Gurmit Singh *Editor*

Oncodynamics: Effects of Cancer Cells on the Body

Springer

Oncodynamics: Effects of Cancer Cells on the Body

Gurmit Singh
Editor

Oncodynamics: Effects of Cancer Cells on the Body

Springer

Editor
Gurmit Singh
Department of Pathology and Molecular Medicine
McMaster University
Hamilton, ON
Canada

ISBN 978-3-319-28556-6 ISBN 978-3-319-28558-0 (eBook)
DOI 10.1007/978-3-319-28558-0

Library of Congress Control Number: 2015960214

Printed on acid-free paper

This Springer imprint is published by SpringerNature
The registered company is Springer International Publishing AG Switzerland

This book is dedicated to students, research fellows, and technical staff who tirelessly commit their energy and time to improve the lives of cancer patients and our understanding of the effects of cancer on the body.

Preface

The Maturing of Oncology: Conceptual Framework

The field of oncology deals with the study of tumours and cancer. As the field has matured over the past century, we have seldom paused to critically examine its structure, and as a result it has grown increasingly murky. The field of oncology is multifaceted: we have been dissecting the circuitry of various cancers to define their signalling pathways and now we are attempting to target pathways that are overwhelming the cell. We have also studied the behaviour of tumour cells as they metastasize and have identified their ability to induce neo-angiogenesis and neo-neurogenesis. Additionally, we have attempted to examine the impact of cancer via psycho-social analysis and therefore study the "quality of life" of individuals. All these studies form the basis of oncology. It is now the time to further clarify these distinctions within oncology to reflect the ongoing maturation of the field. I propose to introduce new terminology to the field of oncology. It provides consistency with terminology used in pharmacology.

I propose that the field of oncology be further subdivided into (i) oncokinetics and (ii) oncodynamics.

(i) *Oncokinetics*: This defines the mechanics of the tumour cells as they arise and spread in the body. It includes tumour cell signalling, tumour growth, tumour metastasis, and tumour cell apoptosis.
(ii) *Oncodynamics*: This defines the impact of abnormal cues generated by tumours on the physiological functioning of the body. It includes tumour-induced neo-angiogenesis, tumour-induced pain, tumour-induced fatigue, tumour-induced depression, cachexia, and neo-neurogenesis.

The conceptual framework of the subdivision of oncology is based on the field of pharmacology which is also subdivided into (i) Pharmacokinetics—defined by what the body does to the drug including drug metabolism, distribution, and excretion and (ii) Pharmacodynamics—defined by what the drug does to the body. This distinction is appropriate in that it allows the study of pharmacology to be

defined on the basis of the drug and the effects of the drug on the body. Similarly, this further subdivision of oncology is useful in that it defines the abnormality of tumours and cancers and also the impact of abnormal cues from the tumours on the body.

This new terminology is necessary to ensure that research and understanding in oncology is accurately delineated. The use of drugs in treating cancer and the impact of chemotherapy drugs or radiation on the body should not be confused with oncology. Their pharmacological examination should be based on pharmacological principles whereas oncological examination should be based on tumours and cancers. Focusing on psycho-social aspects of cancer largely defines quality of life and should be regarded as a tool to gauge the success of treatment. Oncokinetics has developed its fundamentals over the last fifty years and has provided us with a comprehensive model of tumour circuitry. Its control still eludes us. Conversely, the study of oncodynamics, which is at its infancy, needs attention as it may yet provide a mechanistic basis for the treatment of cancer. This can be exemplified by the complexity of certain signalling pathways such as the mTOR pathway that is elevated in tumour cells while its suppression in the central nervous system can lead to depression. Similarly, a number of other established tumour cell signalling pathways have been identified in other normal physiological functions such as memory. Further subdivision of oncology will only help in the focused future research. It provides a framework on which cancer funding agencies and the pharmaceutical industry can develop strategies in accordance with their priorities.

The impact of oncodynamics is very important from both a cancer patient and a caregiver's perspective. This subfield has a much bigger impact on cancer patient functionality and the resultant societal implications, as it portrays the havoc of cancer on an individual. It is curious that cancer scientists and cancer funding agencies are largely consumed in curing cancer while hoping that psycho-social studies alone will address the issues of quality of life. The yardstick for understanding the oncodynamic approach to cancer research is only now being addressed and requires an active debate and the participation of other disciplines, especially neuroscientists, to engage in collaborative research with cancer biologists. Cancer-induced depression for example could provide interesting and useful models to study major depressive disorders as the origin is more distinctly defined. Similarly, the study of cancer pain can lead to novel therapeutic approaches that are not analgesic dependent.

This terminology should not be confused with other associated branches of oncology such as oncogenetics, oncoepidemiology, etc., which have individual primary disciplines such as genetics, epidemiology, etc. Various aspects of cancer treatment that include medical oncology, radiation oncology, and surgical oncology can be viewed as clinical oncology and have a foundation based on the basic science of tumours and cancers.

Finally, the defining of oncology within its subfields provides for an opportunity for cancer researchers to develop cross-discipline interactions and predict potential consequences of tumours and/or treatment. The conceptualization of tumour–host interactions from a physiological viewpoint is very important and supersedes the

"-omic" influence in understanding tumours. The ultimate goal of oncology is to have an understanding of tumours and their influence on the body. This knowledge will enable us to provide appropriate strategies to deal with cancer and limit the diverse consequences of abnormal cues sent by tumours. Thus we may be able to define novel mechanism-based treatment for oncodynamic effects such as fatigue, pain, and depression associated with cancer. We are at the cusp of making enormous advances in oncology if we embrace methods of progress in other fields of science and acknowledge the complexity beyond "-omics" to develop a framework around physiology.

Gurmit Singh

Contents

Contributors

Norman Buckley Department of Anesthesia, McMaster University, Hamilton, ON, Canada

Allison J. Chen Program in Health Sciences, McMaster University, Hamilton, ON, Canada

Mark Clemons Department of Medicine, University of Ottawa, Ottawa, ON, Canada; Department of Medical Oncology, Ottawa General Hospital, Ottawa, Canada

Jennifer Fazzari Department of Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada

Benicio N. Frey Department of Psychiatry and Behavioural Neurosciences, McMaster University, Hamilton, ON, Canada

Caroline N. Gobran Program in Health Sciences, McMaster University, Hamilton, ON, Canada

Zeinab Khawaja Program in Health Sciences, McMaster University, Hamilton, ON, Canada

Katja Linher-Melville Department of Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada

Tanya Miladinovic Department of Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada

Franziska Miller Singh Lab, Department of Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada

Mina G. Nashed Department of Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada

Sureka Pavalagantharajah Program in Health Sciences, McMaster University, Hamilton, ON, Canada

Snezana Popovic Department of Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada

Patricia Rosebush Department of Psychiatry and Behavioural Neurosciences, McMaster University, Hamilton, ON, Canada

Eric Seidlitz Department of Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada

Gurmit Singh Department of Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada

Tina Y. Tang Program in Health Sciences, McMaster University, Hamilton, ON, Canada

Robert G. Ungard Department of Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada

Kimberly Young Department of Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada

Yipeng Zhang Program in Health Sciences, McMaster University, Hamilton, ON, Canada

Chapter 1
The Disrupted Steady-State: Tipping the Balance in Favour of Cancer

Katja Linher-Melville and Gurmit Singh

Abstract Genetic changes, such as the activation of oncogenes or the repression of tumour suppressors, contribute to the development of cancer, imparting malignant cells with the potential for self-promoting growth and survival in the presence of anti-growth or pro-apoptotic signals. However, while these changes may initiate the process of cancer development, they are not necessarily sufficient for disease progression, given the body's intrinsic ability to regain homeostasis. Cancer initiation, promotion, and eventual progression depend on disruptions in normal homeostasis, as well as subsidiary processes imparted by cells of the tumour microenvironment. Recurring players that have been linked with disrupted homeostasis include inflammation and oxidative stress, which have both been strongly associated with the development of cancer. This chapter discusses the intricate relationship between the body and cancer, and how disruptions in normal physiological processes impact the maintenance of homeostasis and tissue repair, providing a framework for understanding the connection between dysregulated homeostatis and a complex disease such as cancer.

Keywords Homeostasis · Allostasis · Cancer initiation · Hallmarks of cancer · Inflammation · Oxidative stress

K. Linher-Melville
Department of Pathology and Molecular Medicine, McMaster University, 1280 Main Street West, HSC 4N48, Hamilton, ON L8S 4L8, Canada
e-mail: linherk@mcmaster.ca

G. Singh (✉)
Department of Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada
e-mail: singhg@mcmaster.ca

G. Singh (ed.), *Oncodynamics: Effects of Cancer Cells on the Body*,
DOI 10.1007/978-3-319-28558-0_1

Introduction

The concept of homeostasis originated from ancient Greek fundamentals that were established based on early attempts to understand balance, harmony, equilibrium, and the maintenance of a steady-state [1]. The idea that all living things are able to undergo constant change, and that stasis is unnatural, was first proposed by Heraclitus (540–480 BC) [1, 2]. Empedocles (495–435 BC) later hypothesized that matter consists of elements with qualities that are either actively aligned with or opposed to each other, and that their balance is intrinsic to the survival of living organisms. Hippocrates (460–375 BC) compared health to the harmonious balance of the elements, with disease arising due to a disrupted state of these elements [1, 2]. In 1865, Claude Bernard published his theory that maintenance of the body's internal environment, which directly affects the numerous cell populations that comprise a tissue or organ, is essential for an organism's survival [3]. Extrapolating on the work of Bernard, Walter Cannon in 1929 referred to the maintenance of inner balance as "homeostasis" [1, 3, 4], a process that maintains physiological variables such as temperature, pH, and blood pressure within defined parameters, with specific normal ranges that are preserved through synchronized adjustments in the internal environment. Cannon suggested that disruptions in homeostasis originate from physical changes in the external (due to injury or temperature extremes) or internal (due to pain or infection) environments, and could also be of psychological origin (due to emotional distress) [3]. Furthermore, Cannon speculated that the maintenance of homeostasis requires an internal communication network, with sensors that identify deviations from normal ranges and effectors that return any deviation back to within acceptable limits—in effect, a system of negative feedback. Negative feedback reduces the outcome of fluctuations by initiating mechanisms that restore a steady-state. Most biological processes rely on negative feedback, including the maintenance of blood pressure, thermoregulation, and the secretion of endocrine hormones. The latter is exemplified by regulated glucocorticoid release from the adrenal cortex. During this process, the hypothalamus secretes corticotropin-releasing hormone, signalling the anterior pituitary gland to secrete adrenocorticotropic hormone (ACTH), which not only directs the adrenal cortex to release glucocorticoids but also contributes to the control of circadian rhythms (reviewed in [5]). Glucocorticoids functions throughout the body. They also negatively affect the release of additional stimulating secretions from the hypothalamus and pituitary gland, acting in a self-limiting manner when physiologically relevant levels that are required to carry out desired functional effects are attained, including those involved in modulating metabolism and immune responses [5].

Understanding the dynamics between the human body and a complex pathological state such as cancer requires an appreciation of how the body itself maintains balance to ensure that key systems function within acceptable normal ranges. The maintenance of homeostasis is based on the coordinated interaction of several organ systems, including the liver, kidneys, and the circulatory, endocrine, immune, and central nervous systems. Together, their interactions underlie both the physical

and psychological aspects of homeostasis. For example, the liver maintains carbohydrate metabolism and the removal of toxins, while the kidneys regulate what enters into the circulation, filtering and excreting wastes and maintaining blood pH as well as water, salt, and iron levels. Disrupted homeostasis and changes in how negative feedback loops function may lead to the onset and progression of disease, or in severe instances, death. One of the major physiological outcomes arising from sustained activation of the body's main response systems is inflammation, which produces clinically discernible local and systemic effects. A local immune response is generally identified by several well-characterized signs of inflammation, while a systemic response is produced by sustained or permanent imbalances in energy intake and utilization, blood composition, extracellular fluid levels, thermoregulation, and disruptions in circadian rhythms (reviewed in [6]).

In the event of trauma, multifaceted physiological responses that occur simultaneously and often produce synergistic effects are initiated to return the body to a homeostatic state as rapidly as possible. The main factors that elicit a response are direct tissue injury, infection, hypovolemia or volume contraction (decreased blood volume), hypoxia, and hypercarbia (higher than normal levels of carbon dioxide in the blood) (reviewed in [7]). The sympathetic nervous system initiates direct and indirect actions through the release of noradrenaline from sympathetic nerves and adrenaline from the adrenal medulla, producing immunological, metabolic, and cardiovascular effects. Blood is diverted from the skin and visceral organs, heart rate and myocardial contractility increase, bronchodilation occurs, and the motility of the gastrointestinal tract slows. Upon initiation of an immune response due to tissue injury, sepsis, surgery, or other trauma, pro-inflammatory cytokines are released, including tumour necrosis factor alpha (TNF-α), interleukin (IL)-1, IL-2, IL-6, interferon, and prostaglandins. These factors in turn stimulate the production of acute phase proteins such as C-reactive protein (CRP) and complement [8]. The accumulation of cytokines in the systemic circulation may contribute to SIRS, systemic inflammatory response syndrome, thereby affecting the entire body in response to chronic infection [8]. The hypothalamic-pituitary-adrenal (HPA) axis controls the endocrine response, with biological stress or trauma increasing the production of ACTH and cortisol (reviewed in [5, 6]). Steroids and peptide hormones such as cortisol, aldosterone, vasopressin, histamine, and the thyroid hormone triiodothyronine (T3), as well as thyroxine (T4), its prohormone, affect a range of metabolic responses. Their catabolic actions increase protein breakdown, with insulin antagonism and an increase in glucagon levels producing a rise in blood glucose levels. During the cardiovascular response, vascular permeability is affected, and platelet activating factor (PAF) enhances the action of cytokines, nitric oxide, and prostaglandins to induce vasodilatation.

As mentioned, a key aspect of homeostasis is the maintenance of physiological stability in response to change, including stressful stimuli. Beyond maintaining the body's balanced state, this adaptive capacity, called allostasis, represents physiological or behavioural consequences arising due to chronic stress [9]. A measurable phenomenon, allostasis can be assessed by examining chemical imbalances that represent changes in the activity of the autonomic and central nervous systems as

well as the neuroendocrine (via the HPA axis) and immune systems [10]. Together with other stress-mediating physiological events, such as increased myocardial activity, cortisol and epinephrine (the major hormonal mediators of the stress response) have dual effects on the body. Short term, these hormones are required to manage acute threats in order to maintain homeostasis, thereby ensuring adaptation to change and an organism's continued survival. However, over prolonged periods and upon frequent activation, the resulting allostatic load, which can be thought of as "wear and tear" on the body [11], induces tissue damage and accelerates the onset of disease, also weakening the immune system, disrupting circadian rhythms, and inducing changes in otherwise plastic brain structures [12]. One could hypothesize that in a normally functioning, healthy body in which homeostasis is well maintained and the allostatic load is minimized, cancer would not be able to "take root", as cellular turnover, the normal maintenance of tissues and organs, and the return to a steady-state after exposure to stressful stimuli would be in equilibrium. It would be much more likely for cancer cells to initiate a cycle of aberrant division in an "unhealthy" individual, whose body is already experiencing homeostatic imbalances and is attempting to regain a steady-state, or is in the process of adapting to these physiological changes. In particular, failure to "turn off" stress responses due to delayed shutdown and inadequate responses that lead to compensatory hyperactivity of other mediators may increase the allostatic load, contributing to disease progression.

Many diseases arise due to homeostatic imbalances. Examples include congestive heart failure, stroke, arthritis, diabetes, and conditions such as gout and oedema that occur in response to excess levels of toxins that accumulate in the circulation. Prolonged disruption of the negative feedback loop that regulates blood glucose levels can be detrimental, as sustained high circulating levels of glucose may lead to insulin resistance, eventually progressing to diabetes. In addition, high fever and chronic inflammation may induce irreparable tissue damage and scarring that contribute to the onset of numerous diseases. Medical and therapeutic interventions aim to restore homeostasis, and to reverse or possibly prevent further tissue or organ damage, but there are instances when the effects of various traumas are permanent, leading to lasting complications, including cancer.

Recently, there has been considerable speculation into whether inflammation, metabolic syndrome (obesity, diabetes, and atherosclerosis), and cancer are causatively linked. It has proven difficult to establish whether, for example, breast cancer in post-menopausal women contributes to the onset of diabetes, or whether insulin resistance and hyperglycaemia underlie the onset and progression of breast cancer. Certain contagious conditions such as viral infections may also influence the risk of developing cancer. Cancer itself is not typically classified as an infectious disease—it is currently thought that a healthy individual cannot "catch" cancer from someone diagnosed with this malignancy [13], with the potential exception of colorectal cancer, which is induced by specific changes in microbial gut populations. Rather, cancer has been attributed to an individual's genetic predisposition, smoking, diet, physical activity, or exposure to UV rays, radiation, and other environmental carcinogens including chemicals and infectious microbes (viruses,

bacteria, and parasites). Cancer itself could be thought of as an adaptive mechanism —maladaptive, but nevertheless functional. This notion is supported by the finding that, rather than undergoing apoptosis, necrosis, or being cleared by the immune system, precancerous cells continue to divide under what are deemed to be physiologically unfavourable conditions, despite having been exposed to damage by some means or sustaining perturbations in their repair mechanisms. The hallmarks of cancer, summarized in the landmark 2000 work of Hanahan and Weinberg [14], are based on the ability of cancer cells to (1) self-stimulate their proliferation and growth, (2) withstand inhibitory signals that would otherwise stop these processes, (3) resist programmed cell death (apoptosis), (4) promote the development and growth of blood vessels that supply nutrients to tumours (angiogenesis), (5) multiply indefinitely, and (6) invade local tissue and spread to distant sites (metastasis). It has been pointed out that five of these hallmarks are also characteristics of benign tumours, and that the only truly significant property of malignant disease is the ability of cancer cells to metastasize to distant sites and invade tissue [15]. In an updated 2011 review, four additional hallmarks of cancer were proposed [16], including (1) deregulated metabolism (given that most cancer cells generate energy utilizing abnormal metabolic pathways, as first suggested in the Warburg hypothesis [17], a topic that is now gaining renewed research interest [18]), (2) evasion of the immune system, (3) genomic instability, especially since cancer cells accumulate a high number of chromosomal abnormalities during disease progression, and (4) inflammation. It is clear that an individual's genotype, and a genetic predisposition to certain cancers due to mutations in or aberrant expression of key genes such as p53 (among many others) play a major role in the onset and progression of various different cancers. However, regardless of the phenotypic and genotypic properties of a cancer cell, it is the body's adaptation to perturbations in normal physiological processes that induces sustained homeostatic imbalances, which has definitive links with cancer and will be the focus of this chapter.

Chronic Inflammation as an Underlying Cause of Cancer

The body's response to cancer is not based on a single unique mechanism, although there are parallels that can be drawn with the processes of wound healing and tissue inflammation. Each unique tissue microenvironment that is able to sustain the eventual growth of a tumour relies on interactions between epithelial and stromal cells. An array of different cell types are present in and around a tumour, including fibroblasts, vascular cells, mesenchymal support cells, and leukocytes [19], the latter representing innate and adaptive immune cells of the myeloid and lymphoid lineages, respectively. Innate immune cells, including macrophages, granulocytes, mast cells, dendritic cells, and natural killer (NK) cells are thought of as the "first line of defense" against foreign agents that induce tissue damage. Numerous studies have underscored the important causative role of leukocytes in the development of cancer [20–24]. When homeostasis is disrupted, mast cells and macrophages

residing within damaged tissue secrete cytokines and chemokines that attract circulating leukocytes to the injured site, eliciting local inflammation [21, 25, 26]. The recruited innate immune cells are then able to eliminate pathogens directly. In addition, after taking up foreign antigens, dendritic cells migrate to lymphoid organs and present the material to adaptive B or T lymphocytes, which expand clonally and mount a targeted response against the foreign agent [27, 28]. Upon its successful elimination, inflammation typically subsides, concomitant with the restoration of tissue homeostasis. However, the mechanisms that enable the body to mount an immune response may also promote tumorigenesis. Rudolf Virchow was the first to postulate in 1863 that cancer originates at the site of chronic inflammation, which was based on his hypothesis that classes of inflammation-inducing irritants also enhance cell proliferation [20, 29]. Inflammatory and proliferative events only abate following the removal of an irritant, or when the process of tissue repair has been completed. Clearance of damaged cells is generally mediated through the activation of cell death pathways, including apoptosis, with upregulated proliferation serving as a means to support tissue regeneration and the return to homeostasis. The persistent and sustained presence of an irritant may increase the risk of cancer, as continued unneeded cycles of cell death and proliferation within an inflamed environment containing high levels of cytokines and other pro-inflammatory substances promote tumour development and progression [21]. Of note, inflammatory fibroblasts, as well as fibroblasts undergoing senescence, have been shown to contribute to tumour initiation, providing a potential link between inflammation, ageing, and cancer [30, 31]. Upregulated expression of specific sets of genes, especially those encoding secreted proteins such as cytokines, chemokines, and transforming growth factor β (TGF-β), occur in inflammatory and senescent fibroblasts, as well as in tumour-associated fibroblasts. Rheumatoid arthritis-derived inflammatory fibroblasts have been shown to enhance tumour growth and invasion to a greater extent than cancer-associated fibroblasts in a xenograft model of human ductal carcinoma in situ [32].

Greater than 20 % of all malignancies are now thought to arise due to the effects of chronic inflammation [33], supporting the notion that the microenvironment and selection of specific characteristics in "initiated" cells promote their malignant potential. A model for inflammation-associated carcinogenesis has been proposed based on the principles of tumour initiation, promotion, and progression/invasion (reviewed in [34]). Generally, the longer inflammation persists, the higher the risk of developing cancer. Initiation is the period during which sporadic or inherited genetic changes in critical genes irreversibly alter the division, survival, differentiation, or adhesion properties of a normal cell. Initiation may occur when DNA damage is induced by nitric oxide, reactive oxygen species (ROS), or prostaglandin E_2, which are all substances that are derived from inflammatory cells in response to microbial infections or chronic inflammation [33, 34]. Indeed, nitrosative and oxidative stress-mediated signalling mechanisms play a central role in inflammation and tissue injury [35], and have been associated with various disease states including cancer, obesity, heart disease, and diabetes. High levels of reactive nitrogen and oxygen intermediates induce DNA damage directly by oxidation or

indirectly by interfering with DNA repair mechanisms [36]. Inflammation and metabolic changes that culminate in permanent shifts in the cellular energy balance increase oxidative stress due to the accumulation of free radicals and their active intermediates. Once the body's ability to effectively detoxify and eliminate excessive levels of these substances through endogenous antioxidant defense mechanisms is compromised, which may occur as a result of imbalances in the activity of pro-oxidant and antioxidant enzymes, neoplastic transformation proceeds. Accumulating free radicals further recruit inflammatory cells, creating a "vicious" cycle. Reactive substances also affect proteins, carbohydrates, and lipids, with their respective derivative products inducing significant perturbations in intra- and intercellular homeostasis, culminating in lasting genotypic changes. An interesting molecular link between chronic inflammation, DNA damage, and cancer is the aberrant upregulation of microRNA-155 (miR-155), which has been implicated in inflammatory processes and is associated with the development of leukaemia, breast, gastric, and lung cancers [37, 38]. Pro-inflammatory stimuli experimentally increase the expression of miR-155, which in turn increases the spontaneous mutation rate by affecting levels of key DNA repair proteins [39]. The authors of the study hypothesize that, upon exposure to a pathogen or noxious substance, cells react rapidly, producing a robust immune response during which cell cycle checkpoints are "put on hold" due to the upregulation of miR-155. This process serves to effectively clear foreign antigens but may also lead to increased genomic mutations that remain fixed during subsequent cell divisions. The number of steps required to induce tumorigenesis during states of chronic inflammation would thereby be shortened, as levels of miR-155 would remain continuously elevated. Regardless of the contributing mechanisms, accumulating DNA damage increases the chance for a tumour-initiating cell with defects in oncogenes or tumour suppressors to emerge, but it is not sufficient to drive the further development of cancer. To effectively promote tumorigenesis, increased cellular turnover that occurs in response to tissue damage and intercellular communication within a network of diverse cell types are also required. Cellular turnover occurs at a high rate to compensate for cell death, which typically arises in response to both non-infectious injuries and infections. As already mentioned, periods of re-population draw on undifferentiated precursors that survive a disturbance within a given tissue niche. These cells are able to undergo sufficient expansion to re-establish and maintain proper tissue function. Compensatory repair processes that help to restore homeostasis require mediation from inflammatory pathways. During tumour promotion, it is thought that signalling through inflammatory mediators, including cytokines derived from non-cancerous cells, selects for a population of immortalized cells that no longer respond to growth inhibition, apoptosis, or innate immune sensing, leading to tumour progression and invasion (reviewed in [34]). Therefore, cancer cells could be viewed as undergoing a sort of evolutionary process.

Oxidative Stress and Dysregulated Homeostasis

Oxidative stress occurs in response to a disrupted balance between levels of ROS and reactive nitrogen species and the endogenous antioxidant capacity. It underlies ageing and most critical illnesses, and is also associated with a poor prognosis [40, 41]. While free radicals play an important role in normal physiological functions (reviewed in [42]), oxidative stress often leads to chronic inflammation, which in turn affects the onset and progression of disease. The mechanisms underlying these processes converge on common signalling pathways that are activated by ROS, thereby inducing key transcription factors including AP-1, WNT, HIF-1α, NF-κB, STAT3, p53, and PPAR-γ [43–48]. The resulting aberrant changes in expression of inflammatory cytokines, anti-inflammatory molecules, chemokines, growth factors, and cell cycle regulators induces normal cells to change, also affecting their continued survival, proliferation, metastasis, invasion, angiogenesis, and chemo- and radioresistance. A hallmark of viral and bacterial infections, the increased generation of reactive oxygen and nitrogen species modulates host cell permissiveness to viral replication, affects host immune responses, and induces oxidative tissue damage [48]. Obesity also stimulates systemic oxidative stress, which has been linked with aberrant production and release of adipocyte-derived cytokines and the development of metabolic syndrome [49]. Traumatic injury due to a wound caused by an external source also induces oxidative stress, thereby contributing to peroxidation of cellular and vascular structures, protein oxidation, DNA cleavage, and inhibition of the mitochondrial electron transport chain.

Connecting Infectious Microbial Agents and Cancer

Historically, leukocytes found in the proximity of developing tumours were thought to represent the body's first attempt at eliminating transformed cells. It has now been shown that specific populations of leukocytes, including cytotoxic T lymphocytes (killer T cells) and NK cells, play a key role in immunosurveillance and the prevention of tumorigenesis, and it has been suggested that, based on the action of these cell types, the incidence of precancerous growths may be considerably higher than those that eventually progress into malignant forms of the disease [50]. Epidemiologic data support the notion of effective immune system containment, as virally-associated cancers are more prevalent in immunocompromised individuals with acquired immunodeficiency disease (AIDS) or patients who have received an organ transplant [51, 52]. There is also a higher incidence of carcinogen-associated cancers, including melanoma and lung adenocarcinoma, in immunocompromised transplant patients [53, 54]. Interestingly, based on epidemiological evidence, the incidence of breast adenocarcinoma and other epithelial cancers is lower in immunocompromised women [55, 56]. Together, these sets of data suggest that the

overall risk for developing cancer may, in part, be regulated by the state of an individual's immune system.

During the early twentieth century, certain infections were experimentally shown to directly induce cancer in animal models, exemplified by murine moloney leukaemia virus in mice and the formation of sarcomas following injection of Rous sarcoma virus into chickens (reviewed in [57]). More recently, viral, bacterial, and parasitic infections have been identified as potential risk factors that contribute to the development of several types of human cancers. According to the American Cancer Society, up to 20 % of all cancers worldwide have been linked to infectious diseases caused by microbes, with a higher incidence in developing countries due to, at least in part, shortcomings in sanitation practices and access to clean water, as well as inadequate health care and a lack of standardized vaccination protocols. Microbial infections contribute to tumorigenesis in several ways. Foreign microbes are able to induce chronic inflammation, alter cellular DNA, or suppress, and thereby evade detection by, the immune system. In response to infectious microbes, the host immune system initiates a cascade of inflammatory events. As already mentioned, while inflammation serves to control infection, leukocyte-derived substances such as cytokines can induce damage to DNA, proteins, and cell membranes. During a persistent infection, inflammation may become chronic, resulting in continued damage and the accumulation of further genetic changes. Several types of microbes, particularly viruses, invade human cells and directly interact with cellular DNA by incorporating their genome into that of the host. This type of interaction has the potential to activate oncogenes, promoting cancer growth, or to inactivate tumour suppressor genes that act as cellular checkpoints and serve to prevent aberrant cell division. Viruses may also suppress the host immune response, reducing the efficiency of the immune system recognizing cells that are infected with cancer-causing viruses or cancer cells themselves. Microbial infections often enable cells to undergo rapid proliferation and to survive for prolonged intervals, and these changes may eventually "turn" normal cells into cancer cells.

Viruses

Humans are exposed to a multitude of genetically diverse viruses, with new genotypes and strains continuously evolving, and new species still being discovered [58]. Bacterial, plant, and animal cells, and material in the human intestine, also carry viruses. The human virome represents all viruses that are present in the human body at a given time, including those causing acute, chronic, or latent infections, as well as those that are permanently integrated into the host genome [59–61]. The virome of each individual is unique and undergoes rapid changes [60] in a manner dependent on age, geographic location, the season, lifestyle choices, and host disease susceptibility, which may be affected by genetics and pre-existing immunity [62].

Viruses are composed of DNA or RNA encoding a key set of genes surrounded by a protein coat. After gaining entry into a host cell, they utilize the cellular

machinery to replicate. In the case of retroviruses, the viral genetic material becomes integrated into the host genome, potentially altering gene expression in a manner dependent on the chromosomal insertion point. Viruses that cause mucosal infections have been implicated in the onset of type 1 diabetes, inflammatory bowel disease, and asthma [63], among other diseases. These types of infections affect a large percentage of the population, but are generally asymptomatic. Several viruses have also been definitively linked with causing cancer in humans, prompting vaccine development. However, vaccines are only effective when administered prior to an individual's exposure to a cancer-causing virus. Although many viruses are suspected to be associated with cancer, prolonged latency and the contribution of other risk factors to this complex disease has made it difficult to establish definitive causality. Indeed, the majority of viruses do not initially cause disease in healthy individuals, and it is only when the immune system is under- or overactive that symptoms underlying a persisting latent infection become apparent. It is therefore imperative to better understand host interactions with the human virome. An increasing number of common viral infections are now being identified as contributors to, if not causative agents of, the pathogenesis of multifaceted diseases such as cancer, and several relevant examples are discussed here.

Human Papilloma Viruses (HPVs)

Spread by contact, HPVs are a group of more than 100 related DNA viruses that infect keratinocytes of mucous membranes, producing lesions on the skin, mouth, larynx, and genitals [64, 65]. HPVs may be causative of anogenital epithelial cancers and cancers of the head and neck [65] and are the underlying cause of cervical cancer, the second most common cancer in women worldwide. In the majority of cases, the host immune system effectively controls the infection or clears it over time, and most infected individuals never develop cancer [64]. Due to the availability of the Pap test, which detects precancerous changes in cervical cells following HPV infection, the incidence of cervical cancer has declined significantly in developed countries. As there is no effective treatment other than the removal of virally infected cells, it is recommended that women testing positive for HPV be screened more regularly for the presence of abnormal cells. HPV infections often occur concomitant with *Chlamydia trachomatis* [66], suggesting that other microorganisms may have synergistic pathological effects. Two clinically approved vaccines, Gardasil and Cervarix, are currently being used to prevent infection with cancer-causing types of HPV in girls, boys, and young women and men. Because it may take decades for cancer to develop after an initial HPV infection, the efficacy of these vaccines remains to be determined.

Epstein-Barr Virus (EBV)

EBV, also known as human herpesvirus 4, is contracted by the oral transfer of saliva and genital secretions [67]. It infects epithelial cells and B lymphocytes, and in in vitro studies, EBV directly immortalized B cells [68]. The site of sustained infection is thought to be bone marrow, as EBV-positive patients receiving bone marrow transplants from negative donors were also EBV-negative after transplantation [69]. EBV has been associated with infectious mononucleosis [70] and certain autoimmune diseases such as rheumatoid arthritis and multiple sclerosis [71], as well as several types of cancer including Burkitt's lymphoma, Hodgkin's disease, non-Hodgkin's lymphoma, lymphomas, nasopharyngeal carcinoma, and certain cases of stomach cancer, as well as potential breast epithelial malignancies [72]. It may also underlie central nervous system lymphomas associated with HIV [73]. Although most individuals gain adaptive immunity, EBV infection is common and persistent, with the National Center for Infectious Diseases estimating that half of all 5-year-old children in the United States and over 90 % of the global population present evidence of a latent infection. Discovered in 1964 [74], it was the first human virus directly linked to carcinogenesis, although EBV-host interactions are not yet fully understood. Recent genomic studies of the virus have explored its lytic reactivation and regulation of the latent viral episome [75].

Hepatitis B and C Viruses (HBV and HCV)

HBV and HCV, unrelated hepatotropic viruses that cause viral hepatitis, are also linked with chronic infections that increase the risk of developing liver cancer [76, 77]. According to the American Cancer Society, approximately one-third of liver cancers are attributed to HBV or HCV infection in the United States, a number that is considerably higher in other countries where both viral hepatitis and liver cancer are more prevalent. HBV and HCV are transmitted through bodily fluids. Of the two viruses, infection with HBV causes flu-like illness and jaundice, while infection with HCV may not produce symptoms for decades. Most individuals recover completely from HBV infection within several months, with only a small percentage becoming chronic carriers who are at higher risk for developing liver cancer. Therapeutic and preventative measures may be used to slow liver damage and cirrhosis, reducing the risk of developing cancer. Long-term treatment of chronic HCV infection may help to reduce the viral load to near undetectable levels, and several drugs are also used to treat chronic hepatitis B, although they do not eliminate the virus. An effective vaccine against HBV is available, which is recommended for all at-risk individuals including healthcare workers, and in the United States, vaccination to prevent hepatitis B has also been routinely recommended for infants since 1991 [78].

Human Immunodeficiency Virus (HIV)

HIV, a virus that is spread through blood, semen, vaginal fluids, and breast milk, infects and destroys helper T cells and is the causative microbial agent of AIDS. While infection with HIV does not appear to directly cause cancer, other viruses such as HPV and EBV may induce greater levels of cellular damage, potentially contributing to the onset and progression of cancer in a shorter period of time. This is especially relevant given that the immune system plays an important role in attacking and destroying newly formed cancer cells, and compromised immunity likely facilitates the prolonged survival of new cancer cells. HIV infection has been linked with an increased risk of developing Kaposi sarcoma, invasive cervical, lung, liver, mouth and throat, and skin (basal, squamous, and Merkel cell) cancers, as well as Hodgkin, non-Hodgkin, and central nervous system lymphomas [73, 79–82]. Anti-HIV drugs based on antiretroviral therapy potentially reduce the risk of developing these types of cancers.

Human Herpes Virus 8 (HHV-8)

HHV-8 is associated with persistent infections that may present with no outward signs of disease, and is transmitted through sexual contact, blood, and saliva [83]. HHV-8 is also known as Kaposi sarcoma (KS)-associated herpes virus (KSHV) and has been found in a majority of tumours derived from patients with KS, a rare, slow-growing cancer that often appears in the form of visible tumours just beneath the surface of the skin. KS arises when HHV-8 infects the cells lining the blood and lymph vessels. Occurring most commonly in Central Africa and the Middle East, the incidence of KS was initially low in developed countries until it began to be detected in AIDS patients in the early 1980s [51]. The number of infected individuals has since dropped in the United States, most likely due to better treatment options aimed at managing HIV infection [84]. In the United States, the majority of patients who develop KS also present with other conditions that affect immune function, including HIV infection or immunosuppression following organ transplantation. HHV-8 has also been linked to rare blood cancers, such as primary effusion lymphoma, and has been detected in patients with multicentric Castleman disease, an overgrowth of lymph nodes that commonly develops into lymphoma.

Human T-Lymphotrophic Virus-1 (HTLV-1)

HTLV-1, a retrovirus, is spread through the same routes as HIV and has been linked with adult T-cell leukaemia/lymphoma (ATL), a type of lymphocytic leukaemia and non-Hodgkin lymphoma [85]. This cancer is most prevalent in southern Japan, the Caribbean, Central Africa, and parts of South America. Once infected with HTLV-1, the incidence of adult T-cell lymphoma is estimated to be as high as 5 %, usually following a prolonged latency of 20 or more years [86]. As a means to

significantly control HTLV-1 infection, all blood donated in the United States is screened for HTLV-1, a practice that has substantially reduced the risk of infection resulting from transfusion.

Merkel Cell Polyomavirus (MCV)

MCV was discovered in 2008 in tissue samples from several cases of a rare and aggressive type of skin cancer, Merkel cell cancer, which, based on its origin, has been classified as a neuroectodermal tumor [87]. Of note, skin cancer is classified into three common types, basal cell and squamous cell carcinomas of the skin (also referred to as non-melanocytic skin cancer), and cutaneous malignant melanoma. A large number of individuals are thought to be infected with MCV, which is generally asymptomatic, with the vast majority of first exposures and primary infections occurring in early childhood [88]. Although MCV has been detected in normal skin, respiratory secretions, saliva, and the gastrointestinal tract, the route of transmission remains to be definitively established [89]. The correlation between cancer and MCV infection is high, as 8 out of 10 Merkel cell cancers are linked with this infection [87]. Associated with exposure to UV light, Merkel cell cancer arises in only a small number of those infected with MCV, particularly in older or immunocompromised individuals [90]. Interestingly, in patients with Merkel cell cancer, the virus is rendered non-transmissible due to mutations in its DNA [91]. Avoiding excessive sun exposure may help to prevent MCV mutations that are associated with an increased risk of developing Merkel cell cancer among those already infected with the virus, especially since epidemiological and experimental evidence has causatively linked UV radiation via sun exposure with skin cancer [92]. MCV may also be linked with non-melanoma skin cancer [93] and other cancers, including cervical squamous cell and adenocarcinomas [94], extrapulmonary small cell carcinomas [95], as well as Bowen's disease and epidermal growth factor receptor mutation-driven non-small cell lung cancer [96].

Viruses with Uncertain or Unproven Links to Human Cancers

Simian virus 40 (SV40), a DNA polyomavirus that infects monkeys and humans, has been suggested to increase the risk of developing several types of cancer. This notion initially arose due to the finding that batches of polio vaccine prepared from monkey cells between 1955 and 1963 were contaminated with SV40, an incidence that was linked with an increased risk of developing certain brain and bone cancers, lymphomas, as well as mesothelioma, a rare cancer of the lining of the lungs or abdomen [97]. The accuracy of this correlation has since been questioned, and retrospective studies aimed at investigating the potential link between the

contaminated polio vaccines and cancer have failed to establish an increased incidence of mesothelioma or other cancers among individuals who were inoculated as children [97, 98]. Although SV40 does cause mesothelioma and sarcomas in infected hamsters [99], and infection with the large T antigen of the virus can induce brain tumours in rats [100], the role of SV40 in the development of cancer in humans remains under debate.

Parasites

Opisthorchis viverrini and *Clonorchis sinensis* are liver flukes, types of flatworm that have been linked with an increased risk of developing cholangiocarcinoma, or cancer of the bile ducts [101]. Infections with these parasites arise from eating raw or undercooked freshwater fish and occur most commonly in East Asia. Infection with *Schistosoma haematobium* (*S. haematobium*), a flatworm found in the water of developing countries of the Middle East, Africa, and Asia, is estimated to occur in over 200 million people worldwide and has been associated with bladder cancer as well as a rare lesion that can arise in *S. haematobium*-infected patients, resulting in squamous and adenosquamous prostate cancers (reviewed in [102]). Possible links to other types of cancer are also under investigation. The incidence of central nervous system infection with *Toxoplasma gondii* (*T. gondii*), a long-lived protozoan parasite that causes toxoplasmosis, is high in HIV-positive patients [103]. Based on statistical correlation studies, *T. gondii*, which encysts in the brain, eliciting inflammatory responses and inhibiting apoptosis, has been predicted to increase the risk of brain cancer [104, 105]. It is likely that parasitic infections are associated with the onset and progression of various types of cancer based on an individual's immune status, which can be affected by age, geographical location, pre-existing infection with other viruses (an individual's virome), or chronic inflammation.

Bacteria

A potential link between bacteria and cancer was identified as early as two hundred years ago, with Robert Koch and Louis Pasteur reporting the presence of bacteria at the site of tumours. William Russell described his discovery of a cancer parasite in 1890 [106], although his theory that bacteria are able to initiate tumour growth was largely rejected during the early twentieth century due to his findings being based on circumstantial evidence. However, recent research supports his claims that bacteria, by interacting with the host immune system, contribute to the onset and progression of cancer. The interaction between bacteria and the immune system commonly occurs at mucosal interfaces, including the mouth and throat, stomach

lining, digestive and intestinal tracts, and reproductive tract, which are common sites of virally and bacterially-induced tumours.

Helicobacter Pylori (H. Pylori) and Stomach Cancer

Chronic infection inflames and damages the inner lining of the stomach, leading to the development of ulcers. The underlying cause of gastritis and peptic ulcer disease (PUD) was thought to be a bacterium with an affinity for acidic environments including the stomach, which was later identified as *H. pylori* [107]. Left untreated, inflammatory changes that arise due to ulceration may progress to cancer [108]. Approximately one in three adults may be infected with *H. pylori*, and it has been estimated that this bacterium is present in the stomach and upper gastrointestinal tract of approximately 50 % of the global population [109]. *H. pylori* is transmitted by either the faecal-oral route, exemplified by contaminated food or water, or mouth to mouth [109]. *H. pylori* infection has been linked to more than half of all cases of stomach cancer, the fourth most common cancer worldwide, and certain types of stomach lymphomas and adenocarcinomas [110]. In addition, infection with *H. pylori* was recently linked with the formation of colorectal polyps and colorectal cancer [111]. These associations have led the World Health Organization to classify *H. pylori* as a carcinogen. Other factors contribute to the onset of stomach cancer, including the presence of dietary nitrites, which can be converted by bacteria, including *H. pylori*, into compounds that induce stomach cancer in animal models. *H. pylori* infections are effectively treated with antibiotics, and their administration to patients following stomach cancer surgery has helped to prevent the onset of new lesions, also eliminating the infection in individuals with PUD.

Chlamydia Trachomatis (C. Trachomatis) and Increased Cancer Risk

C. trachomatis is a common sexually transmitted bacterium that not only infects the female reproductive system, causing pelvic inflammation and potentially leading to infertility due to scar tissue formation in the Fallopian tubes and other parts of the body, including the eye, in both women and men. Infection with *C. trachomatis* is often asymptomatic, persisting for years until detected and treated with antibiotics. Studies have suggested that women whose blood tests positive for chlamydia may be at greater risk for developing cervical squamous cell carcinoma [112]. While chlamydia itself may not cause cancer, together with HPV it may induce the proliferation of cancer cells by temporally affecting the lytic stage of cancer-promoting HPV in the cervix [113].

Perturbations in Gut Microbes

While a high number of bacteria occupy the human gastrointestinal tract, the normal microbial population does not evoke an inflammatory host immune response. Gut homeostasis is maintained by a symbiotic relationship that initially developed through the co-evolution of distinct mechanisms from both the host and bacterial communities. Intestinal cancers such as colorectal cancer are thought to arise when these homeostatic mechanisms are disrupted, or upon introduction of pathogenic bacteria that secrete specific molecules, inducing tumour-promoting signalling cascades or triggering an inflammatory immune response. It is generally accepted that activated pro-inflammatory pathways sustain the growth of cancer cells. Research also supports that gut bacteria modulate the host immune system, thereby influencing tumour initiation. In a rodent model of colorectal carcinogenesis, intestinal microbes were shown to directly influence host immunity and tumour susceptibility, which was demonstrated in microbe-deficient rats genetically predisposed to cancer [11]. DNA damage and the development of hepatic carcinoma have been attributed to the direct action of deoxycholic acid, a metabolite of *Clostridium Cluster* IX bacteria that has also been linked with obesity [12]. *Fusobacterium nucleatum* (*F. nucleatum*) affects the onset and progression of colorectal cancer by two mechanisms. It indirectly initiates a pro-inflammatory immune response involving interleukin (IL)-6, IL-8, and IL-18 [114]. In addition, *F. nucleatum* directly contributes to intestinal tumorigenesis by promoting epithelial cell proliferation involving the binding of its FadA adhesin to E-cadherin, in turn initiating β-catenin-mediated signalling [115]. Imbalances in the host innate immune system that arise due to defects in pattern recognition receptors (PRRs), which include Nod-like and Toll-like receptors, disrupt host immune sensing. PRRs evolved as a rapid response to pathogens and control the homeostatic balance between diverse populations of gut flora. They prevent certain types of microbes from proliferating excessively, which, if otherwise left unchecked, would contribute to a state of chronic inflammation. An example of a Nod-like PRR is NLRP6, which protects gut homeostasis by defending the host against shifts in bacterial populations [116]. NLRP6 also senses and controls potentially pathogenic species, protecting against the development of colitis-associated cancer [117]. Interestingly, a deficiency in Nod2, another PRR, contributes to inflammation-mediated colorectal cancer in mice presenting with perturbations in their normal gut flora, which was also found to be transmissible [118]. Mice deficient in toll-like receptor 2 or 5 developed an altered gut flora and increased levels of intestinal inflammation [119]. To effectively regulate immune and metabolic homeostasis, several highly conserved pathways have evolved and become integrated to respond to pathogens and changes in nutrient levels. Innate PRRs sense excessive nutrients as potential "danger" signals by influencing the intestinal microbial population, and changes in the interactions between PRRs, gut microbes, and an individual's diet have been linked with obesity and other related diseases [119]. The potential for resident bacteria and viruses that comprise the microflora of the human gut to be altered by dietary changes is exemplified by a study that identified a significant relationship

between diet and the presence of distinct types of bacteriophages. This was accomplished by comparing the distance between bacteriophage gut communities in individuals before and after they initiated consumption of a controlled diet. In individuals on the same diet, convergence of their viromes was observed [120].

Cancer and Metabolic Syndrome: Obesity, Physical Activity, Insulin Resistance, and Atherosclerosis

The association of systemic inflammation with obesity, insulin resistance, diabetes, and atherosclerosis, together referred to as metabolic syndrome, is the subject of intense research. Physical activity, diet, and obesity are common risk factors associated with cancer and several other diseases [121]. Increased abdominal adiposity is associated with type 2 diabetes, cardiovascular disease, post-menopausal breast cancer, colorectal cancer, dementia, and overall mortality in a manner independent of body mass index, therefore also including individuals with an overall normal body weight [122]. The common link underlying these conditions is prolonged systemic inflammation, suggesting that a disrupted steady-state arising in response to chronic inflammatory diseases may be connected to cancer. Research-based evidence suggests that initiation, promotion, and progression of cancers are processes stimulated by the systemic elevation of pro-inflammatory cytokines, as well as an elevation in ROS, thereby contributing to chronic inflammation.

Obesity

Abdominal fat deposition and waist circumference have been significantly correlated with systemic inflammatory responses [123]. Beyond storing excess nutrients, adipose tissue acts as an endocrine organ, with adipocytes secreting hormones and cytokines, thus promoting a state of chronic inflammation [124]. Inflammatory cells produce free radicals and their soluble mediators, examples of which include metabolites of arachidonic acid, cytokines, and chemokines that in turn produce ROS and reactive nitrogen species. In obese individuals, several inflammatory markers are elevated, including TNF-α, IL-6, IL-8, IL-18, leptin, insulin, blood glucose, and CRP [125–128]. Leptin, an adipocyte-derived hormone, is secreted by white adipose tissue. It acts directly or by inducing signalling in numerous brain regions, including the hypothalamus, as a means to signal satiety (to decrease food intake), to increase energy expenditure, to modulate glucose and fat metabolism, and to alter neuroendocrine function, thereby affecting diverse feedback loops and influencing a range of physiological processes in addition to metabolism (reviewed in [129]). CRP, a non-specific inflammatory marker, increases in response to

systemic inflammation and may be one of the primary defense mechanisms of the human body, with even its mild elevation associated with an increased risk of cardiovascular disease, including myocardial infarctions, hypertension, and stroke, as well as muscle weakness and fragility [130]. Like body temperature, it may predict homeostatic imbalances, and it has been proposed as a marker to assess overall wellness or "quality of life", especially given that physical activity and a healthy diet reduce serum CRP levels, with the inverse occurring in those who smoke, present with a high body mass index, or become infected with bacteria and fungi [130].

When subcutaneous fat becomes inflamed, adipocytes undergo apoptosis or necrosis, impairing their ability to store additional fat, which is instead deposited as ectopic visceral fat. This "inappropriate" storage of fat is thought to stimulate systemic inflammation. In a process referred to as lipolysis, expanded fat cells leak their contents or break open, resulting in the mobilization of macrophages that embed themselves into adipose tissue and release TNF-α and IL-6, recruiting more leukocytes and compounding the existing inflammatory state induced by cytokines that have already been released by adipocytes (reviewed in [131]). In this manner, increased adiposity and inflammation form a cycle that dramatically increases IL-6 levels in a resting individual by as much as 10–35 %, with levels increasing further as adiposity increases [132]. Hyperglycemia also induces the production of IL-6 from endothelial cells and macrophages [133], and meals high in saturated fat or with a high caloric content have been associated with increases in inflammatory markers [134, 135]. While inflammatory responses are generally acute and occur in response to an incidence of overeating, inflammation may become chronic if overeating also develops into a chronic behaviour. In clinical studies, obese patients on calorie-restricted diets presented with reduced levels of pro- and increased levels of anti-inflammatory molecules within 4 weeks of initiating dietary intervention [136], and in initially obese women, elevated levels of IL-6 were reduced in both serum and subcutaneous adipose tissue following weight loss [125]. In mouse models of obesity, a state of chronic inflammation develops concomitant with the upregulation of macrophage-specific genes in white adipose tissue, lipolysis and the formation of multinucleate giant cells occur, and circulating insulin levels increase, all contributing to obesity-related insulin resistance [137]. It has been shown in mice that levels of angiopoietin-like protein 2 (Angptl2), which is highly expressed in visceral white adipose tissue, are elevated in obese animals, and circulating Angptl2 has been proposed as an inflammatory mediator linking obesity to insulin resistance, also initiating an inflammatory cascade that causes vascular remodelling and the recruitment of macrophages to fat deposits [138]. This is similar to immune cells amassing at sites where neoplastic cells are present. Interestingly, increased expression of Angptl2 in skin has been shown to not only promote chronic inflammation and oxidative stress but also to accelerate tumorigenesis in a mouse model of chemically induced squamous cell carcinoma, increasing "preneoplastic change" and the "malignant conversion" of normal cells due to disrupted DNA repair mechanisms [139, 140].

Obesity is prevalent in many Western nations, as is the marked increase in the incidence of oestrogen receptor (ER)-positive breast cancers [141] and other cancers linked with this hormone, including endometrial and uterine cancers. Breast cancer has been statistically correlated with obesity and circulating oestrogen levels [142]. Aromatase activity in the adipose tissue of obese post-menopausal women is significantly higher than in their normal-weight counterparts, and these women also present with higher levels of circulating estradiol [143]. However, it should also be noted that studies have emerged demonstrating that exogenously administered oestrogen reduces the incidence of mammary tumours in several genetically distinct mouse models by up to 70 %, providing an age-independent protective effect [144]. In addition, others have shown that a cell does not metabolize all forms of oestrogen and oestrogenic compounds equally, and that the intermediates of "carcinogenic" oestrogens such as 17α-ethinylestradiol produce ROS, thereby inducing cellular DNA damage [145]. A lack of endogenous oestrogen may promote inflammation, which is countered by oestrogen replacement using bio-identical forms of the hormone, suggesting that estradiol opposes the inflammatory process [146]. It is therefore possible that inflammation and "carcinogenic" forms of oestrogen, and not oestrogen itself, may contribute to the onset of breast cancer in post-menopausal women. Indeed, the risks associated with hormone replacement therapy (HRT) remain under debate [147], although synthetic progestins (Provera) may be responsible for many of the detrimental side effects [148].

Obesity and metabolic syndrome have been associated with lower-than-normal levels of oestrogens in post-menopausal women and in ovariectomized animals [149, 150], with HRT countering metabolic dysfunctions [151]. Mutations in the oestrogen receptor-α (ERα) gene lead to energy imbalances that manifest as obesity in women and female mice [152, 153], eliciting effects similar to those reported in post-menopausal women and ovariectomized animals. Genistein, a potent phytoestrogen that binds α and β oestrogen receptors, thereby regulating the divergent intracellular signalling cascades of oestrogen, is also able to competitively inhibit various-ATP utilizing enzymes and has shown promise as a therapeutic option to counter oxidative stress, inflammation, cancer, diabetes, obesity, osteoporosis, and neuropathy [154]. It has been shown that obesity may be initiated by changes in oestrogen/ERα signalling in two distinct neuronal populations of the hypothalamus [155], a brain region that links the nervous and endocrine systems to regulate multiple physiological processes including metabolism, appetite, and the distribution of body fat. In this particular study utilizing four distinct mouse models [155], brain-specific deletion of ERα in female mice resulted in increased food intake and decreased energy utilization, concomitant with increased storage of abdominal fat. Mice lacking ERα in steroidgenic factor-1 (SF-1) hypothalamic neurons expended less energy, indicative of a lower metabolic rate, and presented with increased abdominal adiposity. While increased food intake was observed in mice lacking ERα in pro-opiomelanocortin (POMC) hypothalamic neurons, metabolism and fat distribution remained unchanged. An additive effect occurred in mice lacking ERα in both populations of neurons, with increased food intake and abdominal fat storage together with a decreased metabolic rate. Male mice with a brain-specific ERα deletion also

gained more weight, although different populations of neurons appeared to be involved in mediating the obesity effect [155].

Another important connection between obesity and insulin resistance that may also potentially provide a link with tumorigenesis is hypoxia inducible factor 1 alpha (HIF-1α), a transcription factor that enables cells to survive under low-oxygen conditions. It has been shown that while adipose tissue-specific knockout of HIF-1α in mice fed a high-fat diet does not alter adipocyte survival or the onset of obesity, local inflammation is reduced and insulin sensitivity and glucose tolerance are improved compared to genetically unmodified obese counterparts [156]. Under normal physiological conditions, cells utilize oxygen to produce energy. When oxygen levels drop, as is the case during exercise or exposure to high altitudes, cells switch their metabolism from oxidative phosphorylation to glycolysis and enter hypoxia. As a result of this switch, hypoxic cells produce excess ROS that can induce cellular damage, which is mitigated by the activation of HIF-1α. This protein downregulates the production of ROS, also signalling inflammatory cells to infiltrate hypoxic tissue to eliminate already damaged cells. Organisms temporarily need to be able to adapt to the stress of hypoxia as a defense mechanism until conditions stabilize, and HIF-1α plays a central role during this adaptive process. It has been suggested that in obese individuals, HIF-1α becomes aberrantly and constitutively activated in adipose tissue, thereby contributing to a malignant state that is characterized by chronic inflammation and adipocytes that are persistently hypoxic [156, 157]. Cancer cells undergo similar adaptive changes in their metabolism [158], switching to glycolysis, increasing glycogen synthesis, and using glutamine instead of glucose as the major substrate for fatty acid synthesis. To balance their rapid proliferation with the comparatively low rate at which new blood vessels are generated to provide the tumour with oxygen, hypoxic cancer cells must develop mechanisms to survive the stress of low-oxygen conditions, one of which is the upregulation of HIF-1α (reviewed in [159]). Dysregulated HIF-1α is therefore likely to be one of the "master regulators" linking cancer and the metabolic syndrome. While inhibition of HIF-1α appears to be an attractive therapy, multiple other normal physiological processes rely on this protein to maintain homeostasis.

Physical Activity

Use of a practically applied model has demonstrated an association between physical inactivity and the accumulation of visceral fat [160] as well as persistent systemic inflammation in healthy, young individuals [161]. Although not an absolute requirement, chronic low-grade inflammation promotes the development of insulin resistance, atherosclerosis, neurodegeneration, and tumorigenesis [162]. Exercise may protect against these conditions, inducing long-term anti-inflammatory effects that arise from reducing visceral fat deposits [163]. In addition, proteins produced by skeletal muscle are dependent upon contraction, and physical inactivity may

contribute to an altered myokine response, providing a potential mechanism for the association between sedentary behaviour and chronic diseases [164].

One interesting cytokine/myokine that is produced by both adipocytes and myocytes is IL-6, which may have systemic effects on the liver and the immune system, also mediating intercellular communication between interstitial islet cells in the pancreas [164]. Several studies have reported an inverse relationship between physical activity and basal plasma IL-6 levels [165]. High plasma IL-6 levels have been closely associated with physical inactivity and metabolic syndrome, and intervention studies demonstrate that circulating IL-6 is reduced after exercise training [165]. During bouts of physical activity, IL-6 increases rapidly via its transcriptional upregulation in myocytes in response to a muscle contraction-associated factor without inducing local damage to the working muscles [166]. Given that exercise elicits significant anti-inflammatory effects [161], it seems unlikely that IL-6 is acting as a pro-inflammatory cytokine under these particular conditions, especially since IL-6 stimulates the production of classic anti-inflammatory cytokines, IL-1 and IL-10 [167]. Interestingly, glucose uptake is affected by muscle-derived IL-6 in vitro and in vivo [164], with signalling through adenosine monophosphate (AMP)-activated protein kinase (AMPK) central to this process [168]. In skeletal muscle, IL-6 activates AMPK by increasing cyclic AMP levels, thereby altering the AMP:ATP ratio [169]. Several studies have shown that IL-6 enhances glucose uptake and increases muscle cell [170, 171] or systemic [172] fatty acid oxidation by activating AMPK [170]. IL-6 plays an important role in regulating fat metabolism in muscle, increasing fatty acid oxidation rates and attenuating the lipogenic effects of insulin. IL-6 knockout mice develop mature onset obesity and glucose intolerance [173], supporting the notion that IL-6 induces beneficial metabolic effects through the activation of AMPK. In contrast, TNF-α, another myokine/cytokine, does not affect fatty acid oxidation, instead increasing fatty acid incorporation into diacylglycerol, which may be involved in the development of TNF-α-induced insulin resistance in skeletal muscle [171]. Indeed, levels of TNF-α are elevated in IL-6 knockout animals [174]. Based on these lines of evidence, it has been hypothesized that the development of multiple inflammatory diseases associated with excess visceral adipose tissue is primarily due to the action of TNF-α and not IL-6 [166]. The elevated circulating levels of IL-6 accompanying obesity and physical inactivity have therefore been postulated to represent a compensatory mechanism, which would be in line with hyperinsulinaemia being indicative of insulin resistance, and chronically high circulating levels of leptin potentially reflecting leptin resistance [122]. In this context, IL-6 could be thought of as an energy sensor, affecting adipose tissue by increasing lipolysis, regulating transcriptional responses in abdominal subcutaneous fat as well as lipid oxidation throughout the body [166]. As IL-6 is definitively secreted by both adipocytes and myocytes, its role remains to be determined with regard to its contribution to the aetiology of chronic, systemic obesity-linked inflammation and cancer. It is possible that yet unidentified factors that are also secreted by muscle cells may influence cancer cell growth, and the function of pancreatic cells and adipocytes.

Insulin Resistance and Diabetes

Blood glucose is regulated by insulin and glucagon, which are released from the pancreas when levels are too high or too low, respectively. Insulin mobilizes glucose from the circulation into cells throughout the body, facilitating their utilization of glucose as an energy source. Glucagon promotes glycogen release from the liver, which is converted into glucose, thereby returning blood glucose levels to normal. Diabetes mellitus occurs when the pancreas is unable to produce sufficient amounts of these two hormones. Numerous meta-analyses and research-based studies have indicated that the risk for developing several types of cancer, including liver, pancreatic, kidney, bladder, colorectal, breast, and endometrial, as well as non-Hodgkin's lymphoma, is higher in patients with diabetes, with the strongest association reported for liver and pancreatic cancers (reviewed in [175]). The majority of epidemiological studies have been conducted on patients with type 2 diabetes, and due to certain intrinsic differences in the biology of the two classes of diabetes mellitus, considerably less data is currently available to assess cancer risk in type 1 diabetics [175]. Type 2 diabetes is characterized by an association between excess unused glucose (a state of chronic hyperglycaemia) and hyperinsulinaemia. The latter occurs as a compensatory response to insulin resistance, leading to reduced metabolic effects of insulin in peripheral tissues. This condition may persist for decades before a patient is diagnosed as being diabetic, at which stage most type 2 individuals present with decreased insulin secretion due to the failure of pancreatic β-cells, reflective of type 1 diabetes. It is therefore important to assess the stage and class of diabetes mellitus when investigating the role of hyperinsulinaemia in the onset and progression of cancer in diabetic patients.

The case of hepatocarcinoma exemplifies that diabetes increases the incidence of cancer. Due to the portal circulation, healthy hepatocytes are normally exposed to significantly higher concentrations of insulin than cells residing in other tissues. In individuals with insulin-resistant type 2 diabetes, hepatocyte exposure to insulin increases significantly compared to levels to which the liver of a normal individual are exposed. This dramatic change does not occur in insulin-deficient type 1 diabetics treated with exogenous insulin, as its effect on the liver is diluted after being exposed to all other peripheral tissues. It is therefore unlikely that the mitogenic properties attributed to insulin alone play a major role in the higher incidence of liver cancer in diabetic patients. Cirrhosis and steatohepatitis, which is associated with non-alcoholic fatty liver disease, are implicated with the onset of hepatocellular carcinoma, and these conditions also occur more frequently in all diabetic patients. Additional factors that may favour liver cancer under hyperglycaemic conditions include infection with HBV and HCV, the incidence of which is significantly higher in diabetics compared to non-diabetics [176, 177]. Therefore, although the exact mechanisms underlying the association between liver cancer and diabetes remain to be defined, liver inflammation and sustained hepatocellular damage are likely to be involved in carcinogenesis. Another example is the prevalence of breast and endometrial cancers that occur in a manner independent

from obesity in diabetic women. As already mentioned, oestrogen is a known risk factor associated with female cancers, and it has been proposed that hyperinsulinaemia may lower the levels of circulating sex hormone binding proteins, thereby increasing free oestrogen levels, also potentially stimulating androgen synthesis in ovarian stromal cells [178]. In addition, insulin has mitogenic effects that can be mediated by several distinct mechanisms. This hormone is able to directly bind to and signal through the insulin-like growth factor-1 (IGF-1) receptor and is also able to decrease IGF-1-binding proteins, thereby increasing free IGF-1, another potent growth factor [179]. In cancers, particularly breast cancers, expression of the insulin receptor (IR) is upregulated, with its "malignant" isoform A being predominantly expressed [180], thereby also eliciting more mitogenic effects [181]. In hyperinsulinaemic patients with polycystic ovary syndrome, a condition that is often affiliated with the development of ovarian cancer, aberrant IR activation induces overactivation of the mTOR pathway, also favouring RAS/MEK/ERK-mediated signalling, with both mechanisms contributing to a feedback loop that amplifies this deregulated cascade in the muscle [182]. Deregulated IR-mediated insulin signalling elicits tissue-specific effects, impairing glucose homeostasis by disrupting normal metabolism in classic insulin target cells, including myo-, hepato-, and adipocytes, while increasing proliferation in cancer cells. Metformin, an insulin-sensitizer that reduces insulin resistance primarily in hepatocytes by lowering circulating insulin levels, has been used to treat type 2 diabetes for several decades and has been reported to reduce cancer risk in diabetic patients [183]. A possible mechanism by which metformin elicits its anti-cancer effect is through activation of AMPK, an energy-sensing enzyme that stimulates glucose uptake and fatty acid catabolism [184]. In a panel of breast cancer cells, metformin inhibited cell proliferation and induced cell cycle arrest by inhibiting several key signalling pathways, including those controlled by MAPK, AKT, and mTOR [185]. AMPK activators also block insulin and IGF-1-mediated signalling pathways, thereby reducing their proliferative effects [186].

Inflammatory cytokines may also play a role in diabetes, providing a link between cancer and metabolic syndrome. Evidence suggests that while TNF-α is not itself pathogenic, it directly contributes to the development of metabolic syndrome (reviewed in [163]). Diabetic patients express high levels of TNF-α protein in skeletal muscle and present with increased plasma levels of TNF-α, with adipose tissue being the likely source of circulating TNF-α. In vitro studies demonstrate that TNF-α directly inhibits insulin signalling, and the infusion of TNF-α into healthy human subjects induces insulin resistance in skeletal muscle. It has been proposed that TNF-α indirectly causes insulin resistance, which is supported by evidence that this cytokine increases the release of free fatty acids (FFAs) from adipose tissue in vivo, also increasing lipolysis in human and murine cultured adipocytes. TNF-α has no effect on muscle fatty acid oxidation, rather increasing fatty acid incorporation into diacylglycerol, which may be involved in the development of TNF-α-induced insulin resistance in skeletal muscle (reviewed in [122]).

Atherosclerosis

It is possible that chronic hypertension (high blood pressure) and atherosclerosis may be indicators of other physiological disturbances that, over time, lead to the development of cancer. Vasoconstriction due to chronic conditions including atherosclerotic heart disease, peripheral artery disease, diabetes, high cholesterol levels, or hypertension has been implicated with cancer. The Metabolic Syndrome and Cancer Project revealed that the overall risk of developing lung, colorectal, kidney, and skin cancers was increased by up to 20 % in individuals with higher-than-normal blood pressure, also significantly increasing the risk of dying from cancer in both women and men [187]. One of the common factors linking these conditions is oxidative stress, which plays a major role in the pathogenesis of atherosclerosis [188], as well as diabetes [189] and cancer. While it is known that cancer cells are able to induce and act as a source of ROS (reviewed in [190]), there is also an important relationship between chronic inflammation, oxidative stress, and the process of carcinogenesis [36]. As already mentioned, numerous chronic inflammatory conditions predispose cells to neoplastic transformation. Inflammatory cells, like cancer cells, produce free radicals and soluble mediators that further produce reactive species, thereby recruiting inflammatory cells in a cycle and inducing perturbation in intracellular and intercellular homeostasis until DNA becomes mutated. In a study involving 529 patients, both hypertension and diabetes contributed to increased oxidative stress in polymorphonuclear leukocytes, inflammatory cells that release ROS, and mononuclear cells, which contribute to the onset of atherosclerotic lesions [191]. The study also linked CRP to oxidative stress in mononuclear cells [191]. In addition, oxidative stress may play a role in elevating blood pressure induced by high levels of FFAs in the circulation. Excess FFAs have been shown to directly reduce nitric oxide production by blood vessel endothelial cells, resulting in impaired endothelium-dependent vasodilation and thereby increasing blood pressure. The study supports the notion that endothelial cell oxidative stress is central to hypertension [192].

Hyperlipidaemia, the presence of elevated or abnormal levels of lipids such as FFAs and lipoproteins in the blood, is a major risk factor contributing to cardiovascular disease. A possible link between obesity, insulin resistance, type 2 diabetes, and cancer is deregulated fatty acid synthase (FASN) activity, which increases the de novo production of fatty acids beyond dietary intake, contributing to a “lipogenic state” [193]. FASN is most active in the liver, catalyzing the de novo synthesis of fatty acids. Its expression is upregulated by insulin in human adipocytes [194] and it may be associated with an increased risk of insulin resistance-linked hepatocarcinoma [195].

Other than CRP, the effects of substances such as TNF-α may link insulin resistance to the aetiology of atherosclerosis [196], as activated immune cells play a major role in this process [197]. Interestingly, IL-6 inhibits the production of TNF-α and may thereby inhibit TNF-α-mediated insulin resistance and the onset of atherosclerosis [166]. Given the distinct physiological profiles of TNF-α and IL-6,

and given that TNF-α may trigger the release of IL-6, it is possible that adipose tissue-derived TNF-α "drives" inflammation-induced atherosclerosis and insulin resistance [122], also contributing to tumorigenesis.

Cellular Senescence and Cancer

In humans, the risk of cancer increases in an age-dependent manner, particularly in the case of epithelial carcinomas [198]. Ageing is physiologically characterized by a functional decline in tissues and organ systems due to specific structural and biochemical changes [199], with accumulating genomic damage compromising normal cellular function in tissues that comprise a stem cell niche. This is paired with an impaired ability to respond to injury or stress. During ageing, cells undergo senescence by diverse mechanisms including the shortening of telomeres, limiting aberrant cell divisions and thereby theoretically protecting the body from tumorigenesis [200]. The pattern of gene expression is unique in senescent cells, differentiating them from quiescent or terminally differentiated cells [201, 202]. A further characteristic is a significant increase in the secretion of various proteins, including proteases, growth factors, cytokines, and chemokines, which has been termed as the Senescence-associated secretory phenotype (SASP) [203]. Cells that chronically secrete diverse biological effector molecules such as those represented by the SASP could significantly alter tissue structure and the local microenvironment, and indeed, senescent stromal fibroblasts have been shown to disrupt the normal organization and biologically unique function of mammary epithelial cells [31, 204]. Evidence now supports that the SASP promotes malignancy in vitro, as well as tumour growth in vivo (reviewed in [203]). Given that senescent cells and premalignant cells harbouring various mutations accumulate over a lifetime [205], the xenograft studies lend support to the notion that the SASP of senescent cells could potentially stimulate nearby premalignant cells to undergo tumour progression [203].

Importantly, cellular senescence is not limited to ageing individuals. A process referred to as premature or stress-induced senescence occurs in cells exposed to various stresses, including oxidative stress-inducing agents, constitutive activation of the Ras oncogene, or the transfer of cells isolated in vivo to in vitro conditions (culture shock) (reviewed in [201]). It is therefore plausible that cellular senescence is an adaptive process that has evolved to prolong the survival of an organism under environmentally unfavourable conditions, reducing energy consumption required for cellular turnover as well as processes related to differentiation. Once stresses become chronic, the changes induced in the progenitor cells of younger individuals may be indistinguishable from aged cells [201]. In addition to cancer, senescence has been suggested to be an underlying cause of type 2 diabetes (reviewed in [206]), vascular disease [207], and obesity [12], providing another commonality between cancer and metabolic syndrome.

Conclusion

While many theories have been proposed regarding the mechanisms that underlie tumorigenesis, there is no single unifying hypothesis that adequately explains all the phenomena associated with cancer. However, sustained disruptions in homeostasis that lead to an increase in the allostatic load are common to many chronic diseases, including cancer. As cancers can be clonally derived from a single cell, it is possible that specific tissue progenitors, or stem cells, are targeted during tumorigenesis, and that cancer represents an adaptation of these progenitor cells to a permanently disrupted steady-state induced by chronic stress [208]. This notion is particularly relevant given the ability of adult stem cells to undergo self-renewal, which is an essential property for the normal maintenance and repair of somatic tissues throughout the adult life of an organism. High cellular turnover occurs in blood, endothelial cells of the vasculature, and epithelial cells of the intestine, respiratory tract, and skin, with a lower turnover rate but high regenerative potential in response to injury or disease occurring in bone, the liver, pancreas, and skeletal muscle [209, 210]. The body normally responds to stress by continuously replacing cells, and it is within these sites of either high turnover or high regenerative potential that the majority of human cancers arise. A viable hypothesis, therefore, is that certain cancers may arise from genomic instability or mutations in tissue progenitor cells following recurring cycles of injury or damage, including chronic inflammation or viral infections. These mutations are compounded by the body's response to regain homeostasis as rapidly as possible by maintaining, repairing, and regenerating, with primitive precancerous cells exhibiting "stem cell-like" properties [211], and the local tissue microenvironments continuously modifying the responses of these cells to intracellular changes and extracellular cues. Tissue niches are comprised of diverse populations of cells with distinct extracellular matrices, all being affected by autocrine, paracrine, and endocrine signals that interact with each other. The regulated association between progenitor cells and their unique niches ensures that the appropriate responses take place to meet the normal metabolic requirements of different organ systems and the overall survival of an organism, especially under physiologically challenging conditions such as increased oxidative stress following inflammation or injury [209]. Considering the life span of a fully differentiated cell within a tissue niche and the reliance of the body on a continuous, well-controlled cycle of cellular turnover, it is plausible to suggest that cancer is a disease affecting progenitor cells.

