm of gp130, numerous tyrosine residues undergo phosphorylation. These residues later act as docking sites for proteins that start the IL-6/JAK/STAT3 signaling pathway and additional distinct signaling pathways (Frank et al. 1995; Velazquez et al. 1995; Ferrao and Lupardus 2017).

The STAT family members include 750–900 amino acid residues. The STAT structure consists of a coiled-coil domain (CCD), a linker domain (LD), an amino terminal domain, an Src homology 2 domain, a DNA-binding domain (DBD), and a transactivation domain. STAT3 is closely linked to the stimulation of tumor progression and immune suppression among the STAT protein family members (STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6). STAT3 normally resides in the cytoplasm of cells in the dormant state as a monomer. When JAK enzymes get activated, they subsequently phosphorylate multiple tyrosine residues of growth factor receptors like gp130. This places STAT3 in close proximity to active JAK enzymes, which then phosphorylate STAT3 at Tyr705. The phosphorylation of Tyr705 residues results in dimerization of the STAT3 protein in a head-to-tail manner, which is mediated by the Src homology 2 domain. The STAT3 dimer translocates from the cytoplasm to the nucleus through an importin-α–importin-β1-dependent mechanism (Chang et al. 2013a, b).

The STAT3 dimer attaches to the target genes' promoter consensus response elements inside the nucleus. As a result, transcription of genes in charge of controlling cell proliferation (like cyclin D1 and Myc) and cell survival (like survivin and Bcl-xL) as well as transcription of angiogenesis-promoting genes (like vascular endothelial growth factor (VEGF)), immunosuppressive growth factors, and cytokines are stimulated to be expressed (such as IL-6). The capability to phosphorylate or activate STAT3 is not restricted to only JAKs, as the tyrosine kinases Src and BCR–ABL1 can also directly activate STAT3. In addition, it has been reported that posttranslational changes, including phosphorylation of the Ser727 residues, and methylation, acetylation, SUMOylation, and ubiquitylation of other residues, may influence the stability or function of STAT3. Compared to Tyr705 phosphorylation, Ser727 phosphorylation has received less attention in research. It can be regulated by a number of kinases such as various MAPKs, JNKs, and protein kinase C, which typically increases STAT3 action. When nonmalignant cells are stimulated with IL-6 or other cytokines, STAT3 is momentarily phosphorylated and/or activated (Chang et al. 2013a, b).

To regulate the termination of cytokine signaling transduction few protein families are involved that have been documented quite recently and are known to downregulate the signal transduction that include: Several cellular phosphatases, including dual-specificity phosphatase 22 (DUSP22), Src homology 2 protein phosphatase 1 (SHP1), protein tyrosine phosphatase receptor type D (PTPRD), Src homology 2 protein phosphatase 2 (SHP2), protein tyrosine phosphatase nonreceptor types 1 and 2 (PTPN1 and PTPN2, respectively), and protein tyrosine

phosphatase receptor type T (PTPRT), as well as a minimum of three distinct types of negative regulators comprising suppressors of cytokine signaling (SOCS1, SOCS2, and SOCS3) proteins, protein inhibitors of activated STAT (PIAS) proteins, and cytokine-inducible SH2 (Src homology 2) domain-containing proteins (CISH), can control STAT. All of them function as feedforward regulators, which eventually reduce signaling of IL-6 (Tartaglia et al. 2003; Nozawa et al. 2006; Zhang et al. 2007; Buchert et al. 2016; Peyser et al. 2016). According to reports, mice with mutant gp130 omit their ability to bind to SOCS3, which results in continuous stimulation of STAT3 and STAT1, leading to the onset of cancer and chronic inflammatory illness (Nozawa et al. 2006).

5.6 How IL-6 Regulates Tumor Progression

Sources of IL-6 that are most prevalent in the tumor microenvironment include tumor stromal cells, tumor-infiltrating immune cells, and tumor cells along with $CD4^+$ T cells, TAMs (tumor-associated macrophages), fibroblasts, and MDSCs (myeloid-derived suppressor cells) (Nolen et al. 2008; Oh et al. 2013; Nagasaki et al. 2014; Mehraj et al. 2021a, b, c). In the tumor microenvironment, IL-6 directly affects cancer cells by modulating both their intrinsic and extrinsic actions, thereby directly supporting tumorigenesis and influencing stromal cells, and by indirectly supporting tumorigenesis (Fisher et al. 2014). We can say that IL-6 creates a complex of cytokines, proteases, and growth factors consisting of granulocyte macrophage colony-stimulating factor (GM-CSF), VEGF, and matrix metalloproteinase-1 (MMP-1) in prostate and skin cancer, which promotes the advancement of the malignancy.

As IL-6 is made by tumor cells to aid in their survival and growth, stromal cells' paracrine release of IL-6 is not necessary for the autocrine action of interluekin-6 on tumor cells. However, it is found that interluekin-6 trans-signaling affects tumor growth and metastasis through both paracrine and autocrine processes of IL-6 (Lederle et al. 2011; Fisher et al. 2014). It is generally acknowledged that cancer is driven by inflammation. One of the main factors driving this association is nuclear factor-kappa B (NF-κB) signaling, which plays a major role in the overexpression, and many pro-inflammatory cytokines are stimulated by a range of cell types in the tumor microenvironment. Both adaptive and inborn immunity are regulated by interleukin-6. Certainly, IL-6 knockout mice have altered inborn and acquired immune responses to bacteria, parasites, and viruses. IL-6 regulates the natural immune response in many ways. Upregulation of receptors that recognize patterns, including toll-like receptors (TLRs), causes macrophages, monocytes, or neutrophils to produce IL-6. Furthermore, when pattern recognition receptors are activated, stromal cells such as endothelial cells, fibroblasts, epithelial cells, mesothelial cells, smooth muscle cells, and myofibroblasts produce interleukin-6. IL-6 controls the action of cells of innate immunity as it recruits neutrophils, cells of myeloid lineage, and tissue-resident monocytes and also promotes their differentiation, adhesion, activation, and survival. IL-6's ability to regulate acquired immunity

depends on its capacity to regulate T-helper cell differentiation. There are a few potent cytokines that promote inflammation caused by immune cells of adaptive or innate immunity, which control the progression of cancer cells and tumors (Krishnamoorthy et al. 2007; Zhao et al. 2008; Mitchem et al. 2013; Mehraj et al. 2021a, b, c). IL-6, one of these cytokines, is vital for the development of human cancer and is found to be dysregulated in cancer. It activates oncogenic pathways. In many types of cancers, elevated levels of IL-6 are found, for example, in colorectal cancer(CRC), ovarian carcinoma, prostate cancer, breast cancer, pancreatic cancer, pulmonary cancer, cervical cancer, renal cell carcinoma, multiple myeloma (MM), and lymphomas (Altundag et al. 2004; Culig and Puhr 2012; Waldner et al. 2012; Chang et al. 2013a, b; Dethlefsen et al. 2013; Miura et al. 2015). This shows a strong relationship between the IL-6 cytokine and cancer. It is found that males with higher levels of IL-6 show high susceptibility to the occurrence of a liver malignancy. As opposed to this, IL-6 production is inhibited by estrogen and steroid hormones to guard female mice from developing cancer (Naugler et al. 2007; Wei et al. 2015). The hyperactivation of the JAK/STAT3/IL-6 signaling axis regulates various signaling pathways, which is responsible for survival of cancer cells, and thus promotes tumorigenesis (Landskron et al. 2014).

IL-6 controls almost all hallmarks of cancer, such as cell survival, cell proliferation, angiogenesis, inhibition of apoptosis, and metastasis. It is also known for regulating tumor cell metabolism. The bulk of the characteristics of a tumor are regulated by interluekin-6, including a range of biological abilities developed during tumor growth. IL-6's involvement in controlling cancer cell growth stems from the fact that some regulatory mechanisms that downregulate or negatively influence cell growth are avoided by cancer cells. These mechanisms primarily rely on the activity of tumor suppressor genes like the retinoblastoma gene (Rb) and the p53 gene, which go through either gain of function or loss of function to control cell proliferation and cell growth, respectively (Mir et al. 2020). The Rb protein, which is controlled by phosphorylation, interferes with the cell cycle passage from the G1 phase to the S phase (Fig. 5.6). Normally, the retinoblastoma protein is in its active form in the dephosphorylated state, which is bound by E2F, and leads to cell arrest at the G1 phase. On the other hand, Rb is in its inactive form after phosphorylation. In this state, Rb is unable to bind to E2F that triggers cyclin-dependent kinase (CDK) activation in such a manner that it facilitates cell transition into the S phase (Qayoom et al. 2022; Sofi et al. 2022). IL-6 facilitates the phosphorylation of Rb in multiple myeloma (MM) cells, thereby promoting cell growth. Moreover, in MM cells, phosphorylation of Rb also enhances the generation of interluekin-6 (Urashima et al. 1996; Nevins 2001; Giacinti and Giordano 2006).

In cervical cancer, esophageal carcinoma, gastric cancer, myeloma cells, basal cell carcinoma cells, and myeloma cells, IL-6 controls the process of apoptosis by stimulating NF-κB and STAT3 signaling, which trans-activates many antiapoptotic proteins like B-cell lymphoma-2 (Bcl-2), Bcl-xL, and Mcl-1, among others. Additionally, these pro-survival or antiapoptotic proteins, particularly Bcl-2, encourage cell division. It has been suggested that increased amounts of IL-6, both in IL-6-treated cells and in transgenic mice that express IL-6, may alter the

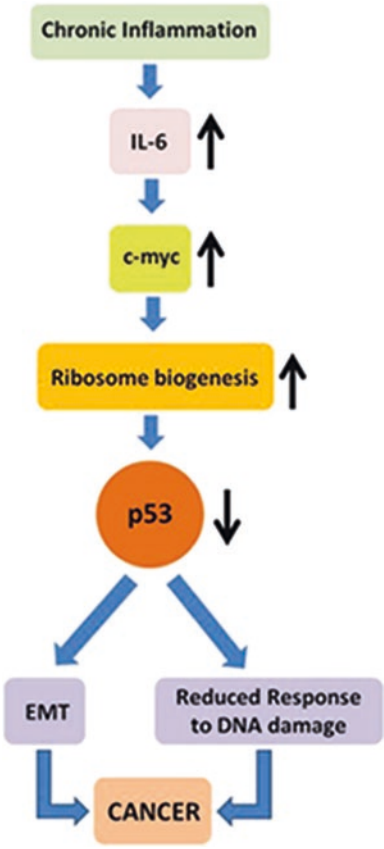

Fig. 5.6 Downregulation of p53 by overexpression of IL-6

proportion of pro- and antiapoptotic proteins, which is crucial for the decision to undergo apoptosis. Moreover, Bcl-2 produced by IL-6 controls interactions between mitofusins and Bak by inhibiting the dissociation of Bak from Mfn2, which, in turn, prevents the interaction of Bak with Mfn1. These are the two major mitochondrial events that determine cell death. Along with Bcl-2 and Bcl-xL, IL-6 promotes the production of survivin by direct binding of STAT3 to the promoter survivin gene. This helps tumor cells survive. Additionally, the expression of the survivin gene is downregulated, which suppresses STAT3, causing tumor cells to undergo apoptosis. NF-κB, MAPK/extracellular signal-regulated kinase (ERK), and PI3K/Akt signaling pathways are all increased by IL-6 in kidney cancer, hepatocellular carcinoma (HCC), prostate cancer, and multiple myeloma cells, which results in an increase in cyclin A1 expression in these cells. Overall, it seems that interleukin-6 primarily promotes tumor growth by reducing cell death and promoting cell proliferation (Gritsko et al. 2006; Brooks et al. 2007; Waxman and Kolliputi 2009; Mir et al. 2023). In addition to promoting the growth of tumor cells, IL-6 is used to create resistance to anticancer treatments that trigger the body's natural death processes. Direct targets of STAT3 upregulation include the bulk of the genes that control cell

differentiation, proliferation, and survival, including p21, Mcl-1, Bcl-xL, Bcl-2, Fas, cyclin E1, and cyclin D1. Moreover, these, including c-Jun, cMyc, and c-Fos, are other transcription factors that promote proliferation and are also targets of STAT3. When STAT3 is activated in tumor cells, p53 expression is transcriptionally repressed, whereas STAT3 inhibition upregulates p53 expression and triggers p53-mediated apoptosis (Hirano et al. 2000; Niu et al. 2005).

5.7 Inflammation Promotes Cancer

Inflammation encourages all phases of carcinogenesis and predisposes to the growth of cancer and promotion of malignant transformation. Many inflammatory mediators like IL-6, TGF-β, IL-10, and tumor necrosis factor-α (TNF-α) have all been implicated in the beginning and progression of cancer. Cancer and inflammation have shown a strong association with cancer prevention, and the use of anti-inflammatory drugs is encouraged. Inflammation related to cancer consists TAMs, white blood cells, and inflammatory mediators such as cytokines (IL-1, IL-6, and TNF-α) and chemokines (CXCL8 and CCL2), which promote tissue remodeling and vasculogenesis (Colotta et al. 2009; Mir et al. 2022a, b, c).

In the tumor microenvironment, interluekin-6 is one of the highly expressed inflammatory mediators, and, in colorectal cancer, IL-6 secretion is associated with STAT3-dependent tumorigenesis and its associated trans-signaling within the tumor milieu induces inflammation. In ulcerative colitis, M2-type macrophages release IL-6 and contribute to the growth of colorectal tumors. These investigations have discovered a connection between IL-6 and inflammation in tumors. Inflammatory cytokines (IL-6, IL-1b, and TNF) as well as transcription factors like NF-κB and STAT3 are the main players in inflammation. Inflammation is heavily regulated by NF-κB, which is dysregulated in many malignancies. IL-6 is a crucial part of the NF-κB/IL-6/STAT3 signaling pathway, which is involved in carcinogenesis, as it is a primary effector molecule of NF-κB that is activated through the JAK/STAT3 signaling pathway. NF-κB activation in tumors must be maintained by STAT3, and IL-6 encourages carcinogenesis by causing inflammation and cell growth. Meanwhile, the stimulation of IL-6-mediated STAT3 signaling promotes inflammation and boosts the progression and development of gastrointestinal cancers, so it is now evident that IL-6, inflammation, and tumor promotion are all strongly associated (Qayoom et al. 2023a).

5.7.1 IL-6 Promotes Invasion, Metastasis, and Angiogenesis

It is common knowledge that inflammation encourages epithelial-to-mesenchymal transition (EMT), which allows cancer cells to spread from the primary tumor site to the nearby tissues and organs. Cancer cells do this by acquiring a mesenchymal phenotype from an epithelial one, which allows them to do so. Tumor cells transform back into epithelial cells after settling into the new organ and multiply to

produce metastatic tumors. It is widely known that inflammation encourages EMT. The terms "epithelial-to-mesenchymal transition" (EMT) and "mesenchymal-to-epithelial transition" (MET) refer to the mechanisms by which cells go from having an epithelial phenotype to bearing mesenchymal characteristics (Kalluri and Weinberg 2009; Ricciardi et al. 2015).

Increased levels of IL-6 in the blood are related to the enlarged size of the tumor as well as invasion (EMT), metastasis, and worse survival rate in people with colorectal cancer. According to reported evidence, IL-6 promotes CXCR4 expression through c-Jun and STAT3, which promotes the metastasis of numerous bone tumors, including breast, pulmonary, prostate, neuroblastomas, adenocarcinomas, multiple myeloma, and melanomas (Ricciardi et al. 2015). By boosting the production of growth factors, such as DNA methyltransferase 1 (DNMT1), VEGF, and MMP-9, IL-6 induces hyperactivation of JAK/STAT3, which is involved in EMT, metastasis, and angiogenesis in bladder cancer. It has also been demonstrated that the autocrine production of IL-6 enhances the ability of tumor cells to infiltrate the extracellular matrix in breast cancer. By increasing the expression of several growth factors, including MMP-9, VEGF, tumor-associated macrophages, and basic fibroblast growth factor (bFGF) in tumor-associated endothelial cells and MDSCs, IL-6/JAK/STAT3 signaling promotes angiogenesis in numerous malignancies (Helbig et al. 2003; Kujawski et al. 2008; Tawara et al. 2011; Chen et al. 2013; Sofi et al. 2023).

5.7.2 At Elevated Levels, IL-6 Acts as a Prognostic Marker for Various Cancers

Nearly 1 pg/ml of IL-6 in present in normal blood circulation, under several conditions. An increase in its level is found during acute hyperglycemia, during/after surgery, after a high-fat meal, during a normal menstrual cycle, and during physical activities. IL-6 levels fluctuate throughout the course of pregnancy, peaking at around 128 pg/ml when the baby is delivered and, immediately afterward, dropping by more than twofold (~58 pg/ml). As mentioned earlier, chronic inflammation promotes carcinogenesis (Sakamoto et al. 1994; Angstwurm et al. 1997; D'Auria et al. 1997; Devaraj et al. 2005; Blackburn et al. 2006; Narbutt et al. 2008; Reihmane and Dela 2014). IL-6 is a potent pro-inflammatory marker, and it exhibits multiple biological activities. Elevated levels of IL-6 in the serum have been reported during cancer; IL-6 acts as a biomarker for various malignant diseases such as gastric cancer, pancreatic cancer, colon cancer, hepatobiliary cancer, prostate cancer, pulmonary cancer, renal cell carcinoma, breast cancer, and ovarian cancer. According to reports, increased IL-6 levels are related to the overall poor prognosis in hepatocellular carcinoma (HCC) patients (Altundag et al. 2004; Groblewska et al. 2008; Culig and Puhr 2012; Waldner et al. 2012; Chang et al. 2013a, b; Dethlefsen et al. 2013; Miura et al. 2015).

5.7.2.1 Colon Cancer

In 32 patients with colorectal cancer, significantly increased levels of IL-6 were seen in comparison to those of normal colorectal tissues, indicating that interluekin-6 plays an important role in tumor development (Komoda et al. 1998). In addition, it has been shown that increased IL-6 levels in the blood are associated with a poorer prognosis in CRC. This is because IL-6 expression is greater in colon rectal cancer tissues than in nonmalignant tissues (Chung and Chang 2003; Zeng et al. 2017). In addition, as compared to cells from a healthy colon epithelium, CRC cells shows high expression of IL-6 and higher levels of STAT3 phosphorylation (Corvinus et al. 2005). Studies have shown that whereas sIL-6R is strongly elevated during carcinogenesis, mbIL-6R is scarcely detectable. This suggests that IL-6 affects CRC through trans-signaling. An increased ADAM17 expression observed in malignant cells in comparison to normal cells serves to authenticate this fact. A sign of this disease's worse prognosis is elevated IL-6 levels (Becker et al. 2005).

5.7.2.2 Breast Cancer

IL-6's effect on breast cancer cell lines has been studied in vitro; the results are inconsistent, displaying tumor-suppressing actions in some models and tumor-promoting activities in others. The results also showed that there was a large concentration of interleukin-6 in Michigan Cancer Foundation-7 (MCF-7)/adriamycin multidrug-resistant breast cancer cell supernatants. However, in parental sensitive cell supernatants, IL-6 was not detectable (Conze et al. 2001). Furthermore, exogenous IL-6 is added to MCF-7 cells, which leads to an increase in their doxorubicin resistance by 8–10-fold, while engineered MCF-7 cells express IL-6, thus showing a 70-fold rise in their sensitivity to various medications. A protein linked to treatment resistance is G96, which was produced by MDA-MB-231 triple-negative breast cancer cells when exogenous interleukin-6 was added to the cells. In line with this, the scientists discovered that IL-6 and G96 protein levels were substantially increased in breast tumors than in normal, noncancerous breast tissues (Haverty et al. 1997; Qayoom et al. 2023).

However, additional research indicates that interluekin-6 may exhibit antitumor activity against a few types of breast cancers. For instance, it has been noted that IL-6 causes MCF-7 breast cancer cells to undergo apoptosis. According to these discoveries, insulin-like growth factor-I (IGF-I), tumor necrosis factor (TNF), and IL-1, along with interluekin-6, are associated with decreased proliferation of MCF-7 cells by suppressing IGF-I, a molecule known to promote DNA synthesis in MCF-7 (Danforth Jr and Sgagias 1993; Chiu et al. 1996). IL-6 can accelerate the migration of breast malignant cells, indicating its function in metastasis, and, despite its contentious role in survival and proliferation, an epithelial-to-mesenchymal transition is induced by IL-6, which then promotes the spread of breast cancer cells. Other findings have shown that IL-6 inhibits the adhesion of breast malignant cells and that this outcome was related to tumor cells' reduced production of E-cadherin (Arihiro et al. 2000).

The IL-6 levels of breast cancer survivors were considerably higher in their blood in comparison to those of healthy women. In patients, elevated blood levels of

IL-6 have been linked to a bad prognosis of this disease, despite the fact that different investigations have produced conflicting consequences concerning the usefulness of the predictive value of IL-6 levels in breast tumor tissues (Bachelot et al. 2003; Salgado et al. 2003; Bozcuk et al. 2004). It was also discovered that as compared to individuals with only one metastatic site, patients with extensive metastatic breast cancer had considerably higher amounts of IL-6 in their blood. Additionally, they discovered that patients with higher IL-6 levels had poorer chemo-endocrine treatment response and lower survival rates (Nishimura et al. 2000; Yokoe and Morishita 2000; Bachelot et al. 2003; Salgado et al. 2003; Bozcuk et al. 2004).

5.7.2.3 Ovarian Cancer

Increased blood levels of the pro-inflammatory cytokine IL-6 is associated with ovarian cancer as well, and a compromised immune function is frequently seen in individuals suffering from advanced epithelial ovarian cancer (EOC), which is linked to overexpression of IL-6. Several research studies have revealed the association of interluekin-6 with the progression of EOC, chemotherapy resistance, and poorer prognosis (Macciò et al. 1998; Hagemann et al. 2007). Advanced epithelial ovarian tumors have elevated expression levels of interleukin-6 in their ascites and cystic fluid in comparison to benign ovarian tumors, and these levels are correlated with the growth/size of the tumor and the ascites volume (Paladugu et al. 1985; Nowak et al. 2010). There is evidence that shows that IL-6 may directly contribute to the progression of ovarian tumors. As discussed earlier, IL-6/JAK/STAT3 signaling hyperactivation upregulates the production of antiapoptotic molecules, for instance, Bcl-xL and Bcl-2 (B-cell lymphoma 2), which promotes EOC cell survival In addition, research has revealed that IL-6 in ovarian cancer cells changes the distribution of the cell cycle. IL-6 also stimulates the release of MMP-9 (matrix metalloproteinase-9), and it adds to increase the invasiveness of ovarian cancer cells. MMP-9 levels were lower in anti-IL-6-treated antibody SKOV-3 ovarian cancer cells in comparison to SKOV-3 ovarian cancer cells in control cultures (Rabinovich et al. 2007; Macciò and Madeddu 2013).

In many ovarian cancer models, IL-6 has also been demonstrated to function as a powerful angiogenic agent. Additionally, it has been noted that ovarian cancer cells, particularly SKOV-3 cells, produce more secreted sIL-6R. This finding suggests the potential role of IL-6 trans-signaling in the development of ovarian tumors and tumor angiogenesis (Nilsson et al. 2005; Macciò and Madeddu 2013).

5.7.2.4 Other Types of Cancers

It is not possible to fully summarize the function of interleukin-6 and its signaling in all forms of cancer in a single chapter. Here, an overview has been provided, including how overexpression of IL-6/JAK/STAT signaling is linked to prolonged inflammation, cancer progression, invasion, tumor cell proliferation, metastasis, and inhibition of expression of proapoptotic genes (Fig. 5.7).

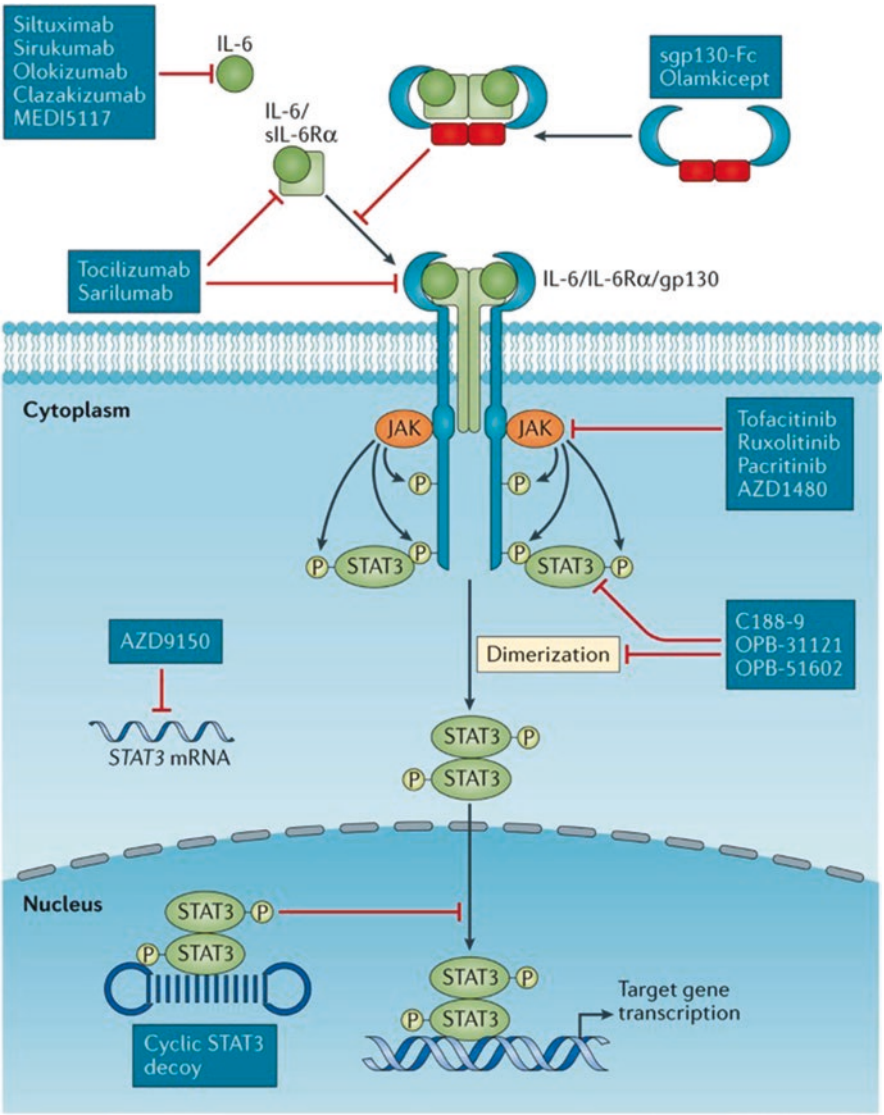

Fig. 5.7 IL-6/JAK/STAT3 inhibitors in cancer targeting (ref: https://www.nature.com/articles/nrclinonc.2018.8)

5.8 IL-6/IL-6R Axis Targeting in Cancer

Accumulated findings have shown that increased levels of interleukin-6 are reported in the pathophysiology of several persistent inflammatory conditions and in various malignancies. By inhibiting IL-6 signaling, it can provide beneficial treatment opportunities for diseases like autoimmune diseases and cancer (Nilsson et al. 2005;

Mir et al. 2022a, b, c). This can be performed by targeting IL-6, its receptor, or its signaling. There are three main approaches that are currently in use for blocking the signaling mediated by IL-6 at either the ligand level or the receptor level. The first strategy is to directly target the ligand interluekin-6 with antibodies like siltuximab; the second strategy is to directly use antibodies like tocilizumab to target IL-6R; and the third strategy is to directly target the complex of IL-6-soluble interluekin-6 receptor utilizing fusion proteins, including sgp130 that directly prevents trans-signaling. Targeting IL-6 and its receptors prevent traditional signaling and trans-signaling; however, when IL-6/sIL-6R complexes are targeted with sgp130 fusion proteins, only trans-signaling is prevented (Klein et al. 1989, 1991; Puthier et al. 1999; Hideshima et al. 2001; Gislén et al. 2003).

In 2014, after siltuximab underwent effective clinical testing, The US Food and Drug Administration (FDA) authorized it as a means of treating multicentric Castleman illness. Siltuximab is presently the most extensively researched therapeutic drug targeting interleukin-6; it is actually a chimeric antibody of mice and humans (Van Rhee et al. 2010, 2014). However, there are no abundant data showing siltuximab's effectiveness in clinical studies against solid tumors. These data show that inhibiting IL-6 by itself may not have much of an impact on patients with solid tumor prognoses (Angevin et al. 2014; Liu et al. 2016). The development of efficient combination medicines is necessary to address this issue, as is the development of a dependable, strong biomarker that can predict a response (Finkel et al. 2016; Liu et al. 2016; Yanaihara et al. 2016).

A humanized monoclonal antibody called tocilizumab targets the 6R and impairs both trans-signaling and classical signaling. The FDA has approved tocilizumab for use in treating pediatric patients, cytokine release syndrome patients, and patients with systemic juvenile idiopathic arthritis and rheumatoid arthritis. Preclinical research results point to tocilizumab's potential as a treatment for ovarian cancer. A monoclonal antibody sarilumab, which blocks the receptor of IL-6, is presently going through clinical research trails.

Patients with tumors that do not express any or little IL-6R may benefit from selective trans-signaling suppression. Proteins that include the sgp130 sequence bind to interleukin-6 and its receptor there by blocking the construction of the IL-6/IL-6R complex, which is capable of precisely inhibiting trans-signaling (Finkel et al. 2016).

5.9 JAK Inhibitors

The most widely examined JAK inhibitors are tofacitinib, ruxolitinib, and pacritinib with numerous other agents under preclinical development. JAK inhibitors have mostly been utilized to treat myeloproliferative neoplasms and chronic inflammatory diseases; patients with solid tumors are currently receiving less attention when taking these medications.

JAK1 and JAK3 are only selectively inhibited by the oral JAK inhibitor tofacitinib, which has a lesser affinity to JAK2. Rheumatoid arthritis can be treated with

tofacitinib, according to the FDA. Other inflammatory disorders being studied clinically for tofacitinib treatment include psoriasis, Crohn's disease, chronic plaque ulcerative colitis, and chronic plaque. Tofacitinib has been FDA-approved for treating individuals at a high or intermediate risk of myelofibrosis and for those with polycythaemia vera that is hydroxyurea-resistant or intolerable. Ruxolitinib is a JAK inhibitor that is oral and selective for JAK1 and JAK2 (Johnson et al. 2018).

5.10 STAT3 Inhibitors

In most cases of human malignancies, STAT3 is hyperactivated and has been shown to have tumor-promoting qualities. It is also frequently linked to a bad prognosis for disease. As a result, the current emphasis is on finding and creating STAT3 inhibitors that can be utilized to treat human malignancies. As an intracellular transcription factor, STAT3 lacks enzymatic activity; it is frequently regarded as an intractable target, and the discovery or development of potential STAT3 inhibitors has proven challenging. Several drugs that block STAT3 expression are now under clinical trials.

The first direct inhibitors of STAT3 were made from peptidomimetics (ISS-610, PM-73G) and tyrosine-phosphorylated peptides (PY*LKTK), which attach to STAT3's SH2 domain and reduce DNA-binding activity (205–208) and STAT3 dimerization. These medications have antitumor and proapoptotic effects on cancer cells that have STAT3 hyperactivation. STA-21, LLL-3, WP1066, STATTIC, and other nonprotein inhibitors of the SH2 domain have also been found, and it has been demonstrated that they can stop the growth of tumors that contain high levels of activated STAT3 (Johnson et al. 2018).

The interactions between the promoter regions of the target genes and STAT3 are competitively inhibited as an alternate strategy for suppressing STAT3 function. It has been demonstrated that a 15-bp, double-stranded oligonucleotide matches the response region of STAT3 and that the FOS promoter competitively reduces DNA binding of STAT3 and inhibits the development of tumors in clinical models of ovary, lung, brain, breast, acute myeloid leukemia (AML), and skin cancer. Antisense oligonucleotide inhibition of STAT3 expression further offers quite a different strategy for reducing STAT3 activity in cells. AZD9150 is a next-generation antisense oligonucleotide inhibitor of STAT3, which outperforms its earlier variants by including ethyl-modified residues with a 2′–4′ constraint (Johnson et al. 2018).

5.11 Conclusions

Interleukin-6 exhibits both anti- and pro-inflammatory activities, upon its interaction with its receptor isoforms via various modes of IL-6 signaling. In addition to aiding in the development of therapeutic resistance, interleukin-6, which is secreted by tumors and a large number of other cells in the tumor milieu, influences and controls almost every characteristic of cancer. This aids in the advancement and

maintenance of tumors. Our knowledge of the possible involvement of IL-6 in the interaction between the tumor and its milieu has improved as a result of recent investigations. The majority of IL-6's actions that promote tumor growth appear to be primarily regulated by the JAK/STAT3/IL-6 signaling pathway. Therefore, neutralizing IL-6 or the IL-6 receptor to stop the beginning of signaling or preventing the completion in the end by reducing the action of two additional members, JAK and STAT3, has demonstrated treatment effectiveness in systemic and cellular carcinoma models. The stimulation of interleukin-6/STAT3 signaling occurs via classical signaling or trans-signaling. Typically, prolonged inflammation encourages various malignant diseases. The hyperactivation of JAK/STAT3/IL-6 signaling is classically linked to patients' overall reduced survival, as it promotes cancer cell survival, angiogenesis, invasiveness, and metastasis, thus severely suppressing the antitumor activity. Employing agents that block interluekin-6 signaling may be potentially therapeutic for cancer.

Further Reading

For further reading, some textbooks mentioned below also provide further understanding of the cytokine and chemokine network.

- *Cytokines and Their Therapeutic Potential* by Manzoor Ahmad Mir
- *The Cytokine Handbook* by Angus W. Thomson and Michael T. Lotze

The below links for video lectures may also be helpful:
https://youtu.be/TAqO0Mq19JQ
https://youtu.be/6wEnYfvoBqk
https://youtu.be/A9fRRCUlMms
https://youtu.be/zIWvmCsKr34

For more insights into the topic, we would suggest detailed findings from the books of Mir et al. (2022a, b, c) https://doi.org/10.1016/C2021-0-02565-7 and https://doi.org/10.1016/C2022-0-00074-X, and Mir (2021) https://doi.org/10.52305/WXJL6770, Mir (2015a, b, c) https://doi.org/10.1016/C2014-0-02898-5, and from the cancer.net website on the following mentioned links:
https://www.cancer.net/cancer-types/breast-cancer/types-treatment
https://discovery.ucl.ac.uk/id/eprint/1472740/
https://www.jmedsciences.com/doi/JMEDS/pdf/10.5005/jp-journals-10045-00138

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The Interleukin-8 Pathway in Cancer

6

Manzoor Ahmad Mir, Mina Rashid, and Nusrat Jan

Abstract

Interleukin-8 (IL-8), a pro-inflammatory chemotactic cytokine encoded by the CXCL8 gene, is mainly released by macrophages and other immune cells and is reported to play a significant role in several malignancies. Both chemotaxis and degranulation of neutrophils are associated with IL-8 that acts as an effective agent of vascularization, increases the proliferation of cancer cells, regulates the androgen-independent proliferation of prostate cancer, metastasis, and tumorigenesis, and infiltrates neutrophils at the tumor site. This chemokine activates multiple downstream signaling pathways by activating a range of effector molecules, including protein kinase C (PKC), phospholipase C (PLC), phosphoinositide 3-kinase (PI3K), mitogen-activated protein kinase (MAPK), Janus kinase (JAK), signal transducer and activator of transcription (STAT), and Rho-GTPase G protein-coupled receptors (cysteine-X-cysteine chemokine receptor (CXCR1, CXCR2). Increased expression of IL-8 and its receptors has been characterized in cancer cells. As a result of the number of effectors and the downstream cascade, CXCL8 signaling initiates tumorigenesis. An inflammatory reaction is brought on by the chemotactic cytokine molecule interleukin-8 (IL-8) that can be used as a patient survival prognostic marker as it can generate main information related to breast gland tumors, prostate cancer, and ovarian cancer. It has been demonstrated that IL-8 signaling confers chemotherapeutic resistance in cancer cells. Decreasing the expression of the IL-8 cascade may be of substantial benefit when targeting the tumor microenvironment for cancer therapy.

Keywords

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6.1 Introduction

The immune system in humans involves many cells, including specialized immune and nonimmune cells, and protects against toxic, infectious agents. The interaction between these cells is important for the proper functioning of the immune system. Contact between cells is preceded by a group of complex soluble factors such as cytokines, chemokines, and interleukins as well as by membrane-bound specific receptors and their ligands, exosomes, endosomes, and membrane vesicles. Each cell type plays a key role in the immune system through direct cell–cell interactions. Moreover, specific receptors bound to membranes and their specific ligands play important roles in immune responses. Interleukins, a subgroup of large signaling cytokines with inflammatory or anti-inflammatory functions, are important for the migration, proliferation, maturation, and activation of immune cells (Waugh and Wilson 2008). In 1979, the term "interleukin" was first coined for those cytokines that were first assumed to be secreted by leukocytes alone. They are a group of peptides or proteins produced by a variety of body cells. The term "interleukin" consists of two parts, "inter" meaning an "interaction between things" and "leukin" meaning "deriving from the fact that many of these proteins are produced by leukocytes and these molecules are released by and act on leukocytes."

Members of this cytokine family are grouped on the basis of several features, which include sequences, receptors, motif structures, cell types that produce them, and also their biological functions. There are about 38 interleukins known to date. These have been approved by a common interleukin nomenclature system for the World Health Organization/International Union of Immunological Societies (WHO/IUIS) in 1994 based on their structures, genes, and peptide sequences and range from IL-1 to IL-38 (Brocker et al. 2010 Jan et al. 2023). They are a cluster of cytokines with immunomodulatory complex functions that elicit a variety of responses by attaching to certain receptors on cell surfaces and activating a particular signaling cascade, therefore acting as autocrine or paracrine molecules and aiding in cell-to-cell communication, cell adhesion, migration, differentiation, cell proliferation, and immune cell activations and mediating physiological responses to infections and disorders (Chung and Barnes 1999; Parker et al. 2016). Interleukins are secreted to address a variety of cellular stresses, injuries, infections, and inflammations that inhibit cancer progression and development. Inflammation is the body's immune system's response against an infection and injury. White blood cells (WBCs) are released to fight infection and repair damage, but, when inflammatory responses are triggered without any injury or infection, it may start damaging healthy cells and lead to chronic or smoldering inflammation that never resolves (Martin and Leibovich 2005). Chronic inflammation is responsible for carcinogenesis, e.g., skin, gastric, pancreatic, and lung cancers, etc. Some interleukins induce signals directly in nonimmune cells, and this event leads to tumor growth, metastasis, and cancer

progression. It is the result of uncontrolled factors (Pahwa et al. 2021). In 1863, Rudolf Virchow was the first to observe that cancer often arises at the site of chronic inflammation. This raises the possibility of a malignancy by increasing the expression of IL receptors at these sites and thus plays a role in guiding metastasis (Demaria and Poli 2012). In humans, more than 50 different interleukins are involved in the functioning of the acquired and native immune systems as they direct immune cells to divide and differentiate by activating a subset of cells with receptors. Earlier, interleukins were considered to be immune function modulators. Now it has been recognized that they are also synthesized by the interaction of host cells that are not involved in immunity but are involved in many other physiological functions (Cekici et al. 2014).

Most of the immunological roles played by interleukins are at least somewhat understood. Interleukin isoforms like interleukin-1, 6, and 8 are linked to many malignancies. Pancreatic cancer cells develop and become more resistant to chemotherapy because of the autocrine synthesis of interleukin IL-1b, which also drives angiogenesis in various cancer forms. For numerous solid malignancies, including non-Hodgkin's lymphoma (NHL), myeloma, renal cell cancer, and bladder and colorectal tumors, IL-6 functions as a growth factor (Nengroo et al. 2022; Qayoom el al. 2023). Similarly, IL-8, a pro-inflammatory cytokine, has been linked to melanoma, ovarian cancer, glioblastoma, and cervical carcinoma, among other tumors, by promoting their growth and metastasis (Xie 2001).

6.2 The Structure of Interleukin-8

In 1987, Yoshimura et al. discovered that IL-8 is a specific chemokine protein that attracts neutrophils but not monokines for eliciting an inflammatory response. It is a secretory protein similar to IL-6 but has a longer half-life of about 99 amino acids, weighs about 11 kDa on Western blot analysis, and cleaves its isoforms at 8 kDa (Holmes et al. 1991). It regulates tumorigenesis and promotes proliferation and metastasis in ovarian cancer, breast cancer, and cervical cancer. CXCL8 (IL-8) is usually expressed in the bone marrow, lymphoid tissues, and kidneys and is secreted by multiple immune cells. Numerous environmental, hormonal, and pharmacological factors control IL-8 expression (tumor necrosis factor (TNF), androgen, estragon). By stimulating angiogenesis, neoplastic development, and inflammation, IL-8 plays a significant role in melanoma. The family of CXC chemokines also contains CXCL8, endothelial cells, and muscle cells. When ligation takes place, the structure of the 99-amino acid CXCL8 protein dimerizes, resulting in an effective CXCL8 protein variant in non-immunogenic cells of 77 amino acids or in monocytes and macrophages and 72-residue peptide fragments in monocytes and macrophages. The gene that produces IL-8 is found on the chromosome 4q13–q21 and is known to activate a positive member of the motif Glu-Leu-Arg: the chemokine cysteine-X-cysteine (Fig. 6.1) (Mukaida 2003).

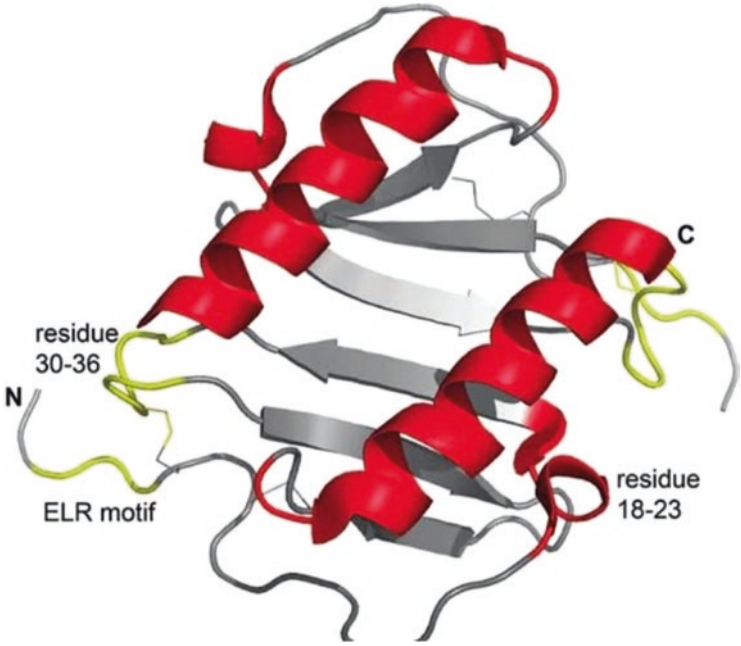

Fig. 6.1 The structure of interleukin-8 (reference: functional aspects of the interaction between interleukin-8 and glycosaminoglycans)

6.3 IL-8 Receptors

6.3.1 CXCR1

The G protein-coupled receptor (GPCR) family member called interleukin-8 receptor A (IL-8RA) has seven transmembrane domains. Both the receptor molecules of CXCR1 and CXCR6 are expressed on mast cells, granulocytes, monocytes, and certain natural killer (NK) cells. Through its N-terminal strand, CXCR1 communicates with CXCL8. The dimer CXCL8 and the CXCR1 N-terminal interaction models simulate tertiary structures. The amino acid residues Lys108, Asp9, Glu12, and Lys120 for CXCR2 and Glu7, Glu12, and Asp9 for CXCR1 may be involved in the binding of CXC chemokines to their receptors (Cummings et al. 1999).

6.3.2 CXCR2

A receptor with a strong affinity toward IL-8 was initially discovered in 1991 [4] and was called interleukin-8 receptor B (IL-8RB). This receptor's genes are found at 2q34–q35. The CXCR1/CXCR2 protein has a 77% resemblance in the amino acid order [5]. The CXCL2 receptor is found on seven CXC molecules: CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, and CXCL8. CXCL6/CXCL8, which

is separated into two groups, is activated at similar concentrations, whereas CXCL1, CXCL2, CXCL3, CXCL5, and CXCL7 are activated at high concentrations (Fig. 6.2). The CXCR2 gene promoter contains a non-classical TATA box and requires additional transcription factors like specificity protein (Sp-1) and activating protein (AP-2) and also contains two NF-κB binding sites at 331bp and 288bp positions. This gene has 11 exons and an open reading frame (ORF) of 1065 bp (Ha et al. 2017). Recent research has found that the increased expression of CXCR2 was observed with a decrease in microRNA (miRNA), which increases metastasis. Hepatocellular carcinoma, stroke, and neuropathic pain are all such diseases associated with a decreased level of miRNAs. Improved CXCR2 appearance on endothelial cells increases their production. The fungus *Cordyceps militaris*, a parasite on moth butterflies, contains miRNAs that decrease the expression of CXCR2 that is used in Chinese medicine. In addition, the expression of CXCR2 is decreased by MALAT1 (metastasis-associated lung adenocarcinoma transcript 1). Some miRNAs can influence CXCR2 activity also, for example, miR-K12-3 directly reduces G protein-coupled receptor kinase 2 (GRK2) expression and the length of CXCR2 by ligand activation, leading to crucial Kaposi sarcoma development (Korbecki et al. 2022).

IL-8 is known to facilitate the abnormal growth of cancer cells by promoting angiogenic response, attracting neutrophils to the location of the tumor, and by other mechanisms like the migration and relocation of cancer tissues.

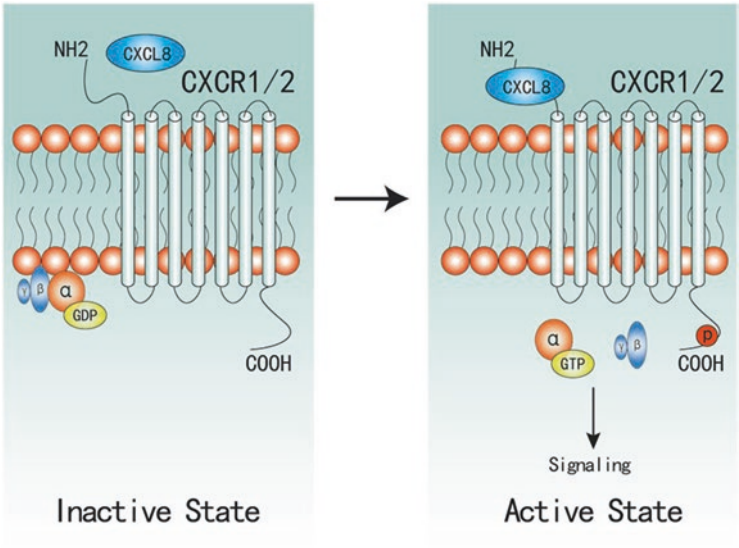

Fig. 6.2 Structures of the CXCR1/2 receptor compound with G-αβγ in the inactive state. When IL-8 attaches to the N-terminal of CXCR1/2, α-guanosine diphosphate (α-GDP) is converted into α-guanosine triphosphate (α-GTP) that detaches with βγ subunits. This subsequently activates related signaling

6.3.3 The CXCL8–CXCR1/2 Signaling Pathway

CXCL8 is induced by several well-characterized downstream signaling pathways that promote the progression of cancer. The expression of CXCR1/CXCR2 receptors in the tumor microenvironment (TME) occurs after the abnormal onset of CXCL8 on tumor-associated macrophages (TAMs) and endothelial cancer cells. When CXCL8 binds to its receptors, CXCR1/CXCR2, it induces a conformational change that leads to the activation of multiple signaling cascades by the heteromeric G-αβγ protein (Fig. 6.3) (Park et al. 2012; Sofi et al. 2023). Activation of CXCR1 occurs in response to the binding of CXCL8 to the granulocytic chemotactic protein (GCP1), and CXCR2 is activated by the growth-related oncogene (GRO), GCP2. The primary functional receptor of CXCL8 is CXCR2, which promotes angiogenesis in prostate cancer, whereas CXCL8/CXCR1 mainly regulates the proliferation in cancer cells. On IL-8 attaching to its specific receptors CXCR1/2, the G protein activates the principal primary effector molecule protein lipase C (PLC) or phosphatidylinositol-3-kinase (PI3K), which plays a vital role in neutrophil attraction, resulting in serine and threonine phosphorylation to activate Akt that modulates tumor survival, angiogenesis, and metastasis. Increased expression of Akt/PI3K interferes in mitogen-activated protein kinase (MAPK) signaling. Once CXCR1/2 is phosphorylated, it leads to two MAPK pathways that are MyD88-dependent: p38 MAPK and extracellular signal-regulated kinase (ERK)–MAPK

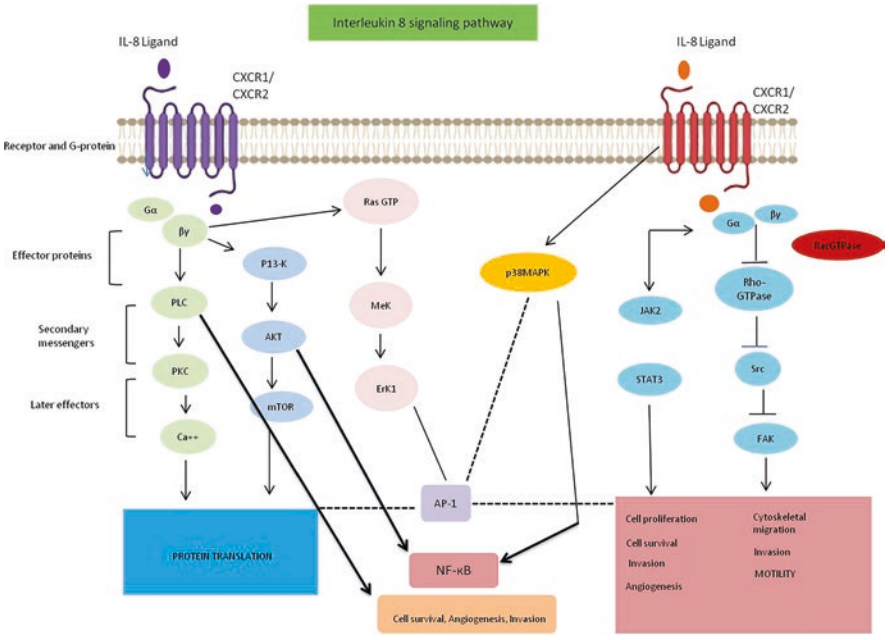

Fig. 6.3 The signaling pathway of IL-8 activating various effector molecules for translation of proteins, showing angiogenesis, cell proliferation, invasion and migration of tumor cells

(Knall et al. 1997). This signaling promotes protein translation for transcription factor regulation. In multiple forms of cancer cell lines, including those that are androgen-independent, angiogenesis, migration, cell survival, and increased Akt activity expression have been detected by IL-8 signaling (MacManus et al. 2007). IL-8 signaling also activates various Rho-GTPase family members' non-tyrosine kinase receptors (Src and focal adhesion kinases (FAK)) and subsequently activates ERK–MAPK signaling (Tobin et al. 2019). As members of the Rho family, both CXCR1 and CXCR2 activate differentially in endothelial cells. CXCR1 promotes rapid induction of Rho-GTPase, whereas CXCR2 signaling results in the delayed activity of Rho-GTPase. MAPK (mitogen-activated protein kinase) is regulated by CXCL8 signaling that represents the wide range of serine/threonine kinases that engage in interactions with proteins that are near cell surface receptors. These kinases are the key components of the Raf-1/MAP/ERK signaling cascade. IL-8 acts as a Ras transcription factor and are associated with the alteration of cell cycle inhibitors such as Cyclin D1/Cyclin B1 increased level is due to IL-8 that activates pi3k/Akt/Raf/Mef to stimulate proliferation. IL-8 also upregulates Bcl-2 (an anti-apoptotic gene) and downregulates caspase-3 (a proapoptotic gene) that suppress apoptosis of Michigan Cancer Foundation-7 (MCF-7) T cells. It also controls the apoptotic pathways by protein kinase B (PKB) and nuclear factor-kappa B (NF-κB) interaction. Protein kinase A (PKA) boosts IL-8 synthesis and PKC inhibition but not PKC activation. CXCL8 activates PLC in tumor cells, and PKC and neutrophils phosphorylate cytoskeletal proteins that mediate cell adhesion, migration, and dynamic alteration. There are three categories of PKC, and all three can be activated by CXCL8 (Li et al. 2021). The stimulation of various transcription factors, together with NF-κB, would be promoted by boosting these upstream signaling pathways, which include PI3K/Akt, MAPK, and PLC/PKC (Hoffmann et al. 2002). NF-κB transcriptional factor activation is the main phenomenon behind inducing CXCL8 secretion and expression. Consequently, there is a positive loop between NF-κB activation and CXCL8 production. According to studies, protein tyrosine kinases such as Src kinases and FAKs are phosphorylated by IL-8. It is currently unclear how these protein tyrosine kinases are stimulated in endothelial cells to increase cell proliferation, invasion, motility, and survival. It has been demonstrated that Src kinases are activated by CXCL8 to cause the phosphorylation of vascular endothelial growth factor receptor 2 (VEGFR-2). IL-8 stimulates time-based initiation of the Rho-GTPase group in prostate cancer cells and endothelial cells. Evidence suggests that CXCL8 can trigger the Janus kinase/signal transducer and activator of transcription (JAK/STAT3) signaling pathway in both tumor and normal cells as well as affect NK cells' role via STAT3 molecules (Wilson et al. 2005; Mir et al. 2023b).

6.4 NF-κB's Role in IL-8 Signaling

The transcriptional regulator NF-κB, which consists of the subunits p50, p52, and p65 (also known as RelA, RelB, and c-Rel) in a structure (homodimeric or heterodimeric), plays a critical role in the inflammation, immunity, and development of a

tumor. IL-8 indication is weakened as a result of the NF-κB effect on Breast cancer (BC) metastasis. TNF-α, oxidative stress, and lipopolysaccharide (LPS) are just a few examples of the signals that might lead to IL-8 gene expression, and NF-κB also plays a vital role in this procedure (Mir et al. 2023a). According to studies, even if NF-κB is a vital factor in the signaling of the IL-8 gene, neither enhancer-binding protein (EBP) nor activating protein-1 (AP-1) is essential for the IL-8 gene's stimulation in BC cells (Shah et al. 2012). According to reports, inhibiting apoptosis, which is a process that kills cells, allows BC cells to survive and is possibly promoted by NF-κB that is essential for directing IL-8 production in the MDA-MB-231 cell line. Several proteins code the regulation of a wide range of cytokines, receptors, and progress influencers (Benoy et al. 2004). The uninterrupted action of NF-κB is related to diverse types of violent tumors. Various pieces of evidence have reported that the transcription factor NF-κB takes part in the enlargement and extension of a malignancy (Xie 2001). NF-κB suppression follows the downregulation of IL-8 production and blockage of abnormal division and spread. Chavey et al. (2008) demonstrated that in mammary cancer cells, IL-8 gene activation demands acetylation and stimulation of NF-κB. Interleukin-8 (IL-8) is upregulated in breast cancer compared with normal breast tissue and is associated with poor prognosis. Moreover, elevated IL-8 concentration upregulates IL-8 RNA, gene synthesis, histone H3 acetylation on the IL-8 promoter, and high action of IL-8 (CXCL8) (Bendre et al. 2003).

6.5 Interleukin-8 (IL-8) and Integrin β3

The signaling cascade of integrins and cytokines is vital for tumor advancement, and there is a positive relationship between IL-8 and integrin β3 (Green et al. 1997). IL-8 downregulation shows a substantial decrease in integrin β3 protein synthesis. To enhance the invasion of the BC cell line, IL-8 upregulates integrin β3 appearance (Green et al. 1997). On the other hand, increased levels of IL-8 and integrin β3 by human BC cell metastasis, via synthesis of integrin β3 through the PI3K/Akt/AP-1 signaling cascade, increases chondrosarcoma cell migration (Green et al. 1997). Studies have reported that in estrogen-negative breast cancer tissues, there is an encouraging correlation between integrin β3 and IL-8. Elevation in the invasion of BC cells happens by the process of IL-8 that activates PI3K/Akt signaling, resulting in the activation of NF-κB, and upregulates integrin β3. Therefore, this study shows that the IL-8/PI3K/Akt/NF-κB/integrin β3 axis may be beneficial for the intervention of BC with a metastatic grade (Green et al. 1997).

6.6 CXCL8 in the Tumor Microenvironment

Tumor cells releasing IL-8 can have a powerful impact on the tumor microenvironment. Cancer cells are supported by their epithelial–mesenchymal transition (EMT) phenotype, which promotes cell death and enhances angiogenesis and invasiveness

(Alfaro et al. 2017). For instance, the survival and multiplication of tumor cells are enhanced by the secretion of IL-8 by cancer cells via autocrine signaling pathways. Additionally, IL-8 signaling encourages angiogenic reactions in endothelial cells (Ha et al. 2017), elevates cancer cell survival and proliferation, myeloid-derived suppressor cell migration, and cancer cell migration. Additionally, tumor cells forming IL-8 stimulate the angiogenesis-promoting effects of endothelial cells in the TME and cause neutrophils to chemotactically enter the tumor site. IL-8 has the ability to encourage TAMs to generate more advanced elements, which, in turn, accelerates the amount of cell division and their migration to the tumor region (Peng et al. 2018). Therefore, in various xenograft and orthotropic in vivo representations, a connection between IL-8 and metastasis, angiogenesis, and tumorigenicity was explored (Wilson et al. 2008). Additionally, it has been demonstrated that chemotherapeutic resistance in cancer cells is conferred by stress and drug-induced IL-8 signaling. Multiple impacts of CXCL8 signaling on diverse cell types are seen in the tumor niche (Fig. 6.4). The TME plays a pivotal role in the development and progression of cancer, and is also a major factor in governing the efficacy of a given therapeutic intervention.

6.7 Cancer Stem Cells (CSCs)/IL-8

Cancer stem cells (CSCs) are a population of tumor cells thought to be responsible for tumor initiation, maintenance, and metastasis. They exhibit a variety of functional traits, including the capacity to start and spread tumors as well as the ability to differentiate into numerous cell types and self-renew (Lawson et al. 2009).

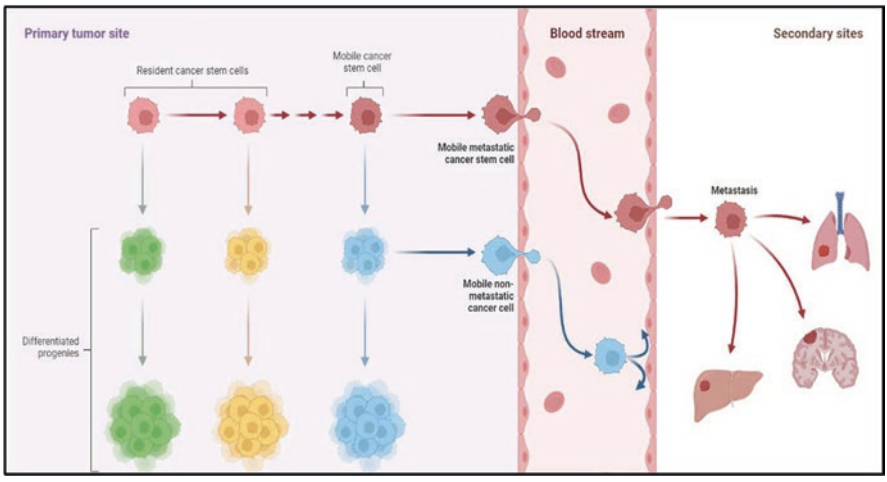

Fig. 6.4 Recruitment of various cells for tumor promotion

Numerous data indicate that the CSC phenotype is driven by the effect of IL-8. For instance, breast CSCs exhibit enhanced CXCR1 expression and increased metastasis formation. CXCL8 levels in metastatic breast cancer cells are also lowered when CXCR is blocked. In addition to other cancers, breast CSC traits are correlated with the IL-8 axis. In colon cancer cell lines, branch marker expression is reduced by IL-8 inhibition, which results in the generation of the colon sphere and the growth of cancer. Increased CSC populations, cell invasion, and tumor sphere development result in pancreatic cancer. In addition, receptor CXCR1/2 restriction reduces lung cancer xenograft, tumor growth, metastasis, and angiogenesis and induces resistance to epidermal growth factor receptor (EGFR) inhibitors and stemness of adenocarcinoma. Collectively, these findings showed that the IL-8-driven cancer axis appears to be a constant process across different forms of tumors. Importantly, these studies also showed that this process can be susceptible to therapeutic intervention by CXCR1/2 suppression (Ruffini 2019).

6.8 Neutrophils/IL-8

Neutrophils are extremely widespread leukocytes, making up 50–70% of white blood cells (Rao et al. 2012). They serve as the "first responders" to acute inflammation and are short-lived, essential cells of the innate immune system in the peripheral human blood. They are prominent for their role in knocking down invading microorganisms, such as bacteria and fungi, through the process of phagocytosis and show activation of cytokines, reactive oxygen species (ROS), and defensins. The part that neutrophils play in tumor biology has not received much attention. A significant part of the immune cells found in tumors can be neutrophils, and, in the majority of investigations on human tumors, tumor-associated neutrophils (TANs) are connected to inferior experimental outcomes, including bronchioloalveolar adenocarcinoma (Jensen et al. 2009; Wang et al. 2014), esophageal epidermoid cell carcinoma (Kuang et al. 2011), neck and head epidermoid carcinoma (Trellakis et al. 2011), liver cancer, skin cancer (Kuang et al. 2011), and renal tumor (Hubert et al. 2011; Reid et al. 2011). In addition, tumor grade is related to a neutrophil increase in a variety of human aggressive cancers. It is significant to note that tumor cells in their niche can themselves promote neutrophil recruitment, and, in preclinical models of Ras-driven malignancy, IL-8 and its efficient receptors CXCL1/2 and keratinocyte-derived chemokine/macrophage inflammatory protein 2 (KC/MIP-2) have been demonstrated to direct neutrophils to the site of infection. An area of the current investigation is how exactly neutrophils affect tumors, and, according to recent research on the plasticity of the TAN phenotype, they can either have antitumor N1 or pro-tumoral N2 phenotypes like tumor-associated macrophages. Using oxidative damage or antibody-dependent cellular cytotoxicity (ADCC), N1 TANs can kill tumor cells directly; however, ROS obtained from neutrophils may be mutagenic and can cause genomic instability. By producing cytokines that attract T cells (CCL-3, 9, 10), inflammatory cytokines (IL-12, TNF-α, granulocyte macrophage colony-stimulating factor (GM-CSF)), and cross-presenting antigens to $CD8^{+}$

cytotoxic T lymphocytes, N1 TANs correspondingly contribute to adaptive antitumor activities (Huber et al. 2020). Contrarily, N2 TANs promote tumor migration by collagenase-IV, heparinase, and neutrophil elastase (extracellular matrix (ECM)-degrading enzymes), thus accelerating both tumor angiogenesis and matrix metalloproteinase (MMP)-9. Additionally, N2 TANs can conquer acquired immunity by binding arginine-deficient T cells with arginase 1 secretion and by engaging regulatory T cells (Tregs) through CCL17 secretion. Early-stage tumors express neutrophils with a more cytotoxic phenotype that can stimulate antitumor T-cell responses, whereas neutrophils from well-known tumors downregulate these functions to acquire pro-tumoral properties. As TGF receptor 1 (TGF-RI) inhibition with the tiny molecule SM16 was discovered to promote N2-to-N1 phenotypic alteration in murine mesothelioma tumors, TGF-β seems to be a crucial deciding factor. Collectively, these data support the idea that IL-8 produced by tumors can aggressively inhibit antitumor immunity by increasing the number of N2 TANs (Labani-Motlagh et al. 2020).

6.9 IL-8/Myeloid-Derived Suppressor Cells (MDSCs)

In the bone marrow, the myeloid cell lines arise from myeloid progenitor cells that differentiate into granulocytes when migrating to peripheral organs, macrophages, and dendritic cells. A population of undeveloped heterogeneous, immunosuppressive cells known as myeloid-derived suppressor cells (MDSCs) are formed when the maturation of these cells is stopped under pathological circumstances. Humans have two distinct subpopulations of MDSCs, namely, granulocytic or monocytic cells. Cancer-suffering individuals have higher levels of circulating MDSCs than do healthy individuals, and the clinical stage is correlated with the number of circulating MDSCs (David et al. 2016). Through the secretion of substances, such as prostaglandins, GM-CSF, IL-6, and VEGF, tumor cells help MDSCs grow within the TME by persistently triggering JAK2/STAT3 signaling in myeloid progenitor cells. Then, after being released by stimulated T cells and intra-tumoral stromal cells, other molecules, such as interferon (IFN-α/β), TGF-β, and IL-4/13, activate MDSCs. MDSCs first suppress the antitumor immune reactions by suppressing T cells using a variety of methods, such as the synthesis of ROS and nitric oxide synthase (NOS), which can disable T-cell receptors and cytotoxic T lymphocyte (CTL)-attracting chemokines, L-arginine, L-tryptophan, and L-cysteine; T-cell nutrients that prevent T-cell initiation and production are depleted by the TME. T-cell homing is disrupted by a disintegrin and metalloprotease 17 (ADAM-17), which also induces Tregs and produces the immunosuppressive molecules TGF-β and IL-10. The absence of a CXCL8 gene in the mouse genome has been found to complicate the function of IL-8 in attracting MDSCs to the TME, according to experimental findings. However, it has been noted how crucial the murine functional homologs of IL-8 and CXCR2 are in controlling MDSC homing in cancer (Dominguez et al. 2017). Asfaha et al. (2013) created a transgenic mouse that carries the whole IL-8 human gene along with regulation-promoting

components carried on a synthetic bacterial chromosome to explore human IL-8. Using this classical system, they demonstrated how IL-8 strongly controls inflammation-related colonic and gastric carcinogenesis through high MDSC staffing. In a scientific investigation of prostate cancer individuals, an elevated range of MDSCs was linked to a more advanced stage, rising serum levels of IL-8 and IL-6, and impaired T-cell action. The human CXCL8 gene was inserted into mice livers using homodynamic gene transfer, and, as a result, MDSCs were drawn to these IL-8-producing livers by chemotactic attraction. This study also observed that MDSCs with a granulocytic phenotype were attracted to IL-8 in vitro by neutrophil extracellular traps and CXCR1/2-expressing MDSCs that were isolated from the mononuclear cells of cancer patients (Fousek et al. 2021).

6.10 IL-8 and Epithelial–Mesenchymal Transition

As a mediator of inflammation, IL-8 has the potential to remodel the TME by accumulating MDSCs and suppressive neutrophils to adapt the antitumor activity. IL-8 also has a strong impact on tumor cells to modulate immune cell chemotactic attraction. These effects can be either pro-tumorigenic or anti-tumorigenic (Acosta et al. 2008; Kuilman et al. 2008), for example, activation of EMT differentiation or CXCR2 signaling that facilitates senescence induced by oncogenes. Epithelial cells lose characteristics like polarity, intercellular contact, intercellular adhesion, invasiveness, motility, and growth inhibition and acquire mesenchymal characteristics such as edge asymmetry; the former is known as EMT and the latter is its opposite, known as MET (mesenchymal–epithelial transition), which occurs during development and tissue regeneration (Kalluri and Weinberg 2009). A similar process is reported in cancer cells without losing their certain characteristics, thus showing a hybrid or metastable phenotype. This triggers the change in tumor phenotype and is also responsible for dynamic alteration in the microenvironment (Tan et al. 2014). Cells that undergo mesenchymalization not only gain motility and invasiveness rate but also greater resistance to cytotoxicity (Sofi et al. 2022a). This transition is controlled by soluble factors that are overexpressed in cancer, including transcriptional factors Slug, ZEB1, ZEB2, zinc-finger protein Snail, Twist1, and Twist2 (helix-loop-helix transcriptional factors) (Thiery and Sleeman 2006). These regulate the intrinsic signaling cascade mediated by oncogenic K-Ras or a range of external stimuli within the tumor microenvironment. Once EMT occurs in cancer cells, there will be secretion of growth factors and cytokines that promote EMT by the autocrine feedback loop. This maintains cells in MET and influences nearby cells for EMT. Both IL-6 and IL-8 share this quality of autoregulation by TGF-β signaling. Overexpression of Snail upregulates IL-8 or CXCR1/CXCR2 expression. Likewise, TNF-α and TNF-β therapy for colon cancer (Savagner 2015) increases the secretion of IL-8. Blockade of its receptors in mesenchymal cancer cells decreases their expression and invasion. This suggests that IL-8 signaling is crucial for the EMT of cancer cells (Fig. 6.5).

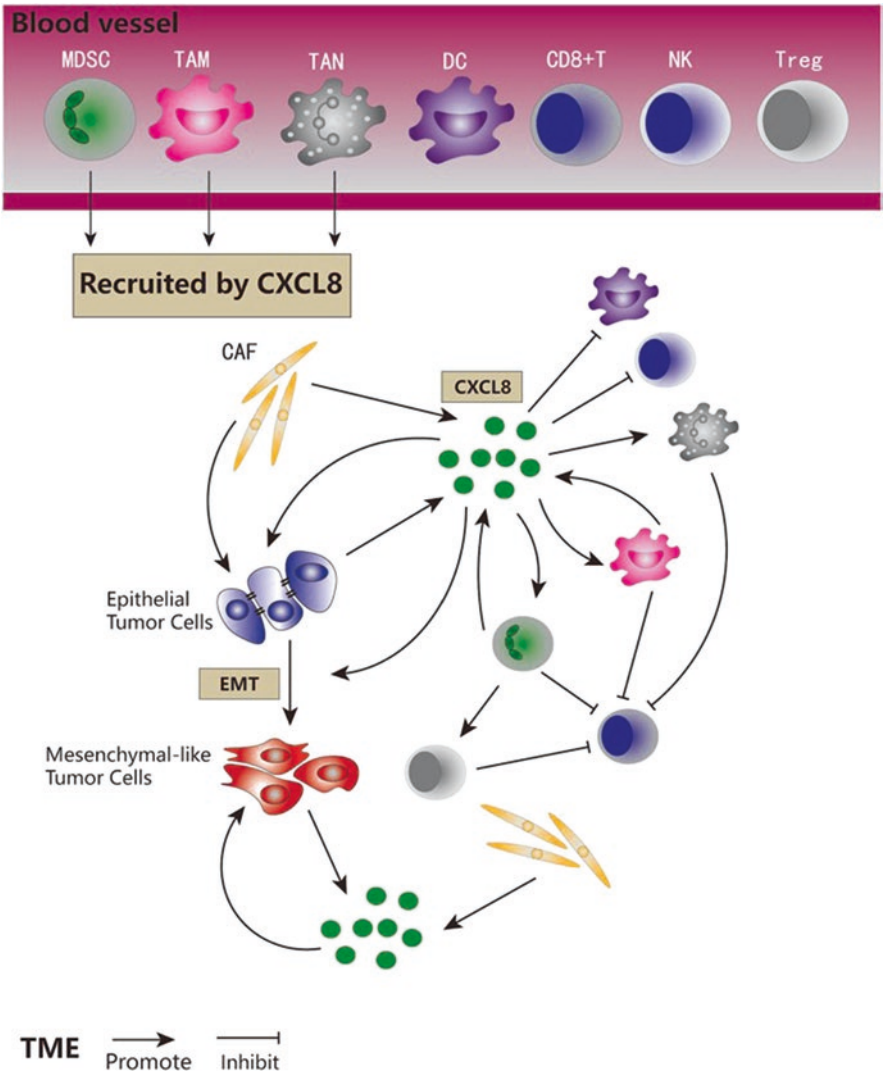

Fig. 6.5 The function of CXCL8 signaling in tumor development. Recruitment of TAMs, TANs, and MDSCs in the TME to promote cell proliferation and EMT directly or indirectly

6.11 Inflammation/Tissue Injury/CXCL8

A group of cytokines known as chemokines encourage the focused movement of leukocytes along with a rise in concentration, which attracts migratory cells to the source of chemokine production. CXCL8, commonly referred to as IL-8, is a CXC pro-inflammatory chemokine that has been identified to play a role in encouraging neutrophil chemoattraction and degranulation (Mir and Albaradie 2014). It

communicates by interacting with CXCR1 (IL-8R) and CXCR2 (cysteine-X-cysteine chemokine receptor 2) (IL-8R). These receptors have different chemokine binding affinities; CXCR1 can only bind CXCL8 and CXCL6, whereas CXCR2 can bind CXCL8, CXCL1, and CXCL2. The chemokine attaching to these receptors causes the initiation of several downstream signaling cascades, such as the PI3K/Akt, PLC/PKC, and MAPK cascades, through FAK, Rho family, JAK (Janus kinase). and STAT (signal transducer and activator of transcription factor). Against any tissue damage, or injury, endothelial, epithelial, and macrophage cells generate the cytokine IL-8 (Mir and Albaradie 2015). One of IL-8's key activities is to cause granulocyte chemotaxis or the movement of neutrophils toward the site of infection. Vascular endothelial cells respond to IL-8 signaling by exhibiting cell division, existence, and relocation, which eventually leads to the development of new bloodstreams. In this way, IL-8 functions to halt the inflammatory stimulation and aid curing (Russo et al. 2014).

6.12 Promoting Tumorigenic Angiogenesis

Angiogenesis, which is crucial for the existence and relocation of a tumor, has been identified as one of the characteristics of cancer. Since CXCL8's impact on tumor angiogenesis has been well-studied, it has already been designated as a chemokine that promotes angiogenesis (Ueda et al. 2022). By default, human vascular endothelial cells show CXCR2. Endothelial cells start to exhibit and secrete MMPs to interrupt the ECM as a result of surface tumor tissues and different kinds of stromal cells producing CXCL8 in the TME. This causes angiogenesis (Liu et al. 2016). Additionally, CXCL8 can show endothelial cell increase, which directly contributes to vascularization. It is interesting to note that CXCL8 and VEGFR-2 have a cross-relationship with angiogenesis. This situation has led to the discovery of a loop in endothelial cells, whereby CXCL8 can increase VEGF-A production while suppressing the expression of VEGFR-2 in endothelial cells. One of the supreme destructive tumors is gastric cancer, with 951594 cases detected globally in 2012 (Mir and Mehraj 2019). Moreover, in 2012, the third principal reason for cancer-related deaths globally was gastric cancer, which caused about 723027 mortalities. Vascularization is the establishment of new blood capillaries from old ones and is responsible for the evolution of dense tumors. The angiogenesis process takes place in several phases during the key step of the malignant progression of the tumor or in tumor invasion and metastasis (Qayoom et al. 2023). Particularly, angiogenesis has been known to be closely associated with diagnosis and hematogenous metastasis of gastric cancer (Chung et al. 2010). Stability in pro-angiogenic and anti-angiogenic factors in a limited situation is vital for the progress of angiogenesis. Interleukin (IL)-8 is a pro-inflammatory chemokine that belongs to the CXC subclass and is an important regulatory factor within the tumor microenvironment. IL-8 is expected to be released by many human cancer cells as well as by gastric cancer cells. It encourages angiogenic reactions in in vivo models as a directly acting angiogenic factor and is associated with tumor angiogenesis, malignant melanoma, hepatocellular

carcinoma, cervical cancer, and nasopharyngeal carcinoma. The function of IL-8 in the initiation of angiogenesis in gastric cancer is unclear, and vascular endothelial growth factor (VEGF)-A binds to its receptor molecules VEGFR-1 and VEGFR-2. As an important intermediator of blood vessel development, VEGF-A is considered a critical controlling protein during angiogenesis and pathological neovascularization. These studies evaluate the effects of IL-8 in angiogenesis and examine the appearance of essential angiogenesis indicators, having VEGF-A, VEGFR-1, and VEGFR-2, using a co-culture model of human gastric cancer SGC-7901 cells and human umbilical vein endothelial cells (HUVECs) (Dikov et al. 2005).