It has been proposed that the events underlying tumorigenesis be considered simply as a process of adaptive evolution that follows the basic principles of population biology, with new geno- and phenotypes continuously arising and interacting with "selective pressures" from the local microenvironment [158]. Experimental evidence in bacteria and yeast demonstrates that environmentally stress-induced mutagenesis generates the occasional "fitter" mutant that further undergoes adaptive evolution, likely contributing to microbial pathogenesis and antibiotic resistance [212]. While it is less clear why multicellular organisms share

these adaptive strategies, stress-induced adaptive mutagenesis is likely to underlie tumorigenesis, as well as cancer progression and chemotherapy resistance. In conclusion, cancer is an adaptive mechanism of adult progenitor cells to a variety of chronic stressors, including systemic inflammation, which occurs to allow the continued survival of an organism. However, as without therapeutic intervention, cancer leads to death, this adaptive plasticity is ultimately not selectively advantageous, but maladaptive.

References

1. Le Moal M. Historical approach and evolution of the stress concept: a personal account. Psychoneuroendocrinology. 2007;32(Suppl 1):S3–9 (Epub 2007/07/31).
2. Clendening L. Sourcebook of medical history. New York: Dover Publications; 1942.
3. Goldstein DS, Kopin IJ. Evolution of concepts of stress. Stress. 2007;10(2):109–20 (Epub 2007/05/22).
4. Buchman TG. The community of the self. Nature. 2002;420(6912):246–51 (Epub 2002/11/15).
5. Dickmeis T, Weger BD, Weger M. The circadian clock and glucocorticoids–interactions across many time scales. Mol Cell Endocrinol. 2013;380(1–2):2–15 (Epub 2013/05/28).
6. Streat SJ, Plank LD, Hill GL. Overview of modern management of patients with critical injury and severe sepsis. World J Surg. 2000;24(6):655–63 (Epub 2000/04/25).
7. Foex BA. Systemic responses to trauma. Br Med Bull. 1999;55(4):726–43 (Epub 2000/04/04).
8. Jaffer U, Wade RG, Gourlay T. Cytokines in the systemic inflammatory response syndrome: a review. HSR Proc Intensive Care Cardiovasc Anesth. 2010;2(3):161–75 (Epub 2010/01/01).
9. Sterling P, Eyer J. Handbook of life stress, cognition, and health. In: Fisher S, Reason JT, editors. Chichester, New York: Wiley; 1988. p. xxxiii, 750 p.
10. McEwen BS, Wingfield JC. The concept of allostasis in biology and biomedicine. Horm Behav. 2003;43(1):2–15 (Epub 2003/03/05).
11. Vannucci L, Stepankova R, Kozakova H, Fiserova A, Rossmann P, Tlaskalova-Hogenova H. Colorectal carcinogenesis in germ-free and conventionally reared rats: different intestinal environments affect the systemic immunity. Int J Oncol. 2008;32(3):609–17 (Epub 2008/02/23).
12. Yoshimoto S, Loo TM, Atarashi K, Kanda H, Sato S, Oyadomari S, et al. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. Nature. 2013;499(7456):97–101 (Epub 2013/06/28).
13. Welsh JS. Contagious cancer. Oncologist. 2011;16(1):1–4 (Epub 2011/01/08).
14. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100(1):57–70 (Epub 2000/01/27).
15. Lazebnik Y. What are the hallmarks of cancer? Nat Rev Cancer. 2010;10(4):232–3 (Epub 2010/04/01).
16. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–74 (Epub 2011/03/08).
17. Warburg O, Wind F, Negelein E. The metabolism of tumors in the body. J Gen Physiol. 1927;8(6):519–30 (Epub 1927/03/07).
18. Targeting Tumour Metabolism. Nature reviews. Drug Discov. 2010;9(7):503–4 (Epub 2010/07/02).

19. Polyak K, Haviv I, Campbell IG. Co-evolution of tumor cells and their microenvironment. Trends Genet TIG. 2009;25(1):30–8 (Epub 2008/12/05).
20. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet. 2001;357 (9255):539–45 (Epub 2001/03/07).
21. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002;420(6917):860–7 (Epub 2002/12/20).
22. Meylan E, Tschopp J, Karin M. Intracellular pattern recognition receptors in the host response. Nature. 2006;442(7098):39–44 (Epub 2006/07/11).
23. de Visser KE, Eichten A, Coussens LM. Paradoxical roles of the immune system during cancer development. Nat Rev Cancer. 2006;6(1):24–37 (Epub 2006/01/07).
24. Balkwill F, Charles KA, Mantovani A. Smoldering and polarized inflammation in the initiation and promotion of malignant disease. Cancer Cell. 2005;7(3):211–7 (Epub 2005/03/16).
25. de Visser KE, Coussens LM. The inflammatory tumor microenvironment and its impact on cancer development. Contrib Microbiol. 2006;13:118–37 (Epub 2006/04/22).
26. Coussens LM, Werb Z. Inflammatory cells and cancer: think different! J Exp Med. 2001;193 (6):F23–6 (Epub 2001/03/21).
27. Osada T, Clay TM, Woo CY, Morse MA, Lyerly HK. Dendritic cell-based immunotherapy. Int Rev Immunol. 2006;25(5–6):377–413 (Epub 2006/12/16).
28. Ghiringhelli F, Apetoh L, Housseau F, Kroemer G, Zitvogel L. Links between innate and cognate tumor immunity. Curr Opin Immunol. 2007;19(2):224–31 (Epub 2007/02/17).
29. David H. Rudolf Virchow and modern aspects of tumor pathology. Pathol Res Pract. 1988;183(3):356–64 (Epub 1988/06/01).
30. Krtolica A, Campisi J. Cancer and aging: a model for the cancer promoting effects of the aging stroma. Int J Biochem Cell Biol. 2002;34(11):1401–14 (Epub 2002/08/30).
31. Parrinello S, Coppe JP, Krtolica A, Campisi J. Stromal-epithelial interactions in aging and cancer: senescent fibroblasts alter epithelial cell differentiation. J Cell Sci. 2005;118(Pt 3):485–96 (Epub 2005/01/20).
32. Hu M, Yao J, Carroll DK, Weremowicz S, Chen H, Carrasco D, et al. Regulation of in situ to invasive breast carcinoma transition. Cancer Cell. 2008;13(5):394–406 (Epub 2008/05/06).
33. Yoshimura A, Naka T, Kubo M. SOCS proteins, cytokine signalling and immune regulation. Nat Rev Immunol. 2007;7(6):454–65 (Epub 2007/05/26).
34. Rakoff-Nahoum S. Why cancer and inflammation? Yale J Biol Med. 2006;79(3–4):123–30 (Epub 2007/10/18).
35. Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB. Reactive oxygen species in inflammation and tissue injury. Antioxid Redox Signal. 2014;20(7):1126–67 (Epub 2013/09/03).
36. Federico A, Morgillo F, Tuccillo C, Ciardiello F, Loguercio C. Chronic inflammation and oxidative stress in human carcinogenesis. Int J Cancer J Int Du cancer. 2007;121(11):2381–6 (Epub 2007/09/26).
37. O'Connell RM, Rao DS, Chaudhuri AA, Baltimore D. Physiological and pathological roles for microRNAs in the immune system. Nat Rev Immunol. 2010;10(2):111–22 (Epub 2010/01/26).
38. Tili E, Croce CM, Michaille JJ. miR-155: on the crosstalk between inflammation and cancer. Int Rev Immunol. 2009;28(5):264–84 (Epub 2009/10/09).
39. Tili E, Michaille JJ, Wernicke D, Alder H, Costinean S, Volinia S, et al. Mutator activity induced by microRNA-155 (miR-155) links inflammation and cancer. Proc Natl Acad Sci USA. 2011;108(12):4908–13 (Epub 2011/03/09).
40. Veglia F, Cighetti G, De Franceschi M, Zingaro L, Boccotti L, Tremoli E, et al. Age- and gender-related oxidative status determined in healthy subjects by means of OXY-SCORE, a potential new comprehensive index. Biomarkers Biochem Ind Expo Response Susceptibility Chem. 2006;11(6):562–73 (Epub 2006/10/24).
41. Roth E, Manhart N, Wessner B. Assessing the antioxidative status in critically ill patients. Curr Opin Clin Nutr Metab Care. 2004;7(2):161–8 (Epub 2004/04/13).

42. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007;39(1):44–84 (Epub 2006/09/19).
43. Eferl R, Wagner EF. AP-1: a double-edged sword in tumorigenesis. Nat Rev Cancer. 2003;3 (11):859–68 (Epub 2003/12/12).
44. Grivennikov SI, Karin M. Dangerous liaisons: STAT3 and NF-kappaB collaboration and crosstalk in cancer. Cytokine Growth Fact Rev. 2010;21(1):11–9 (Epub 2009/12/19).
45. Klaus A, Birchmeier W. Wnt signalling and its impact on development and cancer. Nat Rev Cancer. 2008;8(5):387–98 (Epub 2008/04/25).
46. Koh MY, Spivak-Kroizman TR, Powis G. HIF-1alpha and cancer therapy. Recent results in cancer research. Fortschritte der Krebsforschung Progres dans les recherches sur le cancer. 2010;180:15–34 (Epub 2009/12/25).
47. Gudkov AV, Gurova KV, Komarova EA. Inflammation and p53: a tale of two stresses. Genes Cancer. 2011;2(4):503–16 (Epub 2011/07/23).
48. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: how are they linked? Free Radic Biol Med. 2010;49(11):1603–16 (Epub 2010/09/16).
49. Vincent HK, Innes KE, Vincent KR. Oxidative stress and potential interventions to reduce oxidative stress in overweight and obesity. Diab Obes Metab. 2007;9(6):813–39 (Epub 2007/10/11).
50. Dunn GP, Old LJ, Schreiber RD. The immunobiology of cancer immunosurveillance and immunoediting. Immunity. 2004;21(2):137–48 (Epub 2004/08/17).
51. Boshoff C, Weiss R. AIDS-related malignancies. Nat Rev Cancer. 2002;2(5):373–82 (Epub 2002/06/05).
52. Penn I. Posttransplant malignancies. Transpl Proc. 1999;31(1–2):1260–2 (Epub 1999/03/20).
53. Euvrard S, Kanitakis J, Claudy A. Skin cancers after organ transplantation. N Engl J Med. 2003;348(17):1681–91 (Epub 2003/04/25).
54. Pham SM, Kormos RL, Landreneau RJ, Kawai A, Gonzalez-Cancel I, Hardesty RL, et al. Solid tumors after heart transplantation: lethality of lung cancer. Ann Thoracic Surg. 1995;60 (6):1623–6 (Epub 1995/12/01).
55. Oluwole SF, Ali AO, Shafaee Z, DePaz HA. Breast cancer in women with HIV/AIDS: report of five cases with a review of the literature. J Surg Oncol. 2005;89(1):23–7 (Epub 2004/12/22).
56. Stewart T, Tsai SC, Grayson H, Henderson R, Opelz G. Incidence of de-novo breast cancer in women chronically immuno suppressed after organ transplantation. Lancet. 1995;346 (8978):796–8 (Epub 1995/09/23).
57. Bishop JM. Viral oncogenes. Cell. 1985;42(1):23–38 (Epub 1985/08/01).
58. Woolhouse M, Scott F, Hudson Z, Howey R, Chase-Topping M. Human viruses: discovery and emergence. Philos Trans R Soc Lond B Biol Sci. 2012;367(1604):2864–71 (Epub 2012/09/12).
59. Fontana JM, Alexander E, Salvatore M. Translational research in infectious disease: current paradigms and challenges ahead. Transl Res J Lab Clin Med. 2012;159(6):430–53 (Epub 2012/05/29).
60. Williams SC. The other microbiome. Proc Natl Acad Sci USA. 2013;110(8):2682–4 (Epub 2013/02/08).
61. Wylie KM, Weinstock GM, Storch GA. Emerging view of the human virome. Trans Res J Lab Clin Med. 2012;160(4):283–90 (Epub 2012/06/12).
62. Delwart E. A roadmap to the human virome. PLoS Pathog. 2013;9(2):e1003146 (Epub 2013/03/05).
63. Foxman EF, Iwasaki A. Genome-virome interactions: examining the role of common viral infections in complex disease. Nat Rev Microbiol. 2011;9(4):254–64 (Epub 2011/03/17).
64. Ault KA. Epidemiology and natural history of human papillomavirus infections in the female genital tract. Infect Dis Obstet Gynecol. 2006; Suppl:40470 (Epub 2006/09/14).

65. Mork J, Lie AK, Glattre E, Hallmans G, Jellum E, Koskela P, et al. Human papillomavirus infection as a risk factor for squamous-cell carcinoma of the head and neck. N Engl J Med. 2001;344(15):1125–31 (Epub 2001/04/12).
66. Verteramo R, Pierangeli A, Mancini E, Calzolari E, Bucci M, Osborn J, et al. Human Papillomaviruses and genital co-infections in gynaecological outpatients. BMC Infect Dis. 2009;9:16 (Epub 2009/02/17).
67. Amon W, Farrell PJ. Reactivation of Epstein-Barr virus from latency. Rev Med Virol. 2005;15(3):149–56 (Epub 2004/11/17).
68. Henle W, Henle G. Epidemiologic aspects of Epstein-Barr virus (EBV)-associated diseases. Ann NY Acad Sci. 1980;354:326–31 (Epub 1980/01/01).
69. Gratama JW, Oosterveer MA, Zwaan FE, Lepoutre J, Klein G, Ernberg I. Eradication of Epstein-Barr virus by allogeneic bone marrow transplantation: implications for sites of viral latency. Proc Natl Acad Sci USA. 1988;85(22):8693–6 (Epub 1988/11/01).
70. Weiss LM, O'Malley D. Benign lymphadenopathies. Mod Pathol Off J US Can Acad Pathol Inc. 2013;26(Suppl 1):S88–96 (Epub 2013/01/04).
71. Toussirot E, Roudier J. Epstein-Barr virus in autoimmune diseases. Best Pract Res Clin Rheumatol. 2008;22(5):883–96 (Epub 2008/11/26).
72. Thompson MP, Kurzrock R. Epstein-Barr virus and cancer. Clin Cancer Res Off J Am Assoc Cancer Res. 2004;10(3):803–21 (Epub 2004/02/12).
73. Maeda E, Akahane M, Kiryu S, Kato N, Yoshikawa T, Hayashi N, et al. Spectrum of Epstein-Barr virus-related diseases: a pictorial review. Japan J Radiol. 2009;27(1):4–19 (Epub 2009/04/18).
74. Epstein MA, Achong BG, Barr YM. Virus particles in cultured lymphoblasts from Burkitt's lymphoma. Lancet. 1964;1(7335):702–3 (Epub 1964/03/28).
75. Arvey A, Tempera I, Tsai K, Chen HS, Tikhmyanova N, Klichinsky M, et al. An atlas of the Epstein-Barr virus transcriptome and epigenome reveals host-virus regulatory interactions. Cell Host Microbe. 2012;12(2):233–45 (Epub 2012/08/21).
76. El-Serag HB. Hepatocellular carcinoma. N Engl J Med. 2011;365(12):1118–27 (Epub 2011/10/14).
77. El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology. 2007;132(7):2557–76 (Epub 2007/06/16).
78. Schillie S, Murphy TV, Sawyer M, Ly K, Hughes E, Jiles R, et al. CDC guidance for evaluating health-care personnel for hepatitis B virus protection and for administering postexposure management. MMWR recommendations and reports: morbidity and mortality weekly report recommendations and reports/centers for disease control. 2013;62(RR-10): 1–19 (Epub 2013/12/20).
79. Bonnet F, Lewden C, May T, Heripret L, Jougla E, Bevilacqua S, et al. Malignancy-related causes of death in human immunodeficiency virus-infected patients in the era of highly active antiretroviral therapy. Cancer. 2004;101(2):317–24 (Epub 2004/07/09).
80. Cote TR, Biggar RJ, Rosenberg PS, Devesa SS, Percy C, Yellin FJ, et al. Non-Hodgkin's lymphoma among people with AIDS: incidence, presentation and public health burden. AIDS/Cancer Study Group. Int J Cancer J Int Du Cancer. 1997;73(5):645–50 (Epub 1997/12/16).
81. Lanoy E, Dores GM, Madeleine MM, Toro JR, Fraumeni JF Jr, Engels EA. Epidemiology of nonkeratinocytic skin cancers among persons with AIDS in the United States. AIDS. 2009;23(3):385–93 (Epub 2008/12/31).
82. Sahasrabuddhe VV, Shiels MS, McGlynn KA, Engels EA. The risk of hepatocellular carcinoma among individuals with acquired immunodeficiency syndrome in the United States. Cancer. 2012;118(24):6226–33 (Epub 2012/06/28).
83. Engels EA, Atkinson JO, Graubard BI, McQuillan GM, Gamache C, Mbisa G, et al. Risk factors for human herpesvirus 8 infection among adults in the United States and evidence for sexual transmission. J Infect Dis. 2007;196(2):199–207 (Epub 2007/06/16).
84. Qu L, Jenkins F, Triulzi DJ. Human herpesvirus 8 genomes and seroprevalence in United States blood donors. Transfusion. 2010;50(5):1050–6 (Epub 2010/01/22).

85. Caskey MF, Morgan DJ, Porto AF, Giozza SP, Muniz AL, Orge GO, et al. Clinical manifestations associated with HTLV type I infection: a cross-sectional study. AIDS Res Hum Retroviruses. 2007;23(3):365–71 (Epub 2007/04/07).
86. Poiesz BJ, Papsidero LD, Ehrlich G, Sherman M, Dube S, Poiesz M, et al. Prevalence of HTLV-I-associated T-cell lymphoma. Am J Hematol. 2001;66(1):32–8 (Epub 2001/06/28).
87. Feng H, Shuda M, Chang Y, Moore PS. Clonal integration of a polyomavirus in human Merkel cell carcinoma. Science. 2008;319(5866):1096–100 (Epub 2008/01/19).
88. Chen T, Hedman L, Mattila PS, Jartti T, Ruuskanen O, Soderlund-Venermo M, et al. Serological evidence of Merkel cell polyomavirus primary infections in childhood. J Clin Virol Off Publ Pan Am Soc Clin Virol. 2011;50(2):125–9 (Epub 2010/11/26).
89. Bialasiewicz S, Lambert SB, Whiley DM, Nissen MD, Sloots TP. Merkel cell polyomavirus DNA in respiratory specimens from children and adults. Emerg Infect Dis. 2009;15(3):492–4 (Epub 2009/02/26).
90. Bichakjian CK, Lowe L, Lao CD, Sandler HM, Bradford CR, Johnson TM, et al. Merkel cell carcinoma: critical review with guidelines for multidisciplinary management. Cancer. 2007;110(1):1–12 (Epub 2007/05/24).
91. Shuda M, Feng H, Kwun HJ, Rosen ST, Gjoerup O, Moore PS, et al. T antigen mutations are a human tumor-specific signature for Merkel cell polyomavirus. Proc Natl Acad Sci USA. 2008;105(42):16272–7 (Epub 2008/09/25).
92. Armstrong BK, Kricker A. The epidemiology of UV induced skin cancer. J Photochem Photobiol, B. 2001;63(1–3):8–18 (Epub 2001/10/31).
93. Zur Hausen A. Merkel cell polyomavirus in the pathogenesis of non-melanoma skin cancer. Der Pathologe. 2009;30(Suppl 2):217–20. (Epub 2009/11/19). Das Merkel-Zell-Polyomavirus in der Pathogenese nichtmelanozytarer Hauttumoren.
94. Imajoh M, Hashida Y, Nemoto Y, Oguri H, Maeda N, Furihata M, et al. Detection of Merkel cell polyomavirus in cervical squamous cell carcinomas and adenocarcinomas from Japanese patients. Virol J. 2012;9:154 (Epub 2012/08/11).
95. Hourdequin KC, Lefferts JA, Brennick JB, Ernstoff MS, Tsongalis GJ, Pipas JM. Merkel cell polyomavirus and extrapulmonary small cell carcinoma. Oncol Lett. 2013;6(4):1049–52 (Epub 2013/10/19).
96. Xu S, Jiang J, Yu X, Sheng D, Zhu T, Jin M. Association of Merkel cell polyomavirus infection with EGFR mutation status in Chinese non-small cell lung cancer patients. Lung Cancer. 2014;83(3):341–6 (Epub 2014/02/04).
97. Butel JS. Simian virus 40, poliovirus vaccines, and human cancer: research progress versus media and public interests. Bull World Health Organ. 2000;78(2):195–8 (Epub 2000/04/01).
98. Manfredi JJ, Dong J, Liu WJ, Resnick-Silverman L, Qiao R, Chahinian P, et al. Evidence against a role for SV40 in human mesothelioma. Cancer Res. 2005;65(7):2602–9 (Epub 2005/04/05).
99. Eddy BE, Borman GS, Berkeley WH, Young RD. Tumors induced in hamsters by injection of rhesus monkey kidney cell extracts. Proc Soc Exp Biol Med. 1961;107:191–7 (Epub 1961/05/01).
100. Eibl RH, Kleihues P, Jat PS, Wiestler OD. A model for primitive neuroectodermal tumors in transgenic neural transplants harboring the SV40 large T antigen. Am J Pathol. 1994;144 (3):556–64 (Epub 1994/03/01).
101. Sithithaworn P, Yongvanit P, Duenngai K, Kiatsopit N, Pairojkul C. Roles of liver fluke infection as risk factor for cholangiocarcinoma. J Hepato-Biliary-Pancreat Sci. 2014;21 (5):301–8 (Epub 2014/01/11).
102. Jenkins-Holick DS, Kaul TL. Schistosomiasis. Urol Nurs. 2013;33(4):163–70 (Epub 2013/10/02).
103. Porter SB, Sande MA. Toxoplasmosis of the central nervous system in the acquired immunodeficiency syndrome. N Engl J Med. 1992;327(23):1643–8 (Epub 1992/12/03).
104. Thomas F, Lafferty KD, Brodeur J, Elguero E, Gauthier-Clerc M, Misse D. Incidence of adult brain cancers is higher in countries where the protozoan parasite Toxoplasma gondii is common. Biol Lett. 2012;8(1):101–3 (Epub 2011/07/29).

105. Vittecoq M, Elguero E, Lafferty KD, Roche B, Brodeur J, Gauthier-Clerc M, et al. Brain cancer mortality rates increase with Toxoplasma gondii seroprevalence in France. Inf Genet Evol J Mol Epidemiol Evol Genet Inf Dis. 2012;12(2):496–8 (Epub 2012/01/31).
106. Russell W. An address on a characteristic organism of cancer. Br Med J. 1890;2(1563): 1356–60 (Epub 1890/12/13).
107. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet. 1984;1(8390):1311–5 (Epub 1984/06/16).
108. Dumrese C, Slomianka L, Ziegler U, Choi SS, Kalia A, Fulurija A, et al. The secreted Helicobacter cysteine-rich protein A causes adherence of human monocytes and differentiation into a macrophage-like phenotype. FEBS Lett. 2009;583(10):1637–43 (Epub 2009/04/28).
109. Brown LM. Helicobacter pylori: epidemiology and routes of transmission. Epidemiol Rev. 2000;22(2):283–97 (Epub 2001/02/24).
110. Talley NJ, Fock KM, Moayyedi P. Gastric cancer consensus conference recommends Helicobacter pylori screening and treatment in asymptomatic persons from high-risk populations to prevent gastric cancer. Am J Gastroenterol. 2008;103(3):510–4 (Epub 2008/03/18).
111. Wu Q, Yang ZP, Xu P, Gao LC, Fan DM. Association between *helicobacter pylori* infection and the risk of colorectal neoplasia: a systematic review and meta-analysis. Colorectal Dis Off J Assoc Coloproctol Great Br Irel. 2013;15(7):e352–64 (Epub 2013/05/16).
112. Anttila T, Saikku P, Koskela P, Bloigu A, Dillner J, Ikaheimo I, et al. Serotypes of Chlamydia trachomatis and risk for development of cervical squamous cell carcinoma. JAMA, J Am Med Assoc. 2001;285(1):47–51 (Epub 2001/01/10).
113. Samoff E, Koumans EH, Markowitz LE, Sternberg M, Sawyer MK, Swan D, et al. Association of Chlamydia trachomatis with persistence of high-risk types of human papillomavirus in a cohort of female adolescents. Am J Epidemiol. 2005;162(7):668–75 (Epub 2005/08/27).
114. Kostic AD, Chun E, Robertson L, Glickman JN, Gallini CA, Michaud M, et al. Fusobacterium nucleatum potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. Cell Host Microbe. 2013;14(2):207–15 (Epub 2013/08/21).
115. Rubinstein MR, Wang X, Liu W, Hao Y, Cai G, Han YW. Fusobacterium nucleatum promotes colorectal carcinogenesis by modulating E-cadherin/beta-catenin signaling via its FadA adhesin. Cell Host Microbe. 2013;14(2):195–206 (Epub 2013/08/21).
116. Anand PK, Kanneganti TD. NLRP6 in infection and inflammation. Microbe Inf Inst Pasteur. 2013;15(10–11):661–8 (Epub 2013/07/03).
117. Liu XX, Li XJ, Zhang B, Liang YJ, Zhou CX, Cao DX, et al. MicroRNA-26b is underexpressed in human breast cancer and induces cell apoptosis by targeting SLC7A11. FEBS Lett. 2011;585(9):1363–7 (Epub 2011/04/23).
118. Couturier-Maillard A, Secher T, Rehman A, Normand S, De Arcangelis A, Haesler R, et al. NOD2-mediated dysbiosis predisposes mice to transmissible colitis and colorectal cancer. J Clin Investig. 2013;123(2):700–11 (Epub 2013/01/03).
119. Jin C, Flavell RA. Innate sensors of pathogen and stress: linking inflammation to obesity. J Allergy Clin Immunol. 2013;132(2):287–94 (Epub 2013/08/03).
120. Minot S, Sinha R, Chen J, Li H, Keilbaugh SA, Wu GD, et al. The human gut virome: inter-individual variation and dynamic response to diet. Genome Res. 2011;21(10):1616–25 (Epub 2011/09/02).
121. Haslam DW, James WP. Obesity. Lancet. 2005;366(9492):1197–209 (Epub 2005/10/04).
122. Pedersen BK. Muscle as a secretory organ. Comprehensive Physiology. 2013;3(3):1337–62 (Epub 2013/07/31).
123. Rogowski O, Shapira I, Bassat OK, Chundadze T, Finn T, Berliner S, et al. Waist circumference as the predominant contributor to the micro-inflammatory response in the metabolic syndrome: a cross sectional study. J Inflamm (Lond). 2010;7:35 (Epub 2010/07/28).

124. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab. 2004;89(6):2548–56 (Epub 2004/06/08).
125. Bastard JP, Jardel C, Bruckert E, Blondy P, Capeau J, Laville M, et al. Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. J Clin Endocrinol Metab. 2000;85(9):3338–42 (Epub 2000/09/22).
126. Esposito K, Nappo F, Marfella R, Giugliano G, Giugliano F, Ciotola M, et al. Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: role of oxidative stress. Circulation. 2002;106(16):2067–72 (Epub 2002/10/16).
127. Loffreda S, Yang SQ, Lin HZ, Karp CL, Brengman ML, Wang DJ, et al. Leptin regulates proinflammatory immune responses. FASEB J Off Pub Fed Am Soc Exp Biol. 1998;12 (1):57–65 (Epub 1998/01/23).
128. Mohamed-Ali V, Flower L, Sethi J, Hotamisligil G, Gray R, Humphries SE, et al. beta-Adrenergic regulation of IL-6 release from adipose tissue: in vivo and in vitro studies. J Clin Endocrinol Metab. 2001;86(12):5864–9 (Epub 2001/12/12).
129. Mantzoros CS. The role of leptin in human obesity and disease: a review of current evidence. Ann Intern Med. 1999;130(8):671–80 (Epub 1999/04/24).
130. Kao PC, Shiesh SC, Wu TJ. Serum C-reactive protein as a marker for wellness assessment. Ann Clin Lab Sci. 2006;36(2):163–9 (Epub 2006/05/10).
131. Emanuela F, Grazia M, de Marco R, Maria Paola L, Giorgio F, Marco B. Inflammation as a Link between obesity and metabolic syndrome. J Nutr Metab. 2012;2012:476380 (Epub 2012/04/24).
132. Mohamed-Ali V, Goodrick S, Rawesh A, Katz DR, Miles JM, Yudkin JS, et al. Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. J Clin Endocrinol Metab. 1997;82(12):4196–200 (Epub 1997/12/17).
133. Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. J Clin Investig. 2006;116(7):1793–801 (Epub 2006/07/11).
134. Blackburn P, Cote M, Lamarche B, Couillard C, Pascot A, Tremblay A, et al. Impact of postprandial variation in triglyceridemia on low-density lipoprotein particle size. Metab, Clin Exp. 2003;52(11):1379–86 (Epub 2003/11/19).
135. van Dijk SJ, Feskens EJ, Bos MB, Hoelen DW, Heijligenberg R, Bromhaar MG, et al. A saturated fatty acid-rich diet induces an obesity-linked proinflammatory gene expression profile in adipose tissue of subjects at risk of metabolic syndrome. Am J Clin Nutr. 2009;90 (6):1656–64 (Epub 2009/10/16).
136. Clement K, Viguerie N, Poitou C, Carette C, Pelloux V, Curat CA, et al. Weight loss regulates inflammation-related genes in white adipose tissue of obese subjects. FASEB J Off Pub Fed Am Soc Exp Biol. 2004;18(14):1657–69 (Epub 2004/11/04).
137. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Investig. 2003;112(12):1821–30 (Epub 2003/12/18).
138. Tabata M, Kadomatsu T, Fukuhara S, Miyata K, Ito Y, Endo M, et al. Angiopoietin-like protein 2 promotes chronic adipose tissue inflammation and obesity-related systemic insulin resistance. Cell Metab. 2009;10(3):178–88 (Epub 2009/09/03).
139. Aoi J, Endo M, Kadomatsu T, Miyata K, Nakano M, Horiguchi H, et al. Angiopoietin-like protein 2 is an important facilitator of inflammatory carcinogenesis and metastasis. Cancer Res. 2011;71(24):7502–12 (Epub 2011/11/02).
140. Aoi J, Endo M, Kadomatsu T, Miyata K, Ogata A, Horiguchi H, et al. Angiopoietin-like protein 2 accelerates carcinogenesis by activating chronic inflammation and oxidative stress. Mol Cancer Res Mcr. 2014;12(2):239–49 (Epub 2013/11/22).
141. Glass AG, Lacey JV Jr, Carreon JD, Hoover RN. Breast cancer incidence, 1980–2006: combined roles of menopausal hormone therapy, screening mammography, and estrogen receptor status. J Natl Cancer Inst. 2007;99(15):1152–61 (Epub 2007/07/27).
142. Cleary MP, Grossmann ME. Minireview: Obesity and breast cancer: the estrogen connection. Endocrinology. 2009;150(6):2537–42 (Epub 2009/04/18).

143. Reed MJ, Purohit A. Aromatase regulation and breast cancer. Clin Endocrinol. 2001;54 (5):563–71 (Epub 2001/05/31).
144. Jerry DJ. Roles for estrogen and progesterone in breast cancer prevention. Breast Cancer Res BCR. 2007;9(2):102 (Epub 2007/03/27).
145. Bhat HK, Calaf G, Hei TK, Loya T, Vadgama JV. Critical role of oxidative stress in estrogen-induced carcinogenesis. Proc Natl Acad Sci USA. 2003;100(7):3913–8 (Epub 2003/03/26).
146. Vegeto E, Ciana P, Maggi A. Estrogen and inflammation: hormone generous action spreads to the brain. Mol Psychiatry. 2002;7(3):236–8 (Epub 2002/03/29).
147. Sifferlin A. Hormone-replacement therapy: could estrogen have saved 50,000 lives? Time [Internet] (2013).
148. Bath PM, Gray LJ. Association between hormone replacement therapy and subsequent stroke: a meta-analysis. BMJ. 2005;330(7487):342 (Epub 2005/01/11).
149. Carr MC. The emergence of the metabolic syndrome with menopause. J Clin Endocrinol Metab. 2003;88(6):2404–11 (Epub 2003/06/06).
150. Rogers NH, Perfield JW 2nd, Strissel KJ, Obin MS, Greenberg AS. Reduced energy expenditure and increased inflammation are early events in the development of ovariectomy-induced obesity. Endocrinology. 2009;150(5):2161–8 (Epub 2009/01/31).
151. Wren BG. The benefits of oestrogen following menopause: why hormone replacement therapy should be offered to postmenopausal women. Med J Aust. 2009;190(6):321–5 (Epub 2009/03/20).
152. Heine PA, Taylor JA, Iwamoto GA, Lubahn DB, Cooke PS. Increased adipose tissue in male and female estrogen receptor-alpha knockout mice. Proc Natl Acad Sci USA. 2000;97 (23):12729–34 (Epub 2000/11/09).
153. Okura T, Koda M, Ando F, Niino N, Ohta S, Shimokata H. Association of polymorphisms in the estrogen receptor alpha gene with body fat distribution. Int J Obes Relat Metab Disord J Int Assoc Study Obes. 2003;27(9):1020–7 (Epub 2003/08/15).
154. Saha S, Sadhukhan P, Sil PC. Genistein: a phytoestrogen with multifaceted therapeutic properties. Mini Rev Med Chem. 2014 (Epub 2014/10/31).
155. Xu Y, Nedungadi TP, Zhu L, Sobhani N, Irani BG, Davis KE, et al. Distinct hypothalamic neurons mediate estrogenic effects on energy homeostasis and reproduction. Cell Metab. 2011;14(4):453–65 (Epub 2011/10/11).
156. Lee YS, Kim JW, Osborne O, da Oh Y, Sasik R, Schenk S, et al. Increased adipocyte O_2 consumption triggers HIF-1alpha, causing inflammation and insulin resistance in obesity. Cell. 2014;157(6):1339–52 (Epub 2014/06/07).
157. Wood IS, de Heredia FP, Wang B, Trayhurn P. Cellular hypoxia and adipose tissue dysfunction in obesity. Proc Nutr Soc. 2009;68(4):370–7 (Epub 2009/08/25).
158. Gillies RJ, Gatenby RA. Hypoxia and adaptive landscapes in the evolution of carcinogenesis. Cancer Metastasis Rev. 2007;26(2):311–7 (Epub 2007/04/04).
159. Semenza GL. HIF-1 mediates metabolic responses to intratumoral hypoxia and oncogenic mutations. J Clin Investig. 2013;123(9):3664–71 (Epub 2013/09/04).
160. Olsen RH, Krogh-Madsen R, Thomsen C, Booth FW, Pedersen BK. Metabolic responses to reduced daily steps in healthy nonexercising men. JAMA, J Am Med Assoc. 2008;299 (11):1261–3 (Epub 2008/03/20).
161. Petersen AM, Pedersen BK. The anti-inflammatory effect of exercise. J Appl Physiol (1985). 2005;98(4):1154–62 (Epub 2005/03/18).
162. Handschin C, Spiegelman BM. The role of exercise and PGC1alpha in inflammation and chronic disease. Nature. 2008;454(7203):463–9 (Epub 2008/07/25).
163. Pedersen BK. The diseasome of physical inactivity—and the role of myokines in muscle–fat cross talk. J Physiol. 2009;587(Pt 23):5559–68 (Epub 2009/09/16).
164. Pedersen BK, Febbraio MA. Muscles, exercise and obesity: skeletal muscle as a secretory organ. Nat Rev Endocrinol. 2012;8(8):457–65 (Epub 2012/04/05).
165. Fischer CP. Interleukin-6 in acute exercise and training: what is the biological relevance? Exerc Immunol Rev. 2006;12:6–33 (Epub 2007/01/05).

166. Pedersen BK, Steensberg A, Fischer C, Keller C, Keller P, Plomgaard P, et al. Searching for the exercise factor: is IL-6 a candidate? J Muscle Res Cell Motil. 2003;24(2–3):113–9 (Epub 2003/11/12).
167. Steensberg A, Fischer CP, Keller C, Moller K, Pedersen BK. IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. Am J Physiol Endocrinol Metab. 2003;285(2):E433–7 (Epub 2003/07/15).
168. Steinberg GR, Watt MJ, Febbraio MA. Cytokine regulation of AMPK signalling. Front Biosci (Landmark Ed). 2009;14:1902–16 (Epub 2009/03/11).
169. Kelly M, Gauthier MS, Saha AK, Ruderman NB. Activation of AMP-activated protein kinase by interleukin-6 in rat skeletal muscle: association with changes in cAMP, energy state, and endogenous fuel mobilization. Diabetes. 2009;58(9):1953–60 (Epub 2009/06/09).
170. Carey AL, Steinberg GR, Macaulay SL, Thomas WG, Holmes AG, Ramm G, et al. Interleukin-6 increases insulin-stimulated glucose disposal in humans and glucose uptake and fatty acid oxidation in vitro via AMP-activated protein kinase. Diabetes. 2006;55(10): 2688–97 (Epub 2006/09/28).
171. Bruce CR, Dyck DJ. Cytokine regulation of skeletal muscle fatty acid metabolism: effect of interleukin-6 and tumor necrosis factor-alpha. Am J Phys Endocrinol Metab. 2004;287(4): E616–21 (Epub 2004/05/20).
172. van Hall G, Steensberg A, Sacchetti M, Fischer C, Keller C, Schjerling P, et al. Interleukin-6 stimulates lipolysis and fat oxidation in humans. J Clin Endocrinol Metab. 2003;88(7): 3005–10 (Epub 2003/07/05).
173. Wallenius V, Wallenius K, Ahren B, Rudling M, Carlsten H, Dickson SL, et al. Interleukin-6-deficient mice develop mature-onset obesity. Nat Med. 2002;8(1):75–9 (Epub 2002/01/12).
174. Mizuhara H, O'Neill E, Seki N, Ogawa T, Kusunoki C, Otsuka K, et al. T cell activation-associated hepatic injury: mediation by tumor necrosis factors and protection by interleukin 6. J Exp Med. 1994;179(5):1529–37 (Epub 1994/05/01).
175. Vigneri P, Frasca F, Sciacca L, Pandini G, Vigneri R. Diabetes and cancer. Endocr Relat Cancer. 2009;16(4):1103–23 (Epub 2009/07/22).
176. Chen HF, Li CY, Chen P, See TT, Lee HY. Seroprevalence of hepatitis B and C in type 2 diabetic patients. J Chin Med Assoc JCMA. 2006;69(4):146–52 (Epub 2006/05/13).
177. Davila JA, Morgan RO, Shaib Y, McGlynn KA, El-Serag HB. Diabetes increases the risk of hepatocellular carcinoma in the United States: a population based case control study. Gut. 2005;54(4):533–9 (Epub 2005/03/09).
178. Kaaks R. Nutrition, hormones, and breast cancer: is insulin the missing link? Cancer Causes Control CCC. 1996;7(6):605–25 (Epub 1996/11/01).
179. Kaaks R, Lukanova A. Energy balance and cancer: the role of insulin and insulin-like growth factor-I. Proc Nutr Soc. 2001;60(1):91–106 (Epub 2001/04/20).
180. Frasca F, Pandini G, Scalia P, Sciacca L, Mineo R, Costantino A, et al. Insulin receptor isoform A, a newly recognized, high-affinity insulin-like growth factor II receptor in fetal and cancer cells. Mol Cell Biol. 1999;19(5):3278–88 (Epub 1999/04/17).
181. Frasca F, Pandini G, Malaguarnera R, Mandarino A, Messina RL, Sciacca L, et al. Role of c-Abl in directing metabolic versus mitogenic effects in insulin receptor signaling. J Biol Chem. 2007;282(36):26077–88 (Epub 2007/07/11).
182. Corbould A, Zhao H, Mirzoeva S, Aird F, Dunaif A. Enhanced mitogenic signaling in skeletal muscle of women with polycystic ovary syndrome. Diabetes. 2006;55(3):751–9 (Epub 2006/03/01).
183. Evans JM, Donnelly LA, Emslie-Smith AM, Alessi DR, Morris AD. Metformin and reduced risk of cancer in diabetic patients. BMJ. 2005;330(7503):1304–5 (Epub 2005/04/26).
184. Luo Z, Saha AK, Xiang X, Ruderman NB. AMPK, the metabolic syndrome and cancer. Trends Pharmacol Sci. 2005;26(2):69–76 (Epub 2005/02/01).
185. Alimova IN, Liu B, Fan Z, Edgerton SM, Dillon T, Lind SE, et al. Metformin inhibits breast cancer cell growth, colony formation and induces cell cycle arrest in vitro. Cell Cycle. 2009; 8(6):909–15 (Epub 2009/02/18).

186. Ruderman N, Prentki M. AMP kinase and malonyl-CoA: targets for therapy of the metabolic syndrome. Nat Rev Drug Discov. 2004;3(4):340–51 (Epub 2004/04/03).
187. Stocks T, Van Hemelrijck M, Manjer J, Bjorge T, Ulmer H, Hallmans G, et al. Blood pressure and risk of cancer incidence and mortality in the metabolic syndrome and cancer project. Hypertension. 2012;59(4):802–10 (Epub 2012/02/23).
188. Bonomini F, Tengattini S, Fabiano A, Bianchi R, Rezzani R. Atherosclerosis and oxidative stress. Histol Histopathol. 2008;23(3):381–90 (Epub 2007/12/12).
189. Baynes JW, Thorpe SR. Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. Diabetes. 1999;48(1):1–9 (Epub 1999/01/19).
190. Nashed MG, Balenko MD, Singh G. Cancer-induced oxidative stress and pain. Current Pain Headache Rep. 2014;18(1):384 (Epub 2013/12/03).
191. Yasunari K, Maeda K, Nakamura M, Yoshikawa J. Oxidative stress in leukocytes is a possible link between blood pressure, blood glucose, and C-reacting protein. Hypertension. 2002;39(3):777–80 (Epub 2002/03/19).
192. Wang H, Li H, Hou Z, Pan L, Shen X, Li G. Role of oxidative stress in elevated blood pressure induced by high free fatty acids. Hypertens Res Off J Japan Soc Hypertens. 2009;32 (2):152–8 (Epub 2009/03/06).
193. Menendez JA, Vazquez-Martin A, Ortega FJ, Fernandez-Real JM. Fatty acid synthase: association with insulin resistance, type 2 diabetes, and cancer. Clin Chem. 2009;55(3): 425–38 (Epub 2009/02/03).
194. Claycombe KJ, Jones BH, Standridge MK, Guo Y, Chun JT, Taylor JW, et al. Insulin increases fatty acid synthase gene transcription in human adipocytes. Am J Physiol. 1998;274(5 Pt 2):R1253–9 (Epub 1998/06/27).
195. Caldwell S, Lazo M. Is exercise an effective treatment for NASH? Knowns and unknowns. Annals of hepatology. 2009;8(Suppl 1):S60–6 (Epub 2009/06/25).
196. Yudkin JS, Eringa E, Stehouwer CD. "Vasocrine" signalling from perivascular fat: a mechanism linking insulin resistance to vascular disease. Lancet. 2005;365(9473):1817–20 (Epub 2005/05/25).
197. Matter CM, Handschin C. RANTES (regulated on activation, normal T cell expressed and secreted), inflammation, obesity, and the metabolic syndrome. Circulation. 2007;115(8): 946–8 (Epub 2007/02/28).
198. DePinho RA. The age of cancer. Nature. 2000;408(6809):248–54 (Epub 2000/11/23).
199. Marusyk A, DeGregori J. Declining cellular fitness with age promotes cancer initiation by selecting for adaptive oncogenic mutations. Biochim Biophys Acta. 2008;1785(1):1–11 (Epub 2007/11/06).
200. Collado M, Blasco MA, Serrano M. Cellular senescence in cancer and aging. Cell. 2007;130 (2):223–33 (Epub 2007/07/31).
201. Ishikawa F. Cellular senescence as a stress response. Cornea. 2006;25(10 Suppl 1):S3–6 (Epub 2006/09/27).
202. Blagosklonny MV. Cell cycle arrest is not senescence. Aging. 2011;3(2):94–101 (Epub 2011/02/08).
203. Campisi J, Andersen JK, Kapahi P, Melov S. Cellular senescence: a link between cancer and age-related degenerative disease? Semin Cancer Biol. 2011;21(6):354–9 (Epub 2011/09/20).
204. Tsai KK, Chuang EY, Little JB, Yuan ZM. Cellular mechanisms for low-dose ionizing radiation-induced perturbation of the breast tissue microenvironment. Cancer Res. 2005;65 (15):6734–44 (Epub 2005/08/03).
205. Hasty P, Campisi J, Hoeijmakers J, van Steeg H, Vijg J. Aging and genome maintenance: lessons from the mouse? Science. 2003;299(5611):1355–9 (Epub 2003/03/01).
206. Testa R, Ceriello A. Pathogenetic loop between diabetes and cell senescence. Diab Care. 2007;30(11):2974–5 (Epub 2007/10/30).
207. Brodsky SV, Gealekman O, Chen J, Zhang F, Togashi N, Crabtree M, et al. Prevention and reversal of premature endothelial cell senescence and vasculopathy in obesity-induced diabetes by ebselen. Circ Res. 2004;94(3):377–84 (Epub 2003/12/13).

208. Tez M. Cancer is an adaptation mechanism of the aged stem cell against stress. Rejuvenation Res. 2008;11(6):1059–60 (Epub 2008/12/17).
209. Pekovic V, Hutchison CJ. Adult stem cell maintenance and tissue regeneration in the ageing context: the role for A-type lamins as intrinsic modulators of ageing in adult stem cells and their niches. J Anat. 2008;213(1):5–25 (Epub 2008/07/22).
210. Lagasse E. Cancer stem cells with genetic instability: the best vehicle with the best engine for cancer. Gene Ther. 2008;15(2):136–42 (Epub 2007/11/09).
211. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature. 2001;414(6859):105–11 (Epub 2001/11/02).
212. Galhardo RS, Hastings PJ, Rosenberg SM. Mutation as a stress response and the regulation of evolvability. Crit Rev Biochem Mol Biol. 2007;42(5):399–435 (Epub 2007/10/06).

Chapter 2
Cancer and Angiogenesis

Franziska Miller and Gurmit Singh

Abstract Neoplastic growth is closely linked to neovascularization, as efficient blood supply is necessary to deliver oxygen and nutrients to a tumour. The development of blood vessels in tumours is modulated by pro- and anti-angiogenic factors. Pro-angiogenic factors include those that regulate remodelling of the extracellular matrix (ECM) and changes in perivascular cell structure, as well as those that promote endothelial cell changes and migration, including but not limited to vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and angiopoietin. Anti-angiogenic factors include thrombospondin, 16kDA N-terminal fragments of prolactin and growth hormone, endostatin, vasostatin, and angiostatin. During tumour growth, the balance is tipped in favour of pro-angiogenic factors. This is known as the angiogenic switch and allows for increased tumour progression, a state where proliferation is favoured over apoptosis. The angiogenic switch may thus be considered as the rate-limiting step in the tumour metastasis pathway. Furthermore, this switch is highly dependent on changes in the tumour microenvironment. The tumour microenvironment continues to increase in significance in angiogenesis research and understanding it holds the key to new and more successful anti-angiogenic cancer therapies.

Keywords Angiogenesis · Vascular endothelial growth factor (VEGF) · Endostatin · Basic fibroblast growth factor (bFGF) · Endothelial cells · Microenvironment

F. Miller
Singh Lab, Department of Pathology and Molecular Medicine, McMaster University, 1280 Main Street West, Hamilton, ON L8N 3Z5, Canada
e-mail: franziska.miller@ymail.com

G. Singh (✉)
Department of Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada
e-mail: singhg@mcmaster.ca

G. Singh (ed.), *Oncodynamics: Effects of Cancer Cells on the Body*,
DOI 10.1007/978-3-319-28558-0_2

Abbreviations

ADM	Adrenomedullin
Ang	Angiopoetin
Akt	Protein kinase B
APC	Antigen-presenting cells
bFGF	Basic fibroblast growth factor
CAF	Cancer-associated fibroblasts
CCL2 CC	Chemokine ligand 2
CSF-1	Colony-stimulating factor-1
ECM	Extracellular matrix
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
ERK	Extracellular signal-regulated kinases
FAK	Focal adhesion kinase
HER	Human epidermal receptor
ICAM-1	Intercellular adhesion molecule-1
IL1β	Interleukin 1β
mCAF	Mammary CAF
MDSC	Myeloid-derived suppressor cell
MMP	Matrix metalloproteinase
MT1-MMP	Membrane-type1 matrix metalloproteinase
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NK cells	Natural killer cells
PA	Tissue-type plasminogen activators
PAI-1	PA inhibitior-1
PDGF	Platelet-derived growth factor
PI3K	Phosphoinositide 3-kinase
PlGF	Placenta growth factor
PMP	Platelet-derived microparticles
SDF-1	Stromal cell-derived factor 1
TEM	Monocytes expressing TIE-2 receptors
TGFα	Transforming growth factor α
TGFβ-1	Transforming growth factor β1
TME	Tumour mircoenvironment
TNF-α	Tumour necrosis factor α
TP	Thymididine phosphorylase
Sema4D	Semaphoring 4D
uPA	Urokinase-type plasminogen activator
VCAM-1	Vascular cell adhesion molecule-1
VEGF	Vascular endothelial growth factor
VPF	Tumour vascular permeability factor

Introduction

Neoplastic growth is closely linked to neovascularization, as efficient blood supply is necessary to deliver oxygen and nutrients to a tumour growing larger than 1–2 mm^3 whilst ensuring waste removal [1]. Neovascularization can be categorized into vasculogenesis (development of new capillaries from endothelial progenitor cells as in embryonic development), angiogenesis (development of new capillaries from pre-existing capillaries), vasculogenic mimicry (development of vessels that do not have endothelial cells), and vessel co-option (a process whereby the tumour co-opts host vasculature) [2–5]. Although all these different forms of neovascularization contribute to neoplastic vessel growth, angiogenesis is considered fundamental. Besides aiding tumour growth through adequate perfusion of the tumour, angiogenesis can also be used as an indicator for the tumour's metastatic potential and is often associated with a poor prognosis [6].

The development of blood vessels in tumours is modulated by pro- and anti-angiogenic factors. Pro-angiogenic factors include those that regulate remodelling of the extracellular matrix (ECM) and changes in perivascular cell structure, as well as those that promote endothelial cell changes and migration, including but not limited to vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and angiopoietin [1, 7, 8]. Anti-angiogenic factors include thrombospondin, 16kDA N-terminal fragments of prolactin and growth hormone, endostatin, vasostatin, and angiostatin [8]. During tumour growth, the balance is tipped in favour of pro-angiogenic factors. This is known as the angiogenic switch and allows for increased tumour progression, a state where proliferation is favoured over apoptosis [9, 10]. The angiogenic switch may thus be considered the rate-limiting step in the tumour metastasis pathway. Furthermore, this switch is highly dependent on changes in the tumour microenvironment (TME) [11]. The tumour microenvironment continues to increase in significance in angiogenesis research and understanding it holds the key to new and more successful anti-angiogenic cancer therapies.

Angiogenesis

Angiogenesis can be characterized into sprouting angiogenesis and intussusceptive angiogenesis (see Fig. 2.1) [1, 2]. The sprouting angiogenesis model, whereby new vasculature develops from pre-existing vasculature, was proposed by Folkman and Ausprunk in 1977. This process is initiated when pro-angiogenic factors cause the basement membrane to disintegrate and the intercellular junctions between endothelial cells to loosen. This allows for the rearrangement of endothelial cells, which can now invade the surrounding extracellular matrix (ECM) and produce a new basement membrane lining the new blood vessel (reviewed in [12]). More

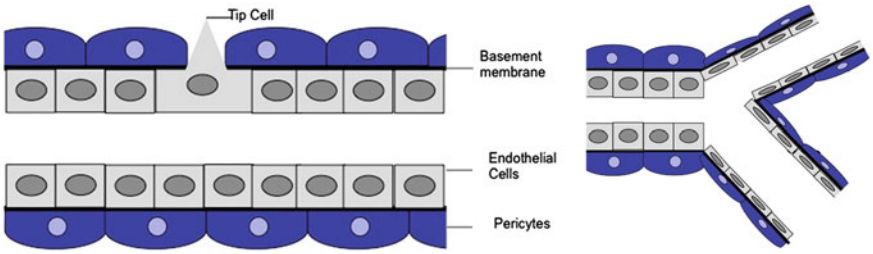


Fig. 2.1 Sprouting (*left*) versus Intussusceptive (*right*) angiogenesis

specifically, endothelial cell function is changed and a single endothelial cell or tip cell is stimulated by pro-angiogenic factors to begin forming a new vessel [13]. Factors secreted by tumour cells and the stroma, including VEGF, FGF, and PDGF, aid this process and serve as chemoattractant signals for the tip cell [1]. The developing sprout maintains its basal–luminal polarity, eventually connects to a neighbouring vessel, and is integrated into the existing vascular system by proliferating pericytes (vascular contractile cells) [14, 15]. The invasive nature of the sprouting process requires the proteolytic action of matrix metalloproteinases (MMPs), which will be discussed in further detail at a later point in this chapter. *In vivo*, sprouting is a slow process and it may take several days for the newly developed capillary to become a functional part of the vascular system [16]. Sprouting angiogenesis is considered the classical model of angiogenesis, but alternative models have been proposed. One such model focuses on intussusceptive or splitting angiogenesis. This type of angiogenesis may occur in response to stress or damage to the vasculature and is achieved by intraluminal growth [reviewed in 12, 17]. In comparison to sprouting angiogenesis, intussusceptive angiogenesis is a much faster process and does not primarily rely on endothelial cell proliferation [17]. Instead, existing vessel walls fold or develop protrusions within the lumen. Opposite endothelial membranes connect, thereby forming the kissing contact, and interendothelial junctions form. At the locus of contact, connective tissue columns or tissue pillars develop and insert themselves between the kissing contact, which results in the formation of two vessels [18]. Tissue pillars are formed when pericytes and myofibroblasts migrate into the collagen pillar core and deposit additional collagenous materials. Tumour cells also have the ability to migrate into developing tissue pillars and contribute to their growth [14]. Intussusceptive angiogenesis is an umbrella term for a process, by which the lumen of an existing vessel is split into two, and includes different subtypes, named for the variable location of the tissue pillars [19].

Although both angiogenic processes have been described separately, it is important to consider that they can occur simultaneously and are not mutually exclusive. Furthermore, a switch from sprouting to intussusceptive angiogenesis may occur, resulting in improved perfusion of the tumour [20].

Differences Between Normal Vessels and Tumour Vessels

Similar to normal vessels, tumour vessels consist of endothelial cells, mural cells (pericytes and smooth muscle cells), and a basement membrane [21]. However, it is important to note that the newly developed vessels in a majority of cancers differ in structure and function from normal vessels (see Fig. 2.2). Such abnormalities can affect the endothelial cells, pericytes, as well as the basement membrane, and result in the formation of tortuous, irregularly shaped, and hyperpermeable vessels [15, 21, 22]. The basement membrane is only loosely associated with the endothelial cells and pericytes, varies in thickness and layer composition, and may invade the stroma of the tumour [21]. However, only the loose association with the endothelial cells and pericytes is unique to tumour cells [23]. Tumour pericytes also play an important role in the abnormality of tumour vessels, as they have abnormal shapes, cannot cover the vessels, and have processes extending away from the vessel wall [24, 25]. This in turn leads to hyperpermeability of the vessels. In addition, the architecture of tumour-derived endothelial cells (TEC) is vastly different from normal endothelial cells, in that they are often leaky with wider junctions and several fenestrations [10, 22]. This architectural change may lead to haemorrhage and increased interstitial fluid pressure, as fluid is no longer contained in the intravascular space. It also hinders cell migration to the tumour site as well as the transport of drugs and oxygen to the tumour, which may aid the cancer in evading detection by the immune system, in becoming less responsive to chemotherapy, and in creating a hypoxic and subsequently acidotic environment. The high interstitial pressure, hypoxia, and low pH in the tumour microenvironment may further alter tumour cells and facilitate the transport of tumour cells through leaky vessels, thus favouring tumour metastasis [22]. In addition to the described differences between normal and abnormal cells, there is also a difference between different tumours in terms of vascular permeability [10]. This complicates targeting angiogenesis in cancer therapy.

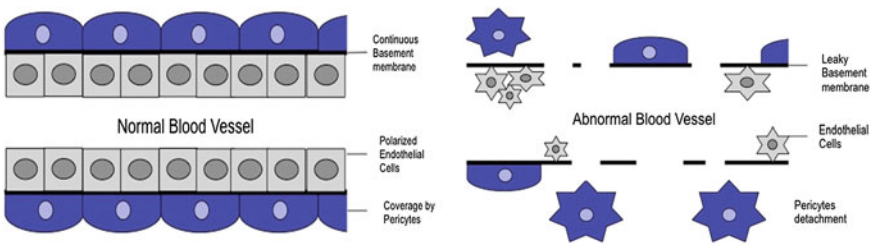


Fig. 2.2 Normal versus tumour vasculature

Molecules Released by Cancer Cells and Tumour Microenvironment

Vascular Endothelial Factor (VEGF)

The most prominent and vastly studied molecule involved in angiogenesis is the vascular endothelial factor (VEGF). VEGF was initially described as an endothelial cell-specific mitogen, while the tumour vascular permeability factor (VPF) was originally shown to increase the permeability of vasculature and to induce fluid accumulation in the peritoneal cavity in a guinea pig tumour model [26, 27]. Senger et al. [26] also stated that the secretion of a permeability factor might be a commonality between all tumour cells [27]. In 1989, cDNA of VPF and VEGF was isolated by two independent research groups [28]. It was subsequently determined that VPF and VEGF were essentially the same molecule and mediated similar biological functions [29]. VEGF can act in a paracrine or an autocrine fashion to regulate normal or abnormal angiogenesis and is expressed in most solid tumours, including cervical cancer, breast cancer, and colon cancer [28, 30, 31].

The human genome contains five genes encoding five VEGF family members: VEGF-A (predominantly known as VEGF), placenta growth factor (PlGF), VEGF-B, VEGF-C, and VEGF-D. These proteins consist of two subunits (120–200 amino acids) and are thus considered homodimers [29]. Out of these five family members, VEGF-A plays a key role in angiogenesis during embryonic development as well as tumour angiogenesis. VEGF-A binds to the Fms-like tyrosine kinase receptor VEGFR-1/Flt-1 and VEGFR-2 [9, 27]. This Fms-like tyrosine kinase receptor contains seven extracellular immunoglobulin domains and exhibits structural similarity to the Fms receptor [29]. VEGF-A can also bind to VEGFR-2 but with lower affinity, despite VEGFR-2 exhibiting a much stronger tyrosine kinase activity than VEGFR-1. VEGFR-2 has been implicated in vascular permeability and is considered a major player in angiogenesis. When VEGF-A (predominantly its splice variants VEGF-A121 or VEGF-A165) binds to VEGFR-2, the receptor is autophosphorylated and a downstream signalling cascade, involving phosphoinositide 3-kinase (PI3K), focal adhesion kinase (FAK), protein kinase B (Akt), and extracellular signal-regulated kinases (ERK), is induced [9]. In particular, the activation of the Rho GTPase pathway seems to be involved in all aspects of angiogenesis ranging from vascular permeability and ECM degradation to lumen formation [9]. Tumour VEGF signalling can be enhanced through inflammatory cytokines, growth factors such as PDGF, as well as hypoxic conditions [9]. In hypoxic areas of tumour masses particularly, VEGF-A expression can directly be upregulated through the hypoxia-inducible factor alpha (HIF-alpha) or indirectly be upregulated through galectin-1 expression, as is the case in human breast cancer [32]. In addition to tumour cells, VEGF-A may be secreted by normal keratinocytes, leukocytes, red blood cells, platelets, and tumour-associated macrophages [33, 34].

VEGF-A was also found to induce the expression of anti-apoptotic proteins Bcl-2 and A1 in human endothelial cells, serine proteases, tissue-type plasminogen activators (PAs), PA inhibitior-1 (PAI-1) in bovine endothelial cells, and metalloproteinases in human umbilical vein endothelial cells [28]. Moreover, VEGF-A is involved in immune responses by promoting the expression of the vascular cell adhesion molecule-1 (VCAM-1) and the intercellular adhesion molecule-1 (ICAM-1) to enhance adhesion of natural killer (NK) cells to endothelial cells via VCAM-1/CD18 and ICAM-1/VLA-4 interactions [28]. VEGF-A also induces monocyte chemotaxis and may inhibit antigen-presenting cells (APCs) from maturing [28]. As such, VEGF-A may facilitate tumour growth by inhibiting antigen-presenting cells from maturing and thus reduce a tumour-targeted immune response. In addition, VEGF-A can aid angiogenesis by increasing the expression of MMPs and plasminogen activators in order to degrade ECM and allow endothelial cells to migrate [35]. It becomes thus quite evident that VEGF-A plays a prominent role in tumour angiogenesis.

PlGF also plays an important role in angiogenesis, as it is secreted in large amounts by activated endothelial cells and in turn regulates the VEGF-mediated angiogenic switch [1]. Furthermore, PlGF has been shown to attract myeloid-derived suppressor cells (MDSCs) and tumour-associated macrophages (TAMs) to the tumour microenvironment (TME) [36]. PIGF is specific to VEGFR-1 and may play a larger role in maintaining tumour blood vessels rather than inducing the development of new vasculature [37].

VEGF-B is arguably the least studied of the VEGF family members. It is a specific ligand to VEGFR-1 as well as neuropilin-1 (NP-1) receptor and has a high sequence homology to VEGF-A but its role in angiogenesis and blood vessel permeability has been controversial [38]. Similar to PIGF, the primary role of VEGF-B seems to centre around the survival of blood vessels in times of stress by inhibiting apoptosis of endothelial cells, smooth muscle cells, and pericytes [37, 38]. These findings suggest that VEGF-B has a survival/anti-apoptotic effect, rather than exhibiting angiogenic activity [37]. Nonetheless, VEGF-B may play a significant role in anti-angiogenic therapy, emphasizing a link between survival/anti-apoptotic and angiogenic activity. As increased coverage by smooth muscles and pericytes confer resistance to anti-angiogenic therapy, limiting the function of VEGF-B may prove to be a viable therapeutic target [38].

Fibroblast Growth Factor (FGF)

Fibroblast growth factors (FGF), among which the basic fibroblast growth factor (bFGF) or FGF2 was the first to be discovered, belong to a family of heparin-binding growth factors and play a key role in the tumour angiogenesis signalling cascade [39]. FGFs are produced by macrophages and tumour cells, among many other cells. They can then bind to their respective receptors, FGR1

and FGR2, on endothelial cells to directly influence the angiogenic process or to indirectly modulate it by inducing the release of pro-angiogenic factors [10, 39]. To be more specific, FGF functions include endothelial cell proliferation, ECM degradation via the upregulation of MMPs, as well as modulation of adhesion proteins including integrins and cadherins [1]. FGFs act synergistically with VEGF-A to promote angiogenesis and may also mediate the resistance to anti-VEGF or anti-EGFR tumour therapy [10, 40].

Angiopoietin

Angiopoietin signalling plays a major role in angiogenesis as well, as it is involved in vasculature development and maturation. In humans, this growth factor family contains three members: angiopoietin-1 (Ang-1), angiopoietin-2 (Ang-2), and angiopoietin-4 (Ang-4) [1]. Ang-1 is produced by pericytes, smooth muscle cells, fibroblasts, and monocytes. Ang-2 is solely produced by endothelial cells, as is the case with cells of the tumour endothelium [41]. The tumour endothelium also facilitates further recruitment of monocytes expressing TIE-2 receptors (TEM) to the tumour site [42]. All members of the angiopoietin family have the ability to bind to TIE-2, a tyrosine kinase receptor expressed on endothelial cells, but can elicit opposing effects. Ang-1 activates TIE-2 signalling and is involved in endothelial cell migration and adhesion as well as pericyte and smooth muscle cell recruitment, whereas Ang-2 serves to inhibit this signalling cascade and functions to decrease vessel stability [43]. Although Ang-1 activates TIE-2 signalling, the role of Ang-1 in tumour angiogenesis is unclear. Depending on tumour type, Ang-1 may enhance or limit growth. Thus, targeting Ang-1 in tumour therapy may not be effective. Ang-2 on the other hand seems to be a more promising target. It is overexpressed in mammary carcinoma, melanoma, and metastatic colorectal cancer leading to non-functional and abnormal blood vessel formation [44, 45]. It has also been suggested that Ang-2 acts together with VEGF-A to further increase tumour vessel permeability and vessel sprouting [7]. It has been thus suggested that VEGF-A anti-angiogenic therapy resistance may be modulated by the upregulation of Ang-2 [46]. In contrast to the hypothesis that VEGF-A and Ang-2 work together to promote angiogenesis, TIE-2 expression on endothelial cells was found to be downregulated via VEGF-A signalling [41]. Therefore, further research is warranted to elucidate the interaction between Ang-2 and VEGF-A. In addition to being a potential therapeutic target, Ang-2 may also serve as a clinical screening marker, as patients with non-small cell lung cancer in comparison to healthy subjects were shown to have higher plasma Ang-2 levels [47]. Plasma levels of Ang-2 may also be used as a biochemical outcome marker to determine metastasis potential and prognosis, as was demonstrated in patients with melanoma and metastatic colorectal cancer [44, 45].

Matrix Metalloproteinases (MMPs)

Matrix metalloproteinases (MMPs), such as MT1-MMP, MMP-2, and MMP-9, belong to a family of zinc-containing calcium-dependent endopeptidases active at neutral pH and have proteolytic functions in the extracellular matrix [48]. The extracellular matrix consists of collagen, proteoglycans, and glycoproteins, and is constantly remodelled by MMPs. MMPs are responsible for the degradation of the basement membrane and matrix proteins in the ECM to allow for new vessel development, as well as modulating the balance between pro- and anti-angiogenic factors. The latter is mainly accomplished by producing protein fragments, which can either inhibit or activate angiogenesis [13]. Thus, MMPs are essential in regulating sprouting angiogenesis. In particular, MMP-9 plays a significant role in angiogenesis, as it can release VEGF-A through the degradation of the ECM [49].

MMP activity is controlled by a membrane-anchored glycoprotein, called the reversion-inducing cysteine-rich protein with Kazal motifs (RECK). RECK expression was found to be decreased in tumours of the liver, pancreas, breast, colon, lung, and skin [49]. In cancer, Ras proteins may downregulate RECK, which may result in an increased secretion of MMP-9 but not MMP-2 and thus inhibit VEGF-A induced angiogenesis [50, 51].

Cells in the Tumour Microenvironment (TME) Involved in Angiogenesis

Although signalling cascades involved in angiogenesis have been studied in the past, it is essential to note that pro-angiogenic factors are not solely secreted by tumour cells. A significant role is played by components of the tumour microenvironment (TME), as pointed out throughout this chapter. Tumour cells have been shown to interact with surrounding inflammatory cytokines, the ECM, platelets, cancer-associated fibroblasts (CAF), tumour endothelial cells (TEC), and tumour-associated macrophages (TAM).

Platelets

Platelets are physiologically involved in hemostasis and wound healing. Upon activation, platelets also secrete pro-angiogenic proteins, such as VEGF-A, FGF, insulin-like growth factor 1 (IGF-1), PDGF, angiopoietins, stromal cell-derived factor-1 (CXCL12), MMP-1, MMP-2, and MMP-9 [33, 52]. Platelets may contain VEGF-A for two reasons: (1) synthesis of VEGF-A and (2) endocytosis of

circulating VEGF-A [33]. The activation of platelets can be mediated by thrombin [53]. The involvement of thrombin in angiogenesis and its link to platelets was further strengthened by Yuan and Liu [54], who found that carcinoma patients had a shorter thrombin time (increased levels of thrombin). Besides being involved in coagulation and wound healing, thrombin can induce angiogenesis by binding to its receptor PAR-1 on endothelial and tumour cells [55]. Targeting PAR-1 in ovarian carcinoma led to a decreased expression of pro-angiogenic factors, including VEGF-A, IL-8, and MMP-2 [54]. This further highlights the role of the tumour microenvironment in angiogenesis.

It was also shown that platelets shed small plasma membrane vesicles, called microparticles. These platelet-derived microparticles (PMP) play a role in angiogenesis and have been associated with tumour aggressiveness and poor prognosis. Their angiogenic function may be mediated by stored growth factors and survival factors [52]. Platelets also express markers on their surface, which mediate tumour angiogenesis. One such marker is CD41, which is involved in mediating the adhesion of platelets to endothelial cells and promoting endothelial-dependent angiogenesis. The expression of CD41 was correlated with increased levels of VEGF-A in ovarian carcinoma, further suggesting that platelets play a role in inducing angiogenesis [54].

Cancer-Associated Fibroblasts (CAF)

Fibroblasts are spindle-shaped cells and are physiologically involved in ECM synthesis, inflammation, and wound healing. Fibroblasts are also found in the tumour microenvironment, where they play a role in tumour growth, angiogenesis, and ECM remodelling [56–58]. These fibroblasts are termed cancer-associated fibroblasts (CAF). In particular, mammary CAF (mCAF) were shown to secrete pro-angiogenic factors, such as bFGF, EGF, VEGF, and a variety of MMP [59, 60]. mCAF also enhance angiogenesis by releasing stromal cell-derived factor 1 (SDF-1) to recruit endothelial progenitor cells into carcinomas [57]. Furthermore, mCAF recruit immune cells to the tumour microenvironment, further contributing to the development of a niche favourable for tumour progression and metastasis [58]. mCAF were also shown to interact with tumour cells for metabolic purposes, as mCAF secrete pyruvate and lactate to provide energy metabolites for the tricarboxylic acid cycle to cancer cells in hypoxic environments, a process termed the reverse Warburg effect [56, 61]. The presence of CAF in the tumour microenvironment is strongly associated with resistance to cancer therapy. CAF mediates resistance to the chemotherapy drug tamoxifen via MMP, among other factors [62]. Despite its key role in cancer and therapy resistance, targeting CAF for cancer therapy is challenging. One reason for this is that CAF express a variety of

molecular markers but none of them are unique to CAF [63]. Targeting CAF may thus produce undesired off-target effects.

Tumour Endothelial Cells (TEC)

Tumour Endothelial Cells (TEC) may have their origin in normal endothelial cells. It is postulated that these endothelial cells change their phenotype as a result of the tumour microenvironment [24]. In renal carcinoma, melanoma, and liposarcoma, TEC have also been found to be aneuploid (containing an abnormal number of chromosomes), which may be the result of factors secreted by the tumour microenvironment [64]. Aneuploidy affected TEC structure, causing the loss of normal apical–basal polarity. Furthermore, TEC showed evidence for altered function and were able to proliferate without senescence *in vitro* [65]. TEC may also be derived from the differentiation of bone marrow-derived circulating or tissue-resident stem cells, as well as tumour cells [24]. Again, the tumour microenvironment may play a significant role in this differentiation, which may occur in a VEGF-dependent or VEGF-independent manner [66].

Furthermore, TEC can express EGFR. Interestingly, EGFR is also expressed by a variety of solid tumours of the breast, colon, lung, pancreas, head and neck, bladder, and brain [67]. The epidermal growth factor receptor (EGFR) belongs to the human epidermal receptor (HER) family, which consists of four receptor tyrosine kinases, and can be bound by its ligands—epidermal growth factor (EGF) and transforming growth factor alpha (TGFα). This elicits a pro-angiogenic response, which is likely mediated by the upregulation of VEGF-A and MMP [68]. As such, EGFR and TEC serve as another target for anti-angiogenic therapy in cancer.

Tumour-Associated Macrophages (TAM)

Tumour-associated macrophages (TAM) are monocytes that have been recruited to the tumour site and that have been primed by molecules secreted by the tumour, including colony-stimulating factor-1 (CSF-1), VEGF-A, and CCL2 [69, 70]. Recruitment may occur in response to hypoxic conditions. Hypoxia can upregulate the expression of pro-angiogenic chemokines secreted by macrophages, including CXCL12, C-C chemokine ligand 2 (CCL2), CXCL8, CXCL1, CXCL13, and CCL5 [71]. TAM phenotype is highly dependent on the tumour microenvironment with a predominance of pro-tumour M2-polarized TAM over antitumour M1-polarized TAM. In models of polyoma virus middle T oncogene (MMTV-PyMT) induced mammary adenocarcinoma, increased presence of TAMs correlated with conditions favourable for angiogenesis and metastasis [6]. TAMs may secrete factors

regulating angiogenesis, including but not limited to VEGF-A, bFGF, EGF, tumour necrosis factor alpha (TNF-α), cytokines (IL-1, IL-2, IL-6, IL-8, IL-12, and IL-17), PDGF, matrix metalloproteinases (MMP-9), nitric oxide, thymididine phosphorylase (TP), urokinase-type plasminogen activator (uPA), adrenomedullin (ADM), and semaphoring 4D (Sema4D) [6, 42]. In particular, TAM expressing CSF-1 receptors infiltrate the stroma of the primary solid tumour, promote increased vessel density, and are thus actively involved in promoting the angiogenic switch [6]. In addition to breast cancer, TAM involvement has been established in animal models of ovarian cancer, melanoma, prostate cancer, cervical cancer, gliomas, lymphomas, and other solid tumours [42]. The importance of TAM research also translates to human cancers, as TAM density has been associated with increased levels of VEGF-A, which is the result of TAM expressing the hypoxia-inducible factor HIF-1α [42]. Besides hypoxia, interleukin 1β (IL1β) and transforming growth factor β1 (TGFβ-1) were shown to induce the HIF-1 controlled VEGF-A expression. Alternatively, tumour-released M-CSF could induce the expression of VEGF-A by TAM through the activation of the nuclear factor kappa-light-chain-enhancer of activated B cells, NF-κB [72, 73]. The release of VEGF-A from the extracellular matrix was also found to be facilitated by MMP-9, another molecule secreted by TAM. Although VEGF-A seems to be highly important in angiogenesis, other factors are equally involved in promoting angiogenesis. One such example is TP, a molecule involved in endothelial cell migration, which in high levels is accociated with poor prognosis in human glioma, gastric cancer, breast cancer, and pancreatic cancer. Interestingly, it has been shown that tumour cells and TAM may act synergistically to amplify the production of pro-angiogenic factors in the tumour microenvironment and thus facilitate the angiogenic switch [42].

Conclusion

Angiogenesis is mediated by a variety of factors, including VEGF-A, FGF, angiopoietin, and MMP. Due to the importance of the angiogenic process in tumour progression and metastasis, it has been long considered as a target for cancer therapy. However, anti-angiogenic therapy has encountered significant challenges due to therapy resistance, which may be facilitated by altered cell characteristics or re-neovascularization. It has been suggested that tumour cells, which have acquired pericyte or smooth muscle coverage confer increased anti-angiogenic therapy resistance [38]. Tumours may also adapt to anti-angiogenic therapy and circumvent the need for angiogenesis by primarily invading locally or co-opting existing blood vessels [43]. Instead of being a cell autonomous process, it is important to remember that tumour progression and angiogenesis is co-mediated by the tumour microenvironment, a process termed oncodynamics. This means that future therapies targeting angiogenesis will have to consider off-target effects and the role of the microenvironment in order to be successful.

References

1. Hillen F, Griffioen AW. Tumour vascularization: sprouting angiogenesis and beyond. Cancer Metastasis Rev. 2007;26(3–4):489–502.
2. Burri PH, Hlushchuk R, Djonov V. Intussusceptive angiogenesis: its emergence, its characteristics, and its significance. Dev Dyn. 2004;231(3):474–88.
3. Folkman J. Tumor angiogenesis: therapeutic implications. N Engl J Med. 1971;285(21): 1182–6.
4. Maniotis AJ, Folberg R, Hess A, Seftor EA, Gardner LMG, Pe'er J, et al. Vascular channel formation by human melanoma cells in vivo and in vitro: vasculogenic mimicry. Am J Pathol. 1999;155(3):739–52.
5. Saharinen P, Eklund L, Pulkki K, Bono P, Alitalo K. VEGF and angiopoietin signaling in tumor angiogenesis and metastasis. Trends Mol Med. 2011;17(7):347–62.
6. Lin EY, Li J-F, Gnatovskiy L, Deng Y, Zhu L, Grzesik DA, et al. Macrophages regulate the angiogenic switch in a mouse model of breast cancer. Cancer Res. 2006;66(23):11238–46.
7. Vajkoczy P, Farhadi M, Gaumann A, Heidenreich R, Erber R, Wunder A, et al. Microtumor growth initiates angiogenic sprouting with simultaneous expression of VEGF, VEGF receptor-2, and angiopoietin-2. J Clin Invest. 2002;109(6):777–85.
8. Ferrara N. Vascular endothelial growth factor and the regulation of angiogenesis. Recent Prog Horm Res. 2000;55:15–35; discussion 35–6.
9. Van der Meel R, Symons MH, Kudernatsch R, Kok RJ, Schiffelers RM, Storm G, et al. The VEGF/Rho GTPase signalling pathway: a promising target for anti-angiogenic/anti-invasion therapy. Drug Discov Today. 2011;16(5–6):219–28.
10. Carmeliet P, Jain RK. Molecular mechanisms and clinical applications of angiogenesis. Nature. 2011;473(7347):298–307.
11. Gacche RN, Meshram RJ. Targeting tumor micro-environment for design and development of novel anti-angiogenic agents arresting tumor growth. Prog Biophys Mol Biol. 2013;113 (2):333–54.
12. Alameddine RS, Hamieh L, Shamseddine A. From sprouting angiogenesis to erythrocytes generation by cancer stem cells: evolving concepts in tumor microcirculation. Biomed Res Int. 2014;2014:986768.
13. van Hinsbergh VWM, Koolwijk P. Endothelial sprouting and angiogenesis: matrix metalloproteinases in the lead. Cardiovasc Res. 2008;78(2):203–12.
14. Paku S, Dezső K, Bugyik E, Tóvári J, Tímár J, Nagy P, et al. A new mechanism for pillar formation during tumor-induced intussusceptive angiogenesis: inverse sprouting. Am J Pathol. 2011;179(3):1573–85.
15. Bergers G, Song S. The role of pericytes in blood-vessel formation and maintenance. Neuro Oncol. 2005;7(4):452–64.
16. Ausprunk DH, Folkman J. Migration and proliferation of endothelial cells in preformed and newly formed blood vessels during tumor angiogenesis. Microvasc Res. 1977;14(1):53–65.
17. Makanya AN, Hlushchuk R, Djonov VG. Intussusceptive angiogenesis and its role in vascular morphogenesis, patterning, and remodeling. Angiogenesis. 2009;12(2):113–23.
18. Auguste P, Lemiere S, Larrieu-Lahargue F, Bikfalvi A. Molecular mechanisms of tumor vascularization. Crit Rev Oncol Hematol. 2005;54(1):53–61.
19. Hlushchuk R, Ehrbar M, Reichmuth P, Heinimann N, Styp-Rekowska B, Escher R, et al. Decrease in VEGF expression induces intussusceptive vascular pruning. Arterioscler Thromb Vasc Biol. 2011;31(12):2836–44.
20. Hlushchuk R, Makanya AN, Djonov V. Escape mechanisms after antiangiogenic treatment, or why are the tumors growing again? Int J Dev Biol. 2011;55(4–5):563–7.
21. Baluk P, Morikawa S, Haskell A, Mancuso M, McDonald DM. Abnormalities of basement membrane on blood vessels and endothelial sprouts in tumors. Am J Pathol. 2003;163 (5):1801–15.

22. Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science. 2005;307(5706):58–62.
23. Morikawa S, Baluk P, Kaidoh T, Haskell A, Jain RK, McDonald DM. Abnormalities in pericytes on blood vessels and endothelial sprouts in tumors. Am J Pathol. 2002;160(3): 985–1000.
24. Bussolati B, Grange C, Camussi G. Tumor exploits alternative strategies to achieve vascularization. FASEB J. 2011;25(9):2874–82.
25. Baluk P, Hashizume H, McDonald DM. Cellular abnormalities of blood vessels as targets in cancer. Curr Opin Genet Dev. 2005;15(1):102–11.
26. Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science. 1983;219 (4587):983–5.
27. De Vries C, Escobedo JA, Ueno H, Houck K, Ferrara N, Williams LT. The fms-like tyrosine kinase, a receptor for vascular endothelial growth factor. Science. 1992;255(5047):989–91.
28. Ferrara N. Role of vascular endothelial growth factor in the regulation of angiogenesis. Kidney Int. 1999;56(3):794–814.
29. Shibuya M. VEGF-VEGFR signals in health and disease. Biomol Ther (Seoul). 2014; 22(1):1–9.
30. Qi L, Xing LN, Wei X, Song SG. Effects of VEGF suppression by small hairpin RNA interference combined with radiotherapy on the growth of cervical cancer. Genet Mol Res. 2014;13(3):5094–106.
31. Rinck-Junior JA, Oliveira C, Lourenço GJ, Sagarra RAM, Derchain SFM, Segalla JG, et al. Vascular endothelial growth factor (VEGF) polymorphism and increased risk of epithelial ovarian cancer. J Cancer Res Clin Oncol. 2014 Aug 5.
32. Piccolo E, Tinari N, Semeraro D, Traini S, Fichera I, Cumashi A, et al. LGALS3BP, lectin galactoside-binding soluble 3 binding protein, induces vascular endothelial growth factor in human breast cancer cells and promotes angiogenesis. J Mol Med. 2013;91(1):83–94.
33. Salven P, Orpana A, Joensuu H. Leukocytes and platelets of patients with cancer contain high levels of vascular endothelial growth factor. Clin Cancer Res. 1999;5(3):487–91.
34. Detmar M, Yeo KT, Nagy JA, Van de Water L, Brown LF, Berse B, et al. Keratinocyte-derived vascular permeability factor (vascular endothelial growth factor) is a potent mitogen for dermal microvascular endothelial cells. J Invest Dermatol. 1995;105(1): 44–50.
35. Bergers G, Brekken R, McMahon G, Vu TH, Itoh T, Tamaki K, et al. Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. Nat Cell Biol. 2000;2(10):737–44.
36. Giordano G, Febbraro A, Venditti M, Campidoglio S, Olivieri N, Raieta K, et al. Targeting angiogenesis and tumor microenvironment in metastatic colorectal cancer: role of aflibercept. Gastroenterol Res Pract. 2014;2014:526178.
37. Li X, Lee C, Tang Z, Zhang F, Arjunan P, Li Y, et al. VEGF-B. Cell Adh Migr. 2009;3 (4):322–7.
38. Zhang F, Tang Z, Hou X, Lennartsson J, Li Y, Koch AW, et al. VEGF-B is dispensable for blood vessel growth but critical for their survival, and VEGF-B targeting inhibits pathological angiogenesis. Proc Natl Acad Sci USA. 2009;106(15):6152–7.
39. Presta M, Dell'Era P, Mitola S, Moroni E, Ronca R, Rusnati M. Fibroblast growth factor/fibroblast growth factor receptor system in angiogenesis. Cytokine Growth Factor Rev. 2005;16(2):159–78.
40. Laschke MW, Elitzsch A, Vollmar B, Vajkoczy P, Menger MD. Combined inhibition of vascular endothelial growth factor (VEGF), fibroblast growth factor and platelet-derived growth factor, but not inhibition of VEGF alone, effectively suppresses angiogenesis and vessel maturation in endometriotic lesions. Hum Reprod. 2006;21(1):262–8.
41. Felcht M, Luck R, Schering A, Seidel P, Srivastava K, Hu J, et al. Angiopoietin-2 differentially regulates angiogenesis through TIE2 and integrin signaling. J Clin Invest. 2012;122(6):1991–2005.

42. Riabov V, Gudima A, Wang N, Mickley A, Orekhov A, Kzhyshkowska J. Role of tumor associated macrophages in tumor angiogenesis and lymphangiogenesis. Front Physiol (Internet). 2014 Mar 5 [cited 2014 May 14];5. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3942647/.
43. Rigamonti N, Kadioglu E, Keklikoglou I, Wyser Rmili C, Leow CC, De Palma M. Role of angiopoietin-2 in adaptive tumor resistance to VEGF signaling blockade. Cell Reports. 2014;8(3):696–706.
44. Helfrich I, Edler L, Sucker A, Thomas M, Christian S, Schadendorf D, et al. Angiopoietin-2 levels are associated with disease progression in metastatic malignant melanoma. Clin Cancer Res. 2009;15(4):1384–92.
45. Goede V, Coutelle O, Neuneier J, Reinacher-Schick A, Schnell R, Koslowsky TC, et al. Identification of serum angiopoietin-2 as a biomarker for clinical outcome of colorectal cancer patients treated with bevacizumab-containing therapy. Br J Cancer. 2010;103(9):1407–14.
46. Daly C, Eichten A, Castanaro C, Pasnikowski E, Adler A, Lalani AS, et al. Angiopoietin-2 functions as a Tie2 agonist in tumor models, where it limits the effects of VEGF inhibition. Cancer Res. 2013;73(1):108–18.
47. Park JH, Park KJ, Kim YS, Sheen SS, Lee KS, Lee HN, et al. Serum angiopoietin-2 as a clinical marker for lung cancer. Chest. 2007;132(1):200–6.
48. Vu TH, Werb Z. Matrix metalloproteinases: effectors of development and normal physiology. Genes Dev. 2000;14(17):2123–33.
49. Alexius-Lindgren M, Andersson E, Lindstedt I, Engström W. The RECK gene and biological malignancy-its significance in angiogenesis and inhibition of matrix metalloproteinases. Anticancer Res. 2014;34(8):3867–73.
50. Takagi S, Simizu S, Osada H. RECK negatively regulates matrix metalloproteinase-9 transcription. Cancer Res. 2009;69(4):1502–8.
51. Takenaka K, Ishikawa S, Kawano Y, Yanagihara K, Miyahara R, Otake Y, et al. Expression of a novel matrix metalloproteinase regulator, RECK, and its clinical significance in resected non-small cell lung cancer. Eur J Cancer. 2004;40(10):1617–23.
52. Varon D, Hayon Y, Dashevsky O, Shai E. Involvement of platelet derived microparticles in tumor metastasis and tissue regeneration. Thromb Res. 2012;130(Suppl 1):S98–9.
53. Brass LF. Thrombin and platelet activation. Chest. 2003;124(3 Suppl):18S–25S.
54. Yuan L, Liu X. Platelets are associated with xenograft tumor growth and the clinical malignancy of ovarian cancer through an angiogenesis-dependent mechanism. Mol Med Rep. 2015;11(4):2449–58.
55. Zigler M, Kamiya T, Brantley EC, Villares GJ, Bar-Eli M. PAR-1 and thrombin: the ties that bind the microenvironment to melanoma metastasis. Cancer Res. 2011;71(21):6561–6.
56. Pavlides S, Vera I, Gandara R, Sneddon S, Pestell RG, Mercier I, et al. Warburg meets autophagy: cancer-associated fibroblasts accelerate tumor growth and metastasis via oxidative stress, mitophagy, and aerobic glycolysis. Antioxid Redox Signal. 2011;16(11):1264–84.
57. Orimo A, Gupta PB, Sgroi DC, Arenzana-Seisdedos F, Delaunay T, Naeem R, et al. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell. 2005;121(3):335–48.
58. Raz Y, Erez N. An inflammatory vicious cycle: fibroblasts and immune cell recruitment in cancer. Exp Cell Res. 2013;319(11):1596–603.
59. Martens JWM, Sieuwerts AM, Bolt-deVries J, Bosma PT, Swiggers SJJ, Klijn JGM, et al. Aging of stromal-derived human breast fibroblasts might contribute to breast cancer progression. Thromb Haemost. 2003;89(2):393–404.
60. Kessenbrock K, Plaks V, Werb Z. Matrix metalloproteinases: regulators of the tumor microenvironment. Cell. 2010;141(1):52–67.
61. Pavlides S, Whitaker-Menezes D, Castello-Cros R, Flomenberg N, Witkiewicz AK, Frank PG, et al. The reverse Warburg effect: aerobic glycolysis in cancer associated fibroblasts and the tumor stroma. Cell Cycle. 2009;8(23):3984–4001.

62. Pontiggia O, Sampayo R, Raffo D, Motter A, Xu R, Bissell MJ, et al. The tumor microenvironment modulates tamoxifen resistance in breast cancer: a role for soluble stromal factors and fibronectin through β1 integrin. Breast Cancer Res Treat. 2012;133(2):459–71.
63. Luo H, Tu G, Liu M, Liu Z. Cancer-associated fibroblasts: A multifaceted driver of breast cancer progression. Cancer Letters (Internet). (cited 2015 Feb 27); Available from: http://www.sciencedirect.com/science/article/pii/S0304383515001214.
64. Hida K, Hida Y, Amin DN, Flint AF, Panigrahy D, Morton CC, et al. Tumor-associated endothelial cells with cytogenetic abnormalities. Cancer Res. 2004;64(22):8249–55.
65. Bussolati B, Deambrosis I, Russo S, Deregibus MC, Camussi G. Altered angiogenesis and survival in human tumor-derived endothelial cells. FASEB J. 2003;17(9):1159–61.
66. Wang R, Chadalavada K, Wilshire J, Kowalik U, Hovinga KE, Geber A, et al. Glioblastoma stem-like cells give rise to tumour endothelium. Nature. 2010;468(7325):829–33.
67. Salomon DS, Brandt R, Ciardiello F, Normanno N. Epidermal growth factor-related peptides and their receptors in human malignancies. Crit Rev Oncol Hematol. 1995;19(3):183–232.
68. Ellis LM. Epidermal growth factor receptor in tumor angiogenesis. Hematol Oncol Clin North Am. 2004;18(5):1007–21, viii.
69. Mantovani A, Bottazzi B, Colotta F, Sozzani S, Ruco L. The origin and function of tumor-associated macrophages. Immunol Today. 1992;13(7):265–70.
70. Qian B-Z, Pollard JW. Macrophage diversity enhances tumor progression and metastasis. Cell. 2010;141(1):39–51.
71. Ding Y, Song N, Luo Y. Role of bone marrow-derived cells in angiogenesis: focus on macrophages and pericytes. Cancer Microenvironment. 2012;5(3):225–36.
72. Eubank TD, Galloway M, Montague CM, Waldman WJ, Marsh CB. M-CSF induces vascular endothelial growth factor production and angiogenic activity from human monocytes. J Immunol. 2003;171(5):2637–43.
73. Wyckoff J, Wang W, Lin EY, Wang Y, Pixley F, Stanley ER, et al. A paracrine loop between tumor cells and macrophages is required for tumor cell migration in mammary tumors. Cancer Res. 2004;64(19):7022–9.

Chapter 3
Cancer-Induced Neurogenesis

Tanya Miladinovic and Gurmit Singh

Abstract This chapter explores what we know about the structure and function of neurons, including the identity and location of adult neural stem cells, the proliferation and specification of neural progenitors, and their suspected involvement in cancer. We begin with a brief review of conventional accounts of neurogenesis and progress toward current issues in the field. Finally, we discuss the potential influence of cancer on the formation and innervation of new neural networks, and the effects of this on metastatic tumour progression. The process of neurogenesis was traditionally believed to occur exclusively during embryonic stages, but recent evidence strongly suggests that neurogenesis occurs in discrete regions of the adult mammalian central nervous system (CNS), and that this process may be upregulated in the presence of cancer. A complex network of biochemical pathways and signalling molecules influence metastatic tumour growth. The dysregulation of these signalling pathways by cancer drives tumour growth and leads to significant symptoms, including pain. Tumour cells secrete growth factors, cytokines, and chemokines and are reported to stimulate adjacent nociceptors. Progressive tumour growth is accompanied by escalating pain behaviours in murine models of cancer-induced bone pain. Neurotrophic factors play an important role in the functionality of nociceptive afferents, and represent a probable link between metastatic tumour growth and pain.

Keywords Neurogenesis · Cancer · Cancer-induced neurogenesis · Neurotrophins · Nerve growth factor · Brain-derived neurotrophic factor

T. Miladinovic
Department of Pathology and Molecular Medicine, McMaster University,
1280 Main Street West, HSC-4N48, Hamilton, ON L8S 4L8, Canada
e-mail: tanyamiladinovic@gmail.com

G. Singh (✉)
Department of Pathology and Molecular Medicine, McMaster University,
Hamilton, ON, Canada
e-mail: singhg@mcmaster.ca

G. Singh (ed.), *Oncodynamics: Effects of Cancer Cells on the Body*,
DOI 10.1007/978-3-319-28558-0_3

Abbreviations

CNS	Central nervous system
BrdU	Bromodeoxyuridine
IHC	Immunohistochemistry
VEGF	Vascular endothelial growth factor
NGF	Nerve growth factor
BDNF	Brain-derived neurotrophic factor
NT	Neurotrophin
NTR	Neurotrophin receptor
CIBP	Cancer-induced bone pain
NSAIDs	Nonsteroidal anti-inflammatory drugs
CGRP	Calcitonin gene-related peptide
NMDA	N-methyl-D-aspartate
AFT-3	Activating transcription factor-3

Introduction

While our understanding of neurogenesis has increased dramatically within the past decade, the field remains relatively elusive, and we are far from a comprehensive understanding. Even more mysterious is the role of neurogenesis in cancer. This is an exciting time to be involved in the field of neurogenesis, as imaging techniques are creating platforms to investigate novel ideas. This chapter will explore what we know about the structure and function of neurons, including the identity and location of adult neural stem cells, the proliferation and specification of neural progenitors, and their suspected involvement in cancer. We begin with a brief review of conventional accounts of neurogenesis and progress toward current issues in the field. Finally, we discuss the potential influence of cancer on the formation and innervation of new neural networks, and the effects of this on metastatic tumour progression.

The Birth of Neurogenesis

Santiago Ramon y Cajal is widely recognized as the father of neuroscience. Traditionally, neurons were believed to be generated exclusively during the prenatal phase of development [79]. “No new neurons after birth” became the central dogma in neuroscience for nearly a century [36]. In the late 1950s, however, a new technique was developed to label dividing cells with [H^3]-thymidine, which incorporates into DNA during the replicative S-phase of the cell cycle and can be detected with autoradiography [95]. In 1961, the generation of new neurons was first reported using this technique on three-day-old mouse brains. Shortly after, Altman and colleagues published a series of reports demonstrating [H^3]-thymidine evidence for new

neurons in the adult rat brain, particularly in the dentate gyrus of the hippocampus [4], neocortex [3], and olfactory bulb [2]. At the time, these studies were seen to lack functional relevance and were not given much attention. In the late 1970s, the issue of adult neurogenesis was revisited when it was demonstrated that newborn neurons in the hippocampus survive for a long period of time [49], receive synaptic inputs [48], and project their axons to target areas [96]. Meanwhile, a series of studies that focused on adult neurogenesis in songbirds provided evidence for functional roles of post-natal neurogenesis in seasonal song learning [72].

Neurogenesis is now a widely studied phenomenon and has known applications that extend beyond simple embryonic proliferation. Research suggests that it is functionally implicated in many mental and physiological illnesses, including cancer.

Neurons

A neuron is an electrically excitable cell that uses chemical signals to transmit information between the brain and body. A typical neuron is composed of a soma (cell body), dendrites, and an axon (Fig. 3.1). Dendrites are thin, branched

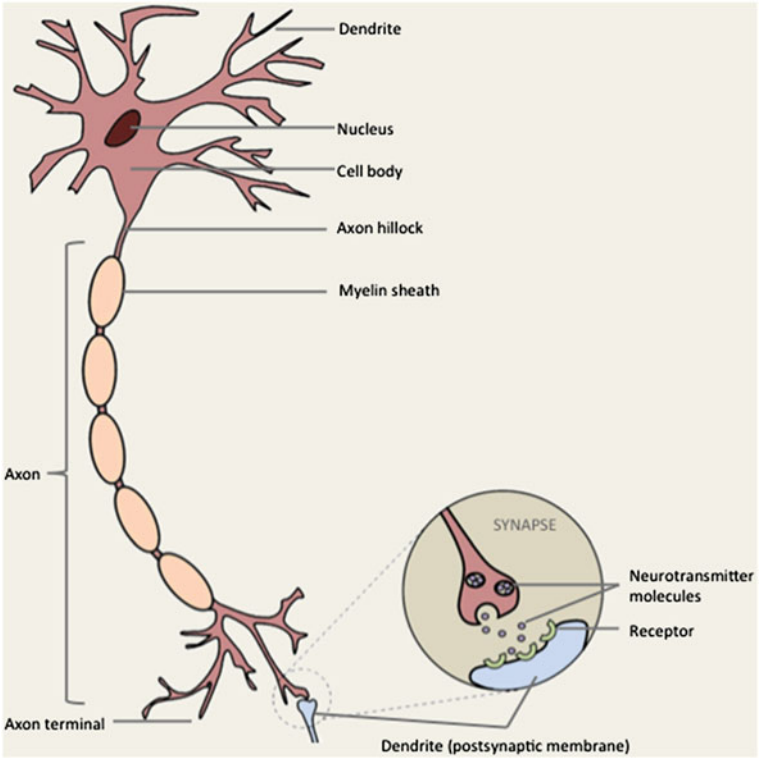


Fig. 3.1 A typical neuron

structures that extend from the cell body and cumulatively form the dendritic tree. Axons also extend from the cell body, but unlike dendrites, each soma gives rise to only a single axon. Axons leave the soma at a swelling called the axon hillock and may be extremely long, extending up to one metre in humans. Electrical signals are transmitted from the cell body, down the axon, to the dendrites of adjacent neurons through a process called saltatory conduction. A layer of electrically insulating material called myelin surrounds axons, creating a myelin sheath that propagates the nerve impulse while preventing the loss of electrical current [103]. At the synapse, the small gap between neurons, signals are transmitted from the axon of one neuron to the dendrites of surrounding neurons via excitatory and inhibitory messengers, called neurotransmitters. Neurons do not undergo cell division, but arise from progenitor cells, or stem cells.

Neurogenesis

Neurogenesis is the process of generating functionally integrated neurons from progenitor cells. Traditionally, this process was believed to occur exclusively during embryonic stages [79], but recent evidence strongly suggests that neurogenesis occurs in discrete regions of the adult mammalian central nervous system (CNS), including two brain regions called the subventricular zone of the lateral ventricle and the subgranular zone of the dentate gyrus in the hippocampus [36, 50, 58]. Beyond these two structures, neurogenesis has appeared to be nonexistent in healthy individuals. However, following trauma and pathological stimulation, non-neurogenic regions in the adult brain appear to support neurogenesis. In an effort to study this phenomenon in adults, neural stem cells were first isolated from the adult CNS of rodents [80] and later from humans [54].

Bromodeoxyuridine (BrdU), a synthetic thymidine analogue and S-phase marker of the cell cycle [34], is detectable by immunohistochemistry (IHC) and can be used for phenotypic analysis and stereological quantification, making IHC the most commonly used technique in the field. Adult neurogenesis has been observed with BrdU incorporation in mammals and human samples [29]. Evidence from combined retroviral-based lineage tracing [76, 84] and electrophysiological studies [12, 20, 101] suggest that newborn neurons in the adult mammalian CNS are functionally and synaptically integrated.

Like angiogenesis, neurogenesis involves the development of intricately branched networks that are regulated by guidance factors and cytokines, including semaphorins and their receptors [111], vascular endothelial growth factor (VEGF), which supports neuronal survival [56], plexins [111], and neuropilins [27], which are involved in tumour vascularization. Multiple clinical observations suggest that angiogenesis and angiogenic factors promote neurogenesis. Seizure- and cerebral ischemia-induced brain injury stimulate both angiogenesis and neurogenesis [37, 74]. Notably, neurogenesis is observed in both human patients and animal

models of neurodegenerative diseases including Huntington's, Alzheimer's, and Parkinson's [35, 47, 104].

Adult neural stem cells are unspecified precursor cells with the ability to proliferate and make new neurons, astrocytes, and oligodendrocytes. Bone marrow-derived $CD34^+$ progenitor cells offer promise for the treatment of various diseases through the repair of damaged tissues. Stem cells differentiate into endothelium, hematopoietic cells, and as reported by some, into neurons, fibroblasts, and muscle [32]. $CD34^+$ and $CD133^+$ differentiate into endothelial cells and thereby participate in neurovascularization, the healing of injured tissues, and promotion of tumour growth and inflammation [7, 21, 40]. In animal models, $CD34^+$ stem cells have been shown to indirectly promote neurogenesis through angiogenesis following stroke, possibly due to a reduction in the G1 phase of the cell cycle [99].

Neurotrophins

Neurotrophins are proteins that regulate neuronal survival, axonal proliferation, synaptic plasticity, and neurotransmission [64, 106]. They are a superfamily of polypeptide growth factors including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophins (NTs) 3–6 [16, 25]. They influence cellular function by activating their respective tyrosine kinase receptors TrkA, TrkB, TrkC, and the common neurotrophin receptor (NTR) p75 [23, 98]. Along with their receptors, neurotrophins are key to the survival, development, and function of the vertebrate nervous system [11, 14, 57], and are also present in non-neuronal tissues [92].

Although classically known for their effects on neurons, neurotrophins have been found to be multifunctional and to affect non-neuronal cells as well. Neurotrophins function to stimulate proliferation and differentiation in various cell types, have been implicated in the pain response, and have receptors that are highly expressed in the central and peripheral nervous systems. Although originally thought to function during the developmental stage only, it is now known that their functionality extends to mature stages of life.

Neurotrophins are constitutively expressed at low levels in adult tissues and are upregulated in inflammatory pain states. The p75 NTR binds all the members of the neurotrophin family with low affinity, while NGF, BDNF and NT-4/5, and NT-3 bind preferentially to TrkA, TrkB, and TrkC, respectively (Fig. 3.2). Under normal physiological conditions, neurotrophins regulate the differentiation, growth, and survival of neurons.

The Trk receptors are tyrosine kinase receptors. Activation by their ligands leads to dimerization of the receptor and phosphorylation of residues that promote the

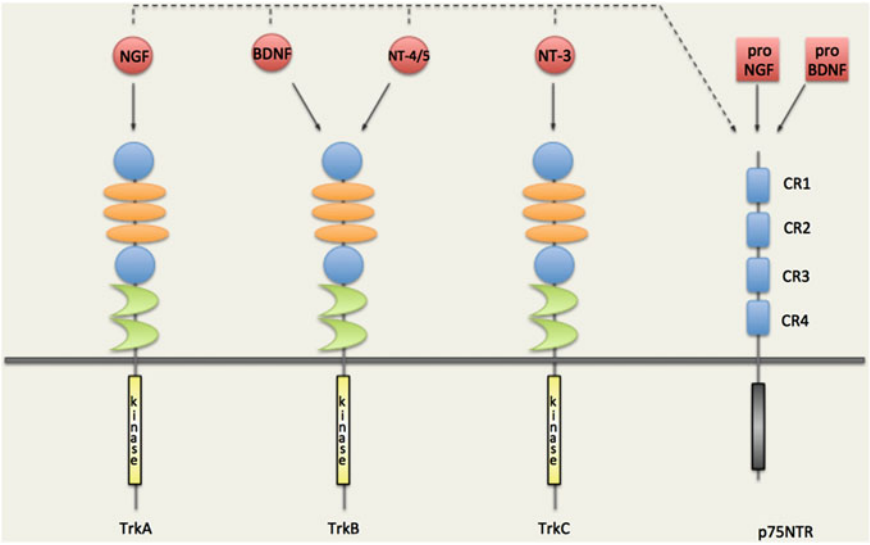


Fig. 3.2 Major neurotrophin–receptor interactions. Proneurotrophins bind p75NTR, but not the Trk receptors. Mature neurotrophins bind and activate p75NTR, and specifically interact with the three Trk receptors. NGF activates TrkA; BDNF and NT4 activate TrkB; NT3 activates TrkC. Ligand-binding specificity is affected by the presence of p75NTR

activation of the Ras-Raf-MAPK, PI3 K-Akt-GSKIII, PLCγ-DAG- PKC, and S6 kinase signalling pathways (Fig. 3.3). The p75 receptor increases the rate of binding of NGF to TrkA, thereby increasing the number of high affinity binding sites [10].

During early development, activation of these pathways blocks apoptosis, thereby promoting cell survival and differentiation. Activation of these pathways in adult neurons regulates neural responsiveness and synaptic function and has important consequences for pain signalling systems.

Regulation of Neurotrophins by System x_C^-

The system x_C^- antiporter exchanges intracellular glutamate for extracellular cysteine at a 1:1 ratio in an effort to protect against oxidative stress. Considerable evidence suggests that glutamate released from system x_C^- is involved in multiple physiological and pathological processes, which may alter neuronal plasticity and can cause cellular toxicity. Glutamate released from activated astrocytes and microglia are capable of killing cortical neurons [30] and granule cells [75], respectively. System x_C^--mediated cystine uptake plays an important role in the regulation of cellular glutathione levels, as glutathione synthesis in the brain is rate-limited by the uptake of cystine [83]. In astrocytes, overexpression of xCT, the functional subunit of system x_C^-, enhances glutathione release and protects

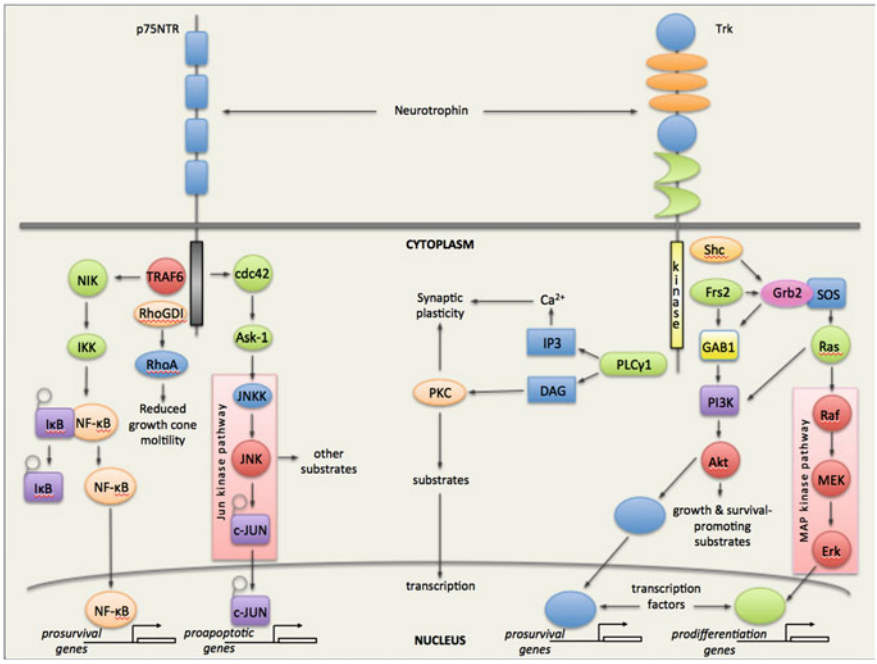


Fig. 3.3 Major intracellular signalling pathways and the interactions of neurotrophins with Trk and p75NTRs. The p75NTR regulates three signalling pathways. NF-kB activation results in transcription of multiple genes that promote neuronal survival. Activation of the Jun kinase pathway regulates activation of several genes, some of which promote neuronal apoptosis. Ligand engagement of p75NTR controls the activity of Rho, which controls growth cone motility. Each Trk receptor controls three major signalling pathways. Activation of Ras activates the MAP kinase signalling cascade, which promotes neuronal differentiation and neurite outgrowth. Activation of PI3 kinase through Ras or Gab1 promotes survival and growth of neurons and other cells. Activation of PLC-g1 results in activation of Ca2C- and PKC-regulated pathways that promote synaptic plasticity. Each of these signalling pathways also regulates gene transcription

neurons from oxidative stress [93]. By overexpressing glutamate, system x_C^- has the potential to cause excitotoxicity.

Cancer cell lines release excess glutamate via system x_C^- [86, 90, 100], and there is considerable evidence for bidirectional signalling between glutamate and neurotrophins. That is, glutamate upregulates neurotrophin expression, while the neurotrophins then upregulate the expression of the system x_C^- transporter [61]. Neurotrophins have many functions within the CNS, including mediating excitotoxicity, oxidative stress, and cellular glutathione levels. Given the high level of overlap between system x_C^- and growth factors, it is perhaps not surprising that some neurotrophic effects may be mediated by the functional regulation of system x_C^-.

Cancer-Induced Bone Pain

Cancer has the propensity to metastasize to the bone microenvironment, causing severe cancer-induced bone pain (CIBP) in patients [69], which is characterized as ongoing or breakthrough pain. Ongoing pain is characteristically dull, persistent, increasing in intensity over time, and is often pharmacologically managed with the use of nonsteroidal anti-inflammatory drugs (NSAIDs). Breakthrough pain is further characterized as "spontaneous pain," without an apparent trigger, or "movement-evoked pain," brought on by movement of the tumour-bearing bone. Exogenous glutamate sensitizes adjacent nociceptors and initiates a pain response in peripheral tissues [18, 19].

CIBP elicits neurochemical changes unique from inflammatory or neuropathic pain states. Bone is innervated with Aβ, Aδ, and C fibres [65]. The acidic tumour environment, along with the secretion of growth factors, cytokines, and chemokines from the tumour cells, are reported to stimulate adjacent nociceptors and evoke pain [71].

Studies have shown that progressive tumour growth is accompanied by pain behaviours in rats [38] and mice [100]. Metastatic tumour growth is influenced by a complex network of biochemical pathways and signalling molecules. The dysregualtion of these signalling pathways by cancer drives tumour growth and leads to significant symptoms, including pain. Neurotrophic factors play an important role in the functionality of nociceptive afferents, and represent a probable link between metastatic tumour growth and pain.

Animal sarcoma models mimic the relative resistance to opioid therapy seen in humans with bone cancer pain, such that 10-fold higher doses of morphine are required to control bone cancer pain as compared to chronic inflammatory pain [63]. Neuropathic pain is also resistant to standard opioid analgesic therapy [108]. Taken together, this information suggests that a potential neuropathic component may be involved in driving bone cancer pain.

Neurotrophins as a Mechanism for Cancer-Induced Bone Pain

Progenitor cells function to repair injured tissues by facilitating new muscle, nerve, and blood vessel formation. Paradoxically, the same progenitors may contribute to tumour growth by promoting angiogenesis and tumour invasiveness.

A tumour is far from an isolated structure within its host organism; it interacts with its environment directly via cell-to-cell contacts. Like native cells, tumours require nutrients and oxygen, as well as a method of excretion for metabolic wastes and carbon dioxide. As an angiogenic response to cancer cells, existing blood vessels are recruited for the host vascular network and new blood vessels form.

It has been hypothesized that neurogenesis significantly contributes to cancer pathology, and that cancer-induced tumours initiate their own innervation by the release of neurotrophic factors in a fashion similar to angiogenesis [28]. That is, tumour cells release neurotrophins, which stimulate adjacent neurons to develop axons that innervate the tumour. These axons then release neurotransmitters, which initiate migratory activity of tumour cells and ultimately foster metastases development [41]. Neural innervation promotes tumour spread along axons, and a major consequence of this innervation is cancer pain.

Neurogenesis clearly has a regulatory mechanism in cancer progression. Notably, neurotrophic activity does not necessarily constitute a sign of malignancy. That is, positive immunostaining for activated neurotrophin receptors in tumour biopsies does not prove that neurotrophins cause metastatic tumours. However, functional experiments provide compelling causative evidence for the involvement of neurotrophins in certain metastatic tumours. Functionally significant neural proliferation has been implicated in neuroblastoma, prostate cancer [8], colorectal cancer [1], esophageal and cardiac carcinoma [62], tumours of the urinary bladder [88], and choroidal melanoma [89]. Together, these studies suggest that the neuroendocrine system plays a major role in metastatic development and cancer progression.

NGF

NGF is important in the modulation of inflammatory [13, 43, 55, 109] and neuropathic [78, 81] pain states, and is expressed by several tumour, inflammatory, and immune cells [26, 102]. Once bound to TrkA, it modulates the expression of the neurotransmitters substance P and calcitonin gene-related peptide (CGRP), receptors, channels, and structural molecules implicated in nociception [39]. It supports nociception through the mechanistic augmentation of afferent neurotransmitter production [5], stimulation of sympathetic fibre ingrowth into dorsal root ganglia [24, 77], and activation of signalling pathways including MAPK [44, 73].

NGF is involved in tumour progression via the generation of a positive microenvironment for cancer cell survival and proliferation [53, 66, 70], and acts as a mediator and modulator of pain in a variety of pain states, including metastatic tumour-induced pain [6, 52, 94]. Humans also report pain at the site of injection after acute administration of NGF [68, 97].

In several malignancies, including breast, prostate, and pancreatic cancers, NGF is implicated in perineural invasion, a process in which cancer cells invade the surrounding nerves [51, 112]. Accordingly, as a potential therapy for cancer pain, researchers have suggested the pharmacological inhibition of NGF and its cognate receptor, TrkA [9].

Early and sustained administration of anti-NGF has been shown to suppress tumour-induced pain and nerve sprouting within tumour-bearing bones [17, 39, 45]. Mouse models of CIBP reveal that nociceptive fibres that innervate bone express TrkA receptors, and treatment with anti-NGF, a selective antagonist, attenuates

behavioural signs of CIBP [17, 39, 45, 46, 67]. Tumour angiogenesis and growth are facilitated by NGF-induced neuronal system development [82]. NGF is a pro-angiogenic factor in breast cancer [82], and neutralization of NGF partially reverses cancer-induced angiogenesis. Together, NGF and its cognate receptor are considered to be major mediator of chronic pain [107].

BDNF

While NGF seemingly has the most prominent influence on CIBP, BDNF also plays a role in tumour pain, although its precise role has not yet been fully elucidated. BDNF is expressed by nociceptors and is upregulated in inflammatory conditions. Increased levels of BDNF are observed in several tumours, including orthotopic hepatocellular carcinoma, multiple myeloma, and neuroblastoma [110]. BDNF released within the spinal cord induces phosphorylation of N-methyl-D-aspartate (NMDA) receptors on adjacent spinal cord neurons, leading to the induction and maintenance of behavioural hypersensitivity following nerve injury [105]. Rats in CIBP groups show microglia and astrocyte activation and upregulation of pro-inflammatory factors, including BDNF, and mechanical allodynia. These phenomena are reversed upon inhibition of the p39 MAPK signalling pathway [60].

Cancer-Induced Neurochemical and Cellular Reorganization

Sarcomas have been shown to induce peripheral changes, including upregulation of activating transcription factor-3 (ATF-3), a marker for injured neurons, and macrophage infiltration of dorsal root ganglion in tumour-bearing femurs [85, 87]. Both mouse and human neoplasms contain few nerve fibres [85, 91], but human studies have revealed abnormal remodelling of adjacent sensory nerve fibres and associated pain in response to tumour growth [15, 22, 59]. Mouse studies show increased periosteal expression of CGRP and substance P, neuropeptides expressed by a subgroup of small neurons that respond to noxious and thermal stimuli.

Spinal cord reorganization is also observed in a fashion similar to central sensitization seen in other pain states, including the upregulation of dynorphin and astrocyte hypertrophy [42, 85, 91]. Interestingly, spinal cord injury patients rarely develop prostate cancer, confirming the significance of nerves in disease progression [31].

NGF stimulates the pathological reorganization of adjacent TrkA sensory nerve fibres. Attempts to systematically prevent the reorganization of sensory nerve fibres reveal the potential mechanisms driving cancer pain [45, 67]. In a mouse model of

prostate CIBP, both preventative and late administration of anti-NGF therapy reduced nociceptive behaviours, sensory and sympathetic nerve sprouting, and neuroma formation [46]. Another study showed that early and sustained inhibition of TrkA markedly attenuated bone cancer pain and significantly blocked the ectopic sprouting of sensory nerve fibres and the formation of neuroma-like structures in the tumour-bearing bone in mice. Late and acute administration of the TrkA inhibitor, however, did not significantly reduce pain or nerve sprouting [33].

Conclusion

Considerable evidence suggests that neurotrophins contribute to tumour growth and cancer pain. NGF acts as a peripheral mediator of pain and is upregulated in inflammatory states. High affinity TrkA receptors are expressed by nociceptors, and NGF sensitizes peripheral nociceptive terminals. Inhibition of this neurotrophin abolishes symptoms characteristic of pain. BDNF is also expressed by nociceptors and is upregulated in inflammatory states. Neutralization of this neurotrophin partially eradicates pain sensitization.

We are in the early stages of understanding the mechanisms that drive metastatic tumour growth, cancer pain, and cancer in general. Neurogenesis appears to contribute to disease progression in a bold way, but more research is needed to elucidate the mechanisms driving sarcomas and to explore treatment options. The use of pharmacological agents to systematically inhibit neurotrophins and their cognate receptors is providing the platform to further investigate promising therapies for controlling tumour proliferation.

References

1. Albo D, Akay CL, Marshall CL, et al. Neurogenesis in colorectal cancer is a marker of aggressive tumor behavior and poor outcomes. Cancer. 2011;117:4834–45. doi:10.1002/cncr.26117.
2. Altman J. Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb. J Comp Neurol. 1969;137:433–57. doi:10.1002/cne.901370404.
3. Altman J. Autoradiographic and histological studies of postnatal neurogenesis. II. A longitudinal investigation of the kinetics, migration and transformation of cells incorporating tritiated thymidine in infant rats, with special reference to postnatal neurogenesis. J Comp Neurol. 1966;128:431–73. doi:10.1002/cne.901280404.
4. Altman J, Das GD. Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. J Comp Neurol. 1965;124:319–35.

5. Amann R, Sirinathsinghji DJ, Donnerer J, et al. Stimulation by nerve growth factor of neuropeptide synthesis in the adult rat in vivo: bilateral response to unilateral intraplantar injections. Neurosci Lett. 1996;203:171–4.
6. Andreev NY, Dimitrieva N, Koltzenburg M, McMahon SB. Peripheral administration of nerve growth factor in the adult rat produces a thermal hyperalgesia that requires the presence of sympathetic post-ganglionic neurones. Pain. 1995;63:109–15.
7. Asahara T, Murohara T, Sullivan A, et al. Isolation of putative progenitor endothelial cells for angiogenesis. Science. 1997;275:964–7.
8. Ayala GE, Dai H, Powell M, et al. Cancer-related axonogenesis and neurogenesis in prostate cancer. Clin Cancer Res. 2008;14:7593–603. doi:10.1158/1078-0432.CCR-08-1164.
9. Bapat AA, Hostetter G, Von Hoff DD, Han H. Perineural invasion and associated pain in pancreatic cancer. Nat Rev Cancer. 2011;11:695–707. doi:10.1038/nrc3131.
10. Barker PA. High affinity not in the vicinity? Neuron. 2007;53:1–4. doi:10.1016/j.neuron.2006.12.018.
11. Bekinschtein P, Cammarota M, Izquierdo I, Medina JH. BDNF and memory formation and storage. Neuroscientist. 2008;14:147–56. doi:10.1177/1073858407305850.
12. Belluzzi O, Benedusi M, Ackman J, LoTurco JJ. Electrophysiological differentiation of new neurons in the olfactory bulb. J Neurosci. 2003;23:10411–8.
13. Bennett DL. Neurotrophic factors: important regulators of nociceptive function. Neuroscientist. 2001;7:13–7.
14. Bibel M, Barde YA. Neurotrophins: key regulators of cell fate and cell shape in the vertebrate nervous system. Genes Dev. 2000;14:2919–37.
15. Black JA, Nikolajsen L, Kroner K, et al. Multiple sodium channel isoforms and mitogen-activated protein kinases are present in painful human neuromas. Ann Neurol. 2008;64:644–53. doi:10.1002/ana.21527.
16. Blasco-Gutiérrez MJ, José-Crespo IJS, Zozaya-Alvarez E, et al. TrkC: a new predictive marker in breast cancer? Cancer Invest. 2007;25:405–10. doi:10.1080/07357900701206349.
17. Bloom AP, Jimenez-Andrade JM, Taylor RN, et al. Breast cancer-induced bone remodeling, skeletal pain, and sprouting of sensory nerve fibers. J Pain. 2011;12:698–711. doi:10.1016/j.jpain.2010.12.016.
18. Cairns BE, Gambarota G, Svensson P, et al. Glutamate-induced sensitization of rat masseter muscle fibers. Neuroscience. 2002;109:389–99.
19. Cairns BE, Svensson P, Wang K, et al. Ketamine attenuates glutamate-induced mechanical sensitization of the masseter muscle in human males. Exp Brain Res. 2006;169:467–72. doi:10.1007/s00221-005-0158-z.
20. Carleton A, Petreanu LT, Lansford R, et al. Becoming a new neuron in the adult olfactory bulb. Nat Neurosci. 2003;6:507–18. doi:10.1038/nn1048.
21. Carmeliet P. Angiogenesis in life, disease and medicine. Nature. 2005;438:932–6. doi:10.1038/nature04478.
22. Ceyhan GO, Bergmann F, Kadihasanoglu M, et al. Pancreatic neuropathy and neuropathic pain–a comprehensive pathomorphological study of 546 cases. Gastroenterology. 2009;136(177–186):e1. doi:10.1053/j.gastro.2008.09.029.
23. Chang L, Karin M. Mammalian MAP kinase signalling cascades. Nature. 2001;410:37–40. doi:10.1038/35065000.
24. Deng YS, Zhong JH, Zhou XF. Effects of endogenous neurotrophins on sympathetic sprouting in the dorsal root ganglia and allodynia following spinal nerve injury. Exp Neurol. 2000;164:344–50. doi:10.1006/exnr.2000.7432.
25. Descamps S, Toillon RA, Adriaenssens E, et al. Nerve growth factor stimulates proliferation and survival of human breast cancer cells through two distinct signaling pathways. J Biol Chem. 2001;276:17864–70. doi:10.1074/jbc.M010499200.

26. Dollé L, El Yazidi-Belkoura I, Adriaenssens E, et al. Nerve growth factor overexpression and autocrine loop in breast cancer cells. Oncogene. 2003;22:5592–601. doi:10.1038/sj.onc.1206805.
27. Ellis LM. The role of neuropilins in cancer. Mol Cancer Ther. 2006;5:1099–107. doi:10.1158/1535-7163.MCT-05-0538.
28. Entschladen F, Palm D, Lang K, et al. Neoneurogenesis: tumors may initiate their own innervation by the release of neurotrophic factors in analogy to lymphangiogenesis and neoangiogenesis. Med Hypotheses. 2006;67:33–5. doi:10.1016/j.mehy.2006.01.015.
29. Eriksson PS, Perfilieva E, Björk-Eriksson T, et al. Neurogenesis in the adult human hippocampus. Nat Med. 1998;4:1313–7. doi:10.1038/3305.
30. Fogal B, Li J, Lobner D, et al. System x(c)- activity and astrocytes are necessary for interleukin-1 beta-mediated hypoxic neuronal injury. J Neurosci. 2007;27:10094–105. doi:10.1523/JNEUROSCI.2459-07.2007.
31. Frisbie JH, Binard J. Low prevalence of prostatic cancer among myelopathy patients. J Am Paraplegia Soc. 1994;17:148–9.
32. Garmy-Susini B, Varner JA. Circulating endothelial progenitor cells. Br J Cancer. 2005;93:855–8. doi:10.1038/sj.bjc.6602808.
33. Ghilardi JR, Freeman KT, Jimenez-Andrade JM, et al. Administration of a tropomyosin receptor kinase inhibitor attenuates sarcoma-induced nerve sprouting, neuroma formation and bone cancer pain. Mol Pain. 2010;6:87. doi:10.1186/1744-8069-6-87.
34. Gratzner HG. Monoclonal antibody to 5-bromo- and 5-iododeoxyuridine: a new reagent for detection of DNA replication. Science. 1982;218:474–5.
35. Greenberg DA, Jin K. From angiogenesis to neuropathology. Nature. 2005;438:954–9. doi:10.1038/nature04481.
36. Gross CG. Neurogenesis in the adult brain: death of a dogma. Nat Rev Neurosci. 2000;1:67–73. doi:10.1038/35036235.
37. Gu W, Brännström T, Wester P. Cortical neurogenesis in adult rats after reversible photothrombotic stroke. J Cereb Blood Flow Metab. 2000;20:1166–73. doi:10.1097/00004647-200008000-00002.
38. Gui Q, Xu C, Zhuang L, et al. A new rat model of bone cancer pain produced by rat breast cancer cells implantation of the shaft of femur at the third trochanter level. Cancer Biol Ther. 2013;14:193–9. doi:10.4161/cbt.23291.
39. Halvorson KG, Kubota K, Sevcik MA, et al. A blocking antibody to nerve growth factor attenuates skeletal pain induced by prostate tumor cells growing in bone. Cancer Res. 2005;65:9426–35. doi:10.1158/0008-5472.CAN-05-0826.
40. Hattori K, Dias S, Heissig B, et al. Vascular endothelial growth factor and angiopoietin-1 stimulate postnatal hematopoiesis by recruitment of vasculogenic and hematopoietic stem cells. J Exp Med. 2001;193:1005–14.
41. Honore P, Mantyh PW. Bone cancer pain: from mechanism to model to therapy. Pain Med. 2000;1:303–9.
42. Honore P, Rogers SD, Schwei MJ, et al. Murine models of inflammatory, neuropathic and cancer pain each generates a unique set of neurochemical changes in the spinal cord and sensory neurons. Neuroscience. 2000;98:585–98.
43. Jaggar SI, Scott HC, Rice AS. Inflammation of the rat urinary bladder is associated with a referred thermal hyperalgesia which is nerve growth factor dependent. Br J Anaesth. 1999;83:442–8.
44. Ji R-R, Suter MR. p38 MAPK, microglial signaling, and neuropathic pain. Mol Pain. 2007;3:33. doi:10.1186/1744-8069-3-33.
45. Jimenez-Andrade JM, Bloom AP, Stake JI, et al. Pathological sprouting of adult nociceptors in chronic prostate cancer-induced bone pain. J Neurosci. 2010;30:14649–56. doi:10.1523/JNEUROSCI.3300-10.2010.

46. Jimenez-Andrade JM, Ghilardi JR, Castañeda-Corral G, et al. Preventive or late administration of anti-NGF therapy attenuates tumor-induced nerve sprouting, neuroma formation, and cancer pain. Pain. 2011;152:2564–74. doi:10.1016/j.pain.2011.07.020.
47. Jin K, Peel AL, Mao XO, et al. Increased hippocampal neurogenesis in Alzheimer's disease. Proc Natl Acad Sci USA. 2004;101:343–7. doi:10.1073/pnas.2634794100.
48. Kaplan MS, Bell DH. Neuronal proliferation in the 9-month-old rodent-radioautographic study of granule cells in the hippocampus. Exp Brain Res. 1983;52:1–5.
49. Kaplan MS, Hinds JW. Neurogenesis in the adult rat: electron microscopic analysis of light radioautographs. Science. 1977;197:1092–4.
50. Kempermann G, Gage FH. New nerve cells for the adult brain. Sci Am. 1999;280:48–53.
51. Kolokythas A, Cox DP, Dekker N, Schmidt BL. Nerve growth factor and tyrosine kinase A receptor in oral squamous cell carcinoma: is there an association with perineural invasion? J Oral Maxillofac Surg. 2010;68:1290–5. doi:10.1016/j.joms.2010.01.006.
52. Koltzenburg M. The changing sensitivity in the life of the nociceptor. Pain Suppl. 1999;6:S93–102.
53. Krüttgen A, Schneider I, Weis J. The dark side of the NGF family: neurotrophins in neoplasias. Brain Pathol. 2006;16:304–10. doi:10.1111/j.1750-3639.2006.00037.x.
54. Kukekov VG, Laywell ED, Suslov O, et al. Multipotent stem/progenitor cells with similar properties arise from two neurogenic regions of adult human brain. Exp Neurol. 1999;156:333–44. doi:10.1006/exnr.1999.7028.
55. Lamb K, Kang YM, Gebhart GF, Bielefeldt K. Nerve growth factor and gastric hyperalgesia in the rat. Neurogastroenterol Motil. 2003;15:355–61.
56. Lazarovici P, Marcinkiewicz C, Lelkes PI. Cross talk between the cardiovascular and nervous systems: neurotrophic effects of vascular endothelial growth factor (VEGF) and angiogenic effects of nerve growth factor (NGF)-implications in drug development. Curr Pharm Des. 2006;12:2609–22.
57. Lewin GR, Barde YA. Physiology of the neurotrophins. Annu Rev Neurosci. 1996;19:289–317. doi:10.1146/annurev.ne.19.030196.001445.
58. Lie DC, Song H, Colamarino SA, et al. Neurogenesis in the adult brain: new strategies for central nervous system diseases. Annu Rev Pharmacol Toxicol. 2004;44:399–421. doi:10.1146/annurev.pharmtox.44.101802.121631.
59. Lindqvist A, Rivero-Melian C, Turan I, Fried K. Neuropeptide- and tyrosine hydroxylase-immunoreactive nerve fibers in painful Morton's neuromas. Muscle Nerve. 2000;23:1214–8.
60. Liu X, Bu H, Liu C, et al. Inhibition of glial activation in rostral ventromedial medulla attenuates mechanical allodynia in a rat model of cancer-induced bone pain. J Huazhong Univ Sci Technolog Med Sci. 2012;32:291–8. doi:10.1007/s11596-012-0051-5.
61. Liu X, Resch J, Rush T, Lobner D. Functional upregulation of system xc- by fibroblast growth factor-2. Neuropharmacology. 2012;62:901–6. doi:10.1016/j.neuropharm.2011.09.019.
62. Lü S-H, Zhou Y, Que H-P, Liu S-J. Peptidergic innervation of human esophageal and cardiac carcinoma. World J Gastroenterol. 2003;9:399–403.
63. Luger NM, Sabino MAC, Schwei MJ, et al. Efficacy of systemic morphine suggests a fundamental difference in the mechanisms that generate bone cancer vs inflammatory pain. Pain. 2002;99:397–406.
64. Lykissas MG, Batistatou AK, Charalabopoulos KA, Beris AE. The role of neurotrophins in axonal growth, guidance, and regeneration. Curr Neurovasc Res. 2007;4:143–51.
65. Mach DB, Rogers SD, Sabino MC, et al. Origins of skeletal pain: sensory and sympathetic innervation of the mouse femur. Neuroscience. 2002;113:155–66.
66. Mancino M, Ametller E, Gascón P, Almendro V. The neuronal influence on tumor progression. Biochim Biophys Acta. 2011;1816:105–18. doi:10.1016/j.bbcan.2011.04.005.

67. Mantyh WG, Jimenez-Andrade JM, Stake JI, et al. Blockade of nerve sprouting and neuroma formation markedly attenuates the development of late stage cancer pain. Neuroscience. 2010;171:588–98. doi:10.1016/j.neuroscience.2010.08.056.
68. McArthur JC, Yiannoutsos C, Simpson DM, et al. A phase II trial of nerve growth factor for sensory neuropathy associated with HIV infection. AIDS Clinical Trials Group Team 291. Neurology. 2000;54:1080–8.
69. Mercadante S. Malignant bone pain: pathophysiology and treatment. Pain. 1997;69:1–18.
70. Molloy NH, Read DE, Gorman AM. Nerve growth factor in cancer cell death and survival. Cancers (Basel). 2011;3:510–30. doi:10.3390/cancers3010510.
71. Mundy GR. Pathophysiology of cancer-associated hypercalcemia. Semin Oncol. 1990;17:10–5.
72. Nottebohm F. The road we travelled: discovery, choreography, and significance of brain replaceable neurons. Ann NY Acad Sci. 2004;1016:628–58. doi:10.1196/annals.1298.027.
73. Obata K, Yamanaka H, Dai Y, et al. Activation of extracellular signal-regulated protein kinase in the dorsal root ganglion following inflammation near the nerve cell body. Neuroscience. 2004;126:1011–21. doi:10.1016/j.neuroscience.2004.04.036.
74. Parent JM, Yu TW, Leibowitz RT, et al. Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. J Neurosci. 1997;17:3727–38.
75. Piani D, Fontana A. Involvement of the cystine transport system xc- in the macrophage-induced glutamate-dependent cytotoxicity to neurons. J Immunol. 1994;152:3578–85.
76. Price J, Turner D, Cepko C. Lineage analysis in the vertebrate nervous system by retrovirus-mediated gene transfer. Proc Natl Acad Sci USA. 1987;84:156–60.
77. Ramer MS, Bisby MA. Adrenergic innervation of rat sensory ganglia following proximal or distal painful sciatic neuropathy: distinct mechanisms revealed by anti-NGF treatment. Eur J Neurosci. 1999;11:837–46.
78. Ramer MS, Kawaja MD, Henderson JT, et al. Glial overexpression of NGF enhances neuropathic pain and adrenergic sprouting into DRG following chronic sciatic constriction in mice. Neurosci Lett. 1998;251:53–6.
79. Ramón y Cajal S. Degeneration and regeneration of the nervous system. London: Oxford University Press; 1913.
80. Reynolds BA, Weiss S. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science. 1992;255:1707–10.
81. Ro LS, Chen ST, Tang LM, Jacobs JM. Effect of NGF and anti-NGF on neuropathic pain in rats following chronic constriction injury of the sciatic nerve. Pain. 1999;79:265–74.
82. Romon R, Adriaenssens E, Lagadec C, et al. Nerve growth factor promotes breast cancer angiogenesis by activating multiple pathways. Mol Cancer. 2010;9:157. doi:10.1186/1476-4598-9-157.
83. Sagara JI, Miura K, Bannai S. Maintenance of neuronal glutathione by glial cells. J Neurochem. 1993;61:1672–6.
84. Sanes JR, Rubenstein JL, Nicolas JF. Use of a recombinant retrovirus to study post-implantation cell lineage in mouse embryos. EMBO J. 1986;5:3133–42.
85. Schwei MJ, Honore P, Rogers SD, et al. Neurochemical and cellular reorganization of the spinal cord in a murine model of bone cancer pain. J Neurosci. 1999;19:10886–97.
86. Seidlitz EP, Sharma MK, Saikali Z, et al. Cancer cell lines release glutamate into the extracellular environment. Clin Exp Metastasis. 2009;26:781–7. doi:10.1007/s10585-009-9277-4.
87. Seidlitz EP, Sharma MK, Singh G. Extracellular glutamate alters mature osteoclast and osteoblast functions. Can J Physiol Pharmacol. 2010;88:929–36. doi:10.1139/y10-070.
88. Seifert P, Benedic M, Effert P. Nerve fibers in tumors of the human urinary bladder. Virchows Arch. 2002;440:291–7. doi:10.1007/s004280100496.

89. Seifert P, Spitznas M. Axons in human choroidal melanoma suggest the participation of nerves in the control of these tumors. Am J Ophthalmol. 2002;133:711–3.
90. Serre CM, Farlay D, Delmas PD, Chenu C. Evidence for a dense and intimate innervation of the bone tissue, including glutamate-containing fibers. Bone. 1999;25:623–9.
91. Sevcik MA, Ghilardi JR, Peters CM, et al. Anti-NGF therapy profoundly reduces bone cancer pain and the accompanying increase in markers of peripheral and central sensitization. Pain. 2005;115:128–41. doi:10.1016/j.pain.2005.02.022.
92. Shibayama E, Koizumi H. Cellular localization of the Trk neurotrophin receptor family in human non-neuronal tissues. Am J Pathol. 1996;148:1807–18.
93. Shih AY, Erb H, Sun X, et al. Cystine/Glutamate exchange modulates glutathione supply for neuroprotection from oxidative stress and cell proliferation. J Neurosci. 2006;26:10514–23. doi:10.1523/JNEUROSCI.3178-06.2006.
94. Shu XQ, Mendell LM. Neurotrophins and hyperalgesia. Proc Natl Acad Sci USA. 1999;96:7693–6.
95. Sidman RL, Miale IL, Feder N. Cell proliferation and migration in the primitive ependymal zone: an autoradiographic study of histogenesis in the nervous system. Exp Neurol. 1959;1:322–33.
96. Stanfield BB, Trice JE. Evidence that granule cells generated in the dentate gyrus of adult rats extend axonal projections. Exp Brain Res. 1988;72:399–406.
97. Svensson P, Cairns BE, Wang K, Arendt-Nielsen L. Injection of nerve growth factor into human masseter muscle evokes long-lasting mechanical allodynia and hyperalgesia. Pain. 2003;104:241–7.
98. Sweatt JD. The neuronal MAP kinase cascade: a biochemical signal integration system subserving synaptic plasticity and memory. J Neurochem. 2001;76:1–10.
99. Taguchi A, Soma T, Tanaka H, et al. Administration of CD34+ cells after stroke enhances neurogenesis via angiogenesis in a mouse model. J Clin Invest. 2004;114:330–8. doi:10.1172/JCI20622.
100. Ungard RG, Seidlitz EP, Singh G. Inhibition of breast cancer-cell glutamate release with sulfasalazine limits cancer-induced bone pain. Pain. 2014;155:28–36. doi:10.1016/j.pain.2013.08.030.
101. Van Praag H, Schinder AF, Christie BR, et al. Functional neurogenesis in the adult hippocampus. Nature. 2002;415:1030–4. doi:10.1038/4151030a.
102. Vega JA, García-Suárez O, Hannestad J, et al. Neurotrophins and the immune system. J Anat. 2003;203:1–19.
103. Virchow R. Ueber das ausgebreitete Vorkommen einer dem Nervenmark analogen Substanz in den thierischen Geweben. Arch für Pathol Anat und Physiol und für Klin Med. 1854;6:562–72. doi:10.1007/BF02116709.
104. Wada K, Arai H, Takanashi M, et al. Expression levels of vascular endothelial growth factor and its receptors in Parkinson's disease. NeuroReport. 2006;17:705–9. doi:10.1097/01.wnr.0000215769.71657.65.
105. Wang L-N, Yang J-P, Ji F-H, et al. Brain-derived neurotrophic factor modulates N-methyl-D-aspartate receptor activation in a rat model of cancer-induced bone pain. J Neurosci Res. 2012;90:1249–60. doi:10.1002/jnr.22815.
106. Wang W, Chen J, Guo X. The role of nerve growth factor and its receptors in tumorigenesis and cancer pain. Biosci Trends. 2014;8:68–74.
107. Watson JJ, Allen SJ, Dawbarn D. Targeting nerve growth factor in pain: what is the therapeutic potential? BioDrugs. 2008;22:349–59.
108. Woolf CJ, Mannion RJ. Neuropathic pain: aetiology, symptoms, mechanisms, and management. Lancet. 1999;353:1959–64. doi:10.1016/S0140-6736(99)01307-0.
109. Woolf CJ, Safieh-Garabedian B, Ma QP, et al. Nerve growth factor contributes to the generation of inflammatory sensory hypersensitivity. Neuroscience. 1994;62:327–31.

110. Yang ZF, Ho DW, Lam CT, et al. Identification of brain-derived neurotrophic factor as a novel functional protein in hepatocellular carcinoma. Cancer Res. 2005;65:219–25.
111. Yazdani U, Terman JR. The semaphorins. Genome Biol. 2006;7:211. doi:10.1186/gb-2006-7-3-211.
112. Zhu Z, Friess H, diMola FF, et al. Nerve growth factor expression correlates with perineural invasion and pain in human pancreatic cancer. J Clin Oncol. 1999;17:2419–28.

Chapter 4
Cancer-Induced Inflammation

Kimberly Young and Gurmit Singh

Abstract The relationship between inflammation and cancer has long been discussed, ever since Virchow first postulated the role of chronic inflammation in the onset of cancer. Though much research since then has focused on inflammation-induced cancer, it is of equal importance to consider the impact tumour cells can have on the immune system. Stemming from the broader concept of "oncodynamics", this chapter will discuss cancer-induced inflammation and immunosuppression caused by the release of tumour-derived factors that act on the body's immune cells.

Keywords Inflammation · Immunosuppression · STAT3 · TAM · Macrophages · MSC · MDSC · NF-κB · Cytokines · Chemokines

Abbreviation

ARG1	Arginase 1
CSF1	Colony stimulating factor 1
CTL	Cytotoxic T lymphocyte ($CD8^+$ T-cell)
DC	Dendritic cell
FOXP3	Forkhead box P3
IFN	Interferon
IL	Interleukin
iMC	Immature myeloid cell
iNOS	Inducible nitric oxide synthase
JAK	Janus kinase
LPS	Lipopolysaccharide
M-CSF	Macrophage colony-stimulating factor
MDSC	Myeloid-derived suppressor cell

K. Young · G. Singh (✉)
Department of Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada
e-mail: singhg@mcmaster.ca

K. Young
e-mail: youngk3@mcmaster.ca

G. Singh (ed.), *Oncodynamics: Effects of Cancer Cells on the Body*,
DOI 10.1007/978-3-319-28558-0_4

MHC	Major histocompatibility complex
MSC	Myeloid suppressor cell
NF-κB	Nuclear factor kappa B
NK	Natural killer
NO	Nitric oxide
NOS2	Inducible nitric oxide synthase
ROS	Reactive oxygen species
STAT	Signal transducer and activator of transcription
TAM	Tumour-associated macrophage
TAN	Tumour-associated neutrophil
TCR	T cell receptor
TGF-β	Transforming growth factor beta
TLR	Toll-like receptor
TME	Tumour microenvironment
TNF	Tumour necrosis factor
T_{reg}	Regulatory T cell
VEGF	Vascular endothelial growth factor

The Link Between Inflammation and Cancer

Rudolf Virchow's discovery of leukocytes in the tumour microenvironment (TME) in 1863 led him to postulate the existence of a link between cancer and inflammation; specifically, that the genesis of cancer is rooted at sites of chronic inflammation [1–4]. Since that initial observation, much research has been conducted in order to investigate the potential role of an inflammatory environment in the onset of cancer. However, although it is important to understand the mechanisms through which immune cells contribute to the progression of cancer, one must not neglect the effects that tumours themselves have on the immune system as they bear equally important implications for cancer treatment and prevention.

The release of various tumour-derived factors, including cytokines, chemokines, growth factors, and transcription factors, recruits a medley of immune cells into the TME. The infiltrating immune cells then go on to have anti-tumour, immunostimulatory capabilities or pro-tumour, immunosuppressive functions. This chapter will explore an assortment of immune cells that are affected by cancer and propagate cancer-induced inflammation.

STAT3

The signal transducer and activator of transcription (STAT) proteins are a family of transcription factors that modulate cell growth. Their activation, in response to growth factors and cytokines, occurs through tyrosine phosphorylation by a

member of the Janus kinase (JAK) tyrosine kinase family [5–7]. Although seven members of the STAT family have been characterized thus far, focus here will be drawn to STAT3 as it possesses especially significant immunosuppressive properties and is a key modulator of inflammation [7–9].

STAT3 is constitutively activated, at high concentrations, in both cancer cells and immune cells in the TME [10, 11]. This persistent activation is the result of an amplification loop where tumour-derived factors interleukin (IL)-6, IL-10, and vascular endothelial growth factor (VEGF) are both activators of and, in turn, upregulated by STAT3 [9, 11–13]. IL-10 is a key immunosuppressive cytokine, affecting an array of immune cells, highly concentrated in the TME [14, 15]. IL-6 is one of the major mediators of acute inflammation and exists in especially high concentrations in patients with colon cancer [14, 16]. The oncoproteins SRC and ABL are also known activators of STAT3, lending its widespread involvement across different types of cancer [11, 13].

The activation of STAT3 in tumour cells suppresses the body's anti-tumour immune response [10, 17, 18]. For example, STAT3 downregulates expression of IL-12, major histocompatibility complex (MHC) class II, and co-stimulatory molecules CD80 and CD86 in dendritic cells (DCs), impeding their functional maturation [11, 17, 18]. Immature dendritic cells are rendered unable to stimulate natural killer (NK) and $CD8^+$ T-cells (also referred to as cytotoxic T lymphocytes or CTLs), though blocking STAT3 signalling reverses these deficits in DC functioning [11, 19]. STAT3 signalling in immature myeloid cells (iMCs) also blocks their functional differentiation into mature DCs [20, 21]. Furthermore, STAT3 contributes to immune evasion by inhibiting the expression of inflammatory mediators and signals necessary for immune activation, including pro-inflammatory cytokines and chemokines [11, 21]. This includes potently inhibiting T helper 1 (T_h1)-type cytokines, like IL-12 and interferon (IFN) γ, limiting T_h1 cell-mediated inflammation [11, 15]. Inhibiting STAT3 activation subsequently upregulates these pro-inflammatory cytokines and chemokines, restoring cytotoxicity to immune cells [11].

STAT3 activity also increases the congregation of tumour-associated regulatory T (T_{reg}) cells, due in part to the fact that immature DCs promote their accumulation in the TME [11]. Immature DCs are also primed to drive the differentiation of $CD4^+$ T cells into T_{reg} cells, furthering the recruitment of T_{reg} cells to the tumour site [21]. STAT3 signalling in T_{reg} cells themselves promotes expression of transforming growth factor beta (TGF-β), IL-10, and forkhead box P3 (FOXP3): TGF-β and IL-10 block the production of IFNγ, resulting in decreased cytotoxicity, and inhibit the proliferation of cytotoxic T cells; FOXP3 halts DC maturation [11, 22, 23]. The implications of high T_{reg} cell concentrations on host immunity are described in greater depth later in the chapter.