6.13 CXCL8 in Tumor Biology

Numerous research studies have found that the serum rate of CXCL8 in cancer individuals can function as a biomarker to support cancer cells, directly and indirectly, by promoting their survival, proliferation, invasion, angiogenesis, and tumor stemness and suppressing their antitumor immunity. Recent research has suggested that CXCL8 may directly donate to the emergence of resistance to immunotherapy, molecularly targeted therapy, and chemotherapy (Mehraj et al. 2021b). As a result, CXCL8 has already been identified as a pro-tumorigenic chemokine that affects tumor cells and modifies the TME to facilitate the growth and metastasis of tumors. CXCL8 can lower intracellular ROS levels because it prevents glycogen synthase kinase 3 (GSK-3) from suppressing prostate cancer cell apoptosis. TME elements can increase levels of cytokines and chemokines, including CXCL8, and play a crucial role in the development and spread of tumors (Xiong et al. 2022).

6.14 The Role of IL-8 in Breast Cancer

One of the major reasons for female mortality worldwide is breast cancer, which constitutes about one-third of all diseases in females. It affects one in eight women in the United States. Similarly, breast cancer is the most persistent cause of death in women in Iran. The accurate and initial observation of BC can extend the survival of the person. Primary recognition of BC with proper authentic treatment procedures makes it easy to differentiate between breast tumors (Hamed et al. 2012). Different signaling cascade deregulations are involved in breast cancer, and early signs of indication can help treat the patient and prevent mortality (Mir et al. 2020). These indicators are capable of providing core details associated with the medical implications of breast tumors (de Campos Zuccari et al. 2012). Interleukin-8 (IL-8) is also elevated in many human tumors (de Andrés et al. 2013). The impact of IL-8 on the relocation and attack of tumor cells is essential for the metastasis and movement of tumors (Čačev et al. 2008). IL-8 emission also drives the appearance of adhesion molecules, such as fibronectin in human inflammatory BC cells. Increased levels of IL-8 occur in neoplastic mammary tissues in contrast to normal tissues. It has been reported that IL-8 expression in individuals with BC is related to

mammary gland metastasis, poor survival, and a more advanced disease (Mehraj et al. 2021a). Thus, an increased rate of IL-8 corresponds with metastatic intrusiveness and primary restenosis. It looks like IL-8 has the required capability to be an analytical and projecting tumor indicator. Interleukin-8 plays a key role in the osteolysis of individuals with BC, and anti-interleukin-8 therapy can be helpful in the diagnosis of skeletal-related incidents with BC, as reported by Kamalakar et al. (2014). However, Zuccari et al. have reported that the low concentration of IL-8 is linked to local reappearance and metastasis in individuals with BC. Thus, the purpose of this research is to estimate the process and action of IL-8 in patients with BC and its association with oxidative stress and estrogen receptors in these individuals (Sofi et al. 2022b). There is evidence of a solid connection between IL-8 secretion and metastasis of breast carcinoma cell lines. Extremely metastatic cell lines release more IL-8. Few reports related to the activity of IL-8 in BC expansion consequences have exposed that with increased BC risk, there is an association of polymorphisms in IL-8 and CXCR2 genes (Qayoom et al. 2021). According to one study, in patients with BC, the serum IL-8 ratio was higher compared to that in healthy individuals. This high serum IL-8 level is associated with the inferior results of a complex tumor load. Advanced IL-8 stages expect the initial metastatic spread of BC. IL-8 production from BC cells in vivo was done on a nude mouse model that had metastasized and displayed that estrogen-receptor negative cell line MDA-231-BSC (metastatic cells isolated from bone metastases) release high rate of IL-8 related with the parental MDA-231 cells. Recent reports have revealed an inverse connection between IL-8 production and metastasis. Less IL-8 levels were observed in women who had undergone post-surgical chemotherapy and radiotherapy. Increased secretion of IL-8 in BC cells is related to BC migration and vascularization (Singh et al. 2013).

6.15 The Role of IL-8 in Ovarian Cancer

Ovarian cancer, the most aggressive type, is the foremost reason for disease in the advanced sphere. About 90% of ovarian cancers arise from epithelial surfaces, which are classified as epithelial ovarian cancer (EOC). EOCs can be categorized as endometrioid, mucinous, serous, clear cell, and transitional cell types. The molecular nature of ovarian carcinomas is heterogeneous and involves multiple pathways of development (Lane et al. 2011). HEY, OVCA429, OVCA433, SKOV3, SNU251, and OVCAR3 are some ovarian cancer cell lines that are studied and have investigated the role of CXCR2 in human ovarian cancer. The receptor of IL-8 is associated with tumor growth. CXCR2 modulates cell cycle regulatory proteins like cyclin A, cyclin B1, cyclin D1, CDK6, and CDK4 that promote cell cycle progression. By suppressing p53, Bcl-xS (adenosine diphosphate (ADP) ribose) CXCR2 inhibits cellular apoptosis and activates Bcl-2 and Bcl-xL. It also stimulates angiogenesis by decreasing the levels of thrombospondin-1 and increasing the levels of VEGF through multiple signaling pathways MAPK and NF-κB (Mir and Qayoom 2023). The higher appearance of CXCR2 leads to ovarian carcinoma and has potential as a

therapeutic target, and it can be used in ovarian cancer diagnosis. Growth-regulated oncogene (GRO1) mediates ovarian cancer, and prostate cancer cells expressing CXCR2 have more attraction toward IL-8 and GRO1 and the least for GRO2 and GRO3; however, this procedure is not fully understood (Yang et al. 2010).

6.16 The Role of IL-8 in Prostate Cancer

Prostate cancer is the second principal reason for tumor-related mortalities in American males (Howlader et al. 2014). Facilitated by its surrounding contacts, it contributes to chronic inflammation—which is commonly seen in the adult prostate and is a permitting characteristic of the tumor (De Marzo et al. 2007). Inflammatory cytokines and single nucleotide polymorphisms (SNPs) in cytokine factors also mediate inflammation-associated prostate carcinogen, cancer risk, and aggressiveness (Kwon et al. 2011). Men with prostate cancer have elevated IL-8 in serum restrained in the stroma surrounding prostate tumors surrounding related to stroma surrounding normal-appearing prostate epithelium. The location of IL-8 synthesis and translation within cells in malignancies versus benign regions is not fully defined. Furthermore, nothing is known regarding the location of cellular interleukin in the prostate niche. It remains to be seen whether immunological or prostatic cells produce these cytokines to a greater extent. Additionally, expression patterns in the prostate vary based on race or an undetermined cancer grade (Yousuf et al. 2022). The most prevalent cytokine identified is IL-8, and it is significant to note that in cases with higher-grade tumors, IL-8 transcription is expanded across both benign and tumor zones. There was no discernible difference in the production of IL-8 between groups. Despite being detected in the serum, IL-1, IL-6, and IL-10 are abundantly expressed inside the TME (Neveu et al. 2014).

6.17 The Role of IL-8 in Nonpathogenic Situations

CXCL8, secreted by multiple cell types, is also known as a neutrophil chemotactic factor, in response to inflammatory invitations, including neutrophils, epithelial cells, monocytes, fibroblasts, mesothelial cells, endothelial cells, and tumor cells. This is why it has a significant impact on inflammation and wound healing. By activating neutrophils, it can also draw T cells and other inflammatory cells into the areas of inflammation. This interleukin's main job is to perform chemotaxis in target cells, mostly neutrophils but also other granulocytes, leading them to gather around the infection site and undergo phagocytosis on arrival. Rise in intracellular calcium, exocytosis (such as the release of histamine), and respiratory burst are only a few of the physiological reactions that IL-8 causes in target cells, which are necessary for migration and phagocytosis. Since it can selectively activate neutrophil granulocytes, IL-8's biological activities set it apart from all other cytokines, as we have committed a transient upgrade in cytosolic calcium stages and the secretion of

enzymes from granules (Fäldt et al. 2002). In this way, IL-8 intensifies the metabolism of ROS (reactive oxygen species), expands chemotaxis, and improves the appearance of adhesion molecules. Thus, IL-8 is also known to be an effective agent of angiogenesis. Comparably, IL-8 is chemotactic not only for neutrophils but also for all known categories of wandering immune cells. Moreover, IL-8 is chemotactic for fibroblasts, speeds up their movement, and excites the expulsion of tenascin, fibronectin, and collagen-I during wound healing in vivo. It also excites α-smooth muscle actin creation in human fibroblasts. On the other hand, IL-8 in vivo powerfully fixes erythrocytes and acts as a mitogen for epidermal cells. This prevents the resistance of leukocytes to stimulate endothelial cells and therefore participates in anti-inflammatory events (Kawarizadeh et al. 2021).

6.18 IL-8 as a Universal Indicator

Due to delayed and challenging diagnosis, tumors are another common cause of high mortality rates universally, necessitating the development of improved biomarkers with high sensitivity and specificity. Used as a noninvasive measure, cytokines are significant mediators of inflammation. A marker for many diseases, including cancers, is IL-8 (Shahzad et al. 2010).

6.18.1 IL-8 as a Marker for Urinary Bladder Cancer

Excess of IL-8 is observed in bladder cancer patients with the tumor located in the outermost layer of the bladder. IL-8 serves as a marker of diagnosis (Koçak et al. 2004).

6.18.2 IL-8 as a Marker for Non-Hodgkin's Lymphoma' (NHL)

According to studies, IL-8 serves as an indicator of NHL (non-Hodgkin's lymphoma'). Since many years, elevated NHL incidences have been seen around the globe, particularly in North American and European nations. Many medical subspecialties are currently using IL-8 as a promising marker to diagnose patients quickly or as a prognostic indicator of a variety of clinical diseases. IL-8 levels are assessed as a result of numerous inflammatory diseases. Therefore, evaluation of the IL-8 levels is necessary to correlate them with the diagnosis or prognosis of the desired clinical condition (Guo et al. 2013). For many clinical illnesses, the one biomarker that includes a useful diagnostic and prognostic tool is IL-8. However, wider research is required to confirm the precision and the results of IL-8's worth and efficiency as a biomarker.

6.18.3 IL-8 as a Marker for Nosocomial Infections

The severity and mortality of microbial diseases like nosocomial infections are liable to increase in neonates. Inquiries, reports, and trials for the estimation of infection are not specific (Franz et al. 1999). Thus, there is a requirement to survey some indicators that sense diseases at the initial phases and therefore aid in the instantaneous onset of antibacterial treatment, to prevent a large death percentage. Franz et al. 1999 studied the function of IL-8 as the first indicator for the analysis of nosocomial bacterial diseases. They concluded that grouping of CXCL8 and C-reactive protein (CRP) is viable to determine cultured nosocomial infections with about 96% accuracy (Kotyza 2012). This grouping of markers is cost-effective and reliable for the initial treatment of nosocomial ailments in neonates. Weitkamp et al. 2002 analogize plasma stages of IL-8 with IL-6 to check their capacity as a marker against infections in neonatal intensive care unit (NICU) individuals. It was concluded that IL-8 is an extra beneficial indicator than IL-6, as it takes less time and trial amount.

6.18.4 IL-8 as an Indicator of Acute Pyelonephritis

For the quick recognition of severe pyelonephritis in youngsters with fever, IL-8 and IL-6 levels in blood and urine may be helpful. This looks particularly promising for pediatric infection of the urinary system, which frequently presents with insufficient clinical symptoms and makes an accurate diagnosis of acute pyelonephritis extremely difficult. Through early detection and treatment of acute pyelonephritis, problems like renal scarring that result in renal dysfunction and high blood pressure can be evaded. When pyelonephritis is severe in minors, a helpful connection was found between the ratios of IL-8, IL-6, CRP, fever, and WBCs in the serum and urine. Urine levels showed 83% IL-8 selectivity, and serum levels had 81% accuracy and 78% delicacy for the treatment of acute pyelonephritis (Ramakrishnan and Scheid 2005).

6.18.5 Recognition of Pulmonary Infections

CXCL8 labelled by 99mTC is helpful to spot respiratory diseases in rabbit models. 99mTc-labeled IL-8 is valued for the initial and accurate prediction of the range and respiratory cancer placement. It is advantageous as it is fast and quick to make, with minimal preparation time for infusion and screening. 99mTc-labeled IL-8 poses less emission problems and is linked to a strong description of contamination foci (Gratz et al. 2001).

6.18.6 Recognition of Osteomyelitis

Speedy analysis of osteomyelitis is a silent, difficult challenge. Analytical variations seem to occur only in the progressive phases of the illness on visualizing sequences. Although complex, 99mTc-methylene diphosphonate (MDP) scintigraphy radiography and bone analysis have the lowest particularities. This necessitates the use of analysis with representations. Stephen et al. (2022) calculated the contribution of 99mTc-labeled IL-8 to the medical scenario of osteomyelitis. Based on their research findings, 99mTc-labeled IL-8 has an adequate screening quality and a low irradiation dose for estimating osteomyelitis. Additional therapy options include the recently developed, highly sensitive, and specific anti-granulocyte monoclonal antibody disintegrate characterized by Tc-99 for treating osteomyelitis (Panteli et al. 2014).

6.19 IL-8-Targeting Immunotherapy

Targeting IL-8/IL-8 signaling is considered to be highly clinically significant in the treatment of tumors in cancer. The two receptors of CXCL8 are excellent beneficial targets as small inhibitor compounds block this pathway (Mehraj et al. 2021b). It has been shown that the noncompetitive inhibitor reparixin of the IL-8 receptors CXCR1/2 can prevent any cellular response by inhibiting the activation of the intracellular signal transduction cascade and blocking CXCR1 and CXCR2 in an inactive state. This inhibitor molecule prevents ligand–receptor interaction (Gonzalez-Aparicio and Alfaro 2020). Additionally, reparixin exhibits a decrease in metastasis when combined with chemotherapy, a reduction in cancer stem cells in triple-negative BC, and a clear tumor-crushing impact. These clinical trials provide an insight into the capability for IL-8 suppression to be used in the experimental setup as the only factor or in combination with chemotherapeutic mediators, as it was done in the I-phase reparixin and paclitaxel trial for the diagnosis of triple-negative breast cancer metastasis and in the II-phase pancreatic islet transplantation. A range of small inhibitor molecules that block CXCR2 signaling in addition to reparixin have been confirmed in pre-medical settings for a range of pathological conditions. IL-8 has mitogenic and angiogenesis-promoting properties. Antibodies against IL-8 have also been created, and two of them, HuMax-IL-8 (formerly known as HuMab10F8) and ABX-IL-8, have undergone extensive research (Qayoom et al. 2022). According to preclinical tests, ABX-IL-8 increased tumor cell apoptosis and lowered angiogenesis and invasion in melanoma tumors, which counteract IL-8. HuMax-IL-8 has also been demonstrated to improve anti-inflammatory responses in people with palmoplantar pustulosis and to reduce neutrophil activation and migration generated by IL-8 in vitro. The tumor-encouraging signals for vascularization, activating neutrophil and MDSC penetration, and EMT, including cancer development and malignancy, may be diminished by limiting IL-8 activity (Citro et al. 2012).

6.20 Future Directions

According to The Cancer Genome Atlas (TCGA) database, the expression of CXCL8 is noticeably greater in many malignancies, and this higher expression is linked to shorter median survival in various cancer types. Numerous pieces of evidence using preclinical models have revealed, via their findings, that a combinational targeting of CXCL8 and CXCR1/2 can exhibit antitumor activity. Investigations also show that CXCL8 is crucial for Immune checkpoint inhibitions (ICIs) response quality. Additionally, the CXCL8–CXCR1/2 axis and its downstream signaling cascade play crucial roles in the existence and foray of cancer. This inhibits antitumor immune reactions in the TME. Additionally, treatments that target this axis are expected to help cancer-suffering individuals by reducing the growth of cancer cells and by modifying or enhancing the immune system's response to the tumor. Medical trials are directed to demonstrate the phenomena; however, it is unclear how it works. The association between CXCL8 and tumor metabolism reprogramming is unclear, and further research is required to examine how CXCL8 affects cancer cell proliferation and pathogenesis, since metabolic reprogramming has now developed as a hallmark of tumors. Additionally, numerous studies have shown that some colorectal cancer cells (CRCs) may release CXCL8, which aids in the EMT of the enduring cells by preventing them from doing so. It is necessary to conduct additional experimental research to determine whether other cancer types manifest comparable phenomena. Just as stated, in the TME, CXCL8 can collect TANs. Unfortunately, no one is actively looking into the connection between CXCL8 and the N2 phenotype. In the meantime, CXCL8 also contributes to the transformation of TAMs into the M2 phenotype. The process, however, is unknown.

6.21 Conclusions

It is well recognized that the chemokine CXCL8 is essential for tumor development, attack, and TME angiogenesis as well as for immune destruction through many intracellular signaling pathways. Numerous mechanisms of CXCL8 now exist in the TME, which mediate the tumor biology discussed above and have been emphasized. Some researchers are indicating that CXCL8 should be regarded as a protumoral feature with double functions, including directly encouraging tumor existence and destroying TME mechanisms to secondarily assist in tumor advancement. Additionally, CXCL8 can serve as a serious indicator of the effectiveness of ICIs in clinical settings. Experimental trials are being conducted to examine the worth and security of combining anti-CXCL8 and ICI treatment as cancer therapy progresses.

Further Reading

For further reading, some textbooks mentioned below also provide further understanding of the cytokine and chemokine network.

- *Cytokines and Their Therapeutic Potential* by Manzoor Ahmad Mir

The below links for video lectures may also be helpful:
https://youtu.be/TAqO0Mq19JQ
https://youtu.be/6wEnYfvoBqk
https://youtu.be/A9fRRCUlMms
https://youtu.be/zIWvmCsKr34

For more insights into the topic, we would suggest detailed findings from the books of Mir (2022) https://doi.org/10.1016/C2021-0-02565-7 and https://doi.org/10.1016/C2022-0-00074-X, Mir (2021) https://doi.org/10.52305/WXJL6770, Mir (2015) https://doi.org/10.1016/C2014-0-02898-5, and from the cancer.net website on the following mentioned links:

https://www.cancer.net/cancer-types/breast-cancer/types-treatment
https://discovery.ucl.ac.uk/id/eprint/1472740/
https://www.jmedsciences.com/doi/JMEDS/pdf/10.5005/jp-journals-10045-00138

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CXCL12–CXCR4 Axis in Cancer Metastasis

7

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Abstract

The malignancy that leads to the most fatalities is characterized by metastasis. Due to continually developing research in the biology of cancer as well as the introduction of novel theories in the research of metastasis, several molecular foundations implicated in the metastatic process have been uncovered. However, the understanding of the process (metastasis) is still very poor. The understanding and naming of some of the fundamental rules guiding the movement of the metastatic cell and flexibility have greatly advanced with the recognition and identification of the interactions that the invading tumor cell has with other cells and proteins on its approach to the target site. The identification of the molecular interactions of metastasis enhanced our understanding of the biological underpinnings of metastatic cells governing their movement and plasticity. The invading cancer cells communicate with their tumor microenvironment to subdue stromal challenges, settle, and colonize influenced by genetic and epigenetic changes in the tumor cell's microenvironment. The underlying biological processes of the metastasis must be established to identify therapeutic windows for effective interventions. Chemokines are crucial regulators of cell adhesion and trafficking because they are tiny proinflammatory chemoattractant cytokines that

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bind to certain G-protein-coupled seven-span transmembrane receptors. Important CXCL12, also known as stromal-derived factor-1, controls both normal and abnormal cell movement by primarily attaching to its specific receptor CXCR4. The control of metastasis is one of the most fascinating and possibly crucial functions performed by chemokines and chemokine receptors.

Keywords

Metastasis · Tumor microenvironment · Cytokines · Molecular interactions

7.1 Introduction

Cancer is still the world's number one cause of death. Cancers are widely divided into two categories, metastatic and nonmetastatic. Metastatic cancers are the principle cause of cancer-related deaths. As opposed to what was previously thought, mounting evidence suggests that metastasis can also occur when a tumor is just starting to grow (Hosseini et al. 2016). While metastatic development is underway, cancerous cells that are escaping from primary tumors acquire biological qualities that enable them to go as well as populate remote tissues (Fig. 7.1) (Gonzalez et al. 2018). An intricate ecosystem of primary and metastatic tumors is composed of extracellular matrix (EM), malignant cells, as well as "accessory" noncancerous cells such as localized endothelial cells, invading inflammatory immune cells and mesenchymal support cells. Growth of tumors is encouraged and maintained through interactions between accessory cells and cancer cells. A highly specialized microenvironment

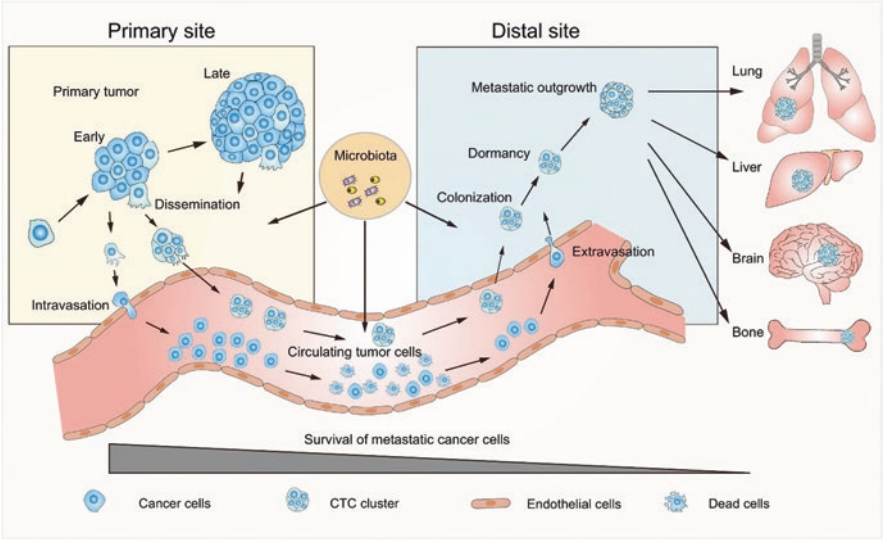

Fig. 7.1 Cancer cells showing metastasis

that is characterized by a weakened EM and persistent inflammation develops as the tumor progresses in the tissue organization (Mir et al. 2022c; Qayoom et al. 2023).

Cancer-related inflammation plays a crucial role in carcinogenesis at various stages, where it influences things like epigenetic changes, genomic instability, amplified cancer antiapoptotic pathways, cancer cell proliferation, and eventually cancer dissemination and angiogenesis (Sofi et al. 2022). Numerous investigations have shown that inflammatory cells actively contribute to the inflammation associated with cancer. To better understand how immunological cells, affect cancer fate at various illness stages, such as initial tumor development, diagnostically observed tumors, therapeutic intervention, and metastatic dissemination, one of the main objectives is to identify the immune cells' role in these processes (Mir 2015b).

The interaction of various cells of immune in the tumor microenvironment as well as their way of interaction affects how a following immunological response turns out is the main determinant in the metastatic process (Mehraj et al. 2021a). Emerging data suggest that immune cells linked to cancer work together to both prevent and promote the progression of tumors. As a result, natural killer (NK) cells play a crucial role in the elimination of tumors. The effector immune response as a whole is improved by the release of XCL1 and CCL5 by natural killer cells, which would additionally encourage the cDCs' recruitment to the TME. As a result, more new antitumor T cell repertoires are primed and activated (Böttcher et al. 2018; Mir and Mir 2022; Moretta et al. 2005). Moreover, effector T cells, antitumor macrophages, and NK cells, all work together to promote CTL differentiation, and NK cell cytotoxicity via secreting IFN and TNF in the tumor site (Showalter et al. 2017; Finisguerra et al. 2015). The N-glycan structures on tumor cells are recognized by macrophages and DCs, which activates the IRF5 pathway and increases the potency of NK cells in NK cell killing (Chiba et al. 2014). Additionally, by luring NK cells to the site of the metastatic disease, CX3CR-1+ patrolling monocytes block the progression of metastatic disease, and IFN produced by these NK cells transforms macrophages into tumor-fighting effector macrophages (O'Sullivan et al. 2012).

The TAMs and tumor-associated Tregs are crucial players in regulating the process, as a result, once the tumors have evaded initial tumoricidal immunity, these inhibit the action of adaptive as well as innate effector immune cells at multiple levels as well as by numerous means. By secreting immune-suppressive chemicals like TGF-β, and IL-10, apart from prostaglandins, for instance, TAMs and Tregs help to increase an immune-tolerant TME (Mehraj et al. 2021b). These reduce the release of IL12 from DCs, which inhibits the genesis of a Th-1 reaction and prevents the exclusion of effector natural killer and T cells (Speiser et al. 2016; Ruffell et al. 2014; Mantovani et al. 2017; Frydrychowicz et al. 2017). Currently, established big metastatic tumors were successfully eliminated in many cancer models by anti-PD-1, recombinant interleukin-2 with a longer half-life, tumor antigen-targeting antibody, and T-cell immunization were used in combination with immunotherapy. Subsequent research showed that the effectiveness required both adaptive and innate immune cells to responds in concert (Mir et al. 2022b; Moynihan et al. 2016).

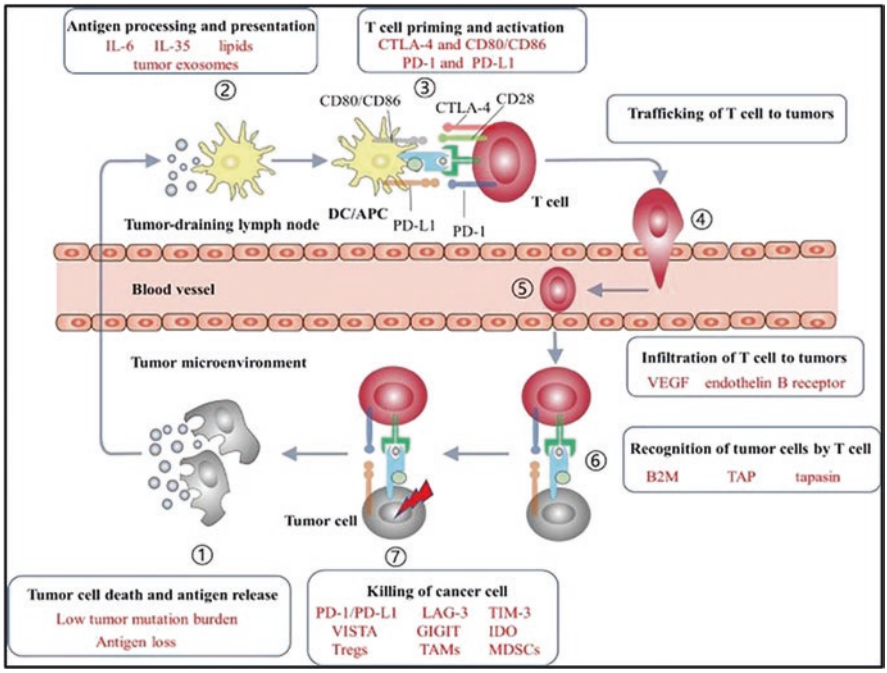

Fig. 7.2 Mechanism of immune escape shown by cancerous cells

7.2 How Tumor Cells Circumvent the Immune System

The available data suggests that there are two methods adopted by cancer cells to escape immune attack as discussed below (Fig. 7.2).

7.2.1 Avoiding Immune Recognition

Cancer cells evade immune detection by ceasing to express tumor antigens on their surface, preventing cytotoxic T cells from recognizing them. Totally, 40% of nons-mall cell lung tumors had been observed to have lost heterozygosity in their HLAs, which results in decreased antigen production and immune evasion. Particularly, HAL loss is connected with poor behavior to a screening inhibition treatment among patients experiencing lungs and carcinoma in addition to susceptibility to T-cell transplant therapy in advanced colorectal cancer (McGranahan et al. 2017; Chowell et al. 2018; Tran et al. 2016). As a result, mutation and deletions may be linked to a mechanism that reduces the production of the antigen, giving T-cell effector chemicals such as TNF-α and IFN-β resistance. In addition, throughout a test metastasis, lung and breast cancer cells reduce the transcription of cell surface NK activators to evade detection by NK cells (Aisha et al. 2021; Malladi et al. 2016; Patel et al. 2017).

7.2.2 Instigating an Immunosuppressive Tumor Microenvironment

Cancer cell-derived substances can activate the immune-tolerant tumor microenvironment as a further method by which cancer cells elude immunological attack. Cancer cells can carry out this strategy in three different ways: by secreting inhibitory elements such as V domain immunoglobulin reducing activation of T-cell and CTLA-4; by expressing inhibitory checkpoint chemicals including TGF-, prostaglandin E2, IL-10, and VEGF (VISTA). CSF1, CCL2, CCL22, CCL5, CXCL8, CXCL12, and CXCL5 are other tumor-derived chemokines that promote the acquisition of Treg, MDSC, and TAM cells (Domínguez-Soto et al. 2011). Together, these strategies produce a complex and effective immune evasion strategy. Therefore, multifunctional drugs that target a variety of the autoimmune system's tolerance to malignancy may enhance the efficacy of continuing immunotherapies (Sofi et al. 2021). Due to this, two recent studies have demonstrated that blocking TGF- promotes tumorigenesis in EMT6 metastatic breast cancer models and the total removal of lymphovascular invasion from a colon cancer model (Mir 2015a; Tauriello et al. 2018; Mariathasan et al. 2018).

The occurrence of other tumors in a bodily region remote from the main malignancy is referred to as "metastasis". Although metastasis is the primary contributor to the failure of cancer therapies and cancer-related mortality, it is still not well understood. Even though cancer patients release a lot of cancer cells into their circulation every day, melanoma research in mice indicates that just 0.1% of tumor cells disseminate (Luzzi et al. 1998). To produce metastases, cancer cells need to depart from their default setting, circulate throughout the body, tolerate intravascular pressure, learn to cope with new biological circumstances inside a remote spot, escape the deadly battle with immune cells, and many other factors (Maitra 2019; Massagué and Obenauf 2016). The encroachment of adjacent tissues as well as the implantation of far-off places to produce metastases remains a crucial element of cancer malignancy. Additionally, metastasis is usually always the leading factor of death in 90% of cancer patients (Steeg 2006). The selection of molecular therapeutic targets that may slow or even stop the spread of cancer will be simpler if the kinetics of this process are understood (Mir et al. 2022d).

7.3 Cancer Proliferation and the CXCR4/CXCL12/CXCR7 Chemokine Axis

Chemokines are crucial regulators of cell adhesion and trafficking because they are tiny proinflammatory chemotactic mediators which attach to certain G-protein-coupled transmembrane domain receptors (GPCRs). CXCL12, also known as stromal-derived factor-1 (SDF-1), binds predominantly to its cognate receptor CXCR4 and controls the movement of both healthy and cancerous cells. Before the discovery of CXCR4, it was believed that CXCL12 could have one receptor only. However, new research has shown that CXCL12 also interacts with CXCR7, another

seven-transmembrane receptor. In addition to their valuable contributions as predictors of tumor behavior and potential therapeutic targets, CXCR4 and CXCR7 are critical in mediating tumor spread in several malignancies. The axis of CXCR4/CXCL12/CXCR7 controls how tumors grow and how they spread metastatically to tissues that produce high CXCL12 concentrations. This leads to the development of secondary cancers (Fig. 7.3) (Sun et al. 2010).

A subclass of cytokine-like proteins known as chemokines connect to and activate several chemokine receptors in the body. There are four families of chemokines (CX3C, CC, C, and CXC) based on four conserved cysteine residue locations, which have been discovered to be present in more than 50 chemokines (Vindrieux et al. 2009). Chemokine transmitters have seven-transmembrane transmitters connected to G-proteins; each having an N-terminus that is external to the cellular membranes in the cytoplasm, and they all have a C-terminus. Inflammatory mediator receptors have interior loops, one of which connects with heterotrimeric G-proteins to mediate the binding of ligands to the receptor, which results in a sequence of signal transduction processes (Ransohoff 2009). Because each chemokine receptor can bind to multiple chemokine ligands with high affinity, the majority of them are promiscuous (CXCR, CCR, XCR, and CX3CR). The chemokine family has a high amount of redundancy as a result of many chemokines binding to the same receptor (Bièche et al. 2007). This quality could be essential for adjusting specific reactions. Contrary to CXC receptors, CC receptors are often more promiscuous (New and Wong 2003). While some inflammatory cytokines exclusively interface with such a particular ligand as well as some receptors only bind one chemokine, other chemokines link to many receptors, some of which may then bind a large number of chemokines.

The control of metastasis is one of the chemokines and chemokine receptors' most intriguing and possibly crucial functions. During all of the crucial phases of

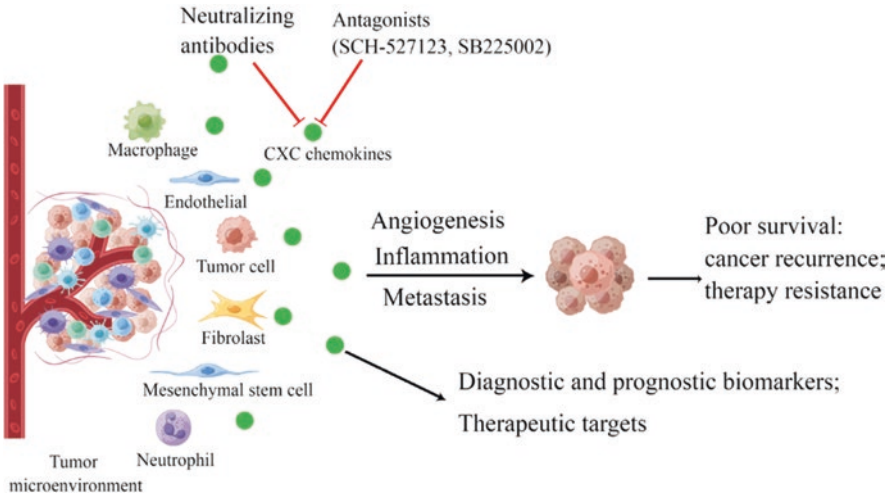

Fig. 7.3 Cancer proliferation and CXC chemokine axis

metastasis, which include tumor cell binding to the endothelium, leakage from blood vessels, metastases colonization, angiogenic, multiplication, and safety from the response of the host, and stimulation of critical survival pathways, like ERK/MAPK, may potentially promote tumor dissemination (Lazennec and Richmond 2010; Keeley et al. 2010; Kruizinga et al. 2009). More focus is being placed on the function of proinflammatory cytokines in boosting neutrophil infiltration and initiation of cancer macrophages (TAMs), and also in facilitating interaction with both cancerous cells and non-neoplastic cells in the tumor microenvironment (TME), like fibroblasts (Mehraj et al. 2021c; Allinen et al. 2004; Hartmann et al. 2004).

The emergence and spread of cancer depend on chemokines, a class of small, secreted cytokines that regulates several biological functions, especially cell motility. They are most recognized by their powers to draw immune cells. Chemotaxis, cell proliferation, migration, and gene expression are only a few of the processes that are significantly impacted by contact of CXCL12 with its receptors. The CXCR4/CXCL12/CXCR7 axis appears to be essential for tumor survival, angiogenesis, metastasis, and the tumor microenvironment, according to mounting evidence. Furthermore, through complex interactions with other pathways, this chemokine axis encourages chemoresistance in cancer therapy (Shi et al. 2020).

Chemokines can be broken down into four subfamilies because of where the two cysteine residues are situated at the amino terminus (CC, CXC, CX3C, as well as C). For chemokines to execute their predetermined activity, G protein-coupled cell surface receptors are necessary. Chemokine receptors are divided into two subfamilies, atypical chemokine receptors (ACKRs) and conventional chemokine receptors (CCKRs), based on the name of their endogenous ligand (chemokines). Chemokines binding to CCKRs would alter the receptor's conformation, resulting in the transmission of intracellular signals. Despite the presumption that chemokine scavengers are signal transduction pathways, ACKRs are not associated with any of them.

The homeostatic CXC chemokine stromal cell-derived factor-1 (SDF-1), also known as CXCL12, exists in seven distinct isoforms. It regulates the organization of secondary lymphoid tissues as well as the movement of hematopoietic cells. It is released by fibroblasts, epithelial cells, and stromal cells in a variety of organs. Increasing data suggest that relationships between tumor stromal cells control the initiation and development of malignancies. Receptors of CXCR4, CXCR7,and CXCL12 are found to be the main drivers in tumor growth. Epithelial ovarian carcinoma expresses CXCL12 at elevated levels. CXCL12 activates the ERK and AKT signaling pathways, which aids in the growth of ovarian cancer cells in culture (Barbieri et al. 2010). Additionally, there is a connection between the expression of CXCL12 and both pathogenic traits and clinical outcomes in human breast cancer.

7.3.1 CXCL12, CXCR4, and CXCR7

SDF-1, or stromal-derived factor, is the name of a CXC chemokine. It was first cloned from a stromal cell line derived from bone marrow, and it was found to function as a factor that stimulates pre-B-cell proliferation (PBSF). Hematopoietic cells

of both immature and adult stages are strongly attracted to CXCL12, which has a widespread expression across different tissue types. Additionally to controlling the survivability and expansion of progenitor cells in both humans as well as in mice, CXCL12 has been linked to the homing of hematopoietic stem cells to the bone marrow (Gillette et al. 2009; Hayakawa et al. 2009; Kyriakou et al. 2008). The two primary isoforms of CXCL12 and are both made through the same chromosome; however, alternative splicing causes CXCL12 to differ at the C-terminal end by four additional amino acids (RLKM) (Tashiro et al. 1993; Dettin et al. 2004). Marrow stromal cells and endothelial cells both emit the most prevalent variant of CXCL12, which are seen in practically all organs. The formation of the isoform increases tissue damage while accelerating blood-borne proteolysis. The isoform, in contrast, is found in the spleen, kidney, and liver and promotes angiogenesis while being more resistant to blood-dependent degradation (Janowski 2009). Production of CXCL12 within or around injured tissues promotes the homing of flowing vascular endothelium tissue-committed stem cells (TCSCs) and the injured tissue, which may lead to repair and regeneration of the tissue (Kucia et al. 2006). There are now four more chemotactic CXCL12 isoforms in humans, all of which have been found. CXCL12 (delta), CXCL12 (gamma), CXCL12 (phi), and CXCL12 (epsilon) are all produced via alternative splicing; however, they all share the same first three exons. The heart and brain, two highly active, poorly vascularized organs that are susceptible to infarction, also contain CXCL12 gamma. It is anticipated that CXCL12 delta will last more than 50% longer than CXCL12 alpha (Yu et al. 2006).

The chemoattractant activity of CXCL12 is greatly decreased when CD34+ cells are pretreated with neutralizing anti-CXCR4 antibodies or neutralizing CXCR4 peptides, demonstrating that CXCL12 and CXCR4 functionally interact with CD34+ cells in the bone marrow. Specifically, the osteoblasts lining the endosteum of bones release CXCL12 (Neiva et al. 2005; Jung et al. 2006; Taichman et al. 2002). SCID mice's repopulating cells were more favorably encouraged to migrate by CXCL12. Human CD34+ cells can regularly restore multilineage hematopoiesis to the marrow of mice (Peled et al. 1999; Ponomaryov et al. 2000). Noteworthy to mention here that CXCL12 expression is increased in both mouse marrow and cultured cells when DNA-damaging chemicals like radiation, cyclophosphamide, or 5-fluorouracil are used (Petit et al. 2002). The promoter of CXCL12 contains two HIF-1-binding sites, and endothelial cells that express HIF-1 produce more CXCL12 than nonexpressed endothelial cells. Increased HIF-1 expression causes CXCL12 levels to rise in hypoxic or damaged tissues, chemoattracting CXCR4+ tissue-committed stem cells (TCSCs) to participate in tissue regeneration (Ceradini et al. 2004).

7.4 C-X-C Motif Chemokine 12 (CXCL12) Receptors

7.4.1 Chemokine Receptor CXCR4

The ligand CXCL12 is bound by the highly conserved transmembrane GPCR CXCR4. CXCR4 has been the subject of extensive study co-receptor for T-tropic (X4) HIV viruses, and it performs this function that attack CD4+ T cells. In response to the participation of CXCL12 in several physiological occurrences, hematopoietic and latent leukocytes progenitors can move through the CXCR4 receptor (Caruz et al. 1998; Gupta and Pillarisetti 1999; Wegner et al. 1998; Zou et al. 1998; Feil and Augustin 1998; Lazarini et al. 2000). CXCR4 is expressed throughout the body's organs as it develops, including the immune and neurological systems. Immature T cells, monocytes, and CXCR4 cells in bone marrow each have high immune system expression levels (Aiuti et al. 1999). The regulation of stem cell trafficking and the preservation of hematopoietic progenitor cells in the bone marrow may be influenced by differences in CXCR4 expression in CD34+ progenitor cells. According to studies, CXCR4/ CXCL12 axis is necessary for growth, and its absence results in problems in the various physiological systems including the immune, hematological, and circulatory systems (Aiuti et al. 1997; Doitsidou et al. 2002; Nagasawa et al. 1996).

G protein's Gi component joins the receptor as CXCL12 binds to CXCR4, forming a complex that blocks the cyclic adenosine monophosphate formation (cAMP) by adenylyl cyclase as well as the mobilization of intracellular calcium. Only a handful of the numerous downstream destinations are activated when the Gi subunit is dissociated from G including MAPK, ERK1/2, AKT, and JNK effectors. The accompanying processes of ligand-stimulated chemotaxis include cytoskeletal reorganizations, polarization, actin polymerization, as well as the forming pseudopodia and other biological substrates (Lee et al. 2007; Lu et al. 2009; Princen et al. 2003; Roland et al. 2003).

Breast cancer, ovarian cancer, prostate cancer, as well as melanoma are among the more than 23 types of cancer that affect people have been shown to overexpress CXCR4, which is among the prevalent receptors of chemokines (Balkwill 2004a, b). CXCR4 is reflected in several tissues; however, many normal tissues, including the ovary and breast, either seldom or never exhibit its presence (Scotton et al. 2001). Through several mechanisms, CXCR4 is expressed more frequently in malignant cells. It has been shown that HIF-1 stimulates CXCR4 expression, which is known to be induced by VEGF, by acting upstream of VEGF. The heterodimeric transcription factor HIF-1 demonstrates a tendency of increasing CXCR4 expression in response to tissue oxygen levels. Therefore, to aid in survival and escape from the initial tumor mass, chemokine receptor levels may be increased in hypoxic regions of developing tumors. In gliomas, it has been shown that HIF-1 stimulates CXCR4, increasing the likelihood of proliferation, making cells resistant to apoptosis, enhancing local invasion, and promoting distant metastasis (Zagzag et al. 2006).

7.5 Chemokine Receptor CXCR7/RDC-1

When it was shown that mouse fetal liver cells from CXCR4 mutant mice can still bind CXCL12, the idea that CXCL12 and CXCR4 interact alone was called into doubt. Additional differences in CXCR4 expression and CXCL12 binding affinity were seen in several human cancer cell types (Burns et al. 2006; Chatterjee et al. 2014). These results suggest the presence of more CXCL12-binding receptors. CXCR7 (also known as RDC-1) is the name of this receptor, which was just recently identified. It was first cloned due to similarities with important GPCR domains (Libert et al. 1990; Balabanian et al. 2005). The genes encoding CXCR2, CXCR4, and CXCR1 are found in human chromosome 2 and mouse chromosome 1, respectively, where the CXCR7/RDC-1 gene is situated. The viral gene ORF74 that codes for a chemokine receptor shares a similarity with CXCR7/RDC-1, which allows it to signal constitutively even in the absence of a ligand. CXCR7 also binds to the chemokine CXCL11 with a low affinity, in addition to CXCL12 (I-TAC). It has been observed that T cells express CXCR7 during chemotaxis mediated by CXCL12. A crucial regulator of B cell growth and differentiation is CXCR7 expression (Jones et al. 2006; Raggo et al. 2005). Memory B cells, which can develop into cells that release antibodies, may be identified by the protein CXCR7. CXCR7 expression is connected with B cells' capacity to develop into plasma cells after activation. Endothelial cells linked to tumors have been found to express CXCR7 more highly. Numerous tumor cell lines, apart from fetal liver cells, vascular endothelium, and placenta express membrane-associated CXCR7. These characteristics show that CXCR7, like CXCR4, is involved when it comes to stem cell control trafficking, angiogenesis, immunology, as well as cancer metastases specific to certain organs (Martínez et al. 2000; Tripathi et al. 2009; Miao et al. 2007; Wang et al. 2008a, b).

But according to new research, CXCR7 is a fake receptor that does not trigger the Gi pathways that cause calcium mobilization or GTP hydrolysis in chemokine receptors. But in other situations, CXCR7 enhances cellular adhesion qualities and significantly increases cell proliferation (Begley et al. 2007). As a result, the issue of whether CXCR7 acts as a GPCR-like mediator of the signal transduction pathway has been raised. As a result, numerous hypotheses regarding CXCR7's mechanism of action have been made. One possible role for CXCR7 is to collect trash or isolate CXCL12, resulting in variations of CXCL12 which alter CXCR4 signaling (Rajagopal et al. 2010; Boldajipour et al. 2008).

Because CXCR4 as well as the receptor when overexpressed in cells form heterodimers that have undergone temporary transfection, the receptor may also act as a co-receptor for CXCR4 and boost CXCL12-mediated G protein signaling. These results show that intracellular signaling molecules drive the ligand-induced crosstalk between CXCR7 and CXCR4 (Mir et al. 2022a; Levoye et al. 2009; Sierro et al. 2007; Hartmann et al. 2008). Recent research has revealed a ligand-dependent interaction between beta-arrestin and CXCR7. CXCR7 presents the possibility that because it is an endogenous beta-arrestin-biased receptor that can signal via beta-arrestin, additional receptors that are now considered to be orphans or decoys may possibly communicate via non-G protein-mediated routes (Kalatskaya et al. 2009).

7.6 Transduction of the C-X-C Motif Chemokine Ligand 12 (CXCL12) Axis

Gene transcription, proliferation, chemotaxis, and cell survival are all regulated by CXCR4/ CXCL12/CXCR7, among other downstream signaling pathways. Based on the type of cell, the precise transduction may vary since various features may vary depending on the tissue. The separation of the Gi component from the G/G dimer is brought about by a three-dimensional conformation shift brought about by CXCL12's association with CXCR4, which starts the conversion of GTP to GDP. Phospholipase C (PLC), which catalyzes the conversion of phosphatidylinositol (4,5)-bisphosphate (PIP2) into the secondary messenger inositol (1,4,5)-trisphosphate (IP3), and diacylglycerol, can be activated by fragmented G/G dimers (DAG). Calcium from intracellular reserves is released into the cytoplasm when it interacts with the IP3 receptor (IP3R).

Mitogen-activated protein kinase and protein kinase C (PKC) are both activated by DAG to enhance chemotaxis (MAPK). Cell cycle progression and gene expression are similarly impacted by Ras-induced ERK/MAPK pathway activation, which also involves the G/G dimer. Based on linked G subunits, four kinds of GPCR signaling pathways may be identified: Gαs, Gαi, Gαq, and Gα12. Initial classification of CXCR4 as a G-protein-coupled receptor. Adenyl cyclase is blocked by the activation of Gαi subunits, which regulates the activity of other downstream effectors and converts 5′-adenosine triphosphate into cyclic adenosine monophosphate via catalysis (cAMP) (Qayoom et al. 2021).

Numerous focal adhesion proteins are phosphorylated as a result of the G/G dimer or the Gi subunit activating phosphoinositide-3 kinase (PI3K), which promotes cell motility. AKT can be activated by PI3K by producing phosphatidylinositol (3,4,5)-triphosphate, and mTOR pathways are crucial for the persistence and growth of cancer cells. In other instances, Gαi is further needed for Rac activation. The G-proteins Gαq and Gα12/13 are two other G-proteins that CXCR4 can use to convey signals. PLC- may couple to the following processes when CXCR4 and Gαq are coupled, boosting the synthesis of IP3 and PKC signaling.

CXCR4 stimulation of Gα12/13 not only inhibits Fas-mediated apoptosis but also fosters Bruton's tyrosine kinase. As a direct Rho activator by altering Rho-guanine nucleotide exchange factors, Gα12/13 is also essential. CXCR4 signaling is influenced by receptor internalization and lysosomal degradation. Immediately after CXCL12 binding, G-protein-coupled receptor kinases (GRKs) phosphorylate the intracellular C-terminus of CXCR4 at serine sites, which causes – arrestin to be recruited and clathrin-mediated endocytosis to occur. Because CXCR4 is unable to interact with the G proteins, β-arrestin selects them for lysosomal degradation.

7.6.1 Cancer-Related CXCL12 and the CXCR7/CXCR4/Axis

The tumor microenvironment is increasingly being shown to be necessary for growth, angiogenesis, metastasis, as well as cancer progression. It is now more

widely understood that proinflammatory cytokines, as well as their receptors, play a critical involvement in creating a microenvironment by promoting communication between stromal cells and tumor cells that is favorable to cancer development along with metastasis, even though they were initially recognized as chronic inflammatory mediators. In many cancer types, the cytokine CXCL12 and its receptor CXCR4 are overexpressed. This abnormal expression greatly encourages proliferation, migration, and invasion through a variety of signaling pathways. The connections among the tumor and stromal cells determine the development and spread of malignancies. CXCL12 and receptors, CXCR4 and CXCR7, are essential components in cancer cells' interaction with their surroundings (Barbieri et al. 2010).

7.6.1.1 Breast Cancer

By showing that breast tumors express high amounts of CXCR4, whereas normal breast tissues do not, CXCR4 and CXCL12 are important factors in controlling metastasis. CXCR4 signaling in response to CXCL12, which also triggers chemotactic and invasive responses, mediates actin polymerization and the formation of pseudopodia. These findings corroborated the idea that cancer cells can move toward chemokine ligands produced in conventional metastatic locations like the lungs, lymph nodes, and bone marrow by using chemokine channels (Müller et al. 2001; Jan et al 2023). The number of bone metastases in vivo was significantly increased when CXCR4 alone was overexpressed, indicating that it is one of a select small gene count favored in a subset of cells of breast cancer that have spread. Demonstrating that siRNAs that suppressed CXCR4 expression reduced the cell expansion by breast cancer inside the test tube thereby stopping metastases in an animal model offered evidence in favor of the notion. It is intriguing to notice that the receptor desensitization and downregulation process appear largely controlled by the CXCR4 carboxy-terminal domain (CTD). Breast cancer cells have increased motility, proliferation, and a downregulation of cell-to-cell contact when the C-terminal domain of CXCR4 is eliminated (Kang et al. 2003; Liang et al. 2005; Ueda et al. 2006).

There have been several significant observations made as a result of the understanding of the CXCR4-centered underlying reasons for breast cancer metastasis and invasion. According to ligand-binding experiments, the quantity and affinities of CXCR4 receptors are equivalent in extremely malignant cells and nonmetastatic cells. When CXCL12 attaches with GDP/ Gαβγ protein complex in metastatic cells, a GTP-for-GDP exchange takes place, activating IκBα, ERK1/2, Akt, JNK, GSK-3αβ, and p38 MAPK. This allows Gi to dissociate from the G subunit. In nonmetastatic cells, CXCR4 may construct a complex on its own with Gαi or Gβγ subunits, but since no Gαβγ heterotrimer could be linked to CXCR4, Gβγ-dependent downstream signaling was not activated. These findings have significance for clinical research that examines the expression of the CXCR4 protein but not receptor activity. Even though the underlying biological mechanism is still not fully understood, there are differences in G protein signaling between metastatic and nonmetastatic cells (Holland et al. 2006; Fulton 2009). The CXCR4 protein is not always a marker of CXCR4-mediated signaling, as was shown in cell lines of breast cancer.

The connection of CXCR4 including several growth factor tyrosine kinase receptors is supported by reliable evidence. It has been shown that IGF-1 exclusively activates metastatic MDA-MB-231 cells rather than nonmetastatic MCF-7 cells. Despite the presence of IGF-1R and CXCR4 in both cell lines, CXCR4 is only expressed when IGF-1R is activated. CXCL12, which is secreted by myofibroblasts connected to breast cancer but not by those in healthy breast tissue, promotes angiogenesis, the development and sustenance of cancerous cells, as well as tumor progression. Breast cancer risk is increased by specific CXCL12 alleles, and CXCL12 has been demonstrated to transactivate Her2/neu, a known breast cancer oncogene (Akekawatchai et al. 2005; Orimo et al. 2005; Razmkhah et al. 2005; Cabioglu et al. 2005). Initial evidence for CXCR7's involvement in cell growth/survival came from the observation that CXCR7-transfected MDA-MB-435s cells proliferated faster. It is experimentally shown that cells expressing CXCR7 have a survival advantage in tissue culture conditions that are not optimal for cell proliferation. In an identical study, it was discovered that CXCR7 transfectant cells considerably outperformed wild-type cells when it came to adhesion to activated human umbilical vein endothelial cells (HUVECs), and they even showed minor adherence to unstimulated HUVECs. Further evidence indicates that TNF-α- and IL-1β-induced in vitro stimulation upregulates CXCR7 expression in the HUVECs. Increased CXCR7 mRNA expression was also seen following HUVEC activation (Burns et al. 2006; Miao et al. 2007).

The use of endothelial cells from the heart, the lung, and several other tissues led to comparable outcomes. The expression of CXCR7 dramatically speeds up the growth of breast tumors originating from cells in the mouse 4T1 cells animal model. Similar to how wild-type or MDA-MB-435s vector-transfected control cells did MDA-MB-435s CXCR7-transfected cells produced larger tumors. Furthermore, the expression of CXCR7 on breast cancer cells enhances their capacity to proliferate and seed in pulmonary metastases. According to a different study, breast cancer contains high levels of CXCR7, which makes it easier for cancer cells to cross the hemato-encephalic barrier and spread to the brain (Salmaggi et al. 2009; Miao et al. 2007).

7.6.1.2 Prostate Cancer (PCa)

Multiple responses may result from the activation of several signaling pathways by the binding of CXCL12 to CXCR4. If CXCL12 functions as a growth factor, this may be one mechanism by which PCa makes use of the CXCR4 receptor. CXCL12 treatment of PC3 human PCa cells resulted in the phosphorylation of MEK, IKK, and IB. This causes NF-κB to localize to the nucleus and then induces CXCR4 transcription and expression (Fernandis et al. 2002). These results suggest that CXCL12-induced CXCR4 expression in PC3 cells depends on the ERK /MEK signaling cascade and NF-κB activation, both of which are essential for tumor cell survival. The CXCL12/CXCR4 axis is crucial to the development of PCA. According to a study, the preferred locations of metastasis for PCa cells include the bone, liver, and kidney, which have higher levels of CXCL12 than organs that are not often affected including the lung, tongue, and eye. The long bones' metaphysis, which is

closest to the growth plate, was where CXCL12 was found, but not in the middle of the bone marrow. Additionally, PCa cells' adhesion to immobilized fibronectin, collagen, and endothelial cell monolayer, as well as to osteosarcoma cells, was enhanced by CXCL12. The integrins α5 and α3 may be upregulated to cause this (Engl et al. 2006; Sun et al. 2005). These findings demonstrated CXCL12/involvement CXCR4's in the PCa tumor localization to the bone marrow (Sun et al. 2003, 2005; Kukreja et al. 2005). It was observed in a study that basal cell hyperplasia and blood vessels linked with PCa tumors both expressed CXCL12. Submucosal spheroids of PC3 cells overexpressing CXCR4 demonstrated significantly increased artery density, functioning, or tumor pervasiveness into the deeper structures in a murine model. Antibodies against CXCR4 suppressed CXCR4-dependent tumor development and vascularization by blocking the interactions of CXCL12/CXCR4. Additionally, using CXCR4 siRNA, it was shown that CXCL12 induced an angiogenic switch by upregulating VEGF and CXCL8, which in turn induced an increase in CXCL12. It is interesting to note that phosphoglycerate kinase 1, a glycolytic enzyme that negatively influenced the expression of VEGF and CXCL8, was downregulated by CXCL12 (Darash-Yahana et al. 2004; Wang et al. 2005, 2007).

The tumor microenvironment contained cultured prostate cancer-associated fibroblasts (CAFs) that were highly CD90(hi) expressed. These CAFs were tested for tumor-promoting activity. TGF-beta increased CXCR4 expression in BPH-1 epithelial cells and reduced apoptosis in BPH-1 cells resulting in co-culture or processed medium by CD90(hi) cells (Zhao and Peehl 2009). Although practically all types of stem cells of the body express CXCR4, it is unclear if PCa stem cells also express this receptor. One research team found a unique subset of CXCR4+ CD133+ cancer stem cells in the invasive front of pancreatic tumors that controls the metastatic phenotype of the particular tumor. Similarly, it is unknown what function CD133+ CXCR4+ and/or CXCR7+ PCa stem cells provide. Only renal progenitor cells have so far been found to express functional CXCR7 (Hermann et al. 2007; Mazzinghi et al. 2008).

CXCR7 is extensively expressed in human PCa cells, as we recently found. High-density tissue microarrays stained to show that aggressive tumors have higher levels of protein CXCR7 expression. Studies on established PCa cell lines also showed that CXCR7 controlled cell proliferation, most likely as a result of improved cell adhesion and survival. Furthermore, CXCR7 boosted the expression of proangiopoietic elements like VEGF and IL8. It is interesting to note that CXCR4 activation affects CXCR7 levels. Additionally, there is proof that CXCR7 signaling in PCa cell lines causes Akt to become phosphorylated. In a mouse model with impaired immunity, PCa cells overexpressing CXCR7 developed more quickly and had a larger density of neovessels (Wang et al. 2008a, b; Mir et al. 2023a).

7.6.1.3 Lung Cancer

Small-cell lung cancer (SCLC) is an aggressive kind of cancer that spreads quickly and is very likely to affect the bone marrow. High numbers of functional CXCR4 receptors were produced by SCLC cells, which also showed that CXCR4 activation caused migratory and invasive responses as well as adherence to stromal cells in the

marrow in a CXCR4- and integrin-dependent manner. The main mediators for SCLC cells adhering to ECM proteins such as collagen I, VCAM-1, and fibronectin are CXCL12/CXCR4 axis. SCLC cells' enhanced adherence to stromal cells in the marrow is a defense against etoposide's ability to cause apoptosis. The SCLC chemosensitivity was affected by the CXCR4 antagonist T140 and its derivates because they prevent CXCR4-mediated adherence to ECM components (Hartmann et al. 2005; Burger et al. 2003; Burger and Kipps 2006). All these findings show SCLC cell expression of CXCR4 promotes integrin activation, which controls tumor cell adherence to the marrow microenvironment, provides resistance towards the drug, as well as promotes cancer cell development. Furthermore, CXCR4 might be responsible for the specific metastatic pattern seen in SCLC patients. Nonsmall cell lung cancer (NSCLC) neoplastic cells may also express CXCR4. It is interesting to note that CXCR4 expression is differentially linked with metastatic potential both in vitro and in vivo, which raises the possibility that CXCR4 levels may influence how quickly NSCLC spreads from its source locations to its metastatic nodes (Allinen et al. 2004; Su et al. 2005). Hypoxia can significantly boost the CXCR4 expression, as has been observed in other malignancies. In samples taken from numerous individuals with squamous cell carcinomas, CXCR7 immunostaining in lung cancer has been demonstrated; nevertheless, its expression can also occasionally be found in lung adenocarcinomas. CXCR7 has been found on a significant portion of the tumor vasculature from patient malignancies in vascular endothelium. Murine breast Lewis lung cancer (LLC) cell lines include CXCR7 on their surface as well, and RNAi targeting CXCR7 inhibits tumor growth more than control transfected cells do (Kijima et al. 2002; Phillips et al. 2005). Mice treated with the CXCR7 antagonist CCX754 develop tumors that are noticeably smaller than those of animals merely given a vehicle. These findings showed that CXCR7 is a protumor factor in lung cancer. Recent research has demonstrated a correlation between greater CXCR7 expression and pathological stage I NSCLC early and metastatic recurrence. The frequent occurrence of EGFR mutations in patients with greater levels of CXCR7 expression is consistent with our findings (Burns et al. 2006; Iwakiri et al. 2009).

7.6.1.4 Other Cancers

Glial and neuronal tumors, colorectal cancer, neuroblastoma cells, brain, esophageal, renal cell cancer, melanoma, rhabdomyosarcoma (RMS), as well cancer of ovarian tissue, all migrate or survive in response to CXCR4 excitation via CXCL12 (RMS) (Jankowski et al. 2003; Balkwill 2004a, b). In cancer of the ovaries, CXCR4 is a chemokine receptor that predominates. CXCL12 has an immediate effect on ovarian cancer cells, promoting their in vitro proliferation. By using CXCR4 inhibitors or neutralizing antibodies against CXCR4, this proliferative effect of CXCL12 can be prevented. Soft agar growth of glioblastoma cell lines with high levels of CXCR4 expression was improved while neurite outgrowth and cellular differentiation were inhibited by CXCR4 expression in the antisense direction (Bertolini et al. 2002; Zhou et al. 2002). Glioblastoma cell lines treated with either CXCL12 or CXCR4 antibodies experienced growth suppression, proving that the CXCR4 gene

is necessary to stop apoptosis following serum withdrawal. Like this, systemic administration of CXCR4 antagonists decreases tumor cell proliferation and boosts apoptosis to slow the growth of cerebral glioblastoma and medulloblastoma xenografts (Zhou et al. 2002; Mir et al. 2023b).

CXCR4 presence in primordial tumor cells is linked to prognosis, regression, and spreading in individuals with colon cancer and melanoma. Interestingly, less bone metastasis occurs in these cancer patients than in those with prostate or breast cancer, the possibility that CXCR4/ CXCL12 can be the only pathway needed for growth as well as localization of tumors toward bone marrow. Rarely, CXCR4 may even be essential for the growth of micro metastases as opposed to the invasion. It was shown that RMS cells express both CXCR7 and CXCR4, each with different as well as overlapping roles in developing metastatic activity (Libura et al. 2002; Grymula et al. 2010; Zeelenberg et al. 2003; Scala et al. 2005; Geminder et al. 2001; Kim et al. 2006). CXCR4 was much more overexpressed among the more malignant pulmonary cell lines compared to the less aggressive ERMS cell cultures, whereas CXCR7 expression is inverse. Other studies have shown that in ERMS cells, hypoxia increases the promoter activity and receptor expression of CXCR4 and CXCR7. Additionally, in the presence of CXCR4 antagonists, CXCL12- and CXCL11-induced responses in CXCR7 RMS cells (T140, AMD3100). Increased tumor cell seeding efficiency was observed in animals given injections of RMS cells that overexpressed CXCR7, whereas the opposite was true when CXCR7 was downregulated (Matsunaga et al. 2005; Sanz-Rodrıguez et al. 2001; Tarnowski et al. 2010). Through activating the EGFR and AKT signaling pathways, CXCR7 promotes tumor cell survival and proliferation. The concept that CXCR7 contributes to the growth of malignancies is also supported by the overexpression of CXCR7 and gene silencing in tumor cells. However, studies on neuroblastoma (NB) revealed that, both in vitro and in vivo, activating CXCR7 dramatically reduced NB cell growth through the ERK1/2 cascade (Miao et al. 2007). In cases of colon cancer, CXCR7 has also been associated to slowing tumor migration and growth. Such finding indicate CXCR7 may play important part in growth of tumor cells as well as raising possibility that CXCR7 may have cell type-specific roles. To create tailored treatment, its role in diverse cancers should be thoroughly investigated (Shi et al. 2020). CXCR7 signaling also prevents apoptosis. The apoptotic fractions of prostate cancer cells that have increased CXCR7 expression are lower, and they are protected from cell death. Similar to this, CXCR7 knockdown increased the MCF7 breast cancer cell line's production.

7.7 C-X-C Motif Chemokine Ligand 12 (CXCL12) Axis in Tumor Cell Growth, Survival, and Tumor Progression

Cancer development has long been associated with chemokines and their receptors. Since then, it has been discovered that the chemokine CXCL12, and its associated receptors are crucial for chemotherapy-induced tumor development, angiogenesis,

invasion, and drug resistance. Cancer metastasis, angiogenesis, and growth are all influenced by CXCL12, either directly or indirectly through indirect effects such as the cancer cells that are CXCR7/ CXCR4-positive are drawn to organs that are CXCL12-expressing. Significant biological effects of the CXCL12 axis support tumor cell survival and expansion. In glioblastoma cell lines, CXCR4 was discovered to be overexpressed, and it was originally shown in 1998 that the expression of CXCR4 antisense inhibits the growth of glioma cells. Additionally, human glioblastoma cell lines exposed to exogenous CXCL12 are stimulated to proliferate in a dose-dependent manner (Sehgal et al. 1998). It has been found that cell lines from several cancers, like breast, lung, prostate, pancreatic, and multiple myeloma, grow when the CXCL12 axis is activated. The CXCR4/CXCL12 relationship enhances the growth of multiple different tumor cell types by directly activating PI3K, MAPK, and Sonic Hedgehog signaling pathways, phosphorylating CXCR4, and promoting calcium flow (Ueda et al. 2006).

The activated MAPK changes the translation of mRNA, which is crucial for cell division, proliferation, and differentiation, in addition to phosphorylating multiple other cellular proteins (c-Myc and RSK kinases). The transcription factor c-Myc may increase the expression of CXCR4, which subsequently activates MAPK. Proliferative signaling is maintained via a positive feedback loop between CXCR4 expression and MAPK signaling. In response to CXCR4 activation, the EGFR transcription signaling proteins also increases, enhancing cell proliferation. Since Wnt signaling affects the morphologies of pancreatic cancer cells and reduces tumor proliferation by inactivating the canonical Wnt pathway, it is crucial in CXCL12-induced tumor cell proliferation (Mir et al. 2020). The CXCL12 axis also induces the phosphorylation and destabilization of IκB-, which is followed by the upregulation of SHH, which heightens NF-κB signaling and increases the nuclear accumulation of NFκ-B. SHH signaling also induces further CXCL12 synthesis and extracellular release involves turning on the stromal cells' protein patched homolog (Ptch), completing a constructive proproliferative feedback loop.

The CXCL12 axis also subtly prevents tumor cell death. The CXCL12/CXCR4 axis, as was already mentioned, stimulates ERK and AKT, it causes an accumulating amount of NF-κB, which can block apoptotic signals. Bcl2-associated activator of cell motility, or BAD, is a proapoptotic protein that is suppressed by CXCL12 through the activation of the MAPK-ERK and PI3K pathways (Katoh et al. 2010). ERK phosphorylating Bad on serine 112 may be the source of this. As a result, Bad is dissociated from Bcl-2, enabling Bcl-2 to show its antiapoptotic characteristics. Similar to this, the CXCR4 /CXCL12 axis encourages ERK to phosphorylate Bim, releasing it from the antiapoptotic proteins Bcl-2 and Mcl-1 so they may bind to BAX and do their antiapoptotic activities.

The Rb or p53 pathways control the CXCL12 axis, which is essential for preventing growth inhibition and thereby encourages longevity as well as division in tumor cells. The CXCR4 gene's proximal enhancer region contains a GFI-1-binding site where wildtype p53 interacts to lessen CXCR4 expression. In p53 mutant cells, treatment with p53 rescue medicines (PRIMA-1, CP- 31398) can reestablish the inhibition of CXCR4 transcription. A common finding in many cancer cell lines is

the loss of functional p53, which is one of the processes driving CXCR4 over expression. The prosurvival proteins NF-B and MDM-2 are produced by the CXCL12/CXCR4 axis, as was already mentioned, by activating AKT.

7.8 C-X-C Motif Chemokine Ligand 12 (CXCL12) Axis in Invasion and Metastasis

Malignant tumors, which are the main cause of death in cancer patients, possess a vital biological characteristic called metastasis. It was once believed that tumor metastasis was initiated by a single tumor cell escaping from a primary tumor. Although previous studies have revealed that the process of tumor metastasis is dynamic and involves several different molecular and cellular pathways. Because of the CXCL12 axis, many malignancies, including as melanoma, and colon cancer spread (Bartolomé et al. 2009; Wang et al. 2008a, b). For instance, antibody neutralization of the receptor and CXCR4 knockdown via shRNA both significantly reduced tumor cell invasion. Additionally, the liver has high levels of CXCL12 expression, which attracts melanoma and CXCR4 (+) cells in particular, accelerating the spread of cancer to the liver. Additional investigation showed multiple pathways that control the CXCL12/CXCR4 axis metastasis. Cancer metastases are linked to a critical pathway called epithelial-to-mesenchymal transition (EMT). CXCR4/CXCL12 signaling induced the SHH signaling, which is connected to EMT and lack of cell attachment. Moreover, CXCL12/CXCR4 transmission increases the activity survivin via the MED/ERK and PI3K/AKT pathways in human sacral chondrosarcoma, resulting in cycle progression (Yang et al. 2015; Kim et al. 2006; Li et al. 2012; Sofi et al. 2023).

Similar results are seen in glioblastoma and hepatocellular cancer. Additionally, CXCL12/CXCR4 signaling via the Wnt/-catenin signaling pathway promotes the invasion and EMT of colorectal cancer cells. An enzyme group known as metalloproteases (MMPs) can help cancer spread by destroying the EM in surrounding healthy tissue. CXCL12 facilitates myeloma cell invasion into the bone by upregulating MT1-MMP and MMP-9 expression (Liao et al. 2017; Hu et al. 2014; Li et al. 2014). Similar to this, CXCR4 increases MMP-9 and MMP-13 synthesis via activating the ERK pathway, which in turn encourages the invasion and movement of oral squamous cell cancer cells. When the CXCL12/CXCR4 axis is activated, additional cell types, such as neuroblasts, can migrate along the corpus callosum and secrete MMP-2 (Parmo-Cabañas et al. 2006; Yu et al. 2011; Mao et al. 2016).

Tumors usually have hypoxia, which leads endothelial cells to produce more CXCL12 as a result of HIF-1. Since they can function as a source of metastasis in the peripheral arteries, CXCR4-positive cancer stem cells are likely recruited to these tissues for this reason. The expression and functionality of integrins on cell surfaces are also altered by CXCL12, facilitating the adherence of tumor cells. In a tumor extravasation or intravasation paradigm, CXCL12, for example, promotes the adherence of PC-three cells to the human umbilical vein endothelial cell monolayer (Engl et al. 2006; Shen et al. 2009). Additionally, prostate tumor cells are induced

by CXCL12 to produce integrins 5 and 3, which makes the tumor cells adhere to the human EM. Similarly to this, CXCL12 promotes the expression of integrin 1 in ovarian carcinoma cells, increasing the adherence of these cells to laminin (Ratajczak et al. 2006; Kukreja et al. 2005).

The development and survival of these tumor cells are favored by enhanced adhesion, which is linked with higher CXCR7 expression. The capacity of breast cancer cells to attach to an endothelial cell of a human umbilical vein is improved by increased CXCR7 expression (HUVECs). Additionally, more aggressive prostate cancer cells are linked to higher CXCR7 expression (Stacer et al. 2016). These findings collectively imply that CXCR7 is an oncogene, but there is still more to learn, especially on the mechanisms behind CXCR7 oncogenic signaling. Increased local breast cancer recurrence in CXCR7-deficient animals after resection suggests that CXCR7 may have tumor-suppressor capabilities associated with the metastatic cascade (Wang et al. 2008a, b; Burns et al. 2006). The fact that oncogenic CXCR7/CXCR4 dimers and tumor suppressor homodimers can heterodimerize with CXCR7 and that the loss of CXCR7 may disturb this delicate balance may certainly explain a seeming dichotomy. The apparent failure of the CXCR7 function indicates that there is still an issue (Shi et al. 2020).

7.9 Conclusion

CXCL12 is critical for the growth of malignancies, as shown by numerous studies. It has been shown that CXCL12 inhibits apoptosis, increases cancer progression and penetration in malignant cells, and stimulates angiogenesis. Several cancer cell signaling pathways may also be activated as a result of it via autocrine or paracrine mechanisms. Additionally, the ligand can attract stromal cells and trigger growth factor discharge that promotes angiogenesis as well as the growth of tumors. Therefore, blocking CXCL12/CXCR4 axis might serve as a novel approach toward cancer-targeted therapy. CXCL12 is expected to play a major role in controlling the transit of hematopoietic stem cells between the bone marrow and other organs. During development, CXCL12 is widely produced in various organs and serves in its capacity as a strong cell adhesion molecule for myeloid cells, enabling them to cross endothelial cell barriers. The CXCL12 chemical, which is generated by stromal cells in the tumor microenvironment, is thought to attract cancer cells by activating the CXCR4 receptor, which is up-regulated in tumor cells. Secondary tumors develop in tissues with high levels of CXCL12 expression, which controls the pattern of metastatic dissemination. Elevated concentrations of CXCL12 attract a population of cancer cells that are strongly tumor promoting and enhance cell survival, replication, angiogenesis, and cancer progression. CXCL12 has many effects on tumor development, but its main purpose seems to be the promotion of tumor cell metastasis or mobilization. Additionally, this may lead to the attraction of hematopoietic cells and the growth of cancer stem cells within the tumor microenvironment.

Further Reading

For further readings, some textbooks mentioned below also give a further understanding of cytokine and the chemokine network.

- Cytokines and their therapeutic potential by Manzoor Ahmad Mir.
- The cytokines of the immune system: The role of cytokines in disease related to the immune response by Zlatko Dembic.
- The cytokine network and immune functions by Jacques Thèze.
- Chemokines and viral infection by T.E. Lane.
- Cytokines and chemokines in infectious diseases.