STAT3 is one of the main transcription factors that promote migration and expansion of myeloid-derived suppressor cells (MDSCs), sometimes referred to as myeloid suppressor cells (MSCs) [20, 24]. STAT3 induces the expression of two S100 calcium-binding proteins, S100A8 and S100A9, which inhibit iMC maturation and play a crucial role in drawing MDSCs, which express S100A8 and S100A9 receptors, to the tumour site [20, 25]. MDSCs themselves also retain the capability

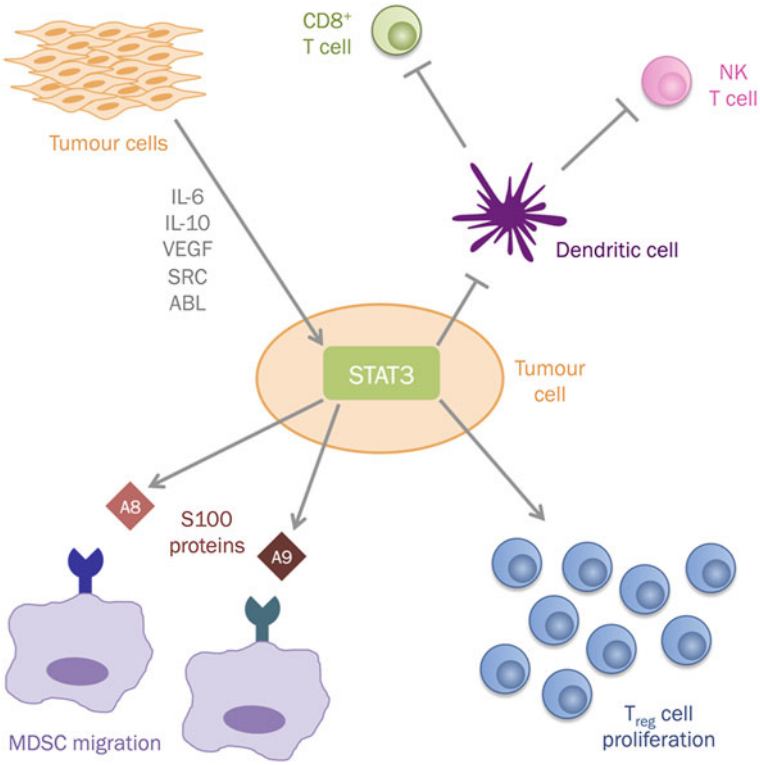


Fig. 4.1 STAT3 transcription factors

to express S100 proteins, completing an autocrine loop that fosters continued recruitment of MDSCs to the TME [18]. MDSCs also express carboxylated N-glycan receptors, which S100A8 and S100A9 also bind to, on their cell surface [20, 26] (Fig. 4.1). The following section will discuss the effects of MDSCs on immunity in further detail.

Myeloid-Derived Suppressor Cells

MDSCs represent a combination of various myeloid progenitor cells and iMCs (which include immature macrophages, granulocytes, and DCs) belonging to the myelomonocytic differentiation pathway [19, 20, 24]. In a cancerous environment, these cells are prohibited from maturing and are left to expand as immature cells. The resulting heterogenous family of iMCs is then induced by T cell- and tumour-derived factors, like IL-10, to become immunosuppressive [18, 20, 21]. In addition to STAT3, tumours recruit MDSCs to the TME and prohibit their

maturation using various factors, including IL-1β, TGF-β, VEGF, and colony stimulating factor 1 (CSF1; also referred to as macrophage colony-stimulating factor, or M-CSF) [14, 18, 24, 27–30].

The main mechanism through which MDSCs negatively affect host immunity involves interfering with the L-arginine pathway in order to suppress T cell proliferation and $CD8^+$ T cell responses [17, 18, 24, 31, 32]. Specifically, MDSCs exploit two enzymes involved in the metabolism of arginine: inducible nitric oxide synthase (NOS2, also abbreviated as iNOS) and arginase 1 (ARG1), both of which are expressed by cancer cells and tumour-associated myeloid cells [33–36]. NOS2 oxidizes L-arginine to yield citrulline and nitric oxide (NO), the latter of which inhibits T cell functionality by blocking phosphorylation of key signalling molecules (including STAT5, Erk, and Akt) that impairs several IL-2 receptor (IL-2R) pathways [18, 20, 21, 33–35]. ARG1 is responsible for converting L-arginine to L-ornithine and urea [20, 21, 33–35]. Depletion of L-arginine, especially through the ARG1-mediated metabolic pathway, results in impaired expression of the CD3 ζ chain, the part of the T cell receptor (TCR) complex responsible for signal transduction and TCR expression [15, 20, 33, 36, 37]. This adversely affects T cell proliferation.

NOS2 and ARG1 activity are usually regulated conversely of each other, where T_h1-type cytokines, like IFNγ, act as inducers of NOS2 activity and suppress ARG1 whilst T_h2-type cytokines, like IL-4 and IL-13, bear an antithetic effect [33, 35]. Both tumour-associated T cells and tumour cells produce these T_h1- and T_h2-type cytokines [20]. However, in tumour environments where there exists a sufficient mixture of both T_h1- and T_h2-type cytokines, NOS2 and ARG1 become concurrently active [33, 35]. In addition to each enzyme's individual effects, the coactivation of NOS2 and ARG1 results in the production of the oxidizing agent peroxynitrite ($ONOO^-$). Peroxynitrites nitrate protein tyrosines, rendering them unable to become phosphorylated. This, in turn, induces T cell apoptosis [15, 33, 37]. Therefore, whether they are acting separately or synergistically, NOS2 and ARG1 greatly impair T cell immunity (Fig. 4.2).

Beyond their effects on T cells, MDSCs retain a plethora of immunosuppressive functions, including inhibiting NK cells, inactivating $CD4^+$ T cells, and expressing reactive oxygen species (ROS) [20, 21, 31]. Finally, in response to tumour-produced factors like IFNγ and IL-10, MDSCs induce and expand T_{reg} cell populations [20, 31]. Regulatory T cells will be the focus of the next section.

T_{reg} Cells

Tumours release factors IL-10 and TGF-β to induce the T_{reg} phenotype in T cells and their recruitment into the TME [11, 38]. Tumour cells and tumour-associated macrophages overexpress the chemokine CCL2; this is especially true in ovarian cancer cell lines. T_{reg} cells, which express the corresponding chemokine receptor CCR4 on their cell surfaces, then migrate towards the tumour site [11]. From there,

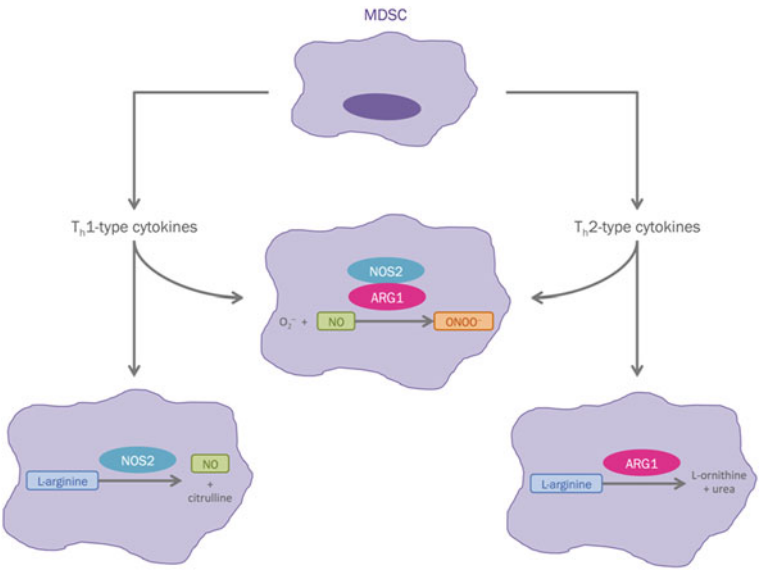


Fig. 4.2 T cell immunity

T_{reg} cells, which are not cytotoxic, suppress adaptive and innate anti-tumour responses from effector T cells and other immune cells like NK cells, T_h1 cells, and DCs [17, 27, 31, 39, 40]. These pro-tumourigenic cells are thought to suppress T cell function by inhibiting their proliferation and IFNγ production, specifically [11].

Tumour-Associated Macrophages

Tumour-associated macrophages, or TAMs, are derived from circulating blood monocytes that are recruited to the TME by tumour-derived chemotactic factor CCL2 [14–16, 37, 41]. Expressed in a diverse range of cancers, levels of CCL2 highly correlate with TAM density at the tumour site and, thus, serve as a reliable indicator of cancer prognosis [15, 32]. It is also of interest to note that TAMs themselves can produce CCL2, hinting at the existence of an amplification loop that sustains TAM accumulation [42]. The acquired monocytes then differentiate into macrophages in response to various tumour-derived factors, including: cytokines VEGF, CSF1, GM-CSF, and IL-3; and chemokines CCL5, CCL7, CCL8, and CXCL12 [15, 32].

A significant source of various cytokines, the main suppressive action of TAMs involves their role in orchestrating the formation of an inflammatory TME [14, 28, 39]. TAMs produce: low levels of inflammatory cytokines, including IL-1β, IL-6, IL-12, and tumour necrosis factor (TNF)-α; high levels of major immunosuppressive and anti-inflammatory cytokines TGF-β and IL-10; and other

factors like NO and prostaglandin E2 (PGE2) [14, 17, 32, 37, 41]. Aside from triggering inflammation around the tumour, these cytokines also suppress adaptive T cell and other anti-tumour responses [20, 28, 37, 41]. IL-10, specifically, induces the expression of programmed death ligand 1 (PD-L1) which interacts with its corresponding receptor, PD-1, on T cells to inhibit their activation and proliferation [31, 32].

TAMs also release several important chemokines into the TME, namely CCL17, CCL18, and CCL22 [37]. CCL18 and CCL22 are released in high concentrations by ovarian carcinoma cells [32, 37]. CCL17 and CCL22 attract T_h2 and T_{reg} cells, specifically, by interacting with their CCR4 receptors [37]. CCL18 mainly recruits naïve T cells, leading to T cell anergy, through interactions with an unidentified receptor [2]. All three aforementioned T cell subsets lack cytotoxic capabilities. Therefore, TAMs are capable of inducing immunosuppressive activity both directly, via cytokines, and indirectly, through the release of chemokines (Fig. 4.3).

When characterizing immune cells, it is important to recognize that one population can be described on a wide spectrum of activated functional states. Depending on how an immune cell is polarized, it can be classified as either immunosuppressive or immunostimulatory. The traditional nomenclature for leukocyte "X" defines X1-type cells as being antitumour and X2-type cells as pro-tumour. Accordingly, X1-polarized cells are classified as "classically" activated whilst X2-polarized cells are deemed "alternatively" activated for their anti-host properties. Macrophages are no exception to this rule, with subtypes organized into M1- and M2-polarized configurations. Macrophages with an M1 phenotype are activated by IFNγ or lipopolysaccharide (LPS) to have cytotoxic effects on tumour cells [18, 37, 39, 43]. They express abundant TNF-α, IL-1, IL-6, IL-12, and IL-23—all of which are

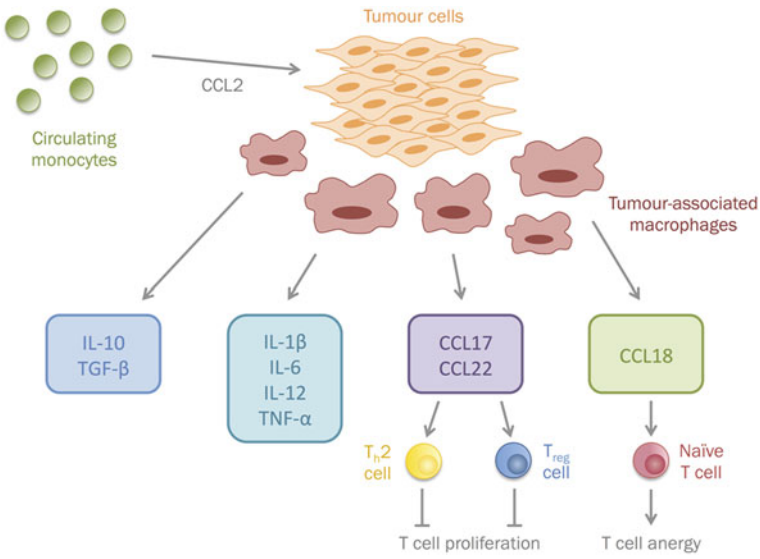


Fig. 4.3 TAMs immunosuppressive activity

pro-inflammatory cytokines [39]. These cytokines help to elicit adaptive host immune responses. On the other hand, macrophages displaying an M2 phenotype act contrarily, suppressing adaptive immunity [15]. This subset is induced by tumour- and macrophage-derived factors including IL-4, IL-6, IL-10, IL-13, PGE2, and TGF-β to become alternatively polarized [15, 39, 44]. Given that TAMs are largely immunosuppressive, it is logical that they are distinguished as an M2-polarized family of macrophages [15, 45, 46].

One final unique characteristic of TAMs to note is their propensity to accumulate in hypoxic and/or necrotic regions of tumours they have infiltrated [14, 15]. Cancer cells release various hypoxia-induced chemoattractants, like VEGF, to attract macrophages to these distinct environments. This unique subset of TAMs then elicits pro-angiogenic properties to propel tumour survival and metastasis [47].

Tumour-Associated Neutrophils

Neutrophils are another important group of leukocytes lured into the TME by tumour-derived CXC chemokines, which are upregulated by the ras oncogene, and cytokines IL-8, TNF-α, and IFNγ [48–51]. Once enlisted into the tumour site, the new TANs, or tumour-associated neutrophils, are induced to polarize into an N1 or N2 phenotype analogous to macrophages. Tumour-secreted cytokine TGF-β is the main enforcer of N2-polarization in neutrophils, after which the TANs act to inhibit effector T cells [49, 50]. The effects of a block in TGF-β expression highlight its crucial role in the initiation of an immunosuppressive phenotype. When TGF-β is no longer active, neutrophils take on the pro-inflammatory and anti-tumourigenic N1 phenotype [50, 52]. N1 neutrophils express immunostimulatory chemokines and cytokines, like TNF-α, IL-1β, and IL-12, and activate $CD8^+$ T cells [48]. This suggests that the immunosuppressive cytokine IL-10, which acts on neutrophils and reduces TNF-α and IL-1β production, may play a role in TAN polarization within the tumoural milieu. Furthermore, it has yet to be elucidated whether N2-type neutrophils are simply less activated forms of their antitumoural counterparts rather than alternatively activated [49].

NF-κB

The nuclear factor kappa B (NF-κB) group of transcription factors, which encompasses all inducible dimeric transcription factors, plays an important role in coordinating inflammation and innate immunity [17, 18, 28, 53]. There are many different stimuli primed to activate NF-κB, as well as two distinct pathways: a classical (which will be the focus here) and an alternative pathway [54]. The classical mechanism involves an array of signals, including TNF-α and IL-1, as well as the TLR-MyD88 signalling pathway [54]. The latter involves the use of toll-like

receptors (TLRs) by immune cells to detect and transmit LPS signals, ultimately leading to the recruitment of the myeloid differentiation primary response gene 88 (MyD88) [57]. In certain varieties of tumours, NF-κB may also present as being constitutively activated [58]. Once NF-κB is activated, it produces various pro-inflammatory cytokines including TNF-α and IL-1, which stimulated it in the first place, as well as IL-6, IL-12, VEGF, TGF-β, and chemokine IL-8 [53–55, 59]. It also regulates the transcription of many genes involved with inflammation and the evelopment of immune cells including T cells, macrophages, and DCs [53, 57, 60].

Conclusion

Cancer cells retain an extremely important role in constructing an inflammatory milieu conducive to their survival. The constitutive activation and expression of an array of tumour-derived factors fosters an environment where immune cells take on adverse functions to benefit tumour growth at the expense of the host.

When considering immunotherapy treatments in combatting cancer, it is important to take into consideration how normal immune cell functions are altered in the tumour microenvironment and how their pathophysiological properties become amplified and perpetuated to allow cancer cells to thrive. Knowledge of how inflammation affects cancer onset and how tumour cells, in turn, shape inflammation arms us with an understanding of the bi-directional dynamics of the immune system. It is through this comprehensive grasp cancer immunology that we can hope to manipulate our body's natural mechanisms to our advantage against cancer.

References

1. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet. 2001;357:539–45.
2. Sica A, Schioppa T, Mantovani A, Allavena P. Tumour-associated macrophages are a distinct M2 polarised population promoting tumour progression: potential targets of anti-cancer therapy. Eur J Cancer. 2006;42:717–27.
3. Bingle L, Brown NJ, Lewis CE. The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies. J Pathol. 2002;196:254–65.
4. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002;420:860–7.
5. Grivennikov SI, Karin M. Dangerous liaisons: STAT3 and NF-κB collaboration and crosstalk in cancer. Cytokine Growth Factor Rev. 2010;21:11–9.
6. Gamero AM, Young HA, Wiltrout RH. Inactivation of STAT3 in tumor cells: releasing a brake on immune responses against cancer? Cancer Cell. 2004:111–2.
7. Groner B, Lucks P, Borghouts C. The function of STAT3 in tumor cells and their microenvironment. Semin Cell Dev Biol. 2008;19:341–50.

8. Fan Y, Mao R, Yang J. NF-κB and STAT3 signaling pathways collaboratively link inflammation to cancer. Protein Cell. 2013;4(3):176–85.
9. Yu H, Pardoll D, Jove R. STATs in cancer inflammation and immunity: a leading role for STAT3. Nat Rev. 2009;9:798–809.
10. Li N, Grivennikov SI, Karin M. The unholy trinity: inflammation, cytokines, and STAT3 shape the cancer microenvironment. Cancer Cell. 2011;19:429–31.
11. Yu H, Kortylewski M, Pardoll D. Crosstalk between cancer and immune cells: role of STAT3 in the tumour microenvironment. Nat Rev Immunol. 2007;7:41–51.
12. Lee H. Kumar Pal S, Reckamp K, Figlin RA, Yu H. STAT3: a target to enhance antitumor immune response. Curr Top Microbiol Immunol. 2011;344:41–59.
13. Kortylewski M, Yu H. Role of Stat3 in suppressing anti-tumor immunity. Curr Opin Immunol. 2008;20(2):228–33.
14. Germano G, Allavena P, Mantovani A. Cytokines as a key component of cancer-related inflammation. Cytokine. 2008;43:374–9.
15. Sica A, Allavena P, Mantovani A. Cancer related inflammation: the macrophage connection. Cancer Lett. 2008;265:204–15.
16. Balkwill FR, Mantovani A. Cancer-related inflammation: common themes and therapeutic opportunities. Semin Cancer Biol. 2012;22:33–40.
17. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature. 2008;454:436–44.
18. Porta C, Larghi P, Rimoldi M, Totaro MG, Allavena P, Mantovani A, et al. Cellular and molecular pathways linking inflammation and cancer. Immunobiology. 2009;214:761–77.
19. Gabrilovich D. Mechanisms and functional significance of tumour-induced dendritic-cell defects. Nat Rev Immunol. 2004;4:941–52.
20. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol. 2009;9:162–74.
21. Qian B, Pollard JW. Macrophage diversity enhances tumor progression and metastasis. Cell. 2010;141:39–51.
22. Kinjyo I, Inoue H, Hamano S, Fukuyama S, Yoshimura T, Koga K, et al. Loss of SOCS3 in T helper cells resulted in reduced immune responses and hyperproduction of interleukin 10 and transforming growth factor-β1. J Exp Med. 2006;203:1021–31.
23. Zorn E, Nelson EA, Mohseni M, Porcheray F, Kim H, Litsa D, et al. IL-2 regulates FOXP3 expression in human $CD4^+$ $CD25^+$ regulatory T cells through a STAT-dependent mechanism and induces the expansion of these cells in vivo. Blood. 2006;108:1571–9.
24. Mantovani A. The growing diversity and spectrum of action of myeloid-derived suppressor cells. Eur J Immunol. 2010;40:3317–20.
25. Ostrand-Rosenberg S, Sinha P. Myeloid-derived suppressor cells: linking inflammation and cancer. J Immunol. 2009;182:4499–506.
26. Sinha P, Okoro C, Foell D, Freeze HH, Ostrand-Rosenberg S, Srikrishna G. Proinflammatory S100 proteins regulate the accumulation of myeloid-derived suppressor cells. J Immunol. 2008;181:4666–75.
27. Chow MT, Moller A, Smyth MJ. Inflammation and immune surveillance in cancer. Semin Cancer Biol. 2012;22:23–32.
28. Allavena P, Garlanda C, Borrello MG, Sica A, Mantovani A. Pathways connecting inflammation and cancer. Curr Opin Genet Dev. 2008;18:3–10.
29. Kusmartsev S, Gabrilovich DI. Immature myeloid cells and cancer-associated immune suppression. Cancer Immunol Immunother. 2002;51:293–8.
30. Zeng Z, Xu X, Zhang Y, Xing J, Long J, Gu L, et al. Tumor-derived factors impaired motility and immune functions of dendritic cells through derangement of biophysical characteristics and reorganization of cytoskeleton. Cell Motil Cytoskelet. 2007;64:186–98.
31. Wang D, DuBois RN. Immunosuppression associated with chronic inflammation in the tumor microenvironment. Carcinogenesis. 2015;36(10):1–9.
32. Qian B, Pollard JW. Macrophage diversity enhances tumor progression and metastasis. Cell. 2010;141:39–51.

33. Bronte V, Serafini P, Mazzoni A, Segal DM, Zanovello P. L-arginine metabolism in myeloid cells controls T-lymphocyte functions. Trends Immunol. 2003;24(6):301–5.
34. Rodriguez PC, Ochoa AC. T cell dysfunction in cancer: role of myeloid cells and tumor cells regulating amino acid availability and oxidative stress. Semin Cancer Biol. 2006;16:66–72.
35. Serafini P, De Santo C, Marigo I, Cingarlini S, Dolcetti L, Gallina G, et al. Derangement of immune responses by myeloid suppressor cells. Cancer Immunol Immunother. 2004;53:64–72.
36. Peranzoni E, Marigo I, Dolcetti L, Ugel S, Sonda N, Taschin E, et al. Role of arginine metabolism in immunity and immunopathology. Immunobiology. 2008;212:795–812.
37. Allavena P, Sica A, Solinas G, Porta C, Mantovani A. The inflammatory micro-environment in tumor progression: the role of tumor-associated macrophages. Crit Rev Oncol Hematol. 2008;66:1–9.
38. Whiteside TL. Immune suppression in cancer: effects on immune cells, mechanisms and future therapeutic intervention. Semin Cancer Biol. 2006;16:3–15.
39. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. Cell. 2010;140:883–99.
40. Balkwill F, Montfort A, Capasso M. B regulatory cells in cancer. Trends Immunol. 2013;34 (4):169–73.
41. Biswas SK, Gangi L, Paul S, Schioppa T, Saccani A, Sironi M, et al. A distinct and unique transcriptional program expressed by tumor-associated macrophages (defective NF-κB and enhanced IRF-3/STAT1 activation). Blood. 2006;107(5):2112–22.
42. Solinas G, Germano G, Mantovani A, Allavena P. Tumor-associated macrophages (TAM) as major players of the cancer-related inflammation. J Leukoc Biol. 2009;86:1065–73.
43. Ruffell B, Affara NI, Coussens LM. Differential macrophage programming in the tumor microenvironment. Trends Immunol. 2012;33(3):119–26.
44. Balkwill F, Charles KA, Mantovani A. Smoldering and polarized inflammation in the initiation and promotion of malignant disease. Cancer Cell. 2005;7:211–7.
45. Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Trends Immunol. 2002;23(11):549–55.
46. Sica A, Larghi P, Mancino A, Rubino L, Porta C, Totaro MG, et al. Macrophage polarization in tumour progression. Semin Cancer Biol. 2008;18:349–55.
47. Balkwill FR, Capasso M, Hagemann T. The tumor microenvironment at a glance. J Cell Sci. 2012;125:5591–6.
48. McGarry Houghton A. The paradox of tumour-associated neutrophils. Cell Cycle. 2010;9(9): 1732–7.
49. Piccard H, Muschel RJ, Opdenakker G. On the dual roles and polarized phenotypes of neutrophils in tumor development and progression. Crit Rev Oncol Hematol. 2012;82:296–309.
50. Fridlender ZG, Albelda SM. Tumor-associated neutrophils: friend or foe? Carcinogenesis. 2012;33(5):949–55.
51. Fridlender ZG, Sun J, Kim S, Kapoor V, Cheng G, Ling L, et al. Polarization of tumor-associated neutrophil phenotype by TGF-β: "N1" versus "N2" TAN. Cancer Cell. 2009;16:183–94.
52. Mantovani A. The yin-yang of tumor-associated neutrophils. Cancer Cell. 2009;16:173–4.
53. Tripathi P, Aggarwal A. NF-κB transcription factor: a key player in the generation of immune response. Curr Sci. 2006;90(4):519–31.
54. Yoshimura A. Signal transduction of inflammatory cytokines and tumor development. Cancer Sci. 2006;97(6):439–47.
55. He W, Liu Q, Wang L, Chen W, Li N, Cao X. TLR4 signaling promotes immune escape of human lung cancer cells by inducing immunosuppressive cytokines and apoptosis resistance. Mol Immunol. 2007;44:2850–9.
56. Karin M, Ben-Neriah Y. Phosphorylation meets ubiquination: the control of NF-κB activity. Annu Rev Immunol. 2000;18:621–63.

57. Li Q, Verma IM. NF-κB regulation in the immune system. Nature Rev Immunol. 2002;2:725–34.
58. Aggarwal BB, Vijayalekshmi RV, Sung B. Targeting inflammatory pathways for prevention and therapy of cancer: short-term friend, long-term foe. Clin Cancer Res. 2009;15(2):425–30.
59. Karin M. Nuclear factor-κB in cancer development and progression. Nature. 2006;441:431–6.
60. Baeuerle PA, Henkel T. Function and activation of NF-κB in the immune system. Annu Rev Immunol. 1994;12:141–79.

Chapter 5
Cancer-Induced Edema/Lymphedema

Jennifer Fazzari and Gurmit Singh

Abstract Cancer patients are often prone to a variety of pathological changes that disrupt normal homeostatic processes in the body. Aside from medical interventions and therapies associated with treating the disease, the cancer itself is a major contributor to systemic disruption of physiological processes. Extracellular body fluid is tightly controlled and monitored by a variety of sensors, hormones, proteins, and organs. Palpable changes in fluid homeostasis can commonly be attributed to inflammation, where the changes in vasculature necessary to facilitate an immune response compromise the vascular endothelial barrier. Such changes, although transient, reveal the consequences of compromised vessel walls, leakage of plasma proteins, and collection of fluid in the interstitial space. Edema represents a pathological form of fluid extravasation into the interstitium and is a common clinical feature in many cases of malignancy. By examining common inflammatory factors secreted by the tumour, it becomes evident that the increased levels of such factors in patient sera could, indeed, influence a pro-edematous state. Therefore, it is the dynamics of the tumour itself in isolation of therapeutic side effects that can influence local and systemic vasculature by promoting a chronic inflammatory state characterized by leaky vasculature and dysregulated fluid homeostasis.

Keywords Edema · Lymphedema · Hydrostatic pressure · Oncotic pressure · Hydraulic conductivity · Coagulation · VEGF · TGF-β · Chronic inflammation · Hyaluronan · Hyponatremia

J. Fazzari
Department of Pathology and Molecular Medicine, McMaster University,
1280 Main Street West, Hamilton L8N 3Z5, ON, Canada
e-mail: fazzarjm@mcmaster.ca

G. Singh (✉)
Department of Pathology and Molecular Medicine, McMaster University,
Hamilton, ON, Canada
e-mail: singhg@mcmaster.ca

G. Singh (ed.), *Oncodynamics: Effects of Cancer Cells on the Body*,
DOI 10.1007/978-3-319-28558-0_5

Dynamics of Transcapillary Flow and Interstitial Fluid Pressures

Body fluid is distributed among three body compartments: the capillaries, lymphatics, and interstitium. The interstitial space is comprised of interstitial fluid (IF) and the extracellular matrix (ECM). IF is responsible for (1) mediating the transport of nutrients and waste products between the vasculature and surrounding tissue, (2) facilitating the transport of signalling molecules between cells, and (3) immune regulation through the transport of cytokines and antigens to the lymph nodes [1]. The interstitium is dynamic and varies depending on the normal and pathological developmental state of the tissue. It is composed mainly of collagen, which provides the structural foundation of the ECM, and glycosaminoglycans [proteoglycans and non-proteoglycans, namely hyaluronic acid or hyaluronan (HA)]. Together, these components make up a gel-like phase that aids in maintaining a concentration gradient and osmotic pressure within the interstitium, allowing it to take in water. This confers the ability of the tissue to resist compression by providing a significant turgor pressure to oppose any constrictive forces. The IF itself also contains plasma proteins and electrolytes [1]. The presence of these interstitial components restricts the presence of macromolecules in the interstitial space, resulting in a volume exclusion effect mediated both by physical and electrostatic exclusion forces. Physiologically, there is free exchange of water, electrolytes, and molecules of a certain molecular size. The site of exchange for these factors is the capillary endothelium.

Fluid homeostasis is maintained by a series of forces arising from cardiac output and protein concentration in the capillaries. Both the capillary and the interstitium exert hydrostatic pressures, where the hydrostatic pressure of the capillary is greater than that of the interstitium, driving fluid out of the capillary at its arterial end. Oncotic pressure exerted by plasma proteins also regulates fluid exchange. Again, both the capillary and the interstitium exert an oncotic pressure, where the oncotic pressure of the capillary is greater than the interstitium, allowing for the reabsorption of water from the interstitium back into circulation at the venous end of the capillary. The net oncotic pressure is dependent on the permeability of the capillary membrane to the proteins that initiate this form of pressure.

In normal physiological conditions, the net movement of fluid out of the capillary is matched by the net movement of fluid into the lymphatic system or back into the blood. Edema is induced by an imbalance in the exchange of fluid between compartments in the tissue [2] with excessive accumulation of IF resulting in the palpable expansion of IF volume [3]. If the filtration rate out of the blood vessels increases, an increase in lymph flow typically compensates for the excess in fluid entering the interstitium. As mentioned, capillary filtration is mediated by both the capillary surface area and the hydraulic conductivity of the capillary, which is the permeability of the capillary to water. The hydraulic conductivity determines the speed at which fluid will move through a vessel under mechanical stress or when subjected to a pressure gradient. Therefore, edema can present when there is excess

filtration out of the capillary or impaired reabsorption of this filtrate. Excess filtration can be induced by (1) increased capillary blood pressure which drives more fluid out of the capillary at the arterial end and (2) increased capillary permeability which raises the osmotic pressure of the IF due to an efflux of plasma protein from the capillary into the interstitium. In a situation of inadequate reabsorption, a decreased concentration of plasma proteins lowers the oncotic pressure in the capillary, preventing fluid uptake at the venous end of the capillary.

Decrease in Colloid Osmotic Pressure

A decrease in the colloid osmotic pressure in the capillary can be the result of a decrease or deficiency in the protein levels in the capillary (albumin), which is responsible for generating the pressure driving fluid into the venous side of the capillary. Second, as seen in inflammation, a leaky vessel wall allows proteins such as albumin to escape the capillary into the interstitium, reversing the concentration gradient and drawing fluid out of the capillary into the extracellular space. Both conditions result in the formation of edema. Observed in cases of idiopathic edema, a large proportion of vascular albumin is found in the extravascular space in combination with a low, total circulating albumin pool leading to increased plasma and IF volume [4]. Not only are these patients in an edematous state, the decrease in plasma volume can result in hypovolemia (low blood volume), if plasma albumin is not regenerated or redistributed. This can further exacerbate edema formation by increasing the production of aldosterone leading to decreased sodium excretion in an effort to retain water and re-establish normal plasma fluid volume. Despite the compensatory action of aldosterone, the additional water retained is still subject to the same disturbance in oncotic pressure due to the leakiness of the blood vessels and the decreased levels of albumin in the blood, exacerbating the increase in IF volume.

Specifically, hypoalbuminemia (low plasma albumin levels) has been associated with critically ill patients experiencing multiple pathologies, including cancer. This has been attributed to increased vascular permeability associated with the disease state. This “leaky” vasculature results in the transcapillary escape of albumin into the interstitium at a rate that far exceeds the normal rate of albumin synthesis and catabolism [5]. In cancer patients, the rate of transcapillary albumin loss is almost doubled relative to that of healthy individuals. This is known as the capillary leak syndrome. Vascular leakage is the movement of solutes and fluids between vascular and extravascular compartments in a pressure-dependent manner. This process is thought to be driven by inflammatory mediators and cytokines, which can both increase vascular permeability and inhibit the transcriptional expression of albumin [6]. In cancer, such factors are subject to overproduction and dysregulation. With higher oncotic pressure in the interstitial space, water will move into the interstitium. It is hypothesized that cancer patients have increased vascular permeability due to tumour-secreted factors, thus presenting an oncological mechanism for edema formation in the absence of therapeutic contributions.

Vascular Permeability

Under normal physiological conditions, the endothelial monolayer lining blood vessels provides a size-selective and semi-permeable barrier between blood plasma and the interstitium [7]. Vascular permeability is normally tightly controlled and regulated by the formation of endothelial cell junctions [8]. Two endothelial transport routes exist for the movement of solutes and ions across the endothelium: paracellular and transcellular mechanisms. The mechanism used is based on solute size where paracellular transport is limited to the movement of solutes under 3 nm in radius [9] and transcellular, vesicle-mediated trafficking selectively transports larger molecules and proteins, including albumin, across the endothelial barrier [10]. These processes regulate fluid homeostasis by controlling the passage of plasma proteins and solutes across the endothelium, and thus modulating the hydrostatic and oncotic pressures across the capillary. Paracellular transport is regulated by tight junctions and adherens junctions, which limit the size of solutes transported. Vascular endothelial (VE)-cadherin proteins are transmembrane components that form these junctions and maintain the integrity of the barrier and limit the diffusion of solutes across the endothelial layer providing a physical interaction between adjacent endothelial cells as well as mediating an intracellular interaction with the actin cytoskeleton via catenin proteins [5]. Disruption of these inter-endothelial junctions can lead to a reduction in oncotic pressure due to albumin loss, resulting in the accumulation of fluid in the interstitium [11]. Pro-angiogenic factors and inflammatory mediators secreted from the tumour cells can destabilize the endothelial layer and result in VE-cadherin internalization and actin–myosin contraction which induces the mechanical disruption of adherens junction [7]. This disruption influences paracellular permeability as endothelial junctions are compromised and as a result fluid balance is altered promoting fluid extravasation and formation of edema.

Mechanisms of Increased Vascular Permeability

Vascular endothelial growth factor (VEGF) is a pro-angiogenic cytokine secreted by tumour cells [12], which induces paracellular permeability through Src-mediated [13] phosphorylation of VE-cadherins [14]. It is a unique endothelial-specific factor known to potently induce vascular leakage [15]. Acting through the VEGFR2 receptor, Src-mediated internalization of VE-cadherins introduces intercellular gaps in the endothelium, compromising the integrity of the paracellular barrier [14]. Several solid tumours show strong VEGF expression [16] including breast [17], colorectal [18, 19], and ovarian [20] carcinomas, with elevated VEGF levels also evident in patient sera [21–27]. Kondo et al. showed that increased levels of VEGF in the bloodstream can be attributed to the presence of tumour itself. They showed that subcutaneous tumour transplantation with HeLa cells induced elevated VEGF

levels in sera of these animals with no change observed when HeLa cells lacking VEGF cDNA was used [22]. In addition to the cancer cells themselves, tumour-associated inflammatory cells (see 28) and peripheral blood cells [27] are known to express VEGF. The concentration of VEGF in lysed whole blood from cancer patients is significantly elevated relative to healthy controls, with the highest concentrations in patients with metastatic cancers [27], therefore, acting as a predictive measure of metastasis [29]. In these patients, elevated VEGF levels correlated to elevated leukocyte and platelet counts, whereas platelet counts did not correlate to VEGF levels in healthy individuals. Elevated VEGF levels have also been associated with platelet–endothelial interactions. Circulating tumour cells adhere to platelets resulting in platelet activation which induces the release of VEGF. This results in the increased vascular permeability required for cellular extravasation and metastasis [30]. Furthermore, platelets are attracted to and can also become activated at these sites of vascular leakage which further propagates the cycle of platelet activation, VEGF expression, and associated vascular leakage which furthers platelet attraction and the cycle repeats [31] (Fig. 5.1). Therefore, tumour cell dissemination as part of the metastatic cascade contributes to the disruption of the endothelial barrier partially mediated by cancer-secreted VEGF [32].

In more detail, changes in the stroma must occur in order to accommodate formation and motility of metastatic tumour cells as a part of malignant invasion. There is a cross-talk between tumour and stroma that is essential for the initial in situ carcinoma (i.e. confined by the basement membrane) to become metastatic. Invading cancer cells must therefore be able to penetrate the collagen-, glycoprotein-, and proteoglycan-rich basement membranes of the ECM to allow for dissemination [33]. The ECM is composed of the basement membrane and interstitial stroma [34]. The process of breaching this barrier therefore involves a cascade of events initiated by tumour-secreted factors that can activate a series of enzymes which are involved in degradation and remodelling of the ECM. These proteinases include matrix metalloproteinases (MMPs), adamalysin-related membrane proteinases, bone morphogenetic protein-1-type metalloproteinases, and tissue serine proteases such as tissue plasminogen activator, urokinase, thrombin, and plasmin [35]. Specifically, MMPs are important components of ECM degradation in many normal and pathological processes such as wound healing, angiogenesis [36], bone dynamics [37] and tumour progression by not only promoting invasion and metastasis through ECM degradation, but also by stimulating tumour growth. Specifically, elevated levels of MMP-9 have been observed in cancer patients with disseminated cancers. MMP-9 is a gelatinase that can degrade collagen in the basement membrane of the ECM and is produced by many cancers. Of significance to vascular disturbances, MMP-9 is also known to induce the release of VEGF from cancer cells [38] which, in turn, can feedback and induce MMP-9 expression in pre-metastatic tissue [39] producing a positive feedback loop that results in pathological levels of vascular permeability-promoting factors. Furthermore, TNF-α has been shown to be secreted by tumour-associated macrophages (TAMs) and also influence the production of MMPs in proximal cancer cells [40].

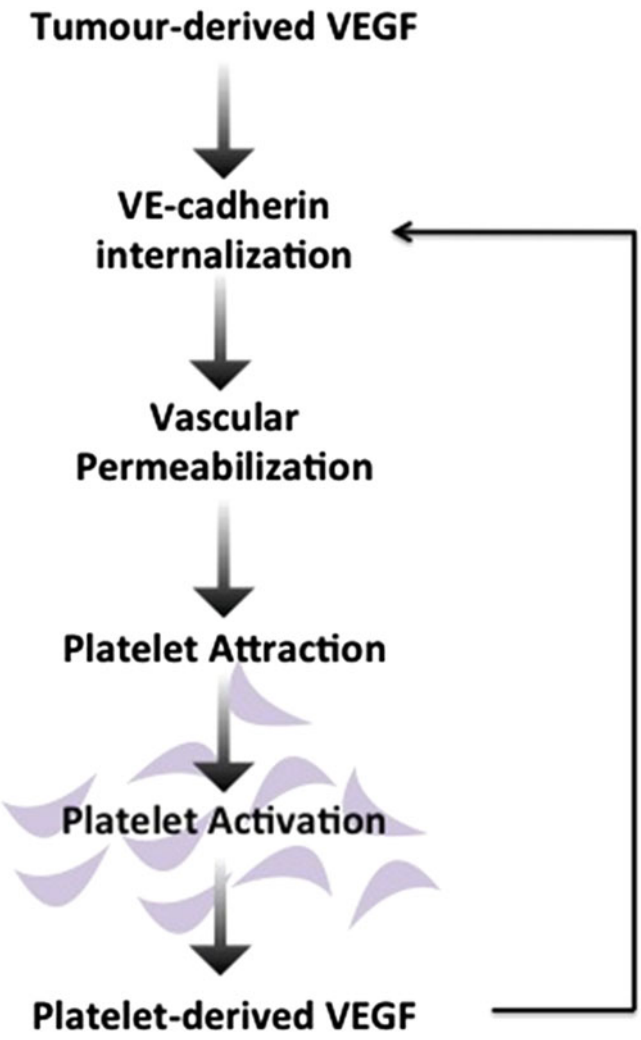


Fig. 5.1 Cycle of vascular permeabilization via platelet activation

If tumour cells and tumour-associated cells of the stroma can induce the production of factors that can induce epithelial membrane degradation as well as stimulate the expression of known permeability factors, it is therefore plausible to hypothesize that cancer dissemination increases the levels of vascular permeability factors that induce leaky vasculature and lead to the presentation of edema.

Degradation of the ECM increases the bioavailability of cytokines and growth factors [41] including TGF-β [42]. In addition, cancer cells themselves are known to secrete TGF-β as well as other cytokines including TNF-α which itself has been shown to play a role in the malignant progression of several cancers [43–45] and

indirectly influence fluid homeostasis. First, TGF-β is a growth factor that has a diverse regulatory control over the ECM, mediating both its synthesis and degradation [42] and has been associated with the induction of vascular permeability in several pathological states. Exogenous (including tumour-derived) TGF-β [46] and TNF-α [47] induce MMP expression in the tumour [48], proximal fibroblasts associated with malignant tissue and stroma [49]. TGF-β also plays another role in upsetting the physical mechanisms controlling fluid permeability. Many studies have associated this cytokine/growth factor with incidences of pulmonary edema (see [50] intro), where pulmonary endothelial monolayers experience a loss of integrity after TGF-β treatment by inducing actin remodelling, TGF-β is known to induce such permeability by inducing changes in endothelial cell shape [51, 52]. This alters the integrity of junctions between endothelial cells forming intracellular gaps and increasing the paracellular transport of macromolecules [53], including albumin. With albumin responsible for generating approximately 70 % of oncotic pressure that maintains fluid homeostasis across the vasculature, loss of albumin to the interstitium, as discussed above, promotes diffusion of water out of the capillary into the interstitium. This process is mediated by the activation of mitogen-activated protein kinase (MAPK) by TGF-β and subsequent phosphorylation of myosin light chain [50]. The resulting actin and cytoskeletal rearrangements induce changes in endothelial cell shape, increasing paracellular permeability [54]. This same MAPK cascade can also be activated by VEGF [55]. VEGF in turn can be activated by TGF-β [56], generating a cyclical signalling cascade that exacerbates vascular permeability (Fig. 5.2).

Angiopoietin–Tie receptor signalling is another important mediator of vascular permeability. Angiopoietin-related protein 4 (ANGPTL4) is yet another target of TGF-β that also plays a role in endothelial barrier disruption and metastasis [57]. Although ANGPLT4 has contradictory roles in the regulation of the integrity of the endothelium and angiogenesis, it has been shown that it can disrupt endothelial barrier integrity by modulating integrin-, VE-cadherin- and claudin-mediated cell contacts [58]. When considering the role of TGF-β discussed so far, TGF-β-mediated expression of ANGPTL4 [57] seems to promote the endothelial disruption associated with metastasis [59]. When put altogether, tumour-derived TGF-β induces a cascade of events that promote vessel permeabilization which, in turn, allows for the escape of normally, capillary-restricted macromolecules namely proteins such as albumin. To recap, leakage of albumin into the interstitium reverses the normal oncotic pressure and drives fluid out of the capillary into surrounding tissues. In conjunction, TNF-α secretion has also been shown to further influence albumin levels by inhibiting its expression at the genetic level [6] which prevents capillary albumin repopulation and further favours disrupted fluid dynamics in a cancerous state (Fig. 5.2).

With tumour-induced signalling increasing vascular permeability and modulating oncotic mediators, fluid is driven out of the capillaries resulting in increased extracellular/interstitial fluid (increased filtration). But why is this fluid state maintained in cancer? Usually, lymph flow can increase in response to an increased filtration rate. However, this increase is limited and a maximal threshold is eventually reached. Therefore, if the increased filtration rate is greater than the maximal

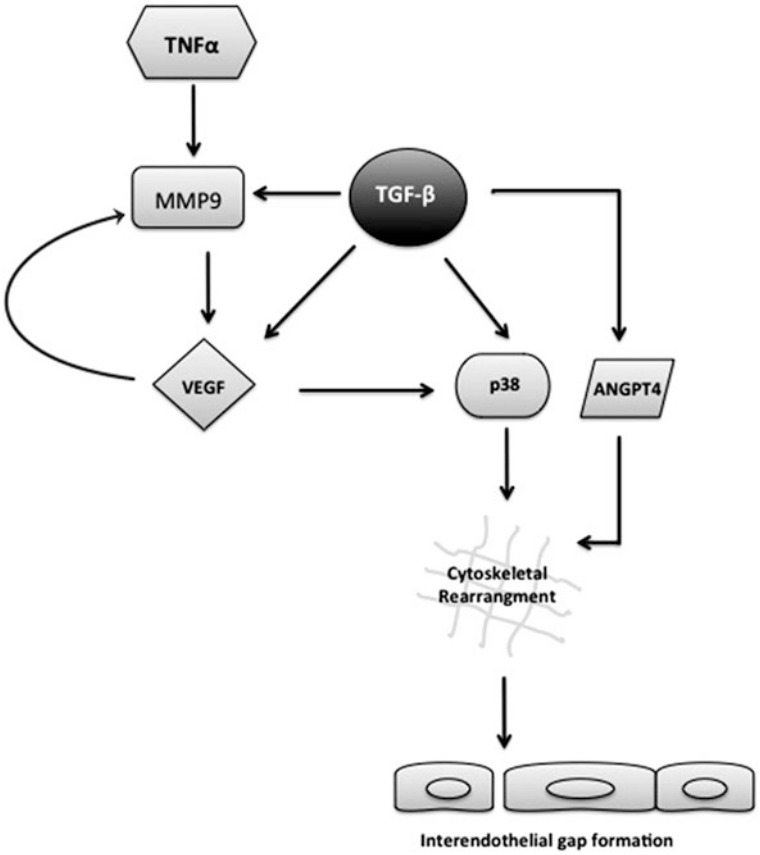


Fig. 5.2 Mechanism of vessel permeabilization by tumour-secreted factors

lymphatic flow, then active fluid accumulation and swelling will occur. This can occur in pathological states where a physical obstruction in the lymphatic system typically results in inadequate IF reabsorption. This is often seen in cases of a malignancy that has disseminated via proximal lymph vessels or from a primary malignancy originating within the lymphatics. Such obstructions impede fluid velocity through the lymphatic vessels and therefore reduce IF flow, that is, elevated IF levels are unable to drain via the lymphatics, and as a result fluid continues to accumulate. Because IF flow is determined by equilibrium between fluid pressures and tissue stresses, changes in IF transport parameters such as fluid velocity have pathological consequences, including secondary lymphedema. Lymphangiogenic responses are initiated in cases of reduced IF flow to compensate for the continuing increase in IF pressure, and to attempt to resolve accumulated extracellular fluid. Another VEGF family member, vascular endothelial growth factor C (VEGF-C), is one such pro-lymphangiogenic factor released in response to such conditions [15].

VEGF-C promotes lymphangiogenesis and lymphatic contraction when acting through VEGFR3 receptors on lymph vessels [60, 61] but also promotes angiogenesis [62] and vessel permeability [63, 64] when acting through the VEGFR2 receptors on blood vessels [65]. Although a reduction in IF velocity induces VEGF-C production, its site of action (lymphatic or blood vessels) is dependent not only on the IF pressure but also on vessel density. In the case of decreased flow towards the lymphatic system, VEGF-C is shown to act on the blood rather than the lymph vessels in regions where there is a high density of blood vessels, but shows lymphangiogenic properties when blood vessel density is low. In cases of chronic exposure to VEGF-C, angiogenesis is more prevalent as seen by an increase in hydraulic conductivity and filtration which is indicative of a greater number of capillaries [66]. When fluid velocity is at a minimum, the static nature of edematous fluid prevents the formation of protease and growth factor gradients that direct endothelial cell migration and tube formation of lymphatics [15]. This further suggests that angiogenesis is the preferred site of action for VEGF-C, especially in areas of high blood vessel density such as the environment surrounding the tumour. There is, however, another caveat to this compensatory mechanism: increased vasculature may increase filtration, but with this follows an increased capillary fluid volume [67]. In conjunction with elevated levels of circulating and local permeability factors such as VEGFs and MMPs, new blood vasculature formed is likely leaky, as seen in tumour, and an increasing surface area of leaky vessels may not adequately restore IF volumes but actually continue to further increase the IF load in response to tumour-secreted permeability factors, to a point where the functional reserve of the lymphatic system is exhausted. This can be further impaired by blockage or damage to lymphatic vasculature or when lymphatic vessel regeneration is not sufficient to cope with increased IF volume which, as mentioned, is commonly the case in static fluid environments [68] (Fig. 5.3).Therefore, both physical and chemical changes in malignancy not only promote impaired fluid dynamics but also prevent physiological adaptation mechanisms from re-establishing fluid homeostasis.

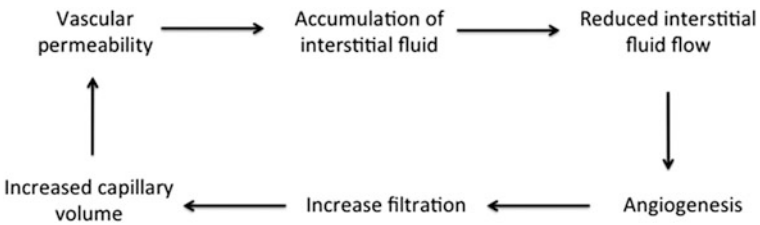


Fig. 5.3 Static fluid accumulation in the interstitium favours angiogenesis which propagates consequences of leaky vasculature

Chronic Inflammatory State

It is understood that cancer patients are in a chronic state of systemic inflammation and subjected to inflammatory mediators that assist not only tumour progression but also propagate vascular permeability physiologically associated with an acute immune response. This chronic inflammatory state is reflected by elevated levels of C-reactive protein (CRP) and chronic endothelial cell activation both of which can indirectly contribute to vascular permeability and therefore contribute to the edematous state. CRP is a non-specific marker synthesized in the liver in response to factors such as IL-6, IL-1, and TNF-α. The course of an inflammatory state can be marked by levels of CRP, with rapid increase after the onset of inflammation and reduction in levels after the inflammation has cleared or continued elevation if inflammation persists [69, 70]. Cancer patients often have elevated serum CRP levels, suggesting that this pathology promotes a state of chronic inflammation [70]. Investigating the immune response associated with malignancy is therefore crucial when assessing susceptibility to disrupted fluid dynamics in these patients. The immune system is a major player in promoting tumour progression and the production of factors that promote fluid dysregulation through vascular permeabilization. Rates of transvascular exchange of solutes and fluid are elevated in inflammatory states to upwards of a 10-fold increase within an hour which can lead to edema formation [71]. It can therefore be deduced that in a state of chronic inflammation this response persists.

Tumour cells produce inflammatory cytokine and chemokine mediators that attract immune cells including leukocytes, which, in turn, contribute to a pro-inflammatory tumour microenvironment. With constant stimuli by exogenous, tumour-derived inflammatory mediators such as TNF-α, IL-6, and IL-8, all of which have been shown to be elevated in the sera of cancer patients, these normally acute inflammation-induced vascular changes become dysregulated. Tumour-secreted IL-6, for example, promotes monocyte differentiation to macrophages, increasing the titre of immune cell infiltrate in tumours. These TAMS also secrete many factors that promote tumour progression including lymphatic metastasis [72]. Activated macrophages express VEGF family members and receptors, which contribute to increased lymphangiogenesis in peritumoural stroma in addition to increasing vascular permeability and tumour dissemination. This, in turn, sustains the increased extracellular osmotic pressure commonly associated with inflammation, promoting further production of pro-inflammatory cytokines which contributes to the state chronic inflammation [73]. As a part of regulatory dysfunction represented by neoplastic formation, malignant cells show a tolerance to inflammatory cytokines and eventually become anergic to any regulatory effect these mediators would have on malignant cell growth and proliferation [74]. Inflammation represents a response to tissue injury and the physiological result of inflammation involves endothelial changes in the vasculature that allows for extravasation of immune cells. This involves the activation of endothelial cells

which transforms the vascular endothelium from anticoagulant to procoagulant. However, the endothelial response to inflammation may play a larger role in addition to facilitating immune cells migration. Clinically, many cancer patients are often present with lower limb edema resulting from deep venous thromboembolisms of the leg which can often preclude a cancer diagnosis [75]. Virchow first described the pathophysiology of thrombus formation in terms of blood vessel wall, blood flow, and the factors in the blood. Abnormalities in this triad can be seen in cancer patients where abnormal blood flow, abnormal blood constituents, and abnormal blood vessel walls present in these individuals predisposing them to a hypercoagulable/prothrombotic state (reviewed by [75, 76]). Many tumours express procoagulant factors and tumour cells, themselves can also interact with platelets to initiate coagulation. Disseminating cancers commonly interact with platelets with increasing platelet activation and aggregation reflected in many cancer patients. Cancer cells can also directly activate the clotting cascade that promotes platelet adhesion and thrombus formation by increasing von Willebrand factor (vWF) with elevated levels commonly seen in cancer patients [77]. Elevated levels of vWF are generally indicative of tissue damage but it has also been shown that tumour-secreted VEGF can directly induce an increase in vWF through VEGF-mediated increases in cytosolic calcium of endothelial cells [77] (Fig. 5.4). Furthermore, other inflammatory cytokines produced by tumour cells influence a prothrombotic state. For example, TNF-α downregulates anticoagulant mechanisms which aid in the transformation to a procoagulant vascular endothelium [78, 79]. Furthermore, in states of persistent inflammation, endothelial cells become activated inducing a procoagulant endothelium. Continuous endothelial cell activation induces the release of proteases like MMPs that, as mentioned above, degrade the ECM and allow endothelial cells to enter the surrounding stroma and initiate new vessel sprouting. Chronically activated endothelial cells show specific expression characteristics indicative of endothelial dysfunction where, for example, the p38

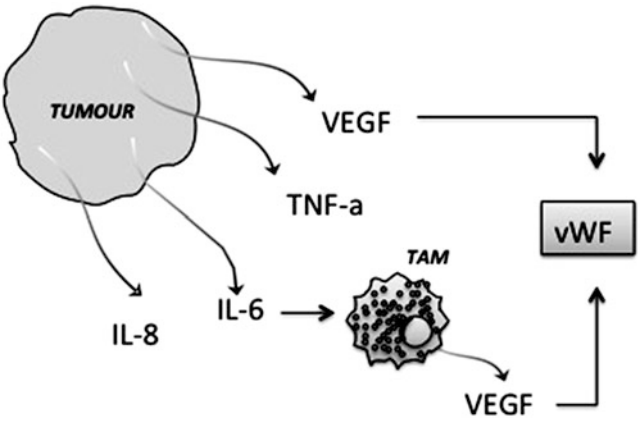


Fig. 5.4 VEGF increases production of von Willebrand factor

MAP kinase cascade switches from anti-angiogenic to pro-angiogenic. It is well known that cancer cells are under oxidative stress (review: Toyokuni et al. [80]). They also show resistance to ROS-induced cell death through the upregulation of antioxidants that promote tumour cell survival allowing oxidative stress to persist (review: Oberley and Oberley [81]). This also contributes to the chronic inflammatory state, which is mediated by TNFα-induced ROS production, subsequent MMP-9 activity and increased vessel sprouting [82]. This represents a hallmark of dysfunctional endothelial activation where physiological angiogenesis becomes negatively regulated by p38 MAPK [83].

Hyaluronan

Hyaluronan (HA), a major glycosaminoglycan component of the ECM, is associated with regulation of lymphatic permeability and promotion of tumour progression and survival. Several carcinomas including those of the breast and colon show ectopic HA expression [84]. HA is overproduced in cancer states, both by the tumour itself and surrounding stroma, and has been shown to disrupt cell–cell junctions in order to facilitate metastasis. In addition, HA attracts water (polar glycosaminoglycan) to hydrate tissue and regulate water homeostasis and ionic exchange [85] in addition to acting as a scaffold and promoter of intercellular adhesions and proliferation (review: Genasetti et al. [86]). This suggests that HA can contribute to tissue swelling and is an important component of edema fluid. Stern et al. [84] review the paradoxical role of hyaluron metabolism where both HA itself and hyaluronidases can be associated with tumour progression. Increased hyaluronidase activity corresponds to low molecular weight (LMW) HA, showing that the activity of HA is dependent on its molecular mass. HA itself is a common substance that promotes cell motility, migration, and tumour progression. LMW HA fragments have been shown to be elevated in sera from individuals with cancer and have recently been correlated with lymph node metastasis in breast and colorectal cancer patients [87, 88]. Hyaluronidase-mediated breakdown of HA generates the LMW form of HA and it is this form that is associated with angiogenesis. Oxidative hydrolysis of HA promoted by the tumour microenvironment also produces LMW HA, possibly due to increased oxidative stress of the tumour and its microenvironment, further promoting immune cell recruitment to the tumour site and continuous production of LMW HA [84]. Furthermore, abnormal stroma surrounding the tumour is generated by paracrine tumour cell signalling, and this includes the abnormal deposition of HA [89]. This is observed in the sera of breast cancer patients, which contains the glycoprotein that stimulates HA activity [90]. Therefore, the deposition of ECM components including HA can promote the oedematous state due to its fluid-retaining properties and its ability to mediate metastatic processes that can compromise vascular integrity.

Electrolyte Disorders: Tumour-Related Hyponatremia

Dysregulated water retention and excretion mechanisms can also promote edema formation. Hyponatremia is a common dysfunction of sodium and water homeostasis and is the most common electrolyte abnormality in cancer patients [91]. Although hyponatremia can present with intact sodium balance, it does not lead to changes in extracellular fluid volume [92]. Therefore, it is a disturbed sodium balance that is of interest when assessing cancer-induced edema. In the cases of disturbed sodium balance, there is increased water absorption from the kidney and increased water and sodium retention, resulting in decreased plasma osmolality, which leads to the accumulation of extracellular fluid (edema/ascites) and may continue even when there is already excessive IF [92]. Furthermore, in a hyponatremic state, hypovolemia can result a state in which there is decreased fluid volume in the capillary due to excessive sodium loss. Under these conditions, perfusion pressure is low, and in an attempt to restore it, antidiuretic hormone (or vasopressin) is released to promote the absorption of more water, further exacerbating the low sodium osmolarity in plasma pushing even more water out of circulation and into the interstitium [93]. Furthermore, the decrease in oncotic pressure associated with increases in capillary permeability activates vasopressin release through a non-osmotic mechanism that drives renal water retention, which could overcome the physiological effect of hypo-osmotic suppression of vasopressin release in a state of hyponatremia [94] (Fig. 5.5). It is therefore important to consider electrolyte disorders when describing how cancer patients are prone to oedema. In conjunction with the physical and mechanical abnormalities surrounding the vasculature of cancer patients, conditions that promote retention of fluid exacerbates a pro-oedematous state.

Cancer Therapeutics and the Edematous State

Therapeutic interventions in the cancer patient can induce edema and lymphedema. Specifically, surgical resection of the lymph node after tumour excision is common to assess the degree of metastasis of the primary cancer. In particular, this is done with breast cancer, as subsequent arm swelling due to lymphoedema is a common side effect. Furthermore, radiation therapy can damage lymph vessels causing fibrosis, which impairs their functional integrity. In clinical practice it is difficult to assess the degree that cancer itself induces certain pathologies like oedema that can also persist due to therapeutic interventions. Presented here is the evidence indicating how the general dysregulation involved in a malignant state can have systemic effects and induce side effects commonly ascribed to cancer treatments rather than disease progression.

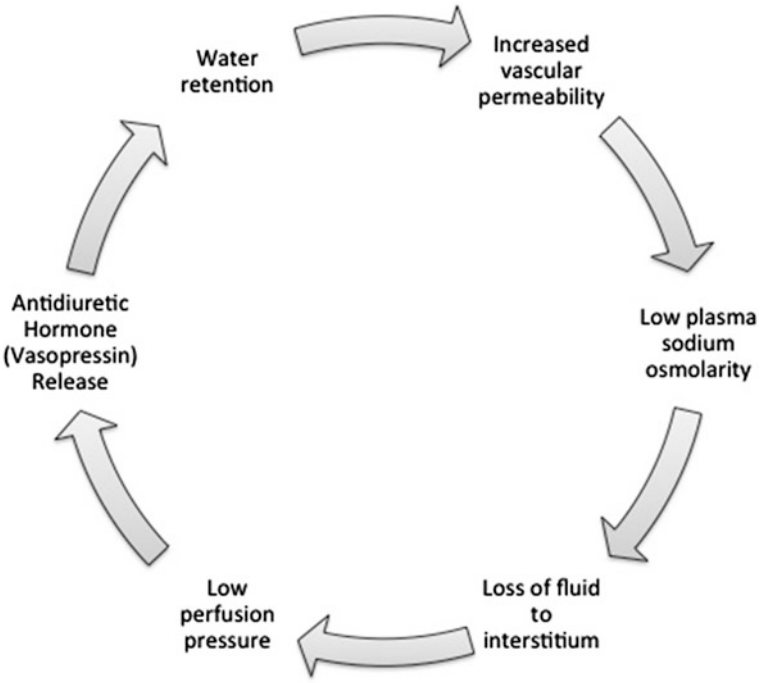


Fig. 5.5 Cycle of electrolyte dysfunction resulting from increased vascular permeabilization

Conclusion

Cancer patients are susceptible to a variety of insults stemming from both dysfunctions associated with the pathology itself, but also from the ongoing therapies directed at killing the cancer. However, when narrowing the investigation to the role the cancer has in disrupting physiological processes, it becomes clear that the tumour is a hub of dysregulated signalling that disrupts normal homeostatic processes including fluid homeostasis. This offers new insight into the pathological state of the cancer patient outside of therapeutic interventions. Investigation into such mechanisms provides knowledge of underlying symptoms experienced by the cancer patient who often go untreated or are overlooked on the basis that these symptoms persist as a result of treatment alone. However, as seen here with fluid dynamics, there is abundant evidence that cancer ultimately promotes the accumulation of IF and this alone can compound the discomforts cancer patients experience with little relief.

Definitions

Capillary hydrostatic pressure	drives fluid out of a vessel as a result of osmosis
Osmotic pressure	pressure exerted by the tendency of water to move from an area of low solute concentration to high solute concentration
Colloid osmotic pressure (oncotic pressure)	pressure resulting from the property causing water to move down a concentration gradient by diffusion through a semipermeable membrane from an area of low concentration to an area with a high concentration of high molecular weight molecules, namely proteins, that are unable to pass through the membrane [95]
Hydraulic conductivity	permeability of a vessel wall to water [95], the speed at which fluid will move through a tissue when a pressure gradient is applied [1]
Protein reflexion coefficient	permeability of a vessel wall to protein [95]

References

1. Wiig H, Swartz MA. Interstitial fluid and lymph formation and transport: physiological regulation and roles in inflammation and cancer. Physiol Rev. 2012;92:1005–60.
2. Starling EH. On the absorption of fluids from the connective tissue spaces. J Physiol. 1896;19:312–26.
3. Scallan J, Huxley VH, Korthuis RJ. Pathophysiology of edema formation. 2010. http://www.ncbi.nlm.nih.gov/books/NBK53445/.
4. Gill JR, Waldmann TA, Bartter FC. Idiopathic edema: I. The occurrence of hypoalbuminemia and abnormal albumin metabolism in women with unexplained edema. Am J Med. 1972;52:444–51.
5. Lampugnani MG, et al. The molecular organization of endothelial cell to cell junctions: differential association of plakoglobin, beta-catenin, and alpha-catenin with vascular endothelial cadherin (VE-cadherin). J Cell Biol. 1995;129:203–17.
6. Brenner DA, Buck M, Feitelberg SP, Chojkier M. Tumor necrosis factor-alpha inhibits albumin gene expression in a murine model of cachexia. J Clin Invest. 1990;85:248–55.
7. Mehta D, Malik AB. Signaling mechanisms regulating endothelial permeability. Physiol Rev. 2006;86:279–367.
8. Goddard LM, Iruela-Arispe ML. Cellular and molecular regulation of vascular permeability. Thromb Haemost. 2013;109:407–15.
9. Pappenheimer JR, Renkin EM, Borrero LM. Filtration, diffusion and molecular sieving through peripheral capillary membranes; a contribution to the pore theory of capillary permeability. Am J Physiol. 1951;167:13–46.

10. Ghitescu L, Fixman A, Simionescu M, Simionescu N. Specific binding sites for albumin restricted to plasmalemmal vesicles of continuous capillary endothelium: receptor-mediated transcytosis. J Cell Biol. 1986;102:1304–11.
11. Fleck A, et al. Increased vascular permeability: a major cause of hypoalbuminaemia in disease and injury. Lancet. 1985;1:781–4.
12. Senger DR, et al. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science. 1983;219:983–5.
13. Gavard J, Gutkind JS. VEGF controls endothelial-cell permeability by promoting the β-arrestin-dependent endocytosis of VE-cadherin. Nat Cell Biol. 2006;8:1223–34.
14. Esser S, Lampugnani MG, Corada M, Dejana E, Risau W. Vascular endothelial growth factor induces VE-cadherin tyrosine phosphorylation in endothelial cells. J Cell Sci. 1998;111 (Pt 13):1853–65.
15. Goldman J, et al. Regulation of lymphatic capillary regeneration by interstitial flow in skin. Am J Physiol Heart Circ Physiol. 2007;292:H2176–83.
16. Senger DR, Perruzzi CA, Feder J, Dvorak HF. A highly conserved vascular permeability factor secreted by a variety of human and rodent tumor cell lines. Cancer Res. 1986;46:5629–32.
17. Brown LF, et al. Expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in breast cancer. Hum Pathol. 1995;26:86–91.
18. Brown LF, et al. Expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in adenocarcinomas of the gastrointestinal tract. Cancer Res. 1993;53:4727–35.
19. Dirix LY, et al. Serum basic fibroblast growth factor and vascular endothelial growth factor and tumour growth kinetics in advanced colorectal cancer. Ann Oncol Off J Eur Soc Med Oncol ESMO. 1996;7:843–8.
20. Olson TA, Mohanraj D, Carson LF, Ramakrishnan S. Vascular permeability factor gene expression in normal and neoplastic human ovaries. Cancer Res. 1994;54:276–80.
21. Yamamoto S, et al. Expression of vascular endothelial growth factor (VEGF) in epithelial ovarian neoplasms: correlation with clinicopathology and patient survival, and analysis of serum VEGF levels. Br J Cancer. 1997;76:1221–7.
22. Kondo S, Asano M, Matsuo K, Ohmori I, Suzuki H. Vascular endothelial growth factor/vascular permeability factor is detectable in the sera of tumor-bearing mice and cancer patients. Biochim Biophys Acta. 1994;1221:211–4.
23. Takano S, et al. Concentration of vascular endothelial growth factor in the serum and tumor tissue of brain tumor patients. Cancer Res. 1996;56:2185–90.
24. Dirix LY, et al. Elevated levels of the angiogenic cytokines basic fibroblast growth factor and vascular endothelial growth factor in sera of cancer patients. Br J Cancer. 1997;76:238–43.
25. Salven P, Mänpää H, Orpana A, Alitalo K, Joensuu H. Serum vascular endothelial growth factor is often elevated in disseminated cancer. Clin Cancer Res Off J Am Assoc Cancer Res. 1997;3:647–51.
26. Yamamoto Y, et al. Concentrations of vascular endothelial growth factor in the sera of normal controls and cancer patients. Clin Cancer Res Off J Am Assoc Cancer Res. 1996;2:821–6.
27. Salven P, Orpana A, Joensuu H. Leukocytes and platelets of patients with cancer contain high levels of vascular endothelial growth factor. Clin Cancer Res Off J Am Assoc Cancer Res. 1999;5:487–91.
28. Vakkila J, Lotze MT. Inflammation and necrosis promote tumour growth. Nat. Rev. Imunol. 2004;4:641–648.
29. Kuroi K, Toi M. Circulating angiogenesis regulators in cancer patients. Int J Biol Markers. 2001;16:5–26.
30. Banks RE, et al. Release of the angiogenic cytokine vascular endothelial growth factor (VEGF) from platelets: significance for VEGF measurements and cancer biology. Br J Cancer. 1998;77:956–64.
31. Salgado R, et al. Platelets and vascular endothelial growth factor (VEGF): a morphological and functional study. Angiogenesis. 2001;4:37–43.

32. Weis S, Cui J, Barnes L, Cheresh D. Endothelial barrier disruption by VEGF-mediated Src activity potentiates tumor cell extravasation and metastasis. J Cell Biol. 2004;167:223–9.
33. Liotta LA, Steeg PS, Stetler-Stevenson WG. Cancer metastasis and angiogenesis: an imbalance of positive and negative regulation. Cell. 1991;64:327–36.
34. Yurchenco PD, Schittny JC. Molecular architecture of basement membranes. FASEB J Off Publ Fed Am Soc Exp Biol. 1990;4:1577–90.
35. Werb Z. ECM and cell surface proteolysis: regulating cellular ecology. Cell. 1997;91:439–42.
36. Stetler-Stevenson WG. Matrix metalloproteinases in angiogenesis: a moving target for therapeutic intervention. J Clin Invest. 1999;103:1237–41.
37. Paiva KBS, Granjeiro JM. Bone tissue remodeling and development: Focus on matrix metalloproteinase functions. Arch Biochem Biophys. 2014;561:74–87.
38. Belotti D, et al. Matrix metalloproteinases (MMP9 and MMP2) induce the release of vascular endothelial growth factor (VEGF) by ovarian carcinoma cells implications for ascites formation. Cancer Res. 2003;63:5224–9.
39. Hiratsuka S, et al. MMP9 induction by vascular endothelial growth factor receptor-1 is involved in lung-specific metastasis. Cancer Cell. 2002;2:289–300.
40. Hagemann T, et al. Enhanced invasiveness of breast cancer cell lines upon co-cultivation with macrophages is due to TNF-alpha dependent up-regulation of matrix metalloproteases. Carcinogenesis. 2004;25:1543–9.
41. Bergers G, et al. Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. Nat Cell Biol. 2000;2:737–44.
42. Roberts AB, McCune BK, Sporn MB. TGF-beta: regulation of extracellular matrix. Kidney Int. 1992;41:557–9.
43. Michalaki V, Syrigos K, Charles P, Waxman J. Serum levels of IL-6 and TNF-α correlate with clinicopathological features and patient survival in patients with prostate cancer. Br J Cancer. 2004;90:2312–6.
44. Zhang GJ, Adachi I. Serum interleukin-6 levels correlate to tumor progression and prognosis in metastatic breast carcinoma. Anticancer Res. 1999;19:1427–32.
45. Dalaveris E, et al. VEGF, TNF-α and 8-isoprostane levels in exhaled breath condensate and serum of patients with lung cancer. Lung Cancer. 2009;64:219–25.
46. Rundhaug JE, Park J, Pavone A, Opdenakker G, Fischer SM. Opposite effect of stable transfection of bioactive transforming growth factor-beta 1 (TGF beta 1) versus exogenous TGF beta 1 treatment on expression of 92-kDa type IV collagenase in mouse skin squamous cell carcinoma CH72 cells. Mol Carcinog. 1997;19:122–36.
47. Yang W-L, Godwin AK, Xu X-X. Tumor necrosis factor-alpha-induced matrix proteolytic enzyme production and basement membrane remodeling by human ovarian surface epithelial cells: molecular basis linking ovulation and cancer risk. Cancer Res. 2004;64:1534–40.
48. Hanemaaijer R, et al. Increased gelatinase-A and gelatinase-B activities in malignant vs. benign breast tumors. Int J Cancer J Int Cancer. 2000;86:204–7.
49. Stuelten CH, et al. Breast cancer cells induce stromal fibroblasts to express MMP-9 via secretion of TNF-α and TGF-β. J Cell Sci. 2005;118:2143–53.
50. Goldberg PL, MacNaughton DE, Clements RT, Minnear FL, Vincent PA. p38 MAPK activation by TGF-beta1 increases MLC phosphorylation and endothelial monolayer permeability. Am J Physiol Lung Cell. Mol Physiol. 2002;282:L146–54.
51. Huot J, Houle F, Marceau F, Landry J. Oxidative stress-induced actin reorganization mediated by the p38 mitogen-activated protein kinase/heat shock protein 27 pathway in vascular endothelial cells. Circ Res. 1997;80:383–92.
52. Vandenbroucke E, Mehta D, Minshall R, Malik AB. Regulation of endothelial junctional permeability. Ann N Y Acad Sci. 2008;1123:134–45.
53. Hurst V, Goldberg PL, Minnear FL, Heimark RL, Vincent PA. Rearrangement of adherens junctions by transforming growth factor-beta1: role of contraction. Am J Physiol. 1999;276: L582–95.
54. Komarova Y, Malik AB. Regulation of Endothelial Permeability via Paracellular and Transcellular Transport Pathways. Annu Rev Physiol. 2010;72:463–93.