The below links for video lectures may also be helpful:
https://youtu.be/TAqO0Mq19JQ
https://youtu.be/6wEnYfvoBqk
https://youtu.be/A9fRRCUlMms
https://youtu.be/zIWvmCsKr34

For more incites about the topic we would suggest detailed findings from the books of (Mir 2022) https://doi.org/10.1016/C2021-0-02565-7, https://doi.org/10.1016/C2022-0-00074-X and (Mir 2021) https://doi.org/10.52305/WXJL6770 (Mir 2015c) https://doi.org/10.1016/C2014-0-02898-5 and from the cancer.net website on the following mentioned below links,
https://www.cancer.net/cancer-types/breast-cancer/types-treatment
https://discovery.ucl.ac.uk/id/eprint/1472740/
https://www.jmedsciences.com/doi/JMEDS/pdf/10.5005/jp-journals-10045-00138

See video links on over all status of Cancer, its various types, current new treatment possible options available
https://www.sciencedirect.com/science/article/pii/S205970292032278X

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CCL5/CCR5 Axis in Cancer

8

Manzoor Ahmad Mir, Aamina Manzoor, and Nusrat Jan

Abstract

Inflammatory chemokines were primarily thought of in the past as essential "gatekeepers" of immunity and inflammation. Recent findings suggest that cancer cells hijack the normal chemokine system, making chemokines and their receptors crucial components of the tumor microenvironment with a variety of functions that promote tumor growth. Several hematological malignancies, lymphomas, and a large number of solid tumors have been found to express CCL5 and the CCL5 ligand, but only a small number of cancers have undergone in-depth research on the function of the CCL5/CCR axis. This study focuses on the roles played by the CCL5/CCR5 axis in the pathological processes of many diseases and the pertinent signaling pathways for the axis' control.

Keywords

Chemokines · Tumor-associated macrophages · Regulatory T-cells · Angiogenesis

8.1 Introduction

Cancer is one of the main causes of death worldwide, yet its impact varies (Forman et al. 2014). The burden of cancer has increased over time due to several complicated variables, including an aging and expanding population, accelerated socioeconomic development, and shifts in the prevalence of related risk factors. These factors are prevalent in both industrialized and developing countries (Forman et al. 2014;

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Do et al. 2020). Cancer is the leading cause of premature death and lowers life expectancy in many nations due to population growth and aging worldwide. The predominance of known risk factors for cancer, such as smoking, being overweight, being inactive, and altering reproductive patterns brought on by urbanization and economic development are all rising along with the population's growth and aging. GLOBOCAN predicts that 8.2 million deaths and 14.1 million new cancer cases globally occurred in 2012. The burden of cancer has migrated over time to less developed nations, which now account for around 57% of cases and 65% of cancer deaths globally (Qayoom et al. 2023). In more advanced nations, breast cancer is still the main cause of cancer-related deaths for women, while lung cancer is now the top killer of males in both more and less developed countries (Mir et al. 2020). In less advanced nations, breast cancer remains the most common reason why women die from the disease. Male prostate cancer and colorectal cancer are two other main causes of cancer mortality in more developed nations. In more developed countries, male prostate cancer and colorectal cancer are two additional major causes of cancer death. In less developed countries, liver and stomach cancer in males and cervical cancer in women are the biggest sources of cancer-related death. Incidence rates for all malignancies combined are around twice as high in more established as in less developed countries in both males and females, even though the mortality rates are only 8–15% higher in more developed countries.

8.2 Chemokines

Small-molecular-weight cytokines called chemokines are important in the physiological regulation of immune cell movement. They are involved in the growth and maintenance of immune cells, the production of cellular and humoral immune responses, and the disease-related pathologic recruitment of immune cells (Mir et al. 2022c). There are now four subfamilies made up of about 50 endogenous chemokines that have been identified in mice and humans (Balkwill 2012; Do et al. 2020). Chemokines CXC, CC, CX3C, and C. Chemokines' cognate receptors, which are cell surface receptors mainly the G-protein-coupled receptor (GPCR) family, are what allow them to function. Cell migration programs are activated by a variety of downstream effectors and signaling cascades that are triggered during activation (Nagarsheth et al. 2017; Aldinucci et al. 2019). But the majority of chemokines receptors exhibit marked promiscuity, having more than one highly-affinity ligand and being redundant for some signaling pathways. Chemokines, which were categorized based on their capacity to cause chemotaxis, play a crucial role in the development of cancer as well as in inflammation and immune surveillance (Allavena et al. 2008; Aldinucci et al. 2020). Chemokines, which are released by tumor cells from primary tumors, metastatic sites, or normal cells that have been recruited and/or locally activated, can operate as growth factors (Ben-Baruch 2006), promote angiogenesis and metastasis development (Meadows et al. 2012), or create an immunosuppressive milieu.). Tumor cells that express the appropriate receptor spread more quickly in response to chemokines found in distant organs (Aldinucci

et al. 2010; Mir et al. 2022b). Additionally, tumor cells develop stronger adhesion, migration, and invasion abilities. Due to the presence of inflammatory cells as reactive leukocytes and the expression of numerous inflammatory mediators, including cytokines, chemokines, and enzymes, in the main tumor are mostly linked to a poor prognosis and the development of metastases. Neoplastic tissues have a range of chemokines and chemokine receptors.

8.3 Role of CCL5 and CCR5 in Invasiveness

The chemokine (C–C motif) ligand 5 (CCL5), which can be released by both tumor cells and mesenchymal stromal cells, has recently been discovered. A crucial node in the bidirectional connection between breast cancer and normal cells, recruited to the tumor, has been found. Cancer cells cause the normal milieu of inflammatory cytokines and chemokines in the tumor microenvironment to become unstable, which has tumor-promoting effects (Dedoni et al. 2018). Cancer cells attract T cells, monocytes, myeloid cells, fibroblasts, and mesenchymal stromal cells (MSCs) from bone marrow or adipose tissue by secreting chemokines and "educate" these cells to develop into immunosuppressive Tregs, tumor-associated macrophages (M2-TAM), myeloid-derived suppressor cells (MDSCs), and cancer-associated fibroblasts (CAF's). As a result, natural killer (NK) cells and anergic or worn-out T cells generate a microenvironment that protects tumors (Aldinucci et al. 2012; Mir and Mehraj 2019). In turn, tumor-educated normal cells promote the migration, invasion, and proliferation of cancer cells, resulting in metastasis (Ridley et al. 2003; Oppermann 2004) (15). Furthermore, through enhancing TAMs' proangiogenic activity, tumor cells both directly and indirectly promote angiogenesis (Mehraj et al. 2021b) (16). Finally, the tumor microenvironment (TME) generates "sanctuaries" that shield cancer cells from therapeutic activity while also preventing medication penetration. The presence of particular invading inflammatory cell types and the expression of various inflammatory mediators (such as cytokines, chemokines, and enzymes) have been related to poor prognosis and metastasis. As a result, inhibiting TME formation and preventing chemokine-mediated effects are now viewed as a second therapeutic option. and the paracrine activity of molecules generated by adipocytes alters the antitumor immune response (Mehraj et al. 2021a).

In neoplastic tissues, inflammatory mediators such as C–C chemokine ligand 5 (CCL5) and C–C chemokine are abundant (19). CCL5 increases antitumor immunity by attracting anti-tumor T cells and dendritic cells to the TME. However, this is a double-edged sword in the context of cancer because it improves the responsiveness to immunotherapy for many tumor types (Casagrande et al. 2019; Sofi et al. 2021). CCL5 (regulated upon activation, normal T cell generated and secreted, Rantes) is a member of the C–C domain chemokine group, which also contains the members CCL3 (macrophage proinflammatory Rantes, CCL4 (MIP-1b) and protein 1-alpha, MIP-1a)) (Aldinucci et al. 2008; Zi et al. 2017). CCL5 binds to its receptor CCR5 with a high affinity (Menu et al. 2006; Trentin et al. 2007). But it also binds CD44, GPR75, CCR1, CCR3, CCR4, and CD44. Nuclear factor

kappa-light-chain-enhancer of activated B cells (NF-B) targets the gene CCL5 (Qayoom et al. 2022; Pervaiz et al. 2015; Halama et al. 2016; Aldinucci and Casagrande 2018; Jiao et al. 2018; Wu et al. 2018). T lymphocytes, macrophages, platelets, synovial fibroblasts, tubular epithelium, and cancerous cells all express it. CCR5, a CC-chemokine receptor, expressed on resting memory/effector T lymphocytes, monocytes, macrophages, and developing progenitor cells (Qayoom et al. 2023). In response to ligand contact, CCR5, a G protein-coupled receptor with seven transmembranes, mediates various signal transduction cascades. It is an active receptor that binds CCL5, CCL3, CCL4, and CCL8 with great affinity. CCR5 engagement leads to G protein activation, the activation of signaling pathways like the Akt and NF-kB pathways, cytoskeleton rearrangement, and chemotactic cell migration. It is crucial to the inflammatory process by directing cells to the inflammatory areas (Ryu et al. 2018). It is a significant coreceptor for humans.

8.4 The CCL5/CCR5 Axis and the Progression of Cancer

The CCL5/CCR5 axis promotes tumor development via a variety of methods. It promotes tumor development, remodels the extracellular matrix, improves tumor cell migration (metastasis formation), promotes the proliferation of cancer stem cells, promotes drug resistance in cancer cells, decreases the cytotoxicity of DNA-damaging agents cellular energetics are deregulated (metabolic reprogramming), encourages angiogenesis, brings in immune and stromal cells, and causes macrophage immunosuppressive polarization (Qayoom et al. 2021; González-Arriagada et al. 2018; Liu et al. 2019) (Fig. 8.1).

8.5 Tumor Growth

Both autocrine and paracrine mechanisms contribute to CCR5 involvement and promote tumor development. The presence of a functioning CCR5 and the secretion of its ligands by cancer cells are characteristics of autocrine growth. CCR5 ligands promote cell proliferation by activating the Jak-STAT pathway (Vaday et al. 2006), stimulating the mTOR (mammalian target of rapamycin) pathway (Murooka et al. 2009), and upregulating the expression of cyclin D1, c-Myc, and Dad-1 (Ding et al. 2016; Gao and Fish 2018). boosting ATP generation, glycolysis, and extracellular acidification while increasing glucose intake.

8.6 Migration and Extracellular Matrix Remodeling

The activation of a series of downstream effects by the binding of CCL5 to its receptors promotes receptor internalization and signal transduction, which in turn causes the activation of integrins (adhesion) and polarisation of the actin cytoskeleton. Through the activation of the v3 integrin by CCL5, PI3K/Akt, IKK alpha/beta, NF-κB, and inhibitor of nuclear factor-B (IKK) are all activated, resulting in cell

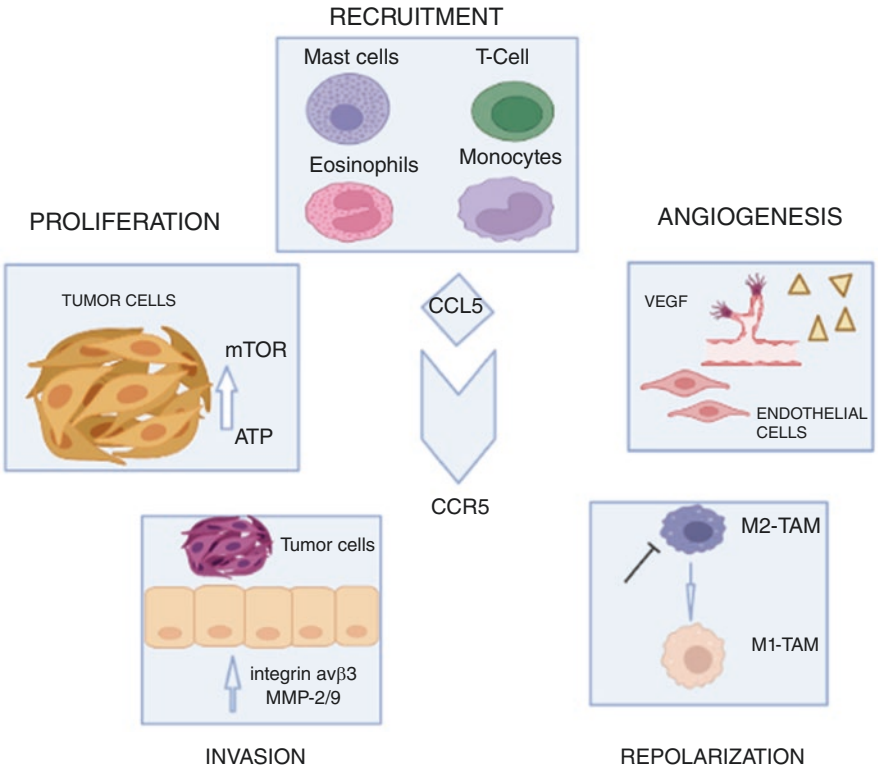

Fig. 8.1 CCL5/CCR5 axis role in cancer progression

migration. Matrix metalloproteinase 1 (MMP-1) and MMP-13 expression are activated by CCL5 to cause collagen breakdown in humans (Wang et al. 2012) rheumatoid arthritis synovial fibroblasts (Huang et al. 2009).

8.7 Drug Resistance, Reduced Cytotoxicity of DNA-Damaging Agents, and Cancer Stem Cell Expansion

In the absence of ligands, the destiny of cancer cells is similarly influenced by CCR5 expression. In mice, CCR5+ breast cancer cells develop mammospheres and tumors more quickly than CCR5 tumor cells, exhibiting characteristics of cancer stem cells. CCR5 expression correlates with more powerful DNA-damaging substances (doxorubicin and -irradiation) that induce repair reactions to DNA damage (Agere et al. 2017). CCR5 inhibitors increase the toxicity of both doxorubicin and beta-irradiation. Breast cancer cells' single-cell research revealed that CCR5 regulates cell survival signaling pathways. T cell-secreted CCL5 reduces prostate cancer chemotherapeutic activity (Singh et al. 2018). Cisplatin reduces the activity of ovarian cancer by causing CAFs to secrete CCL5 (Long et al. 2012).

8.8 Unrestrained Cellular Energy (Metabolic Reprogramming)

A newly found feature of malignancy is the reprogramming of the cancer metabolism (Kato et al. 2013). Cancer cells' impaired glucose metabolism has been linked to both cell growth and resistance to chemotherapy and radiation. Higher glucose absorption rates are needed by tumor cells, and they also have higher use and uptake of catabolites.

8.9 Angiogenesis

By promoting endothelial cell motility, spreading, neovessel formation, and vascular endothelial growth factor (VEGF) secretion, CCL5 enhances proangiogenic activity (Wang et al. 2015). Upon CCL5 activation, tumor cells release VEGF. Tumor cells attract CCR5+ monocytes/macrophages by secreting CCL5, and these cells then stimulate angiogenesis by secreting VEGF (Yi et al. 2013; Zhou et al. 2016; Faubert et al. 2020). Consequently, preventing the recruitment of TAMs, which are currently thought to be the main participant in modulating tumor angiogenesis could be a successful treatment approach. CCL5 may promote lymphangiogenesis.

8.10 Immune and Stromal Cell Recruitment as well as Immunosuppressive Polarization

By secreting CCL5, tumor cells draw in normal cells and eventually take over them (Lin et al. 2020). Monocytes that have been recruited are trained to develop into protumoral immunosuppressive M2-TAMs (Suffee et al. 2012). M2-TAMs express programmed death ligand 1 (PD-L1), CD206, and indoleamine 2,3-dioxygenase, which generate anti-inflammatory cytokines including transforming growth factor (TGF-β), which boosts immunosuppressive Tregs like interleukin (IL) 10 (Wang et al. 2016). Via humoral and cell-cell contact routes, immunosuppressive CD4+ T cells called Tregs control not only effector T cells but also B cells, NK cells, dendritic cells, and macrophages (Mantovani et al. 2017). Tregs have inhibitory effects on T cells that are exhausted or anergic tumor-killing lymphocytes. Immunosuppressive TAM polarization in cancer tissues is regulated by the CCL5/CCR5 axis (Deng 2018). Hematopoietic cells that overexpress CCL5 in the bone marrow evolve into MDSCs, a diverse population of myeloid cells that suppresses effective immune responses to tumors Fig. 8.2.

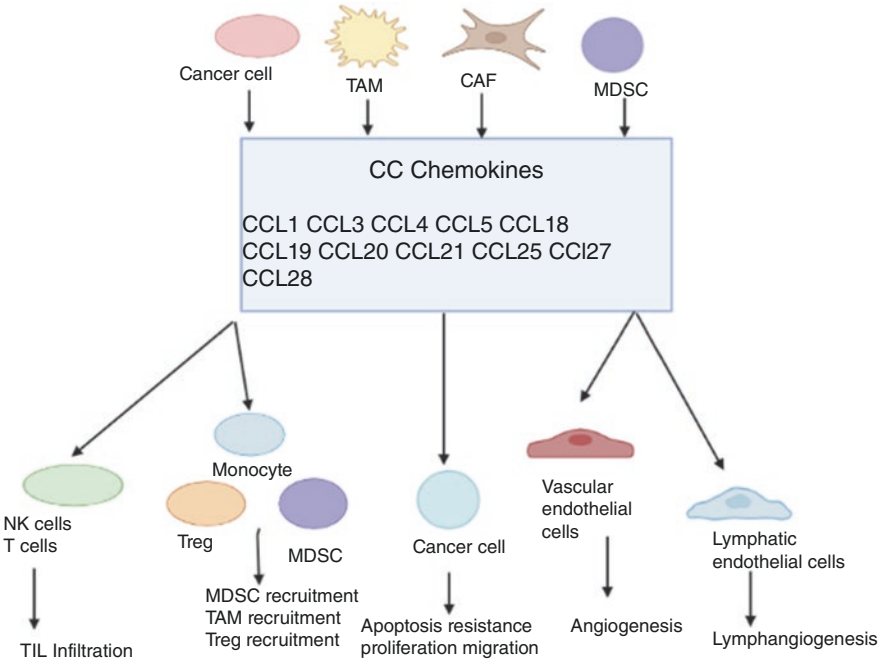

Fig. 8.2 Chemokine's impact on the recruitment of various cells to the tumor microenvironment

8.11 CCL5/CCR5 Axis's Downstream and Upstream Pathways

Cell proliferation, angiogenesis, apoptosis, invasion, division, metastasis, and inflammation have all been related to the downstream CCL5/CCR5 pathway, which encompasses the NF-kB, RAS-ERKMEK, JAK-STAT, PI3K/AKT, HIF-a, and TGF-b-smad pathways. The CCR5/CCL5 axis may help to activate GSK-3b and AKT in the PI3K/AKT pathway, controlling the downstream effects. Particularly, PI3K is phosphorylated following CCL5 binding to CCR5, which helps to phosphorylate the AKT serine 473. After that, the GSK-3 enzyme is phosphorylated by the AKT/PKB complex to stop working. Several downstream proteins' expression is either triggered or stopped for instance, Bcl2's expression level is increased to encourage cell apoptosis, 21 b-catenin is phosphorylated before being destroyed and the level of Cyclin D, a critical protein for controlling the cell cycle, can be inhibited by inactivated GSK-3. CCL5/CCR5 phosphorylates 4E-BP1 in the PI3K/AKT/mTOR pathway, which promotes the mTOR-4E-BP1/S6K1-eIF4F pathway. soon after A tyrosine kinase called mTOR gradually phosphorylates 4E-BP1, S6K1, and eIF4F after receiving signals from CCL5/ CCR5-PI3K/AKT (Barcellos-de-Souza et al. 2016; LeBleu and Kalluri 2018). Then 50-untranslated region (UTR) dissociation and mRNA translation are promoted by activated eIF4F. Additionally, TSC-2's Thr1462 is phosphorylated by CCL5/CCR5-mediated PI3K/AKT, which prevents the formation of the TSC-1/TSC-2 complex. By enhancing the stability

and accumulation of HIF1a, CCL5/CCR5 may also initiate the hypoxia-inducible factor a (HIF-a) pathway, HIF-a triggers angiogenesis in a hypoxic environment through a von Hippel Lindau protein (VHL)-related ubiquitin degradation or HIF response element (HRE). Through the NK-kB pathway, CCR5/CCL5 phosphorylated p65 serine 536 can modify signaling at both the protein and mRNA levels with a co-activator. RAS-ERK-MEK pathway can be started by CCL5/CCR5 by phosphorylating MEK and increasing the kinase activity (Hanahan and Coussens 2012; Cammarota and Laukkanen 2016; Ansems and Span 2020).

8.12 Upstream Regulators of CCL5/CCR5

Various upstream regulators of CCR5 and CCL5 are plasminogen activator inhibitor-1 (PAI-1),47 kruppel-like zinc-finger transcription factors Rig1, SOCS-1, transcription factor 5 (KLF5), etc. For instance, a positive feedback loop could be created by PAI-1 activating CCL5 secretion, which then increases PAI-1 production. As a result, PAI-1 as a CCL5 regulator could encourage cell migration and angiogenesis while inhibiting cell death by secreting MMPs and cytokines at a preferred dose. Additionally, the promoter of the CCL5 gene could bind to activated c-Jun N terminal Kinase and control the transcription's beginning. According to studies, CCL5 and the enhancer of zeste homolog 2(EZH2) level are positively correlated. EZH2 controls histone H3 lysine 27 methylation to control epigenetic gene expression (H3K27). EZH2 may also play a significant role in controlling the migration and invasion of cancer cells by macrophages (Maeda et al. 2012; Ban et al. 2017).

Additionally, HER2, phosphatase, and homolog of tensin, a pair of cancer-related proteins that are intimately linked to metastasis are lost on chromosome 10 (PTEN). Moreover, CCL5, IL-6, and IL-8 secretion will come from an increase in HER2 and a decrease in PTEN.

8.13 The Tumor-Promoting Role of the CCL5/CCR5 Axis

To investigate the CCL5/CCR5 axis in cancer, many approaches have been used, including the generation of CCL5-knockout mice and the reduction of CCL5 production using neutralizing antibodies or gene silencing. CCR5 antagonists maraviroc, vicriviroc, BMS-813160, TAK-779, and anibamine have been studied in preclinical studies. Maraviroc is the most often used CCR5 antagonist, and CCR5 is an antiretroviral medication that has been licensed for the treatment of HIV/AIDS patients because it serves as a co-receptor for HIV-1 entry into host cells and is a target of anti-HIV drugs. Maraviroc reduces HIV entry into T cells and macrophages by disrupting CCL5-CCR5 binding. It binds to the active site pocket of CCR5, deactivating it. Maraviroc is a substrate for the enzyme CYP3A4 and the protein P-glycoprotein, also known as the multidrug resistance protein 1 (MDR1), which inhibits the uptake of drugs by cancer cells or induces drug efflux from them.

MDR1 controls the transport and absorption in the gut and bile (Tupova et al. 2019). The enzyme cytochrome P450 3A4 breaks it down. Vicriviroc is an HIV-active CCR5 antagonist based on piperazine. Vicriviroc was created to attach to CCR5 and prevents HIV from entering CD4 cells. TAK-779 is a nonpeptide CCR5, CCR2b, and CXCR3 antagonist (Zhang et al. 2010). The original natural CCR5 antagonist, anibamine, is a recently discovered distinctive drug pyridine quaternary alkaloid from Aniba panurensis. It has a moderate affinity for CCR5 (Jin et al. 2018; Xia et al. 2020). BMS-813160 is a CCR2 and CCR5 antagonist with potential immunomodulatory and antitumor properties.

8.14 The CCL5/CCR5 Axis in Hematological Malignancies

CCR5 contributes to tumor progression in several hematological cancers, including acute myeloid leukemia, chronic myeloid leukemia, lymphoblastic leukemia (Velasco-Velázquez et al. 2012; Wang et al. 2020), classic Hodgkin lymphoma, chronic myeloid leukemia (Sasaki et al. 2014; Suarez-Carmona et al. 2019), and multiple myeloma.

8.15 Acute Myeloid Leukemia

AML (Acute myeloid leukemia) is a type of cancer that is quite varied. Chemokines have important roles in the initiation, cellular proliferation, migration, survival, and enhancement of AML therapeutic outcomes. The CCR5 receptors together with their ligands have indirect effects on the progression of cancer. Acute myeloid leukemia (AML) development is greatly influenced by the CCL5/CCR5 axis (Haemmerle et al. 2018). CCR5 and its ligands are expressed by AML cells (Cignetti et al. 2003; Wang et al. 2019). AML patients are classified into subgroups based on their chemokine response and chemokine expression pattern (Bruserud et al. 2007). In AML CCL5, CCL4, and CCL3 levels are elevated in the serum of people with the monocytic phenotype (Yazdani et al. 2020). In these patients' CCR5 expression declines and CCL3 levels revert to baseline after the first round of chemotherapy, whereas CCL5 and CCL4 only show a decline. Fms-like tyrosine kinase 3 (FLT3) inhibitor resistance is influenced by CCL5 (Li et al. 2020). FLT3-internal tandem duplication (ITD)-mutated AML cell lines that are resistant to midostaurin, a tyrosine kinase inhibitor approved for the treatment of FLT3-mutant AML, express large amounts of CCL5. Typically, in AML cells, the upregulation of survival/proliferation pathways caused by CCL5 overexpression results in midostaurin resistance.

8.16 Chronic Lymphoblastic Leukemia and Acute Lymphoblastic Leukemia

The most frequent hematological malignancy identified in children is acute lymphoblastic leukemia (ALL), which develops from hematopoietic progenitors dedicated to a T or B cell lineage (Carlo et al. 2005). CCR5 is overexpressed in ALL cells. Maraviroc reduced ALL cell proliferation in vitro, brought on apoptosis, and Jak/STAT signaling was downregulated, along with migration to C-X-C motif chemokine ligand 12 (CXCL13) and CXCL12, adherence to, vascular cell adhesion molecule 1 and fibronectin. Maraviroc further prevented ALL xenograft development. Chronic lymphoblastic leukemia (CLL) cells in vitro, particularly those that express the poor prognosticators CD38 and CD49d, release CCL3 (Varney et al. 2002). From bone marrow biopsies, CCL3 was discovered in the cytoplasm of CLL cells (Abe et al. 2004). Monocyte migration and CD68+ macrophage infiltration are both accelerated by CCL3 is very high in patients with CD38+CD49d+ CLL's bone marrow.

8.17 Hodgkin Lymphoma

Less than 1% of Hodgkin lymphoma (cHL) cells require the assistance of the TME to develop and survive (Carbone et al. 2002). Most of the cells in the TME are normal cells that need to be trained by tumor cells to become protumorigenic (Maggio et al. 2002). The role CCL5 plays in this situation is crucial. In the proliferation of tumors (Buri et al. 2001) and TME development, both tumor cells originating from cHL tissues and cell lines express CCL5 and CCR5 constitutively (Fischer et al. 2003). Macrophages, stromal cells, and lymphocytes of the cHL TME express CCR5 (Kim et al. 2017). Epstein-Barr virus (EBV)-positive HL tissues have higher levels of CCL5 and CCL3 than EBV-negative HL tissues (Mir et al. 2023b; Lentzsch et al. 2003; Roussou et al. 2009), which is in line with the observation that in EBV-negative cell lines, Expression of CCL5 is induced by the EBV gene LMP1, as shown in Fig. 8.3.

8.18 The CCL5/CCR5 Axis in Solid Tumors

Solid tumors of different types often express CCL5 or CCR5. Here, we review current research on how these proteins interact with breast cancer, Oesophageal squamous cell carcinoma, lung cancer, gastric cancer, head and neck cancers, osteosarcoma, colorectal cancer, melanoma, and ovarian, pancreatic, pituitary, prostate and thyroid cancers (Zucchetto et al. 2009).

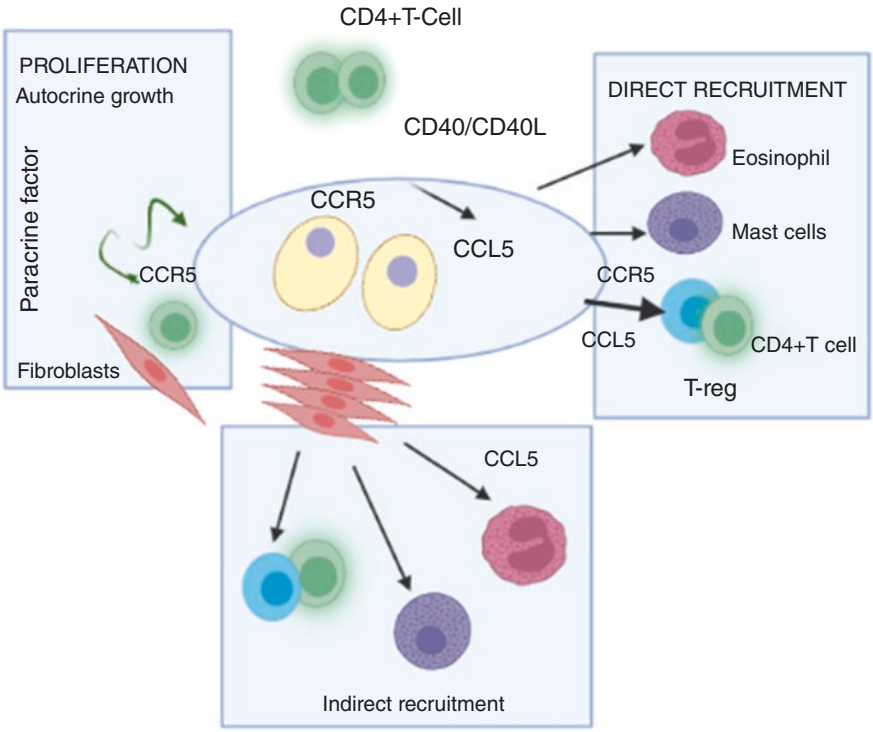

Fig. 8.3 Interactions among cHL cells and the microenvironment

8.18.1 Breast Cancer

Normal breast epithelial duct cells barely express CCL5, while breast carcinoma cells do so heavily at primary tumor sites and local lymph nodes and metastatic locations, demonstrating that CCL5 expression is acquired during malignant transformation and that CCL5 participates in the initiation and/or progression of breast cancer (Lee et al. 2014). Compared to individuals in remission, breast tumor cell CCL5 positivity and expression levels have a strong correlation with the development, recurrence, and/or metastasis. Although the tumor cells themselves are the primary source of CCL5 in this tumor, leukocytes and mesenchymal stem cells (MSCs) that have infiltrated the tumor microenvironment also express CCL5 (Mir et al. 2023a; Vallet et al. 2011). Additionally, CCL5 can be found in the serum, pleural effusions, and interstitial fluids supplying the tumor. A subset of human breast cancer cell lines expresses a functioning CCR5 receptor and exhibits a functional response to CCL5. Additionally, CCR5 expression is induced by oncogene transformation, and cells that express functional CCR5 exhibit enhanced cell motility and invasiveness (Soria and Ben-Baruch 2008; Jiao et al. 2018). The basal and HER-2 genetic subtypes of human breast cancer were shown to have greater expression of CCL5 and its receptor CCR5, but not CCR3 (Karnoub et al. 2007). The lack

of a correlation between CCL5 and CCR5 expression levels in nonneoplastic breast samples, however, suggests that CCL5/CCR5 signaling may be preferentially activated during the development of particular breast cancer subtypes. Triple-negative breast cancer (TNBC) in particular has a strong association with the progression of CCL5 expression, which may represent a potential TNBC immunotherapeutic target (Mir et al. 2022a; Aisha et al. 2021; Dangaj et al. 2019).

8.18.2 Colon Cancer

Due to the overexpression of CCL5 and its receptors in primary tumors, as well as liver and lung metastases, the CCL5/CCR5 axis also plays a role in colon cancer as compared to healthy tissues (Tanabe et al. 2016). Colon cancer cells from both human and animal origins proliferate and move more readily when exposed to CCL5. Additionally, systemic administration of neutralizing anti-CCL5 antibodies to mice reduced the severity of peritoneal carcinosis, liver metastases, and subcutaneous tumors. Chang et al. discovered a unique immune escape mechanism that is mediated by CCL5. CCL5 knockdown in CT26 mouse colon tumor cells reduces the apoptosis of mice tumor-infiltrating CD8+ T cells and blocks tumor development. Here, CCL5 not only encourages T-reg cell migration to tumors but also improves CD8+ T cells' capacity to kill. This enhanced capability is associated with T-reg cells' increased release of TGF (Nishikawa et al. 2019; Jan et al. 2023).

8.18.3 Gastric Cancer

The progression of gastric cancer (GC) is significantly influenced by the CCR5/CCL5 axis. Serum CCL5 levels were found to be higher in GC patients compared to controls (Mencarelli et al. 2013; Sima et al. 2014) and significantly linked with illness stage, decreased survival time, and poor prognosis (Sugasawa et al. 2008). These patients have high CCL5 levels. Immunohistochemical labeling in metastatic lymph nodes (Wu et al. 2019) and malignant tissues (Sen-Sen et al. 2014). High levels of CCL5 are secreted by highly metastatic GC cell lines. Human gastric cancer cell lines with strong metastatic potential also exhibit higher amounts of CCL5, which suggests a tumor-promoting function in gastric carcinoma of CCL5. The interactions between peripheral blood mononuclear cell activity and supernatants from low- and high-metastatic gastric cancer cell lines provide evidence in favor of this hypothesis (PBMC). PBMC activated by supernatants from high-metastatic gastric cancer cell lines expresses more CCL5 than PBMC stimulated by supernatants from low-metastatic cell lines.

8.18.4 Lung Cancer

To boost the expression of integrin v3, the Akt/NF-kB/PI3K pathway is activated by CCL5 to aid in the migration of lung cancer cells (A549 cell line) (Lu and Luan 2020). In nonsmall-cell lung cancer cell lines and tissues, miR-147a has been found to have CCL5 as a target that is downregulated (Zhang et al. 2019). By altering CCL5 in vitro, miR-147 suppresses tumor cell proliferation, migration, and invasion. Additionally, to prevent the development of tumor xenografts, mice were subcutaneously injected with cancer cells that overexpressed the miR-147 gene. This resulted in a reduction in CCL5 expression, invasion, and metastatic development.

8.18.5 Osteosarcoma

Osteosarcomas originate from primitive mesenchymal cells that produce osteoid and/or juvenile bone. High CCL5 levels in osteosarcoma are closely associated with metastasis and a poor prognosis (Sun et al. 2017). CCR5 is expressed by osteosarcoma cells (Oba et al. 2005), and when CCL5 interacts with it, the NF-kB activation drives osteosarcoma cell motility and v3 integrin activation. CCL5 may therefore be a molecular target for osteosarcoma treatment and a biomarker for the disease's prognosis.

8.18.6 Ovarian Cancer

Despite having a low level of expression in ovarian cancer cell lines, CCL5 expression has been found in both benign and malignant ovarian biopsies (Long et al. 2015). Although the types of CCL5 expressed in the biopsies have not yet been identified. In ovarian cancers, infiltrating leukocytes are likely the main source of this chemokine. In ovarian cancer, CD133+ ovarian carcinoma stem-like cells, which can self-renew, trigger tumor relapse, and form metastases, are the main source of expression for both CCL5 and CCR5 (Tang et al. 2016; Mir et al. 2023a). Using ovarian carcinoma cell lines and primary tissues, CCL5 was found to be present in CD133+ ovarian carcinoma stem-like cells and the expression of its receptors CCR5, CCR1, and CCR3 increased. The CCL5/CCR5 axis may be implicated in the ability of ovarian carcinoma stem-like cells to spread, according to research showing that blocking CCL5 or the corresponding receptors reduced the invasiveness of ovarian carcinoma cells. By activating NF-κB and increasing MMP-9 synthesis, both of which are upregulated in ovarian cancer stem-like cells and primary tumors, CCL5 promotes tumor invasion.

8.18.7 Prostate Cancer

Prostate cancer tissues, primary cultures of prostatic adenocarcinoma cells, and human prostate cancer cell lines all express both molecules, suggesting that the CCL5/CCR5 axis is involved in the development of prostate cancer (Luo et al. 2014; Huang et al. 2020). Blood testing revealed high amounts of CCL5, which were linked to poor forecasts (Borghese et al. 2013). Because of its enhanced expression in high-grade prostate cancer, metastatic prostate cancer, and androgen-insensitive prostate carcinoma, prostate-specific membrane antigen (PSMA) is a diagnostic biomarker (Colombatti et al. 2009). PSMA activation boosts the expression of CCL5 and IL-6 and activates the NF-kB via the p38 and ERK1/2 MAPKs cascade (Luo et al. 2015; Sofi et al. 2023). By triggering the STAT5 and cyclin D1 pathways, enhancing tumor invasion, and collaborating with IL-6, CCL5 accelerates tumor growth. TAK-779 prevents tumor cell proliferation and invasion brought on by CCL5.

8.18.8 Thyroid Cancer

Platelets help cancerous cells proliferate by secreting CCR5 ligands. Because platelets play a role in metastasis, high peripheral blood platelet counts are a poor predictor of prognosis for individuals with anaplastic thyroid cancer (Gallo et al. 2012; Hinshaw and Shevde 2019). By upregulating MMP1 and engaging CCR5 expressed by anaplastic thyroid carcinoma, activated platelets increase the migratory and invasive abilities of cancer cells (Daly et al. 2010; You et al. 2018) (Table 8.1).

8.19 Possible Clinical Applications: CCL5 and CCR5 as Therapeutic Targets in Cancer

One of the main objectives of cancer therapy is to break down the pathways that promote the development of tumors or the formation of an immunosuppressive and protumorigenic microenvironment. Consequently, our comprehension of how chemokines work. Novel therapeutic approaches may take advantage of the role of chemokines in the recruitment of lymphocytes or myeloid cells that promote tumor growth as well as receptors in the proliferation and penetration of malignant cells. Since CCR5 is a crucial coreceptor for HIV viral entrance into host cells, it has gained popularity as a target for the creation of anti-HIV therapeutics. Several particular small-molecule CCR5 antagonists are being used as antiviral treatments, including high-throughput screening initiatives that led to the discovery of compounds that not only inhibit CCR5 signal transduction but are also effective (Halvorsen et al. 2016; Pasquier et al. 2018).

Maraviroc and vicriviroc, two CCR5 antagonists, have potent inhibitory effects on chemokine activity and HIV entry (Nie et al. 2019; Mir et al. 2023b). Multiple lines of evidence point to potential clinical uses for CCR5 antagonists in the

Table 8.1 Clinical trials with CCR5 inhibitors

Tumor	Treatment	Effects
Breast cancer	Maraviroc	Inhibited angiogenesis, reduced metastasis, and improved survival in mouse models
	Anti-CCL5 antibodies	Counteracted the ability of MSCs to induce metastasis in tumor xenografts.
		Inhibited collagen VI production by fibroblasts in response to the paracrine coculture of adipose-derived stem cells and tumor cells
Colon cancer	Maraviroc	Decreased CAF and tumor xenograft growth
	Anti-CCL5 antibodies	Decreased in vitro growth and migration of tumor cells, xenograft growth, and liver metastases, sensitized to PDGFRβ therapy
	RNAi-CCL5 silencing	Decreased tumor growth in immunocompetent syngeneic mice and reduced Treg infiltration
Gastric cancer	Anti-CCL5 antibodies	Decreased tumor invasion induced by CCL5 secreted by PBMCs educated by tumor cells. Decreased in vivo growth of tumor cells coinjected with PBMCs
Lung cancer	Anti-CCL5 antibody	Decreased tumor cell migration, invasion, and colony formation; inhibited growth and metastasis in xenografts
Ovarian cancer	Anibamine Anti-CCL5 antibody, shRNA-CCL5 silencing	Inhibited tumor cell growth and intracellular Ca^{2+} flux Inhibited endothelial cell differentiation and tube formation of cancer stem-like cells in vitro and xenografts
Prostate cancer	Anibamine Maraviroc miR-455-5p	Reduced tumor xenograft growth reduced metastasis and improved overall survival (in the presence of chloroquine) Inducted apoptosis, suppressed cellular proliferation, and tumor xenograft growth

management of cancer. Basal breast cancer cells' in vitro invasion is reduced by maraviroc or vicriviroc without affecting viability or proliferation. Maraviroc has already acquired FDA permission for use in humans and prevents the growth of hepatocellular carcinoma in a mouse model. It also lowers lung metastasis in a preclinical animal model of breast cancer (Ruffing et al. 1998; Azenshtein et al. 2002; González-Arriagada et al. 2018), indicating that CCR5 antagonists might be utilized as an adjuvant therapy to lower the risk of metastasis in individuals with the basal breast cancer subtype. An additional mechanism to influence the progression of cancer may be the tumor microenvironment or cancer cells' inhibition of CCL5 secretion.

Developing breast tumors attract MSCs because they can increase the ability of weakly tumorigenic breast cancer cells to metastasize by secreting CCL5. Zoledronic acid significantly affects the secretion of CCL5 and interleukin 6 in MSCs (Blanpain et al. 1999; Ward et al. 2015) suggesting that the drugs could contribute to antitumor activity by affecting the ability of MSCs to interact with breast cancer cells (Mir et al. 2022d).

8.20 Conclusion

Numerous preclinical studies using various cancer models have shown that the CCL5/CCR5 axis has a protumorigenic function in tumor growth and spread as well as in the development of an immune-suppressive and protective TME, consequently, excessive CCL5 expression. The expression of CCR5 by cancer cells has been linked to a decreased activity of DNA-damaging agents, and it has been discovered that the presence of CCR5 by cancer cells or tumor tissues correlates with a poor prognosis. Not many people are aware of how crucial it is to control or target the tumor microenvironment when developing a treatment plan for cancer. Due to the growing importance of immune treatment, the tumor microenvironment has once again received attention. The tumor releases chemokines like CCL5, and by attaching them to the appropriate receptors, they can change the TME. Paradoxically, the CCL5/CCR5 axis may function as a double-edged sword by recruiting immune cells to inflammatory or tumor sites, which might increase proinflammatory responses. On the other side, by increasing MDSC, Tregs, and TAMs help tumor evasion and so produce an immunosuppressive TME, and CCR5 mediates immune suppressive pathways that support the growth of tumors.

Further Reading

For further readings, some textbooks mentioned below also give further understanding of cytokine and chemokine network.

- Cytokines and their therapeutic potential by Manzoor Ahmad Mir.
- The Role of CCL5/CCR5 Signal Transduction in T Cell Function books by Thomas Tsutomu Murooka

The below links for video lectures may also be helpful:
https://youtu.be/TAqO0Mq19JQ
https://youtu.be/6wEnYfvoBqk
https://youtu.be/A9fRRCUlMms
https://youtu.be/zIWvmCsKr34

For more incites about the topic we would suggest detailed findings from the books of (Mir 2022) https://doi.org/10.1016/C2021-0-02565-7, https://doi.org/10.1016/C2022-0-00074-X and (Mir 2021) https://doi.org/10.52305/WXJL6770 (Mir 2015) https://doi.org/10.1016/C2014-0-02898-5 and from the cancer.net website on the following mentioned below links,

https://www.cancer.net/cancer-types/breast-cancer/types-treatment
https://discovery.ucl.ac.uk/id/eprint/1472740/
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CCL2–CCR2 Signaling Axis in Cancer

9

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Abstract

In the present era, one of the common diseases that are considered a highly mortal disease is cancer, and day after day, it is increasing at an alarming rate. In the earlier times, it was not such a common disease, but day after day, new properties of cancerous cells are discovered by which this disease becomes more and more prone to humans. Cancer uses all the molecular pathways and the cell's molecules to grow and invade all tissues and organs of the patient's body. Nowadays, chemokines and cytokines are mainly targeted by the scientists as these are also one of the basic pathways by which cancer grows and develops to other parts of the body, so as to find out new therapeutic targets and as soon as to get rid of such dreadful disease. CCL2–CCR2 pathway helps the cancerous cell to progress and metastasize fully. In this chapter, we will focus on this particular axis and its relation to various cancers and how this axis is used as a therapeutic target.

Keywords

Cancer · Tumor · Macrophages · Chemokines · Cytokines · Receptors · Ligands

9.1 Introduction

A family of small proteins known as chemokines attach onto G-protein-coupled chemokine receptors (GPCRs) which are produced on the surface of the cells to carry out given intended tasks (Sokol and Luster 2015; Hughes and Nibbs 2018;

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Bhusal et al. 2020). Chemokines have been originally defined as agents which promoted immune cell recruitment and invasion into particular inflammatory response areas (Mir 2015a, b). Multiple researches show that these small protein molecules take part in several physiological activities particularly in the growth of numerous invasive cancers (Ono et al. 2003; Mukaida and Baba 2012). These molecules also combine with the cancer cells and microenvironment, thus assisting cancer growth and development (Hanahan and Weinberg 2011). Nowadays, various therapeutic strategies that target chemokines as well as chemokine receptors have been formulated that show better results than available drugs (Xu et al. 2021; Qayoom et al. 2023). In humans, overall 50 chemokines and 19 chemokine receptors have been recognized (Balkwill 2004). There are four subtypes of chemokine proteins including CX3CR, CC, C, and CXC. This classification is based on the number and position of the conserved cysteine residues (Sokol and Luster 2015; Arimont et al. 2017). It has been seen that maximum number of these protein families belong to the CC and CXC subtypes. There are five different monocyte chemoattractant proteins (MCPs) known as CCL2, CCL8, CCL7, CCL13, and CCL12 specified as MCP-1, MCP-2, MCP-3, MCP-4, and MCP-5, respectively. These five proteins belong to the CC-chemokine subtype (Chemokine 2002; Yadav et al. 2010). Out of all these MCPs, CCL2 possess same sequence homology with other MCPs. For instance, CCL7 and CCL8 show nearly 62% and 71% similarity with CCL2 accordingly (Van Coillie et al. 1999).

CCL2 was first identified and extracted out from culture broth of human cancer cell lines and mononuclear cells from peripheral blood in 1989 (Matsushima et al. 1989; Yoshimura et al. 1989a, b). The very first identified and extensively studied CC chemokine is CCL2, which primarily binds to CCR2 receptor (Van Coillie et al. 1999). Past research suggested that CCL2–CCR2 signaling pathway influenced abnormal vasculature, malignant cells persistence as well as expansion, and the attraction of immunosuppressive cells (Kulbe et al. 2004; Lim et al. 2016; Yoshimura and immunology 2018). Hence, both CCL2 and CCR2 provide us ability to investigate the complex mechanisms behind tumor development as well as offer prospective treatments against various types of human cancers. The tumor microenvironment, which has been identified as being a primary cause of cancer, is regulated by chemokines and cytokines (Mehraj et al. 2021a, b; Xu et al. 2021). It has been seen that the cytokines and the chemokines are the key controllers in tumor microenvironment and is considered as one of the indicative driver of cancer or tumor (Hanahan and Weinberg 2011; Mir et al. 2022a, b). Cytokines released by the cancerous cells commonly modify the molecular makeup of the tumor environment in favor of tumor growth (Hanahan and Weinberg 2011; Qian et al. 2011; Mehraj et al. 2021a, b). One of the basic hallmarks of tumorigenesis is inflammation, and a subtype of cytokines commonly called chemokines transport those immune cells that trigger the process of inflammation (Rani et al. 2019). Chemokines cause cellular migration, which is the continuous movement of cells expressing the right chemokine receptors in the direction of increased regional levels of chemokine ligands. The inflammatory reaction is brought on by chemokines, which direct a range of immune cells to the site of tumor formation (Mehraj et al. 2022b; Rani et al. 2019).

Chemokines play important roles in the growth, invasion, vasculature, and metastasis of several kinds of cancers, all of which aid in the advancement of malignancies (Balkwill 2004). Breast, mammary, ovary, and prostate cancer were linked to higher growth and expansion when chemokines like IL-8, CXCL1 (Chemokine (C-X-C motif) Ligand 1), CCL2 (Chemokine (C motif) ligand 2), and CXCL5 concentrations were increased (Begley et al. 2008; Singh and Lokeshwar 2009; Fader et al. 2010; Zhang et al. 2010a, b; Dutta et al. 2018). Along with cancerous cells, numerous cells in the cancer microenvironment such as leukocytes, fibroblast cells, endothelial cells, and adipocytes also produce chemokines and cytokines that aid in cancer development and expansion (Huang et al. 1994; Negus et al. 1995; Hefler et al. 1999; Ueno et al. 2000; Hemmerlein et al. 2001; Ohta et al. 2002, 2003; Niiya et al. 2003; Balkwill 2004; Valković et al. 2005; Cackowski and Roodman 2007; Dwyer et al. 2007; Soria and Ben-Baruch 2008; Soria et al. 2008; Cai et al. 2009; Lu et al. 2009; Tanaka et al. 2009; Vindrieux et al. 2009).

Progressive malignancy, metastasis, and recurrence have also been demonstrated to be connected to the over expression of CCR2 (Nagarsheth et al. 2017). It has been seen that whenever, there is an increased expression of the CCL2, an increase in the growth of various cancers occurs such as mammary cancer (Soria and Ben-Baruch 2008; Soria et al. 2008), ovary cancer (Negus et al. 1995), esophageal cancer (Ohta et al. 2002), gastric cancer (Ohta et al. 2003), kidney cancer (Hemmerlein et al. 2001), lung cancer (Niiya et al. 2003), colon cancer (Huang et al. 1994) as well as papillary thyroid cancer (Tanaka et al. 2009). CCL2 amplification is linked to metastasis, tumor growth, and vasculature in breast cancers (Valković et al. 2005; Mehraj et al. 2022a, b) as well as foretells its outcome and relapse (Ueno et al. 2000). Increased osteolysis and also the production of bone matrix-bound growth factors, such as PDGF, FGF-1, and TGF-β, were caused by CCL2 amplification in breast carcinoma bone metastasis (Cackowski and Roodman 2007). Additionally, numerous investigations showed that individuals having mammary, ovary, and pulmonary cancer had higher serum CCL2 levels, which has been linked to the disease stage (Hefler et al. 1999; Dwyer et al. 2007; Cai et al. 2009). It has been observed that prostate cancer is the second main cause of death among cancer patients in the United States and this type of cancer is one of the leading type of cancer in the United States (Crawford 2003). From the previous 10 years, it has been observed that the mortality of prostate cancer has continued to rise among senior males (Jemal et al. 2007). The aberrations of several cytokines and growth factors as well as other variables like the alteration and/or proliferation of the androgen receptor as well as other oncogenes or the suppression of tumor suppressors, all contribute to the advancement and spread of prostate cancer (Heinlein and Chang 2004; Pienta and Bradley 2006; Saraon et al. 2011). Two very different primary and progressed prostate cancer cells have been found to overexpress CCL2 itself and primary receptors CCR2 (Zhang et al. 2010a, b). It has been observed in 39 patients having prostate cancer by Lu et al. that when CCL2 concentrations elevate there is an close interrelation with different phases of bone cancer, so here it is possible that we can use CCL2 as foretelling biomarker of the different stages of the cancer (Lu et al. 2009; Jan et al. 2023).

9.2 Biological Characteristics of CCR2 and CCL2

CCL2, a member of the class of small molecules known as chemokines, is a low-molecular-weight cytokine having chemotactic action. The prototypical cytokine CCL2 is ubiquitously expressed for physiological purposes, including controlling leukocyte transport from the bloodstream to lymphatic system, and is activated in inflammatory processes where immune complexes are needed for body defense and restoration (Luster 1998). The gene that is responsible for CCL2 is located on the chromosome number 17 and it encode a basic protein composed of 99 amino acids that finally forms a protein composed of the 75 amino acids (Loberg et al. 2007a, b). Originally, CCL2 is reported as a "tumor-derived chemotactic factor," as well as it has since been discovered as a powerful chemotactic agent for a variety of cells like macrophages, NK cells, T cells, as well as immature DCs. As a result of this, CCL2 is thought to be a key mediator of neoangiogenesis and several other proinflammatory consequences (Valente et al. 1988; Allavena et al. 1994; Carr et al. 1994; Sallusto et al. 1998; Peters et al. 2000; Conti and Rollins 2004; Condeelis and Pollard 2006). CCL2 is synthesized by a variety of cells, such as capillaries, epithelial, hematopoietic cells, muscle cells, and fibroblast cells, whether continuously or even after stimulation. It's a powerful chemotactic agent for monocytes, dendritic cells, leucocytes, and natural killer cells (Deshmane et al. 2009; Mir and Mehraj 2019). Basically, CCL2 was classified as MCP-1 because it possess the same structure as other MCPs, like MCP-2, -3, -4, and -5, respectively (Bachelerie et al. 2014a, b). CCL2 predominantly binds with the CCR2 receptor, and this CCR2 receptor is exhibited by several tissues such as spleen, thymus, kidney, heart, brain, blood, lung, liver, ovary, pancreas, spinal cord, etc. Because of the substitutive splicing, CCR2 is categorized into two basic forms: CCR2A and CCR2B, and these two isoforms differ only by 50 bp at C-terminal (Charo et al. 1994). As instance, the growth of the breast gland and the evolution of mammary cancer are both influenced by the interaction between CCL2 and CCR4 (Xiong et al. 2020). As a result, these are repeatedly alluded to as "scavenger receptors" (Bachelerie et al. 2014a, b; Lokeshwar et al. 2020). Apart from this, it has been concluded via various researches that CCR2 is the primary receptor of the CCL2 (Boring et al. 1997). CCR2 is a member of the G-protein-coupled receptor family and possesses seven unique membrane-spanning regions in addition to its N-terminal extracellular domain (Charo et al. 1994; Apel et al. 2019). Numerous inflammatory and neurological disorders, including atherosclerosis, sclerosis, lung allergies, nerve pain, nephrotic syndrome, and cancers, are linked to the CCL2–CCR2 signaling pathway (O'Connor et al. 2015; Lim et al. 2016), due the this fact we can use such pathway as a powerful target for treating such severe disease types.

9.3 CCL2 Amplification by the Activation of the AKT-STAT 3 Signalling Pathway and Its Relation to Cancer Metastasis

By triggering the AKT-STAT3 signaling pathway, elevated PIPKI expression in CRC cancer boosted CCL2 activation and promoted macrophage infiltration (Mir et al. 2022a, b). PIPKI is primarily a phosphoinositide-producing enzyme that is heavily engaged in phosphoinositide metabolism. PIPKI expression is frequently upregulated in primary cancer types. PIPKI has earlier been demonstrated to be increased in pancreatic cancer cell lines, suggesting a pathogenic function for PIPKI in cancerous transformations (Chen et al. 2017). Furthermore, pY639-PIPKI levels are much higher in invasive ductal carcinoma, indicating the relevance of PIPKI in tumor growth (Bunney and Katan 2010). PIPKI plays a key role in a range of biological processes, including focal adhesion construction (Bunney and Katan 2010), ciliogenesis, centriole duplication, and recruitment of leukocytes (Stadtmann et al. 2015). PIPKI has been linked to a variety of oncogenic characteristics, including cell proliferation (Thapa et al. 2015; Li et al. 2017), migration (Choi et al. 2013), invasion (Chen et al. 2015), and epithelial-to-mesenchymal conversion (Thapa et al. 2017) . PIPKI expression levels were shown to be positively linked with greater macrophage infiltration in TCGA cohorts, indicating that PIPKI may operate as a novel regulator of the immunosuppressive milieu in colorectal cancer. In human malignancies, the PI3K/Akt signaling pathways are frequently active (Liu et al. 2009). The production of PI (Aras and Zaidi 2017; Cohen et al. 2019) P3 via PI3K-mediated phosphorylation of PI (Aras and Zaidi 2017; Cohen et al. 2019) P2 initiates these pathways. Thapa et al. previously demonstrated the method by which PIPKI connects with PI3K to initiate PI3K/Akt signaling. Notably, PI3K/Akt signaling and the mTORC1 complex downstream are important STAT3 regulators (Bromberg et al. 1999; Cairns et al. 2011; Mir et al. 2020). The PTEN/mTOR/STAT3 pathway is essential for the survival and maintenance of cancer stem-like cells in breast cancer (Zhou et al. 2007; Sofi et al. 2022). Leukemia cell migration and invasion can be significantly reduced by blocking AKT-mTOR-STAT3 signaling with glycyrrhizic acid (He et al. 2015).

During the initial stages of metastases, cancerous cells acquire an invasive and migratory nature that enables them to destroy the adjacent extracellular matrix (ECM), penetrate nearby tissues, and migrate toward blood or lymph arteries. CCL2 has a role in this early stage by directing cancer cell metastasis by connecting with the CCR2 receptor expressed on tumor cells (Mir and Gul 2022; Monti et al. 2003; Chiu et al. 2012). Furthermore, CCL2 promotes the production of the metalloproteinases MMP2 and MMP9 in cancerous cells, resulting in enhanced invasion (Dagouassat et al. 2010; Tang and Tsai 2012). In a mouse glioma and human glioblastoma xenograft model, inhibition of both tumor and stromal generated CCL2 resulted in a prolongation of mice's survival and a commensurate reduction in TAMs and MDSCs (Zhu et al. 2011).

Several BC cell lines, including 4T1, 4T07, and 67NR, as well as hematopoietic and nonhematopoietic cells in the tumor stroma, express CCL2 at high levels (Rego

et al. 2013; Yoshimura et al. 2013). Through the activation of Smad3 and p42/44 MAPK signaling, CCL2 was found to boost the migration and survival of 4T1, PyVmT, MDA-MD-231, and MCF-7 cells as well as the migration of the breast cancer cell lines MCF-7, T47D, and ZR-75-1 (Youngs et al. 1997). Unexpected negative consequences may result from targeting CCL2–CCR2 signaling, a recent research found. In four mice models of metastatic breast cancer, stopping or interrupting CCL2 inhibition increased metastasis and accelerated mortality. This result was due to increased cancer cell mobilization from the malignancy, accelerated angiogenesis, and increase in number of the metastatic cells.

9.4 CCL2–CCR2 and Cancer Progression

CCL2 (MCP-1) is a member of the CC chemokine family (Deshmane et al. 2009). The earliest description of CCL2 is referred described as a "tumor-derived chemotactic factor" shown to be an effective chemoattractant for a variety of immune cells, such as monocytes and natural killer cells, immature dendritic cells, and memory T cells, therefore triggering a variety of inflammatory effects and neoangiogenesis (Sallusto et al. 1998; Conti and Rollins 2004). CCL2 binds to G-protein-coupled receptor CCR2, to carry out its activity (Zheng et al. 2016). The cross-talk among macrophages, MDSCs, and dendritic cells decreases the ability to enhance the tumor-reactive T cells, creating a strongly immunosuppressant environment that allows for immune surveillance and ongoing cancer growth. This is because the inflammatory TME includes a variety of host cells that are chemoattracted and stimulated by contributing factors of the tumor cells (Ostrand-Rosenberg et al. 2012; Qayoom et al. 2023; Wang et al. 2017).

Numerous inflammatory and neurological disorders, including as atherosclerosis, are linked to the CCL2–CCR2 signaling axis. Consequently, it is investigated in relation to conditions like cancer, neuropathic pain, diabetes, multiple sclerosis, and asthma (O'Connor et al. 2015; Lim et al. 2016), as a possible therapeutic target for various illnesses. Cross-talk among monocytes, MDSCs, and DCs decreases the opportunity to activate tumor-reactive T cells, creating an environment for immune escape and continued tumorigenesis (Table 9.1). The inflammatory tumor microenvironment, which is composed of varied host cells that are chemo attracted as well as stimulated by influencing factors by the tumor, produces a highly immunosuppressant atmosphere (Ostrand-Rosenberg et al. 2012; Wang et al. 2017). Macrophages are a population of myeloid cells that have undergone terminal differentiation as well as tissue-resident cells that are descended from monocytes that circulate in peripheral circulation (Italiani and Boraschi 2014). By controlling adaptive immunity, getting rid of infectious diseases, and speeding up wound healing in healthy people, macrophages help hosts survive (Mosser and Edwards 2008). Numerous studies show that macrophages are utilized to encourage tumor formation when malignancy is present in both mice and people (Mantovani and Sica 2010; Qian et al. 2011). The intricacy of this cell population is illustrated by the continuous range of phenotypically diverse subpopulations seen in the macrophage

Table 9.1 Representation of the relation between CCL2–CCR2 axis and the various types of cancers

S. No	Type of cancer	Role of CCL2–CCR2 axis
01.	Prostate cancer	Promotes cell proliferation, migration, as well as shields such cancer cells from autophagy via the Akt phosphorylation-dependent pathway.
02.	Breast cancer	After the interaction of the CCL2 with CCR2, it promotes cellular migration, as well as recruitment of the immunosuppressive cells toward the TME thus favors cancer progression and metastasis.
03.	Colorectal cancer	It promotes cellular infiltration, as well as recruitment and migration of the immunosuppressive cells toward the TME thus favor cancer progression and metastasis.
04.	Other cancers	Promotes the metastasis in case of various cancers Spreads tumorigenicity Promotes angiogenesis.

population in their tissue microenvironment (Gabrilovich et al. 2012). To distinguish between the various macrophage functional states, the terms "M1 macrophage" and "M2 macrophage" were developed (Mantovani et al. 2002). TAMs increase malignancy and advance multiple critical aspects of tumor growth through immunological and nonimmune processes, such as vasculature, mobility, migration, and T-cell activity suppression (Qian and Pollard 2010). TAMs are also known to diminish the effects of treatments like chemotherapy, radiotherapy, and antiangiogenic drugs (Qian and Pollard 2010; Steidl et al. 2010; Gupta et al. 2018). Aside from cancer, macrophages have been linked to the advancement of a variety of other disorders, including asthma, allergic inflammation, and rheumatic inflammatory diseases (Roberts et al. 2015; Rana et al. 2018). MDSCs, immature myeloid cells that inhibit the immune system, are almost always enhanced in cancer patients and laboratory mice (Ostrand-Rosenberg et al. 2012). A diverse population of immature myeloid cells from the myeloid lineage makes up MDSCs, which are distinguished by coexpression of CD11b and Gr-1. Mice with tumors lack expression of CD11b and Gr-1 as well as characteristics of mature macrophages and dendritic cells. In mice with transplantable and spontaneous tumors, Marvel et al. discovered that stimulation of the immature myeloid cells via a network of regulatory pathways causes the accumulation of MDSCs (Marvel and Gabrilovich 2015; Groth et al. 2019). MDSCs are treated unfairly compared to other myeloid cell types, and they contain, rather than immunostimulatory qualities, severe immunosuppressive actions, interactions between MDSCs to control their activities, immune cell types such as T cells, dendritic cells, macrophages, and natural killer cells (Mir and Mehraj 2019). Growing data indicates that MDSCs contribute to tumor development and immune evasion in cancer via modulating innate immune responses and T-cell antitumor activities, as well as through suppressive nonimmune mechanism (Yang et al. 2004; Nagaraj et al. 2010; Mir 2015a, b). MDSCs as well to accelerate angiogenesis and subsequently encourage tumor development and metastasis through the cytokine expression (Singh et al. 2017). Blood MDSC counts and stage

and metastatic load are correlated in several malignancies (Diaz-Montero et al. 2009) (Fig. 9.1).

According to earlier research, the association of tumor cells with the tumor microenvironment particularly altered cells and immune cells that infiltrate the area, and considerably promotes the development of malignancy (West et al. 2015; Aras and Zaidi 2017). Therapies including PD-1/PD-L1 checkpoint blockade (Cohen et al. 2019) as well as chemokine modulation had completely transformed the immune system's interactions with tumor in terms of rejections or, at the very least, have slowed proliferation (Topalian et al. 2015). But only 20–30% of individuals benefit from immunotherapy therapy (Singh et al. 2015) According to earlier research, immune cell activity and the immunological microenvironment might both be altered by cancer cells. Tumor-associated macrophages (TAMs), which are mostly found in the body, are the most crucial component in this process. TAMs

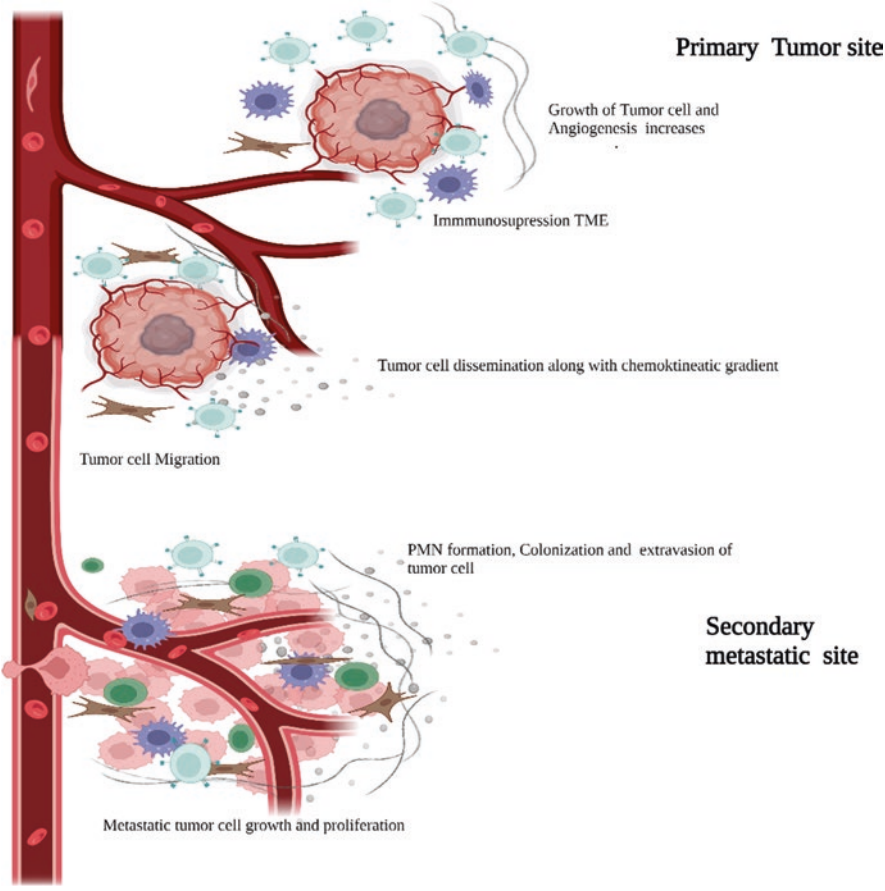

Fig. 9.1 Representation of cancer metastasis by the help of various TME cells and their pathways

may have immunosuppressive properties. Cytokines/chemokines are released, checkpoints ligands are expressed by TAMs and triggering the death of cytotoxic T cells, which cause immunological evasion and immunosuppression activity (Mir et al. 2022b). PIP5K1C encodes type I phosphatidylinositol phosphate kinase (PIPKI), an essential enzyme that controls the production of PI4, 5P2, which is important for many biological functions (Heck et al. 2007; Xue et al. 2017). It has been shown that PIPKI controls cell migration in a variety of ways, including through the EGF receptor (EGFR), which is phosphorylated by receptor tyrosine kinases at position Y639 (RTKs) (Li et al. 2017; Thapa et al. 2017). Through a direct interaction with talin, PIPKI may be able to control the development of neoplastic adhesions at the front edge (Li et al. 2019). Additionally, E-cadherin-based intercellular adhesions may be reformed, and epithelial polarization could be restored by PIPKI binding to AP2, an adaptor of E-cadherin to clathrin (Kahlfeldt et al. 2010). In fact, recent research demonstrates that elevation of PIPKI expression is adversely correlated with patients' overall survival across a range of cancer types (Sun et al. 2010; Chen et al. 2017; Sofi et al. 2023). However, it is still unknown how PIPKI affects the development of the tumor immunosuppressive microenvironment.

9.4.1 CCL2–CCR2 and Prostate Cancer

Prostate cancer cell lines (PC3 and LnCaP) and endothelial cells in the microenvironment of prostate tumors are the main sources of CCL2 expression. CCL2 boosts PC3 and VCaP prostate cancer cells by directly increasing their number and recruiting to other sites through stimulation of first PI3K/Akt and then RacGTPase correspondingly (Loberg et al. 2006). According to a different but related study, PKC was required for CCL2 to increase PC3, LnCaP, and DU145 prostate cancer cells' recruitment to another site. This was accomplished by increasing the expression of the avβ3 integrin on cancer cells (Lin et al. 2013). By triggering the PI3K/Akt/survivin pathway, CCL2 shields prostate cancer cells from autophagic death also enhance their number and recruitment to other site (Roca et al. 2008). Prostate malignancies frequently metastasis to the bone, and inhibiting both tumor and stromal-derived CCL2 had dramatic impacts on the reduction of metastatic lesions of the bone (Loberg et al. 2007a, b; Lim et al. 2009). This conclusion was supported by the observation that CCL2 overexpression in PC3 cells led to an increase in metastatic bone lesions and a parallel rise in the quantity of activated osteoclasts and infiltrating macrophages (Mizutani et al. 2009). CCL2 may also influence bone resorption by stimulating the development of osteoclast-like cells, according to Lu et al. (2007). It has also been demonstrated that CCL2 targeting, either alone or in conjunction with docetaxel, inhibits prostate cancer cell proliferation in the bone (Qian et al. 2010).

9.4.2 CCL2 CCR2 and Breast Cancer

Along with hematopoietic and nonhematopoietic cells in the stroma of the tumor, some breast cancer cell lines, like 4T1, 4T07, and 67NR, express CCL2 at high levels (Rego et al. 2013; Yoshimura et al. 2013). Through the activation of Smad3 and p42/44 MAPK signaling, CCL2 has been demonstrated to facilitate the movement of the MCF-7, T47D, and ZR-75-1 (BC cell lines) (Youngs et al. 1997) , as well as survival and movement of 4T1, PyVmT, MDA-MD-231, and MCF-7 cells (Fang et al. 2012). Aside from its direct impact on tumor cells, CCL2 derived from tumor and stroma promoted metastatic development by attracting several subsets of myeloid cells such as tumor-associated microphages (Saji et al. 2001), MAMs (Kitamura et al. 2015), and monocytes responsible for inflammation (Silzle et al. 2003). Mammary tumor tissue transplant induced macrophage recruitment, and inhibition of CCL2 of stroma with a defensive antibody dramatically decreased infiltration of macrophage and progression of tumor (Fujimoto et al. 2009). The reduced infiltration of macrophages and angiogenesis due to lack of CCL2 in the stroma of tumor, which in turn lowered the frequency of lung cancer (Yoshimura et al. 2013). The impact of stromal CCL2 may surpass that of tumor-derived CCL2. Although the processes are not yet fully understood, the CCL2-derived macrophage infiltration may also result in significant formation of blood vessels in breast tumors (Campion et al. 2009; Arendt et al. 2013), indicating that tumor recruiting myeloid cells might be directly involved in the process of angiogenesis. CCL2 expression from breast cancer cells is enhanced by Twist 1 (Low-Marchelli et al. 2013), and CCL2 is responsible for recruitment of macrophages . MCP-1 along with macrophage are responsible for Twist 1-mediated blood vessels formation. Tsuyada et al. (Tsuyada and Wang 2013) discovered that cocultured human CAFs with BT474, MDA-MB-361, and MCF7 increased CCL2 in malignant cells. Through the STAT3 and NOTCH1 signaling pathways, MCP-1 increased the autorenewing and growth of mammosphere-initiating cancer stem cells, indicating that MCP-1 may give increased truculent stem cell-like malignant attribute. Through STAT3 and NOTCH1 signaling pathways, MCP-1 increases autorenewal and growth of malignant stem cells.

9.4.3 CCL2–CCR2 and Colorectal Cancer

Usually, colorectal tumors travel to the liver, but they can also do so to the lungs, peritoneum, as well as other tissues. When CCL2 expression in cancer cells was knocked down, it temporarily reduced myeloid cell infiltration and temporarily slowed the formation of metastatic tumors in the experimental liver metastasis animal model. However, these differences were not noticeable two weeks after tumor cell injection (Zhao et al. 2013). The outcome of CCR2 inhibition was also unsatisfactory; infiltration of the myeloid cell and the development of metastatic liver tumors were only marginally inhibited in CCR2 knockout mice, indicating that CCL2 may bind chemokine receptors other than CCR2 to promote the progression

of metastasis (Zhao et al. 2013). MCP-1 derived from tumor has been demonstrated to have impact directly on the vessel formation in tumor region, along with infiltration of myeloid cell. In a p38/MAPK-dependent way, CCL2 binding to $CCR2^{+}$ endothelium increased blood vessels penetration and enhanced the extravasation and lung metastasis of colon cancer cells (Wolf et al. 2012).

9.4.4 CCL2–CCR2 and Other Cancers

Several malignancies' and their metastasis has been linked to CCL2–CCR2 signaling. CCL2 increased chondrosarcoma cell invasion and migration in chondrosarcoma, that predominantly proliferates to the lungs, most likely by promoting MMP9 synthesis via Ras/Raf-1 and activating Nuclear factor-κB (Tang and Tsai 2012). Enhanced expression of CCL2 in the gastric cancer cell line TMK-1 dramatically boosted tumorigenicity and caused spread to lymph nodes in ectopic and orthotopic xenograft models of gastric cancer. Cancer cells that overexpressed CCL2 also produced tumors that were more angiogenic than healthy controls (Kuroda et al. 2005). Same results were seen in studies of bladder cancer, where inhibition of MCP-1 or CCR2 prevented T24, J82, and SV-HUC-1 cancer cells from infiltration, decreased production of tumors (Chiu et al. 2012), and prevented versican from promoting the formation of lung metastases (Said et al. 2012). Aside from its direct effects, CCL2 generated by fibrocytes has been linked to the attraction of Ly6C monocytes, which makes B16F10 cells more likely to metastasis to the lung (van Deventer et al. 2013). It has been demonstrated that CCL2 produced by melanoma cells attracts cytotoxic T lymphocytes by interacting to the CCR4 receptor on the lymphocytes (Zhang et al. 2006). In fact, enhanced expression of MCP-1 in B16 melanoma cells increased the production of cytokines from type II helper T cell and decreased the growth of metastatic lung tumors in wild-type C57BL/6 mice, but not in nude mice with impaired acquired immune responses (Hu et al. 2007), suggesting that T cells may be involved in CCL2-mediated antimetastatic effects. As a result, the effects of CCL2 may rely on the binding of a particular receptor (CCR2 as opposed to CCR4) as well as the kind of effector cells involved.