55. Rousseau S, Houle F, Landry J, Huot J. p38 MAP kinase activation by vascular endothelial growth factor mediates actin reorganization and cell migration in human endothelial cells. Oncogene. 1997;15:2169–77.
56. Pertovaara L, et al. Vascular endothelial growth factor is induced in response to transforming growth factor-beta in fibroblastic and epithelial cells. J Biol Chem. 1994;269:6271–4.
57. Padua D, et al. TGFbeta primes breast tumors for lung metastasis seeding through angiopoietin-like 4. Cell. 2008;133:66–77.
58. Huang R-L, et al. ANGPTL4 modulates vascular junction integrity by integrin signaling and disruption of intercellular VE-cadherin and claudin-5 clusters. Blood. 2011;118:3990–4002.
59. Kang Y, et al. Breast cancer bone metastasis mediated by the Smad tumor suppressor pathway. Proc Natl Acad Sci USA. 2005;102:13909–14.
60. Jeltsch M, et al. Hyperplasia of lymphatic vessels in VEGF-C transgenic mice. Science. 1997;276:1423–5.
61. Breslin JW, et al. Vascular endothelial growth factor-C stimulates the lymphatic pump by a VEGF receptor-3-dependent mechanism. Am J Physiol Heart Circ Physiol. 2007;293:H709–18.
62. Cao Y, et al. Vascular endothelial growth factor C induces angiogenesis in vivo. Proc Natl Acad Sci USA. 1998;95:14389–94.
63. Saaristo A, et al. Adenoviral VEGF-C overexpression induces blood vessel enlargement, tortuosity, and leakiness but no sprouting angiogenesis in the skin or mucous membranes. FASEB J Off Publ Fed Am Soc Exp Biol. 2002;16:1041–9.
64. Cao R, et al. Comparative evaluation of FGF-2-, VEGF-A-, and VEGF-C-induced angiogenesis, lymphangiogenesis, vascular fenestrations, and permeability. Circ Res. 2004;94:664–70.
65. Joukov V, et al. A novel vascular endothelial growth factor, VEGF-C, is a ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) receptor tyrosine kinases. EMBO J. 1996;15:290–8.
66. Bates DO, Curry FE. Vascular endothelial growth factor increases hydraulic conductivity of isolated perfused microvessels. Am J Physiol Heart Circ Physiol. 1996;271:H2520–8.
67. Bates DO. An interstitial hypothesis for breast cancer related lymphoedema. Pathophysiology. 2010;17:289–94.
68. Rockson SG. Lymphedema. Am J Med. 2001;110:288–95.
69. Mahmoud FA, Rivera NI. The role of C-reactive protein as a prognostic indicator in advanced cancer. Curr Oncol Rep. 2002;4:250–5.
70. Siemes C, et al. C-reactive protein levels, variation in the C-reactive protein gene, and cancer risk: the Rotterdam study. J Clin Oncol. 2006;24:5216–22.
71. Lum H, Malik AB. Mechanisms of increased endothelial permeability. Can J Physiol Pharmacol. 1996;74:787–800.
72. Schoppmann SF, et al. Tumor-associated macrophages express lymphatic endothelial growth factors and are related to peritumoral lymphangiogenesis. Am J Pathol. 2002;161:947–56.
73. Levy Nogueira, M, et al. Mechanical stress as the common denominator between chronic inflammation, cancer, and Alzheimer's disease. Mol Cell Oncol. 2015;197. doi:10.3389/fonc.2015.00197.
74. Griffioen AW. Anti-angiogenesis: making the tumor vulnerable to the immune system. Cancer Immunol Immunother. 2008;57:1553–8.
75. Lip GY, Chin BS, Blann AD. Cancer and the prothrombotic state. Lancet Oncol. 2002;3:27–34.
76. Blann, AD. Endothelial cell activation markers in cancer. Thromb. Res. 2012;129 Suppl 1:122–126.
77. Brock TA, Dvorak HF, Senger DR. Tumor-secreted vascular permeability factor increases cytosolic Ca2+ and von Willebrand factor release in human endothelial cells. Am J Pathol. 1991;138:213–21.
78. Moore KL, Esmon CT, Esmon NL. Tumor necrosis factor leads to the internalization and degradation of thrombomodulin from the surface of bovine aortic endothelial cells in culture. Blood. 1989;73:159–65.
79. Lindahl A, Boffa M, Abildgaard U. Increased plasma thrombomodulin in cancer patients. Thromb Haemost. 1993;69:112–4.

80. Toyokuni S, Okamoto K, Yodoi J, Hiai H. Persistent oxidative stress in cancer. FEBS Lett. 1995;358:1–3.
81. Oberley TD, Oberley LW. Antioxidant enzyme levels in cancer. (1997). http://digitum.um.es/jspui/handle/10201/18951.
82. Rajashekhar G, et al. Pro-inflammatory angiogenesis is mediated by p38 MAP kinase. J Cell Physiol. 2011;226:800–8.
83. Matsumoto T, Turesson I, Book M, Gerwins P, Claesson-Welsh L. p38 MAP kinase negatively regulates endothelial cell survival, proliferation, and differentiation in FGF-2-stimulated angiogenesis. J Cell Biol. 2002;156:149–60.
84. Stern R. Hyaluronan metabolism: a major paradox in cancer biology. Pathol Biol (Paris). 2005;53:372–82.
85. Henry CB, Duling BR. Permeation of the luminal capillary glycocalyx is determined by hyaluronan. Am J Physiol. 1999;277:H508–14.
86. Genasetti A, et al. Hyaluronan and human endothelial cell behavior. Connect Tissue Res. 2008;49:120–3.
87. Wu M, et al. A novel role of low molecular weight hyaluronan in breast cancer metastasis. FASEB J Off Publ Fed Am Soc Exp Biol. 2014;. doi:10.1096/fj.14-259978.
88. Schmaus A, et al. Accumulation of small hyaluronan oligosaccharides in tumour interstitial fluid correlates with lymphatic invasion and lymph node metastasis. Br J Cancer. 2014;111:559–67.
89. Schor SL, et al. Mechanism of action of the migration stimulating factor produced by fetal and cancer patient fibroblasts: effect on hyaluronic and synthesis. Vitro Cell Dev Biol J Tissue Cult Assoc. 1989;25:737–46.
90. Decker M, et al. Hyaluronic acid-stimulating activity in sera from the bovine fetus and from breast cancer patients. Cancer Res. 1989;49:3499–505.
91. Rosner MH, Dalkin AC. Electrolyte disorders associated with cancer. Adv Chronic Kidney Dis. 2014;21:7–17.
92. Onitilo AA, Kio E, Doi SAR. Tumor-related hyponatremia. Clin Med Res. 2007;5:228–37.
93. Berk L, Rana S. Hypovolemia and dehydration in the oncology patient. J Support Oncol. 2006;4:447–54 (discussion 455–457).
94. Schrier RW. Water and sodium retention in edematous disorders: role of vasopressin and aldosterone. Am J Med. 2006;119:S47–53.
95. Heldin C-H, Rubin K, Pietras K, Ostman A. High interstitial fluid pressure—an obstacle in cancer therapy. Nat Rev Cancer. 2004;4:806–13.

Chapter 6
Oncodynamic Effect of Cancer on Depression

Mina G. Nashed, Benicio N. Frey, Patricia Rosebush and Gurmit Singh

Abstract Depressive disorders are among the most prevalent psychiatric illnesses in the general population. In cancer patients, the prevalence of depression is dramatically increased. In addition to the psychosocial impact of a negative diagnosis, recent evidence suggests that cancer-induced depression (CID) is mediated by biological processes. This oncodynamic effect of cancer on the development of depression is poorly understood, leading to ineffective treatment of CID with drugs that are developed for depressive disorders in the general population. This chapter begins by outlining the clinical profile of major depressive disorder (MDD). We then provide a discussion of the most prominent neurobiological hypotheses of depression, including the monoamine hypothesis, the role of neurotrophins, physiological stress, inflammation, and glutamatergic signalling. The efficacy of current antidepressants is then discussed for depression in the general population and in cancer patients. This leads to a discussion of the biological basis of CID, including the effects of physiological stress, inflammation, and glutamatergic signalling. We conclude that more research is needed to determine oncodynamic events in the development of CID. Development of validated animal models is the first step in delineating contributing biological mechanisms, which will ultimately lead to more targeted drug development and improved efficacy.

M.G. Nashed
Department of Pathology and Molecular Medicine, McMaster University,
1280 Main Street West, Hamilton, ON L8N 3Z5, Canada
e-mail: nashedm@mcmaster.ca

B.N. Frey
Department of Psychiatry and Behavioural Neurosciences, McMaster University,
100 West 5th Street, Suite C124, Hamilton, ON L8N 3K7, Canada
e-mail: freybn@mcmaster.ca

P. Rosebush
Department of Psychiatry and Behavioural Neurosciences, St. Josephs Healthcare,
301 James street South, Fontbonne Building F16, Hamilton, ON L89 3B6, Canada
e-mail: rosebush@hhsc.ca

G. Singh (✉)
Department of Pathology and Molecular Medicine, McMaster University,
Hamilton, ON, Canada
e-mail: singhg@mcmaster.ca

G. Singh (ed.), *Oncodynamics: Effects of Cancer Cells on the Body*,
DOI 10.1007/978-3-319-28558-0_6

Keywords Depression · Cancer · Antidepressants · Prefrontal cortex · Hippocampus · Inflammatory cytokines · Stress · BDNF · Glutamate · NMDA antagonist

Introduction

The psychosocial impact of a cancer diagnosis undoubtedly contributes to co-morbid depression in cancer patients. While depression in the general population occurs with a lifetime prevalence of ~8–12 % [4], it can reach as high as 57 % in breast cancer patients and can be a staggering 95 % in high grade glioma [77]. In addition to the psychosocial contribution, recent preclinical and clinical evidence suggests the involvement of biological mechanisms in cancer-induced depression (CID). This biological underpinning, and the development of the capacity to investigate it at the basic level, has a potentially profound impact on the quality of life of cancer patients. Currently, treatment for CID is limited to therapies developed for non-cancer-related major depressive disorder (MDD) despite lack of convincing evidence for the efficacy of these treatments in cancer patients [73]. A more effective strategy for treating CID begins with the investigation of the oncodynamic effect of cancer on depression at the most basic level. A better understanding of this interaction would provide the framework for developing new pharmacotherapy aimed at novel targets. This chapter will discuss what is currently known about the oncodynamic effect of cancer on depression by first reviewing depression at the clinical and etiological level, then examining cancer signalling events that are likely to contribute to CID.

Depression

The term melancholia (ancient Greek for "black bile") was first used by Hippocrates around 400 B.C. to describe a disease state of persistent fear and despair [101]. According to the humoral theory, this disease state arose from excess black bile—one of the four bodily liquids, or humors. In the early nineteenth century, a "clinico-anatomical" view of disease asserted that symptoms of illnesses could be correlated with anatomical lesions [10]. During the second half of the nineteenth century, this conceptual shift led to greater focus on the brain in an effort to better understand melancholia. Today, insight from preclinical, biochemical, genetic, post-mortem, and neuroimaging studies have led to a greater understanding and classification of mood disorders. In addition to developing cognitive behavioural therapy (CBT), the last several decades have seen a proliferation of psychotropic drugs, which target specific biological pathways, enter the market. In the case of

antidepressants, while the efficacy and tolerance have generally improved, low clinical response rates underscore the importance of continued progress in understanding the neurobiology of depression.

Diagnosis and Classification of Depression

Mood disorders are characterized by persistent periods of intensely reduced or elevated mood that interfere with normal functioning. The subcategory of mood disorders that is defined by reduced mood is termed *depressive disorders*. According to the current fifth edition of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-5) of the American Psychiatric Association (APA), the common feature of this subcategory is the presence of sad, empty, or irritable mood [5]. This can be accompanied by various somatic and cognitive changes that impede day-to-day functioning. Differences between depressive disorders depend on duration and timing of symptoms, as well as presumed aetiology.

In the case of (MDD; commonly called major depression, clinical depression, or simply depression), changes in affect, cognition, and neurovegetative function occur in discrete episodes with inter-episodic remission [5]. Episodes must persist for at least 2 weeks, although typically last considerably longer, and at least one episode is required to make a diagnosis of MDD. If the mood disturbances persist for 2 or more years without periods of remission, a diagnosis of *persistent depressive disorder* (or dysthymia) is given. The depressive episodes required to make a diagnosis of MDD or dysthymia are characterized by the presence of five (or more) of nine symptoms, summarized in Table 1.1. In addition, at least one of the symptoms must be either (1) depressed mood or (2) anhedonia (loss of interest or pleasure).

Table 1.1 Symptoms for major depressive episode

1. Depressed mood most of the day, nearly every day
2. Markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day
3. Significant weight loss when not dieting or weight gain, or decrease or increase in appetite nearly every day
4. Insomnia or hypersomnia nearly every day
5. Psychomotor agitation or retardation nearly every day
6. Fatigue or loss of energy nearly every day
7. Feelings of worthlessness or excessive or inappropriate guilt nearly every day
8. Diminished ability to think or concentrate, or indecisiveness, nearly every day
9. Recurrent thoughts of death, recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide

Neurobiology of Depression

There are several neurochemical and neuroanatomical correlates of depression, which have led to multiple etiological hypotheses. In reviewing these hypotheses, it is worth noting that no single model can sufficiently account for all aspects and variations of depression. Rather than a unified hypothesis of depression, it is likely that the true aetiology of a complex and heterogeneous mental disorder such as depression incorporates components from all current theories.

The Monoamine Hypothesis of Affective Disorders

Monoamine neurotransmitters are a class of neurotransmitters derived from aromatic amino acids, and most notably include serotonin, norepinephrine, and dopamine. In the 1950s, the role of monoamines in mood disorders became apparent through a series of inadvertent discoveries, which eventually culminated in the *monoamine hypothesis of affective disorders* [101]. In 1955, some patients being treated with the antihypertensive agent reserpine were found to become depressed after treatment [48, 95]. It was later shown that reserpine depletes vesicular storage of brain serotonin, which in turn reduces the available serotonin for synaptic transmission [48, 101, 135]. Conversely, the antimycobacterial agent iproniazid was shown to improve mood in tubercular patients with depression [22, 48]. Iproniazid inhibits monoamine oxidase (MAO), the enzyme that degrades free monoamines in the presynaptic nerve terminal. By inhibiting MAO, iproniazid enhances central serotonin and norepinephrine transmission. This discovery prompted the development of other monoamine oxidase inhibitors (MAOIs). Further support for the monoamine hypothesis came when imipramine, a drug initially developed as an anxiolytic for agitated patients with psychosis, was shown to have antidepressant effects [48, 69]. Imipramine, now classified as a tricyclic antidepressant (TCA), acts by blocking monoamine reuptake transporters, thereby increasing the level of serotonin and norepinephrine in the synapse. Together, MOAIs and TCAs constitute first generation antidepressants. In the late 1980s, momentum for the monoamine hypothesis prompted a second generation of antidepressants to enter development. These drugs aimed to increase receptor specificity and, therefore, decrease adverse side effects and increase tolerability. This second generation of antidepressants includes selective serotonin reuptake inhibitors (SSRIs), which are currently the most prescribed class of antidepressants, as well as serotonin–norepinephrine reuptake inhibitors (SNRIs). Although current antidepressants that target monoamine transmission are clinically efficacious for some patients, their delayed antidepressant effect has proven to be problematic for the monoamine hypothesis. SSRIs increase monoamine transmission within hours of administration and begin to cause side effects within hours or days [48, 68]. However, enhanced mood requires weeks of chronic treatment. Additionally, monoamine depletion studies have found that acute reduction of monoamines can

decrease mood in patients with a personal or family history of depression but not in healthy controls [68, 104, 123]. Rather than a direct effect of monoamine neurotransmission on mood state, it is now thought that antidepressants induce secondary transcriptional and translational changes that ultimately lead to synaptogenesis and neurogenesis [68, 101, 113]. For example, the transcription factor CREB (cAMP response element binding protein) is downstream of serotonin receptors and regulates expression of brain-derived neurotrophic factor (BDNF). Clinical studies report decreased levels of CREB in the cortex of depressed patients, and experimentally increased CREB activity in the hippocampus of rodents has been reported to induce antidepressant-like effects on behavioural tests [12, 101]. Additionally, CREB levels in the hippocampus are increased following chronic administration of antidepressants, such as the SSRI fluoxetine [12, 106]. These neuroplastic changes require several weeks and are necessary to achieve behavioural changes, which is consistent with the delayed response to antidepressants. Although the monoamine hypothesis has been the most clinically relevant theory of depression, leading to the development of first and second generation antidepressants, the delayed clinical response to increased monoamines suggests that monoamine deficiency is not a primary abnormality in the aetiology of depression.

Neurotrophins, BDNF, and the Anatomy of Depression

In the brain, the monoamines serotonin and norepinephrine are largely released by the raphe nuclei and the locus coeruleus, respectively. These brainstem structures project to regions in the cerebral cortex and limbic system that regulate emotion, reward, attention, and executive function. Specifically, neuroimaging and volumetric post-mortem studies have identified reduced neural activity and dendritic atrophy in the hippocampus and the prefrontal cortex (PFC) [25, 60, 102, 134]. Although functional imagining studies have produced limited overlap in the brain regions identified in depression, meta-analytic results suggest that the regions with the most consistently reduced neural activity include the PFC, insula, cerebellum, and the parahippocampal gyrus (PHG; the major inflow tract to the hippocampus) [32, 45]. More consistent results have been provided through structural neuroimaging studies. These results were summarized in a meta-analysis, which revealed consistent volume reductions in frontal regions (anterior cingulate, orbitofrontal, and PFC), as well as in the hippocampus and dorsal striatum [45, 63]. Moreover, volume reductions have been shown to be attenuated with antidepressant treatment [134].

The precise mechanism of region-specific volume reductions in depression has not been established. However, the role of BDNF has attracted interest in recent years. Stress-induced downregulation in hippocampal BDNF expression has been well documented in preclinical studies [26]. Conversely, chronic treatment with antidepressants has been shown to upregulate hippocampal and PFC BDNF expression [87]. Post-mortem studies on humans support preclinical results, showing decreased levels of hippocampal BDNF in untreated subjects compared to

subjects treated with antidepressant at the time of death [16, 26, 58, 87]. These correlation studies have prompted investigation into a more causal role of BDNF regulation in depression. To investigate the possibility of a causal association, a single-nucleotide polymorphism (SNP) in BDNF was developed, which substitutes methionine for valine at amino acid 66 (Val66Met), leading to improper storage of BDNF in neurons [30, 68]. Consequently, less BDNF is secreted from the nerve terminals. When implemented into a biological system, knock-in mice with this polymorphism exhibited increased anxiety-related behaviours when exposed to stressors [17, 68]. Antidepressants have also been shown to increase other growth factors in the hippocampus, such as vascular endothelial growth factor (VEGF), likely through the activation of transcriptional regulators such as CREB [68, 143]. However, a direct neuroprotective role of growth factors such as BDNF has not been straightforward to establish due to region-specificity. For example, in the ventral tegmental area (VTA; most notably involved in reward response and drug addiction) and the nucleus accumbens (NAc; also involved in reward processing), infusion of BDNF causes increased depressive-like behaviours in mice [67].

Stress and Cytokines

There is a strong evidence in the literature that dysregulation of the hypothalamic–pituitary–adrenal axis (HPA) is an important factor in the biological aetiology of depression. In response to perceived stress by the cortical regions, the hypothalamus releases corticotropin-releasing hormone (CRH). CRH then stimulates the anterior pituitary gland to release adrenocorticotropic hormone (ACTH), which in turn stimulates the adrenal cortex to release cortisol, a glucocorticoid. In a negative feedback mechanism, excess cortisol inhibits the hypothalamus and anterior pituitary, halting further production of cortisol. Although the first depressive episode usually involves a stressful psychosocial "trigger", later episodes of depression become increasingly "endogenous" as the illness progresses [45]. Even in the absence of exogenous triggers, increased plasma, urine, and cerebrospinal fluid (CSF) cortisol levels have been well documented in a subset of patients with depression [57, 85, 110, 111]. Chronic exposure to elevated levels of glucocorticoids can have a deleterious impact on brain structures involved in cognition and emotional functions [82]. In fact, hypercortisolaemia has been shown to cause structural remodelling in the hippocampus, amygdala, and PFC [90]. In the hippocampus, certain types of acute stress have been demonstrated to suppress neurogenesis in the dentate gyrus, leading to atrophy—an effect that has also been observed in patients with Cushing's syndrome, which is primarily characterized by increased ACTH release from the pituitary gland and hypercortisolaemia [139]. This stress-induced atrophy has been postulated to be the underlying mechanism of the volumetric reductions observed in the hippocampus and PFC of patients with depression. Further support for the role of chronic stress in depression has come from preclinical studies. The most successful and widely used murine models of depression have, in fact, relied on the clinical observations of stress as a risk factor

in depression [103]. Chronic mild stress, chronic unpredictable stress, social defeat paradigms, as well as direct chronic administration of corticosterone have all provided some measure of construct validity in modelling depression by causing anhedonia in the sucrose preference test [39, 103, 114, 146, 147]. These paradigms have also demonstrated face validity by modelling demonstrable symptoms of depression (e.g. decreased investigative and locomotor activity), and predictive validity through the reversal of depressive-like behaviours following chronic antidepressant treatment [103, 146]. It is important to note, however, that true construct validity cannot be achieved in models of depression, as this would require re-creating the disease aetiology, which remains largely unknown. At the molecular level, there is evidence that hypercortisolaemia is associated with modulation of the serotonergic system. The serotonin receptor subtype 5-HT_{1A} has been strongly implicated in depression and anxiety, with reduced receptor numbers and affinity reported in some patients [126]. Recently, preclinical and clinical evidence has suggested a causal role of stress-induced hypercortisolaemia on reduced 5-HT_{1A} receptor downregulation [72, 80].

"Sickness behaviour" constitutes a set of clinically recognized behaviours that human and animal subjects exhibit at the onset of infectious disease [44]. These behaviours, which are due to activation of the inflammatory response, share many characteristics with depression, such as anhedonia and cognitive impairment [45]. Cytokines are the molecular mediators of inflammatory responses. Pro-inflammatory cytokines such as interleukin 1 (IL-1), IL-6, and tumour necrosis factor alpha (TNF-α) have been found to be elevated in the plasma and CSF of patients with depression [151]. In rodents, direct injection of low doses of IL-1 has also been shown to induce "sickness behaviour" [28, 68]. In humans, depressive symptoms have been reported as a common side effect of treatment with interferon alpha (IFN-α), a pro-inflammatory agent, occurring in approximately 30–50 % of patients [52]. Conversely, evidence suggests that anti-inflammatory treatment such as non-steroidal anti-inflammatory drugs (NSAIDs) can be effective adjuvant drugs, particularly for treatment-resistant depression [65]. Despite strong evidence for a possible role of inflammation in the aetiology of depression, the neurobiological mechanism involved remains unknown. Further investigations should focus on the effect of neuroimmunological mediators (i.e. microglia) on surrounding glia and neurons [68].

Glutamate

Glutamate is the anionic form of the amino acid glutamic acid. In the nervous system, glutamate is the most abundant neurotransmitter [92] and plays a key role in cognitive processes that are dependent on synaptic plasticity, such as learning and memory [89]. Peripherally, glutamate is released as a response to induced inflammation and activation of peripheral nociceptive fibres [19, 109]. Additionally, direct injection of glutamate has been shown to increase sensitivity to thermal and mechanical stimuli in murine models [11, 54].

Ketamine is a widely used general anaesthetic, and is pharmacologically classified as an antagonist to N-Methyl-D-aspartate receptors (NMDAR), a type of ionotropic glutamate receptors. In recent years, ketamine has become the focus of accumulating reports assessing its antidepressant effects in both humans and animal models [97, 153]. In 2000, Berman and colleagues carried out the first clinical study that reported on ketamine's rapid antidepressant properties. The antidepressant effects of ketamine were robust for the patients involved in the randomized trial [9] and were then replicated in a larger study involving 18 treatment-resistant patients [153]. Since then, glutamate signalling has become well established as a factor in the neurostructural changes in depression [29, 125], with extensive preclinical [8, 38, 39, 75] and clinical evidence [53, 152] to support the validity of glutamate modulation for treating depression.

In 2010, interested in the potential for new depression therapeutics, Li and colleagues carried out a study on rats that began to elucidate a possible antidepressant mechanism for ketamine. They found that administration of ketamine rapidly activated the mammalian target of rapamycin (mTOR) pathway, leading to increased synaptogenesis in the PFC [74]. Additionally, blocking mTOR signalling effectively blocked ketamine's ability to induce synaptogenesis. It is now suggested that antagonism of NMDA receptors by ketamine causes an increased concentration of extracellular glutamate, resulting in fast excitation of neurons through increased activity of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, another type of ionotropic glutamate receptors [27]. This fast excitation causes an influx of calcium ions through voltage-gated calcium channels, which in turn stimulates the release of BDNF. BDNF subsequently stimulates tropomyosin-related kinase B (TrkB) and downstream signalling pathways including PI3 K-Akt and MAPK. These pathways stimulate mTOR, a serine-threonine protein kinase, which in turn regulates genes that increase the density of synaptic proteins, ultimately leading to synaptogenesis and antidepressant behavioural responses [27]. Although ketamine is also known to interact with other signalling systems, including the dopamine D2 receptors, opioid receptors, and sigma (σ) receptors [66, 119], there is considerable evidence to suggest that the primary antidepressant response of ketamine is mediated by the NMDA receptor. For example, other NMDA antagonists, including MK-801 and CPPene, have also shown effectiveness in inducing anti-depressive effects in animal models [7, 84]. Moreover, the behavioural antidepressant effects of ketamine in animal models of depression have been shown to act independently of σ receptors [119]. In addition to ketamine, the antidepressant action of tianeptine, a clinically used TCA, has recently been attributed to glutamatergic regulation, possibly through the modulation of both AMPAR and NMDAR [91].

Clinically, concentrations of glutamate are elevated in the serum or plasma of patients with MDD [3, 61, 88, 94]. At the brain level, studies using magnetic resonance spectroscopy (MRS) reveal a decreased unresolved glutamate/glutamine signal (Glx) and glutamate alone signal (Glu) in brain regions that are relevant to depression, such as the PFC and anterior cingulate cortex [6, 46].

Antidepressants

Treatment for MDD has improved significantly since the serendipitous discovery of MAOIs and the formation of the monoamine hypothesis of depression in the 1950s. However, with the underlying aetiology of the illness still unclear, efforts to create increasingly targeted therapy has been relatively stagnant. Monotherapy with first and second generation antidepressants often fails to alleviate symptoms, and it may take multiple attempts with different antidepressants and adjunct therapy to achieve clinical efficacy. Treating depression becomes even more difficult when it presents as comorbidity, in part due to a lack of understanding of the relationship between the primary disease and depression. Few studies have examined depression in cancer patients at the basic level, and thus treatment options for CID are limited to those therapies developed for use in non-cancer-related MDD. In this section, we will consider the clinical efficacy of antidepressants in MDD as well as CID.

Antidepressants in Major Depressive Disorder

The Sequenced Treatment Alternatives to Relieve Depression (STAR*D) trial was the largest effort to date on the efficacy of antidepressants. It was commissioned by the National Institute of Mental Health (NIMH) and completed in 2006 [124]. In 2008, data from the trial became available. The study recruited 4041 adult patients (1127 dropped out; 2876 were analyzable) with MDD from primary care and psychiatric settings [50, 124]. As the primary outcome measure for remission, STAR*D used the Hamilton Depression Rating Scale (HAM-D) to measure the severity of depression. The HAM-D is a commonly used 52-item questionnaire that rates severity of depression on a 17-point scale, with scores of 0–7 considered normal [42]. In level 1 treatment, patients received citalopram monotherapy, one of the most prescribed SSRIs, and remission rates were approximately 28 % based on HAM-D scores [51]. In levels 2, 3, and 4 of the trial, patients who did not achieve remission in the previous level were either switched to a different antidepressant or received an augmentation to citalopram treatment. Switches to new antidepressants consisted of other SSRIs, SNRIs, TCAs, or other agents that act on monoamine transmission. In the case of treatment augmentation, a wide range of agents were used, including anxiolytics, lithium, and thyroid hormone T3 [51]. In each level of the trial, the treatment-resistant patients from the previous level were randomized to the new treatment regimens. Remission rates in levels 2, 3, and 4 of the trial were all below 30 %. With only a third of MDD patients responding to initial monotherapy, systematic reviews of randomized control trials (RCTs) have sought to better define the role of antidepressants in the clinical setting. In 2009, Cipriani et al. showed that of the commonly prescribed second generation antidepressants, escitalopram and sertraline were the most efficacious and best tolerated, leading to fewer discontinuations [18]. In another meta-analysis, Fournier et al. investigated

antidepressant efficacy relative to initial symptom severity [33]. They concluded that patients with severe MDD benefit substantially from antidepressant treatment, whereas benefit is minimal in mild or moderate MDD. In addition to pharmacological modulation, CBT has been shown to be beneficial for patients with depression, even in the case of severe MDD [24, 49]. In some cases of severe MDD that is not responsive to antidepressants, electroconvulsive therapy (ECT) may be used. ECT has been extensively shown to be effective in achieving remission in treatment-resistant patients [93]. However, due to the requirement of anaesthetic, ECT is rarely used as a first line of treatment. More recently, repetitive transcranial magnetic stimulation (rTMS) has also been shown to provide some benefit as adjunct therapy in treatment-resistant patients [93].

Antidepressants in Cancer-Induced Depression

In stark contrast to the large-scale and high-quality RCTs available for primary MDD, few studies have investigated antidepressant efficacy and alternative or adjunct therapies in cancer patients. This is surprising considering the high prevalence of depression comorbidity in cancer, a clinical observation that spans decades [13, 31, 35, 62, 78, 144]. Difficulties in studying and treating CID are found at the preclinical and clinical levels. At the preclinical level, the lack of validated animal models for CID has restricted inquiry into the possible biological mechanisms involved. Cancer patients with comorbid depression are, therefore, limited to antidepressant treatment developed for non-cancer patients. Clinically, depression is underdiagnosed and undertreated in cancer patients, largely owing to the psychosocial complication of what might be considered "appropriate sadness" in terminally ill patients compared to treatable psychiatric disease [13, 78, 137]. In addition, factors such as cancer type, cancer stage, and demographic convolute an already complex mental disorder. Thus, in the absence of more precisely tailored treatment, antidepressants (particularly SSRIs) remain the first line of treatment in the oncologic setting.

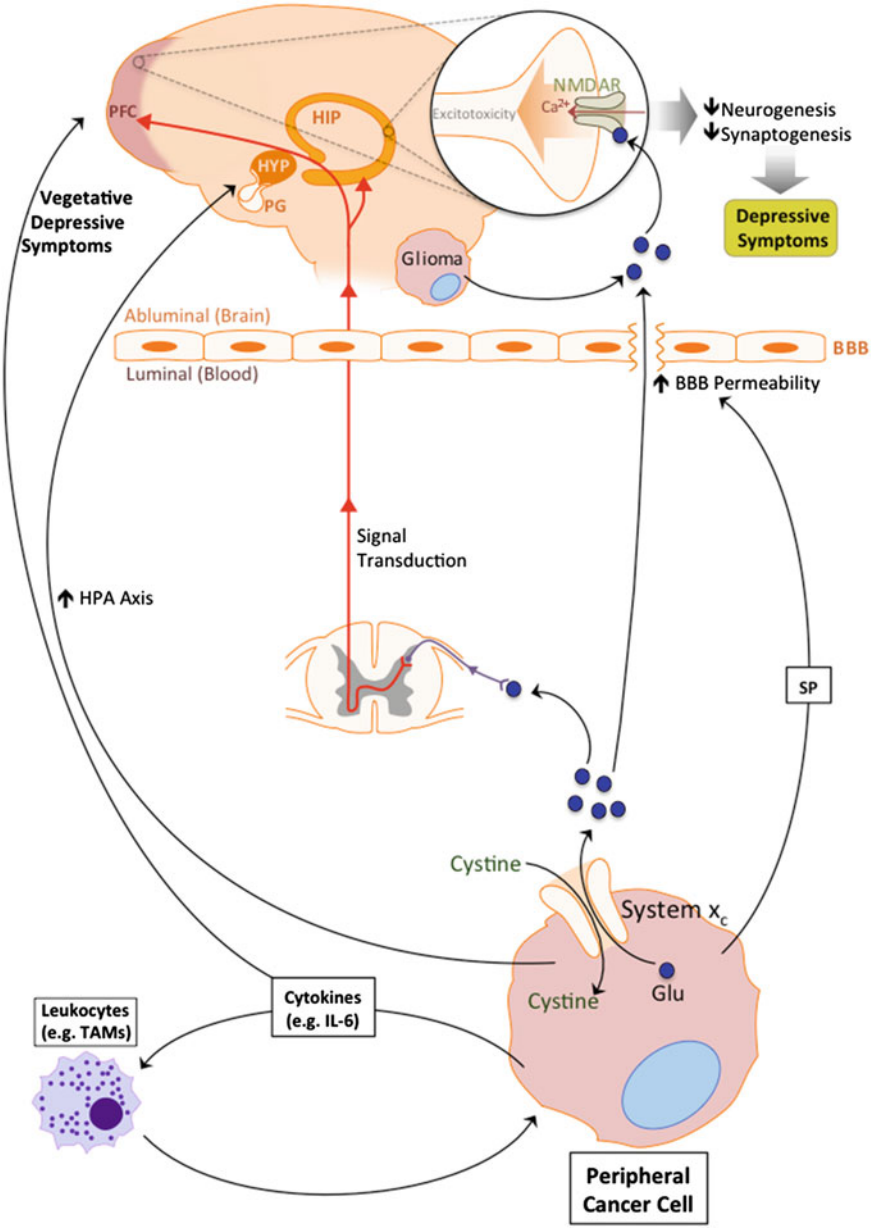
Although few studies have examined the efficacy of antidepressants in CID, a handful of systematic reviews have compiled such studies in an attempt to draw clinical conclusions. In 2006, 2007, and 2011, three groups examined the literature for antidepressant efficacy in cancer. The first review focused on SSRIs and found that four of the five studies reported positive results, and one study using fluoxetine showed no difference in incidence of depression compared to placebo [145]. The second review, which had overlapping studies with the first, also examined the efficacy of mianserin (a tetracyclic antidepressant; TeCA) in two included studies [120]. In this review, three placebo-controlled trials (including the two mianserin studies) showed positive results. Of the remaining four studies, the two placebo-controlled trials did not detect a difference between treatment and placebo, while the two trials comparing active treatments found temporal improvement of

depressive scores but no group differences. The third review in 2011 updated the previous results with one additional study, which did not detect a difference between placebo and paroxetine or desipramine [105]. Underscoring the lack of high-quality studies on the topic, a 2013 Cochrane review found no eligible RCTs, controlled trials, cohort studies or case-control studies investigating antidepressant efficacy in patients with primary brain tumours [122]. Studies under consideration were excluded for a wide range of issues, such as reporting on usual clinical care rather than systematically evaluating specific treatments. Most recently, another systematic review has investigated antidepressant efficacy in breast cancer specifically [15]. This review identified two eligible studies with mixed results, both of which have been included in other systematic reviews [105, 120, 145]. Concerns raised in this review included small sample sizes, and therefore a significant risk of bias. Overall, these systematic reviews highlight the inadequacy of currently available literature on the question of antidepressant efficacy in cancer patients. From these studies, broad clinical conclusions cannot be drawn, which points to a need for larger and better designed clinical trials as well as a capacity to study CID at the basic level.

In addition to pharmacotherapy, psychological interventions such as CBT, supportive psychotherapy, and group psychotherapy may be efficacious for cancer patients either as primary treatment or in combination with antidepressants [2, 73, 79]. However, in clinical trials of antidepressants, physiological interventions, including regular hospice care, may be a confounding variable that can mask antidepressant effect [79]. Therefore, intervention models under investigation need to be well designed and appropriately analyzed to control for such confounds.

Cancer-Induced Depression

Strong clinical and preclinical evidence exists in the literature to support a causal role of cancer on depression. In the introduction to this chapter, the prevalence of depression in the oncologic setting was discussed in comparison to depression in the general population. While the staggeringly high prevalence of depression in cancer patients suggests a strong correlation, the impact of psychosocial factors makes it difficult to establish causation or biological mechanisms. However, early clinical studies reveal that psychological changes relating to depression may in fact *precede* the diagnosis of cancer [40, 55, 112]. More recently, a breast cancer study, which included 428 women, reported that over 25 % of women with breast cancer exhibited symptoms of depression prior to being informed of their cancer diagnosis [142]. Using data from the World Mental Health Survey Initiative, another study performed a retrospective analysis on the mental health of cancer patients, which included 19 countries and more than 52,000 patients [107]. The study found that depression symptoms appear predictive of a later cancer diagnosis. By demonstrating an increased prevalence of depression in patients who have cancer but are


PFC
HIP
HYP
PG
Vegetative
Depressive
Symptoms
Glioma
NMDAR
Excitotoxicity
Ca2+
Neurogenesis
Synaptogenesis
Depressive
Symptoms
Abluminal (Brain)
Luminal (Blood)
BBB
BBB Permeability
Signal
Transduction
HPA Axis
SP
Cystine
System xc
Cystine
Glu
Cytokines
(e.g. IL-6)
Leukocytes
(e.g. TAMs)
Peripheral
Cancer Cell

◀ **Fig. 1.1** Schematic summarizing proposed oncodynamic mechanisms of CID. Glioma cells in the brain release large amounts of glutamate (Glu), which directly cause excitotoxicity of neurons by hyperexcitation of NMDARs. This causes a decrease in neurogenesis and synaptogenesis in brain regions such as the hippocampus (HIP) and PFC, which leads to depressive symptoms. Peripheral cancer cells also release large amounts of glutamate. Substance P (SP) released by cancer cells impairs the blood–brain barrier (BBB), causing increased permeability, which may allow peripherally secreted glutamate to enter the brain. Alternatively, peripherally secreted glutamate may act on the spinal cord through signal transduction pathways that project to brain regions involved in depression. Peripheral cancer cells also secrete cytokines, which may be a causal factor in vegetative depressive symptoms. Tumour burden has also been shown to influence the hypothalamic–pituitary–adrenal (HPA) axis, which leads to chronic physiological stress and depressive symptoms

unaware of their diagnosis, these clinical findings effectively eliminate the confounding psychosocial effect of a cancer diagnosis, and suggest a possible causal role of cancer on mental health at the biological level. In addition to clinical support, this oncodynamic impact of cancer on depression is supported through common biological systems between cancer and depression; namely, inflammation, physiological stress, and glutamatergic dysregulation. In order to investigate the possible causal role of these systems in the induction of depression by cancer cells, validated CID animal models need to be established. In 2009, Pyter et al. reported that peripheral mammary tumours induce behavioural changes such as anhedonia in rats and increase plasma biomarkers such as cytokines and corticosterone [115]. Similarly, in 2011 Lamkin et al. were able to replicate these findings using ovarian cancer in mice [71]. To investigate possible neurological correlates in CID, Yang et al. recently showed that tumour-bearing mice had reduced proliferating and progenitor neurons in the dentate gyrus of the hippocampus when compared to control animals [149]. Although these studies have provided compelling insight into the association between cancer and depression, more rigorous validation of CID models is needed. Behavioural and relevant neuroanatomical comparisons to existing validated models of depression would yield more convincing animal models. In addition, reversal trials using antidepressants on the positive control depressive models would further establish the validity of the behavioural tests used prior to evaluating the CID models. Properly validated CID models would be an essential tool in manipulating inflammatory, stress, and glutamatergic systems in the investigation of the causal oncodynamic effect of cancer on depressive symptoms. To date, only correlative associations have been established between cancer and depression, although a causal relationship has been postulated based on the clinical studies discussed in earlier in this chapter. Expanding on what is currently known about the common biological systems that are involved in cancer and depression, we can discuss the most plausible oncodynamic mechanisms of CID. These proposed mechanisms of CID are summarized in Fig. 1.1.

Oncodynamic Effect Through Inflammation

A well-established characteristic of most cancer cells is their ability to exploit the host's immune system at multiple stages of tumour development and metastasis [1, 20, 21, 34, 41, 43]. Specifically, cancer cells recruit an array of cytokine-producing leukocytes, such as tumour-associated macrophages (TAMs) [20, 100]. Cancer cells themselves are also capable of expressing various cytokines, such as TNF-α and Il-6, that attract more leukocytes [20]. In doing so, cancer cells employ the same mechanisms that are normally activated to repair tissue in response to tissue damage [70]. For example, in order to repair normal tissue damage, the extracellular matrix (ECM) that binds cells together must be broken down in order to allow for the recruitment of new cells to the site of injury. Platelets aggregating at the site of injury release platelet derived growth factor (PDGF), which in turn stimulates fibroblasts to secrete matrix metalloproteinases (MMPs). These enzymes break down the ECM of damaged cells and allow the arrival of new cells [70]. Cancer cells that secrete PDGF can exploit this mechanism by recruiting MMP-secreting fibroblasts to break down the ECM of healthy epithelial cells and by replacing them with multiplying cancer cells [70, 76].

As previously discussed in this chapter, depression is strongly associated with pro-inflammatory mediators in clinical and preclinical studies. The ability of cancer cells to directly secrete pro-inflammatory mediators highlights one possible oncodynamic pathway of CID. We can further postulate on the specific downstream effect of this oncodynamic event through closer investigation of inflammatory consequences in depression. Clinical studies investigating the cytokine profile of cancer patients have shown that IL-6, which is directly secreted by cancer cells [127], is elevated in the plasma of cancer patients who also exhibit depressive symptoms, compared to cancer patients who do not exhibit depressive symptoms [56, 99, 138]. In another study, the increased plasma concentration of IL-6 in ovarian cancer patients was associated with the vegetative symptoms of depression (such as fatigue and weight loss), but not with affective symptoms or overall depression [83]. Similar effects on vegetative, but not affective, depression symptoms have been observed with IFN-α therapy-induced inflammation [14, 70, 98]. Taken together, these results suggest that cancer cell-secreted IL-6 (and possibly other inflammatory mediators) induces an oncodynamic effect on depression, which specifically exacerbates vegetative symptoms.

Oncodynamic Effect Through Physiological Stress

Physiological stress through activation of the sympathetic nervous system is an adaptive response to environmental stressors. As previously discussed, dysregulation of this response is strongly implicated in the aetiology of depression. Undoubtedly, the psychosocial impact of a cancer diagnosis is one source of this dysregulation.

The induction of chronic physiological stress in cancer patients is supported by the clinical observation of increased plasma cortisol in advanced cancer patients [81, 128]. Additionally, plasma levels of cortisol are higher with increased tumour burden, metastasis, and pervasiveness of the cancer [116, 129, 141]. This suggests a direct impact of cancer cells on physiological stress, in addition to the psychosocial contribution. However, the mechanism of cancer-induced activation of the stress response has not been investigated, with the notable exception of adrenal tumours that autonomously produce and secrete cortisol [36]. Other studies have investigated general HPA activation in cancer patients, but not the mechanism of activation, and often in the context of investigating depressive symptoms [83, 138]. Although clinical studies suggest a direct oncodynamic effect of cancer on the dysregulation of the physiological stress response (and ultimately depression), a discussion on the biological mechanisms is lacking in the literature.

Oncodynamic Effect Through Glutamatergic Signalling

As early as the 1980s, results from clinical investigations have demonstrated elevated plasma levels of glutamate in cancer patients [108, 118]. More recently, the mechanism of glutamate release by cancer cells as well downstream consequences of this release have garnered attention in the literature. Initial studies focused on glioma cell lines and found that glutamate secretion into the extracellular environment involved the glutamate/cystine antiporter system x_c^- [59]. This excess glutamate secretion causes excitotoxicity and death of surrounding neurons through over-activation of NMDARs [131, 150]. The same mechanism of glutamate secretion through system x_c^- was later characterized in multiple cancer cell lines, including metastatic breast and prostate cancers, through in vitro and in vivo studies [130–133, 140].

Earlier in this chapter, the emerging role of glutamatergic signalling in the aetiology of depression was discussed. Excess glutamate secretion by cancer cells provides a biologically plausible cause of glutamate dysregulation in depression. This connection is particularly convincing in the case of gliomas, which secrete very high amounts of glutamate and which are also associated with a very high incidence of depression, as previously discussed. Neuronal hyperactivation due to glioma-secreted glutamate would interfere with neuroplastic and synaptoplastic events in the mPFC and the hippocampus, ultimately leading to depression. In peripheral cancers, the effect of glutamate on depression may not be as direct. Because of glutamate's key role in many neuronal signalling events, glutamate distribution and extracellular fluid (ECF) concentrations in the brain are tightly controlled. The vast majority of glutamate in the brain is stored in astrocytes, while glutamate in the ECF is maintained at very low concentrations relative to plasma levels in the periphery [47, 86, 136]. The blood-brain-barrier (BBB) is a crucial structure in the maintenance of this concentration difference between plasma and brain ECF glutamate. Excitatory amino acid transporters (EAATs) on the abluminal

(brain-facing) membrane of the BBB transport glutamate from the ECF to the peripherally circulating blood. The luminal (blood-facing) membrane lacks EAATs, thus preventing the entrance of glutamate from the blood into the brain under normal physiological conditions. However, recent evidence has suggested that pathological conditions disrupt the BBB, leading to increased permeability. Substance P (SP) is a pro-inflammatory neuropeptide that has been implicated in nociception [23], depression [64, 96, 148], and is expressed in breast cancer cells [117]. It was recently shown that breast cancer cell-secreted SP is involved in the transmigration of cancer cells across the BBB [121]. To do this, SP activates an inflammatory response in the endothelial cells that comprise the BBB, which ultimately increases their permeability. Therefore, under pathological conditions such as metastatic disease, tight regulation of brain glutamate may be impaired by breaches in the BBB. This represents one possible mechanism through which glutamate secreted by peripheral tumours can affect brain physiology and induce depression.

An alternative oncodynamic mechanism would be analogous to pain transmission. Glutamate released by peripheral cancer cells causes pain in a model of bone metastasis, which is attenuated using an antagonist of system x_c^- [140]. In this paradigm, glutamate does not need to cross the BBB in order to transmit a pain signal. Nociceptive fibres are activated peripherally and the signal is transmitted through the ascending pathway to cortical regions that perceive pain [37]. Similarly, it is plausible that peripheral glutamate activates CNS pathways indirectly through signal transmission, culminating in brain alterations consistent with depression. Therefore, although a mechanism has not been investigated in the literature, preclinical and clinical evidence suggests that cancer-secreted glutamate imparts an oncodynamic effect on the development of CID. In this section, two biologically plausible mechanisms for this oncodynamic effect have been suggested.

Conclusion

Depression in cancer patients is a highly prevalent comorbidity, which affects quality of life and survivorship. Although psychosocial factors contribute to depression in the cancer setting, the clinical evidence reviewed in this chapter suggests a more causal role of cancer on the induction of depression. Through careful consideration of the overlapping biological mechanisms involved in depression aetiology and cancer physiology, we can postulate on the initial oncodynamic signalling event(s) that lead to the induction of depression. However, a robustly validated preclinical model of CID is lacking in the literature. Therefore, the capacity to investigate the oncodynamic mechanism of CID through manipulation of a valid model has yet to be established. Future direction in this field of research should focus on developing the capacity to investigate the mechanism(s) of CID, while being attentive to advancements in the understanding of depression aetiology.

References

1. Aggarwal BB, Shishodia S, Sandur SK, et al. Inflammation and cancer: how hot is the link? Biochem Pharmacol. 2006;72:1605–21.
2. Akechi T. Psychotherapy for depression among patients with advanced cancer. Jpn J Clin Oncol. 2012;42:1113–9.
3. Altamura CA, Mauri MC, Ferrara A, et al. Plasma and platelet excitatory amino acids in psychiatric disorders. Am J Psychiatry. 1993;150:1731–3.
4. Andrade L, Caraveo-Anduaga JJ, Berglund P, et al. The epidemiology of major depressive episodes: results from the international consortium of psychiatric epidemiology (ICPE) surveys. Int J Methods psychiatr Res. 2003;12:3–21.
5. Association AP. Diagnostic and statistical manual of mental disorders. 5th ed. Arlington: American Psychiatric Publishing; 2013.
6. Auer DP, Putz B, Kraft E, et al. Reduced glutamate in the anterior cingulate cortex in depression: an in vivo proton magnetic resonance spectroscopy study. Biol Psychiatry. 2000;47:305–13.
7. Autry AE, Adachi M, Nosyreva E, et al. NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. Nature. 2011;475:91–5.
8. Banasr M, Chowdhury GM, Terwilliger R, et al. Glial pathology in an animal model of depression: reversal of stress-induced cellular, metabolic and behavioral deficits by the glutamate-modulating drug riluzole. Mol Psychiatry. 2010;15:501–11.
9. Berman RM, Cappiello A, Anand A, et al. Antidepressant effects of ketamine in depressed patients. Biol Psychiatry. 2000;47:351–4.
10. Berrios GE. Melancholia and depression during the 19th century: a conceptual history. Br J Psychiatry: (the journal of mental science). 1988;153:298–304.
11. Bhave G, Karim F, Carlton SM, et al. Peripheral group I metabotropic glutamate receptors modulate nociception in mice. Nat Neurosci. 2001;4:417–23.
12. Blendy JA. The role of CREB in depression and antidepressant treatment. Biol Psychiatry. 2006;59:1144–50.
13. Caplette-Gingras A, Savard J. Depression in women with metastatic breast cancer: a review of the literature. Palliat Suppor Care. 2008;6:377–87.
14. Capuron L, Gumnick JF, Musselman DL, et al. Neurobehavioral effects of interferon-alpha in cancer patients: phenomenology and paroxetine responsiveness of symptom dimensions. Neuropsychopharmacol (official publication of the American College of Neuropsychopharmacology). 2002;26:643–52.
15. Carvalho AF, Hyphantis T, Sales PM, et al. Major depressive disorder in breast cancer: a critical systematic review of pharmacological and psychotherapeutic clinical trials. Cancer Treat Rev. 2014;40:349–55.
16. Chen B, Dowlatshahi D, Macqueen GM, et al. Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. Biol Psychiatry. 2001;50:260–5.
17. Chen ZY, Jing D, Bath KG, et al. Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. Science. 2006;314:140–3.
18. Cipriani A, Furukawa TA, Salanti G, et al. Comparative efficacy and acceptability of 12 new-generation antidepressants: a multiple-treatments meta-analysis. Lancet. 2009;373:746–58.
19. Conn PJ, Pin JP. Pharmacology and functions of metabotropic glutamate receptors. Annu Rev Pharmacol Toxicol. 1997;37:205–37.
20. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002;420:860–7.
21. Coussens LM, Zitvogel L, Palucka AK. Neutralizing tumor-promoting chronic inflammation: a magic bullet? Science. 2013;339:286–91.
22. Crane GE. The psychiatric side-effects of iproniazid. Am J Psychiatry. 1956;112:494–501.

23. De Felipe C, Herrero JF, O'brien JA et al. Altered nociception, analgesia and aggression in mice lacking the receptor for substance P. Nature. 1998;392:394–397.
24. Driessen E, Hollon SD. Cognitive behavioral therapy for mood disorders: efficacy, moderators and mediators. Psychiatric Clin North Am. 2010;33:537–55.
25. Duman RS, Aghajanian GK. Synaptic dysfunction in depression: potential therapeutic targets. Science. 2012;338:68–72.
26. Duman RS, Monteggia LM. A neurotrophic model for stress-related mood disorders. Biol Psychiatry. 2006;59:1116–27.
27. Duman RS, Voleti B. Signaling pathways underlying the pathophysiology and treatment of depression: novel mechanisms for rapid-acting agents. Trends Neurosci. 2012;35:47–56.
28. Dunn AJ, Swiergiel AH, De Beaurepaire R. Cytokines as mediators of depression: what can we learn from animal studies? Neurosci Biobehav Rev. 2005;29:891–909.
29. Duric V, Banasr M, Stockmeier CA, et al. Altered expression of synapse and glutamate related genes in post-mortem hippocampus of depressed subjects. Int J Neuropsychopharmacol [official scientific journal of the Collegium Internationale Neuropsychopharmacologicum (CINP)]. 2013;16:69–82.
30. Egan MF, Kojima M, Callicott JH, et al. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell. 2003;112:257–69.
31. Endicott J. Measurement of depression in patients with cancer. Cancer. 1984;53:2243–9.
32. Fitzgerald PB, Laird AR, Maller J, et al. A meta-analytic study of changes in brain activation in depression. Hum Brain Mapp. 2008;29:683–95.
33. Fournier JC, Derubeis RJ, Hollon SD, et al. Antidepressant drug effects and depression severity: a patient-level meta-analysis. JAMA: (the journal of the American Medical Association). 2010;303:47–53.
34. Franklin RA, Liao W, Sarkar A, et al. The cellular and molecular origin of tumor-associated macrophages. Science. 2014;344:921–5.
35. Fras I, Litin EM, Pearson JS. Comparison of psychiatric symptoms in carcinoma of the pancreas with those in some other intra-abdominal neoplasms. Am J Psychiatry. 1967;123:1553–62.
36. Goh G, Scholl UI, Healy JM, et al. Recurrent activating mutation in PRKACA in cortisol-producing adrenal tumors. Nat Genet. 2014;46:613–7.
37. Gordon-Williams RM, Dickenson AH. Central neuronal mechanisms in cancer-induced bone pain. Curr Opin Support Palliat Care. 2007;1:6–10.
38. Gourley SL, Espitia JW, Sanacora G, et al. Antidepressant-like properties of oral riluzole and utility of incentive disengagement models of depression in mice. Psychopharmacology. 2012;219:805–14.
39. Gourley SL, Taylor JR. Recapitulation and reversal of a persistent depression-like syndrome in rodents. In: Crawley N, et al, editors. Current protocols in neuroscience/ editorial board, Jacqueline Chapter 9: Unit 9 32. 2009.
40. Green AI, Austin CP. Psychopathology of pancreatic cancer. A psychobiologic probe. Psychosomatics. 1993;34:208–21.
41. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. Cell. 2010;140:883–99.
42. Hamilton M. A rating scale for depression. J Neurol Neurosurg Psychiatry. 1960;23:56–62.
43. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144:646–74.
44. Hart BL. Biological basis of the behavior of sick animals. Neurosci Biobehav Rev. 1988;12:123–37.
45. Hasler G. Pathophysiology of depression: do we have any solid evidence of interest to clinicians? World Psychiatry: [official journal of the World Psychiatric Association (WPA)]. 2010;9:155–61.
46. Hasler G, Van Der Veen JW, Tumonis T, et al. Reduced prefrontal glutamate/glutamine and gamma-aminobutyric acid levels in major depression determined using proton magnetic resonance spectroscopy. Arch Gen Psychiatry. 2007;64:193–200.

47. Hawkins RA. The blood-brain barrier and glutamate. Am J Clin Nutr. 2009;90:867S–74S.
48. Hirschfeld RM. History and evolution of the monoamine hypothesis of depression. J Clin Psychiatry. 2000;61(6):4–6.
49. Hoifodt RS, Strom C, Kolstrup N, et al. Effectiveness of cognitive behavioural therapy in primary health care: a review. Fam Pract. 2011;28:489–504.
50. Howland RH. Sequenced treatment alternatives to relieve depression (STAR*D). Part 1: study design. J Psychosoc Nurs Ment Health Serv. 2008;46:21–4.
51. Howland RH. Sequenced treatment alternatives to relieve depression (STAR*D). Part 2: Study outcomes. J Psychosoc Nurs Ment Health Serv. 2008;46:21–4.
52. Hoyo-Becerra C, Schlaak JF, Hermann DM. Insights from interferon-alpha-related depression for the pathogenesis of depression associated with inflammation. Brain Behav Immun. 2014;42:222–231.
53. Ibrahim L, Diazgranados N, Franco-Chaves J, et al. Course of improvement in depressive symptoms to a single intravenous infusion of ketamine vs add-on riluzole: results from a 4-week, double-blind, placebo-controlled study. Neuropsychopharmacology: (official publication of the American College of Neuropsychopharmacology). 2012;37:1526–33.
54. Jackson DL, Graff CB, Richardson JD, et al. Glutamate participates in the peripheral modulation of thermal hyperalgesia in rats. Eur J Pharmacol. 1995;284:321–5.
55. Jacobsson L, Ottosson JO. Initial mental disorders in carcinoma of pancreas and stomach. Acta Psychiatr Scand Suppl. 1971;221:120–7.
56. Jehn CF, Kuehnhardt D, Bartholomae A, et al. Biomarkers of depression in cancer patients. Cancer. 2006;107:2723–9.
57. Juruena MF, Cleare AJ. Pariante CM (2004), The hypothalamic pituitary adrenal axis, glucocorticoid receptor function and relevance to depression. Revista brasileira de psiquiatria. 1999;26:189–201.
58. Karege F, Vaudan G, Schwald M, et al. Neurotrophin levels in postmortem brains of suicide victims and the effects of antemortem diagnosis and psychotropic drugs. Brain Res Mol Brain Res. 2005;136:29–37.
59. Kato S, Negishi K, Mawatari K, et al. A mechanism for glutamate toxicity in the C6 glioma cells involving inhibition of cystine uptake leading to glutathione depletion. Neuroscience. 1992;48:903–14.
60. Kempton MJ, Salvador Z, Munafo MR, et al. Structural neuroimaging studies in major depressive disorder. Meta-analysis and comparison with bipolar disorder. Arch Gen Psychiatry. 2011;68:675–90.
61. Kim JS, Schmid-Burgk W, Claus D, et al. Increased serum glutamate in depressed patients. Archiv fur Psychiatrie und Nervenkrankheiten. 1982;232:299–304.
62. Koenig R, Levin SM, Brennan MJ. The emotional status of cancer patients as measured by a psychological test. J Chronic Dis. 1967;20:923–30.
63. Koolschijn PC, Van Haren NE, Lensvelt-Mulders GJ, et al. Brain volume abnormalities in major depressive disorder: a meta-analysis of magnetic resonance imaging studies. Hum Brain Mapp. 2009;30:3719–35.
64. Kormos V, Gaszner B. Role of neuropeptides in anxiety, stress, and depression: from animals to humans. Neuropeptides. 2013;47:401–19.
65. Kornstein SG, Schneider RK. Clinical features of treatment-resistant depression. J Clin Psychiatry. 2001;62(16):18–25.
66. Kortekaas R, Maguire RP, Van Waarde A, et al. Despite irreversible binding, PET tracer [11C]-SA5845 is suitable for imaging of drug competition at sigma receptors-the cases of ketamine and haloperidol. Neurochem Int. 2008;53:45–50.
67. Krishnan V, Han MH, Graham DL, et al. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. Cell. 2007;131:391–404.
68. Krishnan V, Nestler EJ. The molecular neurobiology of depression. Nature. 2008;455:894–902.
69. Kuhn R. The treatment of depressive states with G 22355 (imipramine hydrochloride). Am J Psychiatry. 1958;115:459–64.

70. Lamkin DM. Inflammatory processes and depressive-like behavior in a syngeneic model of ovarian cancer. Iowa City: University of Iowa; 2010.
71. Lamkin DM, Lutgendorf SK, Lubaroff D, et al. Cancer induces inflammation and depressive-like behavior in the mouse: modulation by social housing. Brain Behav Immun. 2011;25:555–64.
72. Lanzenberger R, Wadsak W, Spindelegger C, et al. Cortisol plasma levels in social anxiety disorder patients correlate with serotonin-1A receptor binding in limbic brain regions. Int J Neuropsychopharmacol/[official scientific journal of the Collegium Internationale Neuropsychopharmacologicum (CINP)]. 2010;13:1129–43.
73. Li M, Fitzgerald P, Rodin G. Evidence-based treatment of depression in patients with cancer. J Clin Oncol: (official journal of the American Society of Clinical Oncology). 2012;30:1187–96.
74. Li N, Lee B, Liu RJ, et al. mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. Science. 2010;329:959–64.
75. Li N, Liu RJ, Dwyer JM, et al. Glutamate N-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure. Biol Psychiatry. 2011;69:754–61.
76. Link CJ Jr, Kohn E, Reed E. The relationship between borderline ovarian tumors and epithelial ovarian carcinoma: epidemiologic, pathologic, and molecular aspects. Gynecol Oncol. 1996;60:347–54.
77. Litofsky NS, Farace E, Anderson F, Jr. et al. Depression in patients with high-grade glioma: results of the Glioma Outcomes Project. Neurosurgery. 2004;54:358–366; discussion 366–357.
78. Lloyd-Williams M. Difficulties in diagnosing and treating depression in the terminally ill cancer patient. Postgrad Med J. 2000;76:555–8.
79. Lloyd-Williams M, Payne S, Reeve J, et al. Antidepressant medication in patients with advanced cancer–an observational study. QJM: (monthly journal of the Association of Physicians). 2013;106:995–1001.
80. Lopez JF, Chalmers DT, Little KY, et al. A.E. Bennett Research Award. Regulation of serotonin1A, glucocorticoid, and mineralocorticoid receptor in rat and human hippocampus: implications for the neurobiology of depression. Biol Psychiatry. 1998;43:547–73.
81. Lundstrom S, Furst CJ. Symptoms in advanced cancer: relationship to endogenous cortisol levels. Palliat Med. 2003;17:503–8.
82. Lupien SJ, Mcewen BS, Gunnar MR, et al. Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nat Rev Neurosci. 2009;10:434–45.
83. Lutgendorf SK, Weinrib AZ, Penedo F, et al. Interleukin-6, cortisol, and depressive symptoms in ovarian cancer patients. J Clin Oncol: (official journal of the American Society of Clinical Oncology). 2008;26:4820–7.
84. Maeng S, Zarate CA Jr, Du J, et al. Cellular mechanisms underlying the antidepressant effects of ketamine: role of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. Biol Psychiatry. 2008;63:349–52.
85. Mahar I, Bambico FR, Mechawar N, et al. Stress, serotonin, and hippocampal neurogenesis in relation to depression and antidepressant effects. Neurosci Biobehav Rev. 2014;38:173–92.
86. Mann GE, Yudilevich DL, Sobrevia L. Regulation of amino acid and glucose transporters in endothelial and smooth muscle cells. Physiol Rev. 2003;83:183–252.
87. Martinowich K, Manji H, Lu B. New insights into BDNF function in depression and anxiety. Nat Neurosci. 2007;10:1089–93.
88. Mauri MC, Ferrara A, Boscati L, et al. Plasma and platelet amino acid concentrations in patients affected by major depression and under fluvoxamine treatment. Neuropsychobiology. 1998;37:124–9.
89. Mcentee WJ, Crook TH. Glutamate: its role in learning, memory, and the aging brain. Psychopharmacology. 1993;111:391–401.

90. Mcewen BS. Physiology and neurobiology of stress and adaptation: central role of the brain. Physiol Rev. 2007;87:873–904.
91. Mcewen BS, Chattarji S, Diamond DM, et al. The neurobiological properties of tianeptine (Stablon): from monoamine hypothesis to glutamatergic modulation. Mol Psychiatry. 2010;15:237–49.
92. Meldrum BS. Glutamate as a neurotransmitter in the brain: review of physiology and pathology. J Nutr. 2000;130:1007s–15s.
93. Micallef-Trigona B. Comparing the effects of repetitive transcranial magnetic stimulation and electroconvulsive therapy in the treatment of depression: a systematic review and meta-analysis. Depression Res Treat. 2014;2014:135049.
94. Mitani H, Shirayama Y, Yamada T, et al. Correlation between plasma levels of glutamate, alanine and serine with severity of depression. Prog Neuropsychopharmacol Biol Psychiatry. 2006;30:1155–8.
95. Muller JC, Pryor WW, Gibbons JE, et al. Depression and anxiety occurring during Rauwolfia therapy. J Am Med Assoc. 1955;159:836–9.
96. Munoz M, Covenas R. Involvement of substance P and the NK-1 receptor in human pathology. Amino Acids. 2014;46:1727–50.
97. Murrough JW. Ketamine as a novel antidepressant: from synapse to behavior. Clin Pharmacol Ther. 2012;91:303–9.
98. Musselman DL, Lawson DH, Gumnick JF, et al. Paroxetine for the prevention of depression induced by high-dose interferon alfa. New Eng J Med. 2001;344:961–6.
99. Musselman DL, Miller AH, Porter MR, et al. Higher than normal plasma interleukin-6 concentrations in cancer patients with depression: preliminary findings. Am J Psychiatry. 2001;158:1252–7.
100. Nashed MG, Balenko MD, Singh G. Cancer-induced oxidative stress and pain. Curr Pain Headache Rep. 2014;18:384.
101. Nestler EJ, Barrot M, Dileone RJ, et al. Neurobiology of depression. Neuron. 2002;34:13–25.
102. Nestler EJ, Gould E, Manji H, et al. Preclinical models: status of basic research in depression. Biol Psychiatry. 2002;52:503–28.
103. Nestler EJ, Hyman SE. Animal models of neuropsychiatric disorders. Nat Neurosci. 2010;13:1161–9.
104. Neumeister A, Nugent AC, Waldeck T, et al. Neural and behavioral responses to tryptophan depletion in unmedicated patients with remitted major depressive disorder and controls. Arch Gen Psychiatry. 2004;61:765–73.
105. Ng CG, Boks MP, Zainal NZ, et al. The prevalence and pharmacotherapy of depression in cancer patients. J Affect Disord. 2011;131:1–7.
106. Nibuya M, Nestler EJ, Duman RS. Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. J Neurosci: (the official journal of the Society for Neuroscience). 1996;16:2365–72.
107. O'neill S, Posada-Villa J, Medina-Mora ME, et al. Associations between DSM-IV mental disorders and subsequent self-reported diagnosis of cancer. J Psychosom Res. 2014;76:207–12.
108. Ollenschlager G, Karner J, Karner-Hanusch J, et al. Plasma glutamate–a prognostic marker of cancer and of other immunodeficiency syndromes? Scand J Clin Lab Invest. 1989;49:773–7.
109. Omote K, Kawamata T, Kawamata M, et al. Formalin-induced release of excitatory amino acids in the skin of the rat hindpaw. Brain Res. 1998;787:161–4.
110. Pariante CM, Lightman SL. The HPA axis in major depression: classical theories and new developments. Trends Neurosci. 2008;31:464–8.
111. Parker KJ, Schatzberg AF, Lyons DM. Neuroendocrine aspects of hypercortisolism in major depression. Horm Behav. 2003;43:60–6.
112. Passik SD, Roth AJ. Anxiety symptoms and panic attacks preceding pancreatic cancer diagnosis. Psycho-oncology. 1999;8:268–72.

113. Pittenger C, Duman RS. Stress, depression, and neuroplasticity: a convergence of mechanisms. Neuropsychopharmacology: (official publication of the American College of Neuropsychopharmacology). 2008;33:88–109.
114. Pollak DD, Rey CE, Monje FJ. Rodent models in depression research: classical strategies and new directions. Ann Med. 2010;42:252–64.
115. Pyter LM, Pineros V, Galang JA, et al. Peripheral tumors induce depressive-like behaviors and cytokine production and alter hypothalamic-pituitary-adrenal axis regulation. Proc Natl Acad Sci USA. 2009;106:9069–74.
116. Rasmuson T, Ljungberg B, Grankvist K, et al. Increased serum cortisol levels are associated with high tumour grade in patients with renal cell carcinoma. Acta Oncol. 2001;40:83–7.
117. Reddy BY, Greco SJ, Patel PS, et al. RE-1-silencing transcription factor shows tumor-suppressor functions and negatively regulates the oncogenic TAC1 in breast cancer cells. Proc Natl Acad Sci USA. 2009;106:4408–13.
118. Rivera S, Lopez-Soriano FJ, Azcon-Bieto J, et al. Blood amino acid compartmentation in mice bearing Lewis lung carcinoma. Cancer Res. 1987;47:5644–6.
119. Robson MJ, Elliott M, Seminerio MJ, et al. Evaluation of sigma (sigma) receptors in the antidepressant-like effects of ketamine in vitro and in vivo. Eur Neuropsychopharmacol: (the journal of the European College of Neuropsychopharmacology). 2012;22:308–17.
120. Rodin G, Lloyd N, Katz M, et al. The treatment of depression in cancer patients: a systematic review. Support Care Cancer: (official journal of the Multinational Association of Supportive Care in Cancer). 2007;15:123–36.
121. Rodriguez PL, Jiang S, Fu Y, et al. The proinflammatory peptide substance P promotes blood-brain barrier breaching by breast cancer cells through changes in microvascular endothelial cell tight junctions. Int J Cancer. (Journal international du cancer). 2014;134:1034–44.
122. Rooney A, Grant R. Pharmacological treatment of depression in patients with a primary brain tumour. Cochrane Database Syst Rev. 2013;5:Cd006932.
123. Ruhe HG, Mason NS, Schene AH. Mood is indirectly related to serotonin, norepinephrine and dopamine levels in humans: a meta-analysis of monoamine depletion studies. Mol Psychiatry. 2007;12:331–59.
124. Rush AJ, Fava M, Wisniewski SR, et al. Sequenced treatment alternatives to relieve depression (STAR*D): rationale and design. Control Clin Trials. 2004;25:119–42.
125. Sanacora G, Treccani G, Popoli M. Towards a glutamate hypothesis of depression: an emerging frontier of neuropsychopharmacology for mood disorders. Neuropharmacology. 2012;62:63–77.
126. Savitz J, Lucki I, Drevets WC. 5-HT(1A) receptor function in major depressive disorder. Prog Neurobiol. 2009;88:17–31.
127. Schafer ZT, Brugge JS. IL-6 involvement in epithelial cancers. J Clin Invest. 2007;117:3660–3.
128. Schaur RJ, Fellier H, Gleispach H, et al. Tumor host relations. I. Increased plasma cortisol in tumor-bearing humans compared with patients with benign surgical diseases. J Cancer Res Clin Oncol. 1979;93:281–5.
129. Schaur RJ, Semmelrock HJ, Schauenstein E, et al. Tumor host relations. II. Influence of tumor extent and tumor site on plasma cortisol of patients with malignant diseases. J Cancer Res Clin Oncol. 1979;93:287–92.
130. Seidlitz EP, Sharma MK, Saikali Z, et al. Cancer cell lines release glutamate into the extracellular environment. Clin Exp Metastasis. 2009;26:781–7.
131. Seidlitz EP, Sharma MK, Singh G. A by-product of glutathione production in cancer cells may cause disruption in bone metabolic processes. Can J Physiol Pharmacol. 2010;88:197–203.
132. Seidlitz EP, Sharma MK, Singh G. Extracellular glutamate alters mature osteoclast and osteoblast functions. Can J Physiol Pharmacol. 2010;88:929–36.
133. Sharma MK, Seidlitz EP, Singh G. Cancer cells release glutamate via the cystine/glutamate antiporter. Biochem Biophys Res Commun. 2010;391:91–5.

134. Sheline YI, Gado MH, Kraemer HC. Untreated depression and hippocampal volume loss. Am J Psychiatry. 2003;160:1516–8.
135. Shore PA, Silver SL, Brodie BB. Interaction of reserpine, serotonin, and lysergic acid diethylamide in brain. Science. 1955;122:284–5.
136. Smith QR. Transport of glutamate and other amino acids at the blood-brain barrier. J Nutr. 2000;130:1016s–22s.
137. Somerset W, Stout SC, Miller AH et al. Breast cancer and depression. Oncology. 2004;18:1021–34; discussion 1035–26, 1047–28.
138. Soygur H, Palaoglu O, Akarsu ES, et al. Interleukin-6 levels and HPA axis activation in breast cancer patients with major depressive disorder. Prog Neuropsychopharmacol Biol Psychiatry. 2007;31:1242–7.
139. Starkman MN, Gebarski SS, Berent S, et al. Hippocampal formation volume, memory dysfunction, and cortisol levels in patients with Cushing's syndrome. Biol Psychiatry. 1992;32:756–65.
140. Ungard RG, Seidlitz EP, Singh G. Inhibition of breast cancer-cell glutamate release with sulfasalazine limits cancer-induced bone pain. Pain. 2014;155:28–36.
141. Van Der Pompe G, Antoni MH, Heijnen CJ. Elevated basal cortisol levels and attenuated ACTH and cortisol responses to a behavioral challenge in women with metastatic breast cancer. Psychoneuroendocrinology. 1996;21:361–74.
142. Van Esch L, Roukema JA, Ernst MF, et al. Combined anxiety and depressive symptoms before diagnosis of breast cancer. J Affect Disord. 2012;136:895–901.
143. Warner-Schmidt JL, Duman RS. VEGF is an essential mediator of the neurogenic and behavioral actions of antidepressants. Proc Natl Acad Sci USA. 2007;104:4647–52.
144. Watts S, Leydon G, Birch B, et al. Depression and anxiety in prostate cancer: a systematic review and meta-analysis of prevalence rates. BMJ Open. 2014;4:e003901.
145. Williams S, Dale J. The effectiveness of treatment for depression/depressive symptoms in adults with cancer: a systematic review. Br J Cancer. 2006;94:372–90.
146. Willner P. Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. Psychopharmacology. 1997;134:319–29.
147. Yan HC, Cao X, Das M, et al. Behavioral animal models of depression. Neurosci Bull. 2010;26:327–37.
148. Yang LM, Yu L, Jin HJ, et al. Substance P receptor antagonist in lateral habenula improves rat depression-like behavior. Brain Res Bull. 2014;100:22–8.
149. Yang M, Kim J, Kim JS, et al. Hippocampal dysfunctions in tumor-bearing mice. Brain Behav Immun. 2014;36:147–55.
150. Ye ZC, Sontheimer H. Glioma cells release excitotoxic concentrations of glutamate. Cancer Res. 1999;59:4383–91.
151. Young JJ, Bruno D, Pomara N. A review of the relationship between proinflammatory cytokines and major depressive disorder. J Affect Disord. 2014;169c:15–20.
152. Zarate CA Jr, Brutsche NE, Ibrahim L, et al. Replication of ketamine's antidepressant efficacy in bipolar depression: a randomized controlled add-on trial. Biol Psychiatry. 2012;71:939–46.
153. Zarate CA Jr, Singh JB, Carlson PJ, et al. A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. Arch Gen Psychiatry. 2006;63:856–64.