9.5 CCL2–CCR2 and TME

From the last decades, many carcinogenic studies basically concentrated on the metastatic cancer cells and the genes and their regulation that is mainly related to such cancer types. From few years back, the idea of tumor microenvironment (TME) came into being and this concept was extensively acknowledged (Mira and ul Haq 2022). The continuous relation between TME and tumor play a significant role in carcinogenesis. On the other hand, these malignant cells consists of the wide variety of cells including mesenchymal stem cells, fibroblast growth factors, endothelial cells and various invading WBCs (Turley et al. 2015) (Fig. 9.2). Many of

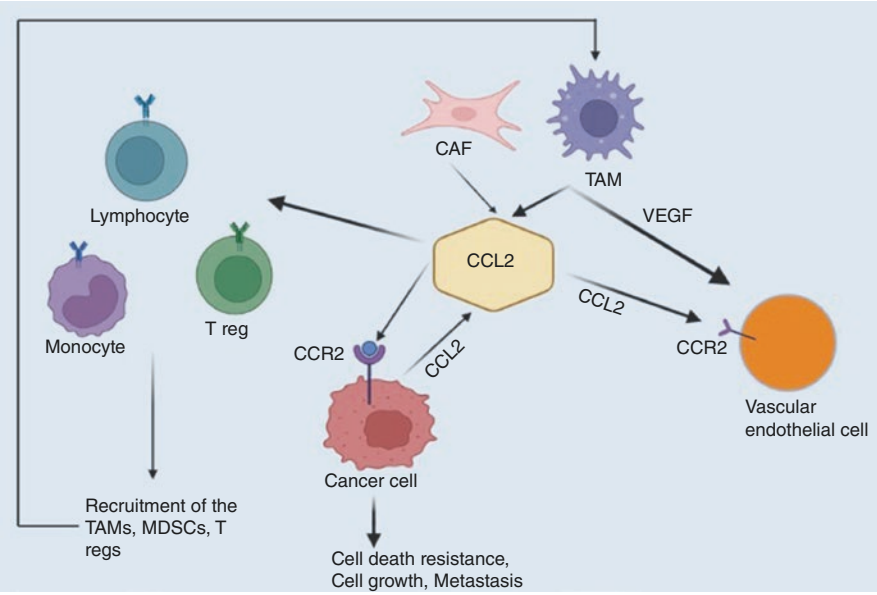

Fig. 9.2 Representation of various cells of the TME and their role in cancer

Table 9.2 Representation of the various cells of the tumor microenvironment and the role of CCL2–CCR2 pathway in tumor microenvironment

S. No.	Cell from TME	Role CCL2–CCR2
01.	Monocytes	CCL2–CCR2 axis assists the infiltration of the TAMs toward the TME
02.	Neutrophils	CCL2 secreting TANs are majority seen in the tumor sites and aid in tumor formations.
03.	Myeloid-derived suppressor cells	CCL2–CCR2 pathways provide functional specialization to these cells This pathway also increases the number of MDSCs inside the TME
04.	T-lymphocytes	CCL2–CCR2 pathway promotes the proliferation and maturity of Th2 effector cells by decreasing the production of IL-12 through APCs Also increases the production of IL-4 by the activated T cells
05.	Fibroblasts	CCL2 promotes the production of the cancer-associated fibroblasts CCL2 production from the CAFs promotes the formation of cancers

these cells have the capability to stimulate the expressions of the CCL2 as well as participates in the cancer metastasis as well as cancer growth. Here we will focus mainly on the CCR2–CCL2 pathway and also noncancerous TME cells including immune cells and FGFs (Xu et al. 2021) (Table 9.2).

9.5.1 Monocytes

Monocytes are attracted toward the areas of cellular injury, infections, and tumors by chemotaxis and develop into macrophages to take part in the body's immunological reaction. These cells divide into 2 subtypes : inflammatory macrophages and wound-healing macrophages celled as M1 and M2 respectively this division process is called as "macrophage polarization" (Funes et al. 2018). Generally, Ariginase-1 is used as a basic indicator to recognize the M2 macrophage, but this indicator is also prompted in case of M1 macrophages. That is why nowadays, researchers favor marker combinations so as to easily recognize macrophage subtypes (Mir et al. 2022; Murray et al. 2014). For example, the regulatory profile of mouse M1 and M2 macrophages was analyzed, and it was discovered that CD38, Gpr18, and formyl peptide receptor 2 (Fpr2) represent helpful indicators for M1, but Early Growth Response Protein 2 (Egr2) and c-Myc are M2 indicators (Jablonski et al. 2015). Functioning as proinflammatory cells, M1 subtype displays antitumor qualities. So opposed to M1, M2 macrophages perform an immunosuppressive function which promotes healing process and aids in the growth of tumors (Lin et al. 2019). Tumor-associated macrophages (TAMs) are a subtype of macrophages that are mostly distinguished of bone marrow-derived mononuclear cells and are gathered in the surroundings of tissue that have been infiltrated by tumors (Lin et al. 2019). Given that these TAMs perform comparable tasks to M2 subtype and promote tumorigenesis by causing immunological suppression, TAMs have been typically regarded as a specialized subtype of M2-like monocytes (Mantovani et al. 2002; Li et al. 2021). CCL2–CCR2 loop assists the migration of monocytes from the blood toward the cancer locations with the peripheral circulation, where these wandering monocytes are distributed separately to become TAMs (Chen et al. 2018; Li et al. 2021). Tumor-associated macrophages hasten the growth of tumors by causing immune evasion, and metastases. It has been revealed that in case of the esophagus cancer, high CCL2 expressing tissues exhibited a comparable elevation in the CD68 expressing cells as a basic marker of tumor-associated macrophages (Yang et al. 2020). Additionally, elevated PD-L2 production in TAMs promoted immunological escapes via the PD-1 axis. Numerous TAMs penetrate the body when colorectal cancer has spread to other organs like liver to promote the TAM-associated immunological suppression that drives the development of aggressive cancerous cells (Grossman et al. 2018). This is interesting to note that tumor-associated macrophages aid in tumor aggression and metastases by producing CCL2, in addition to the CCL2–CCR2 pathway engaging and modifying TAMs to advance tumors (Li et al. 2020). From such studies, it is revealed that the TAMs in the tumor environment aid in the tumor growth and progression to other tissues and finally to whole body.

9.5.2 Neutrophils

Neutrophils are thought of as innate immune system effectors since they are produced from a shared devoted hematopoietic precursor cells. These cells control

various numerous immunological functions like inflammatory responses, autoimmune reactions and cancers (Liew and Kubes 2019). These cells have been seen in the Tumor microenvironment of several malignancies in the meantime. Parallel to TAMs, tumor-associated neutrophils (TANs) contain pro- and antitumor heterogeneities that are controlled by the Tumor environment (Sionov et al. 2015; SenGupta et al. 2019). The substantial TAN invasion was found to be positively correlated well with oral squamous cell carcinoma's late stage and poor diagnosis. Via preventing CCL2 secretion by TANs, melatonin may slow the development of tumor (Lu et al. 2017). Moreover, it has been seen that the TANs secreting CCL2 are primarily seen in the primary cancer sites as well as associated lymph nodes (Fujita et al. 2017). From these studies, we can say that the TANs can aid in cancer progression via CCL2.

9.5.3 Myeloid-Derived Suppressor Cells (MDSCs)

These suppressor cells were originally characterized in the 1980s, and it is seen that they reside in the tumor microenvironment same as TAMs or TANs (Maman and Witz 2018). These cells are a subset of immature immunosuppressive cells that are generally generated out from the bone marrow and are also known for their ability to inhibit T-cell responses (Gabrilovich and Nagaraj 2009; Law et al. 2020). Apparently functional specialization of MDSCs depends upon the CCL2–CCR2 pathway. It has been observed that the CCL2 is the key player in case of colorectal cancer, and it promotes the growth of cancer and also increase the function and number of MDSCs. The stimulation of STAT3 as well as the generation of ROS in PMN-MDSC were the two mechanisms by which CCL2 controlled the T-cell-suppressive behavior that was facilitated by this cell type (Chun et al. 2015). Additionally, the migration of monocytic MDSCs to cancerous tissue as well as the invasive microenvironment was controlled by the CCL2–CCR2 loop (Ugel et al. 2015). Earlier researches shown that the CCL2–CCR2 pathway was crucial for attracting MDSCs toward the tumor microenvironment, thereby shielding cancerous cells from immune-mediated death (Do et al. 2020). Flow cytometry examination of the cancerous tissue in a mouse glioma model revealed that CCR2 is primarily confined to MDSC cells one week since GL261 cell lines had been introduced in animals (Chang et al. 2016). Additionally, MDSCs that are TME stopped the inflow of $CD8^+$ T lymphocytes. Additionally, high levels of $CD8^+$ T lymphocytes reconstituted antitumor resistance to stop the growth of malignancies by increasing the $CD8^+$ T-cell/MDSC ratios inside tumors made by CCL2 knockout mouse (Flores-Toro et al. 2020).

9.5.4 T Lymphocytes

Lymphocytes which display T-cell receptors are known as T cells (TCRs). Both $CD4^+$ helper (Th) lymphocytes and $CD8^+$ cytotoxic cells (CTL), which detect

proteins supplied by MHC I and MHC II, respectively, constitute subtypes of T lymphocytes (Ruffell et al. 2010). One of most prevalent and significant $CD4^+$ T-cell subsets are Th1 and Th2, in addition to other forms as well. Th1-derived mediators promote inflammation and have antitumor properties; however, Th2-secreted cytokines have anti-inflammatory properties and promote the growth of tumors (Ruffell et al. 2010; Chraa et al. 2019). Th2 polarization is linked to CCL2 secretion and production (Gu et al. 2000). The CCL2–CCR2 loop could promote the proliferation and maturity of Th2 effector cells via decreasing the production of IL-12 via APCs and increasing the production of IL-4 by activated T lymphocytes. Furthermore CCR2 could be expressed on T-helper 2 cells (Luther and Cyster 2001). Along with this, CCL2 produced from the tumor microenvironment have the capacity to aggregate the Th2 cells. In accordance with this property, scientists found that the proportions of Th1/Th2 were noticeably reduced inside the cancerous tissues possessing elevated CCL2 expressions (Mandal et al. 2018). Among all the types of cancers, there is an increase in the Treg cells (regulatory T cells). Tregs are a kind of $CD4^+$ T cells that can aid in the cancer growth and development via producing immunosuppressive results (Najafi et al. 2019). It has been demonstrated by various researches that the CCR2–CCL2 pathway is pivotal for the engagement of Tregs and also their growth inside the tumor microenvironment (Ge et al. 2019; Vasanthakumar et al. 2020). The increase in CCL2 following radiation therapy was implicated in tumorigenesis as well as further promoted the recruitment of CCR2-positive Treg in a mouse model of the neck and head squamous cell cancer (Mondini et al. 2019). Additionally, $CCR2^+$ Treg demonstrated strong immunosuppressive effects and was crucial in the inhibition of antitumor activities. Despite the fact that $CCR2^+$ Tregs accumulated in several types of cancers, it may be reduced by limited dose cyclophosphamide, allowing the identification of a potential candidate in enhancing chemotherapeutic efficacy (Loyher et al. 2016).

9.5.5 Fibroblasts

Among the most prevalent stroma elements in the tumor microenvironment is cancer-associated fibroblasts (CAFs). It must be commonly accepted that cancer-associated fibroblasts may be the outcome of the stimulation of nearby tissue-resident fibroblasts, even though their exact cause is still unknown (Chen and Song 2019; Sahai et al. 2020). It has been found by Shen et al. that microRNAs-mediated FOXO3a/VEGF/CCL2 signaling played a pivotal role. According to Shen et al., microRNAs-mediated FoxO3a/VEGF/CCL2 signaling significantly contributed to the transformation of typical fibroblast cells into the cancer-associated fibroblasts (Shen et al. 2016). The researchers also suggested that CCL2 overexpression might promote the formation of CAFs. It's interesting to note that CAFs promote cancer spread by producing CCL2. In cocultured cancer-associated fibroblasts, the amplification of nuclear factor kappa and STAT3 significantly enhanced the production of CCL2 (Li et al. 2014; Yang et al. 2016). Additionally, in pancreatic cancer, cancerous cells instruct fibroblasts by secreting IL-8 and CCL2, which results in the

production of cancer-associated fibroblasts and other metastasis-associated fibroblasts (MAFs) (Pausch et al. 2020). With all of these above researches it is clearly seen that CCL2–CCR2 pathway is elevated during the metastasis and growth of cancer as well as there is an intimate relation between CCL2–CCR2 axis and cancer development and progression. Thus, CCL2–CCR2 pathway can be used as a possible therapeutic target for the treatment of cancer (Mir et al 2022a).

9.6 CCL2–CCR2 Axis as a Therapeutic Target

It has been seen in individuals having BC, prostate cancer , gastric cancer, ovary cancers, colorectal cancers, etc. that the increased levels of the CCL2 relate directly to the development of cancer (Craig et al. 2006). Using statistical analysis of gene expression datasets regarding prostate carcinogenesis, CCL2 has been discovered as the main trigger of prostate cancer formation (Gorlov et al. 2010). There are multiple ways in which the CCL2–CCR2 signaling pathway is involved in a variety of malignancies, and growing scientific data is laying the groundwork for upcoming clinical studies (Fig. 9.3; Table 9.3). The CCL2–CCR2 pathway has also received a lot of interest and has shown remarkable antitumor action in preclinical research and clinical trials. In this section, we will mainly focus on the CCL2–CCR2 pathway and its implication as a therapeutic target against cancer diseases.

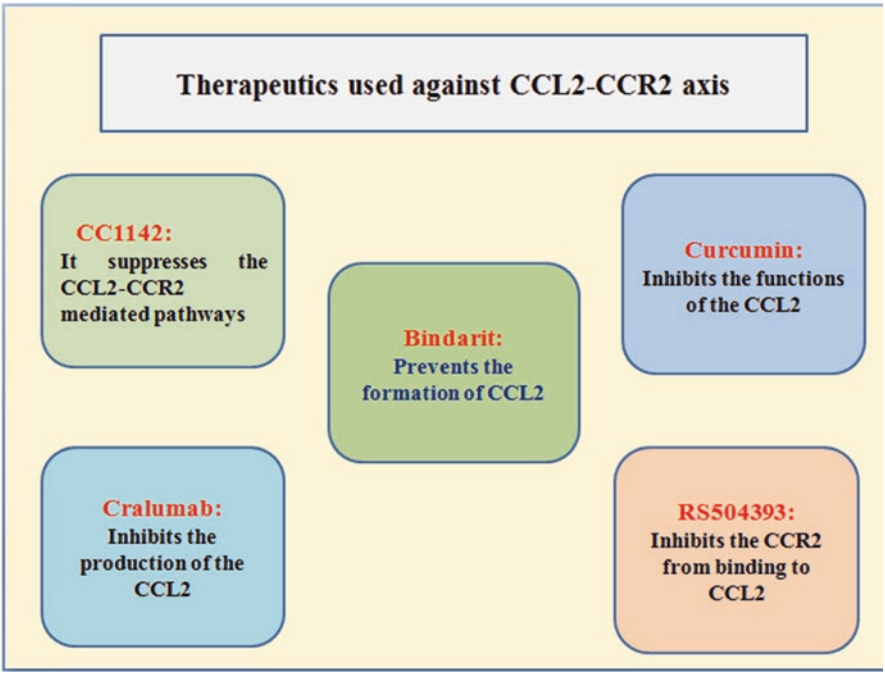

Fig. 9.3 Depiction of various therapeutic strategies against the CCL2–CCR2 pathway

Table 9.3 Depiction of various therapies used against CCL2–CCR2 signaling pathway

S. No.	Type of therapy	Type	Function
01.	CC1142	Murine chimeric antibody	Suppresses the CCL2-mediated signaling pathways Inhibits the cancer growth
02.	Bindarit	Small molecule	Prevents the formation of the CCL2, thus inhibiting the growth of tumors Hinders the endothelium organization Also degrades the tumor microenvironment by limiting MDSC and TAM invasion, thus inhibiting tumorigenesis
03.	Curcumin	Molecule obtained from curcumin	Suppresses the cancer cell growth as well as development of numerous cancers including breast cancer, brain cancer, prostate cancer, colorectal cancer, etc. Inhibits the functions of the CCL2 by suppressing the PKC Inhibits the production of the MMP9, thus inhibiting the growth of tumors
04.	Cralumab	Antibody-based therapy	Inhibits the CCL2 production by restricting the PC-3 cellular division and growth Also used in combination with the docetaxel to regress the growth of cancers
05.	RS504393	Selective CCR2 chemokine receptor antagonist	Slows down the process of tumorigenesis by preventing the immunosuppressive cells from colonizing the tumor locations Inhibits CCR2
06.	RS102895	Chemokine receptor antagonist	It binds on the extracellular side of the CCR2, thus inhibiting CCL2 signaling It also reduces the PC-3 prostate cancer cell infiltration up to 85% Downregulates the MMP9 activity that in turn decreases the proliferation and migration of the breast cancer cells
07.	TC1	CCR2b receptor antagonist	Inhibits the interaction between the CCL2 and CCR2 Inhibits the migration of the cancerous cells It also stops the development of the lung cancer metastasis
09.	Propagermanium	Organogermanium complex (Polymer)	Decreases angiogenesis Inhibits the cancer metastasis

9.6.1 Targeting CCL2–CCR2 Pathway

9.6.1.1 Suppression of CCL2

Numerous CCL2 blockers or antibodies have been employed in experimental investigations to slow the growth of tumors. By suppressing CCL2-mediated signaling pathways, preventing the infiltration of immunosuppressive cells, and raising the frequency of tumor-killing cells, can reduce tumorigenesis in a variety of ways.

C1142: 1142 is a murine chimeric monoclonal antibody that may effectively neutralize CCL2. This antibody has demonstrated promising effects in a variety of cancers, when used singly or in combination with the available drugs (Moisan et al. 2014). It has been seen in the glioma model that when C1142 therapy was administered, there was a significant reduction in the overall number of $CD11b^+$ $Gr1^+$ MDSCs and $CD11b^+$ $CD45^+$ TAMs; it thus inhibited the cancer growth as well as extended the lifespan of mice's with gliomas (Zhu et al. 2011). Additionally, C1142 reduced the proliferation by preventing the invasion of the tumor-associated macrophages along the tumor microenvironment (Loberg et al. 2007a, b). Additionally, C1142 prevented the growth of the tumors by increasing the hepatic natural killer cell cytotoxicity and elevated interferon gamma production (Teng et al. 2017, Mir et al. 2023b). The combination of CCL2 antibodies and also the vaccination may be a potential treatment option for cancers, as C1142 therapy increased $CD8^+$ T-cell counts and decreased tumor size (Fridlender et al. 2010).

Bindarit: An anti-inflammatory indazole derivative known as bindarit has the ability to suppress the growth of tumors by preventing the manufacture of CCL2. According to certain clinical studies, targeting CCL2 with bindarit causes tumor growth inhibition. It has been seen that this drug has the ability to hinder endothelium organization during the development of vascular system in order to prevent tumor growth (Gazzaniga et al. 2007). Additionally, bindarit degrades the tumor microenvironment by limiting MDSC and TAM invasion, therefore restricting tumorigenesis as well as slowing the course of the disease (Zollo et al. 2012; Liu et al. 2018). Moreover, it is also observed that the drug has the capability to inhibit the cancer growth as well as metastasis via negative control of the AKT and NF-κB signaling axis (Zollo et al. 2012).

Curcumin: It has been seen that a polyphenolic compound curcumin obtained from the Curcuma longa has the ability to inhibit the functions of the CCL2. In an experiment, it was observed that plasma CCL2 levels lowered after feeding the mouse having colon cancer with curcumin (Murphy et al. 2011). An essential cytoplasmic regulatory component referred to as PKC is thought to increase CCL2 release and is connected to cancer cell infiltration. Through the suppression of protein kinase C, curcumin dramatically reduces CCL2-induced cell invasions and movements. Additionally, this compound inhibits MMP9 production which is controlled by CCL2, which promotes the growth of tumors (Herman et al. 2009).

Cralumab: It is also called as CNTO 888. Cralumab is an antibody-based therapy against CCL2. It restricts the PC-3 cellular growth and progression in vitro. Researchers discovered that the two drugs named as carlumab and docetaxel when combined induce tumor regression very effectively than either drug individually. Up to date, several clinical trials has been carried out to check the efficacy of this particular drug in order to treat various cancerous diseases (Loberg et al. 2007a, b).

9.6.1.2 Suppression of CCR2

It has been observed that the levels of the CCR2 elevate in the cancerous cells as well as the immunosuppressive cells but the levels of CCR2 are comparatively low

in normal cells, suggesting that the CCR2 can be used as a powerful target in various cancers (Xu et al. 2021).

RS504393: It is a specific CCR2 inhibitor that slows the tumorigenesis via preventing immunosuppressive cells from colonizing tumor locations. It has been proved by the scientists that this particular inhibitor increased the life span of the mice having bladder cancer via obstructing the recruitment of the MDSCs toward tumor microenvironment (Mu et al. 2019). In tumor-bearing mice, overall quantity of tumor-associated macrophages and the tumor size decreased dramatically after treated with RS 504393 (Yang et al. 2019). indicating that RS504393 may be a promising cancer treatment by addressing CCR2 (Yang et al. 2019).

RS 102895: CCR2 inhibitor RS 102895 inhibits CCL2 signaling by occupying a binding site on the extracellular side of CCR2 (Mirzadegan et al. 2000). it has been observed that this inhibitor reduces the PC-3 prostate cancer cell infiltration from 197 to 85% (Lindholm et al. 2015). Additionally, MMP9 activity was downregulated by this particular inhibitor, which might substantially diminish the stimulatory effect of CCL2, which controlled the proliferation as well as migration of BC cells (Han et al. 2018).

TC1: TC1 (Teijin compound 1), designed to prevent the interaction of human CCR2 to CCL2 and acts as a specialized CCR2 inhibitor (Imai et al. 2004). Cancerous cells migration has been seen to be inhibited by TC1 in a trans-endothelial tumor cell migration assay. Along with reducing capillary permeability, this inhibitor also stopped the development of lung metastasis (Roblek et al. 2015).

747: A natural substance from Abies georgei with the designation 747 was previously noted by Yao et al. (2017). As a CCR2 inhibitor with selectivity and sensitivity, it could disrupt the CCL2–CCR2 pathway. While reducing the TAM trafficking as well as increasing $CD8^+$ T-cell counts, 747 demonstrated antitumor efficacy. Furthermore, when it is used in combination with sorafenib, it improves sorafenib's antitumor effectiveness. With such powerful results this drug could become a potential treatment option against various types of cancers by inhibiting CCR2 (Yao et al. 2017).

Antibody-Suppressing CCL2: On the premise that CCL2-neutralizing antibody could interact to CCL2 and prevent the migration and activation of monocytes, CCL2 is indeed the focus of interest. CNTO 888, called as Carlumab, is an antibody-based therapy acting against human CCL2 having the ability to bid with the human CCL2 and manifests wide range of antitumor activities preclinically (Obmolova et al. 2012). Forty-four individuals having metastatic solid tumors participated together in stage I research (NCT00537368) looking at the efficacy of carlumab in patients with solid tumors. Carlumab being typically adequately sustained as no dose-limited toxicities (DLT) was seen in the carlumab-treated participants. Unfortunately, neither of the 33 assessable individuals showed an unbiased response, although four individuals did have permanent stable disease (SD) (Sandhu et al. 2013). Another stage II trial of this particular antibody in case of castration-resistant prostate cancer (NCT00992186) shows that only 34% individuals sustained normal disease for about more than 3 months, and out of 44 diseased individuals, no one showed response. Conversely, the levels of median free CCL2 drastically reduced

from the baseline at 4 hours after carlumab treatment, but overall CCL2 levels upregulated up to thousand folds higher than baseline after 7 days (Pienta et al. 2013). In a similar fashion, scientists determined that carlumab suppressed CCL2 for just a brief period of time in an open-label, multicenter stage Ib trial (NCT01204996) but didn't cause overt antitumor effects (Brana et al. 2015).

Propagermanium: Organogermanium complex, a propagermanium (3-oxygermylpropionic acid polymer), usually inhibits the CCR2 signaling (Yumimoto et al. 2019). Twelve BC patients in aggregate have been participated in a stage I dose-escalation research. It is possible that propagermanium has the capability to inhibit vasculature and stop the spread of malignancy because propagermanium therapy caused a dose-dependent downregulation of the serum IL-6, that actually participated in tumorigenesis. Propagermanium would soon undergo a stage II Trial research to evaluate its antitumor properties (Masuda et al. 2020; Mir et al. 2023a).

There are several other drugs that had been discovered by the scientists some are under clinical trials and some are approved by the US FDA that can be used specifically against various cancers. In the near future, it is believed that potential and specific anticancerous drugs will be available that will drastically decrease the cancer rates and fatalities.

9.7 Conclusion

Cancer by name is the dreadful disease, and every individual in this whole universe is prone to this disease as there is no medicine in the world that can completely cure this specific disease. There are numerous pathways that are hijacked during this disease, and researchers and scientists are using all the pathways in order to curb such disease. CCL2–CCR2 also acts as a mediator pathway in cancer. This pathway helps in the growth and progression of the disease. CCL2 basically takes part mainly as a mediator of inflammation. Several evidences prove it that this CCL2–CCR2 pathway acts as a mediator in several diseases, especially cancers. The CCL2–CCR2 pathway promotes revascularization as well as several signal transductions that support tumor cell growth as well as expansion. So, this is the best therapeutic target to curb this disease and increase the survival rates of cancer patients. Shortly, we are expecting that the CCL2–CCR2 pathway inhibitors can become helpful drugs and therapies to increase the life span of the patients and also to eradicate this disease at certain levels.

Further Reading

For further readings, some textbooks mentioned below also give further understanding of cytokine and chemokine network.

- *Cytokines and Their Therapeutic Potential* by Manzoor Ahmad Mir.
- *The Role of Chemoattractants in the Tumor Microenvironment* by Giovanni Bernardini, Brian A. Zabel.

For more insights about the topic, we would suggest detailed findings from the books of Mir (2022) (https://doi.org/10.1016/C2021-0-02565-7 and https://doi.org/10.1016/C2022-0-00074-X), Mir (2021) (https://doi.org/10.52305/WXJL6770), and Mir (2015a, b) (https://doi.org/10.1016/C2014-0-02898-5) and from the cancer.net website on the following below-mentioned links:

https://www.cancer.net/cancer-types/breast-cancer/types-treatment

https://discovery.ucl.ac.uk/id/eprint/1472740/

https://www.jmedsciences.com/doi/JMEDS/pdf/10.5005/jp-journals-10045-00138

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CXCL9, CXCL10, CXCL11/CXCR3 Axis and Immune Activation

10

Manzoor Ahmad Mir, Tabish Javeed, and Ishfaq

Abstract

Chemokines are proteins that promote tissue extravasation, immune cell differentiation, and chemotaxis. Their applicability in the oncology is particularly fascinating because of these features. Despite the fact that immunotherapy has had clinical success with certain cancer patients, this is not always the case. In the recent years, chemokine signaling has been found as a potential technique for resistance analysis of checkpoint inhibitors. The chemokine axis (CXCL9, -10, and -11/CXCR3) has been found as a viable option that controls different immune-related function (immune cell activation, differentiation, and migration), which aids in suppression of tumor (paracrine axis). However, some information suggests that the other axis plays a part in the growth and spread of tumors (autocrine axis). Therefore, to develop effective cancer management, it is important to understand and comprehend CXCL9, -10, -11/CXCR3 axis. Therefore, the focal purpose of this book chapter is to assess and evaluate the recent trends and outcomes related to the CXCL9, CXCL10, and CXCL11/CXCR3 axis of the immune system and elucidate their impending therapeutic applications.

Keywords

Cancer · Chemokines · Immune activation · Chemokine signaling · Immunotherapeutic applications

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10.1 Introduction

Chemokines are smaller proteins (8–15 kD), that are essential for promoting chemotaxis, leukocyte differentiation, and extravasation of tissue (Eckmann and Bamias 2022; Mehraj et al. 2022a, b, c) (Fig. 10.1). Since then, there has been a lot of interest in learning more about how chemokines affect immune response. Because it regulates how naïve T cells develop into T helper 1 (Th1) cells and instructs immune cells to move to their focus regions (Raza et al. 2022). Therefore, this axis is considered significant for the effective use of the immune system. There is limited information available on the clinical outcomes for cancer patients, despite current evidence emphasizing its clinical value (Battaglia et al. 2022).

The CXCL9, -10, -11/CXCR3 axis controls the migration of cells within the immune system, diversity and differentiation, and stimulation (Zhiming et al. 2018). Immunological reactivity is brought about by the activation of immune cells such as macrophages, natural killer (NK) cells, natural killer T (NKT) cells, and cytotoxic lymphocytes (CTLs) (Qayoom et al. 2023; Wang et al. 2018). Additionally, this axis results in Th1 polarization, which activates immune cells with respect to IFN-γ (Alspach et al. 2019; Mir and Mir 2022) (Fig. 10.2). Different scientific investigations conducted in vivo have reported that this immune axis can sometimes have tumorigenic role and leads to further spread of the tumor. This has prompted the researchers for further investigations, to fully comprehend the role of such axis in tumor environment to determine if it serves as a possible immunotherapy target or a prognostic gauge for the use of existing cancer therapies (Gambardella et al. 2020).

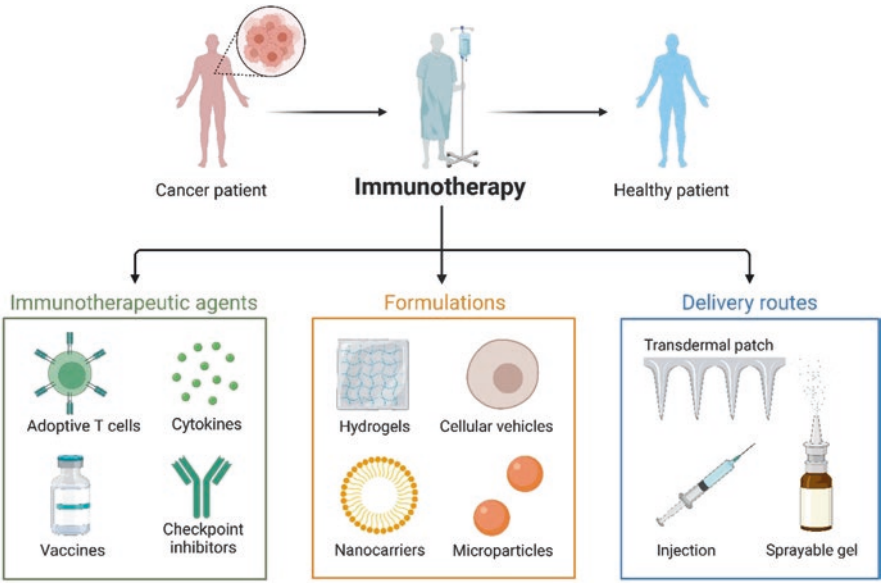

Fig. 10.1 General overview of Immunotherapeutic agents, formulations and delivery routes

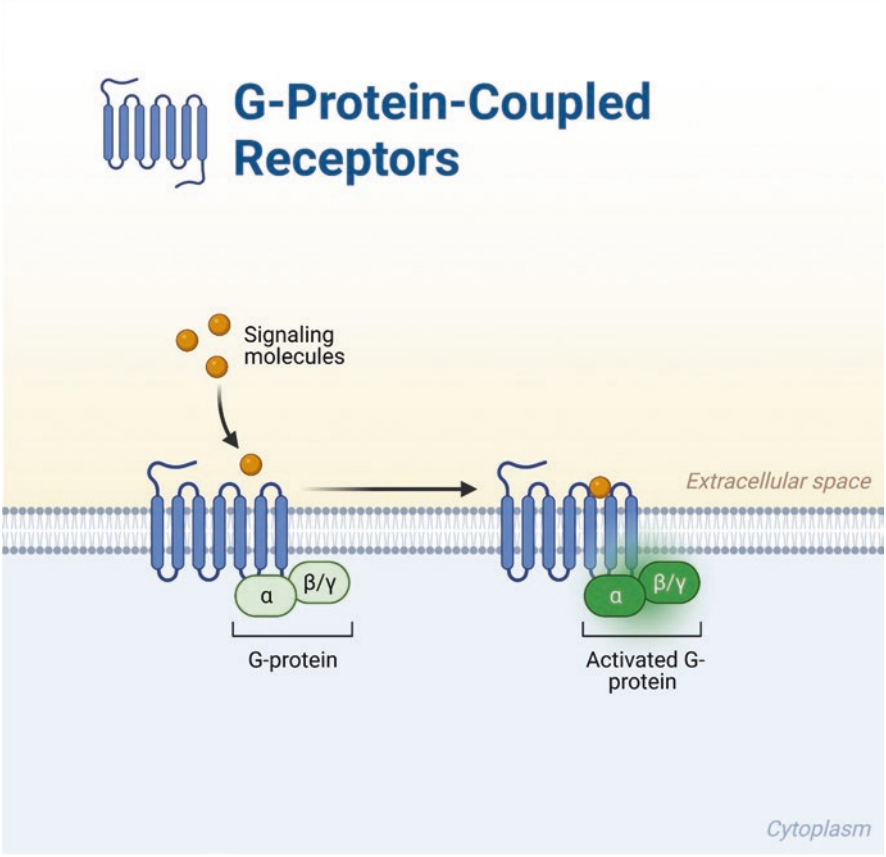

Fig. 10.2 Activation of immune cells with respect to G-protein coupled receptors

Over the last century, the discipline of cancer immunology has built a solid basis (Fig. 10.3) and has shown and proved its critics wrong. After three significant recent findings (Mir et al. 2020), scepticism has diminished in this setting. First, at least in mouse tumor models (Schreiber et al. 2011), it has been convincingly revealed that the cancer develops in the perspective of ongoing interfaces with immunosurveillance, passing through a steadiness, immunoediting, and escape phase. Second, it has been shown that cancer patients' immune responses have a significant impact on their survival (Mir et al. 2022a, b, c). It has been shown that adaptive immune system cell infiltration of tumors have a predictive significance that is greater than that of conventional tumor staging criteria (Galon et al. 2007). The "immune contexture" which is a functional orientation and location of adaptive immune cells that intrude different parts of the neoplastic lesion, refers to these main immunological factors related to patient survival (Galon et al. 2013; Mir 2015a, b). The development of a new scoring system termed "immunoscore" (IS) centred on the occurrence of two different lymphocyte populations (CD3+CD8+ or CD8+CD45RO+ cells) in the tumor centre

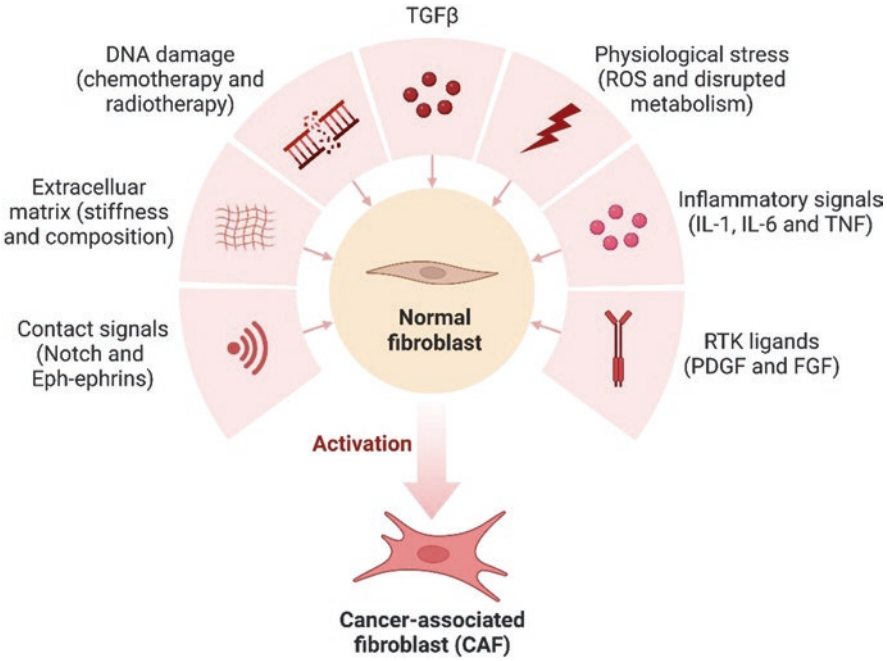

Fig. 10.3 Mechanism of cancer-associated fibroblast activation

(CT) and at its invasive margin was a practical application of these results (Fridman et al. 2012). Third, a number of immunotherapies that rely on spontaneous adaptive immune responses have had incredible success, which has led to a lot of excitement. These include FDA-approved anti-cytotoxic T lymphocyte-associated protein 4 (CTLA4) and monoclonal antibody ipilimumab (Chen and Mellman 2013).

The response of immunodulating therapies with respect to patients is affected by various factors such as density, location and function of immune cells in the tumor microenvironment (Galon et al. 2006) These factors play an important role in melanoma and other cancers and pose a prognostic value. Furthermore, the interactions between homing receptors (HRs) and their ligands on the immune cells causes what is called as immune cell homing (ICH) (Jiang et al. 2018). It has been found that intratumoral infiltration of T cells is closely associated with homing ligands while as intratumoral B cell infiltration shows a strong association with CXCL13 (Uryvaev et al. 2018). In humans, the chemokines promote increased infiltration of T cells in melanomas, thereby causing immune rejection with respect to wide type of cancers (López-Giral et al. 2004; Mir et al. 2022a, b, c). Similarly, chemokines along with their aligned HRs have also been found to be very crucial in B cell trafficking and retention to lymphoid structures (Woods et al. 2017). However, it is recommended that further studies should be conducted to visualize and interpret the contribution of ICH gene signatures in relation to tertiary lymphoid structures and the cross-talk between these pathways.

The significance of the immune axis (CXCL9, -10, -11/CXCR3) in immune response and tumor environment (TME) is elucidated in detail in this chapter along with the potential for innovative therapeutics.

10.2 CXCL9, CXCL10, CXCL11, and CXCR3 Expression and Implication

Chemokines are among the diverse cytokines that regulate immune cell development and rapid chemotactic gradient infiltration of target tissues (Kohli et al. 2022). Due to the presence of surface receptors, immune cells are selected on the basis of their response with respect to chemotaxis (Tian and Zhang 2022). As a result, cytokines and their receptors' interactions must have an effect on how well chemotactic gradients can be distinguished. CXCL9, -10, and -11 are ligands that only work with CXCR3 (Radolec et al. 2022). The ligands typically express at lower stages during homeostasis, but cytokine stimulation causes them to express more. The primary sources of CXCL9, -10, and -11 secretion in response to IFN-γ, which is synergistically increased by TNF-alpha, are monocytes, endothelial cells, fibroblasts, and cancer cells (Farhood et al. 2019).The receptor CXCR3 is mostly articulated on the surface of cancer cells (Tokunaga et al. 2018). CXC chemokines can be distinguished into two categories: ELR (Glu-Leu-Arg) motif causing angiogenic impact and another one that limit angiogenesis (Berg et al. 2022). Normally, angiogenesis is inhibited by the ELR-negative CXC chemokines CXCL9, -10, and -11, which has antitumor effects (Van Raemdonck et al. 2015). It is interesting to note that CXCL9, -10, and -11 encourage the growth and metastasis of tumors (Cambien et al. 2009). Previous studies have shown that, depending on the cell type, these ligands are expressed by various regulatory factors in various cell types in varying temporal and spatial patterns (Metzemaekers et al. 2018). The CXCR3 receptor comes in three different varieties, each of which performs a particular role in the growth of tumors. IFN-γ but not IFN-α/β/induce CXCL9 (Aota et al. 2018) while as CXCL10 and CXCL11 molecules can be found on human chromosome 4. CXCL9, which also prevents tumor growth, is primarily responsible for lymphocytic intrusion into the focal regions (Bronger et al. 2019; Sofi et al. 2023). Gorbachev et al. while conducting an in vivo investigation have revealed that cancer cells that do not have CXCL9 pose greater tumorigenic effects as compared to tumor cells that articulate both CXCL9 and CXCL10 (Gorbachev et al. 2007). Investigations have found that mice lacking CXCR3 and CXCL9 showed less kidney function loss than animals lacking CXCL10, suggesting that intrarenal T cells and macrophages were less responsible for the mice's immune-mediated nephritis (Menke et al. 2008).

IFN-γ, IFN-α/β, and TNF-α all considerably and faintly stimulate the interferon-γ-induced protein 10 (IP-10), also known as CXCL10. It has been shown that CXCL10 may be activated by NF-kB in vitro and that it plays an early role in inflammation brought on by hypoxia (Mir et al. 2020; Xia et al. 2016).

CXCL11, produced by IFN-γ and IFN-β, as well as marginally by IFN-α (Metzemaekers et al. 2018) had the greatest affinity after CXCL9 and CXCL10 of

the three selective ligands for CXCR3 (Cole et al. 1998). The CXCL11-binding domain on CXCR3 is positioned at an unlike place in comparison to CXCL9 and CXCL10 binding domains (Colvin et al. 2004). Furthermore, CXCL11 has the capacity to interact with CXCR7, a receptor associated with invasiveness and a suppressor of tumor cell death (Burns et al. 2006). IFN-γ controls CXCR3 more so than CXCL9, -10, and -11 (Nakajima et al. 2002).

CXCR3 has two distinct intracellular dominions that cause its activation and expression. CXCR3 has been downregulated in naive T cells, but it is promptly upregulated by antigen-presenting dendritic cells, leading to Th1 polarization (Kim et al. 2001). CXCR3A, CXCR3B, and CXCR3-alt are at least three different CXCR3 variants, according to biochemical studies (Lu et al. 1999). CXCR3B, a 52-amino acid splice variant, causes cell death and prevents cell migration; CXCR3-alt, a 101-amino acid shortened variant, mainly mediates CXCL11 activity. It is significant to note that CXCR3B may cohered to CXCL4, which is produced by active platelets during platelet aggregation (Ehlert et al. 2004).

The immunological axis response to host diseases appears to be regulated by both its ligands and various CXCR3 receptor types. CXCR7 binds only to CXCL11 which has the potential to stimulate tumor development. The ligands can also block CCR3, which encourages Th2 polarization.

10.3 Immune Response Axis of CXCL9, CXCL10, and CXCL11/CXCR3

This axis predominantly controls the initiation, differentiation, and migration of cells within the immune system. The types of leukocytes that enter the focal regions have an impact on each disorder's immunological response. Additionally, the axis has a direct impact on cancer cells, promoting their expansion. It has been revealed that in vivo condition, activated lymphocytes are all equally receptive to each of the CXCR3 ligands for immune cell migration, (Campanella et al. 2008). Together, CXCL9, -10, and -11 drive the loss of surface CXCR3 expression on T cells, which express all three CXCR3 variants, and they also cause directed migratory retorts to the pivotal sites (Korniejewska et al. 2011). Chheda et al. (2016) demonstrated that CXCR3 is necessary for CTL mobility using CXCR3 knock-out mice, which demonstrated evident tumor development and lower survival. It has also been established that CXCR3 mutant mice displayed a substantial reduction in tumor-infiltrating NK cells, and that CXCL10-controlled NK cell recruitment was also related to a favourable outcome in addition to tumor cell suppression (Wendel et al. 2008). Additionally, further investigations have asserted that CXCL9/CXCR3 axis-dependent mechanisms regulate the development of T cells, which display actions against the infections or malignancies (Patil et al. 2016). While CXCL4 provides apoptotic indications and showed that T cell migration was not promoted by CXCL4 (Lasagni et al. 2003). This was true even when Akt and ERK were phosphorylated and intracellular calcium was mobilized. CXCL4 may thus have additional functions in T cell function (Korniejewska et al. 2011). Investigations based on disease

models have shown that the absence of the three CXCR3 ligands pointedly impairs cell-mediated immunity (Wendel et al. 2008; Chheda et al. 2016). However, studies have also revealed that by driving Treg migration to focal regions, axis regulates immune suppression (Mulligan et al. 2013).

However, different investigations have revealed immune cell differentiation with respect to the number of activities executed by CXCL9-, -10, and -11 (Yang et al. 2011; Zohar et al. 2014). Consequently, investigations have demonstrated that this axis through CXCR3 resulted in Th1 polarization. Similar to CXCL9, CXCL10 supported enhanced T-bet and ROR transcription, which in turn caused the polarization of Foxp3 type 1 regulatory (Tr1) cells or T helper 17 (Th17) cells from naïve T cells (Zohar et al. 2014). On the other hand, ROR transcription was decreased by CXCL11 but not T-bet, which led to the polarization of naïve T cells into Tr1 or Th2 cells via p70 kinase/mTOR pathways, a mechanism that is comparable to that involving TGF and IL-27 (Apetoh et al. 2010). The variants of CXCR3 were not examined in these studies. However, because CXCL11 plays both tasks and has a high affinity for CXCR3, it could also operate to encourage the spread of cancer. The polarization of tumor-associated macrophages (TAMs), which regulate TME activities, is influenced by the CXCL9, -10, -11/CXCR3 axis, according to several studies (Oghumu et al. 2014; Mehraj et al. 2021). On the other hand, Liu et al. discovered that infiltration of CXCR3-positive B lymphocytes into the cancer site causing hepatocellular carcinoma M2 polarization involving immunoglobulin G-dependent pathways (Liu et al. 2016a, b). Immune cells are activated by CXCL9, -10, and -11 via polarizing and activating Th1 responses. Th1 cells produce IFN-γ, TNF-, and IL-2 and activate CTLs, NK cells, NKT cells, and macrophages to boost antitumor immunity (Mosser and Edwards 2008; Mir et al. 2022a, b, c). Prominently, NK cells function as an origin for the production of IFN-γ and can show immunological action by changing the activity of dendritic cells (Martín-Fontecha et al. 2004). Through the paracrine axis, Th1, CTL, NK cells naturally demonstrate effective action against cells that are cancerous in tumor models (Yang et al. 2006). Autocrine CXCL9, -10, -11/CXCR3 signaling, however, promotes angiogenesis, metastasis, and cancer cell proliferation in cancer cells (Qayoom et al. 2021). Previous research has demonstrated that autocrine signalling from the premetastatic niche may increase the likelihood of metastasis in cancer cells that express the CXCR3 gene both in vitro and in vivo (Zhu et al. 2015). Cancer cells that express CXCR3 may move more easily to ligand-rich metastatic locations attributed to the axis for metastasis. Treatment that concentrates primarily on CXCR3A in axis rather than CXCR3B and CXCR3-alt may be effective in treating metastatic sickness since CXCR3-A plays a significant part in cancer progression (Yang et al. 2016). Clinical cancer samples CXCR3 expression has found a correlation with the prediction and chance of metastasis in patients (Monteagudo et al. 2007). As a result, it is feasible to utilize this axis to forecast the success of a therapy or as a prognostic indication. Even while CXCL10/CXCR3 co-expression was found to have a critical role in increasing metastatic potential and there is still a huge debate regarding the association of ligands with prognosis (Wightman et al. 2015). Lesser levels of CXCR3, CXCL9, and CXCL10 may lessen the incidence of cancer

metastatic spread in melanoma, colon, breast, colon, and other malignancies (Ma et al. 2009). Some organizations concur that the expression of CXCL9 (Blank et al. 2017) and CXCL10 (Liu et al. 2016a, b) is associated with a weaker prediction systems and very low reciprocation to therapy, while other groups contend that the opposite is true for CXCL9 (Bronger et al. 2016) and CXCL10 (Sato et al. 2016). These variations can be attributed to the complex interactions between the ligands as well as the type of cancer. A score-based method on the axis employed by Liu et al. revealed an innovative technique for anticipating the course of a patient therapy (Liu et al. 2016a, b). In the future, it could be required to factor in the expression levels of CXCL9, -10, and -11 in order to predict a patient's prognosis. Chemokine axis activates the paracrine axis, which suppresses the autocrine axis and makes it a desirable target for therapeutic development. Several tumor models have shown the antitumor efficacy of compounds that promote the paracrine expression of CXCL9, -10, and -11 and decrease the expression of CXCR3. Using ligands to entice lymphocytic cells, and M1 macrophages to tumor sites is a viable antitumor approach. According to Zhang et al. (2006), plasmid-borne CXCL9 increased CTL activation and decreased colon and lung cancer when combined with cisplatin. By infiltrating CXCR3+ mononuclear cells, it has been revealed to reduce the tumor progression and angiogenesis in a model infested with tumor while studying the renal cell carcinoma (Pan et al. 2006). Mice implanted with lung cancer cells performed better after receiving intratumor injections of CXCL10 (Arenberg et al. 2001). Retroviral CXCL10 gene transduction has been used to characterize how well CXCL10 overexpression inhibits tumor development different cancer models (Sun et al. 2005). Barash et al. (2014) discovered some intriguing outcomes when they used a CXCL10-Ig fusion protein in a mouse model of multiple myeloma. Similarly, in a mouse model of mesothelioma, CXCL11 has shown to increase certain cells (CTLs and NK cells) and extended longevity but had a little effect on CD4+ T cells (Liu et al. 2016a, b). In a mouse model of autoimmune encephalomyelitis, CXCL11-Ig fusion protein treatment generated fast disease reduction, showing the complexity of targeting CXCL11 through the downregulation of T cell movement and the augmentation of Treg polarization (Zohar et al. 2014). Despite not being connected to these discoveries, CXCR3 variants could be used as therapeutic targets since they cause paracrine signaling.

The anti-CXCR3 therapy appears as an encouraging technique. In animal models, CXCR3 was pharmacologically suppressed to stop tumor development and metastasis. In vivo lung metastasis was decreased by the CXCR3 antagonist AMG487, and in vitro osteosarcoma and colon cancer cell implantation and development were inhibited (Pradelli et al. 2009). The finding that AMG487 might stop local breast cancer development in vivo but not lung metastasis is intriguing (Zhu et al. 2015). AMG487 was also shown to be ineffective to stop the spread of liver metastasis and metastatic tumors (Cambien et al. 2009). These findings imply that the paracrine CXCL9-, -10, -11/CXCR3 axis may decrease tumor growth while adversely affecting the anti-tumor host response. Since AMG 487 inhibits the paracrine axis by targeting all CXCR3 subtypes, tumor development could be

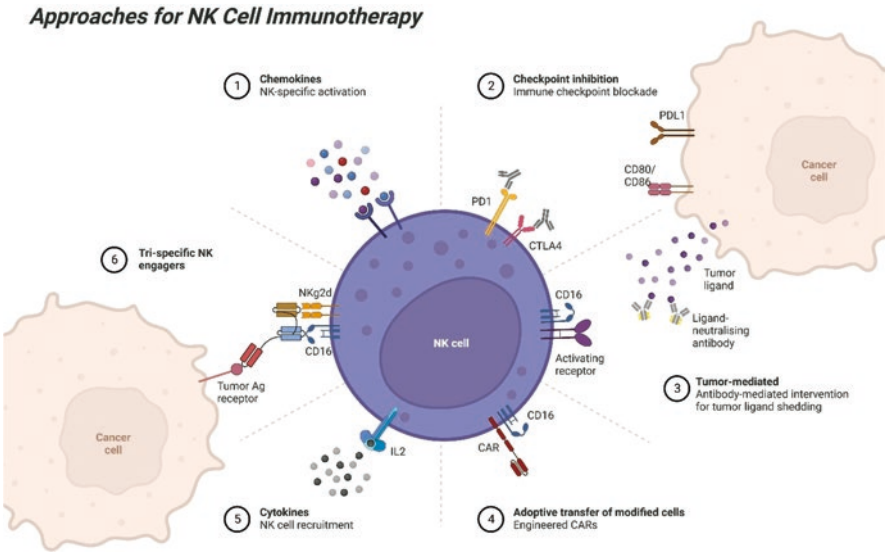

Fig. 10.4 Potential immunotherapeutic approaches for the treatment of cancer using innate lymphocyte natural killer (NK) cell

accelerated. To prevent metastasis, CXCR3A inhibition and the immune activation through ligands can provide a new insight for future research.

10.4 Cancer Treatment and CXCL9, CXCL10, and CXCL11/CXCR3 Axis

The CXCL9-, -10, -11/CXCR3 axis activates the paracrine axis, which suppresses the autocrine axis and makes it a desirable target for therapeutic development (Fig. 10.4). Several tumor models have shown the effectiveness of drugs that promote the paracrine expression of CXCL9, -10, and -11 and suppress the expression of CXCR3 on cancer cells (Tables 10.1 and 10.2).

10.5 Immune Pathway Enhancers and CXCL9, CXCL10, and CXCL11/CXCR3 axis

Although the methodological significance of such axis is still being recognized as an option for cancer treatment, it is precarious to comprehend how such route interacts with other conduits of the immune system (Fig. 10.5).

The relationship between the PDL-1/PD-1 axis and the chemokines axis is the subject of significant investigation. Anti-PD-1 medicine can stop "immune escape" and increase immunological activation because of its higher expression on the T cells that are closer to cancerous cell as compared to cells located on periphery

Table 10.1 Notable works related to cancer treatment and CXCL9, CXCL10, and CXCL11/CXCR3 axis

Target	Approach elucidation	Cancer type	Findings/conclusion	Reference
CXCL9	Cisplatin with low dose and systemic plasmid origin CXCL9	Lung, colon	Microvessel density reduction of tumor was observed as well as increased apoptosis.	Zhang et al. (2006)
CXCL10	Retroviral transduction to tumor cells	Sarcoma, lung	Suppressive tumor growth and mitotic activity	Sun et al. (2005)
CXCL11	Fusion of CXCL-11 with Ig protein	Encephalomyelitis	T-cell migration decreased whereas T-cell polarization were induced	Zohar et al. (2014)
CXCR3	AMG487	Breast	Lung metastasis inhibited but not local cancer growth	Zhu et al. (2015)

(Cichocki et al. 2020). Peng et al. (2013) disclosed that anti-PD-1 may assist not only the T cell-mediated tumor regression but also the production of IFN-γ and CXCL10 by bone marrow-derived cells. In an in vivo model, Chheda et al. (2016) showed a strong link between the anti-PD-1 treatment's effects and CXCR3-induced T cells flocking to the cancer site. Anti-PD-1 therapy may not be successful in the absence of the chemokine axis since it failed to reduce the tumor in CXCR3 deletion animals. Further investigations have shown that if the PDL-1/PD-1 axis in T cells is blocked, the chemokine axis may be used as a constructive feedback loop at the tumor site and hence suggestively upregulated in individuals who had a strong clinical response to ipilimumab during pretreatment of melanoma lesions. A CTLA4 antibody was further employed (Ji et al. 2012). The use of tumor-infiltrating lymphocytes in the treatment of cancer has been considered as a significant step for studying the current oncogenic practices and has received a substantial support and research attention (Mir and Mehraj 2019). The N-terminal of CXCL10 is truncated by the enzyme dipeptidyl peptidase 4 (DPP4), which reduces lymphocyte movement to tumor locations. DPP4 inhibition improved cancer rejection in vivo by increasing lymphocyte homing to tumor locations through the CXCL10/CXCR3 axis and increased immunotherapy effectiveness (Barreira da Silva et al. 2015). According to investigation by Decalf et al. (2016) using a DPP4 inhibitor that has received medical approval, people may still maintain the bioactive version of CXCL10. It has been noted that DPP4 inhibitors pose little or no adverse impacts and hence are considered as safe medications; therefore, they are anticipated to be employed in upcoming treatment techniques (Decalf et al. 2016).

COX-inhibitors are thought to be expressing the chemokine axis (CXCL9, CXCL10) in vitro from the cancer cells but provide anticancer actions in vivo. This provides more evidence of the importance of the CXCL9, -10/CXCR3 axis for the treatment of cancer. In human breast cancer tissue, COX2 and CXCL9 expressions

Table 10.2 Therapeutic approaches associated with chemokine axis as anticancer therapy

Treatment	Target axis	Cancer type	Major findings	Reference
Anti-PD-1	CXCL9,-10	Breast, melanoma	In CXCR3 knockout mice, Anti-PD-1 drug has shown a tremendous potential to reduce the tumor growth.	Chheda et al. (2016)
Anti-CTLA4	CXCL9, -10, -11	Melanoma	T-cell induction and tumor growth reduction	Ji et al. (2012)
Anti-DPP4	CXCL10	Colon, melanoma	Immunotherapy response improved	Barreira da Silva et al. (2015)
COX-inhibitor	CXCL9, -10	Ovarian, breasts	Unselective COX inhibition increases CXCL9 and CXCL10 production, while COX-2-selective COX inhibition decreases that production	Bronger et al. (2012)
Lapatinib along with doxorubicin	CXCL9, -10, -11	Breasts	Induction of T-cell and reduction in tumor growth	Hannesdóttiret al. (2013)
Decarbazine, Temozolomide, Cisplatin	CXCL9, -10,	Melanoma	Improved prognosis with induced T-cells	Hong et al. (2011)
ACT therapy	CXCL9, -10, -11	Melanoma	The response to the therapy was correlated with the expression of CXCL9, -10, and 11 in pretreatment tumors	Bedognetti et al. (2013)
Dendritic vaccine cell therapy	CXCL9, -10, -11	Chronic lymphocytic leukemia	Induced NT and NKT cells	Gustafsson et al. (2011)
IL-7(IL-7/IL-7Rα-Fc	CXCL9, -10	Lung	T-cell and M1 macrophage induction, tumor growth inhibition, and improved prognosis	Sharma et al. (2003)
miR21	CXCL10	Breast	Reduced lymphocyte migration	Wang et al. (2013)

were inversely correlated (Bronger et al. 2012). These findings corroborate major preclinical studies demonstrating a strong negative correlation between overexpression of both COX isoenzymes and tumor-infiltrating lymphocytes (Prima et al. 2017). It has also shown that the amalgamation of COX-2 inhibitor and anti-PD1 controlled utilizing an alginate hydrogel delivery approach synergistically increased the number of Th1 cells, CTLs, and increased CXCL9 and CXCL10 production inside the tumor (Li et al. 2016). It is noteworthy to note that as a result of these effects, the tumor microenvironment contains less Tregs and myeloid-derived suppressor cells (MDSCs) (Mir et al. 2022a, b, c). Because anti-PD-1 therapy alone was unable to reduce Treg and MDSCs inside the tumor, effective pharmaceutical

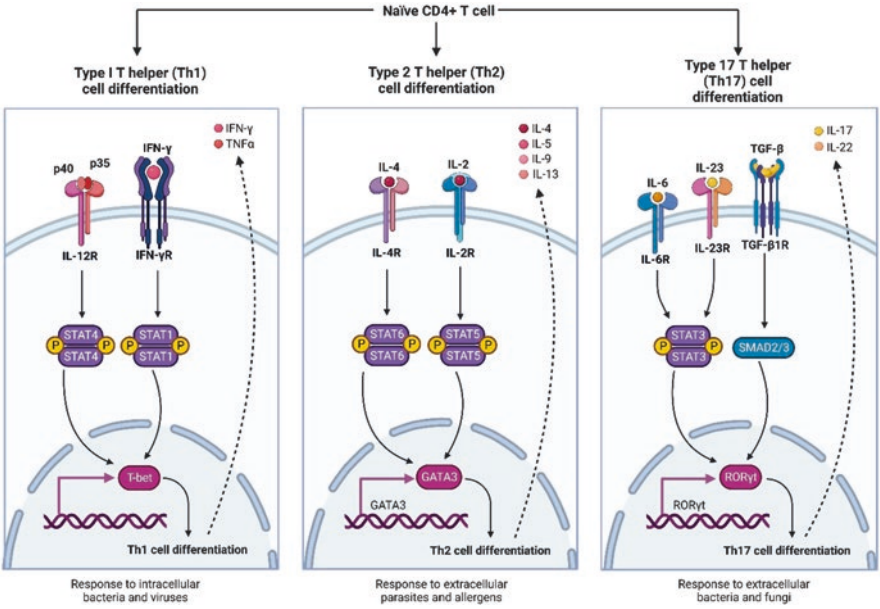

Fig. 10.5 Polarizing cytokines activation and T-cell differentiation and activation

combinations may give greater efficacies (Das et al. 2015). All-trans retinoic acid (ATRA), existing chemotherapy, and other medicines have shown the therapeutic advantages of the chemokine axis; this suggests that stimulating this axis may boost the effectiveness of cytotoxic and targeted therapies (Szabo et al. 2012).

CXCL9, -10, -11, and CCL5 are potential indicators for adoptive T cell transfer treatment in metastatic melanoma (Bedognetti et al. 2013). According to Ulloa-Montoya et al. (2013), it is possible to predict the therapeutic outcome of patients having cancer based on the pretreatment expression of CXCL9, -10 for MAGE-A3 cancer immunotherapy utilizing gene expression signature analysis. The relationship between this axis and immunotherapy was also examined and shown the significance of the chemokine axis in the effectiveness of immunotherapy using dendritic cell vaccination therapy, IL-2, or IL-7 injection therapy (Ulloa-Montoya et al. 2013). The oncogenic miRNA miR21 has been demonstrated to control CCL5 and CXCL10 in breast cancer cells, despite the dearth of research establishing the significance of epigenetics in this axis (Mehraj et al. 2022c; Wang et al. 2013). These cutting-edge approaches to addressing the CXCL9, -10, -11/CXCR3 axis elucidate how crucial synergy is in the oncogenic treatments. It is essential to better understand this route.

Different investigation has clinically revealed that metastasis can be prevented to a large extent by increasing the number of immune cells in tumors (Adams et al. 2019). However, T cell escape from the tumor cells restricts the success for the complete control of metastasis. Immunotherapy has been considered a recent advance for the treatment of cancers, but the lower response rate and clinical

subtypes are the prominent factors that affect the tumor biology (Mir 2015a, b; Santa-Maria and Nanda 2018). Similarly, different biomarkers have been developed from time to time for the immunotherapy purposes such as pembrolizumab can be utilized in microsatellite instability high breast cancers. Although investigations have revealed its lower response rate but higher durability (Nanda et al. 2016).

The intercellular contact between cancer cells and stromal cells in TME, which commonly involves bidirectional transfer of encapsulated chemokines, is of special importance for the current topic. TMVs (tumor microvesicles) have been studied using in vitro cell lines or in vivo animal models, and these studies have highlighted the significance of MVs as key mediators of cancer growth, proliferation, apoptosis, angiogenesis, coagulation, and metastasis, suggesting a paradigm shift toward using TMVs as diagnostic or prognostic biomarkers (Bian et al. 2019; Mehraj et al. 2022a, b, c). TMVs have also been demonstrated to aid in the immunomodulation and chemo-resistance of cancer cells, offering insight on the clinical use of TMV-based or TMV-targeted therapeutic treatments to boost the effectiveness of chemotherapy or immunotherapy (Bian et al. 2019). However, we are just at the very beginning of our understanding of TMVs and TMV-related chemokines (Lee et al. 2011). It is essential to create animal models that allow for precise monitoring and manipulation of the release and absorption of chemokine-containing MVs in order to illustrate the real physiological roles of MVs in vivo. As the mechanism by which MVs facilitate intercellular communication is more understood, MVs are anticipated to transform our understanding of cancer biology, play a significant role in laboratory research, and shed light on innovative treatment approaches for diverse cancer types.

Our knowledge base has been greatly expanded by the integrated molecular studies of breast carcinomas, which have led to the creation of an extensive list of the most likely genetic drivers of the most prevalent breast cancer subtypes (Brigham, Hospital et al. 2012; Sofi et al. 2022). In addition to being consistent with the convergent evolution of gene circuits observed across multiple organisms, our novel finding that different genetic and epigenetic alterations (Mertins et al. 2016) converge phenotypically into four main breast cancer classes is also consistent with models of breast cancer clonal expansion and in vivo cell selection proposed to explain the phenotypic heterogeneity observed within defined breast cancer subtypes (Brigham, Hospital et al. 2012).

Chemokines are associated with longer survival and higher number of immune cell infiltration prediction in breast cancers (Denkert et al. 2010). Therefore, future research regarding the role of chemokines in cancer treatment with their detailed molecular mechanism are warranted.

10.6 Conclusion

The body's chemokine system is an incredibly intricate defensive mechanism made up of a wide variety of interdependent ligands, receptors, and regulatory molecules involved in a variety of cellular functions. Its primary biological role among them is the chemotaxis of immune cells, particularly lymphocytes, although its effects

extend well beyond that. The chemokine/chemokine receptor axis has a tumorigenic function in a variety of cancer models and clinics, as has been discussed conclusively in the book chapter. The major objective of this chapter was to examine the function of the chemokine axis in the tumor environment (TME) and immunological retort. The results obtained from the reviewed investigations revealed that through paracrine signalling, this axis is indispensable for immune activation, which affects the efficacy of cancer therapy. Preclinical studies suggest that the amalgamation of pharmacological ligands and inhibition of CXCR3A may open up newfangled pathways for immune treatments that are more effective and increase the efficiency of traditional chemotherapies. Greater comprehension of this pathway regulation may open the door to more efficient cancer treatment methods. However, there are still many unresolved elements of how chemokines and chemokine receptors function in human disease, necessitating intense efforts in both clinical research and far more fundamental animal investigations.

Further Reading

For further readings, some textbooks mentioned below also gives further understanding of cytokine and chemokine network.

- Cytokines and their therapeutic potential by Manzoor Ahmad Mir.
- Chemokine Receptors in Cancer by Amy M. Fulton 2009

For more incites about the topic we would suggest detailed findings from the books of (Mir et al. 2022a, b, c) https://doi.org/10.1016/C2021-0-02565-7, https://doi.org/10.1016/C2022-0-00074-X and (Mir 2021) https://doi.org/10.52305/WXJL6770 (Mir 2015a, b) https://doi.org/10.1016/C2014-0-02898-5 and from the cancer.net website on the following mentioned below links,

https://www.cancer.net/cancer-types/breast-cancer/types-treatment

https://discovery.ucl.ac.uk/id/eprint/1472740/

https://www.jmedsciences.com/doi/JMEDS/pdf/10.5005/jp-journals-10045-00138

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Role of the CXCL8–CXCR1/2 Axis in Cancer and Inflammatory Diseases

11

Manzoor Ahmad Mir, Masrat Bashir, and Ishfaq

Abstract

Interleukin-8, also known as CXCL8, is a granulocytic chemokine in humans that plays a specific function in the microenvironment of tumors such as attracting cells causing immunosuppression to the tumor cells, facilitating epithelial-to-mesenchymal transition (EMT) as well as angiogenesis. The CXCL8 and the chemokine receptors CXCR1/2 are required for inflammatory mediator's activation and their trafficking, along with metastasis and tumor growth. The function of the CXCL8–CXCR1/2 signaling axis is associated with the development of a number of disorders, including asthma, Chronic obstructive pulmonary disease (COPD), cystic fibrosis, as well as malignancies, growth of cancer, and metastasis depending on interactions between CXCL8, which is expressed by certain malignant cells, and chemokine receptors, CXCR1/2, in the tumor microenvironment. By governing proliferation and self-renewal of cancer stem cell (CSC), the CXCL8–CXCR1/2 signaling might be a key player in tumor development and metastasis. Agents targeting CXCR1/2 and CXCL8 antibodies have been discovered over past 20 years. Such inhibitors are anticipated to be effective in a variety of inflammatory disorders when used alone. According to several preclinical models, some malignancies may respond well to the mixture of CXCR1/2 targeting agents. A number of these agents are presently being tested in advanced clinical studies for metastatic breast cancer, asthma, and COPD. In this chapter, we offer a detailed examination of the CXCL8–CXCR1/2 pathway, its involvement in disease progression as well as role of some coexpressed genes in this pathway. We also go through the most recent improvements in generating small-molecule medications that target this route.

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Immunosuppression · Metastasis · Chemokine · Angiogenesis · Tumor microenvironment · Cancer stem cells

11.1 Introduction

Chemokines are low-molecular-weight secretory glycoproteins (8–12 kDa) that were originally identified as chemotactic cytokines. Chemokines and their corresponding receptors facilitate the activation and migration all through innate and adaptive mechanisms, and they thus play a significant functional role in the biology of white blood cells and other types of cells by regulating recruitment of cells, homeostasis, stimulation, and directing leukocyte movements in basal and inflammatory states (Luster 1998; Bonecchi et al. 2009; Russo et al. 2010). In mammals, chemokine system is complicated, with approximately 50 ligands that are continuously generated by various stimuli and 20 receptors related to class A of the rhodopsin-like G-protein-coupled receptors (GPCRs) by means of seven transmembrane domains. For the structural integrity of chemokines, four cysteine residues are required. Chemokine receptors are found on the cell membrane and function as sensors of the surrounding environment. They are continuously transmitting extracellular chemical signals that lead to activation of cascade of signaling events inside a cell that ultimately alters the cellular response of a cell (Mehraj et al. 2022c; Thelen and Stein 2008; Bachelerie et al. 2014). Chemokines are divided into four distinct classes, CXC, CC, CX3C, and XC based on the sequence of these four cysteine residues (Rollins 1997). The two main classes of chemokines are CC and CXC. On the N-terminus of CXC, the primary amino acid pattern (Glu-Leu-Arg) that comes prior the first cysteine residue is further divided into ELR^+ and ELR^- CXC chemokines, respectively (Hebert et al. 1991).

Arthritis, HIV, multiple sclerosis (MS), irritable bowel syndrome (IBD), reperfusion injury (RI), asthma, tumors, chronic obstructive pulmonary diseases (COPD), cystic fibrosis, lupus, pain, and Crohn's disease are just a few of the illnesses that have been linked to chemokines due to their crucial roles in the immune system and during inflammatory reactions (Horuk et al. 1997). Due to the great importance of chemokine receptors in various disorders, small-molecule inhibitors have been actively developed. There are currently only two chemokine receptor inhibitors that are FDA-approved : Maraviroc, a CCR5 antagonist used to treat HIV-1, and Plerixafor, a CXCR4 antagonist used to mobilize hematopoietic stem cells in multiple myeloma patients as well as in patients of non-Hodgkin lymphoma (Dorr et al. 2005). The role of CXCL8–CXCR1/2 pathway in the pathophysiology of numerous disorders, including as cancer and inflammation are thoroughly discussed in this chapter.

11.2 CXCL8

Ten years after the first chemokine, CXCL4/PF4, was discovered, CXCL8 was isolated, which at the time sparked new interest in the subject. It is one of the first and most extensively investigated chemokines that promotes inflammation. Monocyte-derived neutrophil chemotactic factor, neutrophil-activating factor, and monocyte-derived neutrophil-activating peptide are three names used by distinct groups to refer to CXCL8. Neutrophil activating factor (NAF), a secretory protein, was discovered by Peveri et al. in the late 1980s to be produced by LPS-stimulated blood monocytes and to stimulate exocytosis of neutrophil (granule release) and an oxidative burst, i.e., hydrogen peroxide generation and production of superoxide ion that is mediated by surface receptors of cells (Peveri et al. 1988). In 1987, NAF became the very first chemokine to be isolated and sequenced and after the discovery of further chemokines, it was given the names interleukin-8 (IL8) and CXCL8 (Schröder et al. 1987; Walz et al. 1987; Yoshimura et al. 1987).

11.2.1 Structural Characteristics of CXCL8

In 1991, CXCL8's crystal structure was originally reported. Three antiparallel α-strands and β-strands and one α-helix composed of the C-terminal residues 57–72 form the 72 amino acid peptide known as CXCL8 (Baldwin et al. 1991; Joseph et al. 2015). Two disulfide connections made by Cys7-Cys34 and Cys9-Cys50 stabilize the structure. Since one residue (Gln8) separates the two cysteine residues (Cys7 and Cys9), CXCL8 is categorized as a CXC chemokine. CXCL8 occurs as a dimer stabilized by hydrogen bonds between the first β-strands, as demonstrated by the crystal structure. Although CXCL8 dimer and monomer bind CXCR2 with nearly equivalent affinities, monomer of CXCL8 binds with the CXCR1 receptor with high affinity (Fig. 11.1).

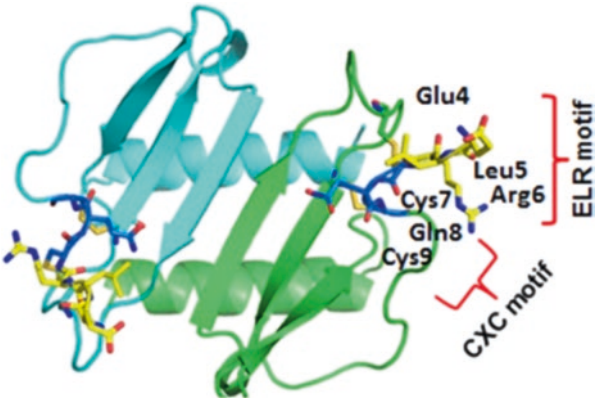

Fig. 11.1 Protein structure of CXCL8

Higher affinities of the receptor for CXCL8 monomer than dimer is a consequence of interactions among CXCR1 N-terminal site-1 and CXCL8. Different signaling outcomes may result from these variations in the selectivity of CXCR1 and CXCR2 for CXCL8 monomer vs. dimer (Rajarathnam et al. 1994; Nasser et al. 2009; Joseph et al. 2015). The crucial N-terminal pattern, Glu-Leu-Arg, was discovered via scanning mutagenesis on CXCL8 in which each of the first 15 amino acid residues of CXCL8 were systematically altered to alanine (ELR). Competitive binding experiments, revealed that mutants had lower affinity toward its receptors and were inactive (Hebert et al. 1991; Clark-Lewis et al. 1991).

11.2.2 Synthesis and Secretion of CXCL8

Multiple cell types, such as alveolar macrophages, blood monocytes, endothelial cells, and epithelial cells, express CXCL8 (Peveri et al. 1988). In unstimulated cells, CXCL8 is nearly invisible. The transcription factors NF-κB and activator protein-1 (AP-1) promote the stimulation of CXCL8 expression by a variety of cytokines, including IL-1, IL-6, CXCL12, and tumor necrosis factor α, reactive oxygen species (ROS), hypoxia, bacterial spores, and other environmental exposure (Brat et al. 2005; Wald et al. 2013). The expression of CXCL8 is upregulated 10 to 100-fold as a result of this stimulation (Podolin et al. 2002) (Fig. 11.2).

The expression of CXCL8 is upregulated by at least three distinct mechanisms working together: The CXCL8 gene promoter is desuppressed, the NF-κB and JNK pathways transactivate CXCL8 synthesis, and the p38 MAPK pathway stabilizes CXCL8 mRNA (Hoffmann et al. 2002).

Three things occur as a result of which the CXCL8 gene promoter is suppressed in unstimulated cells: NRF (NF-κB-repressing factor) binds to the NRE, i.e., a negative regulator element that inhibits the NF-κB-binding site, OCT-1 (octamer-1) works by binding to the complementary sequence of the gene promoter in the direction opposite of the C/EBP binding site, and HDAC-1 (histone deacetylase 1) induces the histone proteins deacetylation, among other mechanisms (Wu et al. 1997; Nourbakhsh et al. 2001; Hoffmann et al. 2002).

The activated p65 subunit of NF-κB translocate to the nucleus and binds to DNA in the presence of a stimuli, like IL1 or TNFα. CREB-binding protein (CBP)/p300 is therefore attracted after C/EBP replaces OCT-1 in the promoter, causing histone acetylation as well as chromatin remodeling. The CXCL8 promoter is thus unrepressed. CXCL8 and other antiapoptotic genes are transactivated by NF-κB proteins and, which are modified by phosphorylation in a signal-dependent manner (Hoffmann et al. 2002; Nourbakhsh et al. 2001; Wu et al. 1997).

Other genes are also important for the production of CXCL8. By controlling site-specific demethylation of DNA as well as histone H3 hyperacetylation, prostaglandin E2 (PGE2) causes desuppression of the CXCL8 gene promoter. IκB (blocker of NF-κB kinase) transactivates NF-kB transcription factor to control CXCL8 production. IκB inhibits nuclear localization signal (NLS) of NF-κB, which inactivates it. IκB is phosphorylated by IKK, a complex made up of two NEMO molecules, IKK

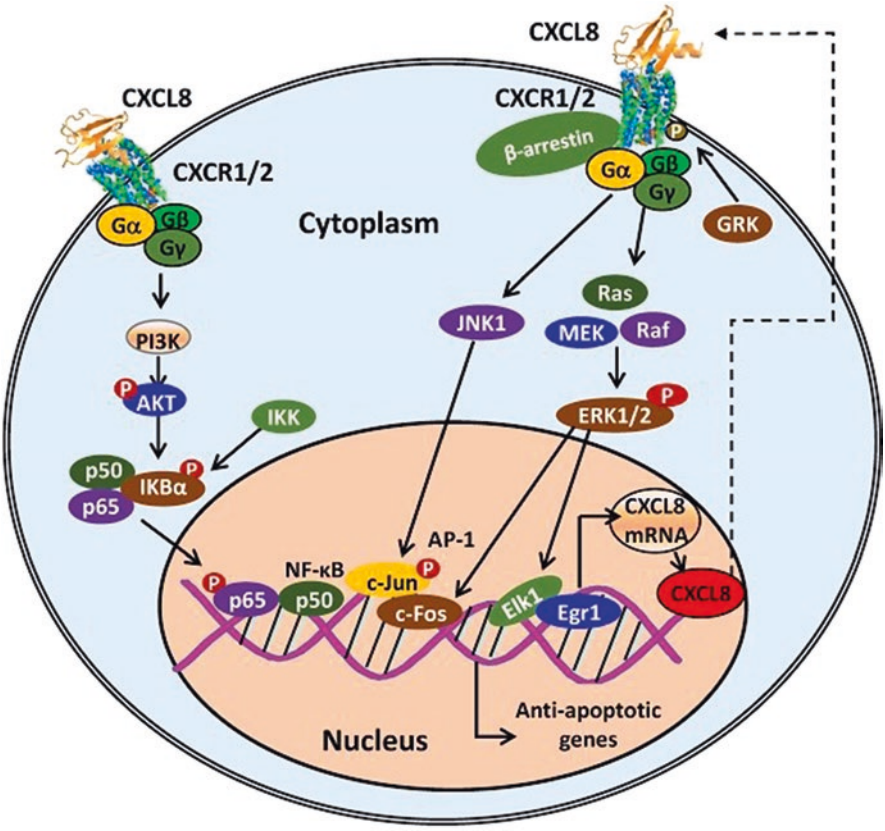

Fig. 11.2 Signalling pathways that activate the synthesis of CXCL8

beta, and/or IKK alpha. The proteasome subsequently breaks down the phosphorylated I-B, enabling the translocation of the NF-κB subunits p50 and p65 into the nucleus (Oeckinghaus and Ghosh 2009).

IKK and NF-κB proteins are activated by the PI3K-AKT signal transduction pathway, which causes production of CXCL8 in human lung epithelial cells (Lin et al. 2011). c-JUN kinase (JNK1) and ERK, these are mitogen-activated protein kinases (MAPK), that stimulates the AP-1 a transcription factor, that transactivates CXCL8 transcription. JNK1 upregulates c-JUN by phosphorylation, which further facilitates its transport into the nucleus and interacts with c-Fos to produce the AP-1 transcription factor, as a consequence of which stimulation of CXCL8 occurs. TWIST1 also contributes significantly to the production of CXCL8 generated by NF-κB. (Hoffmann et al. 2002; Tang et al. 2013).

By enhancing the DNA binding affinity of p65 to the CXCL8 promoter, the TWIST1 carboxy-terminal WR (Trp-Arg) domain promotes the assembly of the TWIST1-p65 complex and increases the gene transcription of NF-κB, hence enhancing the transcription of CXC (Li et al. 2012) The integrity of the CXCL8

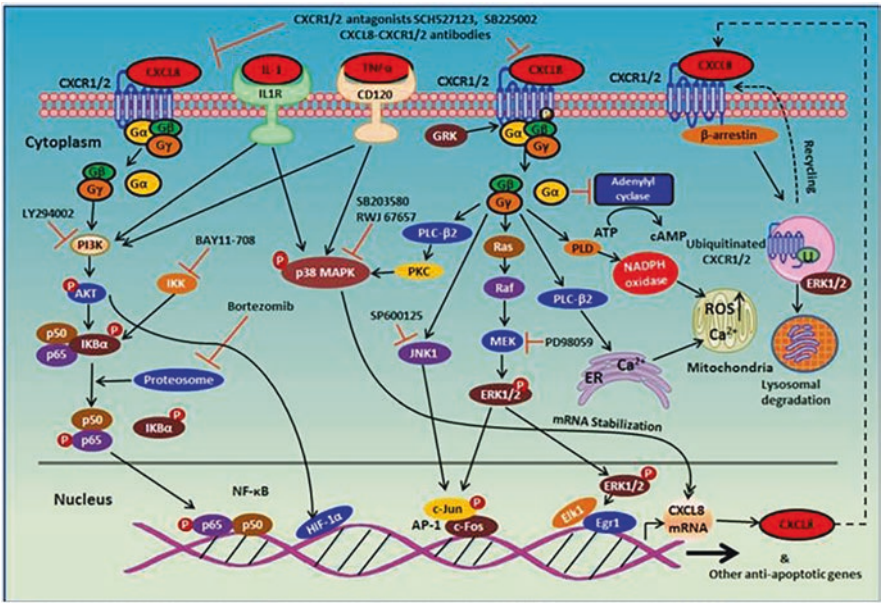

Fig. 11.3 CXCL8–CXCR1/2 signaling cascades and receptor recycling

mRNA, which is quickly destroyed by ARE, i.e., AU-rich cis-elements (ARE) found in its 3′ untranslated region, affects the synthesis of CXCL8. By limiting ARE-based mRNA degradation, p38 MAPK cascades contribute significantly to the post-translational control of CXCL8 generation. Through phosphorylating a tyrosine and threonine residues in the activation loop, a double specificity mitogen-activated protein kinase kinase 6 (MKK6) preferentially triggers p38 MAPK (Winzen et al. 1999; Hoffmann et al. 2002). MKK6-p38 MAPK, a target of MAP kinase-activated protein kinase 2 (MK2), continually maintains the stability of CXCL8 mRNA. The CXCL8 mRNA stabilization caused by MKK6 is hindered by the dominant-negative mutant of MK2 (Winzen et al. 1999) (Fig. 11.3).

11.3 The CXCL8 Receptors: CXCR1 and CXCR2

(CXCR1) C-X-C chemokine receptor type 1 and (CXCR2) C-X-C chemokine receptor type 2 are two G-protein-coupled receptors that CXCL8 binds to extracellularly to mediate its signals. The two receptors have a 76% sequence identity and interact to CXCL8 with a Kd of 4 nM or less. The C-terminal (intracellular) and N-terminal (extracellular) sections of the second extracellular loop are where the two receptors' main distinctions between them are found. All other ELR$^+$ chemokines (CXCL1–3, 5–7) associate with CXCR2 with great affinity, although CXCR1 very weakly binds to them (Murphy and Tiffany 1991; Kunsch and Rosen 1993; Murphy 1994; Winzen et al. 1999; Yang et al. 2000).

11.3.1 Activation of CXCR1 and CXCR2

Significant research has been conducted to describe CXCR1 and CXCR2 receptor signaling and modulation ever since discovery of CXCR1 in 1991. These researches mostly used HEK293 or neutrophils and RBL-2H3 cells that were overexpressed with CXCR1 and/or CXCR2. Following chemokine binding, CXCR1/2 physically interacts with the Gαi subunit of pertussis toxin-sensitive G-protein to bind to it and control a number of signal transduction pathways that control chemotaxis of neutrophils and stimulation (Fig. 11.3) (Damaj et al. 1996; Qayoom et al. 2021). When CXCR1/2 is activated, the G-protein and receptor become dissociated, and the G subunits are released from the G subunit. The stimulation of protein kinase C (PKC), that is essential for chemotaxis of neutrophils, is carried on by the release of the G subunits, which also activates phospholipase C (PLC, -2 isoform). It causes protein kinase C (PKC) activation and calcium transport from the endoplasmic reticulum to the cytoplasm, which are both necessary for chemotactic attraction of neutrophils (Fig. 11.3) (Wu et al. 1993, 2012).

Additionally, extracellular signal-related protein kinase (ERK1/2) and phosphatidylinositide 3-kinase (PI3K)/Akt are rapidly and momentarily phosphorylated in human neutrophils by CXCL8 (Knall et al. 1996; Knall et al. 1997; Xythalis et al. 2002; Fuhler et al. 2005). The Ras-Raf-MEK-ERK signaling pathway includes ERK1/2 (Roskoski Jr 2012). Uncertainty remains about the significance of CXCL8-mediated ERK1/2 activation in neutrophil migration. While other studies found no impact of MEK inhibitor PD098059 on CXCL8-induced chemotaxis of neutrophils (Xythalis et al. 2002; Roskoski Jr 2012), Xythalis et al. demonstrated that the MEK antagonist PD098059 prevented CXCL8-induced neutrophil chemotaxis. Both neutrophils and L1.2 cells excessively CXCR2 had considerably less CXCL8-mediated cell migration when PI3K was inhibited by the small-molecule inhibitor LY294002 (Knall et al. 1997; Lane et al. 2006). Members of the Rho-GTPase family are activated by CXCL8 signaling, which also causes the stimulation of protein kinases including focal adhesion kinase (FAK), and Src. Cellular multiplication and migration were boosted as a consequence of FAK and Src stimulation by CXCL8 signaling (Lane et al. 2006). CXCL8 causes FAK phosphorylation and relocalization in CXCR1- and CXCR2-RBL (rat basophil leukemia) transfected cells. Additionally, it causes actin and β-tubulin to relocalize to facilitate cell proliferation and migration, which is strongly linked to the migratory response generated by CXCL8 (Feniger-Barish et al. 2003; Cohen-Hillel et al. 2006; Waugh and Wilson 2008; Feniger-Barish et al. 2003). By controlling mechanisms involved in cell spreading, adhesion, and dissociation, FAK controls cell motility (Parsons et al. 2000). LIM and SH3 protein 1 (LASP-1) interacts straight to chemokine receptor.

By connecting chemokine receptors to CXCR1–4, LASP-1 may act as an adapter protein that links them to cytoskeleton-related proteins. This relationship is essential for chemotaxis (Raman et al. 2010). Rac-GTPases, which are tiny monomeric GTPases, are another key regulator of polymerization of actin that CXCR2 controls (Schraufstatter et al. 2001, 2003). Inhibiting the enzymatic protein adenylyl cyclase (AC), which converts ATP to cyclic AMP, leads to the G subunit to disassociate

from CXCR2 upon ligand binding, thereby lowers the concentration of intracellular cyclic AMP concentration (Damaj et al. 1996). To explore the effects of CXCL8 and CXCL1 on cyclic AMP levels, Hall et al. stimulated CXCR1/2-overexpressing CHO cells with forskolin (AC activator) in the presence of CXCL8 or CXCL1. Both chemokines In a dose-dependent manner, reduced CXCR2 promoted forskolin-induced cyclic AMP buildup, whereas only CXCL8 inhibited CXCR1-mediated forskolin-induced cyclic AMP accumulation (Hall et al. 1999). Though CXCR1 and CXCR2 cause cell migration and granule release in neutrophils via comparable mechanisms, CXCR1 is the only receptor that activates phospholipase D (PLD) (L'Heureux et al. 1995; Baggiolini 1997; Richardson et al. 1998a, b). PLD is a protein that transforms phosphatidylcholine to phosphatidic acid and choline. Phosphatidic acid promotes neutrophil oxidative burst by activating NAPDH oxidase and producing superoxide anion CXCR1, but not CXCR2, dramatically increased superoxide anion generation in neutrophils, indicating that CXCR1 is required for CXCL8-mediated oxidative burst (Sozzani et al. 1994; L'Heureux et al. 1995; Jones et al. 1996; Baggiolini 1997; Richardson et al. 1998a, b).

11.3.2 CXCR1 and CXCR2 Regulation

To avoid intrinsic signal transduction of CXCR1/2, the G protein-coupled signaling is strictly monitored and rapidly desensitized. Some of the processes that affect receptor desensitization include receptor phosphorylation/β-arrestin1/2 recruitment, AP-2 adaptor protein interaction, and receptor cross-desensitization.

Desensitization to homology (agonist-dependent) In response to ligand stimulation, G protein-coupled receptor kinases (GRKs) phosphorylate CXCR1/2, which then binds to β-arrestin1/2 and AP-2 to facilitate clathrin and dynamin-mediated receptor endocytosis (Barlic et al. 1999; Yang et al. 1999; Richardson et al. 2003; Raghuwanshi et al. 2012). When compared to CXCR1, CXCR2 internalization occurs more promptly and at lower ligand concentrations, indicating that the two receptors' signaling are regulated differently (Prado et al. 1996; Rose et al. 2004). Different GRKs regulate CXCR1 and CXCR2 diversely. CXCR2 is primarily phosphorylated by GRK2, while GRK6 mediates phosphorylation of CXCR2. Through two different mechanisms, receptor phosphorylation attracts arrestin1/2 toward the receptor to suppress G-protein signaling. The affiliation of arrestin1/2 prevents receptor G-protein coupling and brings in the endocytic apparatus, including clathrin and AP-2, to facilitate sequestration and endocytosis of receptor (Luttrell and Lefkowitz 2002).

β-arrestin1/2 may not even be absolutely essential for internalization of receptor CXCR2 (but not CXCR1). Although greatly reduced, receptor internalization was still seen among cells lacking CXCR2 (truncated C-terminal or GRK knockout) and β-arrestin-2, indicating that alternative phosphorylation-independent mechanisms may also be involved in receptor internalization (Fan et al. 2001a, b; Zhao et al. 2004; Su et al. 2005). Increased G-protein signaling caused by phosphorylation or β-arrestin-2 deficiency also causes ROS to be produced, which causes cell death

(Fan et al. 2001a, b; Zhao et al. 2004; Su et al. 2005). Contrary to β-arrestin1/2, AP-2 receptor association does not necessarily involve phosphorylation, and CXCR1/2 internalization involves AP-2 binding site association. When endocytosis occurs, the essential adaptor protein AP-2 allows us to connect membrane-bound receptor sites to the clathrin lattice (Robinson 1994; Kirchhausen et al. 1997; Schmid 1997).

Other receptors that are activated also control CXCR1/2 (heterologous desensitization). By fMLP receptors or complement cleavage product C5a, CXCR1 and CXCR2 are cross-phosphorylated and desensitized to CXCL8 (Richardson et al. 1998a, b). Chemotactic attraction of leukocytes and stimulation are mediated by the potent chemotactic factors fMLP and C5a (Perez 1984; Wittmann et al. 2002). When fMLP and C5a receptors are coexpressed with CXCR1 in RBL-2H3 cells, but not CXCR2, these receptors are also cross-phosphorylated, and thus, desensitization of CXCL8 occurs (Richardson et al. 1998a, b). The capacity of C-terminally shortened CXCR2 b is able to activate PLD to cross-phosphorylate as well as desensitize MLP and C5a receptors, meanwhile, studies suggests that PLD activation affects CXCR1/2's capacity for controlling other receptors (Richardson et al. 1998a, b).

11.4 Receptor Transactivation

Vascular endothelial growth factor receptor 2 (VEGFR2) is transactivated by CXCR1/2 receptor phosphorylation, which is mediated by Src kinases, in endothelial cells after CXCL8 activation of CXCR1/2. Endothelial cell permeability generated by CXCL8 demands EGFR2 transactivation. The NF-κB pathway is used by the CXCL8–CXCR1/2 axis to induce VEGF transcription in endothelial cells, which in turn induces VEGFR2 activation. In order to facilitate endothelial cell migration and capillary tube production, the CXCL8–CXCR2 axis also transactivates the epithelial growth factor receptor (EGFR) through phosphorylation of receptor. Integrin v3 is essential for endothelial cell sustenance and tumor migration during angiogenesis, and CXCL8 increases its expression (Petreaca et al. 2007; Martin et al. 2009; Kyriakakis et al. 2011; Niu and Chen 2011).

11.5 CXCR1 and CXCR2 Structural Features

Their crystallization has been hampered by the complexity of purifying membrane-bound receptors as well as the intrinsic flexibility of GPCRs. Approximately fifteen human GPCR structures are now known, including those of the chemokine receptors CXCR1 CXCR4, and CCR5. Within the next several years, the GPCR Network will develop high throughput structure determination processes to describe 15–25 typical human GPCRs (Niu and Chen 2011). NMR spectroscopy was used to determine the three-dimensional geometry of CXCR1 (PDB code: 2LNL) (Zhang et al. 2015; Park et al. 2012; Stevens et al. 2013).

11.5.1 The CXCR1/2 N-Terminus

Numerous studies involving the CXCR1 and CXCR2 chimaera, receptor truncation, and single amino acid point mutations have uncovered a number of crucial structural features that are important for binding of receptors for its activation, as well as regulation. For ligand binding and specificity as well as for controlling the rate of receptor internalization (Prado et al. 2007), the N-terminus, transmembrane domain 4 (TM4), and CXCR1/2 extracellular loop 2 (ECL2) are essential. Two disulfide bonds are crucial for ligand binding in CXCR1 and are located between Cys30 and Cys277 (connecting the N-terminus to TM7) and Cys187 and Cys110 (connecting TM3 to ECL2). For ligand binding and receptor signal transduction, CXCR1's extracellular loops and transmembrane helices (Lys117 of TM3, Asp85 of TM2, and Glu291 and Asp288 of TM7) include charged residues (Park et al. 2012). CXCR2 ligands bind to overlapping but distinct sites on CXCR2, and ligand affinities are not always correlated with potency in activating the receptor. This implies that the locations where receptors bind and are activated are different (Ahuja et al. 1996). The idea that ligand binding and receptor activation are separate is further supported by the anti-CXCR1 monoclonal antibody 7D9, which suppresses chemotaxis without altering ligand binding to the area of the receptor's first 45 residues (Wu et al. 1996; Prado et al. 2007).

11.5.2 The C-Terminus of CXCR1/2

The phosphorylation, internalization, G-protein coupling, and association with other cytoplasmic proteins of the receptor are all controlled by the C-terminus of CXCR1/2. The intracellular loop 3 (ICL3) of the CXCR1 protein, which extends into the cytoplasm, is crucial for calcium mobilization, G-protein coupling, and cell chemotaxis (Park et al. 2012).

Additionally, a number of CXCR1 amino acid residues, notably Ser132, and Asp134 of TM3 and Phe251 of TM6, Met241, are crucial for G-protein coupling and activation of receptor (Han et al. 2015). Truncation of the C-terminus of CXCR1/2 decreases chemotaxis, enhances G-protein signaling (calcium release), and alters receptor phosphorylation, β-arrestin1/2 interaction, and internalization (Ben-Baruch et al. 1995) Lowered chemotaxis without receptor internalization implies that G-protein signaling pathways negatively regulates chemotaxis and not receptor internalization. Point mutations of alanine show that 342 and 346–348 serine residues are associated in receptor sequestration and desensitization but not involved in phosphorylation of receptor, implying that those certain hydroxylated residues could be associated in phosphorylation of binding site (Mueller et al. 1997).

Furthermore, studies demonstrating serine 346–348's participation in agonist-induced phosphorylation in primary cultured cells derived from mice has been conducted. Several adaptor proteins influence receptor desensitization and endocytosis as they interact to the C-terminus of CXCR2 as it provides a requisite binding site for them. Upon phosphorylation of the receptor, β-arrestin1/2 interacts with CXCR1/2

and facilitates the components of endocytosis such as dynamin and clathrin. In cell lines of HEK293, AP-2 regulates receptor internalization and sequestration by binding to the LLKIL motif on CXCR2 (Fan et al. 2001a, b). The decrease in G-protein activation (measured by GTP-S exchange) that results from C-terminal deletion of CXCR2 suggests that it is also associated in coupling of G-protein (Schraufstätter et al. 1998). CXCR2's third intracellular loop assists in coupling of G-protein and signaling as well (Yang et al. 1997). The serine/threonine phosphatase protein phosphatase 2A (PP2A), that initiates dephosphorylation of receptor and receptor recycling, also interacts directly with CXCR2 on the KFRHGL motif of the C-terminus (Fan et al. 2001a, b). Vasodilator-stimulated phosphoprotein (VASP) is phosphorylated by CXCL8 through PKA- and PKC-mediated signal transduction pathways, which enhances VASP's interaction with the C-terminus of CXCR2. For CXCR2-mediated chemotaxis and polarization, VASP interaction is needed (Neel et al. 2009).

In addition to mediating common GPCR signaling pathways, these receptors also play a part in cellular processes including activation of the Ras/MAPK and PI3K signaling cascades, calcium release, receptor internalization, and chemotaxis. However, it has been demonstrated that CXCR1 but not CXCR2 activates PLD, which thereby promotes the production of ROS and the oxidative burst in neutrophils (L'Heureux et al. 1995; Baggiolini 1997; Richardson et al. 1998a, b). Between the two receptors, there is also a variation in the rate of receptor desensitization. Compared to CXCR1, CXCR2 is absorbed more quickly and with lower ligand concentrations. In contrast, it returns to the surface considerably more slowly than CXCR1. CXCR2 was shown to be able to activate PLD and facilitate CXCL8-mediated superoxide anion generation in investigations using CXCR2 mutants, where the C-terminus was shortened and receptor internalization was hampered (Richardson et al. 1998a, b).

For receptor desensitization as contrary to receptor activation, increased ligand concentrations are needed. Moreover, the tendency of CXCR2 to interact directly with all ELR^+ chemokines leads to the possibility that CXCR2 has a greater impact on chemotaxis as compared to CXCR1 (Chuntharapai and Kim 1995; Hartl et al. 2007). According to prior research using antibodies targeting CXCR1/2 and in vivo testing using $CXCR2^{-/-}$ mice, both CXCR1 and/or CXCR2 were essential for the survival, proliferation, migration, of endothelial cells and also involved in tube formation, and angiogenesis in endothelial cells which is mediated by CXCL8. It is interesting to note that endothelial cells were not affected additively by the combined knockdown of CXCR1 and CXCR2, confirming that the deletion either of receptor is sufficient to modify CXCL8-mediated angiogenesis (Singh et al. 2011).

11.6 Role of CXCL8–CXCR1/2 Axis in Infection

Infection and tissue injury both lead to inflammation, which serves as a defense mechanism (Stadtmann and Zarbock 2012). Neutrophils are recruited to the infected area by the CXCL8–CXCR1/2 axis, which further triggers a neutrophil oxidative burst and granule release to minimize inflammatory stimulation and boost microbial

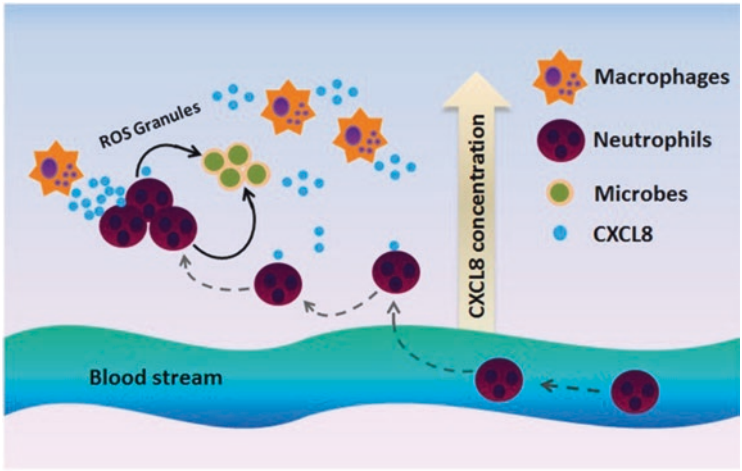

Fig. 11.4 CXCR1 and CXCR2 regulate neutrophil trafficking during infection

clearance (Chuntharapai and Kim 1995; Hartl et al. 2007). Consequently, this axis guards against new infections and tissue damage for the host (Samie et al. 2014). Disturbance in the CXCL8–CXCR1/2 axis might drastically impact the immune defense mechanisms against infectious disease and might even contribute to fatality. In experimental infectious disease models, impaired neutrophil recruitment often results in a decline in bacterial clearance and a lowered survival rate (Fig. 11.4).

11.7 The CXCL8–CXCR1/2 Axis in Cancer

Different cancer cell types secrete and express the CXCL8–CXCR1/2 axis in cancer, which stimulates cancer cell migration and proliferation in an autocrine manner (Wen et al. 2006; Sun et al. 2008; Erreni et al. 2009; Acharyya et al. 2012; Mehraj et al. 2022a, b, c; Mir et al. 2022a, b). According to an oncomine analysis, CXCL8 production is noticeably higher in cancerous tissues than in healthy ones in the case of esophageal, head and neck, pancreatic, and colon cancers (from Thermo 2017). Chemokine expression also directly relates with tumor grade as well as metastatic potential in human tumors. For instance, blood levels of CXCL8 are higher in prostate cancer patients than in healthy individuals, and they are correlated with the metastasis stages (Veltri et al. 1999). CXCL5 production corresponds with clinical stage in pancreatic cancer and is related to reduced survival rates (25.5 months less than those expressing low CXCL5) (Li et al. 2011a, b). CXCL8 is also upregulated in bladder cancer and the increased expression of CXCL8 is related to advanced stage disease. As a whole rate of survival of bladder cancer patients is drastically decreased with elevated expression of CXCL8 (Zhang et al. 2014), upregulation of CXCL1–3 and CXCL8 is linked to the vascular-invasive cancer phenotype, and in turn, our invasion is inhibited by CXCR2 signaling (Sharif et al. 2015). In

endometrial cancer, tumor-associated macrophage-derived CXCL8 promotes invasion of tumor cell as well as metastasis by suppressing estrogen receptor through HOXB13. We compared the levels of gene expression for CXCL8 and its receptors in 1036 cell lines from the Cancer Cell Line Encyclopedia (CCLE) to the NCI-60 cell lines.

Although CXCR1 and CXCR2 are overexpressed in just a small number of cancer cell lines, CXCL8 is upregulated in a variety of cancer cell lines. These genes are highly expressed in immune system tissues in line with their function in the inflammatory response. Using data from the Project Achilles database, we examined the functional significance of the CXCL8–CXCR1/2 axis and found that CXCL8, CXCR1 or CXCR2 knockdown adversely affected cell survival and proliferation (Cowley et al. 2014).

Other chemokine sources, primarily those synthesized by macrophages associated with tumors, can also stimulate cancer cells (Mir et al. 2022a, b). The CXCL chemokines are released by macrophages in response to the hypoxic and stressed tumor microenvironments, which is mediated by NF-κB (Sharif et al. 2015; Mehraj et al. 2021a, b). Endothelial cells that express CXCR1/2 are stimulated by CXCR1/2 ligands, which aids in tumor angiogenesis. A tumor has to develop the ability to stimulate angiogenesis in order to grow past 2–3 mm (Folkman and Hanahan 1991). The vital nutrients and oxygen that the tumor vasculature supplies to the tumor cells aid in their unchecked development and invasion. By cells secrete matrix metalloproteinases (MMPs) to degrade the extracellular matrix (ECM), for instance, endothelial cells stimulated by CXCL8 start the angiogenic process and begin to proliferate and start the construction of capillaries (Li et al. 2003).

The participation of CXCR2 in cancer progression as well as angiogenesis is further illustrated by many in vivo cancer models that showed depletion of chemokines and/or the receptor significantly reduced tumor growth associated with decreased microvessel density. For instance, the CXCR2 knockout mice injected with Lewis lung carcinoma (LLC) display reduced tumor growth, spontaneous, vascular density, metastases in orthotopic tumor models when compared to the wild-type. CXCR2-mutant mice showed smaller tumors and reduced tumor angiogenesis in prostate and pancreatic malignancies, demonstrating similar results. Small-molecule CXCR2 inhibitors have also demonstrated promising anticancer effects in preclinical trials. In colon cancer mouse xenografts, treatment with SCH527123 reduced tumor growth and angiogenesis while improving sensitivity to oxaliplatin (Keane et al. 2004; Shen et al. 2006; Wilson et al. 2008; Li et al. 2011a, b).

SCH527123, on the other hand, suppressed colon cancer hepatic metastases while having no effect on tumor development in mouse xenografts (Singh et al. 2009). CXCR2 deletion experiments employing shRNA and comparatively tiny inhibitors of CXCR2 did not dramatically reduce cancer cell growth in vitro, indicating that CXCR2 and its ligands play a significant role in the tumor microenvironment. Upregulation of CXCL8 also occurs in responses to several anticancer treatments, which may lead to chemoresistance. CXCL8 production mediated by NF-κB signaling, and its secretion was increased in prostate cancer cells treated

with oxaliplatin or 5-FU (Wilson et al. 2008) and in pancreatic cancer cells treated with gemcitabine (Song et al. 2015).

11.8 CXCL8–CXCR1/2 Axis and the Tumor Microenvironment

Tumor progression and metastasis are influenced by the tumor microenvironment (Qayoom et al. 2023). Adipose cells, migratory hematopoietic cells, and fibroblasts are among the nonmalignant stromal cells found in the tumor microenvironment. In addition to these tumor microenvironment components, migratory hematopoietic cells such as tumor-associated macrophages (TAMs), play an important role in tumor growth as well as metastasis (Westra et al. 1993; Mir and Mehraj 2019). Many cancerous cells produce significant quantities of CXCR1/2 ligands without displaying the receptor sites, indicating that CXCR1/2 ligands have a paracrine role in the tumor microenvironment. Moreover, CXCR1/2 ligands enhanced cancer cells proliferation, invasion, and chemoresistance via higher amounts of angiogenic and growth factors generated by tumor-associated neutrophils (Pollard 2004; Houghton et al. 2010) (Fig. 11.5).

CXCL8 is a cytokine that is abundant in TAMs and drives TAM-dependent tumor metastasis and invasion of papillary thyroid cancer (PTC) in vivo (Fang et al. 2014). The CXCL1/8-CXCR1/2 axis recruits obesity-associated adipose stromal cells (ASCs), that worsen specific malignancies including prostate cancer, into the tumor microenvironment (Zhang et al. 2016). In the tumor microenvironment, CXCR2 signaling influences the development and metastasis of breast cancer. $Cxcr2^{-/-}$ mice have lower total tumor cell apoptosis, proliferation, angiogenesis, and metastasis than wild-type mice (Sharma et al. 2015; Mehraj et al. 2022a, b, c). CXCR2 overexpression in the tumor microenvironment corresponds with tumor size in colon cancer, according to optical coherence tomography and laser-induced fluorescence imaging assays (Leung et al. 2015). CXCL8 and CXCR2 in the tumor microenvironment enhanced colon cancer cell proliferation, development, and metastasis (Lee et al. 2012).

In vitro tests show that the lung cancer cells from KrasLA1 mice, LKR-13, are resistant to anti-CXCR2 antibodies. However, the antibody is sensitive to the same cells that are generated as syngeneic tumors in wild-type mice, suggesting the significance of CXCR2 inside the (Chen and Song 2019) tumor microenvironment (Wislez et al. 2006). Through the expression of the CXCR2 receptor on stromal cells in the tumor microenvironment of an osteogenic prostate tumor, CXCL1 induces paracrine activities (Lee et al. 2015). In comparison to PDAC cells alone, pancreatic fibroblasts and pancreatic ductal adenocarcinoma (PDAC) cells together showed quicker tumor formation in mice. In a mixed cell xenograft model, CXCR2 inhibitor therapy or CXCR2 stromal cell knockdown both inhibited tumor growth (Ijichi 2012). These findings confirm the role of the CXCL8–CXCR2 interaction as a critical therapeutic target for cancer and provide more evidence that the connection between stromal CXCR2 and CXCL8 in cancer cells is crucial for tumor growth, invasion, and metastasis.

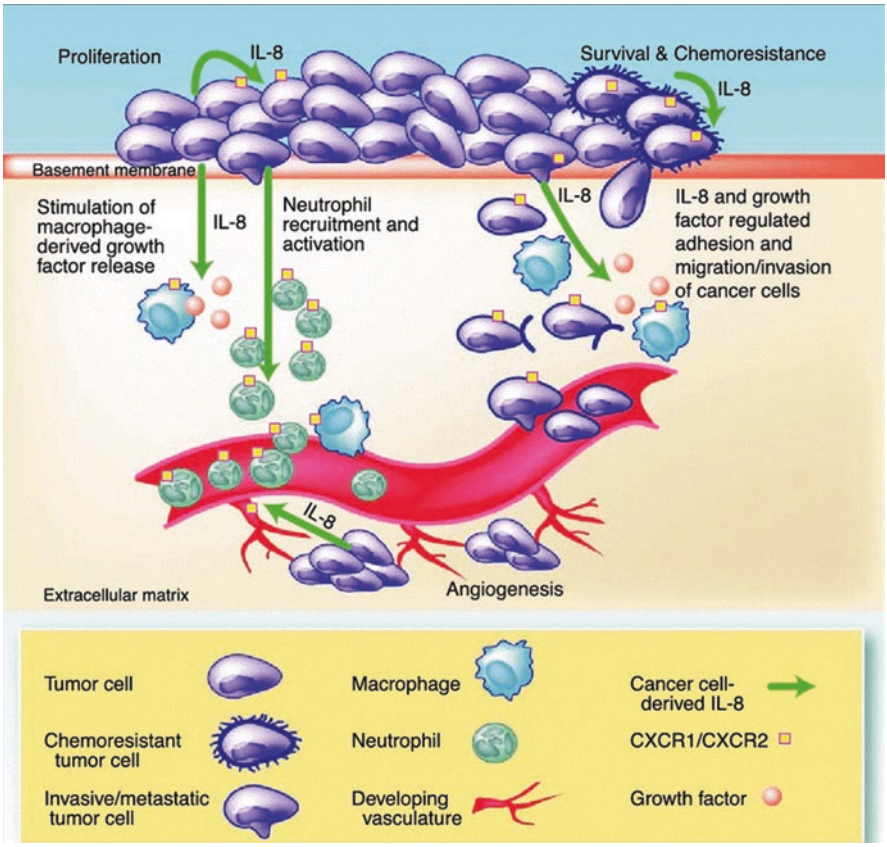

Fig. 11.5 The tumor microenvironment and the CXCL8–CXCR1/2 axis

11.9 Interactions of the CXCL8–CXCR1/2 Axis and CAF

In both metastatic and primary malignancies, CAFs are stimulated fibroblast populations and the main cellular components of the TME. After entering tumor tissue, CAFs have characteristics distinct from those found in normal fibroblasts, including increased proliferation and epigenetic alterations that result in the production of secreted factors (Chen and Song 2019; Öhlund et al. 2014; Wu et al. 2021). Through complicated interactions and intricate signaling with other cell types in the TME, CAFs contribute to desmoplasia and can promote tumorigenesis, tumor cell proliferation, migration, and cancer immunotherapy resistance (Mir 2015; Yoshida 2020; Chen et al. 2021). Due to the possibility of reprogramming many progenitor cell types into CAFs, the population of CAFs is highly diverse. Major CAF progenitor cells are dormant or resident fibroblasts. The putative origin of CAFs is either the pancreas or the liver. Cells from the bone marrow, endothelium, epithelium, and adipocytes can all be reprogrammed to become CAFs. Smooth muscle cells and

mesenchymal stem cells are only two examples of the numerous resident progenitors from which CAFs might develop. However, due to a lack of lineage indicators, the specific origins of CAFs continue to be a mystery. CAFs show properties of both cancer-promoting and cancer-suppressive functions (Mir et al. 2022a, b). It's yet unclear how CAFs work to prevent tumor growth. Promotion of anticancer immunity, tumor-restraining metabolism, tumor-inhibitory signaling, and ECM-related physical obstacles to tumor cell invasion and dissemination are all components of the host defense mechanism (Hutton et al. 2021).

In desmoplastic pancreatic, breast, and lung tumors, connective or fibrous tissue grows. Desmoplastic reaction may result in extensive fibrosis or desmoplasia around the tumor, which will impair its growth and migration (Hutton et al. 2021). The primary regulatory activities of growth factors, cytokines, and chemokines that contribute to angiogenesis, ECM remodeling, abnormal stroma, and an immunosuppressive TME are the mechanisms underlying CAFs' tumor-promoting effects. Two stable and functionally different pancreatic fibroblast lineages were discovered using mass cytometry and single-cell analysis of pancreatic tumor and healthy pancreas tissues (Sahai et al. 2020). Pancreatic fibroblasts that are CD105-positive encourage tumor progression, while CD105-negative fibroblasts are significantly tumor suppressive in a way that depends on adaptive immunity. Specialized secretory activities performed by CAF subsets, such as those of cytokines, chemokines, and collagen I, are involved in ECM remodeling and immunomodulatory activity (Hutton et al. 2021).

The final result of the tumorigenesis process is determined by tumor-fibroblast interactions through soluble factors, which also have an impact on cancer therapy. According to the origins of the cells, the CAF population is heterogeneous, and paracrine substances like CXCL8 and CXCR1/2 ligands can control this functional heterogeneity. By releasing CXCL8, CAFs can draw in monocytes to promote TAM enrichment and inhibit NK cells' activity in colorectal cancer. CXCL8 was shown to be substantially expressed and localized in CAFs in the gastric tumor tissue of chemoresistant patients (Zhai et al. 2019). An increased serum CXCL8 level has been linked with poor response to cisplatin treatment in patients with gastric cancer. CAFs are main sources of chemokines (CXCL1 and CXCL8) which attracts granulocytes (TAM and PMN-MDSC) to tumors. A strong antitumor effect was created by combination of a preferential CSF1R reagent (JNJ-40346527) with a CXCR2 antagonist (SB225002), which was then improved by the addition of antiprogrammed cell death protein 1 (PD-1) (Kumar et al. 2017).

MSCs can be converted to inflammatory CAFs by chronic inflammation and proinflammatory cytokines such tumor necrosis factor (TNF) and interleukin 1 (IL-1). In Luminal-A breast cancer cells, these CAFs release prometastatic chemokines such CXCL6 and CXCL8, which promote migration. CXCL8 can promote normal ovarian fibroblast to CAFs and drive xenograft tumor growth in mice. The ovarian cancer cell buildup was increased by the CXCL8 released from CAFs through the Notch3 signaling pathway (Sofi et al. 2023). Extracellular vesicles from gastric cancer transmit different miRNAs and cause the production of chemokines such CXCL1 and CXCL8 in CAFs. In patients with gastric cancer, abnormal

CXCL1 and CXCL8 production in CAFs was strongly correlated with tumor growth and worse survival (Naito et al. 2019). The basal secretory autophagy in head and neck squamous cell carcinoma is highly expressed in CAFs. The export of cellular inflammatory mediators including IL-6 and CXCL8 is mediated by secretory autophagy. Autophagy inhibition and cisplatin were used in combination therapy, which dramatically decreased tumor volume (New et al. 2017). By producing inflammatory chemokines like CCL2 and CXCL8, stromal cells like MSCs and CAFs improve the triple-negative type of breast cancer metastasis-related properties, comprising migratory, angiogenesis, and invasive abilities (Liubomirski et al. 2019; Sofi et al. 2022). For the stimulation of CXCL8 in tumor–stroma interaction and ultimately for prometastatic actions, Notch 1 activation is necessary (Liubomirski et al. 2019) Prostate cancer cell migration is influenced by androgen receptor activation in CAFs and is facilitated by CXCL8 and CCL2 (Cioni et al. 2018).

CXCR2 is expressed by CAFs, which further enhance CXCR2 ligands such CXCL1, CXCL7, and CXCL8 in response to paracrine signals from pancreatic cancer cells. Angiogenesis was decreased and an anticancer response was produced by CXCR2 deletion in a pancreatic ductal adenocarcinoma syngeneic murine model, while CAF stimulation, fibrosis, and metastasis were enhanced in a mutation-dependent manner (Awaji et al. 2020). The outcome of cancer therapy may be significantly affected by the CAF response to chemotherapy, which is highly variable (Sonnenberg et al. 2008).

Traditional maximum-tolerated dosage chemotherapy stimulates CAF activation and causes ELR motif-positive chemokines like CXCL6 and CXCL8 to be produced and secreted [37] (Chan et al. 2016). These chemokines encourage aggressiveness and treatment resistance by signaling through CXCR2 on cancer cells to turn into tumor-initiating cells. The same overall dosage given as a minimal metronomic chemotherapy increased survival in mice while mostly preventing CAF activation and enhancing treatment effectiveness (Chan et al. 2016). In colorectal cancer, CAF-secreted IL-6 and CXCL8 cause the production of the protein Bromodomain-containing protein 4 (BRD4), which results in chromatin remodeling and BET inhibitor resistance in colorectal cancer (CRC). In a mouse xenograft model for CRC, inhibition of IL-6/CXCL8–JAK2 signaling made BET inhibitors more sensitive (Jobe et al. 2016). In a 3D spheroid invasion test, simultaneous inhibition of IL-6 and CXCL8 can reduce the invasiveness of CAF-induced human melanoma cells (Jobe et al. 2016). Senescent human fibroblasts have been shown to release IL-6 and CXCL8 that aid in the invasion and metastasis of cancer cells. Senescent CAFs are a pathologically significant population of fibroblasts that release too much CXCL8 to encourage the invasion of pancreatic cancer (Wang et al. 2017). By cells that produce IL-6 and CXCL8, a novel subpopulation of $CD10^{+}GPR77^{+}$CAFs promotes the enrichment of cancer stem cells (CSCs) and chemoresistance in cancer. In patients with breast and lung cancer, overexpression of $CD10^{+}GPR77^{+}$CAFs primarily promotes CSCs and is associated with poor prognosis (Su et al. 2018).

11.10 CXCL8–CXCR1/2 Axis in Immunogenic Cell Death

Chemotherapy causes tumor cells to undergo immunogenic cell death (ICD) by releasing damage-associated molecular patterns (DAMP). Multiple steps are involved in ICD process that starts with dying tumor cells secreting "find me" signals like nucleotides, fractalkine, and ATP to chemoattract dendritic cells (DCs) or phagocytes followed by the expression of "eat me" signals like phosphatidylserine and calreticulin that allow phagocytic cells or Dendritic cells to target the tumor cells, and followed by transmission of danger signals like the (HMGB1) high mobility group box 1 protein, which allows tumor cells that are on the verge of dying to lose tolerance (Palombo et al. 2014) (Fig. 11.6).

As a result, antitumor immunity is generated, which results in the death of cancer cells. The suppression of CXCR1 and its ligand CXCL8 inhibits the calreticulin immunogenic translocation in the methotrexate-treated cancer cells, toward the surface of the cell, according to siRNA screening of multiple GPCRs and their ligands. In reality, human cancer cells produced CXCL8 when treated with methotrexate in a lab. ICD is followed by gene transcription of CXCL2, the mouse homolog of CXCL8, according to transcriptome investigations in vivo tumor-bearing mouse

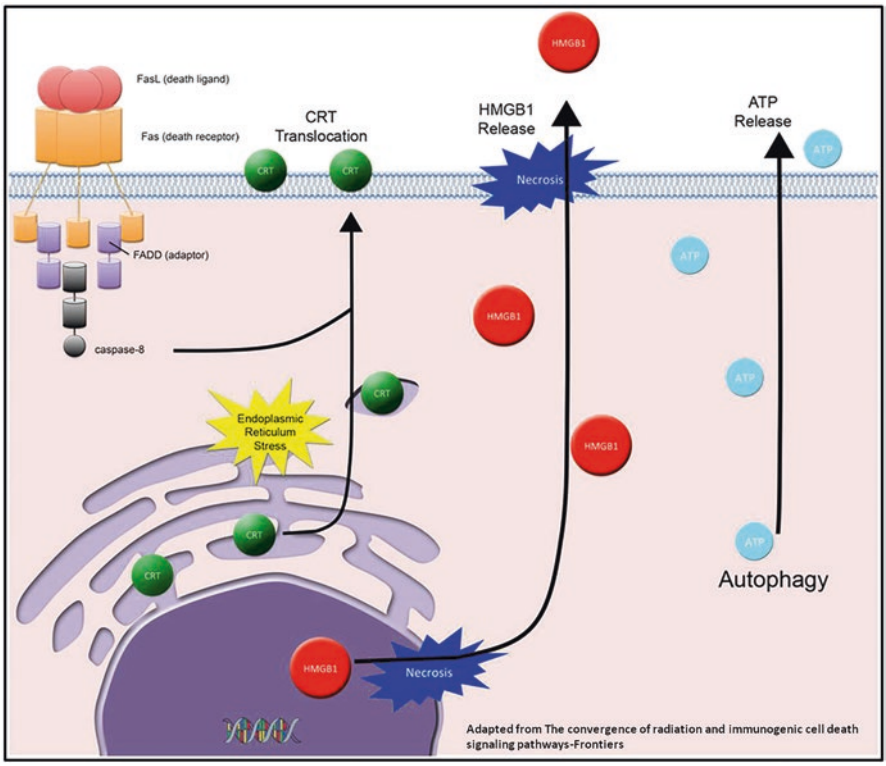

Fig. 11.6 Normal mechanism of immunogenic cell death

models (Sukkurwala et al. 2014) HMGB1 also encourages neutrophils and macrophages to produce CXCL8, a proinflammatory cytokine, speeding up ICD (Krysko et al. 2012). The CXCL8–CXCR1/2 axis may prevent the development of early malignant tumors by inducing cell senescence and promoting the recruitment of innate immune effectors that mediate immunogenic cell death, despite the fact that it promotes tumor progression, invasion, and metastatic spread including both spontaneous and inflammation-driven tumor models. Similarly, depending on the type of cancer, its stage, and particular drug combinations, the therapeutic application of CXCR1/2 agonist or antagonist should be investigated with caution.

11.11 Immunecheckpoint Inhibition and CXCL8–CXCR1/2 Axis

The host's immune system suppression is a significant factor in the development of cancer. There are various immunological checkpoints that let cancer cells evade the immune system by reducing the host's immune response. Many malignancies, including pancreatic and prostate, employ the development of myeloid-derived suppressor cells (MDSCs) as one of these checkpoints to avoid the immune system. These MDSCs are transported to the tumor bed by CXCR2 signaling (Steele et al. 2016; Liu et al. 2016), checkpoints, and the CXCL8–CXCR1/2 axis. Elevated levels of macrophage migration inhibitory factor (MIF), which is produced by CSCs, cause MDSCs to produce more of the immune-suppressing enzyme arginase-1 in a CXCR2-dependent fashion. Antibody-mediated inhibition of the MIF receptor, CXCR2, reduced the synthesis of arginase-1 and prevented tumor cells from evading the immune system (Otvos et al. 2016). The anti-PD-1 antibody inhibited tumor growth during the early stages of tumor progression, whereas anti-PD-1 therapy administered later had limited effects (Highfill et al. 2014). By preventing T-cell infiltration and activation, MDSCs lead to resistance to anti-PD-1 treatment. When combined with anti-PD-1 antibody, CXCR2-blocking antibody or CXCR2 antagonist treatment significantly improved tumor growth inhibition and survival in mouse models of the disease (Highfill et al. 2014). As a result, CXCR2 signaling is crucial for controlling tumor immunological checkpoints, and blocking this signaling pathway with an antagonist or anti-CXCR2 antibody together with chemotherapy may prevent cancer cells from evading the immune system (Fig. 11.7).

11.12 CXCL8–CXCR1/2 Axis and Cancer Stem Cell

Cancer stem cells (CSCs) are cells that have the ability to self-renew and differentiate, and they can initiate and promote the development and spread of cancer. Cancers of the liver, breast, colon, and pancreas are only a few of the tumors for which CSCs have been shown to be significant (Chen et al. 2014) Recent research suggests that via controlling CSC self-renewal and proliferation, the CXCL8–CXCR1/2 axis performs a significant role in tumor development and metastasis (Ginestier et al. 2010; Brandolini et al. 2015). The CXCL8–CXCR1 axis stimulates the development of

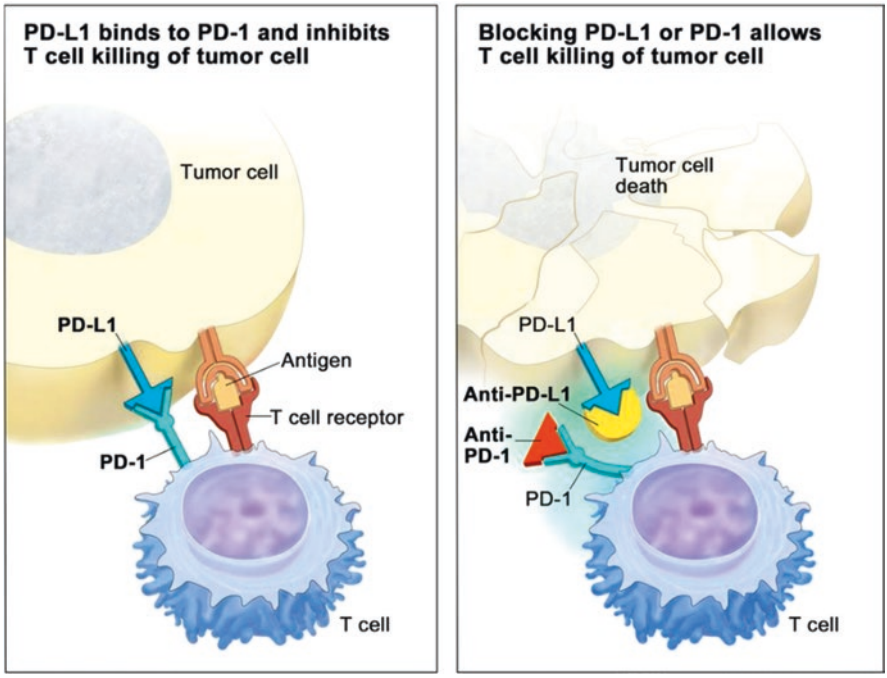

Fig. 11.7 Immunecheckpoint inhibition and CXCL8–CXCR1/2 axis

stem cell-like mammospheres (Chen et al. 2014). In pancreatic cancer, CXCR1 is strongly correlated with the CSC markers CD44 and CD133. These effects on pancreatic CSCs are reduced by blocking antibodies against CXCR1 and CXCL8 (Chen et al. 2014). In an autocrine/paracrine mechanism, in humans, CXCL8 encourages the epithelial–mesenchymal transition (EMT) of breast cancer cells. EMT causes tumor cells to develop metastatic and stem-like properties as well as inherent resistance (David et al. 2016; Fernando et al. 2011).

Human pluripotent stem cells (hPSCs) were treated with siRNA to inhibit CXCR2, which resulted in a decrease in the proliferation and maintenance of stemness characteristics (Mehraj et al. 2021a, b). It also caused a significant increase in the production of the pluripotency markers Nanog, Rex-1, and OCT-4 as well as the germ layer markers NESTIN and GATA3. CXCR2 inhibition prevented hPSC self-renewal and led to a slow but steady rise in differentiation of cell. Glioblastoma stem cells' maintenance and cellular expansion are controlled by the CXCL8–CXCR1/2 signaling. The migration, proliferation, and stem cell properties of CSCs were augmented the 3D scaffold-based approach used to culture glioblastoma endothelial cells upregulated CXCR1/2 as well as increased CXCL8 levels (Infanger et al. 2013).

As CXCR2 knockdown in CSCs eliminated CSCs' in vivo tumor-promoting activities, CXCR2 plays a crucial role in the development of glioblastoma (Infanger et al. 2013; Mir et al. 2020). The population of CSCs and cell self-renewal were raised in

breast cancer cells after recombinant CXCL8 stimulates the activation of CXCR1/2. In metastatic breast CSCs, CXCL8–CXCR1/2 pathways transactivated HER2 signaling via SRC, PI3K, and MEK (Singh et al. 2013). Through the CXCR2 receptor, MSCs (mesenchymal stem cells) in the bone encouraged the migration of breast cancer cells in culture. Antibodies to CXCR2, CXCL1, CXCL5, and a specific molecular inhibitor of CXCR2, SB265610, greatly reduced the ability of PyMT cells to migrate to MSC conditioned medium (Halpern et al. 2011).

A significant role in the development of tumors is played by CXCR2, which is also strongly expressed in populations of stem cells of myelodysplastic syndromes (MDS) and acute myeloid leukemia (Schinke et al. 2015). Given that CSCs are thought to be essential for tumorigenesis, growth, and metastasis, the CXCL8–CXCR1/2 pathway may be a clinically significant target for cancer. Combining HER2 inhibitor with other targeted therapies, such as CXCL8–CXCR1/2 interaction inhibitor, may be a unique treatment option for tumors. CXCR2 controls not just CSCs but also hematopoietic stem cells, human pluripotent stem cells (hPSCs), and CSC self-renewal (HSCs) (Qayoom et al. 2023). Decreased self-renewal as well as pluripotency of potential hPSCs and HSCs was shown by CXCR2 deletion studies in mice and CXCR2 silencing studies in cultured cells (Jung et al. 2015, 2016; Sinclair et al. 2016).

To control endothelial progenitor cell (EPC) migration and angiogenesis, CXCR2 interacts with the PDZ scaffold protein NHERF1. EPC's in vivo angiogenic activity in mice are reduced when this connection is disrupted (Hou et al. 2015). As a result, CXCR2 targeting may be a potential therapeutic approach for conditions linked to angiogenesis.

11.13 Inflammatory Diseases and the CXCL8–CXCR1/2 Axis

Inflammation-mediated diseases such as asthma, cystic fibrosis, chronic obstructive pulmonary disease, inflammatory bowel diseases, psoriasis, and rheumatoid arthritis have been linked to CXCL8 and its receptors abnormal regulation because CXCL8 is an important factor of inflammation-mediated processes (Kulke et al. 1998; Banks et al. 2003; Bento et al. 2008; Grespan et al. 2008; Pickens et al. 2011). It also has a role in the carcinogenesis of several malignancies, including melanoma, breast, ovarian, prostate, lung, and pancreatic (Table 11.1).

11.14 Chronic Obstructive Pulmonary Disorder

Main cause of death and morbidity in developed nations is COPD. COPD is defined by emphysema, which is characterized by destruction of alveolar attachments (fibrosis), constriction of small airways, and gradual and irreversible airway obstruction (Gan et al. 2004; Rogers and Barnes 2006). The development of COPD is aided by CXCL8 in a number of ways. Lung macrophages produce CXCL8 and other chemokines in response to environmental factors (such as cigarette smoke and air

Table 11.1 Role of CXCL8 in common cancers

Cancer type	Function
Pulmonary cancer	Cancer stem cells proliferation, angiogenesis, tumor immune suppression
Colorectal cancer	Proliferation, angiogenesis, cancer stem cells, and migration
Glioma	Invasion and migration
Breast cancer	Cancer stem cells proliferation, migration invasiveness, angiogenesis, tumor immune suppression
Bladder cancer	Cancer stem cells
Osteosarcoma	Invasion and migration
Melanoma	Cancer stem cells proliferation, migration invasiveness, angiogenesis, tumor immune suppression
Ovarian cancer	Cancer stem cells proliferation, migration invasiveness, angiogenesis, tumor immune suppression
B-cell lymphoma	Tumor immune suppression
Pancreatic cancer	Cancer stem cells proliferation, migration invasiveness, angiogenesis, tumor immune suppression
Prostate cancer	Cancer stem cells proliferation, migration invasiveness, angiogenesis, tumor immune suppression

pollution) that control the migration of polymorphonuclear neutrophils (PMN) to the lungs (Barnes et al. 2003; Traves et al. 2004).

The airway epithelium is also stimulated by CXCL8, which causes it to contract and become more permeable to inflammatory cells. A buildup of neutrophils as well as other inflammatory cells produces protease secretion, which causes prolonged and severe tissue injury (Traves et al. 2004; Reutershan et al. 2006). Patients with COPD had higher levels of CXCL8 in their sputum and bronchoalveolar lavage (BAL) than healthy volunteers, which is associated with increased neutrophil recruitment (Keatings et al. 1996). During prolonged exposure to cigarette smoke, p53 increases the expression of CXCL1, CXCL2, and CXCR2 in BAL and increases the expression of plasminogen activator inhibitor-1 (PAI-1) in alveolar epithelial cells (Tiwari et al. 2016).

Neutralizing CXCL8 antibodies also considerably lower the neutrophil chemotactic activity of COPD patient sputum (Beeh et al. 2003). Mice exposed to acute cigarette smoke had less neutrophilic inflammation in their lungs when CXCR2 is inhibited by a small-molecule antagonist, which suggests that CXCL8 is a key factor in lung inflammation and the development of COPD [163] (Thatcher et al. 2005). COPD is influenced by cellular interaction between neutrophils expressing CXCR2 and CXCL8 released by alveolar macrophages. Therefore, it may be advantageous to treat COPD by inhibiting the CXCL8–CXCR1/2 pathway.

11.14.1 Asthma

Episodes of reversible airflow blockage, lung inflammation and bronchial constriction is triggered by allergens are features of asthma (Bousquet et al. 2000). During severe asthma exacerbations, eosinophils and neutrophils are elevated in the epithelium of lungs and sputum along with enhanced expression of ELR^+ chemokines and their receptors (Norzila et al. 2000; Nakagome et al. 2012). Additionally expressed on the smooth muscle lining the airways, CXCR1/2 mediates cell migration and contraction to improve airway sensitivity and remodeling (bronchoconstriction) seen in asthma (Govindaraju et al. 2006). Last but not least, compared to wild-type mice, CXCR2 defective mice show lower by neutrophil mobilization and bronchial hyper-responsiveness produced by ozone challenge (Johnston et al. 2005). Neutrophil recruitment that is CXCL8–CXCR2 dependent is essential for the emergence of asthma, and blocking this axis offer a fresh method of combating the disease.

11.14.2 Cystic Fibrosis (CF)

Cystic fibrosis (CF) is an inherited autosomal recessive illness that results from genetic abnormalities in the CFTR gene, i.e., cystic fibrosis transmembrane conductance regulator gene, which causes improper ion transport of chloride and sodium across the epithelium of tissue organs such as the pancreas and lungs (Clunes and Boucher 2007). The most adversely impacted organs are the lungs organs by CF, showing increased mucus production. Bronchitis and respiratory failure are brought on by repeat cycles of respiratory illness and neutrophilic inflammation (Jacquot et al. 2008). Additionally, CF patients have higher concentration of CXCL8 and other chemotactic cytokines (IL-1, IL-6, and TNF), in their airways. (IL-1, IL-6, and TNF), which regulate neutrophil infiltration, similar to other lung disorders addressed so far (Jacquot et al. 2008; Guan et al. 2016). Sputum from CF patients' dramatically reduced chemotactic activity is inhibited by antibodies against CXCL8 (Richman-Eisenstat et al. 1993). Although the expression of CXCR1 and CXCR2 are roughly equivalent in CF patients' and non-CF patients' smooth muscles airway. CXCL8 causes greater contractions in CF patients' airway smooth muscles, which may be related to more myosin light chains, which help explain the CF's bronchoconstriction (Govindaraju et al. 2008). CXCR1, but not CXCR2, encourages microbial death; however, in CF patients' airways, this function is eliminated. Airway proteases break CXCR1, and the fragments of CXCR1 activate bronchial epithelial cells using Toll-like receptor 2 (TLR2) to release CXCL8 (Hartl et al. 2007). Patients with CF who contain a specific CXCR1–2 HA are linked to reduced CXCR1 and elevated CXCR2 protein and mRNA expression, as well as compromised antibacterial action. In CF patients, these haplotypes are also linked to a decline in lung function (Kormann et al. 2012).

11.14.3 Inflammatory Bowel Diseases

The CXCL8–CXCR1/2 axis, which is important in the pathophysiology of IBD (Khalili et al. 2015), is directed by PMN recruitment. In reaction to CXCL8, neutrophils are quickly attracted to the infection site in the intestine. This is brought on by an increase in PMNs' ability to migrate, which overexpresses CXCR1/2 (Koelink et al. 2014). Epithelial cells release CXCL8 in response to bacterial infection, which facilitates PMN migration from the blood circulation (Koelink et al. 2014) Patients with ulcerative colitis express more CXCL8 mRNA in the colonic mucosa compared to healthy volunteers. (Bruno et al. 2015). By adversely affecting neutrophil activity, the CXCR2 antagonist SB225002 the intensity of DSS-induced experimental colitis in deficient mice the paired immunoglobulin-like type 2 receptor (PILR) alpha was lessened (Kishida et al. 2015). These data confirm the significance of the CXCL8–CXCR1/2 pathway in the pathophysiology of IBD, suggesting that inhibiting this signaling may be a promising therapeutic approach for the management of IBD.

11.14.4 Neuroinflammatory Diseases

Neutrophil infiltration is a hallmark of neuroinflammatory disorders like Neuro-Sweet disease because of aberrant neutrophil chemotaxis facilitated by the CXCL8–CXCR2 axis. CXCL8 levels are higher in the diseased patients than in the healthy controls (Kimura et al. 2008). It has been suggested that CXCR2, which is expressed in neutrophils as well as in oligodendrocyte progenitor cells, promotes demyelination, which causes multiple sclerosis. Remyelination is improved in a mouse model by inhibiting CXCR2 with a CNS penetrating inhibitor or by conditional CXCR2 deletion (Liu et al. 2015a, b). Compared to $Cxcr2^{+/+}$ mice, $Cxcr2^{-/-}$ mice exhibited resistant to cuprizone-induced demyelination (Padovani-Claudio et al. 2006). A CNS-penetrating CXCR2 antagonist (compound 22) demonstrated significant remyelination at 100 mg/kg twice daily for 9 days straight in a mouse model of cuprizone-induced demyelination. Tamoxifen-treated Cxcr2-conditional knock-out mice showed moderately, but significantly, faster remyelination (26.2% PLP-IR area fraction) compared to tamoxifen-treated control mice (24.1% PLP-IR area fraction) after 2 weeks of recovery following 6 weeks of cuprizone feeding (Kishida et al. 2015)

Neutrophils lacking SOCS3 (suppressor of cytokine signaling 3) activate Stat3 and generate significant amounts of CXCL2. Unconventional experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis, is developed in large part by CXCL2. By preventing neutrophils from colonizing the cerebellum and brainstem, CXCR2 inhibition reduced atypical EAE (Liu et al. 2015a, b). Additionally, CXCR1/2 signaling has been linked to a number of neurodegenerative conditions, including stroke and Alzheimer's disease (AD). Neurocognitive problems are linked to the overexpression of CXCL8 in HIV-1 patients' serum and brains. In the A1-42-generated rat model of AD, microglia express higher levels of

CXCR2 and CXCL8, and CXCR2 inhibition decreased microgliosis and provided neuroprotection (Ryu et al. 2015). In patients suffering from essential hypertension, polymorphism of CXCR2 gene is also related to the risk of ischemic stroke (Timasheva et al. 2015). Inhibiting CXCR2 might be a successful neuroprotective tactic because CXCR2 and its ligand CXCL8 are increased in Alzheimer's disease tissues and CXCR2-dependent inflammatory responses are crucial to AD progression.

11.14.5 Vascular Diseases

Reactive oxygen species, increased levels of proinflammatory cytokines and chemokines, and myocardial and cerebral infarction all result in the mobilization of circulatory neutrophils and leukocytes into the ischemic tissues. As a result, the tissue may suffer from ischemia injury brought on by the activated neutrophils. Ischemic damage has been successfully treated by blocking the CXCL8–CXCR1/2 axis (Zhao et al. 2010; Connell et al. 2015). With regard to a mouse model of persistent myocardial infarction, therapy with anti-CXCR2 antibody dramatically reduced the extent of the infarction and inhibited neutrophils infiltration into the infarcted area after prolonged and delayed treatment (Oral et al. 2013). The expression of CXCL5, macrophage migration-inhibitory factor (MIF), and CXCR2 in human atherosclerotic coronary arteries was shown to be increased. According to a theory put forth, interactions between MIF and CXCR2 or CXCL5 and CXCR2 may raise the possibility of developing coronary artery disease (Herlea-Pana et al. 2015). High levels of CXCR2 expression on endothelial progenitor cells (EPCs) govern their homing to regressing plaques and accelerated plaque clearance, in contrast to CXCR2 impact on neutrophil. All of these previously discussed findings point to the relevance of CXCR2 in the progression of coronary artery disease, and pharmaceutical inhibition of CXCR2 could be used as a treatment for this condition by preventing MIF and CXCR2 or CXCL5 and CXCR2 interactions. Furthermore, CXCR2 contributes to the emergence of hypertension. Vascular dysfunction and hypertension are brought on by the infiltration of proinflammatory $CXCR2^+$ cells. In mice, aortic macrophage infiltration and angiotensin II-induced hypertension are reduced by CXCR2 gene knockout or treatment with the CXCR2 antagonist SB265610 (Wang et al. 2016). When $CXCR2^+$ cell infiltration plays a significant role in the development of a disease, therapeutic intervention using a pharmaceutical antagonist of CXCR2 may be an alluring approach for hypertension treatment.

11.14.6 Arthritis

To maintain cartilage homeostasis, CXCR2 signaling is crucial. In inflammatory arthritis, inflammatory cells with CXCR2 expression, such as neutrophils, are attracted by ELR^+ CXC chemokines (Sherwood et al. 2015) CXCR1/2 is responsible for the development of rheumatoid arthritis as a cause of neutrophil adhesion to

the synovial microvascular endothelium and a promoter of neutrophil migration into the joints. Additionally, CXCR2 regulates neutrophil recruitment in arthritis brought on by Brucelas (Lacey et al. 2016). While the pathogenesis of most forms of arthritis, including RA, depends on the activation of CXCR1/2 signaling, that isn't the case during osteoarthritis. In healthy cartilage, ELR^+ chemokines are generated and maintain chondrocytes viability as well as differentiation. Osteoarthritis is primarily brought on by CXCR1/2 signaling disruption, which results in chondrocyte phenotypic instability (Sherwood et al. 2015). Low CXCR1/2 signaling may operate as a preventative measure against the onset of chronic osteoarthritis, whereas excessive CXCR1/2 signaling promotes to the development of chronic arthritis. A healthy amount of CXCR1/2 signaling must therefore be maintained for the articular cartilage.

11.14.7 Psoriasis

An autoimmune skin lesion is an inflammatory disease called psoriasis develops when high epidermal cells accumulate. One of the histological characteristics of psoriasis is CXCR2-directed neutrophils accumulation in the skin. CXCL1, CXCL2, and leukotriene B4 are chemokines that are increased in psoriatic skin and draw neutrophils that express CXCR2 into the lesions. In psoriatic skin, neutrophil infiltration is first triggered by the CXCL1/2-CXCR2 axis and is then sustained by the LTB4–BLT1 pathway (Sumida et al. 2014). Interleukin-17A (IL-17A) stimulates keratinocytes to produce CXCL8. Neutrophil elimination from psoriatic skin is induced and CXCL8 expression is reduced when the antibody secukinumab targets IL-17A (Reich et al. 2015). The CXCR2 antagonist SB225002 also reduces neutrophil infiltration and somewhat reduces psoriatic skin inflammation in a mouse model of psoriatic skin (Sumida et al. 2014). A CXCL8-blocking monoclonal antibody formulation called ABCream (Anogen, Mississauga, ON, Canada) has been authorized in China and is said to be beneficial in treating psoriasis (Bachelerie et al. 2014; Sumida et al. 2014). This is in contrast to ABX-IL-8 (Abgenix, Fremont, CA), an antibody against CXCL8 that has been fully humanized but failed to show effectiveness in phase II clinical studies. Livedoid vasculopathy with atopic dermatitis are two more skin disorders that are exacerbated by CXCR2-mediated neutrophil recruitment. These findings indicate the involvement of CXCR2 and its ligands in the evolution of psoriasis and other skin diseases and suggest that blocking CXCR2 signaling by CXCL8-targeting antibodies or CXCR2 antagonists may be helpful in the treatment of a variety of skin conditions.

11.14.8 Other Inflammatory Disorders

CXCR2 is crucial for various inflammatory diseases. Neutrophil recruitment mediated by CXCR2 is necessary for eradicating microbial spores from diseased lungs. Compared to wild-type mice (Gresnigt et al. 2016), it has been suggested that the

CXCR2 signaling pathway plays a part in nociception. In an animal model of neuropathic pain, upregulation of GRK6 or therapy with CXCR2 siRNA lowers CXCR2 activity in dorsal root ganglion and greatly lessens discomfort (Zhou et al. 2016). Human natural killer (NK) cells control the attraction of MSCs (mesenchymal stem cells) to the site of damage, which is crucial for wound healing. One of the mechanisms promoting healing process or regeneration at the site of damage is the interaction between MSCs that express CXCR2 and NK cells that release NAP-2 (Almeida et al. 2016). CXCL1 and CXCL8 are accountable for the production of Mallory-Denk Bodies in the liver and are markedly elevated in a variety of inflammatory liver disorders, including alcoholic hepatitis. CXCR2 signaling inhibition prevents neutrophil infiltration and lessens liver damage (Roh et al. 2015). The development of pancreatitis is similarly influenced by CXCR2 signaling, and CXCR2 suppression reduces pancreatic inflammation (Steele et al. 2015). Additionally, type 1 diabetes (Citro et al. 2015), Sjogren's disease, and HIV-associated nephropathy are all linked to CXCR2 signaling. For microbial infections, wound healing, and numerous inflammatory disorders, where CXCR2 signaling could be detrimental or beneficial, maintaining CXCR2 regulation is crucial. To better understand the function of CXCR2 signaling and its suppression in various disorders, more research is required.

11.15 Therapeutic Targeting of the CXCL8–CXCR1/2 Axis in Cancer

Both CXCR1 and CXCR2 are activated by the chemokine CXCL8. Their upregulation in chronic or acute inflammatory disorders, including cancer, has been demonstrated in all of them. The main mechanism for the migration of MDSCs and neutrophils is CXCR1 and CXCR2, which may accelerate tumor growth and reduce the effectiveness of immune treatment (Qayoom et al. 2021). An effective treatment method for addressing immune suppression in the TME should be targeting the CXCL8–CXCR1/2 pathway with tiny molecules or antibodies. It was initially discovered that A tiny chemical called SB225002 inhibited CXCL8 that bound to CXCR2. In rabbits, SB225002 effectively prevented CXCL8 stimulated margination as well as neutrophil chemotaxis. In ovarian cancer, breast cancer, nasopharyngeal carcinoma, and acute myeloid leukemia, tumor progression has been shown to be inhibited by blocking CXCR2 with SB225002 (Fig. 11.8).

Reparixin is an allosteric CXCR1 and CXCR2 noncompetitive inhibitor that has the potential to prevent polymorphonuclear cell migration in vivo. Reparixin's action on CXCR1 was 100 times greater than its activity on CXCR2. Breast cancer stem cells have been found to be a druggable target for CXCR1. In rodents and monkeys, the oral CXCR1 and CXCR2 blocker navarixin reduces neutrophil recruitment (Bertini et al. 2004). In a mouse model of melanoma, navarixin reduced tumor cell growth and angiogenesis. A phase II clinical trial (NCT03473925) for Non-small-cell lung cancer (NSCLC), castration-resistant prostate cancer, and colorectal cancer assessed the efficacy and safety of navarixin in combination with anti-PD-1 pembrolizumab. Navarixin's efficacy and safety in combination with anti-PD-1

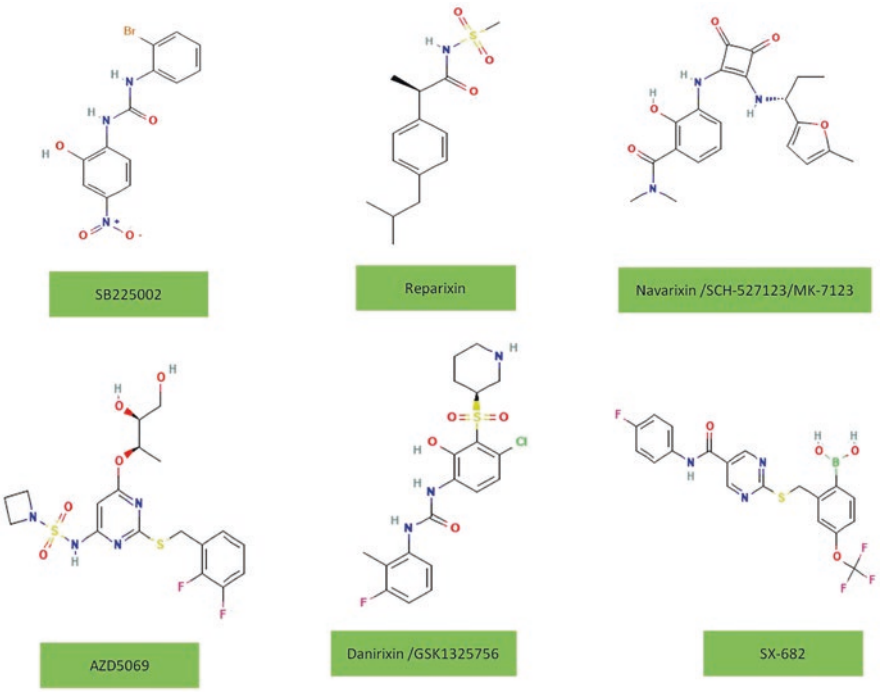

Fig. 11.8 Agents targeting CXCR1/2 signaling impair immunosuppressive cell recruitment and angiogenesis to enhance cancer therapy efficacy

pembrolizumab (Chapman et al. 2007; Nicholls et al. 2015). A reversible CXCR2 antagonist called AZD5069 prevents neutrophil chemotaxis and CXCL8 binding. In advanced prostate cancer, AZD5069 therapy reduces TAM infiltration promotes CD4/CD8 T-cell infiltration, increases vascular development, and slows tumor growth. Combination therapy with AZD5069, anti-PD-1, and durvalumab (MEDI4736) was studied in patients with metastatic pancreatic ductal adenocarcinoma and metastatic squamous cell carcinoma of the neck and head (NCT02499328) (NCT02583477) (Di Mitri et al. 2019).

11.16 Conclusion

It is well known that the CXCL8–CXCR1/2 signaling pathway has a pathophysiological role in inflammatory disorders such as inflammatory bowel diseases, asthma, COPD, cystic fibrosis, psoriasis, Alzheimer's disease, arthritis, and stroke. CXCL8's significant function in PMN recruitment is important for the host's innate defenses against inflammatory disorders. Lung macrophages release CXCL8 reaction to external stimuli, which directs Polymorphonuclear recruitment to the lungs and results in inflammatory disorders of the airways. The development of breast, colorectal, pancreatic, pulmonary, melanoma, ovarian, and prostate cancers has

been linked to the CXCL8–CXCR1/2 axis. More importantly, genes associated with the CXCL8–CXCR1/2 signaling pathway plays an important part in the microenvironment of tumors as well as cancer stem cells, where they effectively contribute to the growth of tumors and the migration of stem cells. By bringing MDSCs, an immunological checkpoint, into the tumor microenvironment, CXCR2 signaling also contributes significantly to the immune evasion of tumor cells and results in resistance to many chemotherapeutic agents. Combining anti-CXCR2 therapy with other forms of chemotherapy, especially anti-PD-1 treatment when treating pancreatic cancer, was more successful than either agent alone. The CXCL8–CXCR1/2 axis participates in the immunogenic cell death caused by chemotherapy as a signaling route that fights cancer.