Chapter 7
Cancer-Induced Pain

Robert G. Ungard, Norman Buckley and Gurmit Singh

Abstract Most commonly, but not exclusively, cancer pain is a result of late-stage metastatic cancers and primary and metastatic cancers that grow in the bone. Cancer pain, like the disease itself, is widely diverse in its quality and extent, and can result from many different causative factors. Many factors have been implicated in the causation and maintenance of cancer pain. Neuropathic pain results from damaged peripheral or central neuronal tissue and from chronically altered neuronal signalling resulting from central and peripheral sensitization. Neuronal tissue can be damaged by direct invasion by tumour cells, as is the case of tumours of the central nervous system (CNS) or by invasion of peripheral neurons in peripheral host tissues. Cancer cells and associated cells also secrete a large number of chemical factors, some of which can directly damage or simulate neurons. Direct physical interaction between the tumour mass and the altered host tissues with neuronal tissue can also cause neuropathic damage through nerve disruption and destruction. Cancer cells and associated cells including stromal and immune cells also secrete a host of chemical signalling molecules that can directly and indirectly stimulate nociceptors. Thermal stimuli of sensory neurons can become pathological following peripheral and central sensitization, which decreases the threshold temperature at which thermally sensitive neurons will respond. Pain is also often a side effect of many treatments of cancer, although the mechanisms of these treatment-induced conditions are beyond the scope of this review. Treatment of cancer pain itself

R.G. Ungard
Department of Pathology and Molecular Medicine, McMaster University, 1200 Main Street West, HSC 4N48, Hamilton, ON L8S 4K1, Canada
e-mail: ungardr@mcmaster.ca

N. Buckley
Department of Anesthesia, McMaster University, 1280 Main Street West, Hamilton, ON L8S 4K1, Canada
e-mail: buckleyn@mcmaster.ca

G. Singh (✉)
Department of Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada
e-mail: singhg@mcmaster.ca

G. Singh (ed.), *Oncodynamics: Effects of Cancer Cells on the Body*,
DOI 10.1007/978-3-319-28558-0_7

largely relies on analgesics and therapies directed against the cancers themselves, although specific treatments for cancer pain are more recently becoming available. It is often the case, however, that cancer pain conditions become intractable, or are poorly controlled. Breakthrough pain which is prevalent in cancer pain is defined by its relationship to treatment where it is an episodic painful event that occurs during a routine of normally effective pain control. Cancer pain is a serious and prevalent oncodynamic effect that arises from a highly variable array of stimuli. The study of cancer pain as a distinct phenomenon is still in its infancy.

Keywords Pain · Cancer-Induced bone pain · Nociception · Neuropathy · Breakthrough pain · Glutamate

Introduction

The ability to sense physiological pain is an essential self-preservational quality of an organism that allows the avoidance of tissue damage and the recognition of damaging pathological states. However, the physiological systems that allow us to perceive pain in a useful manner can also become pathological themselves, either seemingly independently as is the case with some chronic pain conditions, or as the result of an unrelated disease state, such as cancer. The pain produced by cancer can range from mild discomfort to severe, intractable, and self-propagating states of chronic pain.

Some type of cancer-induced pain is estimated to be experienced by 30–50 % of all cancer patients, and by 75–90 % of those with late-stage metastatic cancer [1]. Metastatic cancer-induced bone pain is the most common source of cancer pain reported by patients [2], and has also been the well-studied. Cancer pain can be debilitating and intractable and is a major impediment to the maintenance of quality of life and functional status in cancer patients [3, 4]. And yet, many barriers to the effective management of cancer pain still remain. These include significant sociological and regulatory barriers, but also a deficit of knowledge regarding the mechanisms and control of chronic pain itself, and of cancer pain in particular. It has been recently determined by systematic review that approximately 1/3 of patients undergoing treatment for cancer pain are undertreated, although this number is highly variable globally [5]. This chapter will summarize the molecular mechanisms of cancer-induced pain as an oncodynamic effect of great importance to people living with cancer.

Pain

Pain is defined by the International Association for the Study of Pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage [6]. The human experience of

pain is multifaceted and subjective and difficult to quantitatively study. Mechanistically, pain is subcategorized into three physiological sources; nociceptive, inflammatory and neuropathic pain. In many painful conditions, including many conditions of cancer pain, all three of these pain types will play a contributory role in the overall mechanisms and quality of the experience of pain.

Acute nociceptive pain arises from the stimulation of specialized sensory nerve fibres called nociceptors. This includes the myelinated and rapidly conducting Aβ- and Aδ-fibres, and the unmyelinated, slow-conducting C-fibres. Nociceptors innervate most somatic tissues at differing densities, and exhibit receptors that allow sensitivity to a range of inputs including noxious thermal, mechanical, and chemical stimuli. Most nociceptors in the body remain constitutively inactive until activated with unusual stimuli, as is the case when the distortion of a broken bone stimulates dormant mechanically sensitive nociceptors. The cell bodies of nociceptors that innervate the body lie in the dorsal root ganglia (DRG), lateral to the spinal cord at the vertebral column, or in the trigeminal ganglion for facial nociceptive innervation. The central terminals of nociceptors synapse with second-order neurons in the CNS, usually at the dorsal horn of the spinal cord. Here, these connections are subject to inhibitory, facilitory and other modulatory influence by central descending neurons and by glial cells [7]. Ascending neurons generally pass along the spinothalamic or spinoreticulothalamic tracts to the thalamus and brainstem, and further to the cortex [8]. Multiple brain regions are involved in the perception and processing of pain signalling, including primarily the primary and secondary somatosensory cortex, as well as the insular cortex, anterior cingulate cortex and prefrontal cortex [9]. Nociceptors are widely variable in their structures and functions, including their activating stimuli and thresholds, the extent of their receptive fields, and their speed and frequency of signalling. This heterogeneity allows the sensation of a wide variety and quality of painful sensations at the CNS [10].

Inflammatory pain is pain produced by nociceptors activated by the mediators and molecular products of inflammation. Nociceptors express many receptors for individual products of inflammation, including but not limited to substance P, bradykinin, prostaglandins, adenosine triphosphate (ATP), nerve growth factor (NGF), tumour necrosis factor-α (TNF-α), and protons. These are secreted by the peripheral terminals of nociceptors, and by cells associated with inflammatory states including mast cells, macrophages, and fibroblasts, to the extracellular "inflammatory soup" of pro-inflammatory and algesic signalling molecules that is characteristic of inflammatory sites [11].

Neuropathic pain is pain that arises as a direct consequence of damage or disease affecting the somatosensory system [6]. This can arise from a number of conditions including surgical or traumatic damage, chronic inflammation, and invasive cancer. There is increasing evidence that despite the phenotypic similarities of many conditions of pain, the mechanisms that contribute to the production and maintenance of pain can be significantly divergent. There are peripheral and central mechanistic differences between painful conditions, and between sexes experiencing the same condition that are relevant to treatment [12].

Despite their etiological differences, all pain regardless of the source or any modulation must be transmitted by neuronal cells to the brain in order for perception to occur. This is as true for cancer pain as it is for the pain of any other condition. Also at play, regardless of the source of the pain, is that chronic nociceptive signalling and pathological conditions can produce dramatic reorganization of the structures that transmit and regulate pain signalling. This reorganization includes physiological changes in neurons and glial cells that are associated not only as indicators of a state of chronic pain, but as factors implicit in the maintenance of that pain. Ultimately these pain pathways can transition from acute activation to chronic ongoing activation through the processes of peripheral and central sensitization. Sensitization results in the conditions of hyperalgesia and allodynia, whereby a lower stimulus threshold triggers a nociceptive response and a normally non-nociceptive stimulus becomes painful, respectively. These processes are essential to the physiology of chronic pain conditions, including cancer pain.

Cancer Pain

As befitting such a diverse pathological condition as cancer, pain resulting from cancer can arise from many physical, chemical, and thermal stimuli. Cancer pain can be nociceptive, inflammatory and neuropathic, and is commonly a result of situations such as physical pressure from the tumour itself, damage to or remodelling of tissues in close proximity to the tumour, and peritumoural inflammation. Central and peripheral sensitization renders cancer pain into a chronic condition that can become constant and intractable. Treatments of cancer also often cause pain as a side effect, most notably, chemotherapy-induced peripheral neuropathy (CIPN), and opioid-induced hyperalgesia; however these conditions are not directly oncodynamic, and as such, will not be addressed in this review.

Conditions of cancer pain are defined by the source tissue of the primary cancer, and the host tissue from which the pain emanates. A list of common clinical cancer-associated pain syndromes and their treatment can be found in this review by Portenoy [13]. The quality and intensity of these pain conditions are widely variable, for example the pain emanating from a primary tumour in the breast, if any, presents very differently than the pain of a metastatic breast cancer growing in the spine. One of the challenges of cancer pain management, however, is the inconsistency of the influence of location or tumour type in the generation of pain. One patient's tumour may not cause pain until late stages, whereas a similar tumour in another patient may generate severe pain before the lesion is detectable by other means [14]. This is due to widely differing primary cancers, but also the structures and functions of host tissues in the body, which play a defining role not only in the progression of the invading cancer, but also in the nature and extent of the oncodynamic consequences of that invasion. Despite this, regardless of the host tissue, cancers can cause pain by similar mechanisms. Many cancers secrete a host of algesic chemicals capable of stimulating and sensitizing nociceptors. In innervated

tissue, these chemicals would be expected to be independently capable of nociceptive stimulation, as has been shown to be the case with endothelin-1 (ET-1) which can cause pain following secretion from several different types of cancer cells in multiple tissues [15–18].

Breakthrough cancer pain is a separate condition that is defined by its relationship to pain treatment. It is a transitory exacerbation of pain in excess of the otherwise effective analgesic regimen of the patient [19]. This pain can arise spontaneously or as a result of an action or movement committed by the patient in which case it is labelled as incident pain. The rapid onset and occasional unpredictability of breakthrough pain makes it particularly difficult to control and burdensome for the patient.

Secreted Factors

Many algogenic factors that contribute to cancer pain are secreted from cancer cells and associated stromal cells. Several of these are also mediators of inflammation and inflammatory pain secreted from immune cells recruited to the tumour site. Other classes of secreted factors include neurotrophins, neurotransmitters and cell-signalling molecules including hormones and cytokines. There have been several lines of research focussed on pursuing the importance of particular secreted factors to cancer pain, some of which have shown more potential for treatment than others. It is appearing more evident that targeting a single factor is unlikely to emerge as a valid treatment of cancer pain in isolation. Many secreted factors play complex and intertwined roles in inducing and maintaining cancer pain, and determining their physiological roles and respective importance to cancer pain is an important pursuit.

Nerve Growth Factor

Nerve growth factor (NGF) has recently been found to be an important compound in the development and treatment of multiple pain states including cancer pain, and particularly cancer-induced bone pain. Targeting NGF in cancer pain has accumulated much primary basic and clinical evidence of efficacy, and is emerging as a promising therapeutic avenue. NGF can directly activate nociceptors that bear either the tropomyosin receptor kinase-A (TrkA) receptor or the low-affinity neurotrophin receptor p75. NGF is known to be upregulated in inflammatory pain states, and NGF-TrkA signalling is a mediator of sensitization through action at the spinal cord and DRG [20]. In mouse models of osteosarcoma, NGF promotes the rapid neurogenesis of TrkA positive sensory and sympathetic fibres that eventually reach a pathologic density in the periosteum of tumour-bearing bone [21]. Antibody sequestration of tumour-generated NGF reduces pain and pathological neurogenesis

in animal models of osteosarcoma, prostate cancer, and breast cancer in bone [21–23]. NGF also promotes the development of sensitization through transcriptional upregulation of neuropeptides and ion channels at the DRG in nociceptors, including substance P, calcitonin gene-related peptide (CGRP), and brain-derived neurotrophic factor (BDNF) [21]. BDNF is a neurotrophin that binds the TrkB receptor, and, like NGF, also to p75. The overexpression of BDNF at the spinal cord is likewise involved in the generation of central sensitization in both inflammatory and neuropathic pain states [24]. Microglial production of BDNF is also involved in the development of central sensitization in an animal model of metastatic breast cancer-induced bone pain. Treatment of these animals with a tetracycline inhibitor of microglial activation, minocycline, reduced BDNF at the dorsal horn simultaneously with behavioural evidence of pain [25].

Endothelin-1

Endothelins are vasoactive and nociceptive peptides usually secreted from endothelial cells but also important in the regulation of angiogenesis, bone turnover, and tumour growth. Endothelin-1 (ET-1) can directly stimulate and sensitize nociceptors, and has been found to be secreted by breast and prostate cancer cells [26], fibrosarcoma [15, 16] and oral squamous cell carcinoma [17]. Much research has been focussed on the role of endothelins in cancer pain and they continue to pose a promising, if complex, target for treatment. Inhibition of the endothelin-A receptor (ET_AR) which is expressed by sensory neurons and sensitive to ET-1, has successfully reduced cancer pain in multiple animal models [15–17], however these findings have not yet been validated at clinical trial [27]. Interestingly, inhibition of the endothelin-B receptor (ET_BR) can have the opposing effect of increasing cancer pain in animal models [28].

Acidic Environment

Acidic microenvironments are characteristic of tumours and can directly stimulate nociceptors and induce downstream mediators of pain through several signalling cascades. Acid is a well-characterized mediator of pain. In cancer pain, particularly cancer-induced bone pain, it has been proposed that this acidic microenvironment in bone following tumour growth and osteoclast upregulation may produce sufficient acid to activate the low pH receptors acid-sensing ion channel (ASIC) and transient receptor potential channel-vanilloid subfamily member 1/capsaicin receptors (TRPV1) that are present on nociceptors [29]. In addition, expression of both of these receptors at the DRG is elevated in animal models of cancer-induced bone pain [30, 31], and TRPV1 inhibition has reliably decreased cancer pain in animal models [32].

Glutamate

Many cancer cells secrete the neurotransmitter and cell-signalling amino acid glutamate, including breast, prostate, melanoma and glioma cells. In these cell types, the mechanism of glutamate secretion has been found to be the cystine/glutamate antiporter system x_C^- [33, 34]. Depending on the host tissue or metastatic site, this glutamate release can be a severely a disruptive influence on normal host tissue cell signalling, and can directly activate and sensitize primary afferent nociceptors [35]. In glioma in the CNS, this glutamate release provides a functional advantage to the tumour, promoting malignancy, causing the excitotoxic cell death of neurons, and inducing detrimental oncodynamic side effects including seizures, and possibly headache [33, 36, 37]. In peripheral tissues, glutamate secretion and pain have been investigated in the context of cancer-induced bone pain. Reducing glutamate release from cancer cells by inhibiting the system x_C^- transporter can reduce cancer pain in animal models of breast cancer metastasized to the bone [38]. This outcome may be due to the direct effects of secreted glutamate on the glutamate-sensitive nociceptors in the bone and peritumoural space, or due to differential changes in bone physiology that are susceptible to glutamatergic interference.

There are many other relevant secreted factors to cancer pain. These include but are not limited to: proteases, prostaglandins, bradykinin, TNF-α, interleukins-1 and 6, epidermal growth factor, transforming growth factor-β (TGF-β), and platelet-derived growth factor (Fig. 7.1). These many factors have been detailed in a number of comprehensive reviews [14, 39, 40].

Physical Factors

Visceral pain syndromes often result from physical interference with one or more visceral organs by a tumour mass. Commonly, this pain results from obstructions or distension of the visceral organs due to tumour growth or associated edema, including hepatic distension and intestinal obstructions [13]. The bulk of a growing tumour also poses a risk of physically encountering a sensory neuron that varies with the characteristics and innervation of the host tissue. Physical contact between a tumour and neuron can cause nerve entrapment and injury and induce neuropathic pain states including plexopathies and radiculopathies. In animal models, the leading edge of tumours in bone were found to come into contact, injure and then destroy the distal processes of sensory fibres in conjunction with the development of neuropathic cancer pain states [41]. In addition to stimulating and sensitizing sensory neurons, some of the secreted factors described above, including proteases, can also directly damage neurons, given certain conditions.

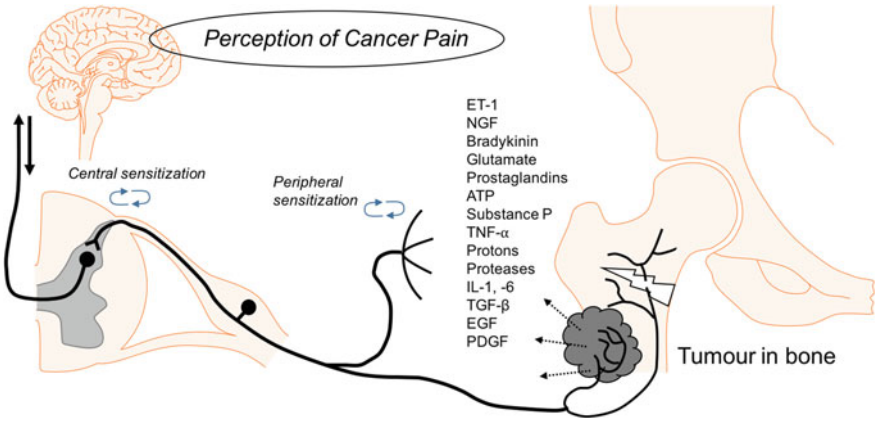


Fig. 7.1 Pain is perceived by transmission through sensory neurons to the central nervous system. Cancer pain is initially stimulated through many mechanisms. This figure illustrates several mechanisms of cancer-induced bone pain, including bone fracture due to weak or degraded bone structures proximal to the tumour, and multiple secreted factors from tumour cells and other cells including immune cells recruited to the tumour site. These secreted factors can modify the tumour itself, the host tissue environment, and can directly stimulate nociceptors. Pain signalling is initiated by sensory neurons in and around in the bone and tumour, and transmitted through the dorsal horn and spinothalamic or spinoreticulothalamic tracts of the spinal cord to the brain. Descending controls from the brain and spinal cord can alter pain signalling and initiate features of central and peripheral sensitization which serve to maintain and amplify pain, leading to intractable chronic cancer pain

Sensitization

Cancer pain, like other enduring pain states, eventually becomes a state of chronic pain through the development of peripheral and central sensitization. Evidence of physiological changes indicative of sensitization in animal models of cancer pain are plentiful, including central sensitization at the dorsal horn [42–45], peripheral sensitization of local primary afferent C nociceptors [15, 46–48], and cellular and neurochemical changes in the DRG neurons and dorsal horn of the spinal cord [41, 45, 49].

Cancer-Induced Bone Pain

Bone pain from cancer is the most common type of cancer pain and despite the transition of several mechanistically targeted therapies into clinical practice, cancer-induced bone pain has remained extremely difficult to manage.

Cancer in bone can be a result of primary cancers of bone tissues and of metastases from distant sites. Bone metastases are extremely disruptive to normal

bone cell metabolism, often resulting in the development of lesions featuring the dysregulated destruction and formation of mineralized bone tissue and the release of pro-inflammatory and algogenic substances into the bone microenvironment. This disruption is responsible for a host of intertwined pathologic consequences including bone fractures and microfractures, spinal cord compression, hypercalcaemia, and severe pain. Cancers of the lung, prostate, kidney, thyroid and breast are the most likely to produce a bone metastasis, with lung, prostate and breast cancer accounting for the vast majority of these cases [50].

Pain in metastatic cancer afflicted bone can arise from a number of stimuli and from any location within the bone. Bones are densely but unevenly innervated with sympathetic and sensory nerve fibres. Aβ-, Aδ- and C-fibres have been identified in the periosteum, as well as throughout mineralized bone and the bone marrow [51, 52]. The densely innervated periosteum is highly sensitive to disruption, however many painful lesions have been found to entirely lack periosteal involvement [1].

Animal models have revealed that cancer-induced bone pain is a unique pain state exhibiting distinct neurochemical and cellular features in the spinal cord and DRG that are not shared with other inflammatory or neuropathic pain states. In particular, changes in the expression of both substance P and CGRP were observed in the dorsal horn of the spinal cord in both inflammatory and neuropathic animal models, but neither neuropeptide was altered in models of bone cancer pain. In addition, bone cancer pain resulted in a much greater increase in glial fibrillary acidic protein (GFAP), a marker of astrocyte proliferation and hypertrophy than other modelled pain states [53].

As discussed above, a number of factors involved in tumour metastasis, growth and lesion formation have the potential to cause pain both directly and indirectly. The confluence of multiple contributing algogenic substances and extensive physical disruption at the tumour site indicate that the mechanisms responsible for cancer-induced bone pain are heterogeneous and complex.

The growing tumour itself contributes to pain generation through pressure on the periosteum or sensory nerves in bone, and through the destruction of sensory neurons. Both osteolytic (net bone resorbing) and osteoblastic (net bone forming) lesions are characterized by weaker bone that is more prone to fracture, compression, and collapse [54]. Microfractures of the bone trabeculae and fractures of the whole bone compress sensory neurons and distort the periosteum, contributing significantly to pain [2].

The mechanisms of pathological bone cell turnover itself have also been linked to cancer-induced bone pain. Osteoblastic lesions commonly arise from prostate cancers and from ~25 % of breast cancers [55]. Their promotion of bone formation in the lesions associated with the metastatic tumour has been associated with the production by the tumour cells of a number of factors that are secreted into the bone microenvironment. The most well-characterized of these many associated factors is the aforementioned ET-1 which is released by typically osteoblastic prostate and breast cancer cell lines, and has been shown to act at ET_AR on osteoblast cells [56]. A number of other tumour associated factors are involved in the promotion of bone volume including osteoprogenetrin (OPG), TGF-β, urokinase, fibroblast growth

factors, and possibly also prostate-specific antigen, all of which are associated with osteoblast cell proliferation [55]. Pathological osteoblast activity associated with bone metastases is not just the overactive production of normal mineralized woven bone or osteons; rather cancerous osteoblastic lesions are typically dysregulated and osteosclerotic tissue that is of poor functional quality and conducive to pain [57].

Many cancers including multiple myeloma and most breast cancer metastases produce primarily osteolytic lesions which extensively degrade mineralized bone and are frequently severely painful. Other conditions including postmenopausal osteoporosis and hormone-ablative therapies in cancer treatment are also associated with pathological osteolysis [58]. Most of the osteolytic degradation associated with metastatic cancer is a result of the pathological activation of osteoclasts by the tumour; however, it has also been demonstrated that tumour cells can directly resorb bone even in the absence of osteoclast cells. Like osteoblastic metastases, osteoclastic bone resorption is stimulated by the tumour through the release of a number of stimulatory factors that upregulate osteoclast proliferation and activity. One released factor, parathyroid hormone related peptide (PTHrP) shares many structural and functional similarities with parathyroid hormone (PTH). At the bone, PTHrP stimulates osteoclast proliferation through osteoblastic production of the receptor activator of nuclear factor-κB ligand (RANKL) [59]. Treatment of animal models of metastatic bone cancer with neutralizing antibodies to PTHrP significantly reduces bone metastasis and resorption [60]. However, PTHrP may have a dual role in bone remodelling, as its expression by prostate cancer cells has conversely been associated with the extent of osteoblastic lesions [61]. Other osteolysis-inducing factors either released directly or induced to be released by tumour cells include macrophage colony-stimulating factor (M-CSF), TGF-β, TNF-α and β, interleukin-1, 6, and 11 [62], and Jagged1 of the Notch signalling pathway [63].

One of the roles of mineralized bone matrix is to act as a reservoir of minerals and growth factors that can be re- released into circulation by osteoclastic bone resorption. Bone resorption in the event of a lytic metastasis results in the pathologic release of these same reserved substances. Ca^{2+} release in this manner is partially responsible for the hypercalcaemia that is characteristic of bone metastases [64], and the release of both mineral and growth factor has been implicated in a positive feedback cycle of tumour growth and bone destruction commonly referred to as the vicious cycle hypothesis. The vicious cycle consists of the release of osteoclast stimulating factors including PTHrP from the metastatic tumour cells which promote osteoclast cells to increase bone resorption, resulting in the release of tumour cell-stimulating cytokines and growth factors from the bone matrix reserves that further stimulate tumour growth and perpetuate the “vicious” cycle. Factors released in this manner from mineralized bone that stimulate tumour cell growth include TGF-β, insulin-like growth factor 1, and Ca^{2+} itself [55].

Bone resorption can also occur independently of osteoclasts through the direct action of cancer cells. This ability has been demonstrated in vitro in several cancer types including breast [65], prostate [66], murine melanoma [67], and giant cell tumour of bone [68]. MMPs secreted from these cancer cells are thought to play a

significant role in this process, particularly MMP-2 and 9 [69], and MMP-13 [68]. Inhibition of MMPs reduced the ability of in vivo human breast cancer cells to degrade bone [69].

Inhibitors of osteoclast activity have reliably been demonstrated to limit bone pain, and the enhancement of resorption has conversely been demonstrated to increase pain, but this could be due to a number of factors [70]. Osteoclastic bone resorption is initiated through the acidification of the resorption compartment of the osteoclast cell at the mineralized bone surface by vacuolar-ATPase H^+ transporters. Due to this process and to the induction of an acidic microenvironment by cancer cells themselves, the extracellular environment of various human tumours becomes progressively acidic as tumours develop [71]. This acidic microenvironment in bone following tumour growth and osteoclast upregulation may produce sufficient acid to activate the ASIC and TRPV1 low pH receptors that are present on nociceptors in bone [29].

Cancer-Induced Bone Pain Treatment

An impediment to the effective treatment of cancer-induced bone pain is that current standard treatments are largely based on principles developed from studies of non-cancer pain [1]. Standard treatment for progressive ongoing pain involves adherence to the World Health Organization (WHO) analgesic ladder following progression from non-opioid analgesics for mild pain through strong opioids in conjunction with non-opioids and adjuvant treatment for moderate to severe pain. Adjuvant treatments in this case are non-analgesics that modify analgesic outcomes. The use of adjuvant treatments in the management of pain is quite common, and standard treatments can include the use of antidepressants or anticonvulsants. In the treatment of cancer-induced bone pain the use of drugs that prevent osteoclastic bone resorption are widely used as adjuvants. Bisphosphonates are a class of antiresorptive compounds with a high affinity to bind Ca^{2+} and therefore to become sequestered in the Ca^{2+} rich bone matrix. When released and absorbed by osteoclasts, bisphosphonates inhibit the enzyme farnesyl diphosphate synthase which then limits the downstream ability of the cell to produce several essential GTP-binding proteins, inducing apoptotic cell death [72]. This limits the extent of osteoclastic resorption in the bone and therefore limits pain from mechanical stress and osteoclast-associated algogenic factors. Bisphosphonate treatment has also been tentatively shown to reduce metastasis to bone and increase survival in breast cancer patients without current bone metastases [73]. These results have fuelled the search for drugs that, like bisphosphonates, inhibit osteoclastic bone resorption. Treatments with OPG, the decoy receptor for RANKL has successfully limited bone pain and tumour growth in animal models [74]. A fully human monoclonal antibody to RANKL, denosumab, has also been developed as a more specific inhibitor of osteoclast activity than bisphosphonates. In multiple phase III clinical trials, denosumab was superior to several bisphosphonates in the prevention of

skeletal-related events including pain in both prostate and breast cancer patients [53]. The inhibition of osteoclasts appears to have several serious side effects that have limited treatment with these drugs. Bisphosphonates are associated with occasional atrial fibrillation, osteomyelitis, and more commonly, osteonecrosis of the jaw of which bisphosphonate treatment is involved in over 90 % of all cases [75]. Standard treatments for cancer in bone can also have an impact on pain including radiotherapy and surgery. Both are applied palliatively with pain control as the primary intention [76]. Recently, a fully humanized monoclonal antibody to NGF, tanezumab has demonstrated clinical efficacy in the treatment of cancer-induced bone pain [77].

Currently, μ-agonist opioids remain the gold standard for the treatment of moderate to severe cancer pain in adherence to the WHO pain ladder. Their efficacy is limited by the occurrence of severe side effects at the doses necessary for adequate analgesia and patient quality of life suffers as a result. Adjuvant treatments are successfully utilized in cancer-induced bone pain management, but reliable pain relief in a manner not independently detrimental to patient quality of life remains elusive.

Current Treatment

The effective management of cancer pain is largely performed in accordance with the principles of the WHO guidelines for cancer pain relief. The core of the guidelines is based upon adherence to the WHO Analgesic Ladder which stipulates a treatment progression from non-opioid analgesics through weak opioids to strong opioids as is necessary to treat progressively worsening pain. Adjuvant drug supplementation and other supplementary interventions including radiotherapy and alternative treatments are applicable throughout as necessary. Adherence to this treatment paradigm has been validated as effective for good or satisfactory pain relief in the majority of cancer patients; however, 24 % of treated patients do not experience complete pain control, with 12 % reporting inadequate pain control [78, 79]. It has also been reported that approximately two-thirds of patients undergoing treatment with opioids experience episodes of breakthrough pain [19]. Episodes of breakthrough pain are treated usually with a “rescue dose” of the patient’s current analgesic, or with a different fast-acting transmucosal μ-opioid agonist [80].

Current analgesic treatment practices are often effective at their priority of reducing the experience of pain for the cancer patient, but that pain relief often comes at the cost of otherwise impairing the patient’s quality of life through treatment side effects. Opioids in particular induce a number of serious dose-limiting side effects including nausea, constipation, vomiting, respiratory depression, sedation, somnolence, and cognitive impairment, and prolonged use can induce the development of physical dependence, tolerance and addiction [81, 82]. Non-steroidal anti-inflammatory drugs (NSAIDs) are most often the first analgesic treatment for cancer pain, and they too are associated with dose-dependent adverse

effects, most predominantly, gastrointestinal and renal side effects [83, 84]. Patient or caregiver concern about treatment-associated side effects or of the consequences of dependence on pain treatment with analgesics can often result in the insufficient control of otherwise manageable pain, as can layers of regulation governing access to controlled pain medications [85, 86]. For these patients who cannot or do not access adequate pain relief, in addition to those patients whose pain cannot be fully controlled with available analgesics, inadequate cancer pain management yet remains a global public health concern.

Conclusion

In conclusion, the oncodynamic effect of cancer pain is a common and severely detrimental consequence for patients living with cancer. As cancer treatments continue to improve, and cancer patients live longer with their disease, strategies of pain control that maintain patient quality of life become ever more valuable, and the understanding and high-quality management of chronic cancer pain becomes a more pressing priority.

References

1. Sabino MAC, Mantyh PW. Pathophysiology of bone cancer pain. J Support Oncol. 2005;3 (1):15–24.
2. Mercadante S. Malignant bone pain: pathophysiology and treatment. Pain. 1997;69(1–2): 1–18.
3. Valeberg BT, Rustøen T, Bjordal K, Hanestad BR, Paul S, Miaskowski C. Self-reported prevalence, etiology, and characteristics of pain in oncology outpatients. Eur J Pain. 2008;12 (5):582–90.
4. Cleeland CS. The impact of pain on the patient with cancer. Cancer. 1984;54(11 Suppl): 2635–41.
5. Greco MT, Roberto A, Corli O, Deandrea S, Bandieri E, Cavuto S, et al. Quality of cancer pain management: an update of a systematic review of undertreatment of patients with cancer. J Clin Oncol. 2014;32(36):4149–54.
6. Loeser JD, Treede R-D. The Kyoto protocol of IASP basic pain terminology. Pain. 2008;137 (3):473–7.
7. Todd AJ. Anatomy of primary afferents and projection neurones in the rat spinal dorsal horn with particular emphasis on substance P and the neurokinin 1 receptor. Exp Physiol. 2002;87 (2):245–9.
8. Basbaum AI, Bautista DM, Scherrer G, Julius D. Cellular and molecular mechanisms of pain. Cell. 2009;139(2):267–84.
9. Borsook D. Neurological diseases and pain. Brain. 2012;135(Pt 2):320–44.
10. Voscopoulos C, Lema M. When does acute pain become chronic? Br J Anaesth. 2010;105 (Supplement 1):i69–85.
11. Gold MS, Gebhart GF. Nociceptor sensitization in pain pathogenesis. Nat Med. 2010;16 (11):1248–57.

12. Sorge RE, Mapplebeck JCS, Rosen S, Beggs S, Taves S, Alexander JK, et al. Different immune cells mediate mechanical pain hypersensitivity in male and female mice. Nat Neurosci. 2015;18(8):1081–3.
13. Portenoy RK. Treatment of cancer pain. Lancet (London, England). 2011;377(9784):2236–47.
14. Mantyh PW, Clohisy DR, Koltzenburg M, Hunt SP. Molecular mechanisms of cancer pain. Nat Rev Cancer. 2002;2(3):201–9.
15. Cain DM, Wacnik PW, Turner M, Wendelschafer-Crabb G, Kennedy WR, Wilcox GL, et al. Functional interactions between tumor and peripheral nerve: changes in excitability and morphology of primary afferent fibers in a murine model of cancer pain. J Neurosci. 2001;21 (23):9367–76.
16. Wacnik PW, Eikmeier LJ, Ruggles TR, Ramnaraine ML, Walcheck BK, Beitz AJ, et al. Functional interactions between tumor and peripheral nerve: morphology, algogen identification, and behavioral characterization of a new murine model of cancer pain. J Neurosci. 2001;21(23):9355–66.
17. Schmidt BL, Pickering V, Liu S, Quang P, Dolan J, Connelly ST, et al. Peripheral endothelin A receptor antagonism attenuates carcinoma-induced pain. Eur J Pain. 2007;11(4):406–14.
18. Yuyama H, Koakutsu A, Fujiyasu N, Fujimori A, Sato S, Shibasaki K, et al. Inhibitory effects of a selective endothelin-A receptor antagonist YM598 on endothelin-1-induced potentiation of nociception in formalin-induced and prostate cancer-induced pain models in mice. J Cardiovasc Pharmacol. 2004;44(Suppl 1):S479–82.
19. Caraceni A, Portenoy RK. An international survey of cancer pain characteristics and syndromes. IASP task force on cancer pain. International association for the study of pain. Pain. 1999;82(3):263–74.
20. Mantyh PW, Koltzenburg M, Mendell LM, Tive L, Shelton DL. Antagonism of nerve growth factor-TrkA signaling and the relief of pain. Anesthesiology. 2011;115(1):189–204.
21. Mantyh WG, Jimenez-Andrade JM, Stake JI, Bloom AP, Kaczmarska MJ, Taylor RN, et al. Blockade of nerve sprouting and neuroma formation markedly attenuates the development of late stage cancer pain. Neuroscience. 2010;171(2):588–98.
22. Bloom AP, Jimenez-Andrade JM, Taylor RN, Castañeda-Corral G, Kaczmarska MJ, Freeman KT, et al. Breast cancer-induced bone remodeling, skeletal pain, and sprouting of sensory nerve fibers. J Pain. 2011;12(6):698–711.
23. Jimenez-Andrade JM, Ghilardi JR, Castañeda-Corral G, Kuskowski MA, Mantyh PW. Preventive or late administration of anti-NGF therapy attenuates tumor-induced nerve sprouting, neuroma formation, and cancer pain. Pain. 2011;152(11):2564–74.
24. Merighi A, Salio C, Ghirri A, Lossi L, Ferrini F, Betelli C, et al. BDNF as a pain modulator. Prog Neurobiol. 2008;85(3):297–317.
25. Wang L-N, Yang J-P, Zhan Y, Ji F-H, Wang X-Y, Zuo J-L, et al. Minocycline-induced reduction of brain-derived neurotrophic factor expression in relation to cancer-induced bone pain in rats. J Neurosci Res. 2012;90(3):672–81.
26. Nelson JB, Hedican SP, George DJ, Reddi AH, Piantadosi S, Eisenberger MA, et al. Identification of endothelin-1 in the pathophysiology of metastatic adenocarcinoma of the prostate. Nat Med. 1995;1(9):944–9.
27. Nelson JB, Love W, Chin JL, Saad F, Schulman CC, Sleep DJ, et al. Phase 3, randomized, controlled trial of atrasentan in patients with nonmetastatic, hormone-refractory prostate cancer. Cancer. 2008;113(9):2478–87.
28. Quang PN, Schmidt BL. Peripheral endothelin B receptor agonist-induced antinociception involves endogenous opioids in mice. Pain. 2010;149(2):254–62.
29. Yoneda T, Hata K, Nakanishi M, Nagae M, Nagayama T, Wakabayashi H, et al. Involvement of acidic microenvironment in the pathophysiology of cancer-associated bone pain. Bone. 2011;48(1):100–5.
30. Pan H-L, Zhang Y-Q, Zhao Z-Q. Involvement of lysophosphatidic acid in bone cancer pain by potentiation of TRPV1 via PKCε pathway in dorsal root ganglion neurons. Mol Pain. 2010;6:85.

31. Qiu F, Wei X, Zhang S, Yuan W, Mi W. Increased expression of acid-sensing ion channel 3 within dorsal root ganglia in a rat model of bone cancer pain. NeuroReport. 2014;25(12):887–93.
32. Ghilardi JR, Röhrich H, Lindsay TH, Sevcik MA, Schwei MJ, Kubota K, et al. Selective blockade of the capsaicin receptor TRPV1 attenuates bone cancer pain. J Neurosci. 2005;25 (12):3126–31.
33. Chung WJ, Lyons SA, Nelson GM, Hamza H, Gladson CL, Gillespie GY, et al. Inhibition of cystine uptake disrupts the growth of primary brain tumors. J Neurosci. 2005;25(31):7101–10.
34. Sharma MK, Seidlitz EP, Singh G. Cancer cells release glutamate via the cystine/glutamate antiporter. Biochem Biophys Res Commun. 2010;391(1):91–5.
35. Cairns BE, Gambarota G, Svensson P, Arendt-Nielsen L, Berde CB. Glutamate-induced sensitization of rat masseter muscle fibers. Neuroscience. 2002;109(2):389–99.
36. Buckingham SC, Campbell SL, Haas BR, Montana V, Robel S, Ogunrinu T, et al. Glutamate release by primary brain tumors induces epileptic activity. Nat Med. 2011;17(10):1269–74.
37. Robert SM, Buckingham SC, Campbell SL, Robel S, Holt KT, Ogunrinu-Babarinde T, et al. SLC7A11 expression is associated with seizures and predicts poor survival in patients with malignant glioma. Sci Transl Med. 2015;7(289):289ra86.
38. Ungard RG, Seidlitz EP, Singh G. Inhibition of breast cancer-cell glutamate release with sulfasalazine limits cancer-induced bone pain. Pain. 2014;155(1):28–36.
39. Jimenez-Andrade JM, Mantyh WG, Bloom AP, Ferng AS, Geffre CP, Mantyh PW. Bone cancer pain. Ann N Y Acad Sci. 2010;1198:173–81.
40. Slosky LM, Largent-Milnes TM, Vanderah TW. Use of animal models in understanding cancer-induced bone pain. Cancer Growth Metastasis. 2015;8(Suppl 1):47–62.
41. Peters CM, Ghilardi JR, Keyser CP, Kubota K, Lindsay TH, Luger NM, et al. Tumor-induced injury of primary afferent sensory nerve fibers in bone cancer pain. Exp Neurol. 2005;193 (1):85–100.
42. Khasabov SG, Hamamoto DT, Harding-Rose C, Simone DA. Tumor-evoked hyperalgesia and sensitization of nociceptive dorsal horn neurons in a murine model of cancer pain. Brain Res. 2007;14(1180):7–19.
43. Urch CE, Donovan-Rodriguez T, Dickenson AH. Alterations in dorsal horn neurones in a rat model of cancer-induced bone pain. Pain. 2003;106(3):347–56.
44. Yanagisawa Y, Furue H, Kawamata T, Uta D, Yamamoto J, Furuse S, et al. Bone cancer induces a unique central sensitization through synaptic changes in a wide area of the spinal cord. Mol Pain. 2010;6:38.
45. Donovan-Rodriguez T, Dickenson AH, Urch CE. Superficial dorsal horn neuronal responses and the emergence of behavioural hyperalgesia in a rat model of cancer-induced bone pain. Neurosci Lett. 2004;360(1–2):29–32.
46. Zhao J, Pan H-L, Li T-T, Zhang Y-Q, Wei J-Y, Zhao Z-Q. The sensitization of peripheral C-fibers to lysophosphatidic acid in bone cancer pain. Life Sci. 2010;87(3–4):120–5.
47. Zheng Q, Fang D, Cai J, Wan Y, Han J-S, Xing G-G. Enhanced excitability of small dorsal root ganglion neurons in rats with bone cancer pain. Mol Pain. 2012;8:24.
48. Hamamoto DT, Khasabov SG, Cain DM, Simone DA. Tumor-evoked sensitization of C nociceptors: a role for endothelin. J Neurophysiol. 2008;100(4):2300–11.
49. Schwei MJ, Honore P, Rogers SD, Salak-Johnson JL, Finke MP, Ramnaraine ML, et al. Neurochemical and cellular reorganization of the spinal cord in a murine model of bone cancer pain. J Neurosci. 1999;19(24):10886–97.
50. Coleman RE. Clinical features of metastatic bone disease and risk of skeletal morbidity. Clin Cancer Res. 2006;12(20 Pt 2):6243s–9s.
51. Mach DB, Rogers SD, Sabino MC, Luger NM, Schwei MJ, Pomonis JD, et al. Origins of skeletal pain: sensory and sympathetic innervation of the mouse femur. Neuroscience. 2002;113(1):155–66.
52. Jimenez-Andrade JM, Mantyh WG, Bloom AP, Xu H, Ferng AS, Dussor G, et al. A phenotypically restricted set of primary afferent nerve fibers innervate the bone versus skin: therapeutic opportunity for treating skeletal pain. Bone. 2010;46(2):306–13.

53. Honore P, Rogers SD, Schwei MJ, Salak-Johnson JL, Luger NM, Sabino MC, et al. Murine models of inflammatory, neuropathic and cancer pain each generates a unique set of neurochemical changes in the spinal cord and sensory neurons. Neuroscience. 2000;98 (3):585–98.
54. Halvorson KG, Sevcik MA, Ghilardi JR, Rosol TJ, Mantyh PW. Similarities and differences in tumor growth, skeletal remodeling and pain in an osteolytic and osteoblastic model of bone cancer. Clin J Pain. 2006;22(7):587–600.
55. Mundy GR. Metastasis to bone: causes, consequences and therapeutic opportunities. Nat Rev Cancer. 2002;2(8):584–93.
56. Yin JJ, Mohammad KS, Käkönen SM, Harris S, Wu-Wong JR, Wessale JL, et al. A causal role for endothelin-1 in the pathogenesis of osteoblastic bone metastases. Proc Natl Acad Sci USA. 2003;100(19):10954–9.
57. Roudier MP, Vesselle H, True LD, Higano CS, Ott SM, King SH, et al. Bone histology at autopsy and matched bone scintigraphy findings in patients with hormone refractory prostate cancer: the effect of bisphosphonate therapy on bone scintigraphy results. Clin Exp Metastasis. 2003;20(2):171–80.
58. Coleman RE. Skeletal complications of malignancy. Cancer. 1997;80(8 Suppl):1588–94.
59. Thomas RJ, Guise TA, Yin JJ, Elliott J, Horwood NJ, Martin TJ, et al. Breast cancer cells interact with osteoblasts to support osteoclast formation. Endocrinology. 1999;140(10): 4451–8.
60. Guise TA, Yin JJ, Taylor SD, Kumagai Y, Dallas M, Boyce BF, et al. Evidence for a causal role of parathyroid hormone-related protein in the pathogenesis of human breast cancer-mediated osteolysis. J Clin Invest. 1996;98(7):1544–9.
61. Liao J, Li X, Koh AJ, Berry JE, Thudi N, Rosol TJ, et al. Tumor expressed PTHrP facilitates prostate cancer-induced osteoblastic lesions. Int J Cancer. 2008;123(10):2267–78.
62. Orr FW, Lee J, Duivenvoorden WC, Singh G. Pathophysiologic interactions in skeletal metastasis. Cancer. 2000;88(12 Suppl):2912–8.
63. Sethi N, Dai X, Winter CG, Kang Y. Tumor-derived JAGGED1 promotes osteolytic bone metastasis of breast cancer by engaging notch signaling in bone cells. Cancer Cell. 2011;19 (2):192–205.
64. Yates AJ, Gutierrez GE, Smolens P, Travis PS, Katz MS, Aufdemorte TB, et al. Effects of a synthetic peptide of a parathyroid hormone-related protein on calcium homeostasis, renal tubular calcium reabsorption, and bone metabolism in vivo and in vitro in rodents. J Clin Invest. 1988;81(3):932–8.
65. Eilon G, Mundy GR. Direct resorption of bone by human breast cancer cells in vitro. Nature. 1978;276(5689):726–8.
66. Sanchez-Sweatman OH, Orr FW, Singh G. Human metastatic prostate PC3 cell lines degrade bone using matrix metalloproteinases. Invasion Metastasis. 1998;18(5–6):297–305.
67. Sanchez-Sweatman OH, Lee J, Orr FW, Singh G. Direct osteolysis induced by metastatic murine melanoma cells: role of matrix metalloproteinases. Eur J Cancer. 1997;33(6):918–25.
68. Mak IWY, Seidlitz EP, Cowan RW, Turcotte RE, Popovic S, Wu WCH, et al. Evidence for the role of matrix metalloproteinase-13 in bone resorption by giant cell tumor of bone. Hum Pathol. 2010;41(9):1320–9.
69. Lee J, Weber M, Mejia S, Bone E, Watson P, Orr W. A matrix metalloproteinase inhibitor, batimastat, retards the development of osteolytic bone metastases by MDA-MB-231 human breast cancer cells in Balb C nu/nu mice. Eur J Cancer. 2001;37(1):106–13.
70. Nagae M, Hiraga T, Wakabayashi H, Wang L, Iwata K, Yoneda T. Osteoclasts play a part in pain due to the inflammation adjacent to bone. Bone. 2006;39(5):1107–15.
71. Gatenby RA. Acid-mediated tumor invasion: a multidisciplinary study. Cancer Res. 2006;66 (10):5216–23.
72. Rodan GA, Martin TJ. Therapeutic approaches to bone diseases. Science. 2000;289 (5484):1508–14.

73. Powles T, Paterson A, McCloskey E, Schein P, Scheffler B, Tidy A, et al. Reduction in bone relapse and improved survival with oral clodronate for adjuvant treatment of operable breast cancer [ISRCTN83688026]. Breast Cancer Res. 2006;8(2):R13.
74. Honore P, Luger NM, Sabino MA, Schwei MJ, Rogers SD, Mach DB, et al. Osteoprotegerin blocks bone cancer-induced skeletal destruction, skeletal pain and pain-related neurochemical reorganization of the spinal cord. Nat Med. 2000;6(5):521–8.
75. Drake MT, Clarke BL, Khosla S. Bisphosphonates: mechanism of action and role in clinical practice. Mayo Clin Proc. 2008;83(9):1032–45.
76. Clines GA, Guise TA. Molecular mechanisms and treatment of bone metastasis. Expert Rev Mol Med. 2008;10:e7.
77. Sopata M, Katz N, Carey W, Smith MD, Keller D, Verburg KM, et al. Efficacy and safety of tanezumab in the treatment of pain from bone metastases. Pain. 2015;156(9):1703–13.
78. Zech DF, Grond S, Lynch J, Hertel D, Lehmann KA. Validation of World Health Organization guidelines for cancer pain relief: a 10-year prospective study. Pain. 1995;63(1):65–76.
79. Mercadante S. Pain treatment and outcomes for patients with advanced cancer who receive follow-up care at home. Cancer. 1999;85(8):1849–58.
80. Casuccio A, Mercadante S, Fulfaro F. Treatment strategies for cancer patients with breakthrough pain. Expert Opin Pharmacother. 2009;10(6):947–53.
81. Benyamin R, Trescot AM, Datta S, Buenaventura R, Adlaka R, Sehgal N, et al. Opioid complications and side effects. Pain Physician. 2008;11(2 Suppl):S105–20.
82. Meuser T, Pietruck C, Radbruch L, Stute P, Lehmann KA, Grond S. Symptoms during cancer pain treatment following WHO-guidelines: a longitudinal follow-up study of symptom prevalence, severity and etiology. Pain. 2001;93(3):247–57.
83. Brater DC. Effects of nonsteroidal anti-inflammatory drugs on renal function: focus on cyclooxygenase-2-selective inhibition. Am J Med. 1999;107(6A):65S–70S; discussion 70S–71S.
84. Wolfe MM, Lichtenstein DR, Singh G. Gastrointestinal toxicity of nonsteroidal antiinflammatory drugs. N Engl J Med. 1999;340(24):1888–99.
85. Cleeland CS, Gonin R, Baez L, Loehrer P, Pandya KJ. Pain and treatment of pain in minority patients with cancer. The eastern cooperative oncology group minority outpatient pain study. Ann Intern Med. 1997;127(9):813–6.
86. Pargeon KL, Hailey BJ. Barriers to effective cancer pain management: a review of the literature. J Pain Symptom Manage. 1999;18(5):358–68.

Chapter 8
Cancer-Induced Fatigue and Cachexia

Yipeng Zhang, Tina Y. Tang, Sureka Pavalagantharajah, Caroline N. Gobran, Zeinab Khawaja, Allison J. Chen and Gurmit Singh

Abstract Cancer-related fatigue (CRF) is a highly prevalent symptom experienced by cancer patients. It is debilitating and has a significant impact on one's physical, mental and social wellbeing. Currently, the ambiguities surrounding CRF have made the standardization of diagnostic and treatment methods difficult. Although there is limited literature on CRF, several hypotheses have been proposed with regard to its underlying mechanisms. These hypotheses include serotonin dysregulation, hypothalamic pituitary adrenal (HPA) axis dysfunction, afferent nerve activation, the basal ganglia hypothesis and muscle wasting. One of the most promising hypotheses is muscle wasting, involving both the degradation of muscle and the inhibition of muscle regeneration. These pathways are initiated through tumour-induced pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α). Following the activation of nuclear factor kappa-light chain enhancer of activated B-cells (NF-κB), its downstream effects further the progression of muscle wasting. This leads to a more aggravated state known as cachexia. As CRF is

Y. Zhang · T.Y. Tang · S. Pavalagantharajah · C.N. Gobran · Z. Khawaja · A.J. Chen
Program in Health Sciences, McMaster University, Michael G. DeGroote Centre for Learning, 1280 Main Street West, MDCL 2102, Hamilton, ON L8S 4K1, Canada
e-mail: zhangyp2@mcmaster.ca

T.Y. Tang
e-mail: tangty3@mcmaster.ca

S. Pavalagantharajah
e-mail: sureka_paval@hotmail.com

C.N. Gobran
e-mail: caroline.gobran@hotmail.com

Z. Khawaja
e-mail: khawaz@mcmaster.ca

A.J. Chen
e-mail: chenaj@mcmaster.ca

G. Singh (✉)
Department of Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada
e-mail: singhg@mcmaster.ca

G. Singh (ed.), *Oncodynamics: Effects of Cancer Cells on the Body*,
DOI 10.1007/978-3-319-28558-0_8

multifaceted, further research into the various hypotheses discussed in this chapter would greatly benefit cancer patients experiencing fatigue.

Keywords Cachexia · TNF-α · NF-κB · Muscle wasting · Muscle regeneration · Central fatigue · Peripheral fatigue · Serotonin · Afferent nerve activation · Basal ganglia

Fatigue

Introduction

Fatigue is one of the most common and under-reported symptoms of cancer. Studies report cancer-related fatigue (CRF) as a persistent and distressing symptom that is prevalent in 60 % to 90 % of cancer patients. However, this symptom is often discounted by physicians [1]. Furthermore, patients report that fatigue has a greater impact on their quality of life in comparison to other cancer-related symptoms. Of patients who experienced fatigue, 91 % reported that it prevented or interfered with normal life functioning and 88 % indicated that fatigue caused an alteration in their daily routine [2].

CRF is defined as a form of fatigue caused by cancer and/or cancer treatments that is unrelated or disproportionate to recent physical or mental exertions and cannot be relieved by rest. CRF is known to be multifactorial, as it is influenced by a combination of psychological and physical factors [3].

CRF can be divided into two categories: peripheral and central fatigue. Central fatigue is associated with problems in the central nervous system. It is characterized by difficulty initiating and sustaining attentional and physical tasks that require motivation and internal cues [4–8]. Due to its effects on motivation and initiation of tasks, the affected physiology is localized to different areas of the brain. Central fatigue hypotheses involve serotonin dysregulation, afferent nerve activation, basal ganglia impairment and alterations to the hypothalamic-pituitary-adrenal (HPA) axis (Fig. 8.1) [1]. The other category, peripheral fatigue, pertains to problems in the neuromuscular junctions and muscle tissues. Peripheral fatigue can limit physical activity due to the consequential inability of peripheral neuromuscular apparatus to respond to central stimuli. Furthermore, with peripheral fatigue, there is little loss of endurance in mental tasks; only physical fatigue is implicated [5, 6]. The current hypotheses involving peripheral fatigue include skeletal muscle wasting and adenosine triphosphate (ATP) depletion (Fig. 8.1) [1]. Cancer-induced fatigue is often caused by a combination of central and peripheral effects.

Symptoms of fatigue can appear before the formal diagnosis of cancer and even continue past cancer treatments [9, 10]. Because of its significant impact on physical, social, and psychological functioning, it has become increasingly important to design and implement effective measures of assessing and controlling fatigue.

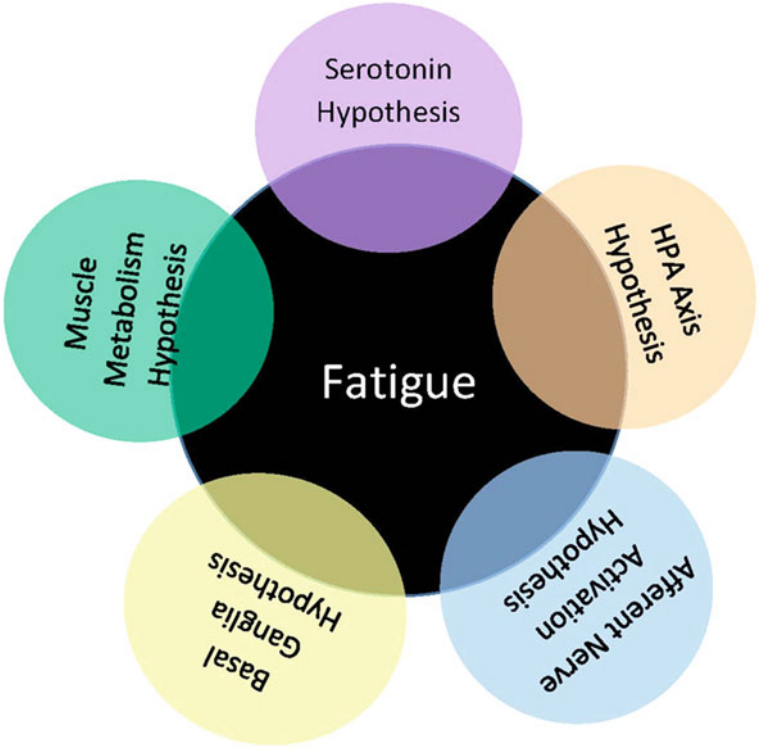


Fig. 8.1 The five main hypotheses to be discussed in this chapter for cancer-induced fatigue

The subjective nature of fatigue has resulted in the development of a variety of different measures to diagnose CRF. Self-reports are typically utilized in clinical trial settings to evaluate a patient's fatigue and the effectiveness of therapeutic interventions [11]. However, there is currently no universally accepted criteria for CRF. One of the greatest challenges in CRF diagnosis is distinguishing the symptom from other psychosomatic and psychological illnesses [11]. For example, measures of fatigue and depression are very strongly correlated, making it difficult to separate CRF from comorbidities. Another challenge arises from the difficulty patients face from reporting the presence and severity of fatigue, or lack thereof.

Tumour microenvironments are complex and involve interactions between several different phenotypes of tumour cells and normal stromal cells, leading to tumour progression and metastasis [11]. The microenvironment of a tumour plays a key role in outlining the possible hypotheses underlying CRF, which are linked by a commonality of proinflammatory cytokines.

Proinflammatory cytokines are known to promote the growth and survival of cancer cells, amongst other functions. They can induce fever, anorexia, cachexia, muscle cramps and severe fatigue. Studies show a correlation between plasma interleukin-1(IL-1), interleukin-6 (IL-6), tumour necrosis factor-α (TNF-α) and CRF, which suggests that proinflammatory cytokines play a role in CRF. Increased

proinflammatory cytokine activity has been found to be linked to several different pathogenic biological processes in CRF, such as rheumatoid arthritis and atherosclerosis [12, 13].

Hypothesis

Serotonin Hypothesis

There is increasing evidence supporting the role of serotonin, or 5-hydroxytryptamine (5-HT), in central fatigue. Serotonin is a neurotransmitter known for its role in mood regulation, sensory perception, sleepiness and appetite, amongst other behavioural and physiological functions [14]. Its actions are mediated by a diverse range of 5-HT receptors, with at least seven known receptor families. Most receptors are G-protein coupled receptors that activate an intracellular secondary messenger cascade. One exception is the 5-HT_3 receptor [15]. The serotonin hypothesis proposes that fatigue is a result of an increase in 5-HT, caused by either increased synthesis or upregulation of the 5-HT receptors [1]. This increase in 5-HT causes decreased motivation and reduced mental capacity to perform tasks, characterizing central fatigue.

The serotonin hypothesis has been proposed based on studies of exercise-induced fatigue. In this context, fatigue is often defined as a failure to continue exercising at a given intensity [16, 17]. 5-HT is unable to cross the blood–brain barrier (BBB) by itself; its synthesis is localized to the brain. Tryptophan, a precursor to serotonin, first crosses the BBB, before being converted to serotonin. During exercise, there is an increased concentration of tryptophan in circulation and branched-chain amino acids (BCAA) are taken up by muscle cells [17, 18]. BCAA and tryptophan share the same BBB transporter and thus compete for entry into the brain. It is hypothesized that due to less circulating BCAA during exercise, more tryptophan is able to enter the brain and be converted into serotonin [19]. This hypothesis is further substantiated by studies examining pharmacological manipulation of serotonin levels. As such, increased 5-HT concentration during prolonged exercise could follow a similar mechanism to the central fatigue experienced by cancer patients.

Studies of patients with chronic fatigue syndrome (CFS) also suggest that changes to 5-HT receptors contribute to fatigue [20]. While the etiology of CFS may not necessarily be identical to those of CRF, much of what is speculated about CRF is based on research done on CFS due to similar symptomatologies. Patients with CFS may have upregulated or hypersensitive postsynaptic 5-HT receptors in the hypothalamus [13]. Current studies often used 5-HT_3 receptor antagonists as a pharmacological treatment for CFS, with marked improvement in the condition of patients [21]. However, there is also evidence for a decreased amount of $5\text{-HT}_{1\alpha}$ receptors or receptor affinity in CFS patients [22]. There have been inconsistent results for the relationship between serotonin levels and central fatigue in CFS.

Proinflammatory cytokines such as TNF-α can also influence 5-HT metabolism. There is a feedback loop between TNF-α and central 5-HT, in which the

synthesized TNF-α increases 5-HT release into the synaptic cleft [23]. Simultaneously, TNF-α works to increase the clearance of 5-HT from the synaptic cleft. This process becomes dysregulated in cancer patients, which may factor into the perception of fatigue [24].

Although the exact relationship between TNF-α and 5-HT has yet to be elucidated, it has been hypothesized that there is an indirect interaction between the TNF-α receptor 1 (TNFR1) and the 5-HT_{2A} receptor. The primary activation of TNFR1 begins with a cascade that activates tumour necrosis factor receptor type 1-associated death domain (TRADD) protein, tumour necrosis factor receptor-associated factor 2 (TRAF2), and receptor-interacting protein 1 (RIP1). Ultimately, nuclear factor kappa-light chain enhancer of activated B-cells (NF-κB) subunits will be released and act as transcription factors to induce the symptoms of fatigue. The 5-HT_{2A} receptor is a G-protein coupled receptor and can stimulate phospholipase C (PLC), a membrane-bound enzyme which assists in the degradation of inositol lipid phosphatidylinositol 4,5-bisphosphate (PIP_2). This will lead to the production of inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG). Further down this pathway, protein kinase C (PKC) can become activated. The 5-HT_{2A} receptor has been found to express mitogenic effects, thus contributing to the metastasis and proliferation of cancerous cells [25]. This all occurs at a proximal step to NF-κB nuclear translocation in the NF-κB pathway, so it is thought that these two pathways may be intertwined [26].

Other findings support the opposite relationship, where fatigue is associated with decreased synaptic levels of 5-HT. Evidence from in vivo studies suggest that proinflammatory cytokines play a role in activating indoleamine 2,3-dioxygenase (IDO), an enzyme that degrades both tryptophan and 5-HT. Furthermore, TNF-α can increase the activity of 5-HT transporters (5-HTT) and increase clearance of 5-HT from the synaptic cleft [27]. Increased activity of both IDO and 5-HTT result in a decreased level of 5-HT [28]. Low synaptic 5-HT levels induce the production of TNF-α to stimulate the release of 5-HT, but because of increased transporter function, the 5-HT is rapidly taken up, resulting in a constant 5-HT deficiency [23]. There have been conflicting findings regarding the relationship between serotonin and central fatigue. This alternative mechanism is also supported by depression symptoms, which are often caused by low 5-HT levels [29]. Fatigue and depression are often associated with one another and cancer patients often experience both. However, selective serotonin reuptake inhibitor (SSRI) treatment is not always capable of reducing fatigue symptoms in cancer patients, suggesting that the mechanism of CRF may also incorporate factors beyond 5-HT levels [30, 31].

The investigation into this hypothesis is currently limited due to the difficulties in accurately measuring 5-HT activity in the brain. In addition, studies have predominantly been conducted on animals and non-cancer patients, making it more difficult to determine whether a clear correlation between 5-HT and CRF exists [32]. Many studies have concluded that it is unlikely that a single neurotransmitter is the sole cause of central fatigue. Since the inception of the 5-HT central fatigue hypothesis, many more proposals for other brain substrates and neurotransmitters have been put forth [17]. The current conflicting research on the relationship

between 5-HT and central fatigue can be attributed to confounding variables such as depression. There is a need for further research in this area.

HPA Axis Hypothesis

The hypothalamic-pituitary-adrenal (HPA) axis involves a system of feedback interactions among neuroendocrine structures located both in the central nervous system as well as the peripheral tissue. The hypothalamus secretes corticotropin-releasing hormone (CRH) in response to stress, which then causes the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland. In turn, ACTH stimulates the adrenal cortex to release cortisol, which negatively feeds back on the axis. Cortisol, a glucocorticoid, also has many effects inside the body, such as regulation of blood pressure, metabolism of carbohydrates and immune function. Dysregulation of the HPA axis is a proposed etiology underlying CRF [1].

Low levels of cortisol and altered HPA axis function have been linked to fatigue in conditions such as Addison's disease and CFS [33]. In addition, low levels of cortisol have been observed in CRF patients. Specifically, it has been found that these patients have significantly lower serum cortisol levels in comparison to non-fatigued patients in the morning, when cortisol levels should peak [34]. Furthermore, studies have also found a flatter diurnal cortisol slope and blunted cortisol response to stress in cancer patients experiencing fatigue [34, 35]. However, the exact mechanism of how low-cortisol levels relate to fatigue has yet to be elucidated.

Although the causal relationship between proinflammatory cytokine-induced microenvironments and alterations in the HPA axis remains unclear, a few possibilities have been proposed. It has been shown in animal models that chronic inflammation decreases the synthesis and release of CRH, ultimately resulting in a decrease in cortisol levels [36, 37]. It has also been observed that 5-HT can stimulate the HPA axis and regulate the release of cortisol at multiple levels such as inducing the release of CRH from the hypothalamus [1, 38, 39]. There is a dysregulation of 5-HT in proinflammatory environments, which can also impact cortisol levels in the body.

Cortisol has a prominent role in the metabolism of glucose, free fatty acids and amino acids. It redirects the source of energy to fatty and amino acid catabolism and away from glucose catabolism. However, the brain is incapable of utilizing free fatty acids and amino acids as a source of energy and solely relies on blood glucose. Cortisol acts to increase blood glucose levels by activating key enzymes involved in gluconeogenesis. A deficiency in cortisol could lead to decreased blood glucose concentrations, limiting the energy source for neurons, which could contribute to fatigue [40, 41].

Glucocorticoids also increase blood pressure through actions involving vasculature and the kidneys. With a cortisol deficiency, blood pressure could drop, resulting in insufficient blood supply to parts of the body. This leads to symptoms, such as light-headedness, which could be perceived as fatigue. Glucocorticoids, like cortisol, increase glomerular filtration rate, proximal tubular epithelial sodium

transport, and free water clearance [42]. It has also been found that cortisol inhibits the effects of vasopressin, also known as antidiuretic hormone [43, 44]. Cortisol deficiency could therefore lead to dilutional hyponatremia, where fatigue presents as a symptom. Unfortunately, there is a limited knowledge on the topic of cortisol deficiency due to a lack of research in this field.

Cortisol suppresses the production of proinflammatory cytokines [45]. It has been proposed that the HPA axis can regulate proinflammatory cytokine production directly, through varying the levels of glucocorticoids produced, or indirectly, through regulating glucocorticoid receptor sensitivity to ligation [46, 47]. The deficits in cortisol observed in CRF patients reduce the suppressive effects of cortisol, allowing cytokine levels to increase. Moreover, it has been shown that there is a downregulation of glucocorticoid responsive genes in the leukocytes of cancer survivors experiencing fatigue, suggesting functional glucocorticoid receptor resistance [46, 48]. The deficits in these two pathways allow for an increase in proinflammatory activity which ultimately leads to fatigue symptoms.

Afferent Nerve Activation Hypothesis

The vagus nerve is the longest of the parasympathetic nerves and is composed of both efferent and afferent fibres. The afferent fibres specifically communicate messages from the viscera to the brain stem [1]. It has been hypothesized that the release of neuroactive agents, such as prostaglandins, cytokines, and serotonin, can activate vagal afferent nerves [1, 51–54]. The induced activation then causes fatigue by stimulating “rest and digest” effects by suppressing somatic muscle activity, or by inducing “sickness behaviour”, which is an organized response strategy to fight infection and inflammation [1, 55].

Furthermore, studies have shown that vagal activation acts to inhibit the somatomotor reflex in animal models [1, 56–62]. It has been suggested that the pulmonary afferent nerve acts to limit exercise when pulmonary congestion is present [1, 60, 63, 64]. Overall, vagal afferent activation results in a decrease in skeletal muscle tone, which causes a feeling that a greater effort is needed to complete a task [1, 65]. However, studies thus far have all been conducted on animal models and the existence of a vagosomatic inhibitory reflex has yet to be confirmed in humans [1]. This area of study would benefit greatly with further research.

Basal Ganglia Hypothesis

There has been growing evidence supporting the involvement of dopamine in central fatigue. A disruption in the dopaminergic system, particularly circuitry in the basal ganglia, can cause the loss of motivation and lack of internal representation of tasks [5].

The basal ganglia receive input from the cerebral cortex and project to the motor cortex via the thalamus, the sensory relay and filtering centre, allowing the basal

ganglia to initiate movement [51]. The basal ganglia are also connected with the neocortex by the motor loop and the association loop. The association loop connects the caudate with cortical association inputs and the basal ganglia with final prefrontal cortex outputs [5, 66]. An interruption of the association loop would suppress cortical activation. An interruption of the basal ganglia would reduce dopamine concentrations, suppressing frontal lobe activation. Both interruptions would result in a loss of motivational influence, ultimately contributing to central fatigue [5].

Additionally, the basal ganglia have been divided into functional categories: the neurologist, psychologist and psychiatrist ganglia. The neurologist ganglia contain putamen-based motor function [5, 67]. The psychologist ganglia contain the caudate-dorsolateral prefrontal circuit responsible for initiating and terminating emotion and cognitive processes, such as attention, memory and planning [5, 51, 67–69]. This is the circuit involved in the dopaminergic loss seen in Parkinson's disease [5, 70]. The psychiatrist ganglia contain the ventral striatopallidal system with the nucleus accumbens that is responsible for behaviour. This portion of the basal ganglia is richly innervated by dopaminergic inputs and converging inputs from the orbitofrontal cortex. These are responsible for motivation and reinforcement, and affective or environmental associations respectively. The ventral striatopallidal system has global regulatory influences on dopaminergic neurotransmission, which affects reward and self-stimulatory behaviour [5, 67]. An interruption of this system would greatly affect the motivational aspects of behaviour and action [5].

The caudate contributes to the determination of oculomotor output, connecting motivational cues with external visual information [5, 71]. The disruption of the caudate-dorsolateral prefrontal circuit at the striatum level has also been shown to impede task execution, causing the lack of an internal representation and cue of tasks. Moreover, the basal ganglia are capable of concurrent processing of motor, cognitive and limbic functions. Initiation of tasks that require internal cues are integrated in the basal ganglia, preparing emotive, moto, and sensory apparatus for subsequent responses [5]. Hence, disruption of the basal ganglia would delay the initiation of performance and prevent the execution of tasks, categorizing central fatigue.

Similar to other CRF hypotheses, the basal ganglia and dopamine mechanisms are affected by chronic inflammation and exposure to proinflammatory cytokines. These alterations contribute to symptoms of fatigue, psychomotor delays and sleep disturbances [72–77]. Specifically, proinflammatory cytokines, such as IFN-α, IL-1 and TNF-α, act to decrease dopamine metabolites in the cerebrospinal fluid and increase presynaptic dopamine reuptake, interrupting reward and self-stimulatory behaviour to ultimately affect the initiation and execution of tasks [78–82].

Muscle Wasting Hypothesis

Muscles are composed of myofibrils, which are compartmentalized into basic contractile units called sarcomeres. Actin and myosin are the two contractile proteins found in sarcomeres, and they allow muscles to contract through cross-bridge

formation [60]. Eukaryotic cells contain regulatory proteins involved in normal muscle degradation. Ubiquitin marks myofibrillar proteins dissociated by calpains for degradation by the 26S proteasome [83–85].

The muscle wasting hypothesis revolves around a decrease in the maximum contractile force of muscle fibres. This can occur through both the degradation of myofilaments, as well as the inhibition of muscle regeneration. The TNF-α in the cancer-induced proinflammatory microenvironment leads to NF-κB synthesis, which enhances the transcription of the aforementioned regulatory proteins [83, 86]. This all leads to increased degradation of myofibrillar proteins. TNF-α also stimulates the expression of myostatin, a protein which inhibits myogenic regulatory factors involved in muscle regeneration, such as MyoD and myogenin. With less contractile proteins and a decreased ability to regenerate these proteins, the force of muscle contraction will decrease, causing peripheral fatigue.

Cachexia

The muscle wasting hypothesis holds great potential in explaining CRF. The muscle wasting hypothesis pertains specifically to peripheral fatigue. Body composition analyses have shown that skeletal muscle (Fig. 8.2) is the major site of protein loss in patients with solid non-haematological tumours [87–89].