Instead of inhibiting CXCL8 expression or secretion, which may be compensated for by alternative chemokine ligands that can still stimulate CXCR1/2 signaling, among the CXCL8–CXCR1/2 axis, inhibiting CXCR1/2 receptors, especially CXCR2, may be the most successful strategy. For inflammatory illnesses, a number of inhibitors and antibodies that target the CXCL8–CXCR1/2 pathway are currently at varying stages of clinical development at this time.

These medications are typically well accepted by people and are particularly well suited for usage in combination with chemotherapy in a few types of cancer. Due to the CXCL8–CXCR1/2 axis' dual roles in promoting and restraining tumor growth, either inhibitors or stimulators of this pathway should be developed with utmost caution. These modulators should be used in accordance with the tumor types, grades, stages, immunogenic circumstances, and concurrent medications. Increased susceptibility to opportunistic infections and neutropenia are two additional negative outcomes that may arise from blocking the CXCL8–CXCR1/2 axis. The most important factor in determining the best anti-CXCR2 therapeutic dosage is reducing the severity of these side effects.

Further Reading

For further readings, some textbooks mentioned below also give further understanding of cytokine and chemokine network.

- *Cytokines and Their Therapeutic Potential* by Manzoor Ahmad Mir.
- *The Role of Chemoattractants in the Tumor Microenvironment* by Giovanni Bernardini, Brian A. Zabel.

For more insights about the topic, we would suggest detailed findings from the books of Mir et al. (2022a, b) (https://doi.org/10.1016/C2021-0-02565-7 and https://doi.org/10.1016/C2022-0-00074-X), Mir (2021) (https://doi.org/10.52305/WXJL6770), and Mir (2015) (https://doi.org/10.1016/C2014-0-02898-5) and from the cancer.net website on the following below-mentioned links:

https://www.cancer.net/cancer-types/breast-cancer/types-treatment

https://discovery.ucl.ac.uk/id/eprint/1472740/

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Chemokine and Cytokine Networks in Tumor Microenvironment

12

Manzoor Ahmad Mir, Sumaya, and Mohd Zahoor Ul Haq Shah

Abstract

A large family of tiny chemotactic cytokines direct the migration of immune cells and is known as chemokines. Chemotactic factors help the immune system relocate when there is cancer because they have a crucial role in the migratory pattern of cells of immune system and have a variety of cells allocated to maintain homeostasis as a result of the elimination of invading infections and defective cells. Chemokine receptors are significant molecules for growth and evolution of the disease in cancer. While cytokines are various groups of soluble glycoprotein and low-molecular-weight peptides that are involved in controlling the growth and cancer spread and may have an autocrine effect on cancer cells like fibroblasts and vascular endothelial cells. Tumor cells in TME frequently exhibit chemokine receptor expression, and chemokines can target directly on non-immune cells. The host releases interferons, which aid in the destruction of cancer cells during cancer treatment. Monocytes and T cells produce interleukins. They can boost the generation of B and T cells. Macrophages produce a cytokine called tumor necrosis factor when they come into contact with an endotoxin. Downregulation of immunological modifying pathways is another modification brought on by chemokine–chemokine receptor interaction. The role of chemokines is important in cancer processes such as immunological infiltration, tumor growth, progression, and metastasis. It is intended that by increasing particular self-associated molecular patterns being expressed (SAMPs), also

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known as don't eat me signals, one might successfully evade immune surveillance. The movement of immune cells requires chemokines to develop and bring an efficient anti-tumor immune response.

Keywords

Cancer · Chemokine · Cytokine · Chemokine receptor · Tumor necrosis factor · Angiogensis · Metastasis · Tumor microenvironment

12.1 Introduction

A chemokine is a broad collection of chemotactic cytokines that control movement of immunological cells and lymphoid tissue. Chemokines contribute to the mechanisms of cancer growth, such as angiogenesis. To maintain homeostasis, which enables the removal of infections from the outside and sick cells, multitude of cells make up the immune system. Immune cells move due to chemokines, which are chemotactic substances (Griffith et al. 2014; Mir 2015a, b; Schulz et al. 2016). The cytokine subfamily is in charge of cell movement and the growth of lymphoid tissue (Schulz et al. 2016; Eckert et al. 2019; Kohli et al. 2022). There are currently more than 50 chemokines that have been announced and are broken down into 4 main types in accordance with position or where cysteine is found: CC, CXC, CX3C, and C (Griffith et al. 2014). All chemokine signals bind to migratory cells like rhodopsin, which is present in heterotrimeric G-protein (Griffith et al. 2014). Each immune cells responds to its environment differently. However, there is a lot of overlap in the way chemokines and chemokine receptors interact. There are various receptors that can bind to cytokine receptors among the 19 traditional chemokine receptors, 14 of which can recognise multiple ligands (Förster et al. 2008; Viola and Luster 2008; Hanahan and Coussens 2012). Because each immune subgroup expresses chemokine receptors in a specific pattern, they respond to chemokines differently and alter in response to their environmental needs (Hanahan and Coussens 2012; Susek et al. 2018). Chemokine ligands and chemokine receptors are necessary for the growth and progression of the cancer. Signalling by chemokines is essential in order to attract immune cells anti-tumor actions like CD8T cells, T helper 1 (TH1), and natural killer cells. TME frequently experiences changes in chemokine ligand synthesis and expression of chemokine receptors (Mir et al. 2022). This frequently results in attracting immune cells that promote tumor growth, like TAN, TAM, myeloid-derived suppressor cells (MDSCs), and T-regulating cells. Additionally, chemokines can directly target tumor cells and vascular endothelial cells in the TME, both of which frequently express chemokine receptors (Mir 2015a, b).

Cytokines are numerous subgroups of soluble glycoproteins and peptides with small molecular weights. Cytokines play a role in controlling the development and spread of cancer (Mir and Mir 2022). In order to create an environment favourable for the formation of cancer, cytokines may operate either directly on cancer cells in an autocrine way or indirectly on supportive tissue like fibroblasts and blood

vessels. Additionally, it has been demonstrated that cytokines are pleiotropic, meaning that they affect various cell types differently.

12.2 Types of Cytokines

12.2.1 Interferon

An interferon is a cytokine and an immodulating agent of sorts. In reaction to pathogens (such as viruses and bacteria) and tumor cells, host cells produce these. Interferons may aid in the death of cancer cells during cancer therapy and prevent cancer cells from developing. They are categorised according to the complementary receptor they share. Interferon is divided primarily into three classes: Type 1, Type 2, and Type 3 of interferons are interferon-alpha and beta, respectively (Interferon Lambda). Type 3 almost behaves in the same ways as Types 1 and 2.

12.2.1.1 Type-1 Interferon

Fibroblasts, endothelial cells, and macrophages can all produce Type-1 Interferon. Type I interferons (IFNs) have diverse effects on innate and adaptive immune cells during infection with viruses, bacteria, parasites and fungi, directly and/or indirectly through the induction of other mediators. Once released by infected cells, they connect to the interferon receptor on that cell as well as nearby cells. Because of the degradation of viral mRNA brought on by this autocrine and paracrine signalling, host and viral protein translocation is prevented from occurring. Additionally, they control the MHC 1 on cell surfaces natural killer cells, too. Therefore, it is evident that natural killer cells and cytotoxic T cells can identify and target virus-infected cells.

12.2.1.2 Type 2 Interferon

Gamma interferon is another name for interferon II. As a result of interleukin-2 and interleukin-18 interferon, neutralising antibodies and cytotoxic T cells can produce beta interferon, which activates macrophages and improves their capacity to kill pathogens by enhancing pinocytosis and lysosome function. Additionally, they increase MHC expression, which improves antigen presentation and phagocytosis.

12.2.2 Interleukin

Interleukins are different cytokines that T-lymphocytes, monocytes, and macrophages produce (Mehraj et al. 2021a, b, c). They serve a variety of purposes, including IL-α/beta, interleukin-4, IL-7, and interleukin-21 production and differentiation of “B” and "T" cells. Using interleukin-2, interleukin-8, or interleukin-12 to activate natural killer cells and neutrophils. Interleukin 6 (IL-6) elevates body temperature (fever) and inhibits microbial development, producing visible indications. Acute phase proteins like C-reactive proteins (CRP), which are associated with

inflammation, are elevated by interleukins as well. Increasing vascular permeability leads to oedema and enables quicker recruitment of immune cells.

12.2.2.1 Tumor Necrosis Factor

When exposed to an endotoxin, macrophages produce a cytokine called tumor necrosis factor. However, more immune cells, like masked cells, B-cells, and T cells, can create it and perform the same roles, such as local apoptosis induction, raises the permeability of nearby arteries, immunoglobulin chemotaxis, creation of a proinflammatory condition, such as when macrophages and the liver produce more prostaglandin E2 and CRP, respectively (Mir and Mehraj 2019). TNF causes fever as well.

12.3 Cytokine Signalling

Cytokine signalling is crucial for the regulation of the human body. In the neurological system, glial cells release nearly all cytokines, which are required for intracellular signalling. The majority of cytokines work locally to regulate lymphocyte awareness and activation. Leptin, the hormone that regulates fat storage, is a component of several cytokine signalling pathways. The body's immune system relies on cytokine signalling to maintain health. Macrophages and dendritic cells consume foreign particles and alert virtually dormant lymphocytes with cytokines.

Some cytokine signals, which are sometimes referred to as hormones, are not local but instead move widely throughout the body. Because cytokines are produced by nervous system glial cells rather than glands, this classification is occasionally modified. Target cells' receptors bind to cytokines, activating a series of intercellular signals. The protein kinase transduction cascade is the most often employed pathway. Inactive protein kinases are triggered by the process of phosphorylation after the cytokine attaches to the receptor ensconced in the cell membrane.

12.4 Chemokine Signalling

In order to precisely control cell placement in space and time during coordinated cell migration, both in health and disease, chemokine signalling is crucial. They are most well known for their capacity to promote cell relocation, especially in WBC's (leucocytes). However, chemokines play crucial role in immune system formation and homeostasis and are engaged in all immunological and inflammatory responses, whether they are beneficial or harmful.

12.5 Building Blocks and Signal Transmission

The chemokines are important protein family, out of which 40 ligands have been found. There is a classification for the chemokines into various classes depending on spaces of the amino acid cysteine residue at NH2 termines. The conserved cysteine motif is separated by an X denoting a non-cysteine. Recently, other chemokine

families have been referred to as CC, CXC, and CX3C, CXC is a class of chemokines that regulates recruitment of neutrophils and T cells, the B cells, T cells and attracting bone marrow and originated cells, e.g. dendritic cells and monocytes are regulated by CC chemokine (Mir et al. 2020).

Chemokine signals to seven transmembrane G-linked receptors at the N terminus trigger phosphorylation of serine/threonine residues at the C terminus, which changes the receptor's shape and activates heteromeric G protein complex attached to the receptor's intracellular domain. In order to activate downstream signalling pathways, GTP must bind to the Gα subunit, causing it to separate from its companions in the Gβ and γ subunits. Cellular functions including proliferation, modification, and the expression of matrix metalloproteinases, and cytokines are regulated by these signalling pathways, which also include PI-3 kinase, the Rho family of GTPases, and MAPK. It is important to remember that chemokine receptors also engage signalling pathways unrelated to G proteins, such as p38MAPK and JAK/stat, to control cellular functions like migration and gene transcription.

It is possible for duplicate signalling to occur because several chemokines can connect to the same receptor and because a single chemokine can bind to numerous receptors. Chemokines have been demonstrated to promote migration through widespread signalling pathways, such as G protein-dependent processes, in in vivo investigations. The possibility of each chemokine ligand/receptor combination having a different function is also supported by research. First, chemokine ligands show that various receptors can bind to the same ligand in distinct ways. Chemokines, for instance, have a higher affinity for CXCR2 than CXCR1. Additionally, various ligands that bind to the same receptor have biological effects that vary depending on the kind of cell. For instance, it has been demonstrated that CCL3, CCL4, and CCL5 drive the migration of activated T cells, while only CCL5 can do the same for resting T cells (Mehraj et al. 2022b). Additionally, research using knockout mice on chemokine receptors and ligands like CCL2 and CCR2 has not revealed compensatory overexpression of other ligands. It is still unknown why there are numerous ligands and receptor binding pairs, but these results suggest that each chemokine and receptor pair may have a specific function that can be used to control how cells react to chemokine signals.

12.6 The Roles of Chemokine Signalling in Cancer Development

As was already indicated, neoplastic transformation frequently results in altered chemokine–chemokine receptor interactions, which causes downregulation in these immune modulatory pathways and has effects on a variety of physiological systems (Sofi et al. 2023). The chemokine involvement in critical processes of cancer, including development of the tumor, immune evasion, metastasis, and angiogenesis, will be discussed here (Fig. 12.1).

Antibody evasion is a sign of the development of cancer (Hanahan and Weinberg 2011). It is known that some self-associated molecular patterns (SAMPs), also known as "don't eat me signals," can help cancer cells successfully evade immune

monitoring. Through the activation of immunological checkpoints, they can immediately reduce the activity of immune cell. They can also cause the development of immune-suppressing cells, which further inhibit the action of anti-tumor cells (Qayoom et al. 2021; Hanahan and Weinberg 2011). In order to develop and convey an efficient tumor-specific immune response, the chemokines are essential in immune cell movement directing. However, the TME frequently experiences altered chemokine production, and an abnormal chemokine profile can help differentiate between them and invasion of immune-suppressive pro-cancerous cells within the tumor, including TAMs, T-cells, and MDSCs (Susek et al. 2018).

A specific subset known as "Treg cells," a subset of CD4+ T cells, is utilised to suppress the immune system, which encourages self-tolerance, prevents exacerbation of immunological responses, and also maintains homeostasis (Kondelkova et al. 2010; Khor 2017). As they can limit the behaviour of anti-tumoral T cells, have been asserted that their infiltration into the TME also results in immunological tolerance and tumor progression (Togashi et al. 2019) . Treg cells express the receptor for chemokines CCR4 far more compared to another CD4+ T cells (Curiel et al. 2004). On the other hand, TAMs as well as primary tumor cells produce CCL22, a chemokine, which is then ingested into the TME. Such increased CCL22 expression is associated with decreased spontaneous and therapy-induced anti-tumor T-cell immunity, which promotes tumor development and adverse patient outcome (Qayoom et al. 2023;

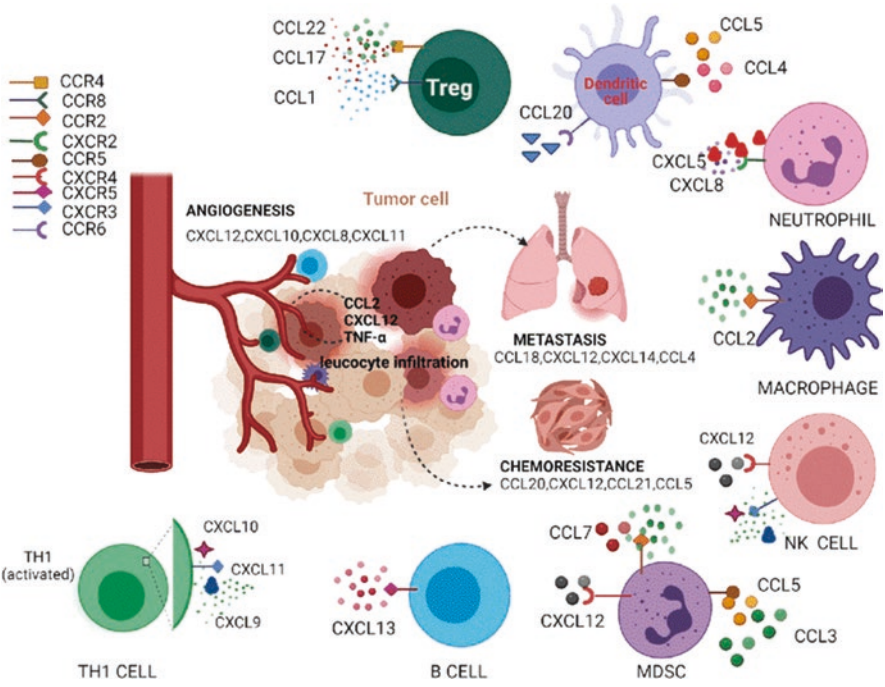

Fig. 12.1 Immuno avoidance and cellular recruitment in an immunosuppressive environment. Multifacted roles of chemokines in cancer development

Curiel et al. 2004; Zou 2006; Mizukami et al. 2008; Maruyama et al. 2010). Other chemokine receptors, for, e.g. CCR10 or CCR5 and its ligand CCL28 is abundant and the hypoxia area of tumor microenvironment can drive Treg cells' infiltration into the TME in addition to CCR4 (Facciabene et al. 2011). The main method through which macrophages are recruited into the TME is the CCL2-CCR2 signalling pathway (Qian et al. 2011). In many cancers, the voicing of CCL2 corresponds with the quantity of TAM and is frequently linked to a bad prognosis for patients (Pollard 2004). The same as T reg cells, TAMs are able to suppress CD8+ T cells specific to tumor-associated antigens (TAA) from becoming activated. The capacity of T cells interact with tumor cells in an antigen-specific manner and promote effector cytokine secretion to promote anti-tumor immunity (Kryczek et al. 2006; Kuang et al. 2009; Wu et al. 2009; Bolitho et al. 2010).

Monocytic and granulocytic cells make up the diverse population of myeloid cells referred to as suppressive cells generated from myeloid that have immunosuppressive features (Gabrilovich and Nagaraj 2009; Mehraj et al. 2021a, b, c). Monocytic MDSCs may undergo TAM-like differentiation at the tumor site, and they may similarly recruit TAMs into the TME via CCL2-CCR2 signalling (Pollard 2004; Kitamura et al. 2015). MDSCs and TAMs with monocytosis are utilised interchangeably when there is cancer due to their similarities in traits and immunosuppressive properties (Kohli et al. 2022). In mouse tumor models, the immunosuppressive properties of monocytic MDSCs have been extensively explored (Rodriguez et al. 2005; Huang et al. 2006; Gabrilovich and Nagaraj 2009; Marigo et al. 2010; Facciabene et al. 2011; Cui et al. 2013; Panni et al. 2014). Through the production of CCL4, they can directly knock down the effector cells and draw in T regulatory cells. Once myeloid cells have invaded the tumor, they can go on in order to make CCL2 the proper ligand and contribute to maintaining or even enhancing the flow of monocytes into tumours (Bolitho et al. 2010; Lee et al. 2018). In breast cancer, increased expression of CCL5 is associated with elevated spread of the illness and TAM invasion (Luboshits et al. 1999; Azenshtein et al. 2002). However, compared to their CCR5 counterparts, CCR5-expressing MDSCs have been reported to be more immunosuppressive (Blattner et al. 2018). Additionally connected to the course of pancreatic illness, increased CCL5 expression (Singh et al. 2018) is seen in gastric (Azenshtein et al. 2002) and ovarian cancer (Tsukishiro et al. 2006). The chemokines CCL7, CCL15, CXCL8, and CXCL12 have also been linked to increased monocyte recruitment to malignancies (Allavena et al. 2008). It is interesting to note that a cascade combining evidence of CCL3 and CCL2 has been found where TAMs, which are produced via MDSCs have been recruited by CCL2, produce CCL3, which also encourages tumor metastatic site and macrophages retention in the tumours (Kitamura et al. 2015; Mehraj et al. 2021a, b, c).

Rare immune cells called plasmacytoid dendritic cells (pDCs) have the capacity to inhibit the immune system that is anti-tumor in nature. Those cells could exist in the human TME and have the capacity to stimulate the growth of CD8+ Treg cells that produce IL-10 and inhibit DC activation of TAA-specific effector cells (Azenshtein et al. 2002; Martin-Orozco et al. 2009). CXCL12, the ligand of the

chemokine receptor CXCR4, is produced by tumor and stromal cells and is expressed by pDCs (Scotton et al. 2001; Curiel et al. 2004; Cui et al. 2013). CXCL12 is therefore the essential substance for pDC signing up for the TME. Additionally, plasmacytoid DCs are shielded by CXCL12. By keeping them from doing going through apoptosis and extending the duration of their immunosuppressive function (Azenshtein et al. 2002; Curiel et al. 2004; Cui et al. 2013).

12.7 Tumor Growth and Progression

The production and creation of signals that promotes growth are tightly regulated in normal cells to preserve cellular homeostasis; these processes are frequently compromised during neoplastic transformation. As a result of interference of the regulatory mechanisms of growth factor signalling and production, tumor cells frequently display uncontrolled growth and proliferation (Qayoom et al. 2023; Hanahan and Coussens 2012). Numerous studies have revealed that the chemokine signalling plays a part in the growth and tumours spread through a variety of methods (Do et al. 2020). Interaction between chemokine receptor-expressing malignant cells and the matching ligands released by fibroblasts which are tumor associated, immune cells and tumor cells that have infiltrated the TME (Mishra et al. 2011; Lau et al. 2014) may initiate signalling pathways such as PI3K/AKT, extracellular signal-regulated protein kinases 1 and 2, and ERK 1/2, which can result in the growth of cancer cells (Balkwill 2004; Teicher and Fricker 2010; Liang et al. 2018). Pathologic upregulation of chemokine receptors on tumor cells and release of chemokine ligands in the TME can both worsen these effects. Additionally, chemokines could avoid tumor apoptosis by balancing the expression of pro- and anti-apoptotic cancerous genes, which include peptides (e.g. by downregulating activating caspase-3 and caspase-9 while expressing Bcl-2) (Murakami et al. 2003; Smith et al. 2004; Mehraj et al. 2022a, b). Despite this, the bit part of chemokines in growth of tumor and development is extremely unclear or puzzling since, besides their procarcinogenic activities, such components can enthusiastically cooperate in a different inhibitory pathways that may either be necessary or challenging to halt tumor advancement (Do et al. 2020). Chemokines can prevent tumor growth and accretion at the beginning of oncogenesis by interfering with the senescence of tumor cells caused by oncogenes. This acts as defence operation opposed to unchecked malignant transformation and growth of cell (Vizioli et al. 2014; Salminen et al. 2018; Sofi et al. 2022). Through NF-β signalling, the CXCL1 and CXCR2 axis encourages ageing, brought on by oncogenes to restrain tumor growth. Aged cells, however, can often operate as a origin of the irritation in more advanced phases of oncogenesis, attracting MDSCs to the tumor site, creating an immunosuppressive milieu, and promoting tumor growth (Lesina et al. 2016; Salminen et al. 2018). The enrollment of particular immune system cells by chemokines can often take part in tumor development. A subset of immune system cells frequently present in the TME called T helper cells secreting interleukin-22 (TH22) has been implicated in supporting carcinogenesis via a number of routes, especially in cancer

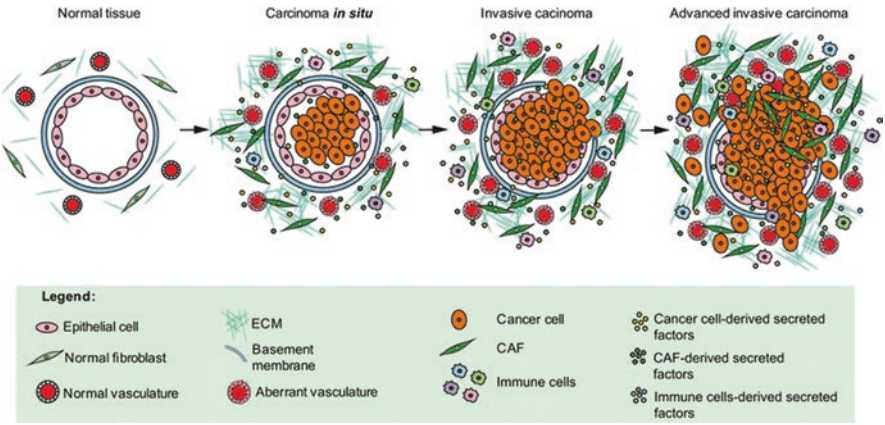

Fig. 12.2 Different immune cells associated with normal and breast cancer tissues

of colon. They point to the receptor of chemokine CCR6 and go toward the TME's ligand CCL20, where they can produce cytokines that boost the cancer stemness and tumorigenic potential of the tissue (Kryczek et al. 2005; Qayoom et al. 2021) (Fig. 12.2).

12.8 Angiogenesis

An essential component for the advancement and expansion of tumours is angiogenesis, which is the development of latest veins and arteries through migration, growth, and differentiation from ones that already exist. Tumor cells require more oxygen, nutrients, and metabolic waste elimination because of their accelerated proliferation or accretion, which necessitates accelerated neovascularisation to meet these demands (Hanahan and Coussens 2012). It has been suggested that chemokines and related substance receptors are crucial inspectors of tumor vasculature and play a dual role in tumor angiogenesis (Singh et al. 2018). Two classes of chemokines CXC are distinguished: ELR + chemokines and E-L- R chemokines based on the ELR (Glu-Leu-Arg) motif's presence at the N-terminus. Overall, ELR+ CXC chemokines, which function by activating both CXCR1 and CXCR2, promote angiogenesis. These chemokines consist of XCL1, XCL2, XCL3, XCL5, XCL6, XCL7, and XCL8. The ELR CXC inflammatory chemokines CXCL4, CXCL9, CXCL10, CXCL11, and CXCL14, on the other hand, considered to be angiogenesis inhibitors. The divide has been not definitive or complete up to this point due to the fact that CXCL12, an ELR chemokine, is regarded to be an angiogenic chemokine (Addison et al. 2000; Keeley et al. 2010; Chow and Luster 2014; Lin et al. 2015). Through direct interaction with endothelial cell-surface chemokine receptors, chemokines can also act as tumor angiogenesis mediators, improving endothelial cell survival, migration, and proliferation (Singh et al. 2007). Additionally, chemokines might influence angiogenesis indirectly via encouraging the inclusion of leukocytes

in the TME that create factor angiogenic. The most eminent pro-angiogenic cells are TANs. Neutrophils have equal amounts of the cellular X receptor 1 and 2, who direct the direction of the ligand CXCL8, and the alternate ligands CCXCL1, CCXCL2, CCXCL5, CCXCL6 (Bai et al. 2004). In several cancer forms, the majority of the ligands are overexpressed., which may be associated with excessive presence of neutrophils, enhanced angiogenic changes, and a negative prognosis (Bai et al. 2004; Zhou et al. 2012; Jablonska et al. 2014; Chao et al. 2016).

Additionally, chemokines can work in conjunction alongside additional angiogenesis-promoting agents, such as VEGF. The expression of vascular-endothelial growth factor was elevated in response to CXCL8 and CXCL12, which may have had a favourable reaction effect, whereby further stimulation from VEGF. Furthermore, chemokines can also collaborate with additional promoters of angiogenesis-like vascular endothelial growth factor (VEGF). CXCL8 and CXCL12 increased the expression of VEGF, which may have had a favourable feedback loop by causing VEGF to boost the production of angiogenic chemokines even more the synthesis of chemokines of angiogenesis (Singh et al. 2007) (Fig. 12.3).

12.9 Metastasis

The multifaceted procedure for cancer tumor transmission of the initial tumor link to the body's far-off regions is known as metastasis, and it is responsible for a sizable fraction of melanoma deaths (Steeg 2016). The invasion metastasis cascade, which consists of several steps, is referred to as tumor metastasis. This sequence of activities includes the initial cancer cells locally invasive into adjacent tissues; harm to these cells, their survival during circulation in the lymphatic or bloodstream, their arrest and extravasation via vascular walls in the parenchyma of far tissues. This parenchyma has developed micrometastatic colonies, and the process of colonisation is the continuous growth of microscopic colonies transformed into a medically visible spreading roughness (Bose et al. 2014). It has been established that the cxcl mechanism is crucial for metastases by numerous research. It' is been observed that cancer cells' chemokine receptor expression is a potential indicator of their secondary location. Therefore, these recurrent locations' production of particular chemokines can improve the movement of cancerous cells in the "premetastatic niche," creating a welcoming atmosphere for the development of metastatic cells (Singh et al. 2007; Krol et al. 2010). Although many CXCL12/CXCR4 axis and chemokines have been linked to metastasis stand out as a key contributor to this phenomena. Their contribution in tumor metastasis had demonstrated in a variety of malignancies, where CXCR4 expression encourages tumor cell migration and expansion into tissue with increased CXCL12 concentrations (Darash-Yahana et al. 2004; Chow and Luster 2014). Metastasis has been linked to a there are numerous chemokines and its receptors; nevertheless, the helix CXCL12/CXCR4 is a key initiator of the process. Their contribution in tumor metastasis has been demonstrated in a variety of malignancies where CXCR4 overexpression is encourages tumor cell migration and expansion into tissues with greater CXCL12 levels (Krol

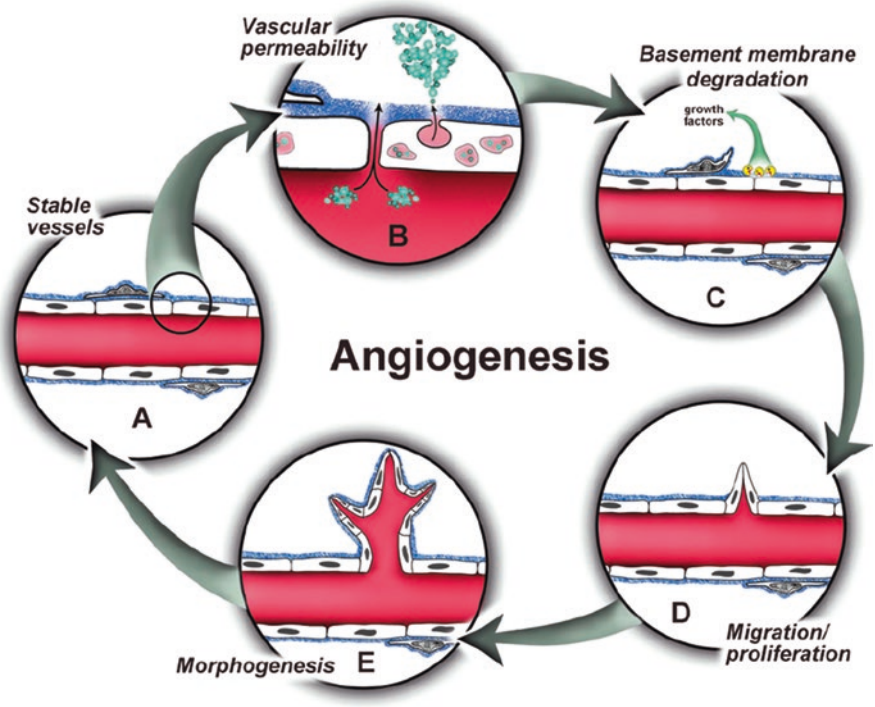

Fig. 12.3 Role of chemokines in angiogenesis

et al. 2010); Expression of the CCR3/CCR10 ligand CCL28 has been linked (Takanami 2003) with breast cancer development and metastatic spread (Yang et al. 2017); signalling of CCR10 and CCL27 holds up melanoma tumor cells' adherence and survivability during invasive dissemination (Ben-Baruch 2008). CXCR5/CXCL13 spread and interlinkage appear to encourage prostate cancer bone metastases (Lillard et al. 2010) (Fig. 12.4).

12.10 Tumor Microenvironment

The presence of non-cancerous cells and other tumor components is indicated by the tumor's microenvironment, which includes chemicals they produce and secrete. In the start, progression, metastasis, and resistance to treatments of tumours, the ongoing tumor microenvironment and tumor cell interactions are clearly important. There has been a lot of research and experimental interest in the environment surrounding tumours as a therapeutic cancer-related target. Here, we will talk about how the tumor microenvironment is now being targeted in both medication research and clinical trials (Mir and Haq 2023).

Along with cancer cells, the tumor microenvironment also contains immune cells, fibroblast, endothelial cells, and numerous other cell populations. It is

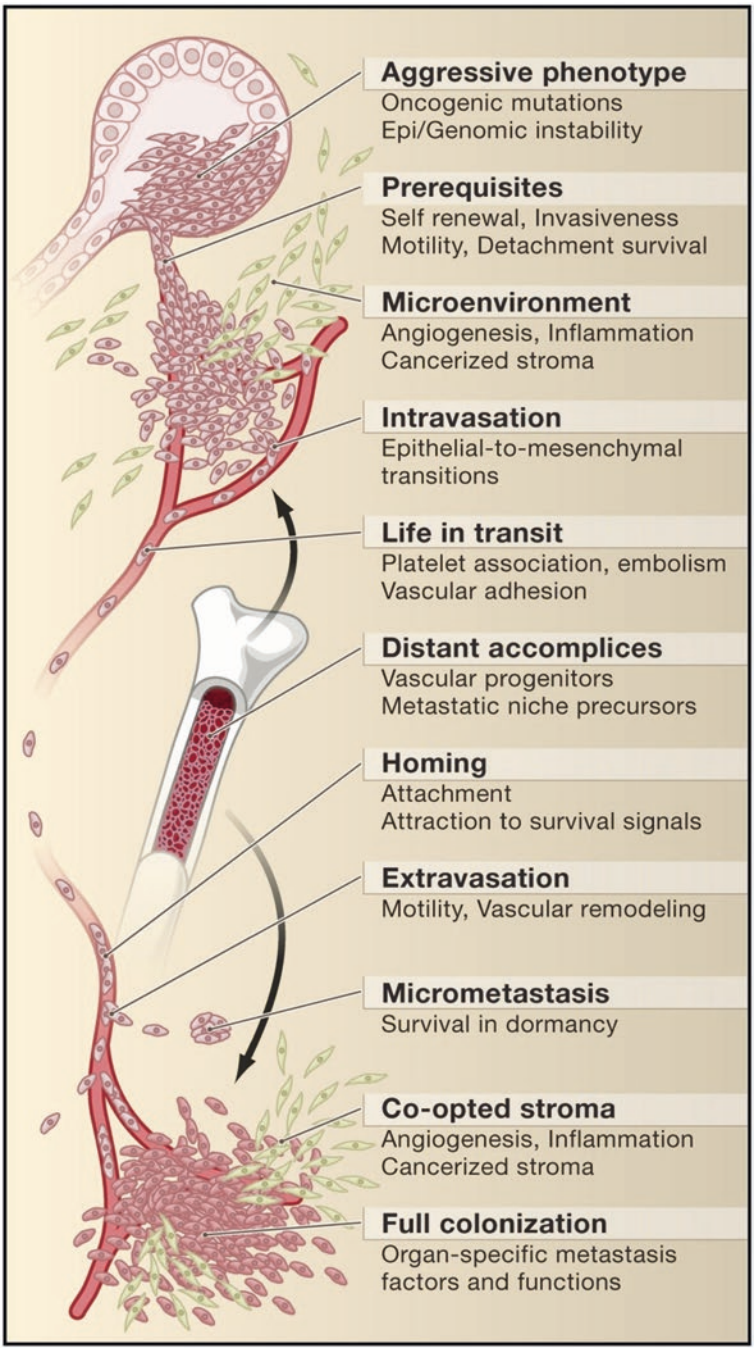

Fig. 12.4 Role of chemokines in metastasis

becoming clearer and clearer that the formation of this support niche is detrimental to the cancer's unchecked, ongoing proliferation. The cancer stemness is maintained by the tumor microenvironment, which also directly promotes angiogenesis, invasion, metastasis, and chronic inflammation. In this chapter, we go over how fibroblasts—more specifically, cancer-associated fibroblasts take a part in the development and spread of cancer. Here, we will concentrate on CAFs' responsibilities in controlling immune cells and responses, which have a wide range of consequences on the development and persistence of cancer. Both immune cell entry into the tumor microenvironment and their operation within the tumor are hampered by CAFs. Finally, we discuss the possibility of modifying CAF activity as a supplemental a plan to improve the effectiveness of immunotherapies for tumor.

12.11 Chemokines in Tumor Microenvironment

Thorough relationships exist between cells of the immune system, stromal cells, and the extra cellular matrix in tumor cells. Those connections encourage the growth of cancers, the spread of metastatic disease, and immune escape via complex processes. Chemokines, a ménage of tiny chemotactic proteins, play a major part in these interactions. Four categories exist for chemokines primary categories in accordance with their protein seq., particularly where the cysteine residues are located at the N-terminus. These four types are C-, CC-, CXC-, and CX3C-chemokines. No matter what class they belong to, chemokines signal when they adhere to the migratory cells' 7-transmembrane G protein-coupled receptors (GPCRs). There are 18 signalling receptors, and 48 chemokines have been discovered in people to yet. All chemokines have a variety of distinct receptors that it can start or activate. According to the unique requirements of each environment, immune cell subgroups variably display chemokine receptors that cause a targeted recruiting of those molecules. Chemokine ligand production within the tumor microenvironment (TME) is also changed, much like it is in healthy tissue recruitment. This supply includes regulatory T cells, tumor-associated neutrophils, tumor-associated macrophages, and myeloid-derived suppressor cells. Such cells enlarge throughout progression of tumor, effector cells, and are related to inferior prognosis in patients with different solid malignancies. Several investigations revealed certain cancer cells form using chemokines in both paracrine and autocrine fashion to actively promote the growth, survivability, and metastasis of cancer cells. Here, we will concentrate on the role played by the chemokine receptors CXCR1/2, CXCR3, and CXCR4 in the employment of tumorigenic and non-tumorigenic immune cells. We high spot the impact of the CXCR3 and CXCR4 axes on increasing effector cell recruitment and the involvement of the CXCR1/2 axis in boosting immunosuppressive cells. Additionally, we summarise the preclinical and clinical research that has shaped the therapeutic promise of chemokine targeting and its relevance to algebraic immunotherapeutic treatment methods.

12.12 Role of Chemokines in Cancer Therapy

Different kinds of cells, comprising fibroblast, the vascular endothelial cells, and neutrophil, express the two CXCRs. While CXCR2 CXCR1 and CXCR2 have a same selectivity for the ligand CXCL6 and CXCL8 (IL-8) and regulate attachment of CXCL1, CXCL2, CXCL3, CXCL5, and CXCL7 (Alfaro et al. 2017). The gene for CXCL8 (IL-8) is absent in mice. Additionally, the human CXCL6 homolog LIX, which is produced by the murine CXCL5 gene, binds both CXCR1 and CXCR2 (Zlotnik and Yoshie 2012). Poor prognosis is associated with cancerous tissue and serum, such as those from ovarian cancer, lung adeno-carcinoma, colon cancer, and pancreatic ductal adenocarcinoma, there are significant concentrations of such chemokine receptors and ligands (PDA) (de Heer et al. 2007). Another reason for poor prognosis is that the CXCR1/ 2 axis preferentially attracts pro-tumorigenic immune cells. Chemokine production can be increased in tumor cells by altered signalling pathways. In ovarian cancer cells, for example, transcriptional factor of activation Snail boosted levels of CXCL1, CXCL2, and CXCL5 via the NF-kB pathway and promoted MDSC recruitment. MDSC cell counts within tumours decreased in response to snail exhaustion or antibody-mediated CXCR2 targeting, but the number of NK cells and For T-cells rose. Similar to this, the generation of cells from breast cancer secretes CXCL1 and CXCL2 led to a rise furthered by the invasion of myeloid pro-tumorigenic cells, enhanced by chemotherapy therapy, resulting in chemoresistance (Acharyya et al. 2012). In models of renal cell cancer (RCC) (Najjar et al. 2017), PDA (Chao et al. 2016), melanoma (Toh et al. 2011; Soler-Cardona et al. 2018), and hepatocellular carcinoma (HCC) (Haider et al. 2019), CXCL5 has also been shown to be involved in attracting CXCR2+ MDSC and TAN. Measures of CXCL5 and CXCL8 within the tumor are associated with elevated invasion by MDSC in RCC patients (Najjar et al. 2017). Targeting CXCR2 enhanced effector T cells while lowering the amount of MDSCs (Najjar et al. 2017). In contrast to immune checkpoint inhibition alone, targeting CXCR2 in combination with it dramatically reduced tumor weight in a mouse RCC model (Najjar et al. 2017). Similar to PDA, increased CXCL5 expression was discovered there, and it promoted the recruitment of CXCR2+ neutrophils (Rodriguez et al. 2005). Neutrophil infiltration was reduced, and the proportion of effector T cells was raised by CXCR2 rejection (Rodriguez et al. 2005). The MDSC was specifically recruited to the tumor site and inhibited T cell activation in hereditarily altered mice that produced human CXCL8 (Rodriguez et al. 2005). These findings collectively show that CXCR1/2 inhibition promotes T and NK cell recruitment while reducing pro-tumorigenic immune cell aggressiveness. By combining CXCR1/2 inhibition with other immunotherapies including checkpoint inhibition and adoptive cell therapy, this support makes an effort to improve immunotherapy. Additionally, CXCR1/2 inhibition aids in overcoming chemoresistance caused by immune cells that promote tumor growth (Acharyya et al. 2012; Nywening et al. 2018). Chemokine signalling in the TME has recently been demonstrated to be very plastic in PDA patients who had previously received treatment with a CCR2 inhibitor, the number of CXCR2+ TAN cells inside tumor samples increased (Nywening et al. 2018).

Cytotoxic CD56dim NK cells have large amounts of CXCR1 and CXCR2 (Morohashi et al. 1995; Lima et al. 2015). Recently, we demonstrated that RCC decreases the expression of CXCR2 on NK cells that invade tumours and genetic modification to restore CXCR2. Increased NK cells are drawn to the location of the tumor (Kremer et al. 2017). Recently, we showed that RCC reduces the statement of CXCR2 on NK cells that have infiltrated tumours, and genetic modification to restore CXCR2 boosted NK cell recruitment to the tumor site (Ali et al. 2014). Patients with stage III melanoma had better prognoses when they had a higher percentage NK cells of all this group, which is located in the middle of every NK cell in the lymphatic vessels with strong flow. Similarly, in mouse melanoma models, genetically altered CXCR2+ T cells showed enhanced in vitro migration (Vallet et al. 2010; Idorn et al. 2018). Patients with metastatic melanoma that has been genetically altered to produce CXCR2+ T cells are participating in a clinical phase I/II investigation.

12.13 CXCR4 and Its Ligand CXCL12

Under physiological conditions, CXCR4 and CXCL12 are necessary for hematopoiesis, cardiogenesis, and neurogenesis. The CXCR4/CXCL12 axis participates in the generation consisting of NK, B, T, and HSC homing and maintenance of cells in the bone (Sugiyama et al. 2006; Noda et al. 2011). When CXCR4 expression is present, it encourages tumor cell proliferation, the migratory and invasive nature of cancer (Müller et al. 2001). Additionally, the tumor-promoting impacts of CXCL12, which is produced within the tumor, might recruit plasmacytoid dendritic cells, MDSCs, and CXCR4+ Treg (Zou 2005, 2006; Obermajer et al. 2011). Solid tumor samples with extremely high CXCR4 expression typically have a bad prognosis. Particularly, CXCR4 expression in breast cancer was substantially linked to distant metastasis, lymph node metastases, and shorter overall survival (Zakikhani et al. 2008). Similar results could be made with lung cancer, melanoma, and prostate cancer (Sun et al. 2005; Choi et al. 2018).

CXCR4 expression levels within N-K and T-cells vary depending on subset and stage of maturation, as well as their hiring to various organs is also reliant on how some chemokines co-express with one another (Kumar et al. 2006; Carrega et al. 2014) CXCR4 desensitisation is required for NK cells to exit the bone marrow, whereas high CXCR4 expression on NK cells is linked to combination within the bone marrow compartment (Bernardini et al. 2008). Several variables can alter the immune cells' repertoire of chemokine receptors: For instance, training human NK cells with TGF-1, generated from neurons in neuroblastoma, greatly a rise in CXCR4 and CXCR3 expression while considerably lowering the NK cell's CX3CR1 expression (Castriconi 1950). Due to this, NK cells developed a characteristic that is kept in the marrow as opposed to being sent to tumours and external organs tissue (Youn et al. 2011). According to a different study, PGE2 regulates CXCL12 levels in malignant ascites from ovarian cancer patients and CXCR4 expression on MDSC (Obermajer et al. 2011). Investment of PGE2 stopped MDSC from migrating a

pathological ascites. Accordingly, lung cancer that is not small cell (NSCLC) specifically recruit CD4+CD69+CXCR4+ T cells that express large quantities of CXCL12 in this way and have an increase in the proportion of regulatory T cells (Wald et al. 2006). Amount of NK cells within the tumor tissue dropped, despite the fact that the percentage of CD8+ T cells remained unchanged. As mentioned, regulatory T cells can move along the CXCR4-CXCL12 axis and are retained in the bone marrow (Zou et al. 2004) . Imatinib and nilotinib, two tyrosine kinase inhibitors (TKIs), have been demonstrated to specifically enhance CXCR4 on the cell surface of NK cells and monocytes in in vivo investigations utilising NK cells from neuroblastoma patients. This information relates to the CXCR4 expression modification by pharmacological means (Bellora et al. 2017).

There have been several methods investigated to target this axis, several of them have participated in clinical trials, with various outcomes. Two anti-CXCR4 inhibitors, TN14003 and AMD3100 (Plerixa), have been examined in xenografts made from patients of breast cancer and have demonstrated anti-cancer activity in the HER2 isoform (Lefort et al. 2017). It is interesting to note that neither inhibitor appeared to be able to stop tumor development nor prevent the spread of metastatic disease in triple-negative PDX, highlighting the intricacy of the many breast cancer subtypes and their individual T-M-Es Moreover, AMD3100 has been investigated, either alone or together with immunological checkpoint antagonists, in a mouse model of human pancreatic cancer (Feig et al. 2013). In this study, AMD3100 was successful in promoting T-cell mobility in vitro and blocking CXCR4 signalling. The combination of AMD3100 and an anti-PD-L1 monoclonal antibody demonstrated enhanced anti-tumor efficacy (Brahmer et al. 2012). As a result of CXCR4's high expression in colorectal cancer, CXCR4 targeting has a therapeutic justification (Brahmer et al. 2012). By inhibiting CXCR4-mediated signalling in colon cancer cells in vitro, a CXCL12-KDEL retention protein, a remarkable reduction in the spread of metastatic cancer was seen (Zeelenberg et al. 2003). In this model, AMD3100 has also been looked at, and similar favourable preclinical results have been obtained (Li et al. 2016). Oncolytic viruses and NK cells that have been genetically altered are further techniques for controlling the CXCR4-CXCL12 axis. In instance, administering an oncolytic virus that inhibits CXCR4 decreased regulatory T cell recruitment, decreased metastatic dissemination, and the pathogenic signalling was restored in a mouse ovarian cancer model (Gil et al. 2014). However, in a glioblastoma tumor model, NK cells modified to co-express a chimeric antigen receptor (CAR) as well as CXCR4, a chemokine receptor, improved N-K cell invasion and tumor cell death. Last but not least, Spiegelmer aptamers with tremendous potential for modifying the TME of solid tumours are the CXCL12-targeting NOX-A12, also known as Olaptesed Pegol, to promote immunological cell invasion, make tumours more sensitive inhibitors of checkpoints, and hinder tumor repair pathways in metastatic colorectal and pancreatic malignancies, while clinical trials are still underway (Noxxon Pharma). Oesophageal and gastric cancer are two further examples of solid tumours that may profit from suppression of the axis CXCR4–CXCL12.

12.14 Conclusion

A subpopulation of cells in many cancers has stem cell traits, mediates metastasis, and contributes to treatment resistance. These cells are considered as cancer stem cells (CSCs). CSC properties of tumor cells are immensely regulated by close interactions with tumor microenvironment components such as mesenchymal stem cells, tumor-related fibroblasts, adipocytes, endothelial cells, and immune cells via the intricate network of cytokines, chemokines, and growth factors. Inflammatory cytokines including interleukin (IL)-1, IL-6, and IL-8 play a major role in these interactions via the activation of signal transduction pathways like Stat3/NF-κB in stromal and tumor cells. The activation of these pathways increases the release of more cytokines, resulting in positive feedback loops which help in CSC self-renewal. The pathways controlled by these cytokine loops are similar to those that are active during chronic inflammation and wound healing, suggesting that they might have critical role in establishing relationship between inflammation and cancer. Anti-inflammatory drugs have been identified to inhibit these cytokines and their receptor-mediated pathways. These agents have the potential to target CSCs by inhibiting signals from the tumor microenvironment and considered to be a potential candidate for future therapeutics.

Further Reading

For further readings, some textbooks mentioned below also gives further understanding of cytokine and chemokine network.

- Cytokines and their therapeutic potential by Manzoor Ahmad Mir.
- The role of chemoattractants in the tumor microenvironment by Giovanni Bernardini, Brian A. Zabel.

For more incites about the topic we would suggest detailed findings from the books of (Mir et al. 2022) https://doi.org/10.1016/C2021-0-02565-7, https://doi.org/10.1016/C2022-0-00074-X and (Mir 2021) https://doi.org/10.52305/WXJL6770 (Mir 2015a, b) https://doi.org/10.1016/C2014-0-02898-5 and from the cancer.net website on the following mentioned below links:

https://www.cancer.net/cancer-types/breast-cancer/types-treatment

https://discovery.ucl.ac.uk/id/eprint/1472740/

https://www.jmedsciences.com/doi/JMEDS/pdf/10.5005/jp-journals-10045-00138

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Prognostic and Diagnostic Significance of Chemokines and Cytokines in Cancer

13

Manzoor Ahmad Mir, Aanisa Ishrat, and Nusrat Jan

Abstract

A cooperated, powerful, but self-constrained immune reaction to a goal antigen is produced by the immune system's cells in conjunction with cytokines, which act as molecular messengers. Increased efforts to identify cytokines and use of their extensive signalling networks to create cancer treatments have coincided with the increased interest in using the resistant system to fight cancer during the past two decades. The main cytokines used in cancer immunotherapy will be reviewed, along with their fundamental biology and therapeutic uses, in this chapter. The chapter will also go through novel distribution strategies, biological agent sequences, new cytokines in diagnosing research and potential future directions for cytokine-based research in the future. Immune cell chemotaxis and the growth of lymphoid tissues are mediated by chemokines, which are chemotactic cytokines. Recent research has shown that chemokines and their corresponding receptors are essential for the growth of cancer and the inflammation that results from it. As a result of these discoveries, the chemokine system is now a current possible goal for cancer therapy. The chemokine system regulates the flow of immune cells into the tumor microenvironment, producing both pro- and anti-immunity and promoting or inhibiting tumor growth and angiogenesis, metastasis and proliferation. Growing evidences support the chemokine system's positive predictive importance in cancer patients. Depending on the kind of cancer, CCL10, CXCL2 and CX3CL1/CX3CR1 might be one of the two favourable or unfavourable prognostic variables; however, CCL14 and CXCL1 have strong predicting value. More chemokines with poor prognostic value include CXCL1, CXCL8 and CXCL12. Despite significant advancements in our comprehension

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of the complicated complexion of the system chemokine in tumor biology, our grasp of the chemokine system's diverse functions in many cancers is still restricted. Additional research is required to clarify the many functions of the system chemokine in the development of cancer and to confirm their potential utility in cancer prognosis.

Keywords

Prognostic indicators · Cancer · Chemokines · Interleukin-2 · Immunotherapy · Cytokines · Interferon

13.1 Introduction

In order to create a coordinated, potent, but self-limited response to a particular antigen, immune system cells must be able to interact with one another via molecules called cytokines. While many immune system communications involve direct cell-to-cell interaction, the production of cytokines promotes the quick spread of immunological signalling in a complex and effective way. An increase in attempts to describe cytokines and utilise their extensive signalling networks to create cancer treatments has coincided with the previous two decades' increased interest in utilising the immune system to fight cancer. Cytokines improve cytotoxic effector cell recognition of cancer cells by directly activating immune effector cells and stromal cells at the site of the tumor. There are several cytokine-based techniques for cancer therapy because numerous studies using animal tumor models have shown that cytokines have extensive anti-tumor activity. For persons who have developed cancer, several cytokines, including GM-CSF, IL-7, IL-12, IL-15, IL-18 and IL-21, have recently entered medicinal research. Preclinical research is still being done to support the idea that blocking suppressive cytokines like IL-10 and TGF-β may boost anti-tumor immunity (Mir 2015a, b). Additionally, improvements in complementary cell treatment rely on the use of cytokines to produce an in vitro, tightly balanced environment for the best maturation of anti-tumor T cells. For the medical check-up of metastatic melanoma and renal cell carcinoma, high-dose, bolus IL-2, and for the adjuvant therapy of stage III melanoma, IFN has received FDA approval as single medicines. Because it can result in long-lasting complete responses in a minority of patients with renal cell carcinoma and metastatic melanoma, high dosage IL-2 is the greatest illustration of how cytokines can be employed in cancer immunotherapy (Mir et al. 2022a, b, c). However, the substantial pleiotropism and redundancy of cytokine signalling, as well as the double roles of many cytokines in immune stimulation and immune repression, pose remarkable provocations to our capability to attain relevant anti-tumor reactions without creating treatment-limiting toxicities a conundrum that is also well illustrated by the low reaction rates and well-known toxicities of IL-2. To create effective immunotherapeutic cancer treatments, it is essential to comprehend the intricate, diverse roles that cytokines play in the triggering and control of the anti-tumor response. The main cytokines used in

cancer immunotherapy will be surveyed in this chapter, along with their fundamental biology and therapeutic uses, for discussion. The study will also include new cytokines that are in pre-clinical growth, biological agent consolidations, novel delivery systems and possible lines of inquiry for cytokine-based research in the future.

Through tight interactions with tumor cells throughout the entire course of cancer development, from tumourigenesis and development to metastasis, the immune system can either suppress or promote cancer (Qayoom et al. 2021). The stability of these activities characterise the final results, that, in the case of clinically poor results, are the results of malignancies' immune elusion (Vinay et al. 2015). The environment around the tumor (TME) is made up of surrounding stromal cells, immunological cells and tumor cells. It is interesting to note that cancer cells can use these immune cells to their advantage in order to bypass the host immune system. The chemokine system also controls the recruitment of various immune cell subsets into the TME, as well as the spatiotemporal control of tumor immune responses and cancer-related inflammation (Foxman et al. 1997). Chemokines are a wide group of chemotactic cytokines with modest molecular weights (8–14 kDa) (Bose and Cho 2013; Mehraj et al. 2022a, b, c, d). They are well known for their functions in lymphoid tissue development and immune cell recruitment (Moser and Loetscher 2001). In the TME, chemokines can also have a direct effect on tumor cells and endothelial cells to control the development and proliferation of tumor cells as well as their new vessel formation, cancer stem-like features, spreading and metastasis. Chemokines therefore have a significant impact on cancer therapy and result by directly and indirectly influencing tumor immunity and growth of cancer (Mehraj et al. 2022c; Nagarsheth et al. 2017). Recent advancements in cancer immunotherapy that targets the chemokine system include numerous successes. An anti-CCR4 antibody called mogamulizumab has received clinical approval to treat adult T-cell leukaemia-lymphoma that has relapsed and become resistant to treatment (Fuji et al. 2018). Additionally, the CXCR4 antagonist plerixafor (also known as AMD3100) was authorised for the mobilisation of hematopoietic stem cells for displacement in victims with multiple myeloma (MM) or non-lymphoma Hodgkin's (NHL) (Micallef et al. 2018). Because of these developments, chemokine receptors and chemokines are now recognised as favourable goals for cancer immunotherapy (Qayoom et al. 2023). Consequently, comprehensive understanding of the functions and method of the chemokine system in cancer is essential for the evolution of medicines for the therapy of cancer. Not all patients benefit from the current standard therapy for the majority of malignancies. As a result, finding useful predictive biomarkers has significant clinical relevance for both enhancing therapy target outcomes and generating new therapeutic targets. The system chemokine is a prospective label that may be able to forecast outcomes for cancer patients due to its substantial influence on cancer progression. The future usefulness of the system chemokine, which was studied in several types of cancer, is discussed in the current chapter.

13.2 Cytokine General Characteristics

Cytokines are membrane-bound or released proteins that mediate intercellular signalling to control the immune system's balance. Inborn and acquired immune cells create them in reaction to pathogens and tumor antigens. Individual cytokines' effects on immunity are influenced by a number of variables, such as the concentration of cytokines in the area, the pattern of cytokine receptor impression and the integration of various signalling pathways in reacting immune cells (Sofi et al. 2023; Mir 2015a, b). The greater occurrences of spontaneous tumours observed in mice genetically defective in type I or type II interferon (IFN) receptors or constituents of downstream IFN receptor signal transduction has been used to illustrate the importance of cytokines in tumor immunosurveillance (Kaplan et al. 1998; Shankaran et al. 2001; Picaud et al. 2002). Pleiotropism, or the capacity of one cytokine to operate on a diversity of cell types to induce various and perhaps conflicting effects, is a significant feature of cytokine signalling. Due to its dual function as a powerful activator of both the T regulatory chamber and the T effector compartment, this has appeared to be one of the main deficiencies of IL-2 therapy. The stage of redundancy, or how many cytokines can have the same functional consequences, is another significant characteristic of cytokine signalling (Qayoom et al. 2023). The medicinal modulation of cytokines can be relatively tough due to this redundancy because altering a cytokine one can make other cytokines compensate. When it comes to the immune system, a host's defence and tumor immunobiology, cytokines play complex and frequently conflicting roles. To develop cytokine-based immunotherapy as a cancer treatment, it is crucial to comprehend the biological functions and methods of action of these components.

13.3 Cytokine and Cytokine Receptor Classification

A number of shared and common receptors that cytokines employ to communicate have helped to classify cytokines more functionally. There are now seven different cytokine receptor groups. Type I and Type II receptor cytokines, superfamily of immunoglobulin receptors, tumor necrosis factor (TNF) receptors, coupled G protein receptors, transforming growth factor (TGF-β) and the just newly discovered IL-17 receptors are some examples of cytokine receptors. Because they have the most potential for use in clinical settings right away, the chapter will concentrate on cytokines that communicate using the Type I and II cytokine receptor groups (Fig. 13.1).

13.4 Cytokine Receptor Type I

The ordinary chain (c), which combines with a cytokine-distinct component to originate intracellular signals, is a signalling subunit shared by the Type I cytokine receptors, which comprise receptors for IL-4, IL-2, IL-9, IL-7, IL-21 and IL-15.

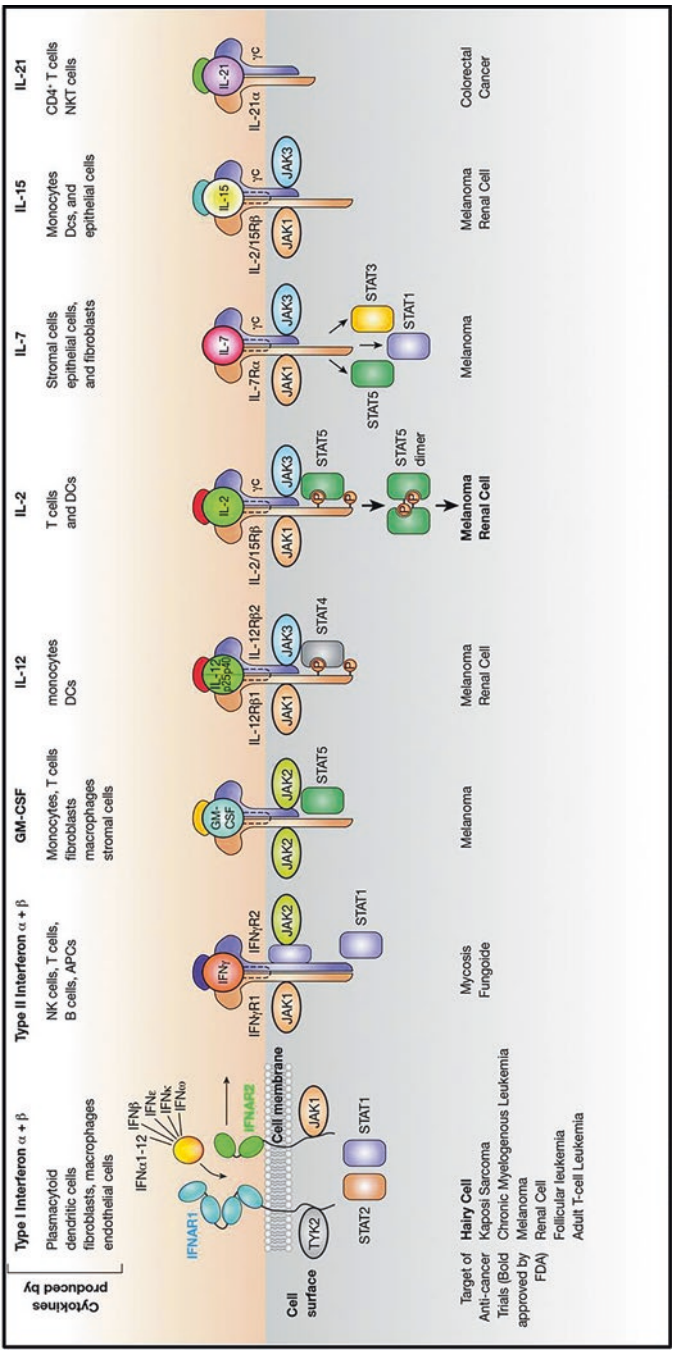

Fig. 13.1 Structural diagram of the main cytokine receptor families

These receptors work in concert with Janus kinases (JAK) 1 and 3 and signal transducers of operated T (STAT) molecules (Mir and Albaradie 2014; Rochman et al. 2009). Other Type I cytokine receptor subgroups include the GM-CSF and IL-6 receptor families. These receptor group share a gp130 receptor component that conducts sophisticated variable-pathway signal activity in their destination cells (Kishimoto et al. 1995; Beekman et al. 2009). Numerous receptor complexes that have unessential and multiple phenotypic expression impacts on the immunological, hematopoietic and neurological systems, such as IL-11, IL-6, leukaemia inhibitory factor (LIF), endostatin M, cardiotrophin-1 and ciliary neurotrophic factor, make use of the gp130 signal transduction component (Hermanns et al. 1999). Similarly, receptors of a different subfamily of GM-CSF receptors that identify IL-5, IL-3 and GM-CSF also do so (Sakamaki et al. 1992). These receptors have an ordinary chain that forms a complex with the cytokine-distinct chain.

13.5 Receptors for Type II Cytokines

The receptor cytokine II, that is made up of a signalling chain and a ligand-binding chain, arbitrates the actions of IFN-α, IFN-β, IFN-γ and IL-10. The II Type receptor cytokine sequences resemble in collaboration with domains Ig-like, and the inner cell regions are frequently linked to a kinase Janus (JAK) family tyrosine kinase (Kotenko and Pestka 2000).

13.5.1 Superfamily of Immunoglobulin Receptors

The immunoglobulin superfamily receptors, which also include the IL-18, IL-1, stem cell factor and monocyte colony-stimulating factor receptors, have extracellular immunoglobulin domains (Hanlon et al. 2002).

13.6 Current Immunotherapy Cytokines

An attempt has been made to involve cytokines in this section which are either earlier well-established in clinical practise or have a solid preclinical foundation for indicating the medicinal efficacy in cancer patients.

13.7 The Interferons (IFN)

According to their capacity to cohere to certain receptors, the interferon are classified into following groups (Hervas-Stubbs et al. 2011).

13.7.1 Interferons I Class

The most clinically effective type I IFNs for the medication of cancer are IFN-alpha and IFN-beta. They are almost universally released by the body's cells and take part in a vital role in cellular immune responses to aggressive infections (Constantinescu et al. 1994; Müller et al. 1994). Major Histocompatibility Complex (MHC) class I molecules are indicated on tumor cells as a result of type I interferons (IFNs) as well as the progression of a fraction of dendritic cells (DC) (Basham et al. 1982; Ameglio et al. 1983; Dolei et al. 1983). Additionally, they have the ability to operate macrophages, natural killer (NK) cells and cytotoxic T lymphocytes (CTLs) (Jewett and Bonavida 1995; Siegal et al. 1999). Besides to their immunologic impacts, Type I IFNs can have cytostatic and potentially apoptotic effects on tumor cells as well as anti-angiogenic effects on tumor neo vasculature (Tsuruoka et al. 1988; Wagner et al. 2004). It is suggested that type I IFNs are crucial for tumor immunosurveillance since mice with a specific removal of the Type I IFN receptor develop cancer more frequently and grow tumours more quickly in transplantable tumor models (Dunn et al. 2005a, b).

IFNs trigger the JAK-STAT signalling system. IFN-α and IFN-β cause IL-4 release and successive B-cell operation by promoting the activities of the JAK1 and TYK2 proteins, STAT1 and STAT2, and JAK1 and TYK2 tyrosine phosphorylation (Schandené et al. 1996; Decker et al. 2005). IFN-α also induces straight apoptosis of tumor cells in a caspase-dependent manner, which may devote to the familiar abilities of type II and I IFNs to increase the expression of tumor cell antigens as well as co-stimulatory and co-inhibitory receptors, which are crucial for the immune response type that occurs among tumor and essential cells (Chawla-Sarkar et al. 2003). IFN-α has anti-angiogenic properties at low concentrations (Kerbel and Kamen 2004). IFN-α outdated the most extensively studied cytokine for treating human cancer and may show to be a key part of connectional techniques for immunotherapy of solid tumours, even though the precise method of IFN-conciliated tumor refusal in animal models have not been completely understood.

13.7.2 IFN-α Clinical Applications

On series based of collaborative category, multi-organisational clinical tests, the only adjuvant medication currently licenced for individuals with dangerous II stage or III stage melanoma is IFN-α. Although these tests at first appeared improvements in both relapse-free survival and general survivorship, later continuing investigated details revealed that with prolonged investigated time, the tails of the curves for relapse-free and especially all-inclusive survival no longer revealed notable interest for IFN-α over the comparison (Kirkwood et al. 1996, 2000, 2004). IFN-α was linked to an advancement in aseptic survival in 10 out of 17 contrast and an improvement in overall survival in four out of 14 comparisons in a more recent meta-analysis of 14 randomised clinical trials including 8122 patients over an 18-year term (Mocellin et al. 2010). IFN-α should continue to be the gold excellence for

additional treatment in melanoma patients at dangerous, according to these authors' findings. A review from the Hellenic Oncology Group showed a powerful correlation amidst favourable result and the emergence of additional autoimmune events (thyroid abnormalities or, vitiligo or serologic evidence) in dangerous melanoma patients during or after adjuvant IFN-α treatment (Gogas et al. 2006), even though successive examination from US and European studies failed to discover this correlation (Bouwhuis et al. 2009). While high-dose IFN-α is the most frequently utilised regimen, when adjuvant IFN-α is given to melanoma patients in the United States, there is a huge need for treatments with a superior therapeutic index, and a range of alternative remedies are now being investigated in this scenario. It is another indication of the ongoing debate about the genuine value of this adjuvant therapy because some large studies did not include an IFN-containing 'control' arm while others did.

In addition to being used to treat melanoma, IFN-α is also approved to treat specific haematologic cancers, Kaposi's sarcoma linked to AIDS and advanced kidney carcinoma when combined with bevacizumab in an anti-angiogenic regimen. Cochlear leukaemia (HCL) and long-term myelogenous leukaemia are two conditions for which IFN has proven to be especially successful as a treatment (CML). IFN-2b treatment for HCL produced a 77% overall reaction rate with a 5% accomplished reaction rate when given subcutaneously at a short dose of 2 million units/m^2 three times per week for a year. This low dose was also well tolerated. The significance of newly cytokine therapy in this illness is shown by the fact that patients with an undamaged spleen emerged to acquire higher complete response rate of 25–35% in follow-up studies. Retreatment results in remissions in the majority of patients, even though HCL relapses are prevalent after IFN-therapy (Golomb et al. 1986; Quesada and Gutterman 1986). However, the introduction of nucleoside analogues, with an accomplished reaction rate close to 90% and resistant remissions in the majority of patients has reduced the use of interferon treatment to second-line treatment in patients with refractory illness or in those who have contraindications to nucleoside analogue medications (Goodman et al. 2003). The ability of IFN-α to improve tumor antigen acceptance by boosting MHC class I appearance is a well-known function, despite the fact that its complicated mechanisms of action are still poorly understood. A fascinating analogy recently drawn between HCL and melanoma—each of two which possess a change in the BRAF gene—implies the special significance of this effort (Tiacci et al. 2011). BRAF mutations in melanoma appear to facilitate immune equivocation by inhibiting the production of melanoma different antigens, that are generally the focus for anti-tumor T cells (Boni et al. 2010). BRAF restrains are able to re-establish this antigen's expression. If the BRAF change is also discovered to result in a suppression of tumor antigens in HCL, this is used to offer a convincing clarification for why two diseases that appear to be unrelated are among the uncommon responders to IFN-α. There would be justification for additional research into IFN-α in connection with BRAF suppressors if it turns out that IFN-α's primary therapeutic mechanism is an increase in tumor antigen expression.

13.7.3 IFN-β Clinical Potential

IFN-β is a protein that is made by both leukocytes and some tumours (Satomi et al. 2002); however, in a number of preclinical models, it has been shown to have therapeutic potential in immunomodulatory strategies for suppressing autoimmune reactivity and for stimulating the immune system in the treatment of malignancy. Four various thesauruses of the medicine are allowed for the treatment of multiple sclerosis. IFN-β is very effective than IFN-α2 in eliciting anti-suppressive impacts in preclinical cancer models, according to comparative assessments of the antitumor causes of the IFNs I Type (Borden et al. 1982; Johns et al. 1992; Chawla-Sarkar et al. 2001). Despite having a higher anti-proliferative potential than IFN-α2, IFN-β's clinical use in cancer therapy has been constrained by its reduced bioavailability and long-lasting by-products. This, yet, may be remedied by conveying through various ways and at various times.

13.7.4 Interferons II Type

IFN-γ, the sole member of this family, attaches to a different complex receptor (IFN-R2 and IFN-R1). IFN-γ is produced by NKT cells, NK cells, Th1 $CD8^+$ T cells, $CD4^+$ T cells, antigen-presenting cells (APCs) and B cells (Frucht et al. 2001; Lighvani et al. 2001; Mir et al. 2022a, b, c). MHC class I, MHC class II and co-stimulatory fragments are indicated on APCs as a result of macrophage activation by IFN-γ (Boehm et al. 1997). Furthermore, IFN-γ causes proteasome modifications that improve antigen presentation (Groettrup et al. 2001a, b). Additionally, IFN-γ encourages $CD4^+$ T-cell development into Th1 cells and prevents B-cell isotype flipping that is IL-4 dependent (Snapper and Paul 1987). IFN-γ phosphorylates JAK1 and JAK2 proteins, creating a STAT1 recruiting site, activating the JAK-STAT signalling cascade.

13.7.5 IFN-γ Clinical Applications

Given that controls do not have an increased risk of spontaneous or chemically induced tumours, mice with targeted deletion of IFN γ or the Type II IFN receptor do (Street et al. 2002), numerous mouse studies first showed that IFN-γ plays a significant role in tumor immunity. Some cancerous cells can be killed by IFN-γ, and it also has some anti-angiogenic properties (Friesel et al. 1987; Coughlin et al. 1998a, b; Mir et al. 2022a, b, c). IFN-γ can potentially have a significant role in controlling anti-tumor task brought on by additional cytokines, most notably IL-12 and presumably IL-2 (Elhilali et al. 2000; Koziner et al. 2002). IFN-γ, on the other hand, has shown only very little clinical utility in the treatment of cancer, which may be partly explained by its low therapeutic index and immune management function in the stimulation of myeloid-derived elimination cells. Even though IFN-γ had limited clinical utility, its production, or the assertion of its gene in effector

lymphocytes is frequently applied as a deported in laboratory tests for specific-antigen effector cell purpose.

13.7.6 Interferons III Type

IFN-2, IFN-1 and IFN-3 belong to this recently identified family and activate 10-IL receptor subgroup 2 and 28-IL receptor subgroup complexes (Vilcek 2003). The therapeutic benefits of III Type IFNs have not still been identified; however this subgroup may turn out to be significant in the future. Contrary to popular belief, III Type IFN receptor assertion is primarily confined to origin epithelial cells (such as the lung and liver) and is conspicuously away from hematopoietic cells and the central nervous system. Type III IFNs and type I Type IFNs contribute various biological tasks as a result of a similar signalling pathway. This result has sparked ongoing research to see if type III IFNs can show IFN-like anticancer effects in the absence of corresponding haematologic and neural lethality (Steen and Gamero 2010).

13.8 Interleukin-2

Interleukin 2 (IL-2) and another T-cell growth factors in the IL-2-related family, such as IL-7, IL-4, IL-15, IL-9 and IL-21, utilise a similar receptor signalling mechanism to activate and expand $CD4^+$ and $CD8^+$ T cells. The IL-2 receptor is a trimeric compound made up of (CD122), (CD25) and (CD132) chains that mediates the biological causes of 2-IL, a 15.5 kDa protein that is alternately glycosylated and has four antiparallel α-helices. The specific-ligand chain only participates in cytokine attaching, whereas the end chains are involved in signalling. Despite the fact that the and chains are indicated on B cells, NK cells and T cells (Begley et al. 1990; Mir and Mir 2022), the chain is instinctive and indicated primarily by T cells, but it is adjacent on a number of phenotypically and efficiently diverse classes of T lymphocytes. The Th1 fraction of CD4 T cells is the most cellular source of 2-IL, and this subset's primary physiological function is to stimulate the autocrine and paracrine invigoration and generation of NK and T cells (Voss et al. 1989). NK cells show the transitional affinity 2-IL receptor (no subgroup), in comparison to T cells. When NK cells are exposed to IL-2, they multiply, exhibit increased cytolytic activity and release other cytokines. Additionally, B cells have transitional affinity IL-2 receptors and are capable of secreting IL-2 in conjunction with other cytokines to promote B-cell differentiation and proliferation. The elimination of T-cell reactions is mostly mediated by 2-IL. Self-reactive T-cells are suppressed by a $CD4^+$ T-cell subset that is characterised by strong stages of CD25 and the transcription factor FoxP3. As soon as effector T-cell responses are activated, these managing T cells (Tregs) keep resistance in check and guard against autoimmunity (Sakaguchi 2000). By encouraging the purpose of $CD8^+$ cytotoxic T cells, $CD4^+FoxP3^+$ Treg depletion promotes tumor non-acceptance and upgrade therapeutic reactions to cancer

vaccines in mouse models (Shevach et al. 2001). Uncertainty surrounds the processes by which $CD8^+$ CTLs are inhibited from functioning by Tregs. However, recent in vivo investigations demonstrate that Tregs restrict $CD8^+$ T-cell proliferation and effector development via competition for IL-2 (McNally et al. 2011). Moreover, the absence of 2-IL signalling causes a generalised inflammatory disease and autoimmune colitis, which is frequently fatal, as shown in mice with a focused deletion of 2-IL or the 2-IL receptor (McHugh et al. 2001; Golgher et al. 2002; Thornton et al. 2004). This provides additional proof that IL-2 serves as both an important mediator of immune tolerance and an immune response activator. The use of 2-IL in the therapeutic context requires to be reassessed in light of this relatively recent discovery that it functions more as a regulatory cytokine than as a T-cell growth factor. A more thorough examination of the dosage, scheduling and kinetics of IL-2 treatment on particular T-cell subsets will be a significant focus of future research.

13.9 IL-2 Clinical Applications

Metastatic melanoma and renal cell carcinoma patients are treated with 2-IL, which is crucial. Malignant melanoma is a melanocyte-derived tumor, and variety of prime cutaneous melanomas show histologic reversion and NK and T-cell infiltration at the course of clinical diagnosis (Barnetson and Halliday 1997). Cytotoxic chemotherapy has not generally been effective against melanoma. The development of efficient immune reaction in melanoma patients was the focus of early studies. Initial research at the National Cancer Institute's Surgery Branch discovered that adoptively transferring peripheral blood mononuclear cells that had been IL-2 activated and had the phenotype and effective properties of operated NK cells, hold up by the concurrent management of 2-IL in over doses, caused a notable tumor reversion in patients chosen for usual organ purpose and virtuous presentation status. These optimistic outcomes prompted further research which revealed a small group of patients receiving large doses of IL-2 alone might experience therapeutic benefit.

Five to 7% of individuals with advanced melanoma experience persistent full responses after receiving high-dose IL-2, while 15–20% of these patients experience objective clinical responses (Atkins et al. 2004). A range of adjustments to the higher-dose IL-2 regimens, such as dose, timing and path changes and also chemical modifications to IL-2's molecular structure that change its cellular destination, have been examined (Atkins 2002) in melanoma patients in an attempt to lessen the side effects associated with the drug. There have also been experiments with other alterations, such as the insertion of toxicity modulators including medicines having anti-inflammatory or anti-angiogenic capabilities. Unfortunately, none of these adjustments have improved the therapeutic index, and present approaches are revisiting the idea of looking at host and tumor biochemical and immunologic characteristics that can predict benefit and enable the selection of patients with a better therapeutic index. More work is being done to create immunomodulators that mix cytokines with other substances to improve antitumor immunity. IL-2 continues to

be a crucial T-cell growth factor in adoptive T-cell therapies (as a part of the T-cell expansion process and given after cell infusions), but as the benefits and potential synergies of other cytokines like IL-15, IL-21 and IL-7 are better understood, they may eventually replace or supplement IL-2. Another tumor with innate resistance to cytotoxic treatments and demonstrated response to immune modulators like IFN- and IL-2 is metastatic renal carcinoma, particularly the clear-cell histology that accounts for the majority of cases. Metastatic renal cell carcinoma patients who receive high-dose intravenous bolus IL-2 have a response rate of about 25%, which is comparable to that of melanoma patients and has a rate of durable complete response in the range of 7%. With median response lengths of 24–54 months and over 80% of complete responders being long-term survivors, the clinical responses to high-dose IL-2 have been generally very persistent (Fisher et al. 2000). In order to treat advanced renal cell carcinoma, the clinical oncologist should continue to use high-dose IL-2. To give the best treatment for all patients with this condition, it is important to comprehend how IL-2 interacts and what sequence is most effective when combined with tyrosine kinase inhibitors, which are now frequently utilised for the first and subsequent treatment of advanced renal carcinoma.

13.10 IL-2 Predictive Biomarkers

Discovering biomarkers that can predict patient response to IL-2 therapy for both RCC and melanoma has garnered a lot of attention. Patients with Stage IV melanoma who had the deletion in the CCR5 gene (CCR532) had a worse chance of surviving after receiving IL-2 treatment than those who did not have the polymorphism (Mir et al. 2020; Ugurel et al. 2008). An inadequate response to IL-2 and a reduced overall survival were related with pre-treatment serum levels of vascular endothelial growth factor (VEGF) and fibronectin that were elevated (Sabatino et al. 2009). Initial studies suggested that patients with renal cell carcinoma who had tumours exhibiting elevated levels of carbonic anhydrase IX (CAIX) had a better response to IL-2 therapy than patients whose tumours had normal or low levels (McDermott and Atkins 2006; Atkins et al. 2005), but larger trials that included additional characteristics raised the possibility of more discriminating markers that are still being researched. In other investigations, the quantity, phenotypic traits and functional status of $CD4^{+}FoxP3^{+}$ Tregs were evaluated in melanoma and renal cell carcinoma patients receiving routine high-dose IL-2 therapy. Patients who responded to IL-2 showed a drop in Tregs to normal levels within four weeks of finishing IL-2 treatment, whereas the number of Tregs increased following exposure to IL-2 and remained elevated in patients with illness progression. In the end, it will be crucial to find predictive indicators that can be tested in sizeable patient cohorts that are specific to the intervention rather than just prognosticators for the disease's natural history.

13.11 Cytokines Related to IL-2

13.11.1 Interleukin-7

One of the IL-2-related cytokines that affects T-cell survival, proliferation and homeostasis is IL-7, a member of the small four-helix bundle family of cytokines. IL-7 signals through the c receptor subunit. Because IL-7 receptor mutations result in a lack of T cells and the emergence of severe combined immunodeficiency (SCID), it has been determined that IL-7 plays a crucial role in T-cell development (Puel et al. 1998). Homeostatic cytokine IL-7 serves as a limited resource for resting naive and memory T cells by continuously signalling them (Schluns et al. 2000). When lymphopaenia occurs, IL-7 then builds up, increasing T-cell proliferation as well as T-cell repertoire variety. IL-7 also contributes to the maturation of B cells, and immature B-cell progenitors contain its receptor. The ability of IL-7 to boost $CD8^+$ T-cell populations while sparing $CD4^+FOXP3^+$ regulatory T cells may be a therapeutic advantage over IL-2 (Rosenberg et al. 2006). Recombinant IL-7 has been demonstrated in mouse models to enhance antigen-specific T-cell responses following immunisation and adoptive cell treatment (Melchionda et al. 2005; Colombetti et al. 2009), and two clinical trials are currently being conducted in people to test this hypothesis (Mackall et al. 2011). The possible involvement of IL-7 in enhancing T-cell recovery following chemotherapy or hematopoietic stem cell transplantation is a significant topic of inquiry. Recombinant IL-7 has been shown in early stage clinical trials to be well tolerated with minimal toxicity at physiologically active dosages (where the number of circulating $CD4^+$ and $CD8^+$ T lymphocytes increased by 3–4 fold), suggesting a wide therapeutic index.

13.11.2 Interleukin-15

Another cytokine that signals through the c receptor subunit and shares structural similarities with IL-2 is IL-15α, which has just started to be studied in clinical trials for cancer and haematologic malignancies (Lorenzen et al. 2006). While IL-2 and IL-15β both serve as early stimulators of T-cell growth and activation, IL-15 works to prevent the apoptosis that IL-2 causes (Waldmann and Tagaya 1999; Marks-Konczalik et al. 2000; Prlic et al. 2003). In addition, IL-15 promotes memory $CD8^+$ T-cell survival, which may be crucial for sustaining long-term anti-tumor immunity (Ku et al. 2000). A number of pre-clinical murine cancer models have shown that IL-15 has strong therapeutic efficacy (Di Carlo et al. 2000). The direct activation of $CD8^+$ effector T lymphocytes mediates these effects in a way that is independent of the presence of an antigen (Meresse et al. 2004). Importantly, although sharing receptor subunits with IL-2, IL-15 must be delivered to $CD8^+$ T cells on the high affinity IL-15R in reverse orientation, which may explain some of its variations in activity from IL-2 (Waldmann 2006). IL-15 has recently begun Phase I clinical trials in humans, and it is anticipated to be useful in a range of immunotherapeutic

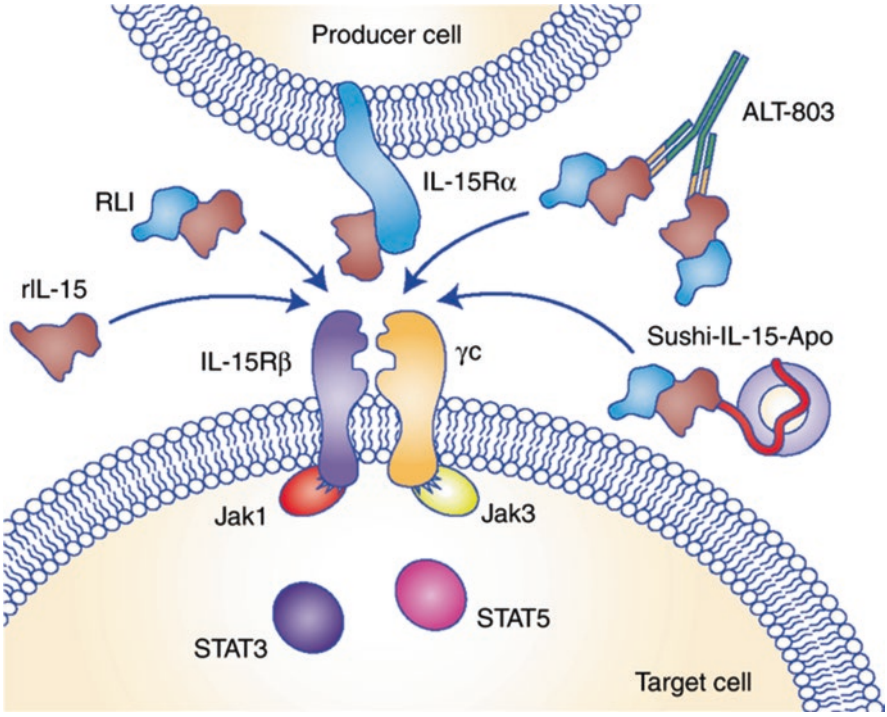

Fig. 13.2 Modified forms of the interleukin-15 (IL-15)

approaches, such as both in vivo and ex vivo approaches for adoptive cell therapy (Fig. 13.2).

13.11.3 Interleukin-21

IL-21 is a type I cytokine that closely resembles IL-2, IL-4 and IL-15. It also shares a cytokine-specific receptor and the c receptor subunit with IL-7 and IL-9. The primary activation of STAT1 and STAT3 distinguishes IL-21 signalling from that of other c cytokines (Parrish-Novak et al. 2000). IL-21, which is largely produced by activated $CD4^+$ T cells, exhibits pleiotropic effects, including the encouragement of $CD4^+$ and $CD8^+$ T-cell proliferation and augmentation of $CD8^+$ T-cell and NK-cell cytotoxicity without boosting activation-induced cell death (Brandt et al. 2003a, b; Habib et al. 2003). Although it is unknown how IL-21 affects Th1/Th2 differentiation, it is necessary for regular humoral responses and is thought to have an impact on the shift from innate to adaptive immunity (Sivakumar et al. 2004). Recently, Phase I clinical trials for IL-21 were started, with modest initial results (Kishida et al. 2003; He et al. 2006). IL-21 has shown therapeutic activity in murine tumor models of melanoma. Based on its capacity to greatly increase the ex vivo production and affinity of antigen-specific T cells, IL-21 has just lately begun to play a role

in adoptive T-cell techniques, similar to the other cytokines of the c family (IL-2, IL-7 and IL-15) (Li et al. 2005).

13.12 Additional Cytokines with Clinical Use

13.12.1 Interleukin-6

The usual sources of the type I cytokine IL-6 are macrophages, endothelial cells and certain activated T cells (Mir and Mehraj 2019). It uses the gp130 signal transducing component, which is also found in a subfamily of cytokines that includes IL-11 and IL-27, to transmit signals through a receptor (Abbas et al. 2014). IL-6 performs a variety of tasks, but among them, it mediates inflammation and is essential for haematopoiesis, stem cell multiplication and differentiation (Trikha et al. 2003). Numerous cancers, including breast, renal cell, colon, prostate and bladder cancers as well as B-cell malignancies, most notably myeloma, appear to be influenced by the overexpression of IL-6. Furthermore, it has been demonstrated that all of these tumor types have a worse prognosis when there are higher serum levels of IL-6. Some cancer cells, like myeloma, start secreting large amounts of IL-6 as an autocrine growth factor to aid in their own survival. In fact, the inhibition of IL-6 by corticosteroids is probably one of the reasons why they are effective treatments for myeloma. IL-6-conjugated toxins and monoclonal antibodies to IL-6 and the IL-6 receptor have been used in treatment efforts as a result of our understanding of IL-6 as a pro tumorigenic cytokine (Trikha et al. 2003). However, this strategy is constrained by the widespread expression of IL-6R by many normal cells. Using monoclonal antibodies against IL-6, there have been a few early-stage clinical trials with varying degrees of success. In one of the largest studies, a chimeric human-mouse IL-6 antibody was used to treat 12 patients with refractory myeloma; 11 of these patients experienced disease stability, but none showed a clinically significant response (defined as a decrease in M protein of more than 50%) (Zaanen et al. 1998). Anti-IL-6 medication, however, might have a more advantageous function in the treatment of cancer-related, inflammatory-related symptoms such cachexia, fever and pain (Blay et al. 1997).

13.12.2 Interleukin-18

IFN-inducing factor IL-18, a 24 kDa, non-glycosylated polypeptide, was first discovered and is structurally linked to IL-1 (Okamura et al. 1995; Tsutsui et al. 1997; Tomura et al. 1998a, b). NK and $CD8^+$ T cells' IFN secretion is stimulated by IL-18, which also increases the cytotoxicity of these cells (Tomura et al. 1998a, b). In addition to these roles, IL-18 also promotes angiogenesis, Th1 helper $CD4^+$ T-cell growth, enhanced FasL expression on lymphocytes and macrophage activation (Okamura et al. 1998; Park et al. 2001). Increased serum IFN and GM-CSF levels were discovered in patients after receiving intravenous IL-18 in phase I clinical

studies, which also confirmed the drug's safety. Only two objective responses were seen in 26 patients in one trial and three patients with stable disease in another investigation, indicating that the clinical responses were not particularly robust (Lee et al. 2004). Finding a place for IL-18 in cancer immunotherapy techniques will require more research.

13.13 GM-CSF or Granulocyte-Macrophage Colony-Stimulating Factor

Initially recognised as a modulator of haematopoiesis and monocyte-macrophage differentiation, GM-CSF is a heterogeneously glycosylated 14–35 kD polypeptide (Lee and Margolin 2011). The GM-CSF receptor shares a common chain with the highly pleiotropic cytokines IL-3, which promotes multi-lineage myelopoiesis, and IL-5, which is the main growth factor for eosinophils. Similar to IL-3 and IL-5 receptors, GM-CSF receptors are made up of a ligand-specific and a common subunit (Shearer et al. 2003). Dendritic cell maturation is accelerated by the production of GM-CSF, which is made by monocytic cells and T cells. Numerous tumor models have demonstrated the ability of GM-CSF to activate immune responses, such as a murine melanoma where transgenic expression of GM-CSF protected over 90% of the animals from recurrence of tumor challenge (Dranoff et al. 1993). Additionally, promising outcomes have been seen in other tumours when GM-CSF was used alone or in combination with other immunomodulators like checkpoint-blocking antibodies (Small et al. 1999; Hodi et al. 2002). GM-anti-tumor CSF's effect seems to be correlated with its capacity to stimulate dendritic cells and macrophages (Grabstein et al. 1986a, b; Mehraj et al. 2021). In addition to maturing DCs, GM-CSF also increases co-stimulatory molecules and CD1d receptors, which are important for antigen presentation (Leslie et al. 2008). Initial research revealed that $CD4^+$ and $CD8^+$ T cells were responsible for mediating GM-CSF-stimulated antitumor immunity; however, more recent studies employing CD1d-deficient animals have shown that NKT cells play a crucial part in GM-CSF-stimulated antitumor immune responses. More recently, it has been proposed that GM-CSF may play a more regulating role in the induction of DC-mediated T-cell immunity through intricate interactions with milk fat globule-8 (MFG-8), a glycoprotein on antigen-presenting cells that helps to regulate immunologic responses resulting from their interactions with T-cell subsets under various biological conditions (Jinushi et al. 2007). After receiving induction chemotherapy, patients with acute myelogenous leukaemia were given recombinant GM-CSF to speed up neutrophil recovery and lower their risk of infection. Additionally, GM-CSF is utilised to promote engraftment and myeloid reconstitution following autologous and allogeneic bone marrow transplants as well as to mobilise hematopoietic progenitor cells into peripheral blood for leukapheresis collection. As a result of the significant preclinical research proving this molecule's immunostimulatory characteristics, particularly through its transgenic expression in tumor cells to produce a promising tumor vaccine (Dranoff 2003; Mir et al. 2023), numerous immunotherapeutic techniques have been

thoroughly tested. Direct injection of GM-CSF, a single drug, into metastatic lesions has been shown to have anticancer efficacy in melanoma (Ridolfi et al. 2001).

13.14 Chemokines and Chemokine Receptors

The four primary chemokine subfamilies (CC, CXC, CX3C and C) are based on the number and placement of the highly conserved cysteine residues at the N-terminus of the chemokine ligands, as well as the presence or absence of intervening amino acids. The first and third of the four conserved cysteine residues are absent from the C chemokines, in contrast to the CC, CXC and CX3C chemokines, which each include zero, one and three non-conserved amino-acid residues between the first two cysteine residues, respectively. The chemokine ligands belonging to the CC, CXC, CX3C and C subfamilies have been given the names CCL, CXCL, CX3CL and XCL, respectively, under a naming scheme. Seven transmembrane-domain G protein-coupled receptors (GPCRs) that identify these chemokines have the classifications and names CCR, CXCR, CX3CR and XCR, respectively, depending on the sources of the chemokine ligands they bind to.

13.15 Chemokine System's Impact on Cancer Prognosis

In this section, we will talk about how chemokines affect cancer patients' prognosis in connection to how they affect the course of the disease.

13.15.1 CCL2

It's interesting to note that one theory proposed that CCL2 is produced by tumor cells to increase the recruitment of monocytes or macrophages, which may then destroy tumor cells by secreting pro-inflammatory cytokines (Qayoom et al. 2023; Zhang et al. 2013). Although CCL2 has angiogenesis-promoting properties in vivo, the activity is only seen at specific levels and not at greater dosages (Salcedo et al. 2000). Nevertheless, a number of findings point to the possibility that the CCL2-dependent signalling pathway may support tumor cell survival (Brummer et al. 2018; Yao et al. 2019), enhance metastasis and increase angiogenesis (Mir et al. 2020; Ueno et al. 2000). Patients with increased CCL2 or CCR2 expression had significantly improved OS in NSCLC but lower OS and progression-free survival in diffuse large B-cell lymphoma (DLBCL) (Li et al. 2019), despite the paucity of data about the dual roles of the CCL2/CCR2 axis in DLBCL. Clarification of the significance of CCL2/CCR2 as a prognostic factor in a variety of cancer types requires further research.

13.15.2 CCL5

Through the enlistment and control of inflammatory cell activity, as well as the subsequent creation of an immunosuppressive environment containing TAMs and MDSCs in BC, the CCL5/CCR5 axis has been shown to promote cancer cell migration (Sofi et al. 2022; Soria et al. 2012). According to data from a study on BC patients, those who had higher serum levels of CCL5 were more likely to develop lymph node metastases (Wan et al. 2017). Similar to this, stage II BC patients had a considerably higher risk of disease progression when their tumours expressed CCL5 positively, either on their own or in combination with the absence of oestrogen receptors (Yaal-Hahoshen et al. 2006). As a result, CCL5 is a poor predictor of outcome for BC, especially in stage II patients.

13.15.3 CCL14

Uncertainty exists over CCL14's roles in the development of cancer. CCL14 encourages apoptosis, reduces HCC cell proliferation and development by obstructing cell cycle progression via the Wnt/-catenin signalling pathway and helps HCC patients live longer. High CCL14 gene expression consistently increased HCC patients' survival rates (Zhang et al. 2017). Contrarily, CCL14 was found to encourage macrophage polarisation, infiltration and proliferation in the bone marrow, which was thought to be related to chemoresistance in MM (Li et al. 2015; Mehraj et al. 2022a, b, c, d). More research is required to understand how this chemokine contributes to the growth of cancer and how it affects prognosis in HCC and other cancer types.

13.15.4 CCL20

According to studies, the CCL20/CCR6 axis is important for the tumor-chemokine network and accelerates tumor growth in HCC and CRC. This axis has been demonstrated to enhance metastasis (Mehraj et al. 2021; Rubie et al. 2006; Cheng et al. 2014), induce EMT via the PI3K/AKT and Wnt/-catenin pathways (Hou et al. 2015) and stimulate cell proliferation, likely through controlling the expression of p21, p27 and cyclin-D1 (Liu et al. 2014a, b). The CCL20/CCR6 network drives immune suppression and promotes Treg activity to mediate the death and spread of cancer cells. These pro-tumor effects led to a negative correlation between patient outcomes and CCL20 expression in HCC and CCL20 and CXCL8 co-expression in CRC (Cheng et al. 2014).

13.15.5 CCR7

It is generally known that CCR7 actively encourages the growth of tumours in many different cancer types. By reducing IFN-mediated inflammation in melanoma,

CCR7 can reduce the host's anti-tumor immunity (Takekoshi et al. 2012). Additionally, it has been shown that CCR7 stimulates proliferation and inhibits apoptosis in NSCLC by accelerating the G2/M phase through the ERK1/2 pathway (Xu et al. 2012a, b). By encouraging the production of VEGF-C in prostate cancer, CCR7 also stimulates tumor angiogenesis (Chi et al. 2015). Additionally, it boosts angiogenic capability via the NF-B/VEGF pathway in oesophageal squamous carcinoma cells (ESCC) and promotes endothelial cell proliferation and migration (Cai et al. 2017). Additionally, it has been discovered that CCR7 induces EMT in PDAC and lung cancer (LC) (Li et al. 2016; Zhong et al. 2017), encourages MMP-2 and -9 expression in bladder cancer (Mo et al. 2015) and promotes the spread, migration and eventual creation of metastases in tumor cells (Legler et al. 2014). The expression of CCR7 or CCL21 has been found to be substantially linked with poor survival due to the strong pro-tumor activities (Zu et al. 2019). Consequently, CCR7 may develop into a diagnostic marker for a variety of malignancies, such as colorectal liver metastases.

13.15.6 CXCL1

By stimulating glycolysis, CXCL1 has been shown to have a significant role in the progression and metastasis of CRC (Zhuo et al. 2018). Another ELR$^+$ CXC chemokine called CXCL1 binds to CXCR2 to promote angiogenesis. In order to promote liver metastases in CRC, CXCL1, which is generated by TAMs, also attracts CXCR2$^+$ MDSCs for the pre-metastatic niche. Additionally, in pancreatic cancer, CXCL1 directly inhibits T-cell infiltration and reduces sensitivity to immunotherapy (Li et al. 2018). High CXCL1 expression has repeatedly been found to be associated with a poor overall survival (OS), advanced tumor, node, and metastatic stage, and lymph node metastasis because of the tumor-promoting actions. The prognostic relevance of CXCL1 for a variety of malignancies, including CRC, pancreatic cancer and others, is strongly shown by this data (Zhang et al. 2019). Additionally, it has been proposed that CXCL1 promotes radio resistance in ESCC by controlling the DNA damage response in a ROS-dependent manner (Zhang et al. 2019).

13.15.7 CXCL8

For its roles in fostering cancer, CXCL8 has been thoroughly studied. In CRC, cervical cancer and lung cancer, CXCL8 can promote proliferation and survival by autocrine activation (Zhu et al. 2004; Ning et al. 2011; Liu et al. 2014a, b), whereas in NSCLC, it can do so through the ERK1/2 pathway. Additionally, CXCL8 causes EMT in CRC (Shen et al. 2017), stimulates angiogenesis and cell migration, draws MDSCs to create NETs and aids tumours in evading the immune system (Gonzalez-Aparicio and Alfaro 2019). High expression of CXCL8 may be a poor prognostic indicator in a variety of human malignancies because of the aforementioned

pro-tumor actions, including lung adenocarcinoma (Sunaga et al. 2014), cervical cancer (Yan et al. 2017) and CRC (Di Caro et al. 2016). CXCL8 has consistently also aflibercept therapy for metastatic CRC (Lambrechts et al. 2015) and chemoimmunotherapy (Tsukinaga et al. 2015) both use this factor as a reliable indicator of a poor prognosis

13.15.8 CXCL10

On TME, CXCL10 has opposite effects. On the one hand, local CXCL10 production draws CTLs into ESCC tissue and is likely helpful for patient survival (Liu et al. 2015). On the other hand, an in vitro investigation revealed that monocytes, mediated by CXCL10, induce metastasis by promoting the invasion and migration of tumor cells in B-cell precursor acute lymphoblastic leukaemia (ALL) (Lee et al. 2012). Due to the aforementioned conflicting roles, higher expression of CXCL10 has been linked to better results in ESCC (Sato et al. 2016), but an adverse prognosis in DLBCL (Ansell et al. 2012).

13.15.9 CXCL12/CXCR4

The well-studied axis CXCL12/CXCR4 is crucial for the development of tumours at all stages in a variety of cancers. In HCC and malignant pleural mesothelioma, CXCL12/CXCR4 contributes to immunosuppressive actions, tumor-related inflammation and the recruitment of Treg cells into the TME. The ERK1/2 and AKT pathways, as previously mentioned, are used by the CXCL12/CXCR4 combination to encourage the growth of tumor cells and the division of glioblastoma cells. Additionally, the axis participates in tumor angiogenesis in glioblastoma (Ansell et al. 2012) and BC (Ansell et al. 2012) via VEGF-dependent pathways. Along with encouraging metastasis, this axis also induces EMT (Li et al. 2014), pseudopodia development and actin polymerisation (Sun et al. 2010). Furthermore, in drug-resistant NSCLC, CXCR4 is essential for CSC self-renewal (Jung et al. 2013). Numerous studies have consistently demonstrated that the expression of the CXCL12/CXCR4 axis is linked to a poor prognosis for patients with oesophageal cancer (Uchi et al. 2016), acute myelogenous leukaemia (Spoo et al. 2007), BC (Andre et al. 2009; Xu et al. 2013), HCC (Hu et al. 2015) and NSCLC (Zhang et al. 2015). This is because of the axis' potent pro-tumor actions. In addition, CXCR4 expression promotes a more aggressive phenotype in papillary thyroid cancer and correlates negatively with the extent of tumor infiltration (Torregrossa et al. 2012), as well as being a poor prognostic indicator for NHL treatment response (Mazur et al. 2014).

13.15.10 CX3CL1/CX3CR1

Depending on the type of cancer, the CX3CL1/CX3CR1 has both suppressive and cancer-promoting effects on the development of cancer. This has an impact on the prognosis, which can be either good or bad. Patients with gastric cancer who express more CX3CL1 have a better prognosis due to increased $CD8^+$ T- and NK-cell recruitment (Hyakudomi et al. 2008). Additionally, CX3CL1/CX3CR1 modulates the cancer cell cycle and tumor growth in HCC patients via the autocrine or paracrine systems, which is linked to successful results (Hyakudomi et al. 2008; Sofi et al. 2022). Contrarily, it has been demonstrated that CX3CL1/CX3CR1 promotes tumor growth, proliferation and resistance to apoptosis by activating the AKT/NF-B and Pathways for JAK/STAT signalling in PDAC As a result, a PDAC patient research provided evidence that elevated expression of the CX3CL1/CX3CR1 axis in tumor tissues was associated with a poor OS prognosis (Xu et al. 2012a, b; Mehraj et al. 2022a, b, c).

13.15.11 XCL1

$CD4^+$ and $CD8^+$ T lymphocytes, neutrophils and NK cells are all attracted by XCL1 (Hedrick et al. 1997; Cairns et al. 2001). It likely increases antitumor responses, making it important for some malignancies' gene transfer immunotherapies. Curiously, higher blood XCL1 levels upon diagnosis and their gradual reduction after chemotherapy were linked to longer survival in ALL (Gutiérrez-Aguirre et al. 2017). This may be explained by the tumor's response to cancer therapy, which has shown a steady reduction in the leukemic load. This finding should be taken into account, though, as individuals with good predictive indicators tended to be younger and have lower white blood cell counts. Future research should look more closely at how XCL1 affects cancer progression and how it affects cancer prognosis.

Several chemokines and chemokine receptors, in short, can serve as prognostic indicators for people with cancer. Depending on the kind of cancer, CCL2, CXCL10 and CX3CL1/CX3CR1 can function as both positive and negative prognostic indicators. While CCL14 and XCL1 have only demonstrated high predictive significance, other chemokines, including CCL5, CCL20/CCR6, CCR7, CXCL1, CXCL8 and CXCL12/CXCR4, have repeatedly served as poor prognostic indicators in many cancer types. Intriguingly, it has been found in various clinical trials (Ciombor et al. 2014; Noonan et al. 2016; Jamal et al. 2017) that high levels of several chemokines are closely linked to worse patient OS. Further research in diverse clinical trials of anticancer treatments is required in order to identify and validate several chemokines as potential and predictive tumor-based biomarkers for patient outcomes (Table 13.1).

Table 13.1 The chemokine system's functions as cancer prognostic indicators

Chemokines/ receptors	Cancer types	Sites of expression	Study types
Good prognostic markers			
CCL2	Non-small cell lung cancer (NSCLC)	Tissue	Retrospective
CCL14	Hepatocellular carcinoma (HCC)	Tissue	Retrospective
CXCL10	Oesophageal squamous cell carcinoma	Tissue	Prospective
CX3CL1/ CX3CR1	HCC	Tissue	Prospective
CX3CL1	Gastric adenocarcinoma	Tissue	Prospective
XCL1	Acute lymphoblastic leukaemia	Serum	Prospective
Poor prognostic markers			
CCL2/CCR2	Diffuse large B-cell lymphoma (DLBCL)	Tissue	Prospective
CCL5	Breast cancer (BC)	Serum	Prospective
	Stage II BC	Tissue	Prospective
CCL20	Colorectal cancer (CRC)	Tissue	Prospective
CCL20/CCR6	HCC	Tissue	Prospective
CCL21/CCR7	Colorectal liver metastasis	Tissue	Prospective
CCR7	Solid tumours	Tissue	Meta-analysis
CXCL1	Various cancers	Tissue, urine, serum	Meta-analysis
CXCL8	CRC	Tissue	Prospective
	CRC	Serum	Prospective
	Cervical cancer	Tissue	Prospective
	Lung adenocarcinoma	Tissue	Prospective
CXCL10	DLBCL	Serum	Prospective-retrospective
CXCL12	Oesophageal cancer	Tissue	Prospective
CXCR4	Acute myelogenous leukaemia (AML)	AML cells	Prospective
	Early BC	Tissue	Prospective-retrospective
	BC	Tissue	Meta-analysis
	HCC	Circulation and/or tissues	Meta-analysis
	NSCLC	Tissue	Meta-analysis
CX3CL1/ CX3CR1	Pancreatic ductal adenocarcinoma	Tissue	Retrospective

13.16 Conclusions

A variety of cytokines are needed to generate effective, targeted and long-lasting anti-tumor immunity. These cytokines control crucial processes related to the

equilibrium between tumor rejection by antigen-specific effector cells and suppressive mechanisms that enable tumours to evade immunologic detection. In murine models and in the clinical management of a number of human cancers, the cytokines have shown therapeutic anti-tumor activity. They are essential for tumor immunosurveillance. Melanoma and renal cell carcinoma can now be treated with single-agent IFN and high-dose IL-2. Based on promising mouse tumor models, other IL-2-related cytokine family members are being thoroughly investigated for potential anti-tumor uses. Additionally, a number of cutting-edge methods have been created that use cytokines to promote effective anti-tumor immunity. These methods include bifunctional molecules like antibody-cytokine fusions, expression of cytokines in recombinant viral vectors, or irradiated whole tumor cells as vaccines. The continued development of efficient cytokine-based cancer treatments will depend on a deeper knowledge of the molecular signalling pathways used by cytokine receptors as well as the temporal and kinetic pattern of receptor expression. An important area for future study is the quest for predictive biomarkers to help in the identification of individuals who are most likely to respond, given the low response rates and substantial toxicities of IL-2 and IFN. The significant pleiotropy and redundancy of cytokine signalling indicate that the future of cytokine-based therapy may involve combination regimens that target many pathways to increase the anti-tumor response while suppressing the regulatory mechanisms and while reducing toxicities. Recent developments in molecularly focused treatments, including BRAF inhibition, have already sparked interest in a novel use of cytokines in conjunction with these treatments. There is little doubt that cytokines will continue to play a significant role in the advancement of cancer immunotherapy given their shown efficacy in the treatment of cancer.

Depending on the type of cancer, chemokines such as CCL2, CXCL10 and CX3CL1/CX3CR1 can either be positive or unfavourable prognostic indicators. CCL14 and XCL1, however, are only effective prognostic indicators for the prognosis of cancer patients. the chemokines and their receptors, such as CCR7, CXCL1, CXCL8 and CXCL12/CXCR4, which specifically promote carcinogenesis, may subsequently function as unfavourable prognostic indicators for cancer patients. Despite significant advancements in our understanding of the intricate interplay between the chemokine system and tumor biology, little is still known about the complex functions of chemokines and their prognostic significance in various cancers, particularly in response to various anticancer therapies. However, a sizeable number of chemokine receptor inhibitors that target various chemokine signalling pathways are now being assessed in a number of pre-clinical investigations and clinical trials, which when combined with immune checkpoint treatment or chemotherapy shows good results. Further comprehensive research are required to understand their unique functions and the action mechanisms involved in cancer progression, which will help validate certain chemokines and their receptors as prognostic indicators of specific cancer types.

Further Readings

For further readings, some textbooks mentioned below also give further understanding of cytokine and chemokine network.

- *Cytokines and Their Therapeutic Potential* by Manzoor Ahmad Mir.
- *The Cytokines of the Immune System: The Role of Cytokines in Disease Related to Immune Response* by Zlatko Dembic.
- *The Cytokine Network and Immune Functions* by Jacques Thèze.
- *Chemokines and Viral Infection* by T.E. Lane.
- *Cytokines and Chemokines in Infectious Diseases*.

The below links for video lectures may also be helpful:
https://youtu.be/TAqO0Mq19JQ
https://youtu.be/6wEnYfvoBqk
https://youtu.be/A9fRRCUlMms
https://youtu.be/zIWvmCsKr34

For more insights about the topic, we would suggest detailed findings from the books of Mir et al. (2022a, b, c) (https://doi.org/10.1016/C2021-0-02565-7 and https://doi.org/10.1016/C2022-0-00074-X), Mir (2021) (https://doi.org/10.52305/WXJL6770), and Mir (2015a, b) (https://doi.org/10.1016/C2014-0-02898-5) and from the cancer.net website on the following below-mentioned links:
https://www.cancer.net/cancer-types/breast-cancer/types-treatment
https://discovery.ucl.ac.uk/id/eprint/1472740/
https://www.jmedsciences.com/doi/JMEDS/pdf/10.5005/jp-journals-10045-00138

For diagrammatic illustrations and descriptive tables, see http://www.eurekaselect.com/article/49928

See video links on overall status of Cancer, its various types, and current new treatment possible options available:
https://www.sciencedirect.com/science/article/pii/S205970292032278X

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Therapeutic Implications of Cytokines and Chemokines Network in Cancer

14

Manzoor Ahmad Mir, Nusrat Jan, and Iqra Noor

Abstract

Innate and adaptive immune cells, as well as non-immune cells and tissues, communicate with one another through interleukins and related cytokines. Interleukins belong to a family of cytokines and play a crucial role in the initiation, progression, and management of cancer. Interleukins are vital for a successful tumor-directed immune response, but they can also promote conditions that encourage and enable the progression of cancer. These belong to a class of cytokines and can be used to enhance immunotherapies and increase their efficiency while reducing negative effects. The use of cytokines to stimulate cancer patients' immune systems has long been a significant therapeutic approach and is still having a significant impact on clinical cancer research today. To increase their antibody-dependent cellular cytotoxicity or sustain cellular responses and anticancer efficacy, cytokines are currently being studied clinically with different engineered cytokine mutants (superkines), chimeric antibody-cytokine fusion proteins (immunokines), anticancer vaccines, CPIs, and cancer-directed monoclonal antibodies. An increase in attempts to describe cytokines and utilize their extensive signaling networks to create cancer treatments has coincided with the previous two decades' increased interest in using the immune system to fight against cancer.

Keywords

Cytokines · Chemokines · Interleukins · Interferons · Tumor microenvironment · Superkines

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14.1 Introduction

A cytokine network is made up of the signaling molecules known as cytokines, which also mediate and control hematopoiesis, inflammation, and many other cellular processes. Cytokines can also affect nonimmune cells, and it was first recognized as immunocellular products that operate as facilitators and regulators of immunological processes. Glial cells of the neurological system are also capable of secreting cytokines (Qayoom et al. 2023b). Various other names are mentioned based on their alleged role namely secretory cell, or intended target of action; cytokine is a broad term. For instance, one leukocyte makes interleukins, which act upon other leukocytes, whereas lymphokines are another name for the cytokines produced by lymphocytes. Cytokines that have chemotactic characteristics are known as chemokines (Mir et al. 2019).

Over the past four decades, cytokines and their respective receptors have been thoroughly studied as potential targets regarding cancer or cancer treatments. Therapeutic approaches that enhance interferons and interleukin's growth-inhibitory and immunostimulatory characteristics, such as IL-2, -7, 12, and IL-15, or that reduce cytokines' protumor and proinflammatory properties like TNF, IL-1, and interleukin-6 are supported by a strong preclinical rationale (Mir 2015). The findings revealed that the expression of cytokines has changed and is dysregulated in all types of human cancer. Clinical tests of numerous cytokines or cytokine antagonists triggered by these findings revealed pertinent biological activity but only modest therapeutic effectiveness. Although it may not be the optimum circumstance for cytokine-based therapy, patients with advanced illnesses made up the majority of trial participants (Qayoom et al. 2023a). New strategies for utilizing cytokine networks to treat cancer have emerged because of the development of immunotherapies that are more effective and deeply know the tumor microenvironment. Among the various treatments, the use of cytokine-based treatment to target early-stage malignancies reduces the immune-related negative effects (Mir and Mir 2022).

14.2 Overview of Cytokines

A wide family of proteins known as cytokines plays a crucial role in cell signaling (Qayoom et al. 2021). They are made by several types of cells, viz., endothelium, fibroblast, and stromal cells, including immunological cells such as mast cells, T lymphocytes, B lymphocytes, and macrophages (Mehraj et al. 2021a, b, c). Cytokines also include colony-stimulating factors, tumor necrosis factors, growth factors, chemokines, ILs, and interferons (Fig. 14.1).

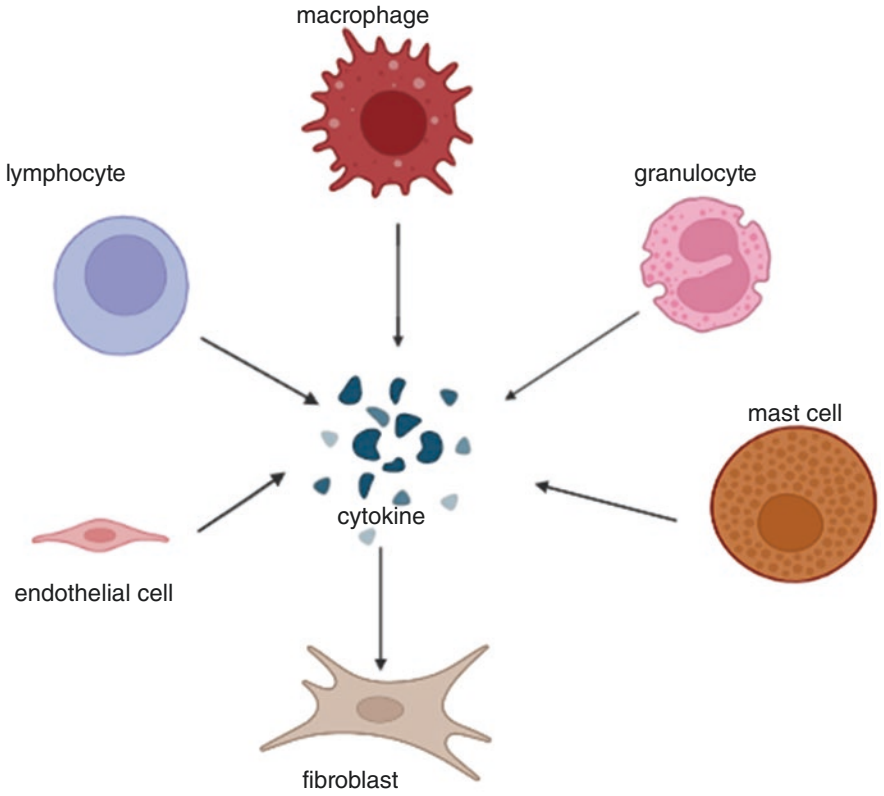

Fig. 14.1 Major types of cells secreting cytokines

14.3 Family of Cytokines

Cytokines are soluble protein that belongs to a diverse family having a low molecular weight of (5–20 kDa) and are produced by a variety of cells in response to immunogens, mitogens, or other stimuli. These are known to mediate communication between cells. Numerous studies have shown that cytokines function as immunomodulating agents and have roles in host defense, inflammation, and immunity including tumor immunobiology. Cytokines act via endocrine, paracrine, and autocrine systems of signaling (Mir and Albaradie 2014; Tisoncik et al. 2012) (Fig. 14.2). Structure and function are the two main guiding principles used to categorize cytokines.

The cytokines are either produced as membrane-bound proteins or as secreted proteins (Dranoff 2004) and consist of a major subgroup that includes chemokines, interleukins, colony-stimulating factors, tumor necrosis factor (TNF), including interferons. Redundancy and pleiotropy are the characteristics exhibited by cytokines; with different cytokines displaying functional similarity. Cytokines induce their effects via interacting with the family members of cytokine receptors. These

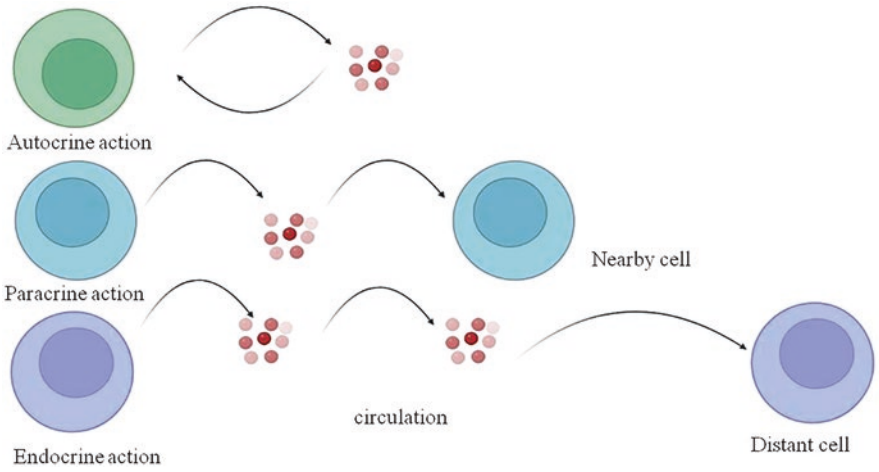

Fig. 14.2 Immunomodulatory function of cytokines

include G-protein coupled (chemokines), TNF, TGF-β, and IL-17 receptors including type I, and type II immunoglobulin superfamily (Lee and Margolin 2011). On target cells cytokines upon binding to receptors activates a sequence of downstream proteins, which result in gene expression patterns alteration and induce a desired response in target cells (Altan-Bonnet and Mukherjee 2019).

14.3.1 Interleukins

Interleukins, commonly known as ILs, were first discovered in the 1970s and are a class of cytokines that are essential for regulating cell functions (like cell proliferation, motility, and differentiation) and eliciting immunological reactions. Interleukins allow immune system cells to communicate with each other and generate a coordinated specific response to a target antigen. Immune system cells secrete interleukins and bind to a target hostile cell via specific cell surface receptors. Upon attachment within the target cell, it causes a cascade of events resulting in the alteration of the cell's behavior. In addition, several immunomodulatory functions are possessed by interleukins that help the cells of the immune system in differentiation, adhesion, maturation, and migration. So far, numerous interferons have been discovered which include IL-2, IL-3, IL-4, IL-6, IL-19, etc. (Anestakis et al. 2015).

14.3.2 Interferon

Interferons are glycoproteins produced by some cells in response to viral infections and certain chemical inducers including immune stimulation. Within the immune

system and with the various effects, they act in a paracrine manner and mimic the diversity of the endocrine system. In humans, twenty interferons have been investigated. Interferons are classified into 3 main groups which include alpha, beta, and gamma. Both the interferons alpha and beta were originally named according to their early method of production, like leukocyte-derived alpha and fibroblast-derived beta. T-cells solely secrete the gamma interferon, which is a lymphokine. Interferons are utilized for cell-to-cell interactions and in the activation of the immune system's defensive mechanisms that aid in the eradication of infections, including interferons (Zeng et al. 2002).

14.3.3 Growth Agent

A naturally occurring steroid hormone or protein known as growth factor promotes tissue healing, growth of cells, and differentiation. Growth factors, which might be released by nearby cells, organs, and glands or possibly the actual tumor cells, are crucial for controlling a range of biological activities. Several growth factors are required for the proliferation and viability of normal cells. Growth factors can stimulate the cells via endocrine, paracrine, and autocrine manner (Hafez et al. 2000).

14.3.4 Tumor-necrosis Factor (TNF)

TNF a multifunctional cytokine is known to play a crucial role in numerous cellular events, which include cell proliferation, differentiation, survival, and death. TNF is a proinflammatory cytokine that is secreted by inflammatory cells. It is known to be involved in inflammation-associated carcinogenesis. Activating different signaling pathways which include NF-κB and JNK, TNF maintains its biological activity (Lebrec et al. 2015).

14.3.5 Chemokine

Chemokines are a large family of chemotactic cytokines that regulate the positioning and movement of immune cells. These are also known to be involved in many pleiotropic functions including activation of inflammatory responses, migration of leukocytes, and also in the regulation of tumor growth (Frederick and Clayman 2001; Mehraj et al. 2022a, b). These act by coupling to seven transmembrane protein receptors, known as G-protein coupled receptors (GPCRs). Around fifty known chemokines and twenty GPCRs have been investigated in humans (Frederick and Clayman 2001).

14.4 Cytokine Receptor

Cell-surface transmembrane glycoproteins called cytokine receptors often have many subunits and bind to cytokines selectively to transmit their signals. The cytokine receptors are classified into the following subtypes: hematopoietic cytokine receptor superfamily, immunoglobulin superfamily (IGSF), chemokine receptor superfamily, nerve growth factor receptor, the related Toll-like receptors (TLRs), IL-17 receptors, receptor tyrosine kinases, interferon family cytokine receptors, tumor necrosis factor (TNF) family receptors, interleukin (IL)-1 receptor, and the transforming growth factor-β (TGF-β) family receptor serine kinases. Many cell functions are regulated by members of the cytokine receptor superfamily (O'Shea et al. 2019).

14.4.1 Superfamily of Chemokine Receptors

Chemokine receptors belong to the seven transmembrane domain G-protein coupled rhodopsin superfamily. These regulate the migration of leukocytes, chemotaxis, and cell adhesion through binding to chemokine receptors. Twenty distinct chemokine receptors in humans have been found. Depending on the pattern of chemokine/ligand interaction that they bind, chemokines are grouped into four subfamilies, which include CC, CXC, CX3C, and XC (Frederick and Clayman 2001).

14.5 A Cytokine Network during the Development of a Metastatic Niche

There are multiple processes in the development of tumor cell growth, two of which are tumor cell detachment from the primary tumor and migration to the target organs through the lymphatic or blood circulatory systems and this is a process known as metastasis (Fares et al. 2020; Sofi et al. 2022). Organotropism refers to the predisposition of some tumors to metastasize to organs, and tumor cell homing is determined by the development of a favorable metastatic microenvironment (Fig. 14.3). A growing body of research suggests that primary tumors may be able to prime distant organs' microenvironments for metastatic colonization (Fares et al. 2020). Numerous investigations have shown that certain organs are prone to metastases in particular malignancies because a first-stage metastatic niche has developed in them (Joyce and Pollard 2009).

14.6 Cancer and Cytokine

Endothelial cells, stromal fibroblasts, tumor cells, infiltrating leukocytes like macrophages, T lymphocytes, and tumor-associated dendritic cells (TADC) are just a few of the types of cells that cancer cells recruit and reprogram to establish a

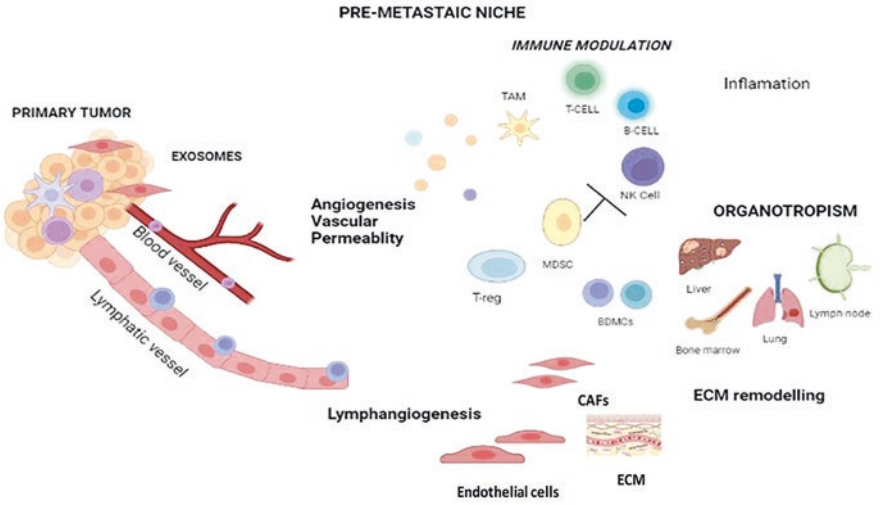

Fig. 14.3 Premetastatic niche formation: A role for cytokines and chemokines for ultimate spreading to additional organs and tissue locations, primary tumors provide a favorable microenvironment. In initial tumors, a lively interaction between TDSFs, BMDCs, regulatory/suppressive immune cells, and stromal components creates an efficient niche milieu for the proliferation and spread of the tumor cells and inflammation

tumor-friendly environment during the development of cancer. Additionally, some tumor forms contain granulocytes, eosinophils, B cells, and natural killer cells (Mehraj et al. 2021a, b, c).

The pathogenesis of cancer is significantly influenced by a variety of cytokines that are released by diverse cells in the immediate tumor environment (Sheu et al. 2008). Cancer formation and progression can be slowed down as a result of cytokines, which are produced in response to infection, inflammation, and immune responses. The following cytokines, namely IL2, 6, 8, 10, 12, 18, TNF, TGF, IFN, CXCR4, and macrophage migration inhibitory factor (MIF) have been discovered to be studied in cancer patients in accumulating clinical investigations (Lippitz 2013). We chiefly concentrate on how these cytokines work in cancer in this chapter.

14.6.1 IL-2 and Cancer

IL2, which is mostly produced by CD4+ T cells, is an antigen-stimulated T lymphocyte proliferation factor and oversees clonal proliferation of T cells in acquired immunity following antigen recognition. IL-2 is a crucial cytokine that affects the immune system in a variety of ways. Very low interleukin-2 concentrations or increased levels of soluble interleukin-2 receptors were linked to disease progression or a poor prognosis in cancer (Lippitz 2013). Numerous studies have shown that IL2 increases tumor development in melanoma cell lines in an autocrine manner (Ito et al. 1993). The cytokine receptor type I interleukin-2 mediates the

biological effects of IL-2 (Waldmann 1987). The three subunits and chains make up the trimeric IL-2 receptor complex. While the chain merely participates in cytokine binding and is involved in signaling. Multiple signaling pathways are activated when IL-2 binds to its receptor; the first signal transduction step is to draw the JAK1 and JAK3 to the cytoplasmic domains of IL-2R or IL-2R. STAT-1, 3, 5A, and STAT5B are recruited and phosphorylated by activated JAK kinases. The STAT signaling pathway, the MAPK, and the PI3K-AKT signaling route are the three important downstream signaling pathways that are active.

14.6.2 IL-6 and Cancer

Numerous tumors secrete IL-6, an incredibly pleiotropic cytokine that plays a crucial role in tumor promotion and inhibition of cancer. It is a significant component that is present in cancer in high amounts and is known to be dysregulated (Hong et al. 2007; Kumari et al. 2016). Nearly all varieties of tumors have been reported to overexpress it. The elevated levels of IL-6 in the tumor milieu, which regulate all characteristics of cancer and several signaling pathways, such as survival, apoptosis, proliferation, metastasis angiogenesis, and invasiveness, are indicative of the significant correlation between inflammation and cancer (Mehraj et al. 2022a, b). Additionally, through promoting repair and the production of counter-signaling (antioxidant) and prosurvival (antiapoptotic) pathways, cancer cells are protected by IL-6 against DNA damage, apoptosis, and oxidative stress brought on by treatment. Perhaps most significant is the robust STAT-3 activation by IL-6, a TF essential for interactions between a tumor and its environment, tumor evasion from immune control, and angiogenesis (Grivennikov and Karin 2008).

14.6.3 IL-8 in Cancer

Interleukin-8, sometimes referred to as CXCL-8, is a chemokine generated by several human cancer cells. TNF-, LIF, IL-1, IL-6, IL-8, or IFN-, all increase IL-8 mRNA, implying a crucial involvement in the development of malignancies. Additionally, it has been suggested that localized inflammation in the cancer milieu promotes the growth and spread of tumors. The immune regulatory function of interleukin-8 to cancer is demonstrated by a variety of human cancer models (Solomayer et al. 2000; Liang et al. 2007; Peng et al. 2007). Polymorphonuclear neutrophil (PMN) mobilization and activity are regulated by the chemokine IL-8. When melanoma cells are co-cultured with PMNs, IL-8 expression is increased. IB-degradation is induced by melanoma cells in PMNs, demonstrating that NF-Kb signaling is necessary for interleukin-8 release in PMNs.

14.6.4 Cancer and IL-10

Since tumor cells appear to regularly express IL-10, which has a high immunosuppressive effect, most human malignancies tend to have higher circulating serum concentrations of IL-10. IL-10's impact on the growth of tumors is complicated. Many studies claim that IL-10 prevents the growth and spread of tumors, whereas others show that it is pro-tumorigenic. Proinflammatory substances include TNF-, IL-12, and IL-6 cytokines that can be inhibited by IL-10 via blocking NF-κB activation (Hoentjen et al. 2005). Particularly interleukin-10 has been demonstrated to regulate cell death and inhibit vascularization during tumor regression (Stuelten et al. 2005). Mammary and ovarian cancer xenograft expression of IL-10 reduces tumor development and metastasis (Kohno et al. 2003). Major histocompatibility complex class I expression is downregulated by IL-10, which enhances NK cell-mediated tumor cell lysis and slows the growth of tumors. The fact that IL-10 inhibits the production of VEGF, TNF, and IL-6 may potentially be responsible for this effect on the tumor stroma (Huang et al. 1999; Sofi et al. 2023).

14.6.5 IL-12 and Cancer

The heterodimeric proinflammatory cytokine interleukin-12 is made of 35 kDa and 40 kDa subunits. For T cells and Natural killer cells to produce interferons, interleukin-12 is required. A physiological immune response should result in increased IL-12 expression. IL-12 expression seems to be downregulated in several cancer types, especially when the disease is more advanced and in its later stages, such as renal-cell cancer, glioblastoma, head and neck squamous-cell carcinoma, colorectal cancer, malignant melanoma, and others. Many cancer patients seem to be adversely affected by this step. In patients having gastric and colorectal cancer with advanced disease, stimulated peripheral blood mononuclear cells produce considerably less of the cytokine interleukin-12.

14.6.6 IL-18 and Cancer

Apart from IL-12, IL-18 is mostly produced by DCs and macrophages and has an impact on interferon production. Interleukin-18, a member of the interleukin-1 family of chemokines, is a cytokine that works as a receptor in target cells and shares structural similarities with this inflammatory mediator. The roles of interleukin-18, like IL-12, offer crucial physiological connections among cells and actions of innate and acquired immunity, promoting the formation of T-helper1 cells and memory CD8 T cells. In clinical trial models, IL-18 alone exhibited an anticancer effect, nonetheless, combos with IL-2 or IL-12 showed more promising potential. Intravenous IL-18 induced several minor reversible metabolic and hematological abnormalities as well as flu-like and inflammatory side effects in Phase I research in patients with advanced cancer (Li et al. 2005; Margolin 2008).

14.6.7 TNF-Alpha and Cancer

Tumor necrosis factor (TNF) is a multifunctional cytokine that is crucial for various cellular processes, viz cell survival, proliferation, cell death, and differentiation. Inflammatory cells release TNF, which may play a part in inflammation-related carcinogenesis. When it comes to cancer, TNF is a double dealer. It facilitates the growth, proliferation, invasion, and vascularization of cancer cells, indicating that it might serve as a natural tumor promoter. On the other side, TNF may be able to defeat cancer and cause cancer cells to die, making it a potential cancer treatment, albeit significant work needs to be done to lessen its toxicity for routine TNF dosing (Wang and Lin 2008).

14.6.8 TGF-Beta and Tumor

Growth factor TGF has multiple effects on target cells. It can stimulate or impede distinct cell proliferation and has significant control over how tissues and cells differentiate. TGF has two functions for cancer. On the one hand, TGF is crucial in controlling the onset and spread of cancer. TGF, on the other hand, switches functions in more advanced invasive tumors, where it promotes tumor formation, despite initially serving as a tumor suppressor in early carcinomas and normal cells (Neel et al. 2012).

14.6.9 Interferon-gamma and Cancer

NK cells, CD4+ T cells, and CD8+ T cells are the main secretors of IFN gamma, and it increases the upregulation of MHC class 1, and class II, and co-stimulatory molecules on APCs and activates macrophages. IFN's antitumor effects in murine models provided evidence that it might be useful against a variety of cancers. IFN has shown little clinical promise in the therapy of human cancer, although it may be essential for other cytokines' in vivo effects. TGF, prostaglandin E2, and other immunosuppressive substances are inhibited by IFN while IL-4, IL-10, and TGF operate as negative regulators of IFN production.

14.6.10 CXCR4 and Cancer

One of the most extensively researched chemokine receptors CXCR4 is also known to engage solely with CXCL12 and was previously identified as a co-receptor for HIV entrance. Recent investigations have revealed that around 23 different forms of human cancer have shown elevated CXCR4 expression. Overexpression of CXCR4 in cancer cells promotes tumor development, invasion, angiogenesis, metastasis, recurrence, and resistance to treatment. It has been demonstrated that CXCR4 antagonism impairs interactions in tumors and stroma, makes cancer cells

more susceptible to cytotoxic agents, and inhibits tumor expansion and metastatic burden (Chatterjee et al. 2014).

14.6.11 Cytokines in the Treatment of Cancer

Because of cytokines immune cells may interact with each other at short distances, which are significant controllers of innate as well as adaptive immunity. The use of cytokines to stimulate cancer patients' immune systems has long been a significant therapeutic approach and is still having a significant impact on clinical cancer research today. For the adjuvant therapy of high-risk melanoma patients who have undergone total resection as well as for some resistant cancers, interferon-alpha (IFN) is approved. Although melanoma and metastatic renal cell cancer can both be treated with high-dose interleukin-2 (HDIL-2), these treatments are currently less popular due to the discovery of newer drugs. The effects of GM-colony-stimulating factor, Interferon-gamma, interleukin-7, interleukin-12, and interleukin-21 were studied in clinical and in experimental trials. A combination trial using antitumor antibodies or checkpoint inhibitors (CPIs) has been started after the initial single-agent clinical studies using the eagerly anticipated interleukin-15 were completed. However, the efficacy of cytokines in monotherapy has not lived up to the promise of preclinical studies. They are frequently accompanied by significant toxicities that are dose limited and controllable with careful dosing. To overcome these challenges, cytokines are clinically studied with novel engineered cytokine mutants (superkines), anticancer vaccines, chimeric antibody-cytokine fusion proteins (immunokines), CPIs, and cancer-directed monoclonal antibodies to boost their cellular cytotoxicity induced by antibodies or maintain their cellular responses and effectiveness against malignancy. Here in this chapter, we give a general overview of the current level of knowledge and therapeutic applications of cytokines, either on their own or in conjunction with other biological agents. We place a strong emphasis on discussing potential future lines of inquiry into these cytokines to make them useful tools in the fight against metastatic cancer. Innate and adaptive immune system molecules called cytokines allow paracrine and autocrine immune system cell communication over short distances. There has been a lot of interest in using cytokines to treat cancer during the past few decades due to the immune system's ability to detect and destroy cancerous cells. Several murine cancer models have shown success in preclinical studies using IFN alpha, GM-CSF, interleukin-2, interleukin-12, interleukin-15, and interleukin-21 (Goldstein and Laszlo 1988; Dranoff 2004; Lee and Margolin 2011). Hairy cell leukemia (HCL) was the first type of human cancer to be treated with IFN in 1986. High-dose IL-2 (HDIL-2) was granted a license in 1992 for the treatment of metastatic renal cell carcinoma (mRCC) and metastatic melanoma (MM) following the study of several therapeutic options. The symptoms of adjuvant melanoma, follicular lymphoma, mRCC coupled with AIDS-related Kaposi's sarcoma, and bevacizumab have been added to IFN after its initial approval. However, cytokines used alone have not lived up to the original excitement they generated. The effectiveness of these immune therapeutics has been

restricted due to several issues. The immune response is not focused on a specific tumor by the introduction of cytokines but rather calls for the host to start an immunological response that the cytokine can then supplement. A further obstacle for cytokines like IL-2 is that the stimulation of immunological checkpoints such as programmed death protein-1 (PD-1, CD279), cytotoxic T-lymphocyte-associated 4 (CTLA-4), PD-1 ligands PD-L1 (CD274, B7-H1) and PD-L2 coincides with the beneficial effects of cytokines like IL-2 (CD272, B7-DC) (Sckisel et al. 2015). Although cytokines like IL-2 and IL-15 significantly boost the amount of natural killer (NK) cells that are activated. These effector cells are blocked by the interaction of killer-cell immunoglobulin (Ig)-like receptors (KIRs) and self-class I-A, B, major histocompatibility complex (MHC) molecules. Moreover, MHC self-class I-E with NKG2A, which is increased in response to immunological activation. Normal paracrine or autocrine action of soluble cytokines at short distances (Rochman et al. 2009). Acute renal failure, respiratory failure, hypotension, and neuropsychiatric symptoms are only a few of the systemic toxicities that can occur when cytokines are administered parenterally at larger dosages to obtain effective intratumoral concentrations. The ineffectiveness of cytokine monotherapy has also been addressed by the use of local or cavitary cytokine injection and the transformation of stimulating or effector cells with genes producing the cytokine by plasmid or viral delivery. Another innovative strategy is cytokine engineering using structure, which produces "superkines" with enhanced anticancer responses by an increased binding affinity for certain receptors and proportionally lessen Treg activation. When compared to native molecules, the tumor localization and pharmacokinetics of cytokines are improved by making chimeric antibodies and cytokine fusion proteins and by the infusion of anticytokines with cytokines. Additional cancer-specific monoclonal antibodies are injected with cytokines in clinical investigations, anticancer vaccines, and anti-CTLA-4 or anti-PD-1/PD-L1 checkpoint inhibitor (CPI) antibodies to increase these antibodies' anticancer effectiveness and antibody-dependent cellular cytotoxicity (ADCC) (Becker et al. 1996; Carter 2001; Schrama et al. 2006; Mir et al. 2023).

14.7 Interferons

Isaacs and Lindeman in 1957 first described interferons and categorized them into 3 types: type I (IFN alpha and IFN beta), type II (IFN gamma), and the more recently discovered type III. Different types of cells produce type I IFNs, a family of cytokines, as a result of immunological activation, virus-mediated infection, and some chemical stimulants. So far, 20 interferons have been investigated in humans (Goldstein and Laszlo 1988). Most of the subtypes fall into the IFN alpha category, although there are also two beta (I and II) and one T-type, IFN gamma subtypes. IFNAR1 and IFNAR2 are a pair of receptors that allow type I IFNs to signal (Müller et al. 1994). These Interferon receptors send signals that trigger the phosphorylation of STAT1 and STAT2 through the receptor-associated JAK1 and TYK2 proteins (Darnell Jr et al. 1994). IFN enhances NK cell cytotoxicity and survival, increases

MHC class I surface molecule levels, stimulates apoptosis, which is caspase dependent in some cancers, inhibits angiogenesis in tumors, polarizes immune responses toward Th1, causes the development and survival of memory CD8 T cells, and CTLs and accelerates dendritic cell (DC) maturation.

14.7.1 Application of IFN Alpha in Medicine

There are three isoforms of recombinant IFN formulations that are used in clinical settings (alfa-2a, alfa-2b, and alfa-2c). IFN and Peg interferon alpha 2b are approved as first-line therapies for patients with mRCC (alfa-2a and alfa-2b in combination with bevacizumab), AIDS-related Kaposi's sarcoma, follicular lymphoma, HCL (alfa-2a, alfa-2b), and chronic myelogenous leukemia (Philadelphia chromosome-positive alfa-2a) (Gutterman et al. 1980; Kirkwood and Ernstoff 1984). IFN has, however, frequently been supplanted by new drugs or drug combinations or otherwise relegated to second line and beyond treatments (Boyman and Sprent 2012; Wang et al. 2005).

14.7.2 IL-15 in the Treatment of Cancer

Interleukin-15 is a 14–15 kDa member of the 4-alpha-helical bundle family of cytokines. It is primarily produced by DCs, monocytes, and epithelial cells (Wang et al. 2005). The IL-15R is the component of the IL-15 receptor, which is specific to IL-15, the IL-2/IL-15R which is shared with IL-2, and the common gamma subunit c which is shared with IL-2, 4, 7, 9, and IL-21. Through JAK1, JAK3, STAT5, and, to a smaller degree, STAT3, this receptor transmits signals. Both interleukin-15 and interleukin-2 have roles in the immunological response while also making unique contributions (Fehniger et al. 2002; Steel et al. 2012). In several mouse models of neoplasia, IL-15 showed promise as a treatment (Dubois et al. 2008). It was discovered that in many instances, the antitumor impact was heavily reliant on the improvement of NK cell cytotoxicity and NKG2D-mediated activation of NK cells. Rhesus macaques were utilized to assess the IL-15's safety (Lugli et al. 2010). Recombinant human (rh) IL-15 produced in *E. coli* was administered to rhesus macaques on a daily regimen of 12 IV bolus infusions at doses of 10, 20, and 50 mcg/kg/d. The only biologically significant laboratory aberration was grade 3/4 transitory neutropenia. The amount of circulating NK, stem, central, and effector memory T cells increased 4- to 8-fold after a 12-day bolus of IV injection of 20 mcg/kg/day of IL-15 to rhesus macaques. The amount of circulating NK cells increased by about 10 times, while the proportion of effector memory CD8 T cells increased by 80–100 times, following a CIV injection of IL-15 at a dosage of 20 mcg/kg/day for 10 days (Sneller et al. 2011). After receiving subcutaneous infusions of 20 mcg/kg/day for ten days, it was found that the number of circulating effector memory CD8 T cells had increased by a more moderate factor of ten.

14.8 Approaches to Improve Cytokine-Based Therapy

Recent attempts have concentrated on creating novel cytokines and cytokine mutants, including "superkines," due to the drawbacks of cytokine monotherapy. With the emphasis on the immunotherapeutic cytokines interleukin-2, interleukin-4, interleukin-15, and interferon, designing cytokines depending on their structure has created new opportunities for the use of cytokines as drugs (Wang et al. 2005). The changes to interleukin-2 were made to lessen its contact with interleukin-2R alpha, which could decrease its stimulation of Tregs and interaction with IL-2R alpha in the blood vessels to lessen capillary leak syndrome and pulmonary edema. Interleukin-2 mutants F42K and R38A, which have modified interleukin-2R-binding domains, were created by Heaton and colleagues in early research. While having a much-reduced affinity for IL-2R, they nevertheless have an affinity for IL-2R and that is comparable to that of native IL-2. These IL-2 mutants prevented the high amounts of proinflammatory cytokines (IFN-γ, IL-1, and TNF-α) from being produced when LAK cells were triggered, which decreased the toxicities brought on by the spike in proinflammatory cytokines viewed with traditional interleukin-2 therapy at a high dose. Additionally, these IL-2 mutants avoided attaching to endothelial cells that expressed the IL-2R and blocked the VLS (Hu et al. 2003). Additionally, these mutants were less effective at encouraging the growth of Tregs.

The Laboratory named Garcia also created an IL-2 "superkine" with enhanced binding affinity for IL-2R beta, eliminating the functional necessity of IL-2 for IL-2R alpha production (Levin et al. 2012). The cytokine's core mostly underwent evolutionary alterations that made a helix in the IL-2R-binding region less flexible and improved the receptor binding confirmation so that it resembled that when bound to CD25. By causing STAT5 phosphorylation, the alterations in interleukin-2 superkine were analogous to CD25's functional role. With less Treg expansion and less pulmonary edema than IL-2, the superkine caused increased cytotoxic T-cell expression, which improved anticancer responses in murine models (Sofi et al. 2023).

The actions of interleukin-15 have been enhanced to be both agonistic as well as antagonistic inhibitory (Plet and Jacques 2006). When the extracellular region of IL-15R was fused to IL-15, it stabilized the development of the signaling complex, activating IL-15R-deficient cells similarly to how the IL-2 superkine activates cells without IL-2R. The proliferative and antiapoptotic properties of IL-15 were improved by the IL-15 fusion protein. Zhu and colleagues produced IL-15 agonists by stabilizing the IL-2R beta interface by raising the cytokine's affinity for the receptor (Zhu et al. 2009). IL-15 engineering has also focused on creating antagonists to block its immunostimulatory effects. The Q108 residue of IL-15 was found to be essential for yc interaction and deletion of this residue prevented cytokine-mediated proliferation (Pettit et al. 1997). Therefore, synthetic versions of IL-2 and IL-15 may be useful for treating a variety of immune regulation-related conditions.

DNA shuffling has been used to try to increase IFN antiviral activity. DNA sequences from every IFN-alpha subtype were joined to create a shuffled library, which was then tested for an antiproliferative and antiviral activity to determine

whether it had any functional purpose (Stemmer 1994). With a 20 to 70-fold increase in antiviral potency relative to IFN-alpha2, this screening discovered 2 shuffled proteins namely B9X25 and B9X14. In comparison to wild-type IFN-alpha2, the shuffled proteins' affinities for the IFNAR complex increased 9 to 100-fold, respectively. Unfortunately, the immunogenicity caused by the multiple mutations detected in the rearranged Interferon products, which produced additional potential T cell epitopes, prevented this shuffled IFN from being developed further for clinical use.

14.9 Cytokine Network, Breast Cancer Stem Cells, and Tumor Microenvironment

A fraction of cells that exhibit stem cell characteristics, facilitate metastasis, and contribute to treatment resistance is responsible for maintaining many malignancies, including breast cancer (Mehraj et al. 2021a, b, c). Through complex interactions with the elements of the tumor microenvironment, such as mesenchymal stem cells, adipocytes, tumor-related fibroblasts, endothelial cells, and immune cells, these cancers stem cells (CSCs) are controlled (Mehraj et al. 2021a, b, c). These elements make intriguing treatment targets since they have a direct impact on CSC characteristics. The genetic defects that cause and fuel cancer have been the subject of extensive investigation, but there is now convincing proof that the microenvironment also has a significant impact on how tumorigenic cells behave. Decades of research have led to the conclusion that metastasis and growth of tumors are driven and promoted by the interaction of many cell types within these organ-like structures. Some stem cells in normal tissues can self-renew and differentiate into a variety of lineages. A similar hierarchy appears to control various malignancies, including breast cancer, as the body's evidence for this point grows (Mir et al. 2022). Cancer stem cells (CSCs) are operationally defined by their capacity to develop tumors in immunocompromised mice upon serial passage, a proof of self-renewal, as well as their capacity to specialize into the nonself-renewing cells composing the tumor bulk (Al-Hajj et al. 2003; Korkaya et al. 2008). CSCs are controlled by, and in turn control, cells in the tumor microenvironment, like how regular stem cells are controlled by their "niche." Through networks of growth factors and cytokines, these cells, which include mesenchymal stem cells (MSCs), tissue-associated fibroblasts, and endothelial cells, communicate with CSCs. Additionally, immunomodulatory cells like T cells and macrophages can stimulate and inhibit CSCs (Mir and Mir 2022). New therapeutic targets are made possible by the interplay between CSCs and their microenvironment. The effective targeting of this cell type is essential because CSCs may contribute to treatment resistance and relapse because of their relative resistance to chemotherapy and radiation therapy (Mir et al. 2020; Wicha et al. 2006; Li et al. 2008). An important method of enhancing patient outcomes is to employ strategies that particularly target the relationship between the CSC and its microenvironment. Although tumor heterogeneity has long been understood, it has lately been clear that tumor cells can differentiate into hierarchical cellular

structures. Human acute myelogenous leukemia (AML) has a cellular hierarchy that resembles the hierarchy found in healthy haematopoiesis, according to a study conducted in 1997 by Bonnet and Dick (1997). Leukemic stem cells are recognized at the top of this hierarchy due to their expression of stem cell markers, particularly that they are CD34+/CD38−. Leukemic cells (which arise from a pool of leukemic stem cells) result in leukemia in immunosuppressed mice but not from the majority of leukemic blast cells (Mehraj et al. 2021c; Bonnet and Dick 1997). Recent studies have revealed that a person's leukemia may be driven by numerous clones of leukemia stem cells and that the majority of CD34+ AMLs develop from progenitor cells rather than hematopoietic stem cells (Qian et al. 2002). These and other recent findings indicate that CSC heterogeneity may be higher than previously thought (Taussig et al. 2008). Subsequently, similar CSCs were found in a variety of human solid tumors, including those of the breast, brain, colon, prostate, and pancreas (Singh et al. 2003; Li et al. 2007). Due to their relative resilience to radiation and chemotherapy, further investigations have shown that these CSCs drive tumor spread and contribute to relapse after therapy. This emphasizes how crucial it is to comprehend the processes that control this cell population. CSCs, like their normal counterparts, are controlled by intrinsic and extrinsic signals that originate in the tumor microenvironment (Vera-Ramirez et al. 2010), and changes in epigenetic status and genetic expression that take place during carcinogenesis affect these regulatory signals (Baylin and Herman 2000; Jaenisch and Bird 2003). The Notch, Hedgehog, Wnt, PI3K, NF-κB, and Jak/STAT pathways are among the stem cell regulatory pathways that are typically dysregulated in tumor cells (Korkaya et al. 2009; Iliopoulos et al. 2010). These pathways like in breast cancer are sometimes dysregulated by signals from the tumor microenvironment, but in some malignancies, essential regulatory components are mutated to activate them (Ma et al. 2009). There is growing evidence that the microenvironment's various biological components, including the tumor cell hierarchy, co-evolve during the carcinogenesis process (Polyak et al. 2009). Bidirectional paracrine signals govern CSCs and other tumor-causing cell types (Karnoub et al. 2007; Liu et al. 2011). In turn, tumorigenic cells produce substances that draw and control the wide range of cell types that make up the tumor microenvironment. It is interesting to note that many of the pathways involved in tumor growth are also involved in normal wound healing, including the transcription factor NF-κB and cytokine loops. Additionally, immunomodulatory cells influence the development of tumors both positively and negatively (Mantovani 2009; Yu et al. 2009). In this section, we will go over the connections between cancer and inflammation with a focus on cytokine networks and NF-κB signaling. We will also go over how these methods to control the CSC population may be impacted by the various varieties of cell types in the tumor microenvironment. The elucidation of these networks is crucial for clinical purposes since it could lead to the discovery of new therapeutic targets and the creation of methods for specifically targeting BCSC (breast cancer stem cell) populations.