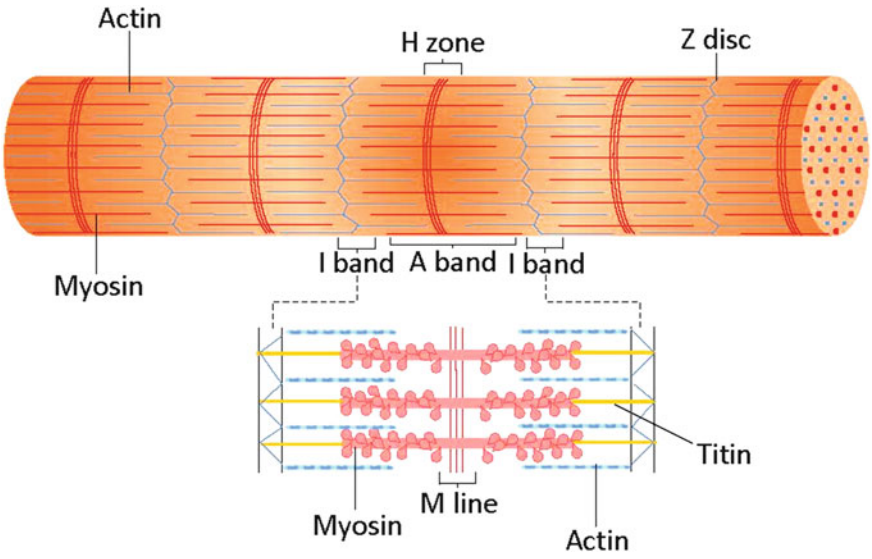


Fig. 8.2 Sarcomeres are the rudimentary contractile units of myofibres. Z disks directly anchor actin and indirectly anchor myosin through titin and nebulin

There are two factors that contribute to muscle wasting. The first involves the degradation of muscles, which can further develop into a state of cachexia. The second involves inhibition of muscle regeneration. The cancerous microenvironment can increase the translation of proteins that work to inhibit muscle regeneration, thereby exacerbating fatigue.

Degradation Pathways

Muscles can be degraded through either the lysosomal pathway or the ubiquitin-proteasome pathway. The lysosomal pathway involves lysosomal enzymes, such as cathepsin-D and β-glucuronidase, both of which are involved in programmed cell death. A positive correlation has been found between muscle wasting and increased activity of these two enzymes in skeletal muscle tissues of patients with malignant tumours. Cathepsin-D has been shown to be involved in cytokine-induced programmed cell death of muscle cells by acting as a carboxyl proteinase to break down intracellular proteins [90, 91].

Another pathway for muscle degradation is the proteolytic ubiquitin-proteasome pathway, which has shown to be more important in the proteolysis of myofibres than the lysosomal pathway. Ubiquitin is a small protein cofactor that marks a protein for degradation. A chain of five or more ubiquitin molecules links to a protein substrate, effectively marking it. This marked protein is then recognized and rapidly degraded by a large proteolytic complex known as the 26S proteasome. This degradation requires energy from ATP. IL and TNF from activated macrophages and endothelial cells are primarily involved in the activation of this pathway, and their release results in increased ubiquitin mRNA transcription [92–95].

The ubiquitin-proteasome pathway is initiated when cancer cells induce a proinflammatory microenvironment, causing the release of TNF-α [96–98]. Primarily, the production of TNF-α by monocytes and lymphocytes is induced by structural elements on microbial pathogens that bind to toll-like receptors (TLRs). TLRs transcriptionally induce proinflammatory cytokines through an NF-κB signalling pathway [99–101].

TNF-α mediates its effects through two transmembrane receptors: TNF receptor 1 (TNF-R1) and TNF receptor 2 (TNF-R2). TNF-R1 is universally expressed on all cells and has a broader role than TNF-R2 in NF-κB activation. TNF-R2 is only expressed on endothelial and immune cells and is shown to mediate signals that promote tissue repair and angiogenesis. A significant difference between TNF-R1 and TNF-R2 is the presence of a death domain on TNF-R1. Thus, TNF-R1 requires the binding of a TNF-associated death domain (TRADD) protein before it can bind TNF receptor associated factor 1 (TRAF1) and TNF receptor associated factor 2 (TRAF2). Compared to the role of TNF-R2, the role of TNF-R1 is more understood in the NF-κB pathway [99–101].

TNF-α binds to TNF-R1, causing TRADD to bind to the death domain. TRADD then recruits TRAF2 and receptor interacting protein1 (RIP1) [83, 99–102]. TRAF2

recruits the kinase of inhibitor of NF-κB complex (IKK), which phosphorylates the inhibitor of NF-κB (IκB) complex. IκB is then polyubiquitinated and subsequently degraded by the 26S proteasome causing the release and nuclear translocation of NF-κB [83, 102].

Upon arrival at its binding sites, NF-κB increases the production of inducible nitrogen monoxide synthase (iNOS) mRNA, which increases cytosolic levels of nitrogen monoxide and reactive nitrogen species (RNS) [83, 102]. RNS causes an increased concentration of hypoxia inducible factor-1α (HIF-1α) through two different mechanisms. The first mechanism is S-nitrosation, an important post-translational protein modification that regulates protein function and cell signalling. HIF-1α has a Cys-800 residue with a reactive thiol group, which is needed for the recruitment of a p300 co-activator. This co-activator is then required for HIF-1α complex transcriptional activity. The HIF-1 heterodimer, composed of HIF-1α and HIF-1β, binds to the hypoxia-response element on the HIF-1α promoter to increase transcription. The second mechanism through which RNS increases HIF-1α is the prevention of HIF-1α degradation. Normally, when HIF-1α is undergoing oxygen dependent hydroxylation of the proline residues, it binds to the Von-Hippel-Lindau tumour suppressor protein (pVHL). The binding of HIF-1α to pVHL tags HIF-1α for ubiquitination and its subsequent degradation. However, RNS inhibits the activity of prolyl hydroxylase domain enzymes (PHDs), which prevents the hydroxylation of the proline residues, a process necessary for pVHL binding [103–105].

The resulting increased levels of HIF-1α propagate hypoxic signals, producing reactive oxygen species (ROS), which then causes endoplasmic reticulum (ER) stress [106–108]. ER stress initiates the unfolded protein response, a homeostatic signalling network that orchestrates the recovery of ER function. It is mediated by the activation of stress sensors such as the protein kinase-like endoplasmic reticulum kinase (PERK). These stress sensors transmit information about protein folding status in the ER to the nucleus and cytosol, buffering fluctuations in unfolded protein load and restoring protein folding capacity. Normally, the chaperone protein BiP binds to the N-termini of PERK, preventing its activation. However, ER stress allows BiP to release PERK, thereby activating it [109–111].

After its activation, PERK phosphorylates and inactivates a protein called the eukaryotic translation initiator factor-2α (eIF-2α), which plays a key role in regulating mRNA translation. The inactivation of eIF-2α causes a global shutdown of mRNA translation, reducing the protein load on the ER, thereby lowering ER stress. However, during this process, certain mRNA gain selective advantage for translation [109, 112, 113].

The mechanism regulating activating transcription factor-4 (ATF4) expression involves the differential contribution of two different upstream open reading frames (uORFs), shown in Fig. 8.3. The first uORF, uORF1, is a positive-acting element that facilitates ribosomal scanning and reinitiation at downstream coding regions in the *ATF4* mRNA. When eukaryotic initiation factor 2 (eIF2) is active, there is an abundance of eIF2 bound to the energy carrier guanosine triphosphate (eIF2-GTP) in the non-stressed cells. The eIF2-GTP binds to the start tRNA, allowing its

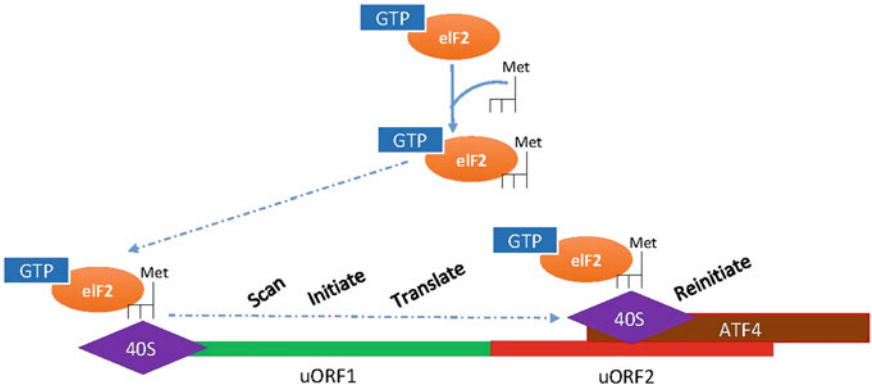


Fig. 8.3 The eIF-ATF4 mechanism of action

association with a ribosome. This causes the ribosome to scan downstream of uORF1 and reinitiate at the next coding region, uORF2. uORF2 is an inhibitory element that blocks the translation of ATF4. However, during ER stress, the phosphorylation of eIF2 is accompanied by a reduction in eIF2-GTP levels. The eIF2-GTP still binds to a start tRNA, allowing its association with a ribosome, but the reduced amount of eIF2-GTP present due to phosphorylation of eIF-2α increases the time required for the scanning ribosomes to reinitiate downstream. This delay in reinitiation allows for the ribosome to reinitiate at the ATF4-coding region allowing for the translation of ATF4 and bypassing the inhibitory effects of uORF2 [109, 112, 113].

ATF4 then activates the *CCAAT-enhancer binding protein homologous protein (CHOP)* promoter, allowing the initiation of the production of CHOP mRNA, which will then be translated into the protein. CHOP is a transcription factor, and one of its direct gene targets is the *ER oxidoreductin-1α (ERO-1α)* gene, which codes for the ERO-1α protein. ERO-1α is an enzyme that catalyzes the formation and isomerization of disulfide bonds in the ER. One of the primary targets of ERO-1α is the protein-disulfide isomerase (PDI) family. Within the PDI family, the endoplasmic reticulum protein-44 (ERp44) has a high affinity for and preferentially interacts with ERO-1α. ERp44 binds onto a lumenal domain of inositol-1,4,5-triphosphate receptor 1 (IP3R1), inhibiting the receptor. The dissociation of ERp44 from IP3R1, caused by ERO-1α, activates IP3R1. The binding of IP3 to IP3R1 opens the receptor channel, allowing an intracellular increase in calcium levels. The calcium then binds to calcium-binding domains on calpains, which are proteolytic enzymes, resulting in their activation [114–118]. Increased calcium levels induce calpain activity, which initiates digestion of myofibrillar proteins like titin and nebulin, and leads to myofilament dissociation and sarcomere disassembly [84, 118–121].

The free actin and myosin then undergo ubiquitination. This process involves four enzymes: ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme

(E2), ubiquitin ligase (E3), and ubiquitin chain assembly factors (E4). When a protein is ubiquitinated, it can either be mono-ubiquitinated or poly-ubiquitinated. The number of ubiquitin molecules and the lysine residue that is ubiquitinated determines whether a protein is degraded. The ubiquitination of lysine-29 and −48 signal for degradation. A chain of at least five ubiquitin molecules is required to mark myofibrillar proteins for degradation. Ubiquitin is activated by E1. It is then transferred to E2, which carries ubiquitin until E3 transfers the ubiquitin to a lysine residue on the substrate protein. E4 promotes the polymerization of polyubiquitin chains. There are over one thousand types of E3 enzymes, allowing for high specificity. The E3 enzyme implicated in muscle degradation is the muscle RING finger-1 (MuRF-1) protein. The exact function of MuRF-1 is still under investigation, but it is thought to aid in the ubiquitination of myosin heavy chains. It has been found that an increase in NF-κB leads to an increase in MuRF-1. Another E3 that is involved in muscle atrophy is the muscle atrophy F-box (MAFbx). MAFbx becomes involved later in the process of muscle wasting as it works to inhibit muscle regeneration. Ubiquitination by MuRF-1 can then signal for the degradation of actin and myosin by the 26S proteasome [118, 120, 122–125].

These ATPases bind to the proteins to be degraded and use ATP hydrolysis to unfold and translocate the protein into the 20S particle in a process known as linearization. Linearization of the folded protein is essential for it to be translocated through the gated entry channel into the 20S particle. Even in its open state, the pore is too narrow for globular proteins. The ATPases also act as a "key in a lock" to cause the opening of the gated substrate entry channel of the 20S outer ring. The 20S particle is a hollow cylinder with four hollow rings. There are two identical outer α-rings and two identical inner β-rings, each containing seven distinct but related subunits. Three of the subunits in the β-rings contain the proteolytic active sites that are positioned on the interior face of the cylinder. The three main enzymes at these active sites are the chymotrypsin-like, trypsin-like, and peptidyl-glutamyl peptide hydrolase (PGPH) enzymes. They all cleave peptide bonds in different locations. Initially, the chymotrypsin-like enzyme cleaves peptide bonds formed between aromatic residues, such as tyrosine and tryptophan. This generates fragments that are further cleaved by the other active sites in the core. The PGPH cleaves peptide bonds that occur immediately after acidic residues or BCAAs. Finally, the trypsin-like enzyme cleaves peptide bonds that follow basic residues. The 26S proteasome can only break down the myofilaments if they are free, meaning that the calpains must first release them from the sarcomeres [102, 112, 118, 126]. Sarcomere component degradation leads to decreased force-generating capacity, characterizing fatigue [121].

Inhibition of Regeneration

Myostatin has been found to play an important role in skeletal muscle wasting by increasing protein degradation and decreasing muscle synthesis. Myostatin is an

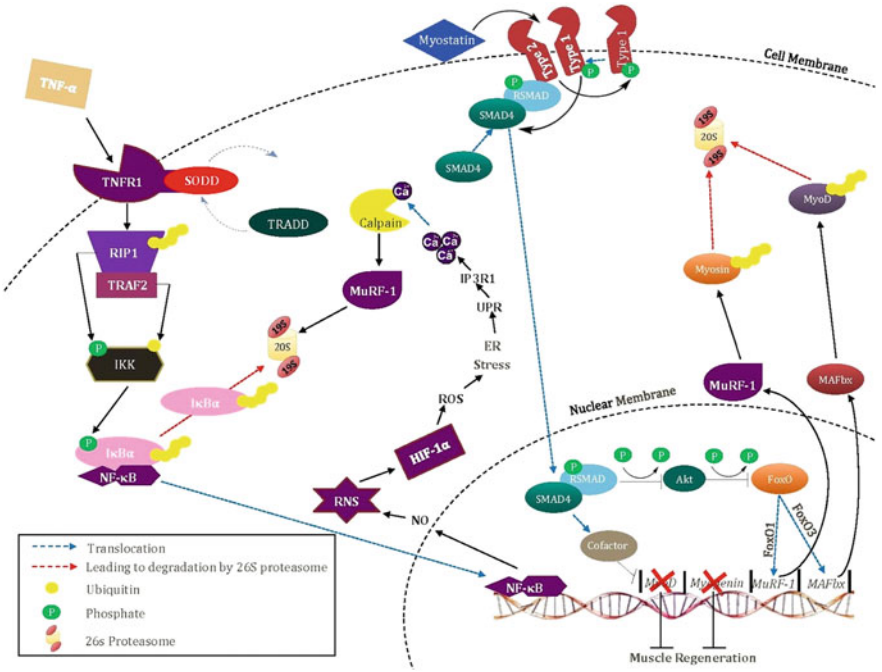


Fig. 8.4 The hypothesized cellular signalling pathways for cancer-induced skeletal muscle wasting causing fatigue

inhibitor of myogenesis, a process regulating proliferation and differentiation of myoblasts [127, 128]. The inhibition of muscle regeneration begins with increased levels of TNF-α in the cancer microenvironment, resulting in increasing levels of myostatin. Myostatin then binds to its type II receptor, which then transphosphorylates and activates the type I receptor by forming a heterotetrameric receptor complex (Fig. 8.4). The activated complex then phosphorylates receptor bound SMADs or R-SMADs. The phosphorylation of the R-SMADs allows them to oligomerize with SMAD4. The R-SMAD and SMAD4 complex then translocates into the nucleus and interacts with cofactors to regulate transcription [129]. This interaction subsequently causes the upregulation and increased phosphorylation of SMAD2 and 3. The increased activity of these SMADs inhibits protein kinase B (Akt), which results in the activation of forkhead box (FoxO). Additionally, activation of SMAD can inhibit the transcription of both myoD and myogenin [127, 128].

FoxO usually binds to the DNA, but when it is phosphorylated by active Akt, its binding site for the 14-3-3 regulatory protein is exposed. This process masks the nuclear localization signal, causing FoxO to exit the nucleus without binding to DNA. Therefore, when Akt is inhibited, FoxO is not phosphorylated and the 14-3-3 binding site is not exposed. This allows FoxO to bind to DNA and produce its downstream effects [130].

FoxO is a transcription factor that binds to promoters to upregulate transcription. FoxO1 binds to the FoxO binding element on the MuRF-1 gene, while FoxO3 binds to the *MAFbx* gene promoter. Both of these genes code for their respective ubiquitin ligase enzymes. MAFbx ubiquitinates MyoD, signalling for its degradation by the 26S proteasome. This results in decreased muscle synthesis. Ultimately, fatigue is aggravated as muscle fibres cannot be adequately produced to replace those that are degraded [127, 128].

Other Cytokines

Like TNF-α, IL-1 and IL-6 are also largely implicated in the inflammatory response and have been linked to breast cancer progression [131]. There are three IL-1 signalling pathways leading to NF-κB activation: the transforming growth factor β-activated kinase 1 (TAK1)-dependent pathway, the TAK-1-independent/protein kinase C (PKC)-dependent pathway, and the TAK-1-independent/mitogen-activated protein kinase kinase kinase 3 (MEKK-3) dependent pathway. IL-6 can influence muscle atrophy and fatigue through the Janus kinase-signal transducers and activators of transcription (JAK-STAT) signalling pathway.

IL-1 first binds to its receptor, interleukin 1 receptor type I (IL-1RI), as seen in Fig. 8.5. This binding allows for the association of IL-1RI with IL-1 receptor accessory protein, forming a signalling receptor complex [132, 133]. The complex allows for the recruitment of myeloid differentiation primary response gene 88 (MyD88) [134]. The recruitment causes the translocation of complex IL-1 receptor-associated kinase (IRAK-1) and the adaptor protein Tollip into the IL-1RI complex [135, 136]. Structurally, IRAK-1 contains an N-terminal death domain, a proline, serine, and threonine-residue (ProST) rich region, a serine/threonine-specific protein kinase domain, and a C-terminal domain with three TNF receptor-associated factor 6 (TRAF6) consensus motifs [137–139]. TRAF6 and IRAK-4, an IRAK-1 related kinase, are recruited following the activation of the receptor complex, forming complex I [140–142]. IRAK-4 then undergoes autophosphorylation, allowing for its kinase activity [108]. Once IRAK-4 is activated, IRAK-1 is phosphorylated in the ProST region, resulting in dissociation from MyD88 and Tollip. At this point, it is still associated with TRAF6 [138, 140]. The dissociated IRAK1-TRAF6 complex interacts with TAK1-binding protein (TAB1), TAB2 or TAB3 membrane complex to form complex II [58, 143].

TRAF6-TAK1-TAB1-TAB2/3 complex translocates to the cytosol, where IRAK-1 becomes polyubiquitinated and phosphorylated [143, 144]. In the cytoplasm, TRAF6 interacts with an E2 complex, forming complex III [145]. The E2 complex interacts with the RING finger domain of TRAF6, causing polyubiquitination of TRAF6, binding of TAB2, and activation of TAK1 [137, 145]. This is necessary for IKK activation [146]. The activation of TAK1 phosphorylates IKKβ, which phosphorylates IκBα, and triggers proteasome-dependent degradation [137]. NF-κB is then able to translocate to the nucleus to initiate transcription.

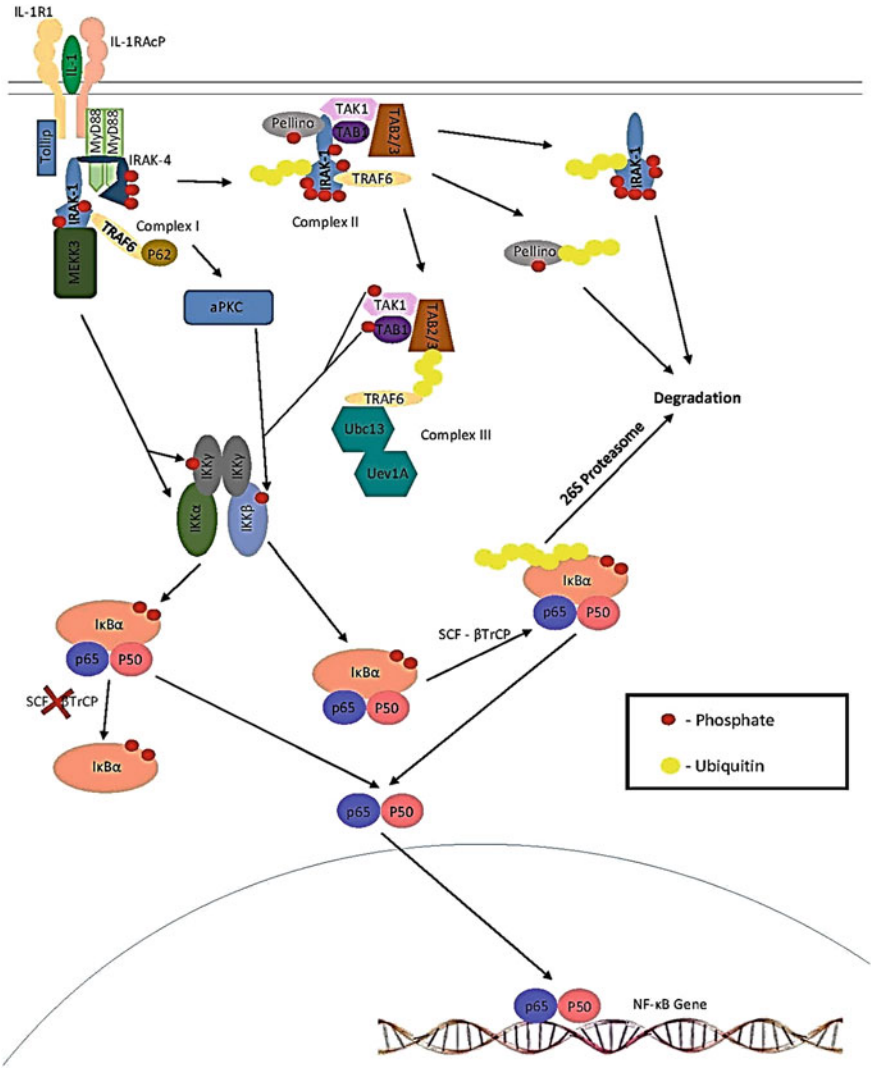


Fig. 8.5 The cellular signalling pathways of IL-1

There are also possible routes within that pathway that are independent of TAK-1, such as the TAK-1-independent/PKC-dependent pathway. After IL-1 is stimulated, TRAF6 also interacts with p62, an atypical PKC-interacting protein, causing PKC activation and phosphorylation of IKKβ [147]. As in the TAK-1 dependent pathway, IKKβ subsequently phosphorylates IκBα, leading to NF-κB activation.

Another pathway leading to NF-κB activation is the TAK-1-independent/MEKK-3-dependent pathway that involves IKKγ phosphorylation and IKKα

activation. This pathway was determined as IL-1-induced IκBα phosphorylation, which leads to NF-κB translocation, and is only completely terminated when both TAK1 and MEKK3 are impaired [148]. After IL-1 is stimulated, IRAK-1 and TRAF6 also interact with MEKK-3, activating IKKα. IKKα then phosphorylates IκBα and the phosphorylated IκBα dissociates away from NF-κB, allowing for its nuclear translocation and binding onto the responsive genes [144].

IL-6 exerts its effects through a JAK-STAT pathway, leading to muscle atrophy and reduced maximum force-generating capacity [149]. Many studies have pointed at the role of IL-6 in causing muscle wasting through an imbalanced growth factor-related signalling pathway that ultimately favours a catabolic profile. In particular, IL-6 infusion has been shown to have resulted in a preferential loss of myofibrillar proteins and muscle atrophy, and can stimulate skeletal muscle breakdown [150]. However, IL-6 has a controversial impact on muscle, as it plays an anti-inflammatory role through the inhibition of TNF-α [146, 151, 152].

Through JAK's tyrosine kinases, STAT is phosphorylated and consequently activated [146, 153]. The activated STATs then translocate to the nucleus and participate in transcriptional regulation [153]. Studies have supported the role of STAT3 in IL-6 infused muscle [146]. Increased STAT3 signalling inhibits the growth related factor STAT5 and causes an increase in suppressor of cytokine signalling 3 (SOCS-3) mRNA [146]. A negative feedback loop is initiated, as SOCS-3 is known to mediate downregulation of the IL-6 receptor, and is the likely agent that attenuates growth hormone (GH) signalling [154]. This attenuated GH and/or IGF-1 receptor signal is a possible mechanism leading to muscle fatigue. Furthermore, IGF-1 infusion, which causes ribosomal protein S6 kinase 1 (S6K1) phosphorylation, is also associated with skeletal muscle hypertrophy. Decreased S6K1 phosphorylation may also be an indicator that one of the catabolic impacts of elevated IL-6 is a decrease in its translational capacity [155]. In sum, IL-6 muscles initiate a SOCS feedback mechanism causing increased STAT protein phosphorylation, favouring a catabolic profile [146].

Feedback and Metastasis

The human body uses feedback loops to regulate levels of various enzymes, proteins and hormones within the body. Some of these feedback loops exist within the NF-κB ubiquitin-proteasome pathway. By inducing the transcription of iNOS, TNF-α, and HIF-1α, NF-κB works to enhance the effects of the aforementioned pathways, thus increasing the detrimental effects of fatigue [156].

The ubiquitin-proteasome pathway also has many molecules that contribute to fatigue and can feed back, causing cancer to metastasize, ultimately resulting in systemic effects (Fig. 8.6). In this way, the pathway works as a positive feedback loop.

The first of these loops involves hypoxia-induced acidosis, which allows for evasion of the immune system and metastatic invasion by cancer. There are two

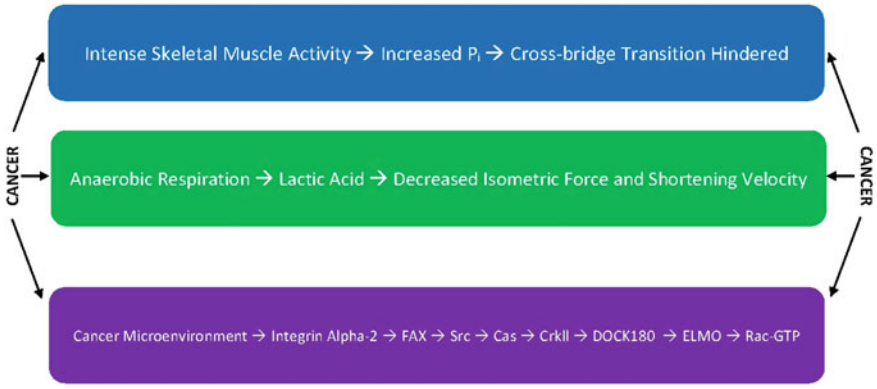


Fig. 8.6 The pathways that cancer can exacerbate fatigue

hypothesized exercise-fatigue mechanisms. The classic hypothesis involves acidosis induced by lactic acid from exercise-induced anaerobic respiration. The acidification reduces isometric force and shortening velocity of muscle fibres. However, fatigue does not always correlate with the occurrence of acidification. The second hypothesis, inorganic phosphate (P_i) accumulation, is a major contributor to exercise-induced fatigue. The concentration of P_i increases during intense skeletal activity through the phosphocreatine cycle. The release of P_i in cross-bridge models promotes the transition from low-force, weakly attached states to high-force, strongly attached states. The increase in P_i hinders this transition [157–159]. Increased myoplasmic P_i decreases force production through direct action on cross-bridge function, thus increasing the effects of fatigue. It may also reduce myofibrillar sensitivity. The presence of P_i also reduces the maximum calcium activated force by inhibiting calcium uptake and the formation of calcium-P_i precipitation. P_i acts directly on the sarcoplasmic reticulum to increase the probability of calcium release channels being open. The calcium-induced calcium release increases tetanic calcium, which often occurs in the early stages of fatigue. P_i also inhibits ATP-driven sarcoplasmic reticulum calcium uptake. The short-term increased tetanic calcium causes calcium accumulation in other organelles, substantially reducing the amount of calcium available for release, and reducing latter tetanic contractions. Lastly, the P_i that enters the sarcoplasmic reticulum forms calcium-P_i precipitation, decreasing the amount of calcium available for release. The calcium concentration available for release is thus reduced in fatigued muscle fibres [157–159].

Another way the tumour metastasizes and the effects of the pathway are enhanced is through calpains [160]. Through the tumour microenvironment, cytoskeletal changes, involving talin, vinculin, and alpha-actinin, decrease or increase integrin expression patterns. The change in expression of integrin α-2 leads to the activation of calpains, which then cleaves focal adhesion kinase (FAK) to activate it [161]. FAK, along with associated integrin cytoplasmic tails, recruits

Src-family kinases through phosphorylation [159]. Src is dephosphorylated and activated by protein tyrosine phosphatase-1B and later recruits Crk-associated substrate (Cas) [162]. Paxillin recruits Crk II to Cas, which then recruits dedicator of cytokinesis (DOCK180) and engulfment and cell motility (ELMO) complexes, stimulating RhoA-GTPase (Rac-GTP) activity loading and actin polymerization. Rac-GTP leads to intravasation and extravasation [163, 164]. This process ultimately leads to metastasis, and thus systemic fatigue.

Skeletal Muscle and Uncoupling Proteins

During ATP synthesis, under normal conditions, electrons are transferred down the electron transport chain, using oxygen in a series of redox reactions. The energy produced by this process is then used to pump protons out of the inner mitochondrial membrane, creating a proton gradient that has potential energy. Protons will travel back across the mitochondrial membrane, down the gradient, through the enzyme ATP synthase [165]. This enzyme uses the potential energy to generate ATP.

Uncoupling proteins (UCP) dissipate the proton gradient before it can be used. This increases the permeability of the inner mitochondrial membrane, allowing protons that have been pumped into the intermembrane space to return to the mitochondrial matrix. This reduces the proton gradient, so there is less potential energy and less ATP synthesis, thereby uncoupling respiration from ATP synthesis [166]. Instead of being converted into potential energy, the energy from respiration is instead released as heat and this is known as non-shivering thermogenesis.

UCP1, UCP2, and UCP3 cause non-shivering thermogenesis. UCP1 is found in brown adipose tissue and UCP2 is expressed ubiquitously within the body, whereas UCP3 is expressed in large quantities in skeletal muscle exclusively [167, 168]. Both UCP2 and UCP3 mRNAs are elevated in skeletal muscle during tumour growth and in addition to UCP1, they are both activated by ROS [169, 170]. ROS accumulation is involved in a tumour-induced muscle metabolism pathway, therefore providing an explanation for the decreased energy efficiency seen in cancer patients.

UCP2 leads to decreased insulin secretion and increased glucagon secretion in the pancreas, causing reduced glucose uptake by muscles and increased gluconeogenesis in the liver [171, 172]. The use of tumour-derived lactate for gluconeogenesis is highly inefficient, consuming six molecules of ATP per cycle, instead of the usual four [166]. As such, this process involves not only excessive usage of ATP, but also ineffective uptake of the additional glucose produced by muscles to synthesize ATP.

The protein degradation observed in cancer patients is the body's attempt to compensate for the lack of ATP. Muscle degradation reduces the amount of UCP3, thereby decreasing uncoupling of ATP synthesis from oxidative phosphorylation. It also provides non-tumour-derived amino acids for gluconeogenesis, so that less ATP is used in this process.

Conclusion

Although it is unclear whether all CRF cases are a result of cancer or cancer treatments, there are some cases where it is clear that the fatigue is a result of cancer, as 40 % of patients report CRF at diagnosis. Furthermore, animal studies have shown that CRF was present in cancer models with the absence of psychosomatic effects. Although cancer may not be the sole cause of CRF, these studies demonstrate that cancer itself directly contributes to the symptom of fatigue [98].

Current research points towards muscle degradation and inhibition of muscle regeneration as contributing factors to muscle wasting. Muscle degradation occurs due to the ubiquitin-proteasome pathway and the lysosomal pathway. Other proinflammatory cytokines like IL-1 and IL-6 can activate NF-κB through the ubiquitin-proteasome pathway as well. Furthermore, aggravated muscle degradation can develop into cachexia, which is present in up to 80 % of cancer patients in advanced stages and is characterized by weight loss that cannot be reversed through nutritional supplementation [164]. MyoD, myogenin and myostatin work to exacerbate the problem by inhibiting muscle regeneration. The current lack of research efforts dedicated to CRF has left much to be elucidated for these pathways.

Given the high prevalence and debilitating nature of this symptom, future directions in this field of research should focus on developing a better understanding of the mechanisms underlying CRF and elucidating the causality between cancer and fatigue. Such understanding will allow for increasingly refined means of diagnosing and treating this debilitating symptom.

References

1. Ryan J, Carroll J, Ryan E, Mustian K, Fiscella K, Morrow G. Mechanisms of cancer-related fatigue. Oncologist. 2007;12(1):22–34.
2. Curt G. Impact of cancer-related fatigue on the lives of patients: new findings from the fatigue coalition. Oncologist. 2000;5(5):353–60.
3. Berger A, Gerber L, Mayer D. Cancer-related fatigue. Cancer. 2012;118(S8):2261–9.
4. Bower J, Bak K, Berger A, Breitbart W, Escalante C, Ganz P, et al. Screening, assessment, and management of fatigue in adult survivors of cancer: an American Society of clinical oncology clinical practice guideline adaptation. J Clin Oncol. 2014;32(17):1840–50.
5. Chaudhuri A, Behan P. Fatigue and basal ganglia. J Neurol Sci. 2000;179(1–2):34–42.
6. Swartz M. The chronic fatigue syndrome—one entity or many? N Engl J Med. 1988;319 (26):1726–8.
7. Cimprich B. Symptom management: loss of concentration. Semin Oncol Nurs. 1995; 11(4):279–88.
8. Smith A, Behan P, Bell W, Millar K, Bakheit M. Behavioural problems associated with the chronic fatigue syndrome. Br J Psychol. 1993;84(3):411–23.
9. Hofman M, Morrow G, Roscoe J, Hickok J, Mustian K, Moore D, et al. Cancer patients' expectations of experiencing treatment-related side effects. Cancer. 2004;101(4):851–7.
10. Schwartz A, Nail L, Chen R, Meek P, Barsevick A, King M, et al. Fatique patterns observed in patients receiving chemotherapy and radiotherapy. Cancer Invest. 2000;18(1):11–9.

11. Yellen SB, Cella DF, Webster MA, et al. Measuring fatigue and other anemia-related symptoms with the Functional Assessment of Cancer Therapy (FACT) measurement system. J Pain Symptom Manage. 1997;13:63–74.
12. Ribatti D, Vacca A. The role of microenvironment in tumor angiogenesis. Genes Nutrition. 2008;3(1):29–34.
13. Dinarello C. Proinflammatory cytokines. Chest. 2000;118(2):503–8.
14. Jager A, Sleijfer S, Rijt C. The pathogenesis of cancer related fatigue: could increased activity of pro-inflammatory cytokines be the common denominator. Eur J Cancer. 2008;44 (2):175–81.
15. Cooper J, Bloom F. The biochemical basis of neuropharmacology. New York: Oxford University Press; 1996.
16. Mazak K, Doczy V, Kokosi J, Noszal B. Proton speciation and microspeciation of serotonin and 5-hydroxytryptophan. Chem Biodivers. 2009;6:578–90.
17. Fernstrom J, Fernstrom M. Exercise, serum free tryptophan, and central fatigue. J Nutr. 2006;136(2):553–9.
18. Meeusen R, Watson P, Hasegawa H, Roelands B, Piacentini M. Central fatigue. Sports Med. 2006;36(10):881–909.
19. Blomstrand E. A role for branched-chain amino acids in reducing central fatigue. J Nutr. 2006;136(2):544–7.
20. Newsholme EA, Blomstrand E. Tryptophan, 5-hydroxytryptamine and a possible explanation for central fatigue. Adv Exp Med Biol. 1995;384:315–20.
21. Davis J, Bailey S, Jackson D, Strasner A, Morehouse S. Effects of a serotonin (5-HT) agonist during prolonged exercise to fatigue in humans. Med Sci Sports Exerc. 1993;25 (Supplement):S78.
22. Späth D, Welzel L, Färber M. Treatment of chronic fatigue syndrome with 5-HT3 receptor antagonists—preliminary results. Scand J Rehabil Med. 2000;29(113):72–7.
23. Cleare AJ, Messa C, Rabiner EA, et al. Brain 5-HT1A receptor binding in chronic fatigue syndrome measured using positron emission tomography and [11C]WAY-100635. Biol Psychiatry. 2005;57:239–46.
24. Clement H, Buschmann J, Rex S, Grote C, Opper C, Gemsa D, et al. Effects of interferon-a, interleukin-1a, and tumor necrosis factor-a on the serotonin metabolism in the nucleus raphe dorsalis of the rat. J Neural Transm. 1997;104(10):981–91.
25. Morrow G, Andrews P, Hickok J, Roscoe J, Matteson S. Fatigue associated with cancer and its treatment. Support Care Cancer. 2001;10(5):389–98.
26. Sonier B, Arseneault M, Lavigne C, Ouellette R, Vaillancourt C. The 5-HT2A serotoninergic receptor is expressed in the MCF-7 human breast cancer cell line and reveals a mitogenic effect of serotonin. Biochem Biophys Res Commun. 2006;343(4):1053–9.
27. Mössner R. Enhancement of serotonin transporter function by tumor necrosis factor alpha but not by interleukin-6. Neurochem Int. 1998;33(3):251–4.
28. Pelletier M, Siegel R. Wishing away inflammation? New links between serotonin and TNF signaling. Mol Interv. 2009;9(6):299–301.
29. Milak M, Parsey R, Keilp J, Oquendo M, Malone K, Mann J. Neuroanatomic correlates of psychopathologic components of major depressive disorder. Arch Gen Psychiatry. 2005;62 (4):397.
30. Morrow G. Differential effects of paroxetine on fatigue and depression: a randomized, double-blind trial from the University of Rochester Cancer Center community clinical oncology program. J Clin Oncol. 2003;21(24):4635–41.
31. McNair DM, Lorr M, Doppelman LF. Profile of Mood States (POMS) manual; education and industrial testing sevices, San Diego, 1971; 24–25.
32. Morrow G, Roscoe J, Hickok J, Smith B, Qazi R. An SSRI antidepressant reduced depression but not fatigue in ninety-six breast cancer patients [abstract]. Support Care Cancer. 2001;9:319.
33. Davis M, Alderson N, Welsh R. Serotonin and central nervous system fatigue: nutritional considerations. Am J Clin Nutr. 2000;72(2):573s–8s.

34. Lindeberg S. An epidemiological study of exhaustion in the context of chronic stress. 2011.
35. Bower J, Ganz P, Aziz N, Fahey J. Fatigue and proinflammatory cytokine activity in breast cancer survivors. Psychosom Med. 2002;64(4):604–11.
36. Bower J, Ganz P, Dickerson S, Petersen L, Aziz N, Fahey J. Diurnal cortisol rhythm and fatigue in breast cancer survivors. Psychoneuroendocrinology. 2005;30(1):92–100.
37. Bower J, Ganz P, Aziz N. Altered cortisol response to psychologic stress in breast cancer survivors with persistent fatigue. Psychosom Med. 2005;67(2):277–80.
38. Harbuz M, Rees R, Eckland D, Jessop D, Brewerton D, Lightman S. Paradoxical responses of hypothalamic corticotropin-releasing factor (CRF) messenger ribonucleic acid (mRNA) and CRF-41 peptide and adenohypophysial proopiomelanocortin mRNA during chronic inflammatory stress. Endocrinology. 1992;130(3):1394–400.
39. Harbuz M, Leonard J, Lightman S, Cuzner M. Changes in hypothalamic corticotrophin-releasing factor and anterior pituitary pro-opiomelanocortin mRNA during the course of experimental allergic encephalomyelitis. J Neuroimmunol. 1993;45(1–2): 127–32.
40. Chaouloff F. Physiopharmacological interactions between stress hormones and central serotonergic systems. Brain Res Rev. 1993;18(1):1–32.
41. Heisler L, Pronchuk N, Nonogaki K, Zhou L, Raber J, Tung L, et al. Serotonin activates the hypothalamic-pituitary-adrenal axis via serotonin 2C receptor stimulation. J Neurosci. 2007;27(26):6956–64.
42. Arlt W, Stewart P. Adrenal corticosteroid biosynthesis, metabolism, and action. Endocrinol Metab Clin North Am. 2005;34(2):293–313.
43. Exton J. Regulation of gluconeogenesis by glucocorticoids. Monogr Endocrinol. 1979;12:535–46.
44. Marver D. Evidence of corticosteroid action along the nephron. Am J Physiol. 1984;246(2): F111–23.
45. Papanek P, Raff H. Physiological increases in cortisol inhibit basal vasopressin release in conscious dogs. Am J Physiol. 1994;266(6):R1744–51.
46. Raff H. Glucocorticoid inhibition of neurohypophysial vasopressin secretion. Am J Physiol. 1987;252(4):R635–44.
47. Petrovsky N, McNair P, Harrison L. Diurnal rhythms of pro-inflammatory cytokines: regulation by plasma cortisol and therapeutic implications. Cytokine. 1998;10(4):307–12.
48. Bower J, Lamkin D. Inflammation and cancer-related fatigue: mechanisms, contributing factors, and treatment implications. Brain Behav Immun. 2013;30:S48–57.
49. Bower J. Cancer-related fatigue—mechanisms, risk factors, and treatments. Nat Rev Clin Oncol. 2014;11(10):597–609.
50. Bower J, Ganz P, Irwin M, Arevalo J, Cole S. Fatigue and gene expression in human leukocytes: Increased NF-κB and decreased glucocorticoid signaling in breast cancer survivors with persistent fatigue. Brain Behav Immun. 2011;25(1):147–50.
51. Blackshaw LA, Grundy D. Effects of 5-HT on discharge of vagal mucosal afferent fibres from the upper gastrointestinal tract of the ferret. J Auton Nerv Syst. 1993;45:41–50.
52. Blackshaw LA, Grundy D. Effects of 5-HT on discharge of vagal mechanoreceptors and motility in the upper gastrointestinal tract of the ferret. J Auton Nerv Syst. 1993;45:51–9.
53. Ek M, Kurosawa M, Lundeberg T, et al. Activation of vagal afferents after intravenous injection of interleukine-1β: Role of endogenous prostaglandins. J Neurosci. 1998;18: 9471–9.
54. Niijima A. The afferent discharges from sensors for interleukin-1β in the haptoportal system in the anaesthetized rat. J Auton Nerv Syst. 1996;61:287–91.
55. Dantzer R. Cytokine-induced sickness behavior: mechanisms and implications. Ann N Y Acad Sci. 2001;933(1):222–34.
56. Schweitzer A, Wright S. The anti-strychnine action of acetylcholine, prostigmine and related substances, and of central vagus stimulation. J Physiol. 1937;90:310–29.
57. Coleridge JC, Coleridge HM. Afferent vagal C fibre innervations of the lungs and airways and its functional significance. Rev Physiol Biochem Pharmacol. 1984;99:1–110.

58. DiCarlo SE, Collins HL, Chen C-Y. Vagal afferents reflexly inhibit exercise in conscious rats. Med Sci Sports Exerc. 1994;26:459–62.
59. Kalia M. Effects of certain cerebral lesions on the J reflex. Pflugers Arch. 1973;343:297–308.
60. Paintal AS. Vagal sensory receptors and their reflex effects. Physiol Rev. 1973;53:159–227.
61. Pickar JG, Hill JM, Kaufman MP. Stimulation of vagal afferents inhibits locomotion in mesencephalic cats. J Appl Physiol. 1993;74:103–10.
62. Pickar JG. The thromoboxane A2 mimetic U-46619 inhibits somatomotor activity via a vagal reflex from the lung. Am J Physiol. 1998;275:R706–12.
63. Davies SF, McQuaid KR, Iber C, et al. Extreme dyspnoea from unilateral pulmonary venous obstruction. Demonstration of a vagal mechanism and relief by right vagotomy. Am Rev Respir Dis. 1987;136:184–8.
64. Paintal AS. Sensations from J receptors. New Physiol Sci. 1995;10:238–43.
65. Andrews PLR, Morrow GR, Hickok JT, et al. Mechanisms and models of fatigue associated with cancer and its treatment: evidence of pre-clinical and clinical studies. In: Armes J, Krishnasamy M, Higginson I, editors. Fatigue in cancer. Oxford: Oxford University Press; 2004. p. 51–87.
66. Stahl S. Basal ganglia neuropharmacology and obsessive-compulsive disorder: the obsessive compulsive disorder hypothesis of basal ganglia dysfunction. Psychopharmacol Bull. 1988;24:370–4.
67. Taylor A, Saint-Cyr J, Lang A. Subcognitive processing in the frontocaudate "complex loop". Alzheimer Dis Assoc Disord. 1990;4(3):150–60.
68. Lange K, Robbins T, Marsden C, James M, Owen A, Paul G. l-Dopa withdrawal in Parkinson's disease selectively impairs cognitive performance in tests sensitive to frontal lobe dysfunction. Psychopharmacology. 1992;107(2–3):394–404.
69. Kawagoe R, Takikawa Y, Hikosaka O. Expectations of reward modulates cognitive signals in the basal ganglia. Nat Neurosci. 1998;1:411–6.
70. DeLong M, Georgopoulos A. Motor functions of the basal ganglia. Handb Physiol. 1981;2:1017–61.
71. Capuron L, Ravaud A, Miller A, Dantzer R. Baseline mood and psychosocial characteristics of patients developing depressive symptoms during interleukin-2 and/or interferon-alpha cancer therapy. Brain Behav Immun. 2004;18(3):205–13.
72. Capuron L, Miller A. Cytokines and psychopathology: lessons from interferon-α. Biol Psychiatry. 2004;56(11):819–24.
73. Capuron L, Fornwalt F, Knight B, Harvey P, Ninan P, Miller A. Does cytokine-induced depression differ from idiopathic major depression in medically healthy individuals? J Affect Disord. 2009;119(1–3):181–5.
74. Kumai T, Tateishi T, Tanaka M, Watanabe M, Shimizu H, Kobayashi S. Effect of interferon-α on tyrosine hydroxylase and catecholamine levels in the brain of rats. Life Sci. 2000;67(6):663–9.
75. Sammut S, Goodall G, Muscat R. Acute interferon-α administration modulates sucrose consumption in the rat. Psychoneuroendocrinology. 2001;26(3):261–72.
76. Song C, Horrobin D, Leonard B. The comparison of changes in behavior, neurochemistry, endocrine, and immune functions after different routes, doses and durations of administrations of IL-1β in rats. Pharmacopsychiatry. 2006;39(3):88–99.
77. Felger J, Alagbe O, Hu F, Mook D, Freeman A, Sanchez M, et al. Effects of interferon-alpha on rhesus monkeys: a nonhuman primate model of cytokine-induced depression. Biol Psychiatry. 2007;62(11):1324–33.
78. Spencer T, Madras B, Bonab A, Dougherty D, Clarke A, Mirto T, et al. A positron emission tomography study examining the dopaminergic activity of armodafinil in adults using [11C] altropane and [11C]raclopride. Biol Psychiatry. 2010;68(10):964–70.
79. Urban N, Slifstein M, Thompson J, Xu X, Girgis R, Raheja S, et al. Dopamine release in chronic cannabis users: a [11C]raclopride positron emission tomography study. Biol Psychiatry. 2012;71(8):677–83.

80. Felger J, Miller A. Cytokine effects on the basal ganglia and dopamine function: the subcortical source of inflammatory malaise. Front Neuroendocrinol. 2012;33(3):315–27.
81. Fu H, Lin Y, Fatimababy A. Proteasomal recognition of ubiquitylated substrates. Trends Plant Sci. 2010;15(7):375–86.
82. Hasselgren P, Fischer J. Muscle cachexia: current concepts of intracellular mechanisms and molecular regulation. Ann Surg. 2001;233(1):9–17.
83. Smalle J, Vierstra R. The ubiquitin 26S proteasome proteolytic pathway. Annu Rev Plant Biol. 2004;55(1):555–90.
84. Wu Y, Zhou BP. TNF-alpha/NF-kB pathway in cancer cell migration and invasion. Br J Cancer. 2010;102:639–44.
85. Hakim J. Reactive oxygen species and inflammation. C R Seances Soc Biol Fil. 1993; 187(3):286–95.
86. Reid M. Free radicals and muscle fatigue: of ROS, canaries, and the IOC. Free Radic Biol Med. 2008;44(2):169–79.
87. Florida Institute for Human and Machine Cognition. [Internet]. 2015 (cited 2 March 2015). Available from: http://cmapspublic3.ihmc.us/rid=1HS44MGZQ-1QH5BBJ-NBK/types%20of%20muscle.jpg.
88. Deiss L, Galinka H, Berissi H, Cohen O, Kimchi A. Cathepsin D protease mediates programmed cell death induced by interferon-gamma, Fas/APO-1 and TNF-alpha. EMBO J. 1996;15(15):3861–70.
89. Lundholm K, Bylund A, Holm J, Scherstén T. Skeletal muscle metabolism in patients with malignant tumor. Eur J Cancer. 1965;12(6):465–73.
90. Ciechanover A. The ubiquitin-proteasome proteolytic pathway. Cell. 1994;79(1):13–21.
91. Costelli P, Carbó N, Tessitore L, Bagby G, Lopez-Soriano F, Argilés J, et al. Tumor necrosis factor-alpha mediates changes in tissue protein turnover in a rat cancer cachexia model. J Clin Invest. 1993;92(6):2783–9.
92. Epstein F, Mitch W, Goldberg A. Mechanisms of muscle wasting—the role of the ubiquitin-proteasome pathway. N Engl J Med. 1996;335(25):1897–905.
93. Hershko A. The ubiquitin system for protein degradation. Annu Rev Biochem. 1992; 61(1):761–807.
94. American Cancer Society. Cancer Facts & Figures 2015 [Internet]. Atlanta; 2015. Available from: http://www.cancer.org/research/cancerfactsstatistics/cancerfactsfigures2015/index.
95. Pusztai L, Clover L, Cooper K, Starkey P, Lewis C, McGee J. Expression of tumour necrosis factor alpha and its receptors in carcinoma of the breast. Br J Cancer. 1994;70(2):289–92.
96. Leek R, Landers R, Fox S, Ng F, Harris A, Lewis C. Association of tumour necrosis factor alpha and its receptors with thymidine phosphorylase expression in invasive breast carcinoma. Br J Cancer. 1998;77(12):2246–51.
97. Moelants E, Mortier A, Van Damme J, Proost P. Regulation of TNF-α with a focus on rheumatoid arthritis. Immunol Cell Biol. 2013;91(6):393–401.
98. Sethi G. TNF: A master switch for inflammation to cancer. Front Biosci. 2008;Volume (13):5094.
99. Wajant H, Pfizenmaier K, Scheurich P. Tumor necrosis factor signaling. Cell Death Differ. 2003;10(1):45–65.
100. Goldberg A. Importance of the ATP-ubiquitin-proteasome pathway in the degradation of soluble and myofibrillar proteins in rabbit muscle extracts. J Biol Chem. 1996;271 (43):26690–7.
101. Sumbayev V, Yasinska I. Mechanisms of hypoxic signal transduction regulated by reactive nitrogen species. Scand J Immunol. 2007;65(5):399–406.
102. Shimoda L. 55th Bowditch Lecture: effects of chronic hypoxia on the pulmonary circulation: Role of HIF-1. J Appl Physiol. 2012;113(9):1343–52.
103. Metzen E. Nitric oxide impairs normoxic degradation of HIF-1 by inhibition of prolyl hydroxylases. Mol Biol Cell. 2003;14(8):3470–81.
104. Koh M, Spivak-Kroizman T, Powis G. HIF-1 regulation: not so easy come, easy go. Trends Biochem Sci. 2008;33(11):526–34.

105. Turrens J. Mitochondrial formation of reactive oxygen species. J Physiol. 2003;552(2): 335–44.
106. Clanton T. Hypoxia-induced reactive oxygen species formation in skeletal muscle. J Appl Physiol. 2007;102(6):2379–88.
107. Xu C. Endoplasmic reticulum stress: cell life and death decisions. J Clin Invest. 2005;115 (10):2656–64.
108. Ron D, Bertolotti A, Zhang Y, Hendershot L, Harding H. Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. Nat Cell Biol. 2000;2(6):326–32.
109. Harding H, Zhang Y, Bertolotti A, Zeng H, Ron D. Perk is essential for translational regulation and cell survival during the unfolded protein response. Mol Cell. 2000;5(5):897–904.
110. Hetz C. The unfolded protein response: controlling cell fate decisions under ER stress and beyond. Nat Rev Mol Cell Biol. 2012;13(2):89–102.
111. Wek R, Cavener D. Translational control and the unfolded protein response. Antioxid Redox Signal. 2007;9(12):2357–72.
112. Marciniak S, Yun C, Oyadomari S, Novoa I, Zhang Y, Jungreis R, et al. CHOP induces death by promoting protein synthesis and oxidation in the stressed endoplasmic reticulum. Genes Dev. 2004;18(24):3066–77.
113. Higo T, Hattori M, Nakamura T, Natsume T, Michikawa T, Mikoshiba K. Subtype-specific and ER lumenal environment-dependent regulation of inositol 1,4,5-trisphosphate receptor type 1 by ERp44. Cell. 2005;120(1):85–98.
114. Anelli T, Alessio M, Bachi A, Bergamelli L, Bertoli G, Camerini S, et al. Thiol-mediated protein retention in the endoplasmic reticulum: the role of ERp44. EMBO J. 2003;22 (19):5015–22.
115. Masui S, Vavassori S, Fagioli C, Sitia R, Inaba K. Molecular bases of cyclic and specific disulfide interchange between human ERO1 protein and protein-disulfide isomerase (PDI). J Biol Chem. 2011;286(18):16261–71.
116. Bosanac I, Michikawa T, Mikoshiba K, Ikura M. Structural insights into the regulatory mechanism of IP3 receptor. Biochim Biophys Acta. 2004;1742(1–3):89–102.
117. Hanna R, Campbell R, Davies P. Calcium-bound structure of calpain and its mechanism of inhibition by calpastatin. Nature. 2008;456(7220):409–12.
118. Powers S, Kavazis A, DeRuisseau K. Mechanisms of disuse muscle atrophy: role of oxidative stress. AJP: regulatory, integrative and comparative. Physiology. 2004;288(2): R337–44.
119. Williams A, Decourten-Myers G, Fisher J, Luo G, Sun X, Hasselgren P. Sepsis stimulates release of myofilaments in skeletal muscle by a calcium-dependent mechanism. J Fed Am Soc Exp Biol. 1999;13(11):1435–43.
120. Passmore L, Barford D. Getting into position: the catalytic mechanisms of protein ubiquitylation. Biochem J. 2004;379(3):513.
121. Bodine S, Baehr L. Skeletal muscle atrophy and the E3 ubiquitin ligases MuRF1 and MAFbx/atrogin-1. Am J Physiol—Endocrinol Metab. 2014;307(6):469–84.
122. Kravtsova-Ivantsiv Y, Ciechanover A. Non-canonical ubiquitin-based signals for proteasomal degradation. J Cell Sci. 2012;125(3):539–48 (Web 27 Mar 2015).
123. Kulkarni S, Saido TC, Suzuki K, Fox JEB. Calpain mediates integrin-induced signaling at a point upstream of rho family members. Am Soc Biochem Mol Biol. 1999;274(30):21265–75.
124. Vierstra R. The ubiquitin–26S proteasome system at the nexus of plant biology. Nat Rev Mol Cell Biol. 2009;10(6):385–97.
125. Stitt T, Drujan D, Clarke B, Panaro F, Timofeyva Y, Kline W, et al. The IGF-1/PI3K/Akt pathway prevents expression of muscle atrophy-induced ubiquitin ligases by inhibiting FOXO transcription factors. Mol Cell. 2004;14(3):395–403.
126. Lokireddy S, McFarlane C, Ge X, Zhang H, Sze S, Sharma M, et al. Myostatin induces degradation of sarcomeric proteins through a SMAD3 signaling mechanism during skeletal muscle wasting. Mol Endocrinol. 2011;25(11):1936–49.

127. Kollias H, McDermott J. Transforming growth factor- and myostatin signaling in skeletal muscle. J Appl Physiol. 2008;104(3):579–87.
128. Hay N. Interplay between FOXO, TOR, and Akt. Biochimica et Biophysica Acta (BBA)—Molecular Cell Res. 2011;1813(11):1965–70.
129. Goldberg JL, Schwertfeger K. Proinflammatory cytokines in breast cancer: mechanisms of action and potential targets for therapeutics. Curr Drug Targets. 2010;11(9):1133–46.
130. Greenfeder SA, Nunes P, Kwee L, Labow M, Chizzonite RA, Ju G. Molecular cloning and characterization of a second subunit of the interleukin 1 receptor complex. J Biol Chem. 1995;270:13757–65.
131. Huang J, Goa X, Li S, Cao Z. Recruitment of IRAK to the interleukin 1 receptor complex requires interleukin 1 receptor accessory protein. Proc Natl Acad Sci USA. 1997;94:12829–32.
132. Wesche H, Henzel WJ, Sillinglaw W, Li S, Cao Z. MyD88: an adapter that recruits IRAK to the IL-1 receptor complex. Immunity. 1997;7:837–47.
133. Burns K, Clatworthy J, Martin L, Martinon F, Plumpton C, Maschera B, Lewis A, Ray K, Tschopp J, Volpe F. Tollip, a new component of the IL-1RI pathway, links IRAK to the IL-1 receptor. Nat Cell Biol. 2000;2:346–51.
134. Zhang G, Ghosh S. Negative regulation of Tolllike receptor-mediated signaling by Tollip. J Biol Chem. 2002;277:7059–65.
135. Lamothe B, Besse A, Campos AD, Webster WK, Wu H, Darnay BG. Site-specific Lys-63-linked tumor necrosis factor receptor-associated factor 6 auto-ubiquitination is a critical determinant of IkB kinase activation. J Biol Chem. 2007;282:4102–12.
136. Kollewe C, Mackensen AC, Neumann D, Knop J, Cao P, Li S, Wesche H, Martin MU. Sequential autophosphorylation steps in the interkeukin-1 receptorassociated kinase-1 regulate its availability as an adapter in interleukin-1 signaling. J Biol Chem. 2004; 279:5227–36.
137. Ye H, Arron JR, Lamothe B, Cirilli M, Kobayashi T, Shevde NK, Segal D, Dzivenu OK, Vologodskaia M, Yim M, et al. Distinct molecular mechanism for initiating TRAF6 signalling. Nature. 2002;418:443–7.
138. Cao Z, Henzel WJ, Gao X. IRAK: a kinase associated with the interleukin-1 receptor. Science. 1996;271:1128–31.
139. Lye E, Mirtsos C, Suzuki N, Suzuki S, Yeh WC. The role of interleukin 1 receptor-associated kinase-4 (IRAK-4) kinase activity in IRAK-4-mediated signaling. J Biol Chem. 2004;279:40653–8.
140. Jiang Z, Ninomiya-Tsuji J, Qian Y, Matsumoto K, Li X. Interleukin-1 (IL-1) receptor-associated kinasedependent IL-1-induced signaling complexes phosphorylate TAK1 and TAB2 at the plasma membrane and activate TAK1 in the cytosol. Mol Cell Biol. 2002;22:7158–67.
141. Qian Y, Commane M, Ninomiya-Tsuji J, Matsumoto K, Li X. IRAK-mediated translocation of TRAF6 and TAB2 in the interleukin-1-induced activation of NF-kB. J Biol Chem. 2001;276:41661–7.
142. Verstrepen L, Bekaert T, Chau T, Tavernier J, Chariot A, Beyaert R. TLR-4, IL-1R and TNF-R signaling to NF-κB: variations on a common theme. Cell Mol Life Sci. 2008;65 (19):2964–78.
143. Deng L, Wang C, Spencer E, Yang L, Braun A, You J, Slaughter C, Pickart C, Chen ZJ. Activation of the IkB kinase complex by TRAF6 requires a dimeric ubiquitin-conjugating enzyme complex and a unique polyubiquitin chain. Cell. 2000;103:351–61.
144. Wang C, Deng L, Hong M, Akkaraju GR, Inoue J, Chen ZJ. TAK1 is a ubiquitin-dependent kinase of MKK and IKK. Nature. 2001;412:346–51.
145. Sanz L, Diaz-Meco MT, Nakano H, Moscat J. The atypical PKC-interacting protein p62 channels NF-kB activation by the IL-1-TRAF6 pathway. EMBO J. 2000;19:1576–86.

146. Yao J, Kim TW, Qin J, Jiang Z, Qian Y, Xiao H, Lu Y, Qian W, Gulen MF, et al. Interleukin-1 (IL1)- induced TAK1-dependent versus MEKK3-dependent NFkB activation pathways bifurcate at IL-1 receptor-associated kinase modification. J Biol Chem. 2007;282:6075–89.
147. Haddad F, Zaldivar F, Cooper DM, Adams GR. IL-6-induced skeletal muscle atrophy. J Appl Physiol. 2005;98:911–7.
148. Goodman MN. Interleukin-6 induces skeletal muscle protein breakdown in rats. Proc Soc Exp Biol Med. 1994;205:182–5.
149. Janssen SP, Gayan-Ramirez G, Van den Bergh A, Herijgers P, Maes K, Verbeken E, Decramer M. Interleukin-6 causes myocardial failure and skeletal muscle atrophy in rats. Circulation. 2005;111:996–1005.
150. Bodell PW, Kodesh E, Haddad F, Zaldivar FP, Cooper DM, Adams GR. Skeletal muscle growth in young rats is inhibited by chronic exposure to IL-6 but preserved by concurrent voluntary endurance exercise. J Appl Physiol. 2009;106:443–53.
151. Heinrich PC, Behrmann I, Haan S, Hermanns HM, Muller-Newen G, Schaper F. Principles of IL-6-type cytokine signaling and its regulation. Biochem J. 2003;374:1–20.
152. Alexander WS. Suppressors of cytokine signaling (SOCS) in the immune system. Nat Rev Immunol. 2002;2:410–6.
153. Haddad F, Adams GR. Inhibition of MAP/ERK kinase prevents IGF-I induced hypertrophy in rat muscles. J Appl Physiol. 2004;96:203–10.
154. Boston University Biology,. NF-Kb Target Genes» NF-Kb Transcription Factors | Boston University. N.p., 2015. Web. 16 Mar. 2015.
155. Westerblad H, Allen D, Lannergren J. Muscle fatigue: lactic acid or inorganic phosphate the major cause? Physiology. 2002;17(1):17–21.
156. Gillies R, Robey I, Gatenby R. Causes and consequences of increased glucose metabolism of cancers. J Nucl Med. 2008;49:24–42.
157. Larsen M, Tremblay M, Yamada K. Phosphatases in cell-matrix adhesion and migration. Nat Rev Mol Cell Biol. 2003;4:700–11.
158. Gabarra-Niecko V, Schaller M, Dunty J. FAK regulates biological processes important for the pathogenesis of cancer. Cancer Metastasis Rev. 2003;22:359–74.
159. Sawhney R, Cookson M, Omar Y, Hauser J, Brattain M. Integrin α2-mediated ERK and calpain activation play a critical role in cell adhesion and motility via focal adhesion kinase signaling. J Biol Chem. 2006;281:8497–510.
160. Deakin N, Turner C. Distinct roles for paxillin and Hic-5 in regulating breast cancer cell morphology, invasion, and metastasis. Mol Biol Cell. 2011;22(3):327–41.
161. Wyckoff J, Jones J, Condeelis J, Segall J. A critical step in metastasis. in vivo analysis of intravasation at the primary tumor. Cancer Res. 2000;60:2504.
162. Gullett N, Mazurak V, Hebbar G, Ziegler T. Nutritional interventions for cancer-induced cachexia. Curr Probl Cancer. 2011;35(2):58–90.
163. Senior AE. ATP synthesis by oxidative phosphorylation. Physiol Rev. 1988;68:177–231.
164. Argilés J, Busquets S, Felipe A, López-Soriano F. Molecular mechanisms involved in muscle wasting in cancer and ageing: cachexia versus sarcopenia. Int J Biochem Cell Biol. 2005; 37(5):1084–104.
165. Gimeno RE, et al. Cloning and characterization of an uncoupling protein homolog: a potential molecular mediator of human thermogenesis. Diabetes. 1997;46:900–6.
166. Boss O, et al. Uncoupling protein-3: a new member of the mitochondrial carrier family with tissue-specific expression. FEBS Lett. 1997;408:39–42.
167. Busquets S, Sanchís D, Alvarez B, Ricquier D, López-Soriano F, Argilés J. In the rat, tumor necrosis factor α administration results in an increase in both UCP2 and UCP3 mRNAs in skeletal muscle: a possible mechanism for cytokine-induced thermogenesis? FEBS Lett. 1998;440(3):348–50.
168. Murphy MP, et al. Superoxide activates uncoupling proteins by generating carbon-centered radicals and initiating lipid peroxidation: studies using a mitochondria-targeted spin trap derived from alpha-phenyl-N-tert-butylnitrone. J Biol Chem. 2003;278:48534–45.

169. Pi J, Collins S. Reactive oxygen species and uncoupling protein 2 in pancreatic β-cell function. Diabetes Obes Metab. 2010;12:141–8.
170. Diao J, et al. UCP2 is highly expressed in pancreatic alpha-cells and influences secretion and survival. Proc Natl Acad Sci USA. 2008;105:12057–962.
171. Portenoy R, Itri L. Cancer-related fatigue: guidelines for evaluation and management. Oncologist. 1999;4(1):1–10.
172. Moss E, Simpson J, Pelletier G, Forsyth P. An open-label study of the effects of bupropion SR on fatigue, depression and quality of life of mixed-site cancer patients and their partners. Psycho-Oncol. 2006;15(3):259–67.

Chapter 9
Oncodynamic Changes in Skeleton

Eric Seidlitz, Snezana Popovic, Mark Clemons and Gurmit Singh

Abstract When cancers are present in bone, a number of complex changes occur that can alter the physiology and structure of the skeleton. To properly understand these *oncodynamic* processes—how the bone changes in response to cancer cell invasion—it is necessary to define the types of cells that are present in normal bone, to explore the main physiological functions of these cells and of the bone itself, and to describe the types of cancers that often grow in bone. To properly characterize the functional and anatomical responses of bone cells, a broader definition of what cell types are present in bone is required. Using a more comprehensive and inclusive definition of bone cells, adaptations that result from cancer cell invasion can be categorized on the basis of the signalled functional and structural changes that occur between all involved cells in the bone environment. These pathological responses will be integrated with what is known about the chemical mediators that may be involved. This analysis of the normal signalling environment in bone and the potential interactions between cell types will help to better characterize the complex oncodynamic processes that can occur when cancer invades bone and disrupts this carefully balanced microenvironment.

E. Seidlitz · S. Popovic · G. Singh (✉)
Department of Pathology and Molecular Medicine, McMaster University,
1200 Main Street West, Hamilton, ON L8N 3Z5, Canada
e-mail: singhg@mcmaster.ca

E. Seidlitz
e-mail: seidlit@mcmaster.ca

M. Clemons
Department of Medicine, University of Ottawa, Ottawa, ON, Canada
e-mail: mclemons@toh.on.ca

M. Clemons
Department of Medical Oncology, Ottawa General Hospital,
501 Smyth Road, Ottawa k1H 8L6, Canada

G. Singh (ed.), *Oncodynamics: Effects of Cancer Cells on the Body*,
DOI 10.1007/978-3-319-28558-0_9

Keywords Bone metastasis · Bone · Cancer · Osteoblast · Osteoclast · Osteocyte · Primary bone cancer · Signalling

Introduction

Cancers growing in bone, whether due to a primary tumour that develops in the bone or a distant cancer that has spread to the bone, elicit a variety of physiological and structural changes that can lead to significant clinical morbidity. The role of therapy in the majority of patients with metastatic bone disease is palliative [1], with a strong focus on symptoms such as pain, fractures, fatigue, or bone marrow suppression. As some patients present with bone-related symptoms *prior* to treatment, it is these directly *signalled* changes in bone that are what we consider as *oncodynamic* responses—how the body changes in response to cancer cell invasion—and they have been given little focus in the cancer literature. Oncodynamics is a conceptual framework that parallels that of pharmacodynamics, the study of how drugs affect the body. With the primary clinical motivation being to identify how to either alter or kill the cancer cells, we need to re-conceptualize what occurs in the cancer bone microenvironment to better understand how to prevent the changes that result, independently of those elicited by cancer therapy. Looking just at the host changes that are elicited by cancer cells allows us to better view these effects as normal responses to altered environmental conditions due to the unique physiological perturbation created by the cancer. With this perspective in mind, we can then begin to focus specifically on identifying what the underlying biological mechanisms are that initiate the pathology. Successful treatment depends on identifying and manipulating the most appropriate target that is causatively linked to the dynamic changes occurring in this complex environment. An understanding of the oncodynamic environment is just the first step in developing effective therapeutics for bone cancers.

This chapter is written from the perspective of the bone and its response to the invasion by cancer cells. The main goal of this review is to answer the question: "*What does cancer do to bone physiology and anatomy?*" To accomplish this, it is necessary to explore the main physiological functions of these cells, to define the types of cells that are present in normal bone, and to describe the cancers that frequently occur in bone. With this framework, bone adaptations that result from cancer cell invasion can then be categorized by way of the functional and structural changes that occur. These changes will also be integrated with what is known about the chemical mediators involved in these pathological processes. As with all oncodynamic effects, *context* is the critical feature to understand when examining the changes in normal physiology that occur due to cancer. Thus, the current local environment and normal functions are particularly important to consider. This chapter is all about context. What cancer cells do is determined as much by where they are located and what the normal physiological functions are in that location, as they are by the nature of the tumours themselves. As many changes in bone cell

functions are the natural and expected biological responses of these cell types to the perturbations caused by cancer, these fundamental mechanisms must be understood before that any effective approach to reversing or preventing the changes can be considered. To understand the scope of changes that can occur, it is necessary to characterize both the anatomical relationship between cell types in bone and their physiological/structural roles. In this respect, the most logical starting point for an understanding of bone is to identify the functions of the bone and the different types of cells of which it is comprised.

Functions of Normal Bone as a Tissue System

Bone is a very metabolically active and dynamic connective tissue system, and is composed primarily of a mineralized matrix and type I collagen. As reviewed by Wagner and Aspenberg [2] and others [3], internal skeletons may have evolved as an adaptation to provide enhanced movement and sensory capacity over the earlier exoskeleton format. Another important adaptation that likely favoured an internal skeleton is the use of calcium phosphate rather than calcium carbonate as a structural matrix. The reasoning for this is argued to be related to changes in the phosphate content of the oceans [4] or that calcium phosphate is more chemically stable in systems with higher metabolic activity [5]. It is agreed, however, that acellular mineralized bone evolved first, with cellular components emerging phylogenetically later [6]. The appearance of calcium sensing receptors appears to only have evolved in vertebrates, and the development of structures to detect extracellular calcium levels parallels the advent of G-protein coupled receptors and their correspondingly complex signal transduction mechanisms [7].

In addition to the mineralized structural components, there is a collection of cell types in bone that work in concert to perform a variety of functions. Those functions, however, are not limited to simply providing structural *support* for the body, to *protect* internal organs, and to allow for *movement*, but also to serve as a *storage* system for calcium and phosphate (and other factors such as sodium, potassium, magnesium, sulphur, copper, and fat), and a location for *hematopoiesis* [8]. An extensive body of work has also identified the bone as being an important endocrine regulator [9–12] that has significant impact on cellular energy metabolism [13], fertility [14–16], and neural functions [17].

Cell Types in Bone and Their Functions

When considering skeletal anatomy and physiology, three primary cell types are typically described as the ‘functional cells’ of bone. These are the osteoclasts (Oc), osteoblasts (Ob), and osteocytes (Ocyt). However, many cell types other than these *classic three* do in fact exist in bone, and each of these has very specific functions in bone homeostasis. Furthermore, each of the numerous cell types can respond

uniquely when cancer is present, and these responses are critical in determining the overall resulting pathological effects.