14.10 Cytokine Networks as Important Regulators in Breast Cancer Stem Cells

Virchow originally hypothesized the connection between inflammation and cancer in 1864 after noticing that inflammatory cells regularly infiltrated the tumor stroma (Balkwill and Mantovani 2001). There is a lot of clinical data linking inflammatory conditions to the development of cancer, such as the correlations between chronic pancreatitis, ulcerative colitis, and malignancies of the colon, liver, and pancreas, respectively (Balkwill and Mantovani 2001). Women's chance of developing breast cancer again following initial therapy relates to levels of chronic inflammation as measured by blood C-reactive protein or amyloid. Expression of these markers indicates chronic inflammation, which may be mediated by cytokines including IL-1, IL-6, and IL-8 (Coussens and Werb 2002). Individuals with certain cytokine gene polymorphisms are more likely to develop cancer (Michaud et al. 2006) (Table 14.1).

The growth and spread of tumors may be aided by these inflammatory cytokines, stimulation of CSC self-renewal. IL-6 and IL-8 have been linked to both persistent inflammation and the development of tumors (Mehraj et al. 2021a; Scheller and Rose-John 2006; Waugh and Wilson 2008). Numerous cell types, including mesenchymal cells, macrophages, and immune cells, release IL-6 and IL-8 in the tumor microenvironment. Additionally, both cytokines serum levels have been linked to a bad prognosis for breast cancer patients (Benoy et al. 2004; Yao et al. 2007). IL-6 has been demonstrated to encourage tumorigenicity, angiogenesis, and metastasis in some preclinical models (Ara and DeClerck 2010). The established link between serum IL-6 levels and a worse patient outcome in breast cancer patients supports the clinical importance of this research. BCSC self-renewal has been demonstrated to be directly regulated by IL-6, and this process is mediated via the IL-6 receptor/GP130 complex by activating STAT3 (Iliopoulos et al. 2009). Recently, it was shown that gradients of IL-6 attract bone marrow-derived MSCs to areas of developing breast tumors using mouse xenografts (Liu et al. 2011). These MSCs and BCSCs participate in a positive feedback loop, in which IL-6 is a crucial part. Additionally, Sethi et al. recently showed that IL-6-mediated Jagged1/Notch signaling encourages bone metastases in breast cancer (Sethi et al. 2011). According to this research, IL-6 and its receptor provide promising therapeutic targets. It was recently discovered that the IL-8 receptor CXCR1 was highly expressed in BCSCs using gene expression profiling and that IL-8 was able to encourage self-renewal (Ginestier et al. 2010). By drastically reducing the number of BCSCs, blocking this receptor in mice xenografts reduced tumorigenicity and metastasis. The NF-κB

Table 14.1 Cytokines produced by tumor microenvironment cells

Cell types	Factors	Pathways activated
Mesenchymal cells	CCL5, IL6, CXC5, IL8	P13K/AKT
Fibroblasts	TGF-beta, CXCL12, FGF, IGF, PDGF	P13K/AKT
Endothelial cells	VEGF	MAPK
Immune cells	IL8, IL6	P13/AKT, STAT3

signaling system controls the synthesis of inflammatory cytokines including IL-6 and IL-8. The NF-κB pathway is essential for the development of both inflammation and cancer. The five transcription factors that make up the NF-κB family are p50, p52, RelA (p65), c-Rel, and RelB (Pn 2005; Hoffmann et al. 2006). NF-κB proteins are mostly found in the cytoplasm of resting cells, where they are linked to the IB family. When NF-κB is activated by various signals, IB is degraded by ubiquitin ligase, and NF-κB protein complexes are translocated to the nucleus. NF-κB stimulates the production of several cytokines, including IL-6 and IL-8 (Barnes and Karin 1997). Additionally, it has recently been demonstrated that a positive feedback loop keeps tumor cells in a chronic inflammatory state. It is interesting to note that this loop includes the microRNA let7 and Lin28, a component of embryonic stem cell self-renewal (Iliopoulos et al. 2009). By activating STAT3, which in turn activates NF-κB and its downstream targets Lin28 and let7, IL-6 maintains this feedback loop (Iliopoulos et al. 2009). Both the development of cancer and normal breast physiology may be significantly influenced by NF-κB. Suppression of NF-κB in mammary epithelium decreased the breast stem cell compartment in a HER2-neu model of mammary carcinogenesis, delaying the onset of HER2-neu-induced tumors and reducing angiogenesis and macrophage infiltration (Liu et al. 2010). Additionally, NF-κB is connected to the control of mouse mammary stem cells during pregnancy. Differentiated breast epithelial cells produce RANKL in response to elevated progesterone levels during pregnancy. Through the activation of NF-κB in these cells, RANKL in turn promotes breast stem cell self-renewal (Asselin-Labat et al. 2010; Joshi et al. 2010). The activation of comparable mechanisms in BCSCs may contribute to the higher prevalence of aggressive breast cancers linked to pregnancy (Sofi et al. 2022; Peck et al. 2002). Due to the recognized association between obesity and inflammation, epidemiologic studies show a considerable rise in postmenopausal breast cancer among those who are obese. Proinflammatory cytokines, such as TNF-, IL-6, and C-reactive protein, circulate at higher levels in patients with type 2 diabetes mellitus (Wellen and Hotamisligil 2005). Additionally, the activation of NF-κB has been associated to these increased levels of proinflammatory cytokines (Cai et al. 2005). The diabetes medication metformin lowers blood glucose levels, improves insulin sensitivity, and lessens hyperinsulinemia brought on by insulin resistance. Interestingly, metformin has been demonstrated to specifically inhibit BCSC self-renewal in preclinical mice models, lowering the proliferation of these cells in breast tumors.

Breast cancer is one solid tumor that is made up of diverse cell populations that interact in intricate networks. Tumor cells interact with these biological components, which in turn control the hierarchy of tumor-genic cells, just as they do in developing organs. At metastatic sites, metastatic tumor cells also establish intricate cellular microenvironments. Paget put up the "seed and soil" theory of tumor spreading more than 120 years ago (Fidler 2003). In a more contemporary setting, the CSCs are the seeds, and the rich microenvironment is the network of different cell types that interacts with the tumor cells via networks of growth factors and cytokines. These networks control the CSCs and the offspring that make up the majority of tumors. Understanding these pathways may reveal fresh targets for drug

development. Some of these chemicals and pathways, such as the cytokines IL-6 and IL-8 and their receptors, IL-6R and CXCR1, have already been the subject of research efforts. In mouse breast cancer xenograft models, blockade of these mechanisms decreases BCSC growth (Ginestier et al. 2010). In these models, the tumor development and metastasis are decreased as a result. Additionally, NF-κB is a desirable target. Early-stage clinical trials for the treatment of leukemia are being conducted utilizing the NF-κB inhibitor parthenolide (Hassane et al. 2010) due to encouraging in vitro and preclinical findings. These studies will ascertain whether anticancer treatments are more effective when CSCs and their microenvironment are simultaneously targeted.

14.11 Current Use of Chemokines in Cancer Therapy

Given the role of chemokine expression in the development, invasion, and metastasis of cancer, it is crucial to produce inhibitors of these chemokines or their related receptors. Recently, research studies conducted have utilized a variety of chemokine and chemokine receptor inhibitors and have demonstrated success in curing solid tissue cancer in preclinical models. A huge number of studies are now moving forward with clinical trials for patient care. Targeting CXCL12 and its receptor CXCR4 has been found to control cancer cell homing to distant regions, best illustrated in breast cancer, is one promising approach in the treatment of metastatic illness in solid tumors (Mir et al. 2020; Müller et al. 2001). AMD3100, one of the substances that have received the most research, is expected to particularly inhibit CXCR4 signaling (Burger et al. 1999). This substance has demonstrated effectiveness in murine models by delaying the spread of mammary carcinoma cells to the lungs (Smith et al. 2004), reducing the spread of ovarian cancer cells (Kajiyama et al. 2008), and slowing the growth of gastric cancer tumors (Yasumoto et al. 2006). While AMD3100's mode of action is still unknown, it has been demonstrated to prevent CXCL12-stimulated breast cancer (Cabioglu et al. 2005), ovarian cancer (Scotton et al. 2002), and gastric cancer cell migration (Ohira et al. 2006) as well as lessen the invasiveness of prostate cancer cells (PC3 cell line) (Zhang et al. 2008). In vitro tests on this substance have also been conducted on a variety of cell types, including pancreatic, colorectal, osteosarcoma, and malignant melanoma (Burger and Peled 2009). These studies show that AMD3100 slows down the growth of tumors in part by preventing the proliferation, migration, and invasion of tumor epithelial cells. AMD3100 has been proven in recent experiments to mobilize hematopoietic cells into the peripheral circulation (Azab et al. 2009). By reducing their affiliation with the bone marrow, the enhanced mobilization makes leukemia and myeloma cells more susceptible to chemotherapeutic intervention (Nervi et al. 2009). Thus, the potential advantages of AMD3100 are currently being examined in patients with blood malignancies who are also receiving other chemotherapies (Cashen et al. 2008). AMD3100's impact on hematopoietic cells in solid tumors is still unknown, though further research should be done on the functional effects of AMD3100 on the tumor microenvironment given the role hematopoietic cells play

in the development of solid tumors (Pollard 2004). Other CXCL12/CXCR4 pathway inhibitors have demonstrated encouraging outcomes in preclinical models and early clinical studies. Early clinical trials of the peptide analog of CXCL12 and active ligand inhibitor CTCE-9908, which stabilized illness in late-stage cancer patients, showed promising outcomes (Hotte et al. 2007; Evans and Krolicki 2008). This compound has been utilised in preclinical research to treat osteogenic sarcoma in mice and has been observed to reduce osteosarcoma cell proliferation, adhesion, migration, and invasion (Kim et al. 2008). Treatment with CTCE-9908 reduced the frequency of gross metastatic lung nodules by 50% and significantly decreased micro-metastatic disease when these cells were afterwards injected into the tail veins of mice. Melanoma cells have also demonstrated comparable outcomes, but only when the cells were pre-treated with the inhibitor prior to injection (Qayoom et al. 2023a; Kim et al. 2008). While AMD3100 inhibits CXCR4 and CTCE-9908 targets CXCL12 specifically. Both drugs prevented the metastatic spread of different tumors in preclinical animals, highlighting the significance of CXCL12/CXCR4 signaling in metastatic illness.

CCL5/CCR5 is another chemokine receptor/ligand combination that has been investigated in the development and spread of cancer. This combination of proteins, like CXCL12/CXCR4, has been connected to the development and spread of numerous malignancies, including breast cancer and multiple myeloma. According to one study, mesenchymal stem cells increased MDA-MB-231 breast cancer cells in vitro by blocking CCL5 signaling with monoclonal antibodies to CCR5. Additionally, systemic administration of anti-CCR5 to animals with tumors prevented the metastatic dissemination of MDA-MB-231 cells (Karnoub et al. 2007). These results show that CCR5 antagonists greatly prevent interactions between mesenchymal stem cells and tumor epithelial cells throughout the development of tumors. In other investigations, daily systemic administration of a functional antagonist to CCL5 (met-RANTES or met-CCL5) reduced macrophage infiltration into the tumor and inhibited the development of 410.4 breast cancer cells transplanted into mice (Robinson et al. 2003). However, Met-CCL5 was unable to lessen the infiltration of additional immune cells, such as neutrophils, which have previously been demonstrated to promote tumor growth in breast cancer (Robinson et al. 2003; Queen et al. 2005). Another option is that alternative CCR5-binding ligands could make up for the absence of CCL5 activity. These cells may express additional chemokine or cytokine receptors that could react to additional ligands in the tumor microenvironment. Therefore, it is probable that redundant chemokine receptor signaling will lessen the effectiveness of these chemokine antagonists in treating cancer. Progress was lacking in Phase IIA clinical trials for the treatment of rheumatoid arthritis (Vergunst et al. 2008). These data suggest that one potential strategy for treating metastatic cancer involves decreasing stromal: epithelial interactions mediated by CCL5. According to Vaday et al. (2006), TAK-779, another CCR5 antagonist, was discovered to block CCL5-induced prostate cancer cell invasion in a concentration-dependent way (Vaday et al. 2006). This finding suggests that CCR5 antagonists also have an impact on autocrine signaling in cancer cells. It is yet unknown how this molecule works and whether it is effective against other tumors in in vitro and

in vivo trials. Together, these data suggest that the CCL5/CCR5 constitutes yet another important chemokine signaling route that cancer treatments may wish to focus on.

Other chemokine antagonists have additionally demonstrated promise for use in cancer therapy. For instance, it has been demonstrated that the CXCR2-selective antagonist AZ10397767 reduces the ability of androgen-independent prostate cancer cells to withstand oxaliplatin via an NF-κB-dependent mechanism (Mehraj et al. 2021a; Wilson et al. 2008). The capacity of AZ10397767 to prevent TRAIL and IL-8-induced elevation of c-FLIP is most likely what caused the co-administration of this inhibitor with TRAIL to improve the sensitivity of PC3 cells (Wilson et al. 2008). These studies suggest that using chemokine antagonists in conjunction with other treatments may have some advantages. Recent investigations have discovered that pharmacologic inhibitors used to target other molecules also serve as chemokine inhibitors, in addition to those that have been specially created to target chemokines and their receptors. For instance, it has been demonstrated that patients with metastatic breast cancer who take the TNF-α inhibitor Etanercept (Enbrel), which is often used to treat arthritis, had much lower systemic levels of CCL2 (Madhusudan et al. 2004). Additionally, the endometrial cancer cell line (EFE184) has been shown to express less CCL2 mRNA and protein when tamoxifen is administered (Wang et al. 2006). These results show that treatments meant to focus on particular molecules also subtly influence the expression of other molecules. CCL2 and numerous other chemokines have been demonstrated to play tumor-promoting roles in various cancers. These undesired side effects may be utilized in the treatment of cancer (Table 14.2).

It is feasible that existing medication therapies intended to treat inflammatory conditions or certain tumors could act as chemokine signaling inhibitors and can be used in the treatment of other cancers. This course of treatment, however, needs more research and comprehension of how conventional medicines impact chemokine expression and function at the molecular and cellular levels. In conclusion, even though few chemokine antagonists have advanced to clinical trials, chemokine antagonist development for cancer treatment is still a viable field to research.

Table 14.2 Various chemokine targets in several cancers

Chemokine target	Agonist	Cancer	Clinical trials
CCR2	MLN1202	Breast cancer	Preclinical
CXCR12	AMD3100	Leukemia and lymphoma	Phase2
CXCL12	CTCE-9908	Late-stage ovarian, gastric, colorectal	Phase1/2
CXCR2	AZ10397767 SB225002	Prostate, Oesophageal	Preclinical
1CCR5	TAK-779	Multiple myeloma, prostrate	Preclinical
CCL5	Met-CCL5	Breast cancer	Preclinical

14.12 Future Role of Chemokines

During tumor progression, chemokine signaling controls a variety of activities, including primary tumor growth, tumor angiogenesis, and metastatic spread. According to recent research, chemokines control tumor growth and invasion via mediating stromal: epithelial interactions in the primary tumor microenvironment. Additionally, research shows that chemokines control how cancer cells with chemokine receptors homing to distant regions during metastatic illness. Cancer stage grade and patient survival studies have shown that chemokine expression patterns in the cancer stroma may be a good indicator of the host's reactions to the tumor. Chemokine expression has been linked to immune cells in some malignancies, which might indicate a good or worse prognosis (depending on the tissue type) (Qayoom et al. 2023c; Kleine-Lowinski et al. 1999). The soluble substances present in the tumor microenvironment play a role in how well immune cells can create an antitumor response (Lewis and Pollard 2006). Chemokine production in cancer may therefore have a variety of effects on the development of the disease by controlling the recruitment of immune cells to the primary tumor. The functional effects of chemokine signaling on the recruitment of immune cells in cancer should be further studied; however, the substantial connections between the production of chemokines in the stroma of cancer cells and tumor malignancy point to potential use for chemokines as prognostic indicators. Research on mice models of invasive cancer has demonstrated that chemokine antagonists are successful in treating the disease. There are several problems with the therapeutic targeting of chemokines that need to be resolved and completely understood before they can be used to treat cancer. Delivery of chemotherapeutic medications that target certain molecules always takes the likelihood of harmful consequences in normal tissues into account (Chari 2008; Wysocki et al. 2008). Given that chemokine and its receptors are expressed in both healthy and malignant tissues, this problem is particularly crucial to consider. Chemokine antagonists' ability to precisely target cancer tissues may be made more difficult by the promiscuous binding of ligands to a variety of receptors. While CCL2/CCR2 signaling demonstrates that some chemokine ligand/receptor combinations have distinct roles, the prospect of duplicate signaling may make the use of chemokine antagonists in cancer even more challenging.

14.13 Conclusion

Cytokines are significant immunological regulators, and cytokine-based medicines present a wide range of therapeutic options. Since the cDNA sequences for the majority of cytokines are publicly available, large quantities of cytokines can be easily synthesized utilizing eukaryotic or prokaryotic expression systems, which makes cytokines appealing for novel medication development. However, statistical findings show that many clinical studies with cytokine-based medications failed to provide published results, primarily due to their poor efficacy, severe side effects, and antagonistic immunoregulatory activities. In animal studies, these issues can be

partially resolved by administering viral oncolytic agents or nanoparticles, alternatively by the combo of immunotherapies, chemotherapy medications, or all in a clinical setting. Future clinical trials would employ and enhance such tactics. Clarifying the immune-regulatory mechanisms of cytokines can also increase the efficiency and security of those substances in curing cancer.

The proportion among rejection of tumors via suppressor mechanisms and effector cells that are specific for the antigen that enable tumors to evade the defense mechanism is regulated by many cytokines, which are involved in antitumor-specific immunity. In the treatment of various human tumors in clinical settings and mice models, cytokines have proven to have therapeutic anticancer effects. The use of IFN and interleukin-2 has also been suggested for the treatment of certain cancers. However, cytokines have not lived up to their early promise to take center stage in cancer immunotherapy. To increase the activity of cytokines, however, numerous novel ways are being created while considering our understanding of the immune response's regulation mechanisms, which have been demonstrated in animal studies. In recent years, genetic engineering has altered the make-up of IL-2 to produce IL-2-like "superkines" with altered IL-2R alpha binding domains. These altered "superkines" have increased affinity for the IL-2Rbeta gamma complex while significantly decreasing binding affinity to IL-2Ralpha, increasing action on effector T cells, and less attachment with Tregs. Additionally, in preclinical experiments, pulmonary edema was decreased due to the absence of interaction with IL-2R. IL-2, for example, pharmacokinetics and effectiveness have been enhanced by the use of specific anticytokine antibodies in conjunction with the cytokine and it's PEGylation. Antibody–cytokine fusion proteins were an additional strategy to increase the specificity of activity and the effectiveness of the action. Clinical trials are evaluating antibody-interleukin-2 fusion proteins that contain interleukin-2, interleukin-12, interleukin-21, tumor necrosis factor, and IFNs to direct cytokines selectively to tumor sites where they trigger an anticancer response without causing systemic damage. To improve tumor targeting, these conjugates contain anti-DNA and antiphosphatidylserine. Yet in other attempts, cytokines have been combined with substances that relax immune system checkpoints. Studies are being started, for instance, using the simultaneous inclusion of anti-CTLA-4 and anti-PD-L1 with IL-15 to produce a combination that showed extended animal survival in murine models. The number and level of activation of NK cells are greatly increased by cytokines like IL-2 and IL-15 in particular. However, the binding of an inhibitory KIR or NKG2A receptor with self-class MAC A or B or E, respectively, which are expressed on the tumor cells, prevents the action of these cells. To overcome this obstacle, tumor-directed monoclonal antibodies are co-administered with IL-15 to increase the natural killer cell mediated-antibody dependent cellular cytotoxicity of these anticancer monoclonal antibodies. Gamma cytokines also cause the production of CIS and SOCS3, which results in insufficient CD4 support and the formation of "helpless" CD8 T cells, further preventing their utilization. Insufficient CD4 cells can be replaced by antagonistic anti-CD40 monoclonal antibodies or CD40 ligands, which promote the development of antigen-specific CD8 cytotoxic T cells. A method that is being applied in clinical trials increased tumor-specific CD8 T cells

and antitumor activity in mice models by combining an agonistic anti-CD40 monoclonal antibody with IL-15. With these cutting-edge combination strategies, it is thought that cytokines will eventually play an important role in cancer immunotherapy.

Further Reading

For further readings, some textbooks mentioned below also gives further understanding of cytokine and chemokine network.

- Cytokines and their therapeutic potential by Manzoor Ahmad Mir.
- The Role of Chemoattractants in the Tumor Microenvironment by Giovanni Bernardini, Brian A. Zabel.

For more incites about the topic we would suggest detailed findings from the books of (Mir et al. 2022) https://doi.org/10.1016/C2021-0-02565-7, https://doi.org/10.1016/C2022-0-00074-X and (Mir 2021) https://doi.org/10.52305/WXJL6770 (Mir 2015) https://doi.org/10.1016/C2014-0-02898-5 and from the cancer.net website on the following mentioned below links:

https://www.cancer.net/cancer-types/breast-cancer/types-treatment

https://discovery.ucl.ac.uk/id/eprint/1472740/

https://www.jmedsciences.com/doi/JMEDS/pdf/10.5005/jp-journals-10045-00138

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Chemokines in Cancer Therapy

15

Manzoor Ahmad Mir and Iqra Noor

Abstract

Chemokines are small cell signalling proteins corresponding to family of cytokines that aid in movement of leukocytes. They are low-molecular-weight proteins (8-10KDa), usually having four cysteine residues, categorised into four families, viz., CC, CXC, CX3C, and C. Initially, it was believed that they have role in immune cell infiltration, but they help in migration of non-immune cells also and that could be a tumor cell hence cause tumor progression and metastasis. Also, they have direct role in cell proliferation. So, there is a balance between tumor induction and tumor inhibition. It is becoming more and more clear that chemokines have dual functions during tumor development, acting to both suppress and promote tumor growth. Therefore, for the development of effective chemokine therapies against cancer, several pre-clinical research and clinical trials have been done to target chemokines in treating cancer.

Keywords

Chemotactic cytokines · Metastasis · Chemokine receptor · Tumor microenvironment · Targeted therapy

15.1 Introduction

Chemokines are intercellular messengers that cause migration of leukocytes. The chemokine expression over time and space closely control the guided migration of cells (Griffith et al. 2014). Twenty chemokine receptors and roughly fifty

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chemokines are present. The receptors are GPCR receptors; however, some non-GPCR receptors are also there commonly called atypical chemokine receptors. Four families make up the chemokine classification, namely CC, CXC, CX3C, and C, according to the position of first two cysteine repeats at amino terminus of a polypeptide, discussed later in the chapter. Chemokines have direct role in establishing tumor microenvironment. Differential chemokine receptor expression on leukocytes causes chosen cell-type recruitment under distinct circumstances, resulting in suitable and effective immunological responses catered to the invading pathogen or external stimulus. They are now understood to have significant function in the maturation of the cells of the immunity, their coordinated migration to the site of inflammation, the formation of lymphoid tissues, and the production and delivery of adaptive immunological responses (Griffith et al. 2014). A vast spectrum of human disorders, including autoimmune and inflammatory conditions, cancer, are thought to be caused by dysregulated production of chemokines and their related receptors (Balkwill 2004; Charo and Ransohoff 2006). More and more, it is understood that tumors are complex microenvironments composed of several kinds of cells that coexist and interact with one another through a convoluted signalling network. It is now becoming more widely acknowledged that understanding the role of every kind of tumor cell and how each kind of cell types communicate and interact within the tumor microenvironment (TME)is necessary to completely comprehend cancer. Understanding how chemokines and cytokines, which are exchanged among tumor cells, affect the immune system and metastasis is part of this. Chemokines have a significant impact on cell migration and cell–cell communication, which has a substantial effect on the formation of tumours (Qayoom et al. 2023a). The release of a range of unique chemokines by tumor cells and tumor-associated host cells in the tumor milieu leads to the recruitment and activation of many cell types that mediate the balance between tumor promoting and anti-tumor responses. Chemokines are involved in number of different processes that have a connection to tumor, including angiogenesis, metastasis, and tumor cell proliferation, additionally to their fundamental function as chemo attractants (Balkwill 2004). From the past four decades, cytokines and their corresponding receptors have been thoroughly examined as potential targets for cancer as well as for cancer therapies. Therapeutic approaches that increase both growth resistance and immunostimulatory properties of interferons and interleukins, such as IL-2, 7, 12, and IL-15, or that reduce the inflammatory and cytokines' tumor-promoting effects like TNF, IL-1, and Interleukin-6 are supported by a strong pre-clinical rationale. The fact that altered and dysregulated expression of cytokines has been found in every kind of human cancer supports this theory. As a result of these discoveries, many cytokines or cytokine antagonists were tested in clinical studies, which showed pertinent biological activity but rather little therapeutic effectiveness. Although, most of the experiments conducted included individuals with progressed disease condition, which may not be the best circumstance for cytokine-based therapy. New strategies for using cytokine networks in curing cancer have emerged as a result of the greater immunotherapy efficacy and a deeper comprehension of the tumor microenvironment. These strategies incorporate the application of cytokine-based therapies to boost the action or lessen

the immune linked side effects of other treatments and to target early-stage cancers (Qayoom et al. 2023b). There are still more issues to be resolved, particularly regarding delivery mechanisms, context requirements, and the pleiotropic, redundant, and frequently competing activities of several cytokines.

15.2 Chemokines

Chemokines pertain to a category of intercellular mediators or signalling molecules called cytokines, having molecular weight of about 8–10 kilodaltons. They help in localization of leukocytes towards the site of infection. They are also called chemotactic cytokines. They have a basic secondary structure of proteins with alpha helices, beta sheets, and loops and usually have 4 cysteine residues that form inter disulphide bonds at amino terminus. There are about 50 different chemokines with twenty receptors (Zlotnik and Yoshie 2000). They can be broadly divided into four divisions CC, CXC, CX3C, and XC. Although they were first designated for particular roles, a systematic nomenclature that consists of a subfamily designation (e.g., CC, CXC, CX3C, or XC), followed by the letter L (denoting "ligand") and then a number based on when the gene was first discovered, was developed in 2000 (Murphy et al. 2000). An amino terminal signal peptide is present in the production of all chemokines, but it is eliminated once the chemokine has been guided to the endoplasmic reticulum for secretion. Two chemokines, CX3CL1 and CXCL16, possess a long C terminal that includes a transmembrane domain and a stalk that resembles a mucin (Matloubian et al. 2000). These chemokines are held by this, although it can be broken by proteolysis to free the chemokine component into the extracellular environment (Garton et al. 2001). The prolonged N terminus of other chemokines, such as CCL6, 9, and CCL23, can be cut-off via proteolysis to improve receptor activation abilities (Berahovich et al. 2005). Formyl peptide receptor-like 1 (FPRL1), a G protein-coupled receptor that is not categorised as a cCKR 20, can be activated by an N-terminal peptide that has been cleaved off a CCL23 variant. Various chemokine variations can result from alternatively spliced transcripts. For instance, six different human CXCL12 isoforms have been identified, each with a unique C terminus (Yu et al. 2006) and unique biological characteristics (Fig. 15.1) (Janssens et al. 2018).

15.2.1 CC Chemokine

The CC chemokine's amino terminus contains two contiguous cysteine repeats. For mammals, at least 27 different CC chemokine ligands—contributors to this group—have been mentioned, CCL 1 to CCL 28. CCL 10 and CCL 9 are equivalent. Most members of this family have 4 cysteine residues (C4-CC), although a little number also have six (C6-CC). C6-CC CCL 1, CCL 15, CCL 21, CCL 23, and CCL 28 are examples of chemokines. Monocytes and other cells like NK cells move around due to CC chemokines. Monocyte chemoattractant proteins MCP-1 and CCL2, which

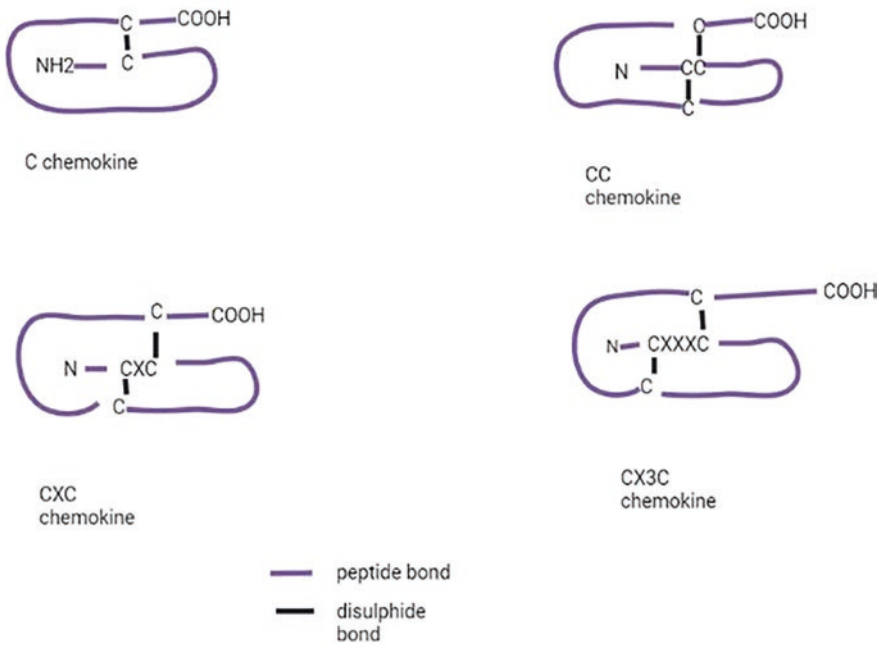

Fig. 15.1 Chemokine subclasses

may result in monocytes leaving the circulation and entering the surrounding tissue to transform into macrophages, are two examples of CC chemokines. CCL5 (RANTES) attracts T cells, basophils, etc that display the receptor CCR5. Elevated levels of CCL11 in blood cause ageing and decreased neurogenesis in both mice and humans. These chemokines all act as ligands for the CCR1-10 family of CC motif chemokine receptors. CC chemokines are essential for the immune system cells to operate, just like the other chemokines. They do, however, exhibit some pro-cancer traits in addition to their anti-cancer properties, hence play a significant role in neoplasia. Certain characteristics of chemokines are present in the tumor. These qualities can be categorised as pro- and anti-cancer. The former is mainly linked to the recruitment of tumor-infiltrating lymphocytes that are anti-cancer and kill cancer cells (Iannello et al. 2013; Lança et al. 2013). Consequently, chemokines' pro-cancer qualities include attracting cells that aid in the growth of tumours, also promoting or inducing the proliferation, localization, and invasion of cancer cells. A certain chemokine frequently, if not always, exhibits both pro- and anti-cancer effects.

The tumor microenvironment includes CC chemokines as a key element. CC chemokines, which are secreted by tumor cells and tumor-associated cells like CAF, TAM, and TAN, promote cancer cell proliferation, migration, and infiltration in addition to resistance to drugs. A circulating cancer cell that expresses a particular CC chemokine receptor will go to tissues that have high levels of the receptor's ligand. Like this, CC-type chemokines draw tumor-associated cells into the tumor

niche; however, this method of action is confined and is reliant on additional elements in the tumor microenvironment. However, some of the recruited tumor-associated cells (namely, TIL) do possess anti-cancer characteristics even though they can accelerate the growth of a tumor. This demonstrates the significance of tailoring the therapeutic strategy to the environment in which a particular CC chemokine act. Therapies should strive to utilise a given chemokine's anti-cancer characteristics or reduce its pro-cancer features. For instance, they might boost a particular chemokine's expression in a tumor before using immunotherapy, which causes cancer fighting immune cells to gather in the tumor through this chemokine. However, they may alternatively focus on preventing the chemokines that cause tumor immune escape and only then apply immunotherapy.

15.2.2 C Chemokines

The C chemokines have only 2 cysteines; one at N terminal side and one downstream. Two chemokines have been mentioned namely XCL1 (lymphotactin-alpha) and XCL2 (lymphotactin-beta). This entry refers to chemokine XC receptor 1 (XCR1), a lymphotactin receptor (Yoshida et al. 1998). Lymphotactin is the sole member of the C (or XC) chemokine family that is known to exist. It is secreted by specific T cell subsets and natural killer cells, and it also has chemotactic properties for these cell types (Kelner et al. 1994). XCR1 is only discovered at very low levels in peripheral blood leukocytes, is substantially expressed in the placenta, and is expressed at lower levels in the spleen and thymus. CD8+ T cells and NK cells are the only ones that can express within these tissues (Kennedy et al. 1995). The pertussis toxin-sensitive stimulation of calcium mobilisation and migration by lymphotactin binding to XCR1 suggests connection of the receptor to Gi-type proteins (Yoshida et al. 1999). The chemokine may be involved in leukocytes' self-recruitment as evidenced by the same expression patterns of lymphotactin and its receptor (Yoshida et al. 1999).

15.2.3 CXC Chemokines

The two adjacent cysteines at N terminus have any amino acid in between other than cysteine which is represented by X. There are about 17 different CXC chemokines mentioned in mammals and are further sub-grouped into two, one being ELR positive and other is ELR negative. The former chemokines are those which have a specific sequence of glutamic acid-leucine-arginine (ELR) prior to the CXCs cysteine motif. Those which lack ELR motif are called ELR negative. The ELR positive cause movement of neutrophils and interact with CXCR1 and CXCR2 receptors. Examples include interleukin 8 causes neutrophil to leave blood stream and enter surrounding tissue. ELR negative such as CXCL13 attracts lymphocytes. Seven CXC chemokine receptors, CXC1-7, have been identified till now to which CXC chemokines can bind.

15.2.4 CX3C Chemokines

The fourth chemokine family has three other amino acids in between cysteines. They are also called d-chemokines. Fractalkine, or CX3CL1, is the only CX3C chemokine that has been identified. They are either secreted or bound to cell surface therefore can act as chemoattractant and adhesion molecule**.**

15.3 Chemokine Receptors

There are two classes of chemokine receptors to which chemokines bind as ligands and transduce signals downstream to run intracellular cascade of reactions. They are conventional chemokine receptors are GPCR receptors in nature. GPCR is a cell surface receptor linked to G protein with 7 domains crossing the membrane, an extracellular domain that bind to ligand also an intracellular G protein-coupled region on cytoplasmic side. G proteins are guanine nucleotide-binding proteins having 3 subunits that act as GTPase and hydrolyse GTP to GDP.

15.3.1 A Conventional Chemokine Receptor

Certain chemokines bind to ACKRs via extracellular domain, i.e. loops and N terminus of the receptor. Negative charge on N terminus can be enhanced by polysialytion, glycosylation, etc. Polysialytion of CCR7 is mandatory for its activation by CCL21. Once ligand binds to ACKR conformational change is there, the signalling mechanism is much complex beyond the scope of this chapter. There are 18 ACKRs named based on chemokine to which they bind R receptor is then followed by the number for their order of discovery. There are 10 CC receptors, 6 CXCRs, single CX3CR and XCR.

15.3.2 A Typical Chemokine Receptor

Atypical chemokine receptor is a non-GPCR receptor is a heptad spanning receptor with missing or modified canonical motif DRYLAIV within intercellular groove and are not able to induce classical GPCR signalling cascade. Currently, the ACKR have 5 receptors duffy antigen receptor for chemokines DARC, D6, CXCR7, and CC-chemokine receptor like 1 and 2 (CCLR1 and CCLR2).

15.4 Tumor Microenvironment and Chemokines

The tumor microenvironment refers to the cellular environment where tumor stem cells reside. It comprises of tumor cells, resident host cells, secreted signalling factors, extracellular matrix, immune cells, and blood vessels. A specific environment

develops as the tumor grows because of the interactions it has with the host. The tumor has a constant grip over it and moulds it, which orchestrates the molecular as well as cellular activities occurring in the surrounding tissues. Effectors of innate immunity such macrophages, polymorphonuclear leukocytes, and infrequently natural killer (NK) cells, as well as T lymphocytes, dendritic cells, and occasionally B cells, are immune cells found in the tumor microenvironment (Kaufman and Wolchok 2007). Blood vessel development into the tumor mass is necessary for tumor growth and metastasis formation. This abnormal angiogenic mechanism benefits from the tumor microenvironment. Endogenous angiogenesis inhibitors like endostatin can be found in the extracellular matrix and basement membranes. Contrarily, a variety of extracellular matrix components can encourage angiogenesis by stabilising blood arteries and enclosing growth substances that are pro-angiogenic. Fibroblasts make up most of the stromal cells in carcinomas. Normal fibroblasts do not exhibit the same phenotypic as cancer-associated fibroblasts do. Although the exact processes by which tumor-associated fibroblasts control angiogenesis are not really clear, it is assumed that they are a significant source of the growth factors and cytokines that attract endothelial cells. Another source of chemokines, growth factors, and other substances that control angiogenesis is the immune system, notably macrophages and neutrophils. Together, the numerous cell types and extracellular matrix that make up the tumor microenvironment form a complex, disorganised tissue that can control the pathological angiogenic switch particularly cancer progression.

15.5 Cells of the Immune System in Tumor Milieu

The tumor microenvironment depends heavily on immune cells, and they have a dichotomous impact on the tumor microenvironment, either promoting or suppressing tumor growth, depending on the situation (Fig. 15.2). In several cancer forms, including colorectal, hepatic, and cervical carcinoma, tumor growth is frequently mediated by persistent inflammation brought on by chronic infection. Immune cells are broadly categorised as innate immune cells (macrophage, neutrophil, and dendritic cells) and adaptive cells of the immunity like T-cell and B-lymphocyte.

15.5.1 Natural Killer Cells

Normally, natural killer cells scour the circulation for tumor cells and virally infected host cells. Natural killer cells can be split into two kinds centred on how they function: those that directly take part in cell-mediated tumor cell death and those that release inflammatory cytokines. Natural killer cells are extremely effective at eliminating tumor cells in the bloodstream and can help prevent metastasis, but they are less effective in eliminating cells in the tumor microenvironment.

Nearly all tumor infiltrates, even precancerous tumours lack Natural Killer cells, which facilitate innate immune response and are rich in granules made up of

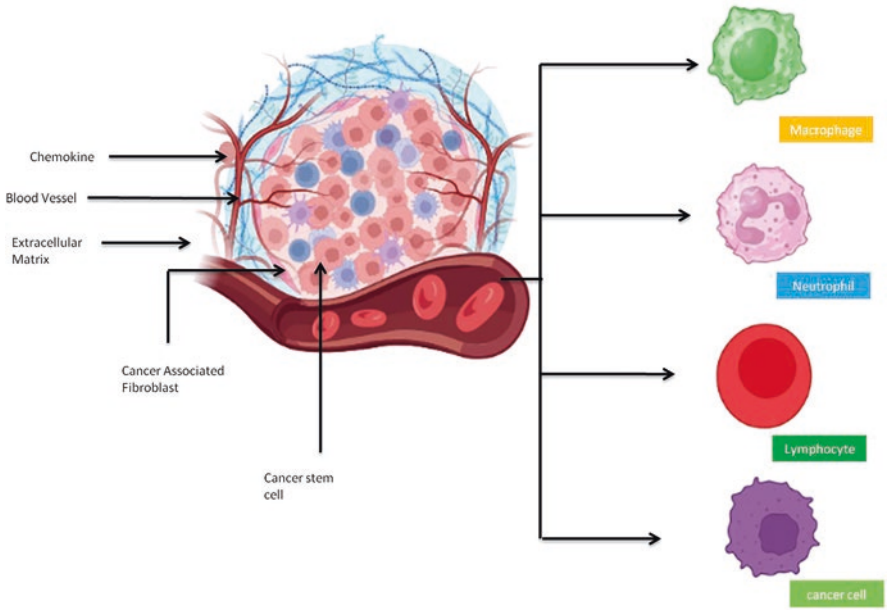

Fig. 15.2 An illustration of the tumor microenvironment

perforin or granzyme (Whiteside et al. 1998). Even though tumor cells commonly downregulate HLA antigen expression and include an abundance of MICA and MICB molecules, NK cells are uncommon in the tumor microenvironment despite being "the first line" of defence (Lanier 2003) also mediate powerful cytotoxicity against tumor in vitro (Chang et al. 2005). According to Lee et al. (2004), these attributes make the tumor vulnerable to cytotoxicity mediated by NK cells, and their low prevalence in infiltrating tumours might be an illustration of an evasion strategy inhibiting localization of NK cells to the tumor location.

15.5.2 Macrophages

The two types of monocyte-derived macrophages are immune-suppressive M2 macrophages, which aid in wound healing, and inflammatory M1 macrophages, which phagocytose and kill cells (Mir and Albaradie 2015). The tumor microenvironment supports the M2 phenotype through hypoxia and the release of cytokines (such I L-4) to support tumor growth and progression, despite the presence of both types of macrophages within a tumor (Mir and Mehraj 2019). A tumor's mass may include up to 50% macrophages, which can make up a significant portion of some tumor types. High macrophage infiltration is typically linked to a bad prognosis for patients with a many different types of malignancies such as breast, lung, and stomach cancers. In the tumor microenvironment, macrophages are frequently seen encircling

blood vessels where they release VEGF, which promotes the creation of new blood vessels/angiogenesis (Mehraj et al. 2021a).

15.5.3 Dendritic Cells

In the tumor microenvironment, stimuli that support either tolerance or an immune response against tumor influence the fate of DC. Although they are biologically designed to just be anti-tumorigenic, the tumor microenvironment can coax them into promoting the growth of tumours. Tumor microenvironment-secreted cytokines cause dendritic cells to tolerate the presence of tumor cells also prevent the triggering of an immunological response.

15.5.4 Neutrophils

In light of the nature of cancer and its stage of progression, neutrophils can either operate to restrict or encourage tumor expansion. As a tumor develops, they are drawn to the microenvironment of tumor wherein they release cytokines and reactive oxygen species that encourage tumor cell death and exacerbate inflammation. When a tumor is further advanced, neutrophils aid in its growth by altering the extracellular matrix. They do this by secreting VEGF and generating the enzyme matrix metalloprotease (MMP)-9, which promotes angiogenesis, tumor progression, and local invasion.

Chemokines have been associated with several human disorders including cancer, metastasis, immunological dysfunction, and chronic inflammation. The chemokine expression in the tumor surrounding, their promotive and inhibiting functions in tumor development, vascularization, spread, and tumor immunity are covered in this chapter. The many functions of chemokines particularly (CXCL9–11, CXCL4, and its variation CXCL4L1) and their corresponding 2-receptor chemokine CXCR3 isoforms, namely CXCR3–A and CXCR3–B, are also given specific attention. These two different isoforms have two different functions in tumours: CXCR3-A promotes tumor growth, whereas CXCR3-B inhibits it. They have an impact on tumor cells directly, but they also have an impact on tumor immunity and angiogenesis indirectly. To further support the use of these chemokines with their corresponding receptors as cancer biomarkers or treatment targets, a thorough understanding of their mechanisms of action is essential.

15.6 A Summary of cxc and cc Chemokines' Impact on Cancer and Cancer Milieu

Research have shown that chemokines are released by tumor cells either autocrinally to directly increase tumor cell proliferation, survival, and spread or by paracrine manner to turn on cells of stroma that assist new blood vessel formation in tumor, spread, and immune evasion. In fact, the two chemokines CC and CXC control

tumor growth, invasion, and leukocyte movement into the tumor, make up the majority of the tumor microenvironment (Freire and Van Dyke 2013).

15.6.1 Direct Tumor Promoting Role

These chemokines have been shown to have effects that promote tumor growth. A well-established prostate cancer cell line resistant to cabazitaxel was identified to have increased CCL2 expression and secretion. Under cabazitaxel treatment, the growth of certain prostate cancer cell lines which were chemo resistant was inhibited using a CCR2 (specific receptor of CCL2) antagonist (Mir and Albaradie 2014). As a result, the CCL2-CCR2 axis seems to be a major factor in the development of cabazitaxel resistance in prostate cancer cells (Natsagdorj et al. 2019). The lymphatic sinuses of lymph nodes secrete CCL1, which encourages metastasis by luring CCR8+ (the CCL1 receptor specifically expressed on tumor cells) cells to the lymph node. Recombinant CCL1 encourages the movement of tumor cells that express CCR8, and inhibiting CCR8 or CCL1 prevents in vitro tumor cell invasion of lymphatic endothelial cells. Blocking CCR8 or knocking it down with shRNA dramatically reduced lymph node metastases in 3 mice breast cancer and melanoma models (Das et al. 2013). It has been determined that a crucial barrier to the spread of metastases into lymph nodes is the CCL1-CCR8 axis. Non-small cell lung cancer (NSCLC) has a strong expression of CCR9 (Li et al. 2015). Additionally, it was demonstrated that CCL25-CCR9 axis promotes the growth of NSCLC tumours by inhibiting tumor cell death.

15.6.2 Immune Regulatory Functions

The chemokines CC and CXC been proven to affect leukocyte movement and immunological response. These chemokines generated by tumor as well as stromal cells influence the make-up of the leukocyte infiltration in many malignancies. Expression of the chemokines CC and CXC may affect the development of tumours by regulating the immune cell population that infiltrates. To draw immune cells like myeloid-derived suppressor cells and tumor-associated macrophages, CCL2 is necessary (Arenberg et al. 2000). NK (natural killer) cells are crucial for anti-tumor immunity. Highly cytotoxic Natural killer cells accumulate in lymph nodes as malignant melanoma spreads to lymph node metastases, and the production of CXCL8 in the tumor microenvironment stimulates the recruitment of this NK cell population (Ali et al. 2014). Additionally, necessary for NK cell penetration into malignancies is the CXCR3-CXCL10 axis. The quantity of NK cells in tumours increased as a result of forced CXCL10 expression, and NK cell-dependent survival was extended (Wendel et al. 2008). According to Harlin et al. (2009), malignancies including T cells preferentially express a fraction of six chemokines such as (CCL2, 3, 4, 5, CXCL9, and CXCL10). They provided proof of that, in a mouse xenograft model, CXCL9 and 10 are essential in the tumor microenvironment of melanoma also capable of attracting effector CD8 T cells (Harlin et al. 2009). By enlisting T cells, CCL5 could also be related to more effective anti-cancer immune response

(Nesbeth et al. 2009). High CXCL12 expression was associated with enhanced tumor inflammation and regulatory T cell (Treg) recruitment in lung adenocarcinoma tumours (Wald et al. 2006). According to Facciabene et al. (2011), tumor hypoxia in ovarian cancer promotes the production of chemotactic factors such CCL28. They demonstrated that the recruitment of Tregs in vivo via their cognate receptor CCR10 is directly responsible for the direct relationship between tumor CCL28 overexpression and increased tumor development.

15.7 Chemokine Organotropism

One can wonder if specific CC or CXC chemokines prefer an organ's specific expression and function. These are often extensively dispersed not likely to exhibit any organotropism. For instance, CXCL10 functions as a chemotactic and tumor-promoting agent in the lung (Pradelli et al. 2009) and the brain (Doron et al. 2019), respectively. However, the function may be inhibiting in breast cancer because of an overabundance of NK cells (Wendel et al. 2008). Additionally, CXCL12 affects the pancreatic (Roy et al. 2015), prostate (Saha et al. 2017), and breast (Huang et al. 2009) by either promoting or inhibiting tumor growth.

15.8 Role of Chemokines in Tumor Growth and Progression

Chemokines are chemotactic cytokines guide movement of immune cells (Fig. 15.3). Interaction between chemokine and chemokine receptors facilitates tumor growth and metastasis. Tumor cells secrete soluble molecules as chemical messengers such as VEGF-A (vascular endothelial growth factor), TNF-α etc that work on myeloid and endothelial cells, inducing the expression of S100 chemokine, a non-classical one. S100 chemokine target tumor cells to the premetastatic location and are involved in metastasis. Also, S100A8 and S100A9 activate P38 MAPK pathway (mitogen-activated protein kinase) causing increased formation of pseudopodia that eventually enhance invasive property of tumor cells (Rafii and Lyden 2006).

VEGF correlated chemokine-1 VCC-1 is a new one displayed in colon cancer and breast cancer (Liston and McColl 2003). VCC1 levels are positively correlated with increased VEGF at tumor locations, especially in endothelial cells producing tubes in vitro. In comparison to cells transfected with a control vector, the chemokine VCC-1 overexpression in NIH3T3 cells accelerates the formation of tumours in mice (Mir and Albaradie 2015). Therefore, VCC-1 possibly mediate its effect via multiplied angiogenesis (Liston and McColl 2003).

The chemokines released by tumor cells influencing the microenvironment by encouraging vascularization, luring infiltrating leukocytes, and/or boosting proliferation also activating their unique receptors, which change the possibility of tumor cells to adhere, move through the bloodstream, colonise surrounding organs, and extravasate (Table 15.1).

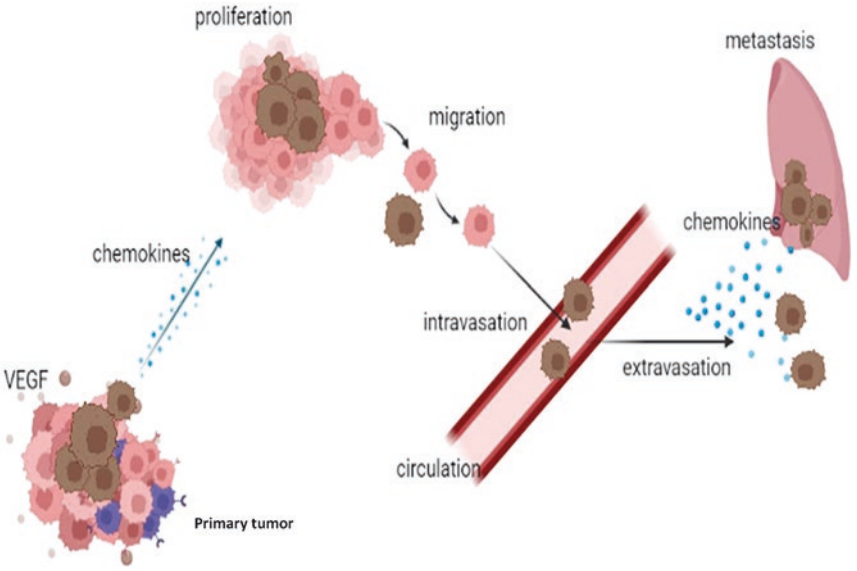

Fig. 15.3 Chemokines' complex roles in tumor development, invasion, and metastasis

Table 15.1 The chemokine and their respective receptors known to have role in tumor progression, metastasis, and vascularization

Systematic name	CXC chemokines	Chemo-receptors	P/M
ELR+ chemokines	HUMANS		
CXCL1	MGSA-ALPHA/GRO-ALPHA	CXCR2>CXCR1	P
CXCL2	MGSA-BETA/GRO-BETA	CXCR2	P
CXCL3	MGSA-GAMMA/GRO-GAMMA	CXCR2	P
CXCL4	PF4	UNKNOWN	A
CXCL5	ENA-78	CXCR2	P
CXCL8	IL-8	CXCR1, CXCR2	P
ELR – chemokines			
CXCL9	MIG	CXCR3	A
10	IP10	CXCR3	A
11	I-TAC	CXCR3	A
12	SDF-1 ALPHA/BETA	CXCR4, CXCR7	M, P
CXCL13	BCA1	CXCR5	
CXCL16	SR-PSOX	SXCR6	

P prog1ression, *A* angiogenesis, *M* metastasis

Chemokines' function in tumours inflammation is a crucial element of the tumor microenvironment and one of the primary characteristics of cancer (Mantovani et al. 2008). A class of tiny, secreted, structurally similar cytokines known as chemokines plays a key role in immunity and inflammation (Griffith et al. 2014). Being present at the site of the tumor for already existing chronic inflammatory diseases while simultaneously being a target of cancer causing pathways, they are also important mediators of inflammation connected to cancer (Mantovani et al. 2010).

They were discovered to have direct control over cancer cell proliferation and metastasis after being initially recognised as playing a significant role in shaping the makeup of tumor stroma (Caronni et al. 2016).

15.8.1 Leukocyte Recruitment

The expression of chemokines in space and time controls the correct migration of the cells of immune system. At the tumor site, inflammatory CC chemokine (CCL2, 3, 5) and CXC (CXCL2, 5, 6, and CXCL8) attract CCR2+ monocytes and CXCR2+ neutrophils, which distinguish into TAMs and TANs, which possibly promote or inhibit tumor effect (Balkwill 2012). Some chemokines that are present at the tumor site can change how leukocytes are activated; for example, in solid tumours, CXCL16 influencing CXCR6 causes macrophage (Cho et al. 2016). Chemokines like CXCL9 and CXCL10 greatly influence the (T helper 1) Th1 immune response by attracting Natural Killer cells, CD4+ T helper 1, and CD8+ cytotoxic lymphocytes that trigger anti-tumor response (Wendel et al. 2008). Additionally, CCL20 and CCL5 are effective dendritic cell (DC) attractants (Scarpino et al. 2000). CCL21 and CCL19 not only draw regulatory T cells (Tregs) but also CCR7+ DC (Correale et al. 2012). CCL17 and CCL22 that act on CCR4 are capable of direct recruitment of T regulatory and T helper 2 cells that enhance tumor development and expansion (Gobert et al. 2009).

15.8.2 Angiogenesis

The roles of CXC and CC chemokines in tumor angiogenesis are crucial for tumor growth and metastatic spread (Ridiandries et al. 2016). Depending on ELR motif's presence at the amino-terminus of chemokine polypeptide, CXC chemokines are classified as ELR+ chemokines with angiogenic effects and ELR- chemokines with angiostatin effects. The survival of endothelial cells and tumor angiogenesis are promoted by CCL2, 11, 16, 18, and CXCL8 (Keeley et al. 2010). Additionally, interaction between CXCL16 and CXCR6 serves as a powerful angiogenic agent. The binding of CCL2 and CXCL12 to their corresponding receptors such as CCR2 and CXCR4 exhibited by tumor arteries directly stimulate angiogenesis and block endothelial cell death or alternatively encourage leucocyte recruitment (Sozzani et al. 2015). On the other hand, chemokines, like CCL21 and ELR negative (CXCL4, CXCL9, and CXCL11), impede the growth of endothelial cells and angiogenesis (Strieter et al. 2005).

15.8.3 Tumor Development and Spread

Chemokines released by cancer cells itself, infiltrating leucocytes, fibroblasts linked to tumours bind to receptors presented by tumor cells (Mehraj et al. 2021b), and directly cause tumor cell proliferation via the activation of tremendous signalling

pathways like PI3K/AKT/NF-B as well as MAPK/ERK pathways (Zlotnik et al. 2006; Teicher and Fricker 2010). Further, they can increase the survivorship of tumor cells by blocking their death and controlling the ratio of apoptosis promoting to anti-apoptotic molecules (for example, by downregulating the manifestation of Bcl-2 or inhibiting the stimulation of caspase-3 and caspase-9) (Murakami et al. 2003).

15.8.4 Metastasis

Cancer cells that contain chemokine receptors assist their migration to metastatic locations (Zlotnik et al. 2011). There are many chemokines and their receptors implicated in this phenomenon: When CCL19 and CCL21, the tumor cells' ligands are produced in lymph nodes, and CCR7 facilitates their movement there (Takanami 2003). The CCR10/CCL27 axis promotes melanoma cell adherence and survival during metastatic dissemination (Ben-Baruch 2008). Through the MAPK/ERK pathway, CCL28 encourages breast cancer growth and metastatic dissemination (Yang et al. 2017). Finally, bone metastases in prostate cancer are supported by the chemokine receptor CXCR5 and its ligand CXCL13 (Lillard et al. 2010). However, the CXCL12/CXCR4 axis is the primary participant in this process. Cancer cells with CXCR4 expression have the potential to go into organs that secrete a lot of CXCL12 and spread there (Darash-Yahana et al. 2004).

Chemokines are crucial to the development of tumours. Tumor growth is encouraged by persistent inflammation (Fig. 15.4). Chemokines are cytokines produced by both stromal and cancer cells. These support tumor cell development through autocrine and paracrine process, promote angiogenesis, and aid in evading immune monitoring by immunoediting. Leukocyte movement into the tumor milieu and tumor angiogenesis are encouraged by the chemokine receptor CXCR2 and its ligands. Tumor cells increase their expression of CXCR4 when exposed to harsh acidic and hypoxic microenvironmental circumstances which enables them to move up a gradient of CXCL12 created by CAFs (Qayoom et al. 2023). The CCL21 -CXR7 chemokine ligand receptor combination supports lymph node metastasis, while the CXCL12- CXCR4 axis makes it easier for metastasis to spread to distant organs. These two chemokine-ligand receptors systems serve as essential targets for chemotherapy since they are frequent major mediators of tumor cell metastasis for a variety of cancers.

Chemokines with trophic and angiogenic capabilities are produced by tumours, especially CXC chemokines with an ELR motif that bind to CXCR2 (CXCL1, 2, 3, CXCL8). They also release chemotactic chemicals that draw macrophages, especially CCR2 ligands like MCP-1 and CCL2. By releasing pro-angiogenic and tumor-stimulating chemokines and other angiogenic substances, tumor luring macrophages significantly support the expansion of the tumor (bFGF, VEGF). An amplificatory loop of this process is defined by the release of macrophage-recruiting chemokines by tumor-associated macrophages. CXCL9, 10, CXCL11, and mouse CCL21, on the other hand, exhibit anti-angiogenic capabilities through direct

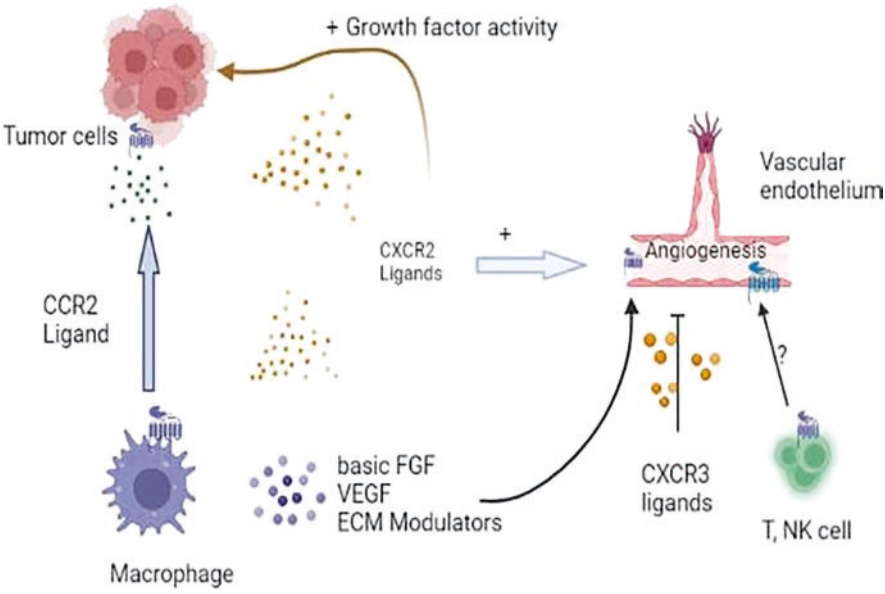

Fig. 15.4 Chemokines regulate angiogenesis and tumor growth

contact with the endothelium and/or the recruitment of T or NK cells that can destroy the vasculature (Strieter et al. 1995).

15.9 Chemokines in Cancer Therapy

The immune system can be targeted as a practical cancer prevention strategy (Papaioannou et al. 2016). Numerous methods have been developed since Coley's toxin was created in 1893 to increase leukocytes' anti-cancer activity (Balkwill and Mantovani 2012). Chemokines and their receptors have been associated with multiple aspects of cancer biology; therefore, numerous pre-clinical research and clinical trials have looked at how they might be targeted to treat cancer (Mir et al. 2020). A monoclonal antibody called mogamulizumab, which is anti-CCR4 monoclonal antibody, and a chemokine receptor inhibitor called AMD3100, which is CXCR4 antagonist/inhibitor, are already used in clinical practise for haematological malignancies (Qayoom et al. 2022).

15.9.1 CCR1

Myeloid cells are the primary target of CCR1 inhibition, which slows the spread of cancer. The CCR1 antagonist CCX721, which targets osteoclasts and their progenitors, inhibited tumor growth and osteolysis in multiple myeloma (MM) mice

models (Vallet et al. 2007). Blocking the CCR1 ligand CCL3, which MM cells abundantly generate, had the same result. The CCR1 antagonist BL5923 decreased liver metastases in a mice model of colon cancer by restricting the enrolment of immature myeloid cells (Kitamura et al. 2010). Recently, a mouse breast cancer model treated with anti-PD-L1 and the CCR1 receptor antagonist CCX9588 shown a synergistic anti-tumoural impact by decreasing the myeloid infiltration (Jung et al. 2015). CCR1 antagonists are the best options for modifying the myeloid infiltration in treatments like combination therapies because they did not exhibit any negative effects when administered to autoimmune disease patients (Horuk 2009).

15.9.2 CCR2 and CCL2

Because fewer monocytes, promoting tumor and metastatic activities, are recruited, CCL2/CCR2 axis interference has anti-tumoural effects in various malignancies. Pancreatic tumours are the subject of a wealth of information. The oral CCR2 blocker PF-04136309, when combined with the chemotherapeutic medication Gemcitabine (GEM), lowers the quantity of macrophages connected with tumours and had a minimal impact on tumor development in a pre-clinical model (Mitchem et al. 2013). A Phase Ib/II trial using PF-04136309 in conjunction with nab-paclitaxel [(PTX), a nanoparticle albumin-bound version of PTX capable of inducing tumor-associated macrophage activation toward an M1 like phenotype], and GEM on patients with pancreatic cancer produced encouraging findings (NCT02732938) (Noel et al. 2017). Patients with locally advanced or borderline respectable pancreatic ductal adenocarcinomas were treated with the same inhibitor in a different clinical trial (NCT01413022) in addition to the conventional treatment FOLFIRINOX (FX). The combined therapy boosted the percentage of objective responses, according to preliminary findings (Nywening et al. 2016). CCX872 is a highly promising CCR2 inhibitor in the context of pancreatic cancers. It increased the effectiveness of the anti-PD-1 treatment (Jung et al. 2016) in a pre-clinical context, and when combined with FX, positive outcomes were also attained in a clinical trial (NCT02345408) (Linehan et al. 2018). CCR2 targeting with the antagonists RDC018 or 747 in conjunction with Sorafenib reduced tumor development and metastasis with a commensurate decrease in macrophage infiltration in mice models of hepatocellular carcinoma (HCC) (Yao et al. 2017).

15.9.3 CCR4

Many hematopoietic cancers, such as Adult T-cell leukaemia and cutaneous T-cell lymphoma, overexpress CCR4. The anti-CCR4 human antibody Mogam1ulizumab, which is now being used in Japan to treat relapsed/refractory ATL, kills tumor cells by antibody-dependent cellular cytotoxicity (ADCC) (Fuji et al. 2018). According to a global phase III trial, it is also regarded as the best treatment for Cutaneous T-cell Lymphoma patients who have already had treatment (Mollica Poeta et al.

2019). Additionally, chimeric antigen receptor-T cells made against CCR4 were successful in treating a variety of T cell malignancies in pre-clinical investigations (Perera et al. 2017).

In addition, CCR4 is seen as a viable target for solid tumours due to its ability to regulate leukocyte infiltration, particularly through reducing Tregs. Affi 5, a CCR4 blocking mAb, inhibited tumor growth in a pre-clinical model of renal cancer by altering the phenotypic of myeloid cells and boosting the amount of invading NK cells (Berlato et al. 2017). Patients with renal cancer are now thought to have CCR4 as a target (Allison 2017). However, there are significant worries regarding the safety of using mAbs against CCR4, particularly in individuals who have had an allogenic bone marrow (BM) transplant in the past. For a short period of time, anti-CCR4 mAbs are also depleting Tregs, raising the risk of graft versus host illness (Fuji and Shindo 2016). Small chemical CCR4 antagonists are being developed as a result, and one of them, AF399/420/1802, significantly increased the effectiveness of cancer vaccines in various pre-clinical tumor models (colon cancer, lung, and melanoma) by suppressing the formation of Tregs (Beziaud et al. 2018).

15.9.4 CCR5

It is still debatable whether CCR5 has tumor promoting or tumor inhibiting role in cancer, based on the type of cell where it is expressed. When a tumor expresses CCR5, it promotes their metastasis and development, whereas when it is exhibited by T cells, it amplifies anti-tumor responses (González-Martín et al. 2012). For example, in breast cancer, the receptor has been linked to metastasis and cancer progression in addition to its dual role in stimulating anti-tumor immune responses (Velasco-Velázquez et al. 2014). The mobilisation of myeloid cells with pro-tumoural activity is caused by CCR5 in more recent studies (Hawila et al. 2017), and results from clinical trials and clinical colorectal cancer (CRC) models show using the negative allosteric inhibitor Maraviroc encouraged macrophages to polarise towards an anti-tumoural state and promoted the polarisation of macrophages towards an anti-tumoural state. Interestingly, three out of five patients who underwent Maraviroc (NCT01736813) and chemotherapy exhibited an objective partial response (Halama et al. 2016). These findings imply that whereas CCR5 activity on T cells needs to be conserved in other malignancies for proper immune response formation, inhibiting CCR5 may have a significant anti-cancer effect in tumours that are CCR5 positive and have a common myeloid infiltrate with immunosuppressive activity (Mir and Qayoom 2023).

15.9.5 CCR7

CCR7 inhibitors have a lot of potential as a medical treatment. Many cancers overexpress CCR7, which promotes both tumor growth and metastatic spread. With the aid of siRNA technology, CCR7 inhibition reduced the number of colon cancer

metastases (Shuyi et al. 2008) and stopped the growth of prostate cancer (Chi et al. 2015). Additionally, lowering the expression of CCR7 in breast cancer prevented metastasis (Kim et al. 2012), and T-cell acute lymphoblastic leukaemia brain metastases were reported to be resistant to single-chain antibodies that block CCR7 (Cunningham et al. 2014).

15.9.6 CXCR2

Numerous tumor cells express CXCR2, which plays a role in chemotherapy resistance in various pre-clinical cancer models. CXCR2 deletion improved paclitaxel responsiveness in breast cancer cells (Sharma et al. 2013). While the CXCR2 inhibitor SB225002 improved the anti-angiogenic therapy sorafenib in a model of ovarian tumor, the CXCR2 inhibitor navarixin synergized with MEK inhibition in a model of melanoma (Devapatla et al. 2015). Finally, the CXCR1 and CXCR2 inhibitor reparixin improved the effectiveness of 5-fluorouracil in treating human gastric cancer (Wang et al. 2016). Targeting CXCR2 also has an impact on myeloid cell infiltration, which slows tumor growth. CXCR2 inhibition inhibited metastatic progression and enhanced response to anti-PD-1 in pancreatic cancers by preventing the generation of neutrophils that trigger the T cell response (Steele et al. 2016). It is interesting to note that using both CXCR2 and CCR2 inhibitors at once reduced TAMs' compensatory response, boosted anti-tumor immunity, and enhanced FX responsiveness. Last but not least, CXCR2 inhibition by SB265610 in a prostate cancer model reduced myeloid cell recruitment and improved Docetaxel-induced senescence, restricting tumor growth (Di Mitri et al. 2014). A phase II clinical trial with the CXCR2 inhibitor AZD5069 is currently being conducted in patients with pancreatic cancer because of these encouraging pre-clinical results (NCT02583477). Additionally, double-blind research with these medications for metastatic triple-negative breast cancer is being conducted, and the safety of using reparixin in combination with paclitaxel was evaluated (NCT02370238) (Schott et al. 2017).

15.10 Chemokine Therapy and Breast Cancer

Breast cancer is regarded as a diverse illness, with various biochemical and phenotypic characteristics that make diagnosis and treatment difficult. Breast cancer growth and occurrence are complicated by the chemokine network, which is made up of chemokines and chemokine receptors (Sofi et al. 2022a). Some chemokines can stop breast cancer cells from growing, whereas other chemokines enable breast cancer cells spread and develop into tumours. CXCL9, CXCL10, and CCL16, for instance, can suppress breast cancer (Liang et al. 2005), but CCL2, CCL5, CXCL8, and CXCL12 can promote it (Goldberg-Bittman et al. 2004; Sofi et al 2023). Chemokine research offers a novel approach for the treatment of breast cancer and

other malignant tumours, and related research using chemokine as a target for cancer is now being conducted (Qayoom et al. 2021). CXCR4 has a positive expression rate of up to 60% in breast cancer. The role of CXCR4 in the pulmonary metastasis of breast cancer has been established (Abdou et al. 2019; Dayer et al. 2018). Drugs that target CXCR4 may therefore be effective in treating breast cancer. There are now additional options for chemokine target treatment because more chemokines have been identified in breast cancer (Yousuf et al. 2022). Inhibiting the expression of CXCL13 led to a considerable reduction in IL-1, TNF-α, and TGF-β1, as well as a decrease in cell proliferation and an increase in apoptosis rate. The suppression of the ERK signal in breast cancer cells is what causes the anti-tumor effect (Ma et al. 2018). The results of this study point to CXCL13 inhibition as a potential strategy for slowing the spread of breast cancer. Breast cancer patients who have high levels of CCR5 and its ligands (CCL4, CCL5) enhance tumor growth. Asim and his colleagues discovered that inhibiting CCR5 expression dramatically reduced bone metastases in naked mice implanted with MDA-MB-231 cells in addition to promoting the expansion and death of metastatic breast cancer cells (Pervaiz et al. 2019). The findings imply that the treatment focus for advanced breast cancer may be CCR5. Another challenge in treating tumours is the possibility of recurrent tumours. Tumor recurrence following treatment is the main factor in more than half of breast cancer-related fatalities (Sofi et al. 2022b). After treatment, residual tumor cells are still alive. It is crucial to understand how the tumor microenvironment and tumor recurrence interact. The distinct tumor microenvironment induces immunosuppression in the remaining tumor cells, and a significant number of immune cells linked to tumours are enlisted to take part in tumor recurrence process, promoting the ongoing growth and potentially malignant progression of the tumor. Because CCL5 attracts macrophages that express CCR5 receptor, this could contribute to the build-up of residual tumours, Andrea and her colleagues have discovered that one of the best ways to preclude breast cancer incidence may be to disrupt the tumor necrosis factor-CCL5 macrophage axis (Walens et al. 2019).

15.10.1 Studies on Drugs that Target Chemokines and Their Receptors in bc

Additionally, the utilisation of chemokines and chemokine receptors as efficient biomarkers for the early detection and prognosis of breast cancer is possible. The findings demonstrated that CCL2 and CCR2 levels were significantly greater in breast cancer patients, suggesting the potential utility of these molecules as molecular markers for early detection of the disease (Table 15.2).

Clinical evidence suggested a connection between CXCR4 expression and the overall survival rate of invasive patients. Reza and his co-workers discovered that invasive breast cancer samples have an upregulated CXCL12/CXCR4 chemokine axis in comparison to healthy surrounding lesions (Sharma et al. 2013). Chemokines and chemokine receptors may serve as indicators to determine breast cancer prognosis and early diagnosis.

15.11 Conclusion

Inflammatory and immunological responses have a considerable influence on the genesis and progression of cancer. Leukocyte infiltration, neovascularization, and fibrosis are examples of inflammatory reactions, whereas immunological responses were used by immunological cells such dendritic cells and lymphocytes. Chemokines significantly affect the cells involved in both immunological and inflammatory reactions. Additionally, a few chemokines have an immediate impact on the invasiveness and proliferation of tumor cells. As a result, chemokines play important roles in the growth and progression of tumor cells by influencing both malignant and healthy cells. It is embarrassing that the same chemokine can, depending on the situation, both protect against a tumor and cause it to progress. We can better comprehend the pathophysiological mechanisms behind tumor growth and progression by elucidating the multifaceted functions that chemokines play in carcinogenesis. This will also open new opportunities for the development of chemokine-targeted anti-cancer therapies. Therefore, they make an excellent target for immunotherapy since leukocyte infiltration and tumor cells both express chemokines and chemokine receptors. To prevent potential adverse effects, a deeper comprehension of their functions in various cancers is still required. Targeting overexpressed chemokine receptors in haematological malignancies kills tumor cells directly but may cause unintended immunological responses (e.g., CCR4). Chemokine receptor inhibitors are showing promising effects in the treatment of solid tumours when combined with chemotherapy or antibodies against immunological checkpoints. It is therefore plausible to imagine the use of chemokine receptor inhibitors in the future to modify the stromal portion, resolve chemotherapy resistance, and enhance patient immunological responses.

Table 15.2 Chemokines and their inhibitors

Target	Blocker	Consequences	Citations
CCR2	CCX9588 + anti-PD-L1	Reduce the amount of MDSCs., impede development and pulmonary metastases	Jung et al. (2015)
CCR5	Maraviroc	impede the metastasis of bone	Walens et al. (2019)
S			
CCL2	CNTO888+radiotherapy	Encourage metastasis and angiogenesis	Bonapace et al. (2014)
CXCR4	Reparixin + PTX	Minimise the quantity of MDSCs, and impede metastasis	Schott et al. (2017)
			Pernas et al. (2018)
	GST-NT2 1MP	Reduce migration and growth and reduce metastasis	Yang et al. (2014)
CXCL12	LYG202	halt endothelial cell activation and vascularization	Zhao et al. (2018)

Further Reading

For further readings, some textbooks mentioned below also gives further understanding of cytokine and chemokine network.

- Cytokines and their therapeutic potential by Manzoor Ahmad Mir.
- The Role of Chemoattractants in the Tumor Microenvironment by Giovanni Bernardini, Brian A. Zabel.

For more incites about the topic we would suggest detailed findings from the books of (Mir 2022) https://doi.org/10.1016/C2021-0-02565-7, https://doi.org/10.1016/C2022-0-00074-X and (Mir 2021) https://doi.org/10.52305/WXJL6770 (Mir 2015) https://doi.org/10.1016/C2014-0-02898-5 and from the cancer.net website on the following mentioned below links,

https://www.cancer.net/cancer-types/breast-cancer/types-treatment

https://discovery.ucl.ac.uk/id/eprint/1472740/

https://www.jmedsciences.com/doi/JMEDS/pdf/10.5005/jp-journals-10045-00138

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