Bone remodelling occurs due to the coordinated actions of Oc, Ob, and Ocyt cells which together form the traditional *bone remodeling unit* or *basic multicellular unit* [18, 19]. Within this temporary anatomical structure, bone is formed by Ob, maintained by Ocyt, and degraded by Oc. These cells maintain a functional balance through a complex combination of paracrine [20–22] and physical interactions [18, 23]. However, by defining 'bone cells' only as those cells which are involved in making or breaking down of mineralized bone unnecessarily limits our ability to understand how the bone responds to cancer cell invasion. The bone is a complex microenvironment of multiple cell types, and each of these cellular partners contributes to the overall structure and function of the system. For example, all of the three classic bone cell types arise from osteoprogenitor cells—these progenitors are unequivocally present in the bone environment yet are often not mentioned when discussing bone physiology. Thus, osteoprogenitor cells represent an entire category of cells that are clearly resident in the bone environment, are critical for overall bone maintenance, but yet are often overlooked as major players in bone homeostasis. Florencio-Silva et al. [24] provide an excellent review of the major bone cell types and their functions and include a comprehensive set of histological images.

Widening the definition of what we call 'bone cells' is vital for a proper evaluation of oncodynamic effects, as numerous different cell types can be located in the bone environment at any single point in time, including those which may only be present transiently. Therefore, the following is an expanded list of cell types in bone that should be considered when evaluating oncodynamic effects. These cell types are *Oc*, *Ob*, *Ocyt*, bone lining cells (BLC), *stromal* or medullary cells (including osteoprogenitors, adipocytes, and fibroblasts), blood and *hematopoietic* stem cells (including macrophages), *chondrocytes*, *blood vessel-related* cells (e.g., endothelial cells, smooth muscle cells), and *neurons*. Although this list is admittedly incomplete and includes some overlap between categories, these cell types have been selected based on their abilities to respond to the physiological perturbations caused by cancer cell invasion.

Osteoclasts

Oc are the cells responsible for degrading mineralized bone matrix. These multinucleated cells are formed from the fusion of hematopoietic progenitor cells of the monocyte/macrophage lineage [25]. Oc cells generate an acidic environment by secreting protons onto the surface of the bone to demineralize the hydroxyapatite structure, while other secreted enzymes digest the non-mineralized components [26]. Specialized transport mechanisms within the Oc move the degraded material away from the bone surface for disposal—a process called transcytosis [27]. A number of helpful reviews are available that elegantly describe the functions and cellular anatomy of the Oc [28–32]. These cells work in balance with Ob cells to constantly maintain stable bone mass under normal conditions.

Osteoblasts

Ob cells primarily serve to build new bone. They are generally cuboidal in shape with a single nucleus and they are derived from mesenchymal stem cell precursors that exist in the bone marrow or the periosteum. Once the precursor cells begin to express alkaline phosphatase activity, they are classified as *preosteoblasts*. The preosteoblasts then proliferate and mature, and characteristically begin to secrete bone matrix proteins such as type I collagen, bone sialoprotein I and II, and osteocalcin [33]. Bone formation proceeds in a two-step process with the secretion of osteoid toward the surface followed by mineralization of the newly formed matrix. Several good reviews are also available which describe Ob cell differentiation and functions in new bone synthesis [34–37].

In addition to bone formation, Ob cells also function to actively modulate Oc cell formation and hematopoietic stem cell homeostasis via several different signalling systems. Secreted osteocalcin can act locally in the bone or in an endocrine manner to modulate other functions such as male fertility or whole-body energy metabolism [9, 34]. Once Ob cells have performed their bone synthesis role, some may senesce and die by apoptosis. However, Ob cells are actually the precursors to two other types of cells—the Ocyt and BLC. These cell types are vital to the overall maintenance and functioning of normal bone.

Osteocytes

Ocyt cells are terminally differentiated Ob that are incorporated directly into the matrix of newly formed bone [38, 39]. These cells were originally thought to be quiescent Ob with the limited role of holding the bone together, although they are now considered as the master coordinators of bone synthesis and resorption. They have also been described as integrators and transducers of mechanical information [10, 40–42]. These long-lived [41, 43] multifunctional cells comprise about 90 % of all bone cells [42]. Their cell bodies reside in the lacunae of the bone and have dendritic-like processes (usually $\sim$50 for each cell) that reach through the canaliculi to form a complex network with other cells via gap junctions [44]. It has been estimated that the total number of Ocyt in the human skeleton is $\sim$42 billion and that the total number of Ocyt dendritic projections from these cells is $\sim$3.7 trillion—thus leading to a staggering 23 trillion direct connections between cells [45].

In addition to their gap junctional connections, Ocyt cells secrete a variety of proteins that modulate both bone formation and bone degradation. For example, Ocyt cells express a protein called *sclerostin* that acts as an effective inhibitor of the Wnt signalling pathway and Ob bone mineralization [46]. Neuropeptide Y (NPY) is also secreted by the Ocyt and this peptide can directly repress Ob function [47]. To control bone resorption, Ocyt cells are a major source of receptor activator of nuclear factor kappa-B ligand (RANKL) [48], the predominant cytokine involved in

stimulating Oc differentiation [49]. They can also indirectly modulate Oc-mediated bone resorption, as parathyroid hormone (PTH) causes a decrease in sclerostin expression [50] which subsequently results in increased Ob mineralization and decreased Oc bone resorption.

Ocyt cells appear to act as mechanosensors to detect mechanical forces and they transmit this information to other cells in the bone environment for encoding as a structural change. Ocyt cells are the primary mechanosensory cells in bone and the sensation process likely involves Wnt/β-catenin signalling [51]. Mechanical transduction is also facilitated through paracrine mediation of bone cells via specific glutamate transporter [52–54] and receptor systems [55], in addition to nitric oxide, prostaglandins, and osteopontin [56]. Ocyt cells that are mechanically stimulated also begin to secrete factors that alter mesenchymal stem cell migration [57] and these prompt newly formed osteoprogenitor cells to migrate and replace the exhausted Ob.

Bone Lining Cells

BLC serve as a protection for the bone, and like Ocyt, these cells are derived from flattened Ob. BLC are quiescent cells that cover the bone surfaces wherever resorption and bone formation are not occurring. Coincident with their bone protection role, BLC are important in the regulation of calcium movement in and out of bone under the control of paracrine factors such as PTH and calcitonin [58, 59]. There are two types of BLC, based primarily on anatomical location—*endosteal* and *periosteal* cells. Endosteal cells line the marrow cavities, and as such, they maintain close contact with hematopoietic cells of the bone marrow. The endosteum has significantly less sympathetic innervation compared to the periosteum [60]. The highly innervated periosteum covers the entire surface of long bones except for the articular surfaces. The periosteum has an outer layer of fibroblasts, collagen, neurons, and microvessels, and an inner layer of mesenchymal progenitor cells, osteoprogenitor cells, Ob, fibroblasts, sympathetic neurons, and microvessels [61]. Both the periosteum and endosteum have numerous resident macrophages that are likewise involved in modulating bone metabolism at these surfaces [62]. For more detail on this cell type, Franz-Odendaal et al. [63] present a comprehensive review of how Ob become Ocyt.

Stromal Cells

This broad category incorporates a number of different cell types and their precursors and is essentially a definition of anatomical location, including many cell types residing in the medullary or bone marrow space. Arbitrarily defined, these are cell types which are not directly involved with the main function of bone marrow—

hematopoiesis. The bone marrow itself is a densely cellular heterogeneous tissue found in the interior of most bones. Bone marrow stromal cells (sometimes called mesenchymal stem cells) give rise to the non-hematopoietic cells [64, 65]. In this space you can also find the precursors to the Oc, Ob, and Ocyt—called the osteoprogenitor cells. Among many others, the major types that are found in the marrow are adipocytes, fibroblasts, and macrophages. Although many of these cell types have functions critical to bone maintenance, some may only temporarily reside in the bone marrow.

Osteoprogenitor Cells

Although not often considered as true bone cells, the osteoprogenitors that differentiate into Oc, Ob, Ocyt, and BLC cells are significant players in overall bone functioning. As described above, Oc progenitors are formed in bone marrow from hematopoietic stem cells of the monocyte/macrophage lineage. Bone marrow stromal cells are the precursors to Ob cells, which can further differentiate into Ocyt or BLC. Although it is difficult to identify osteoprogenitor cells on the basis of anatomic features alone, these cells can be defined by their differential expression of a variety of surface marker proteins [66]. A good review of the history relating to the identification of osteoprogenitor cells is presented by Modder and Khosla [67].

Adipocytes

Bone marrow in particular has a large number of adipocytes. Adipocytes and Ob are derived from a common mesenchymal progenitor cell, with specific environmental conditions and transcriptional regulation factors determining the fate of the mesenchymal precursors. Several important factors can alter the differentiation pathway to switch between adipocytes or Ob, and these include *zinc finger protein 521* [68] and extracellular glutamate levels [69].

Fibroblasts

Fibroblasts are a heterogeneous group of differentiated cells of mesenchymal origin that synthesize precursors of the extracellular matrix—particularly collagen—and have different appearances depending on their anatomical location. Their primary function is to maintain the integrity of connective tissues [70]. In many parts of the body, fibroblasts generate robust cellular connections with other fibroblasts [71]. Although differentiated, fibroblasts can be reprogrammed to become other cell types via controlling the expression of specific transcription factors and growth conditions [72].

Blood and Hematopoietic Stem Cells

This diverse category includes red blood cells, macrophages/monocytes, lymphocytes (Natural killer cells, T cells, and B cells), and hematopoietic stem cells that differentiate into some of the cell categories discussed previously. Although many of the cells in this group have their primary functions in other parts of the body, important functional interactions between bone-resident cells and hematopoietic cells occur in the bone. A review by Taichman [8] provides the context to understand the many two-way interactions between classical bone cells and the processes relating to blood cell synthesis.

Macrophages are derived from hematopoietic precursors in the bone and are found throughout the body. These cells typically function as immune surveillance cells and will actively phagocytose cellular debris, regardless of their location. Macrophages that remain in bone, usually called *osteomacs*, are anatomically found near to the periosteal or endosteal BLC [62]. These cells are involved in both the degradation and synthesis processes for maintenance or repair of damaged bone [73, 74]. Osteomacs form a temporary and protective canopy-like cover over active Ob to aid in their generation of mineralized bone [70, 75]. Macrophages phagocytose old red blood cells and thus also serve as a regulator of iron levels for haemoglobin production [76].

Lymphocytes, or white blood cells of the immune system, include natural killer cells, T cells, and B cells. These originate in the bone marrow space and interact frequently with other cells in this environment. A number of factors secreted by lymphocytes are known to alter bone synthesis and degradation. For example, RANKL produced by activated T cells is important in normal bone metabolism by stimulating Oc differentiation [48], at least in young animals.

Hematopoietic stem cells are the precursors for the synthesis of virtually all blood cells, including myeloid cells, lymphoid cells, red blood cells, and platelets (or thrombocytes) [77, 78]. In adults, they reside primarily in the bone marrow space near blood vessels and the endosteum, with some evidence suggesting that their location may be partly related to oxygen availability [79]. These stem cells maintain typical stem cell features such as the abilities to self-replicate and to differentiate into non-hematopoietic cell types [4]. Regulation and maintenance of hematopoietic stem cells, however, appears to be under the control of bone marrow stromal cells [80], further demonstrating how the bone microenvironment can operate as a highly interconnected network.

Chondrocytes

Chondrocytes are derived from mesenchymal stem cell precursors and are important cells for the generation of cartilage and fully formed bone. The long bones of most vertebrates develop primarily through a process called endochondral

ossification, in which new cartilage is formed by hypertrophic chondrocytes then subsequently mineralized by the addition of hydroxyapatite crystals. How the mineralization occurs is still controversial, although Ob secretion of osteoid against new cartilage may be involved [81]. Another view is that chondrocytes differentiate into Ob-like cells [82], which then switch from collagen synthesis to begin expressing alkaline phosphatase [83]. The switchover from synthesis of collagen to the development of alkaline phosphatase activity may be related to the redox balance of chondrocytes [84]. Chondrocytes can either respond to external signals themselves or produce them to control other cell types, and it has been noted that they have all the appropriate mechanisms needed for fully functional glutamate signalling [85]. Vesicular glutamate release has been demonstrated to be signalled by activation of AMPA receptors on chondrocytes [86], and glutamate can inhibit chondral mineralization via enhancement of chondrocyte apoptosis [87].

Blood Vessel-Related Cells

All the cell types associated with blood vessels and the lymphatic system are represented in this category. These include vascular smooth muscle, endothelial cells, pericytes, etc. [88]. Although the blood vessel adventitia is primarily composed of collagen and connective tissue, it incorporates many cellular components of the types discussed above—macrophages, mast cells, progenitor cells, T cells, microvascular endothelial cells, and adipocytes [89]. Although there is some evidence that lymphatic vessels appear in normal bone, they are restricted to the outer fibrous layers of the periosteum [90]. It is likely that lymph vessels do not play a major role in bone function.

Neurons

Often forgotten, neurons are clearly present in bone and are particularly important for the regulation of bone metabolism. The bone has a very dense network of sensory [60] and sympathetic neurons [91] that are closely associated with blood vessels, trabecular bone, and near hematopoietic cells [92]. The periosteum, specifically, has an exceptionally high neuron density [60]. The sympathetic nervous system (SNS) exerts primary control over bone metabolism [93], with evidence of catecholamine signalling to Ob being well established [94]. Some bone compartments may also use cholinergic signalling systems [93]. Bone, bone marrow, and periosteum are densely innervated with peptide-rich sensory neurons/C-fibres (unmyelinated) (substance P and CGRP) [95]. Myelinated Aβ- and Aδ-fibres also are present [96].

Demonstrating the impact of the SNS, sympathectomy after administration of guanethidine to neonatal rats resulted in significantly increased numbers of Oc at

the surface of mineralized bone; removing just the sensory C-fibre innervation (with capsaicin treatment) caused a decreased number of Oc in the same locations [97]. These experiments suggest that sensory functions, in addition to sympathetic regulation, may also contribute to the feedback control over bone remodelling. In fact, the neurotransmitter glutamate is highly expressed particularly near bone cells, suggesting that glutamatergic control over bone functioning may be essential in normal bone metabolism [92], perhaps independently of the sympathetic modulation. Many of the primary afferent sensory neurons innervating mineralized bone express acid-sensing ion channels such as the vanilloid receptor, and these may be present to respond to the acidic microenvironment caused by osteoclast functions [60]. It is interesting to note that, although most bone structures deteriorate over time, the sensory neuron density apparently does not decline with age [98].

What Cancers Appear in Bone?

Many cancer types can appear in bone, and they may originate from a variety of sources. Cancers may originate in skeletal structures (primary cancers such as chondrosarcoma, osteoma, multiple myeloma), others migrate from other distant sites (metastatic cancers such as breast, prostate, lung cancers), while some may invade into bone from nearby structures (e.g., head and neck cancers). Once in the bone, many cancers cause similar alterations in the bone microenvironment as they interact with the same host cell environments, although in different ways.

Primary Bone Cancers

The most frequent primary bone cancers can be divided into solid tumour and non-solid (haematological) tumour types. This categorization is purely arbitrary, although it does allow the haematological cancers to be defined as a bone cancer type mostly due to the location of the affected cells in the bone marrow. The three most common solid bone tumours are osteosarcoma, Ewing's sarcoma, and chondrosarcoma. Osteosarcomas develop from uncontrolled proliferation of osteoprogenitors, and it has been argued that Ocyt cells may actually be the aberrant progenitor [99]. Ewing's sarcoma is thought to be of ectodermal origin although new evidence suggests that this cancer may derive from mesenchymal stem cells in the bone marrow [100]. Furthermore, genomic analysis demonstrates a potential relationship to chondrocyte progenitor cells [101]. Ewing's sarcoma is associated with severe bone pain with significant periosteal reaction but with little evidence for cortical bone changes. Similarly, chondrosarcomas are cancers of the chondroid matrix-producing cells. Although typically a primary cancer, chondrosarcoma can also become metastatic and move to other sites [102, 103].

Several non-solid tumours or haematological cancers develop partly in the bone marrow space, and these include leukaemia, lymphoma, and multiple myeloma. Leukaemias are cancers of myeloid or lymphoid cell lines, while multiple myeloma is a cancer originating in the white blood cell type called *plasma cells* [104], an important mediator of adaptive immunity. Although these cancer cells are present in the bone marrow for only some portion of their life cycle, the most prominent bone-related symptoms that occur are likely due to their overgrowth and disruption of bone marrow functioning.

Metastatic Bone Cancers

Cancers that spread or metastasize from distant sites in the body can preferentially find refuge in the bone environment. The identity of the primary tumour is an important oncodynamic factor to consider since it may determine some of the metabolic properties of the metastatic cells and these properties determine the responses of local cells in the bone.

The most common metastatic cancers that spread to bone are breast and prostate [105]. Breast cancer's predilection to seek bone was initially described by Paget in 1899 in which he suggested that the properties of the cancer cells (the seed) were as important as the properties of the bone (the soil) in determining this preferential localization [106]. Frequent bone localization in breast cancer metastasis is therefore not a chance phenomenon. In fact, 73 % of breast cancer patients were found to have bone metastases on *post mortem* examination [107]. The same occurs in prostate cancer, with about 68 % of patients having bone metastases [107]. Although at a lower frequency (between 35 and 42 %), other tumours that spread to bone include lung [108], kidney [107], thyroid, and gastrointestinal cancers [107]. Some cancers can metastasize to bone but only do so rarely. These include melanoma [109], neuroblastoma [110], cervical [111], and ovarian cancers [112, 113].

What Bone Functions Change When Cancer Cells Are Present?

Virtually, all of the cell types residing in bone can respond to cancer invasion with changes in their normal physiological functions. The resulting clinical symptoms experienced by patients with bone cancer can include bone pain, fractures, impaired mobility, impaired haematological functions, and hypercalcemia [105]. How these symptoms occur in patients is based mostly on a composite of the individual cellular responses that transpire within the bone environment. Since the maintenance of bone requires a delicate balance between bone synthesis and bone degradation processes, one common response to cancer invasion is a change in the inherent structure of the bone that results from disruption in one or both of these

processes. Although there may be either increased bone loss or enhanced bone synthesis, many cancers result in simultaneous and sometimes quite subtle alterations in both processes.

Understanding oncodynamic effects is vital to enable clinicians to effectively identify and treat cancer in the bone. To demonstrate this more clearly, take as an example the presentation of a patient with breast cancer bone metastasis showing significant Oc-mediated bone resorption. A common therapeutic intervention for this apparent osteolysis problem, in addition to starting standard anticancer chemotherapy or endocrine therapy, is to inhibit Oc function with drugs such as a bisphosphonate (e.g., zoledronic acid) or reduce Oc differentiation using a RANKL inhibitor (e.g. denosumab) [114, 115]. However, even though it is agreed that there is increased bone degradation, the treatment choices for this metastatic bone disease may be better informed by determining the fundamental cellular signalling mechanisms driving the oncodynamic effects that result in the observed bone resorption. Many cancers may appear to present as a simple increase in Oc activity, yet the real effect could easily be due to a variety of factors—including inhibition of Ob differentiation [116], alteration of Ocyt control over the balance maintained between Oc and Ob functions [40], or an enhancement of Oc-precursor cell survival [117]. The overall osteolytic result would likely appear the same in each case. Although Oc inhibition has proven effectiveness in bone metastasis [118, 119], many clinicians agree that the solution to the problem is not to just get rid of the Oc effector cells, as this may miss what is really happening. By perceiving the system as an oncodynamic process, then identifying the actual changes that are occurring in bone, novel therapeutic targets may be identified. To better understand the specific oncodynamic effects that can occur in bone, the cellular responses will be described within the context of the essential functions of bone itself.

Changes in Bone Structure

Cancer-induced alterations in bone metabolism can directly impact the three structural functions of bone—namely support, protection, and movement. A disruption in homeostasis often leads to a reduction in bone strength and potential changes in its anatomical configuration. Considering that there are two distinct but interconnected processes used for bone maintenance—degradation and synthesis—theoretically, there are three possible changes that can be imagined. Although perhaps an oversimplification, these abstract categories are important for logically defining the problem. The three possibilities are (a) disruptions of the bone degradation processes alone, (b) changes in the processes of bone synthesis alone, or (c) alterations in both processes at the same time. Since Oc and Ob functions are normally very tightly coordinated, it appears most likely that both cell type functions are impacted by cancer at the same time, and that it is the sum of these effects that will be the determining factor in classifying the bone pathology as predominantly *osteolytic* (decreased bone mass), *osteosclerotic* (increased bone mass), or

mixed (having a combination of both processes). To further complicate this model of structural modification, each of these inherent processes can have two opposing directions—either an increase or a decrease.

Increases in Bone Degradation

If the overall 'symptom' of cancer invasion is a loss of bone, increases in the functions and numbers of Oc responsible for degrading bone may be the culprit. These effects can be due to direct mature cell functional changes or to alterations in the differentiation of bone cell progenitors. Many cancers present with a predominantly degradative bone phenotype, including breast cancers, leukaemias, lung, thyroid, renal, multiple myeloma, and metastatic neuroblastoma. However, a few of these are solely the result of enhancement of Oc functions. More commonly, cancers alter the survival or growth/differentiation rate of the Oc progenitor cells.

A more effective technique to achieve osteolysis is to induce alterations in both Oc and Ob functions simultaneously. An example is *metastatic neuroblastoma*. In this cancer, osteolysis is mostly due to stimulation of osteoclastogenesis, although there is also some inhibition of Ob precursor differentiation. As a consequence of the relative increase in numbers of Oc cells, the result is enhanced resorption of bone [120]. However, most osteolytic phenotypes are more complex than this. Multiple myeloma cells can indirectly achieve the same goal by stimulating the secretion of factors from host cells in the bone environment which in turn stimulate Oc functioning [121, 122]. These myeloma cells also can cause a direct physical disruption of the bone remodelling unit which prevents bone formation from occurring normally [18]. Other examples of complex mechanisms achieving and osteolytic phenotype include breast cancer, in which the cancer cells secrete cytokines that both enhance the development and the survival of Oc progenitor cells, and thus indirectly stimulate bone resorption by sustaining an increased number of Oc cells [123]. Non-metastatic neuroblastoma [120] cells take a different approach and do this by actively suppressing osteoblastogenesis from Ob precursors. Of course, breast cancer cells also secrete factors that reduce the survival of monocyte Ob progenitor cells [124], but this further supports that the reality that parallel oncodynamic effects often occur in both the synthesis and degradation processes. The result from each of these different mechanisms is the same—increased Oc-mediated bone resorption.

Increases in Bone Synthesis

Prostate cancer is widely considered as the best example of a tumour that frequently elicits a net increase in bone mass [125]. However, this newly formed bone in prostate cancer is usually atypical in appearance, with incomplete mineralization

and osteoid deposition that creates what is sometimes called woven bone [126]. Woven bone may not be as structurally sound as normal trabecular bone [127]. As a parallel to the multiple options described in cases of predominant osteolysis, the pathological enhanced bone deposition in prostate cancer may be due to higher Ob activity, an indirect increase in Ob number (due to changes in osteoblastogenesis), or via corresponding decreases in Oc activity and Oc numbers.

In vitro evidence supports that a soluble secreted protein from prostate cancer cells called prostatic acid phosphatase (PAP) causes direct activation of bone mineralization by mature Ob cells [128], separate from effects on Ob differentiation. Rabbani et al. also identified that urokinase-type plasminogen activator (uPA) secreted by prostate cancer cells acted as a mitogenic agent for Ob cells to stimulate their mineralization functions [129]. Similarly, we observed that the amino acid glutamate results in increases of alkaline phosphatase activity and bone mineralization in Ob cells independently of cell differentiation or proliferation [130]. Although perhaps not as common, direct inhibition in Oc functioning has been observed. When in contact with bone, prostate cancer cells secrete *endothelin-1* (ET-1) and this peptide will signal to the Oc cells and reduce their functioning by directly impairing cell mobility [131].

More subtle indirect changes can occur, with prostate cancer cells enhancing Ob precursor differentiation via secreted factors that stimulate this process—leading eventually to enhanced bone formation [132]. Although inhibiting Oc activity directly, the secreted ET-1 is also received by Ob and will stimulate their functions [133], with the specific endothelin-A receptor mediating this effect [134] through a secondary cytokine-based signalling mechanism. Secondary activation of bone formation by reducing Oc proliferation rather than by direct inhibition of functioning is very well established in the literature. *Osteoprotegerin* (OPG), a soluble decoy receptor for RANKL, is produced by Ob, and this factor inhibits RANKL stimulation of Oc differentiation, thus decreasing Oc numbers [135, 136].

Uncoupling of Ob and Oc

As seen above, many cancer-signalled changes in bone metabolism involve simultaneous changes in both Oc and Ob cell functions. Being so delicately balanced, the functioning of these two cell types appears to be easily perturbed when cancer cells invade. The homeostasis of normal bone is maintained by complex intercellular communications between all the cellular partners in the bone environment, and anything that disrupts this signalling can lead to uncoupling of the Oc and Ob and result in the observed bone pathologies. In such a complex system, it is quite unlikely that a unilateral change in a single cell type will result in changes in bone structure.

Prostate cancer can be used as an example to emphasize this point more clearly. Although prostate tumours typically result in an overall osteosclerotic phenotype, there is considerable evidence that Oc-mediated bone degradation is still critical in

the development of the net bone formation effect [137]. Without degradation, the tumour-enhanced Ob cells may have few locations at which to build new bone. In fact, a purely osteoblastic phenotype of prostate cancer may be a rarity. In a study of prostate cancer patient samples, not a single patient had bone lesions that could be categorized into either purely osteosclerotic or purely osteolytic [126]. Other cancers that generally show a predominant phenotype also show evidence of changes in both degradation and synthesis. This includes breast cancers that typically evoke an osteolytic response—these cells actually secrete factors such as bone morphogenetic proteins (BMPs) that enhance the mineralization activities of Ob [138]. BMPs in turn can alter both Ob and Oc differentiation, although the overall result in breast cancer bone metastases is osteolytic. In multiple myeloma, bone marrow stromal cells maintain their capacity to differentiate into Ob cells (given the correct environmental signals) even though this is almost always an osteolytic disorder [139], suggesting that uncoupling of Ob and Oc in this case may be related to disruption of communication between cells rather than pathological changes to the individual cell types directly. This type of effect is the basis of our hypothesis that the cancer cell secreted factor glutamate may be acting to uncouple Oc and Ob by disruption of critical glutamate signalling mechanisms between cell types in the bone [130, 140].

Changes in Other Bone Functions

Bone performs many functions other than as a connective tissue. The bone's storage, endocrine, and hematopoiesis activities are also important tasks that can be perturbed when cancer invades. Thus, identifying oncodynamic effects relating to these functions is critical to fully understand the implications of cancer invasion of bone.

Changes in Storage Functions

The bone stores a variety of different factors within its hydroxyapatite matrix, including calcium, phosphorous, and numerous growth factors. When bone is degraded by Oc, whether pathological or not, these factors may be released to have local autocrine or paracrine effects or distant endocrine-like responses. This is a normal process that occurs all of the time, with the bone acting as a primary storage mechanism for a number of different substances. When cancer induces oncodynamic effects, a number of changes occur in bone degradation and formation to result in a different rate of release of stored components than normal. Pathologies can result when the rate of release of those stored factors is important for the normal balance maintained in the bone. These altered release dynamics are typically observed to result in direct pathological effects in the bone or at distant sites in the body.

Calcium

Being the principal mineral component of the bone matrix and a critical ionic signalling molecule throughout the body, the maintenance of calcium balance is a tightly regulated multisystem process. Vitamin D enhances intestinal absorption of calcium in response to low circulating calcium levels. If absorption from the diet is insufficient to maintain the proper circulating levels, the high vitamin D will signal the bone to move calcium into circulation [141]. This signalling leads to Oc, Ob, and Ocyt cells all responding together to achieve a net increase in bone resorption either directly, or indirectly via stimulation of hormone signalling from the parathyroid glands [142, 143]. When cancers invade bone and drive an overall increased bone resorption, hypercalcemia can become a significant clinical problem. Although mostly due to Oc-related bone resorption, additional signalling by endocrine factors secreted by the cancer cells that are received by the kidney can cause increased renal reabsorption of calcium, resulting in even higher circulating levels. A dysregulated serum calcium level, regardless of the aetiology, can have profound neurological and psychiatric consequences [144, 145].

Phosphate

Like calcium, phosphorous is stored as a component of the calcium phosphate bone matrix and its availability in circulation is regulated through a number of endocrine and paracrine mechanisms. In addition to being a structural component of bone, phosphate is also critically important for all phases of cellular energy metabolism. Phosphate balance relies on several systems and endocrine factors secreted primarily by Oc and Ocyt cells [146] and the parathyroid glands will regulate phosphate secretion by the kidney. For a review of what is known about normal phosphate homeostasis, see a review by Eleanor Lederer [147]. When cancer appears in bone, additional factors secreted by the tumour cells disturb this delicate balance and often leads to hypophosphatemia and tumour-induced osteomalacia—a 'softening' of the bones as a result of inadequate bone mineralization. This change in bone strength can also be accompanied by pain, fatigue, and muscle weakness. A recent review of cancer-induced osteomalacia describes some of the factors involved in the development of this disruption in phosphate homeostasis [148].

Other Stored Factors

Numerous growth factors, hormones, and cytokines are stored within bone matrix and these can be released during normal bone remodelling or cancer-induced bone resorption. Much of the literature on bone metastasis emphasizes the paracrine

stimulatory effects of these factors on the invading tumour cells directly, although these released substances evoke important responses from host cells in the bone environment. An example here is transforming growth factor β (TGF-β) which is released from bone in a latent form [149], and the acidic conditions accompanying Oc-mediated matrix resorption will activate it [150]. Although TGF-β has effects on the growth of cancer cells, it also directly and indirectly alters the functions of Oc and Ob. Specific TGF-β receptors are expressed by Oc and the resulting stimulation of this receptor may secondarily lead to other growth factors being secreted by the Oc [151]. There is some evidence that suggests that TGF-β signalling of Oc also assists in stimulating Ob-mediated bone formation [152], as part of the coupling mechanism that normally regulates Oc and Ob functions.

We have taken advantage of the storage function of bone in our own work. Since we anticipated that metastatic breast cancer cells would prompt Oc to degrade the bone, we pre-administered the tetracycline drug *doxycycline* and allowed it to accumulate in the bone matrix. When the expected oncodynamic responses occurred, we observed an overall decrease in bone resorption and a reduced tumour burden as the high local concentrations of doxycycline released from its storage in the matrix effectively inhibited both tumour growth and osteolysis [153–155].

Changes in Endocrine Functions

The storage and endocrine functions of bone are intimately linked, as many factors released by osteoclastic bone degradation lead to responses locally as well as elsewhere in the body. Many of the factors that are released by bone when it is degraded can also be considered as endocrine mediators. These or the cancer cells may secondarily stimulate host cells to produce other substances—all of these may lead to oncodynamic responses in other parts of the body. The prime example of such a factor is the calcium that is released from degrading bone—this calcium has well-established roles as a mediator in the parathyroid glands and the intestinal tract, as discussed above.

Changes in Hematopoiesis Functions

An important function of bone is to be a location for the development of blood cells. One of the most direct oncodynamic effects is the simple displacement of these cells from the marrow space by the cancer, and this essentially prevents the complex interactions between hematopoietic precursor cells and the bone environment from occurring. In addition to this compartment-based displacement, some cancer cells may lead to a paracrine factor-mediated “reprogramming” of bone marrow cells to produce a generalized immunosuppression—presumably by

altering the progression of hematopoietic stem cells preferentially toward the myeloid lineage [156]. Since the bone provides a safe environment for hematopoietic cells, other cancers like chronic myelogenous leukaemia take advantage of this relative safety to proliferate in the endosteal niche, enhancing the growth of Ob which directly support the growth of the cancer cell [157]. One example of a significant hematopoietic disruption by cancer is the disruption of the immune system cells that initially develops in the bone. Various cytokines (e.g., TGF-β) that cause functional responses in Ob and Oc also cause inhibition of T cell and natural killer cell proliferation, and this results in reduced immune surveillance in the bone [158]. Evading the immune system is an important factor in cancer cell survival.

Other Signalled Changes

A vital function of bone that is often overlooked relates to its sensory activities. Bone incorporates a number of different cell types with the ability to sense a variety of stimuli other than the secreted chemical signals. The bone changes its structural configuration in response to mechanical stimulation by altering the balance between Ob and Oc activities. Multiple signalling molecules are definitely involved, although glutamate intercellular communication appears prominently in the literature [51, 55, 57, 159]. The effector cells for the adaptive degradation or formation of bone are the Oc and Ob, but the Ocyt cells have been revealed as the master modulator of these changes [52]. We have proposed that the presence of cancer cells that secrete high concentrations of glutamate into the bone environment is able to disrupt this control system [140, 160], and have some evidence to demonstrate that this glutamate mechanism may be related to the sensation of bone pain in cancer [161].

Although the mediators of bone cancer pain are not well understood, the sensation of pain is strongly associated with cancer invasion into the bone. Acute lymphoblastic leukaemia [162], multiple myeloma [163], and metastatic breast [164] and prostate cancers [165] are all associated with significant bone pain. As described previously, the bone has numerous sensory fibres within its structure, and these sensory neurons can respond to chemical and mechanical stimuli which may be perceived as pain. In addition to traditional signalling, cancer cells can cause direct damage to neurons [96] and eventually lead to a neuropathic type of pain. Mechanistically, many believe the Oc to be critically involved in cancer-induced bone pain. However, although protons secreted by the Oc to demineralize bone are associated with pain sensation [166, 167], Oc are not the only players, as therapeutic ablation of Oc function does not stop pain in later stages of the disease [168]. It should be noted, though, that signalling from the bone to the nervous system is not the only direction possible—there is evidence demonstrating that substances released from sensory neurons also play a role in coordinating the functional adaptation of bone cells to strain and mechanical loading [169].

What Are the Signalling Mediators?

With all the anatomical and functional complexity of the skeletal system, it is not reasonable to expect that a single mediator molecule could be solely responsible for a specific oncodynamic effect. In fact, as many of the mediators can arise from numerous sources within the bone environment, it is often difficult to confirm whether the signals derive from the cancer or the host cells—or both at the same time. Combined with frequent secondary responses to the same or related mediators, the sophisticated interplay between cell types in the bone remains the greatest obstacle for understanding and treating bone cancers. By taking an oncodynamic approach, it allows a different perspective to be applied to this problem. An attempt at reviewing current knowledge of cancer-derived or cancer-induced signalling from the viewpoint of the cell types present in bone may help to make some sense of the complex interactions that can occur.

An admirable attempt at integrating the many mediator molecules controlling bone homeostasis in breast cancer metastasis is provided in a recent review by Rusz and Kahán (see Table 1 in Ref. [170]). This table identifies many of the mediators that are known to be involved in changing Oc, Ob, and tumour cell functions. However, the authors appear to approach the problem from the perspective of how the bone responses will continue the *vicious cycle* that many groups characterize as being a fundamental feature of bone metastasis [171–175]. This cycle directly connects the bone cell responses back to the growth and survival of the cancer cells in a positive feedback loop.

To better fit with our oncodynamic interpretation of bone cancer, and to concentrate primarily on changes induced by the cancer cells, we have developed a similar tabular format but have instead organized the signalling molecules by the cell types present in bone that are impacted by those mediators. This is clearly a non-exhaustive list (see Table 9.1, sorted alphabetically within each cell type), but it provides some of the signalling context for a better understanding of oncodynamic responses in bone.

By examining the list of mediators in Table 9.1, a few general patterns begin to emerge. The most striking pattern is how frequently some of the mediators appear as modulators of different cell types. For example, glutamate appears repeatedly as a mediator and it impacts almost all cell types in bone. This, however, should not be overly surprising since glutamate is a highly conserved chemical signalling molecule that is phylogenetically quite ancient. In fact, eukaryotes used glutamate (a simple and easily accessible amino acid) as a signalling molecule *before* they evolved discrete nervous systems [176]. Most cell types in bone, including cancer cells [177], express various glutamate receptors and transporters [160] and thus have the requisite capacity to communicate via glutamate signals. From the oncodynamic perspective, we have found that glutamate alters the differentiation and functions of Ob and the differentiation (but not the functions) of mature Oc [130]. Glutamate also appears to cause direct stimulatory and inhibitory effects in addition to the more enduring and slower to achieve effects on cell differentiation, further supporting its relevance to normal bone homeostasis.

Table 9.1 Oncodynamic mediators and effects by bone cell type

Cell type	Signalling substance	Source	Effect
Osteoclasts and progenitors	bFGF, FGF-1, FGF-2	Cancer, host, bone	Enhances proliferation
	BMP	Cancer, host, bone	Enhances differentiation
	ET-1	Cancer, host	Directly impairs mobility
	Glutamate	Cancer, host	Enhances differentiation
	Interleukins (multiple)	Cancer, host	Enhances differentiation, survival, and function
	MCP-1	Cancer, host (Ob)	Enhances maturation
	M-CSF	Cancer, host	Enhances differentiation and proliferation
	microRNA	Cancer, host	Enhances differentiation
	OPG	Cancer, host (Ob)	Indirectly inhibits differentiation
	PDGF	Cancer, host, bone	Enhances differentiation
	PTHrP	Cancer	Enhances differentiation
	RANKL	Cancer, host (Ocyt)	Enhances differentiation and survival
	sICAM1	Cancer	Enhances differentiation
	TGF-P	Cancer, host, bone	Enhances function
	TNF-a	Cancer, host	Enhances differentiation
	VEGF	Cancer, host	Enhances differentiation
Osteoblasts and progenitors	BMP	Cancer, host, bone	Directly enhances functions; enhances differentiation
	DKK1	Cancer	Inhibits terminal differentiation
	ET-1	Cancer, host (Ob)	Enhances proliferation and functions
	FGF23	Host (Ob, Ocyt)	Secondarily regulates mineralization
	Glutamate	Cancer, host	Direct activation of functions and differentiation
	Interleukins (IL-18)	Cancer, host (Ob)	Enhances functions
	microRNA	Cancer	Inhibits differentiation
	NPY	Cancer, host	Directly inhibits functions
	PAP	Cancer	Direct activation of functions
	PTH, PTHrP	Cancer, host	Inhibits Ob functions; inhibits differentiation
	Semaphorin 3A	Cancer	Enhances differentiation
	TGF-P	Cancer, host, bone	Indirectly enhances function
	uPA	Cancer	Direct activation of functions

(continued)

Table 9.1 (continued)

Cell type	Signalling substance	Source	Effect
Osteocytes	Glutamate	Cancer, host	Disrupts control over Ob and Oc
	Interleukins (various)	Cancer, host	Stimulates FGF23 release
Bone lining cells	bFGF	Cancer, host, bone	Enhances endosteal bone formation
Stromal cells	bFGF	Cancer, host, bone	Alters functions
	GRP78	Cancer	Enhances activation
	VEGF	Cancer, host	Polarizes macrophages
Hematopoietic cells	DKK1	Cancer, host	Inhibits proliferation
	NPY	Cancer, host	Stabilizes and regulates (hibernation)
	TGF-P	Cancer, host, bone	Inhibits proliferation
	TNF-a	Cancer, host	Inhibits differentiation
	VEGF	Cancer, host	Activates macrophages
Chondrocytes	FGF23	Cancer	Alters cartilage formation
	Glutamate	Cancer, host	Inhibits endochondral ossification and enhances apoptosis
	Protons	Cancer, host	Enhances chondrocyte apoptosis
Blood vessel-related cells	ET-1	Cancer, host (Ob)	Contracts vascular smooth muscle
	NO	Cancer, host	Relaxes vascular smooth muscle; enhances angiogenesis
	VEGF	Cancer, host	Enhances angiogenesis
Neurons	Glutamate	Cancer, host	Nociception; stimulates neurogenesis
	NGF	Cancer, host	Enhances neuron growth
	NPY	Cancer, host	Alters signalling; prevents nerve injury
	Protons	Cancer, host	Nociception

This is a non-exhaustive list of signalling mediators known to alter bone cell functions when cancer invades bone, organized by bone cell type. The mediators are sorted alphabetically within each cell type and both the source(s) and the potential effect(s) of the mediator are noted. Many factors are generated by cancer cells (*cancer*) as well as by host bone cells (*host*), with several also being stored in the bone matrix (*bone*) and released upon bone degradation

Abbreviations: bFGF: basic fibroblast growth factor; BMP: Bone morphogenetic proteins; DKK1: dickkopf 1 protein (a Wnt inhibitor); ET-1: endothelin-1; FGF23: fibroblast growth factor-23; GRP78: glucose-regulated protein-78 (a heat-shock protein); MCP-1: monocyte chemoattractant protein-1; M-CSF: macrophage colony stimulating factor; NGF: nerve growth factor; NO: nitric oxide; NPY: neuropeptide Y; Ob: osteoblast; Oc: osteoclast; Ocyt: osteocyte; OPG: osteoprotegerin; PAP: prostatic acid phosphatase; PDGF: platelet-derived growth factor; PTH: parathyroid hormone; PTHrP: parathyroid hormone-related protein; RANKL: receptor activator of nuclear factor kappa-B ligand; sICAM1: soluble intercellular adhesion molecule-1; TGF-β: transforming growth factor-beta; TNF-α: tumour necrosis factor-alpha; uPA: urokinase-type plasminogen activator; VEGF: vascular endothelial growth factor

There are numerous growth factors and cytokines that appear in multiple locations on the list, and these include vascular endothelial growth factor (VEGF) [178], nerve growth factor (NGF) [179], tumour necrosis factor-alpha (TNF-α) [180], and fibroblast growth factor 23 (FGF23) [146]. These and other similar growth factors are derived from either the invading cancer cells or the various classes of host cells in the environment. Also, included are those that can also be stored in the bone matrix (thus being available from at least three separate sources), including *TGF-β* [158], basic fibroblast growth factor (bFGF) [181], platelet-derived growth factor (PDGF) [182], and BMP [138]. A good list of these bone matrix-derived growth factors is available in a review by Mohan and Baylink [183]. This class of mediators is involved in many of the fundamental processes of bone metabolism, but appears to be also critical for reactive processes related to immune responses and inflammation. In more general terms, many of these cytokines act as predominantly stimulatory or enhancing mediators, and often have effects on multiple cell types simultaneously, implying that they cause more generalized effects rather than being involved in specific cell-type homeostatic control. Furthermore, these effects also impact both mature cell functioning and differentiation of progenitors in very complex ways. A more detailed discussion of the oncodynamic implications of cytokines and growth factors is described in other chapters of this volume.

PTH and *PTHrP* are well-characterized endocrine factors that are secreted by either the cancer cells or the host (typically from the parathyroid glands) and have specific effects that relate to whole-body mineral homeostasis. The effects are described here as being more specific as they induce increases in Oc differentiation and they inhibit Ob both differentiation and function, thus leading to increased bone resorption and calcium mobilization from the skeleton [184]. This is particularly important in osteolytic metastatic breast cancer, as these cells are unable to alter Oc functions directly, and thus use PTHrP to inhibit the opposing cell type (Ob) to achieve the same net result. Roodman provides an excellent review of PTHrP in bone metastasis that is well worth reading [185].

Similar to PTHrP, where the control over functioning is accomplished by skewing the balance between Oc and Ob, the *RANKL* and *OPG* system is understood in considerable detail. In breast cancer, this system operates similarly in many ways to ensure an overall induction of Oc activity. Breast cancer cells can sometimes produce RANKL directly, and this leads to increased Oc differentiation secondarily through its interaction with the receptor RANK expressed on Oc precursors—this receptor–ligand interaction essentially permits other growth factors in the environment to elicit the required Oc differentiation [186]. However, in prostate cancer this system operates differently. Prostate cancer cells secrete OPG which acts as a decoy receptor for RANKL, preventing the soluble RANKL signal from binding to permit the growth factors from causing Oc differentiation. As discussed previously, prostate cancers often cause mixed osteoblastic and osteolytic lesions, and this is partly due to these cancer cells also producing the RANKL signal, interleukin-1, and TNF-α, all of which are associated with enhanced osteoclastogenesis [187]. Often, it is the ratio of RANKL to OPG in the bone environment that determines the resultant phenotype [188].

Another repeating pattern is that there appear to be several highly specific and direct effects on mature Oc and Ob functions that result from small protein-based signals arising from cancer cells. As discussed above, ET-1 is a protein produced by some cancers, which causes direct inhibition of Oc functions by interfering with cell mobility [134] along with a concomitant direct enhancement of Ob functions [133]. The previously described prostate cancer-derived factor uPA also has direct Ob enhancing properties [129]. Direct inhibition of Ob functioning can also be achieved by other small peptides, such as NPY [47] and PAP [128]. These specific and immediately functional responses from exogenous agents stand out as being an unusually precise effect in such a complex system with multiple redundant control systems. By recognizing this pattern, these highly specific proteins and their responses distinguish themselves as being potentially accessible and specific targets for future therapeutic strategies. More typical, however, are the innumerable examples of effector molecules that result in the slower, yet potentially longer lasting changes in cell numbers—that is, by the enhancement or inhibition of precursor differentiation processes.

There are many examples of mediators that serve to enhance Oc differentiation. This strategy for manipulating bone homeostasis may be viewed as a means of amplifying the effectiveness of a small quantity of signal to eventually generate an enduring and robust functional response. This is in contrast to the highly specific and direct effects on a very small number of cells discussed in the previous paragraph—where a small quantity of signal will achieve a small functional effect. In the small molecule category, various microRNA molecules derived from both host and cancer cells have been reported to enhance Oc differentiation. These small ribonucleotide molecules fulfil their communication goals by entering the receiving cell and altering or initiating transcriptional and translational processes in that cell. One specific example of this is *miRNA-223*, and this RNA fragment appears to be critical for Oc differentiation changes [189, 190]. Another small molecule called soluble intercellular adhesion molecule-1 [191] (sICAM1) similarly results in a generalized (but not rapid) increase in Oc number, eventually causing greater osteolysis. An example of a mediator working in the opposite direction to enhance Ob differentiation is the prostate cancer-derived molecule called *semaphorin 3A* [132]. The stimulation of Ob precursor proliferation effectively increases the number of Ob cells and thus increases bone formation. What is most interesting here is that *semaphorin 3a* is a member of a class of chemorepulsant protein inhibitors that are most often described in relation to the nervous system [192]. A protein that normally inhibits or repulses cell movement, in this case, acts as an activator of Ob precursor differentiation. Precursors to cell types other than Oc and Ob are also sensitive to oncodynamic manipulation. Glucose-regulated protein-78 (GRP78) is secreted from cancer cells and can stimulate/activate bone marrow fibroblasts to become cancer-associated fibroblasts [193]. GRP78 is also known as an endoplasmic reticulum chaperone and heat-shock protein when intracellularly located, so its effects (like that of microRNA), although specific, appear to not be a classic receptor–ligand interaction.

In contrast to the numerous enhancers of bone cell precursors, very few mediator molecules have been characterized that inhibit precursor differentiation, although, as described above, prostate cancer-derived OPG achieves this Oc-precursor inhibitory function indirectly. Since Oc cells are derived from hematopoietic progenitor cells in the busy bone marrow space, it is possible that the multipotent precursors that may eventually become Oc cells are not a very specific or practical target for such a subtle modulation. There are, however, a few molecules that can inhibit Ob progenitor development, and one example is dickkopf-1 (DKK1), a secreted protein known to be a Wnt signalling inhibitor. DKK1 is produced by myeloma and Ocyt cells, and it inhibits Ob differentiation to reduce the total number of Ob and thus decrease bone deposition [116]. This protein also has an 'enhancing' function with endothelial cell progenitors, causing these cells to have greater angiogenesis potential [194]. It is likely that precursor redundancy or anatomical location may be important factors in determining how easy it is to interfere with cell-specific precursor development.

Several nontraditional signalling molecules also are involved in oncodynamic bone cell responses. However, these molecules generate what may be described as more non-specific responses in comparison to the highly specific protein-based mediators described above. A good example is the response by bone cells to low levels of nitric oxide (NO), often generated by activated macrophages. This gaseous mediator can cause vessel relaxation and angiogenesis [195], which may sufficiently change the physiological environment to achieve functional responses. Even more atypical stimulation occurs from simple hydrogen atoms, or *protons*, which are liberated by many metabolic reactions in the bone. Chondrocytes possess G-protein coupled receptors that sense protons and this, in essence, becomes an acid-sensing system to detect levels of Oc-mediated bone resorption. These receptors, combined with the correct calcium environment, then promote chondrocyte apoptosis in advance of Ob bone mineralization [196]. Protons are also quite relevant to the sensory functions of neurons in the bone. It is commonly thought that the highly acidic environment (high numbers of protons) generated by Oc may be an initiator for the perception of bone pain due to excessive osteolysis [167, 197, 198]. This model suggests that acid-sensing ion channels present on the sensory neurons in bone receive these protons and respond electrically to be perceived eventually in the brain as pain [166]. However, we also suggest that the ubiquitous glutamate molecule secreted by cancer cells, also being an amino acid and a copious proton donor, could also serve the same function in nociception. This process could easily be signalled via acid-sensing ion channels and/or through specific glutamate receptor systems expressed by the peripheral sensory neurons present throughout the bone environment.

The overall patterns of mediators for oncodynamic processes in bone discuss herein seem to fall into at least five discrete categories. These are [1] simple and redundant amino acid signalling systems that are involved in normal bone homeostasis, but can be co-opted by cancer cells to disrupt effective communication between cells; [2] multifunctional but somewhat non-specific growth factor/cytokine-like mechanisms affecting many cells simultaneously; [3]

well-characterized endocrine and paracrine factors that are intimately involved with normal bone homeostasis but can be also be leveraged by cancer cells to change the environment; [4] specific and direct functional effects on mature bone cells by proteins (usually) that are produced by cancer cells or are already present in the bone environment (both inhibitory and stimulatory effects); and [5] enhancement of differentiation of bone cell precursors, frequently by small molecules, but only with limited examples of inhibition of bone cell precursor differentiation.

These emerging patterns suggest that there are some fundamental processes that may be more easily targeted in the bone, and depending on the nature of the invading cancer cells, these processes will be impacted differentially. Cell-type specific effects certainly can occur, but most frequent are enhancements of differentiation rather than interference with proliferation. This may be a result of anatomical or physiological barriers that make precursor inhibition a less controllable effect. Use of the precursor route for achieving functional changes can be viewed as an efficient adaptation that maximizes the response with the smallest mediator intervention. Although highly specific and direct functional responses occur, these may actually represent the most accessible targets for therapeutic interventions.

Conclusions

Oncodynamics, or how the body responds to the invasion of cancer cells, is a theoretical construct that parallels the concept of pharmacodynamics. By taking the view of examining the effects of cancer on normal physiological and anatomical processes from the perspective of the host cells, the oncodynamic approach may provide novel insights into how cancer may be treated. The bone is a frequent target of cancer, whether as a primary site for the development of a tumour, or a destination in which cancers take up residency. This chapter provides the basic context for the bone as an environment in which cancers can grow. This is first achieved by defining the types of cells that are in bone and by redefining which cell types should be included on that list. Followed by a brief description of the functions of bone as an organ/tissue system, it reviews the cancers that frequently are associated with the bone. The corresponding changes that occur in bone functions following cancer invasion are then characterized, based primarily on the functions of bone and the cell types involved in those processes. Perhaps, the most valuable aspect of the oncodynamic approach was to provide a fresh look at not only the chemical mediators that participate in bone cell responses, but also the emerging patterns of mediator-response associations that appear to occur with higher frequencies in bone cancers. This integration of dynamic bone responses and mediators revealed that there are several fundamental strategies that are used to realize functional changes in bone metabolism. These strategies may not have been recognized if a traditional cancer cell-centric viewpoint was used, since the advantage of oncodynamics is in

simplifying the variables to focus specifically on what a cancer cell does to the host. With these insights, novel therapeutic strategies may be more successful if they address the more readily targetable and specific disruptions in bone functions that occur, rather than the indirect and subtle changes that involve bone cell progenitor differentiation. Oncodynamics appears to be a very useful approach for identifying potential opportunities to exert control over pathological disruptions in bone homeostasis, and this is achieved by pursuing a better understanding of the cells, processes, and mediators that maintain normal bone structure and functions.

References

1. Van PC, Somerfield MR, Bast RC, Cristofanilli M, Goetz MP, Gonzalez-Angulo AM, et al. Use of biomarkers to guide decisions on systemic therapy for women with metastatic breast cancer: American Society of Clinical Oncology Clinical Practice Guideline. J Clin Oncol. 2015;33(24):2695–704.
2. Wagner DO, Aspenberg P. Where did bone come from? Acta Orthop. 2011;82(4):393–8.
3. Donoghue PC, Sansom IJ. Origin and early evolution of vertebrate skeletonization. Microsc Res Tech. 2002;59(5):352–72.
4. Sahni A. Biomineralization: some complex crystallite-oriented skeletal structures. J Biosci. 2013;38(5):925–35.
5. Ruben JA, Bennett AA. The evolution of bone. Evolution. 1987;41(6):1187–97.
6. Donoghue PC, Sansom IJ, Downs JP. Early evolution of vertebrate skeletal tissues and cellular interactions, and the canalization of skeletal development. J Exp Zool B Mol Dev Evol. 2006;306(3):278–94.
7. Herberger AL, Loretz CA. Vertebrate extracellular calcium-sensing receptor evolution: selection in relation to life history and habitat. Comp Biochem Physiol Part D Genomics Proteomics. 2013;8(1):86–94.
8. Taichman RS. Blood and bone: two tissues whose fates are intertwined to create the hematopoietic stem-cell niche. Blood. 2005;105(7):2631–9.
9. Oldknow KJ, MacRae VE, Farquharson C. Endocrine role of bone: recent and emerging perspectives beyond osteocalcin. J Endocrinol. 2015;225(1):R1–19.
10. Dallas SL, Prideaux M, Bonewald LF. The osteocyte: an endocrine cell … and more. Endocr Rev. 2013;34(5):658–90.
11. Hu MC, Shiizaki K, Kuro-o M, Moe OW. Fibroblast growth factor 23 and Klotho: physiology and pathophysiology of an endocrine network of mineral metabolism. Annu Rev Physiol. 2013;75:503–33.
12. Schwetz V, Pieber T, Obermayer-Pietsch B. The endocrine role of the skeleton: background and clinical evidence. Eur J Endocrinol. 2012;166(6):959–67.
13. Zhang Q, Riddle RC, Clemens TL. Bone and the regulation of global energy balance. J Intern Med. 2015;277(6):681–9.
14. Karsenty G, Oury F. Regulation of male fertility by the bone-derived hormone osteocalcin. Mol Cell Endocrinol. 2014;382(1):521–6.
15. Oury F, Sumara G, Sumara O, Ferron M, Chang H, Smith CE, et al. Endocrine regulation of male fertility by the skeleton. Cell. 2011;144(5):796–809.
16. Oury F, Ferron M, Huizhen W, Confavreux C, Xu L, Lacombe J, et al. Osteocalcin regulates murine and human fertility through a pancreas-bone-testis axis. J Clin Invest. 2013;123(6):2421–33.
17. D'Amelio P, Panico A, Spertino E, Isaia GC. Energy metabolism and the skeleton: reciprocal interplay. World J Orthop. 2012;3(11):190–8.

18. Andersen TL, Sondergaard TE, Skorzynska KE, Dagnaes-Hansen F, Plesner TL, Hauge EM, et al. A physical mechanism for coupling bone resorption and formation in adult human bone. Am J Pathol. 2009;174(1):239–47.
19. Kular J, Tickner J, Chim SM, Xu J. An overview of the regulation of bone remodelling at the cellular level. Clin Biochem. 2012;45(12):863–73.
20. Hinoi E, Takarada T, Yoneda Y. Glutamate signaling system in bone. J Pharmacol Sci. 2004;94(3):215–20.
21. Palumbo C, Ferretti M, Ardizzoni A, Zaffe D, Marotti G. Osteocyte-osteoclast morphological relationships and the putative role of osteocytes in bone remodeling. J Musculoskelet Neuronal Interact. 2001;1(4):327–32.
22. Skerry TM. The role of glutamate in the regulation of bone mass and architecture. J Musculoskelet Neuronal Interact. 2008;8(2):166–73.
23. Stains JP, Civitelli R. Cell-to-cell interactions in bone. Biochem Biophys Res Commun. 2005;328(3):721–7.
24. Florencio-Silva R, Sasso GR, Sasso-Cerri E, Simoes MJ, Cerri PS. Biology of bone tissue: structure, function, and factors that influence bone cells. Biomed Res Int. 2015;2015:421746.
25. Horowitz MC, Lorenzo JA. The origins of osteoclasts. Curr Opin Rheumatol. 2004;16 (4):464–8.
26. Baron R. Polarity and membrane transport in osteoclasts. Connect Tissue Res. 1989;20 (1–4):109–20.
27. Morimoto R, Uehara S, Yatsushiro S, Juge N, Hua Z, Senoh S, et al. Secretion of L-glutamate from osteoclasts through transcytosis. EMBO J. 2006;25(18):4175–86.
28. Blair HC, Athanasou NA. Recent advances in osteoclast biology and pathological bone resorption. Histol Histopathol. 2004;19(1):189–99.
29. Teitelbaum SL. Bone resorption by osteoclasts. Science. 2000;289(5484):1504–8.
30. Kylmaoja E, Nakamura M, Tuukkanen J. Osteoclasts and remodeling based bone formation. Curr Stem Cell Res Ther. 2015;19.
31. Cappariello A, Maurizi A, Veeriah V, Teti A. The great beauty of the osteoclast. Arch Biochem Biophys. 2014;15(558):70–8.
32. Charles JF, Aliprantis AO. Osteoclasts: more than 'bone eaters'. Trends Mol Med. 2014;20 (8):449–59.
33. Malaval L, Liu F, Roche P, Aubin JE. Kinetics of osteoprogenitor proliferation and osteoblast differentiation in vitro. J Cell Biochem. 1999;74(4):616–27.
34. Capulli M, Paone R, Rucci N. Osteoblast and osteocyte: games without frontiers. Arch Biochem Biophys. 2014;1(561):3–12.
35. Aubin JE. Regulation of osteoblast formation and function. Rev Endocr Metab Disord. 2001;2(1):81–94.
36. Schaffler MB, Kennedy OD. Osteocyte signaling in bone. Curr Osteoporos Rep. 2012;10 (2):118–25.
37. Rosenberg N, Rosenberg O, Soudry M. Osteoblasts in bone physiology-mini review. Rambam Maimonides Med J. 2012;3(2):e0013.
38. Krishnan V, Dhurjati R, Vogler EA, Mastro AM. Osteogenesis in vitro: from pre-osteoblasts to osteocytes: a contribution from the Osteobiology Research Group, The Pennsylvania State University. Vitro Cell Dev Biol Anim. 2010;46(1):28–35.
39. Kamioka H, Sugawara Y, Honjo T, Yamashiro T, Takano-Yamamoto T. Terminal differentiation of osteoblasts to osteocytes is accompanied by dramatic changes in the distribution of actin-binding proteins. J Bone Miner Res. 2004;19(3):471–8.
40. Bellido T. Osteocyte-driven bone remodeling. Calcif Tissue Int. 2014;94(1):25–34.
41. Neve A, Corrado A, Cantatore FP. Osteocytes: central conductors of bone biology in normal and pathological conditions. Acta Physiol (Oxf). 2012;204(3):317–30.
42. Bonewald LF. The amazing osteocyte. J Bone Miner Res. 2011;26(2):229–38.
43. Tomkinson A, Reeve J, Shaw RW, Noble BS. The death of osteocytes via apoptosis accompanies estrogen withdrawal in human bone. J Clin Endocrinol Metab. 1997;82 (9):3128–35.

44. Tanaka-Kamioka K, Kamioka H, Ris H, Lim SS. Osteocyte shape is dependent on actin filaments and osteocyte processes are unique actin-rich projections. J Bone Miner Res. 1998;13(10):1555–68.
45. Buenzli PR, Sims NA. Quantifying the osteocyte network in the human skeleton. Bone. 2015;75:144–50.
46. Winkler DG, Sutherland MK, Geoghegan JC, Yu C, Hayes T, Skonier JE, et al. Osteocyte control of bone formation via sclerostin, a novel BMP antagonist. EMBO J. 2003;22 (23):6267–76.
47. Igwe JC, Jiang X, Paic F, Ma L, Adams DJ, Baldock PA, et al. Neuropeptide Y is expressed by osteocytes and can inhibit osteoblastic activity. J Cell Biochem. 2009;108(3):621–30.
48. Fumoto T, Takeshita S, Ito M, Ikeda K. Physiological functions of osteoblast lineage and T cell-derived RANKL in bone homeostasis. J Bone Miner Res. 2014;29(4):830–42.
49. Xiong J, Piemontese M, Onal M, Campbell J, Goellner JJ, Dusevich V, et al. Osteocytes, not osteoblasts or lining cells, are the main source of the RANKL required for osteoclast formation in remodeling bone. PLoS ONE. 2015;10(9):e0138189.
50. Bellido T, Ali AA, Gubrij I, Plotkin LI, Fu Q, O'Brien CA, et al. Chronic elevation of parathyroid hormone in mice reduces expression of sclerostin by osteocytes: a novel mechanism for hormonal control of osteoblastogenesis. Endocrinology. 2005;146(11): 4577–83.
51. Lara-Castillo N, Kim-Weroha NA, Kamel MA, Javaheri B, Ellies DL, Krumlauf RE, et al. In vivo mechanical loading rapidly activates beta-catenin signaling in osteocytes through a prostaglandin mediated mechanism. Bone. 2015;76:58–66.
52. Brakspear K, Mason D. Glutamate signalling in bone. Front Endocrinol. 2012;3(89).
53. Huggett JF, Mustafa A, O'neal L, Mason DJ. The glutamate transporter GLAST-1 (EAAT-1) is expressed in the plasma membrane of osteocytes and is responsive to extracellular glutamate concentration. Biochem Soc Trans. 2002;30(Pt 6):890–3.
54. Mason DJ, Suva LJ, Genever PG, Patton AJ, Steuckle S, Hillam RA, et al. Mechanically regulated expression of a neural glutamate transporter in bone: a role for excitatory amino acids as osteotropic agents? Bone. 1997;20(3):199–205.
55. Szczesniak AM, Gilbert RW, Mukhida M, Anderson GI. Mechanical loading modulates glutamate receptor subunit expression in bone. Bone. 2005;37(1):63–73.
56. Nomura S, Takano-Yamamoto T. Molecular events caused by mechanical stress in bone. Matrix Biol. 2000;19(2):91–6.
57. Brady RT, O'Brien FJ, Hoey DA. Mechanically stimulated bone cells secrete paracrine factors that regulate osteoprogenitor recruitment, proliferation, and differentiation. Biochem Biophys Res Commun. 2015;459(1):118–23.
58. Norimatsu H, Yamamoto T, Ozawa H, Talmage RV. Changes in calcium phosphate on bone surfaces and in lining cells after the administration of parathyroid hormone or calcitonin. Clin Orthop Relat Res. 1982;164:271–8.
59. Norimatsu H, Wiel CJ, Talmage RV. Morphological support of a role for cells lining bone surfaces in maintenance of plasma calcium concentration. Clin Orthop Relat Res. 1979;138:254–62.
60. Mach DB, Rogers SD, Sabino MC, Luger NM, Schwei MJ, Pomonis JD, et al. Origins of skeletal pain: sensory and sympathetic innervation of the mouse femur. Neuroscience. 2002;113(1):155–66.
61. Allen MR, Hock JM, Burr DB. Periosteum: biology, regulation, and response to osteoporosis therapies. Bone. 2004;35(5):1003–12.
62. Chang MK, Raggatt LJ, Alexander KA, Kuliwaba JS, Fazzalari NL, Schroder K, et al. Osteal tissue macrophages are intercalated throughout human and mouse bone lining tissues and regulate osteoblast function in vitro and in vivo. J Immunol. 2008;181(2):1232–44.
63. Franz-Odendaal TA, Hall BK, Witten PE. Buried alive: how osteoblasts become osteocytes. Dev Dyn. 2006;235(1):176–90.

64. Sanchez-Ramos J, Song S, Cardozo-Pelaez F, Hazzi C, Stedeford T, Willing A, et al. Adult bone marrow stromal cells differentiate into neural cells in vitro. Exp Neurol. 2000;164 (2):247–56.
65. Chamberlain G, Fox J, Ashton B, Middleton J. Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. Stem Cells. 2007;25(11):2739–49.
66. Abdallah BM, Al-Shammary A, Skagen P, Abu DR, Adjaye J, Aldahmash A, et al. CD34 defines an osteoprogenitor cell population in mouse bone marrow stromal cells. Stem Cell Res. 2015;15(3):449–58.
67. Modder UI, Khosla S. Skeletal stem/osteoprogenitor cells: current concepts, alternate hypotheses, and relationship to the bone remodeling compartment. J Cell Biochem. 2008;103 (2):393–400.
68. Addison WN, Fu MM, Yang HX, Lin Z, Nagano K, Gori F, et al. Direct transcriptional repression of Zfp423 by Zfp521 mediates a bone morphogenic protein-dependent osteoblast versus adipocyte lineage commitment switch. Mol Cell Biol. 2014;34(16):3076–85.
69. Takarada-Iemata M, Takarada T, Nakamura Y, Nakatani E, Hori O, Yoneda Y. Glutamate preferentially suppresses osteoblastogenesis than adipogenesis through the cystine/glutamate antiporter in mesenchymal stem cells. J Cell Physiol. 2011;226(3):652–65.
70. Chang Y, Li H, Guo Z. Mesenchymal stem cell-like properties in fibroblasts. Cell Physiol Biochem. 2014;34(3):703–14.
71. Langevin HM, Cornbrooks CJ, Taatjes DJ. Fibroblasts form a body-wide cellular network. Histochem Cell Biol. 2004;122(1):7–15.
72. Yamamoto K, Kishida T, Sato Y, Nishioka K, Ejima A, Fujiwara H, et al. Direct conversion of human fibroblasts into functional osteoblasts by defined factors. Proc Natl Acad Sci USA. 2015;112(19):6152–7.
73. Wu AC, Raggatt LJ, Alexander KA, Pettit AR. Unraveling macrophage contributions to bone repair. Bonekey Rep. 2013;2:373.
74. Alexander KA, Chang MK, Maylin ER, Kohler T, Muller R, Wu AC, et al. Osteal macrophages promote in vivo intramembranous bone healing in a mouse tibial injury model. J Bone Miner Res. 2011;26(7):1517–32.
75. Hume DA, Loutit JF, Gordon S. The mononuclear phagocyte system of the mouse defined by immunohistochemical localization of antigen F4/80: macrophages of bone and associated connective tissue. J Cell Sci. 1984;66:189–94.
76. Korolnek T, Hamza I. Macrophages and iron trafficking at the birth and death of red cells. Blood. 2015;125(19):2893–7.
77. Boisset JC, Robin C. On the origin of hematopoietic stem cells: progress and controversy. Stem Cell Res. 2012;8(1):1–13.
78. Mosaad YM. Hematopoietic stem cells: an overview. Transfus Apher Sci. 2014;51(3):68–82.
79. Nombela-Arrieta C, Pivarnik G, Winkel B, Canty KJ, Harley B, Mahoney JE, et al. Quantitative imaging of haematopoietic stem and progenitor cell localization and hypoxic status in the bone marrow microenvironment. Nat Cell Biol. 2013;15(5):533–43.
80. Anthony BA, Link DC. Regulation of hematopoietic stem cells by bone marrow stromal cells. Trends Immunol. 2014;35(1):32–7.
81. Millan JL. The role of phosphatases in the initiation of skeletal mineralization. Calcif Tissue Int. 2013;93(4):299–306.
82. Zerega B, Cermelli S, Bianco P, Cancedda R, Cancedda FD. Parathyroid hormone [PTH (1-34)] and parathyroid hormone-related protein [PTHrP(1-34)] promote reversion of hypertrophic chondrocytes to a prehypertrophic proliferating phenotype and prevent terminal differentiation of osteoblast-like cells. J Bone Miner Res. 1999;14(8):1281–9.
83. Bianco P, Cancedda FD, Riminucci M, Cancedda R. Bone formation via cartilage models: the “borderline” chondrocyte. Matrix Biol. 1998;17(3):185–92.
84. Shapiro IM, Golub EE, Kakuta S, Hazelgrove J, Havery J, Chance B, et al. Initiation of endochondral calcification is related to changes in the redox state of hypertrophic chondrocytes. Science. 1982;217(4563):950–2.

85. Piepoli T, Mennuni L, Zerbi S, Lanza M, Rovati LC, Caselli G. Glutamate signaling in chondrocytes and the potential involvement of NMDA receptors in cell proliferation and inflammatory gene expression. Osteoarthritis Cartilage. 2009;17(8):1076–83.
86. Wang L, Hinoi E, Takemori A, Yoneda Y. Release of endogenous glutamate by AMPA receptors expressed in cultured rat costal chondrocytes. Biol Pharm Bull. 2005;28(6):990–3.
87. Wang L, Hinoi E, Takemori A, Nakamichi N, Yoneda Y. Glutamate inhibits chondral mineralization through apoptotic cell death mediated by retrograde operation of the cystine/glutamate antiporter. J Biol Chem. 2006;281(34):24553–65.
88. Islam A, Glomski C, Henderson ES. Endothelial cells and hematopoiesis: a light microscopic study of fetal, normal, and pathologic human bone marrow in plastic-embedded sections. Anat Rec. 1992;233(3):440–52.
89. Majesky MW, Dong XR, Hoglund V, Mahoney WM Jr, Daum G. The adventitia: a dynamic interface containing resident progenitor cells. Arterioscler Thromb Vasc Biol. 2011;31 (7):1530–9.
90. Edwards JR, Williams K, Kindblom LG, Meis-Kindblom JM, Hogendoorn PC, Hughes D, et al. Lymphatics and bone. Hum Pathol. 2008;39(1):49–55.
91. Masi L. Crosstalk between the brain and bone. Clin Cases Miner Bone Metab. 2012;9 (1):13–6.
92. Serre CM, Farlay D, Delmas PD, Chenu C. Evidence for a dense and intimate innervation of the bone tissue, including glutamate-containing fibers. Bone. 1999;25(6):623–9.
93. Bataille C, Mauprivez C, Hay E, Baroukh B, Brun A, Chaussain C, et al. Different sympathetic pathways control the metabolism of distinct bone envelopes. Bone. 2012;50 (5):1162–72.
94. Takeda S, Elefteriou F, Levasseur R, Liu X, Zhao L, Parker KL, et al. Leptin regulates bone formation via the sympathetic nervous system. Cell. 2002;111(3):305–17.
95. Jimenez-Andrade JM, Mantyh WG, Bloom AP, Xu H, Ferng AS, Dussor G, et al. A phenotypically restricted set of primary afferent nerve fibers innervate the bone versus skin: therapeutic opportunity for treating skeletal pain. Bone. 2010;46(2):306–13.
96. Peters CM, Ghilardi JR, Keyser CP, Kubota K, Lindsay TH, Luger NM, et al. Tumor-induced injury of primary afferent sensory nerve fibers in bone cancer pain. Exp Neurol. 2005;193(1):85–100.
97. Hill EL, Turner R, Elde R. Effects of neonatal sympathectomy and capsaicin treatment on bone remodeling in rats. Neuroscience. 1991;44(3):747–55.
98. Jimenez-Andrade JM, Mantyh WG, Bloom AP, Freeman KT, Ghilardi JR, Kuskowski MA, et al. The effect of aging on the density of the sensory nerve fiber innervation of bone and acute skeletal pain. Neurobiol Aging. 2012;33(5):921–32.
99. Sottnik JL, Campbell B, Mehra R, Behbahani-Nejad O, Hall CL, Keller ET. Osteocytes serve as a progenitor cell of osteosarcoma. J Cell Biochem. 2014;115(8):1420–9.
100. Lin PP, Wang Y, Lozano G. Mesenchymal stem cells and the origin of Ewing's sarcoma. Sarcoma. 2011;2011.
101. Tanaka M, Yamazaki Y, Kanno Y, Igarashi K, Aisaki K, Kanno J, et al. Ewing's sarcoma precursors are highly enriched in embryonic osteochondrogenic progenitors. J Clin Invest. 2014;124(7):3061–74.
102. Pinder MA, Dibardino DJ, Collier AB, Knudson JD. Chondrosarcoma presenting with pulmonary embolism in a 9-year-old girl: a case report. Cardiol Young. 2015;25(2):384–7.
103. Rodriguez A, Roubertie F, Thumerel M, Jougon J. Pulmonary metastatic chondrosarcoma with massive extension into left atrium and left ventricle: outcome of surgical management in emergency. Eur J Cardiothorac Surg. 2013;44(5):e341–2.
104. Kyle RA, Rajkumar SV. Criteria for diagnosis, staging, risk stratification and response assessment of multiple myeloma. Leukemia. 2009;23(1):3–9.
105. Coleman RE. Skeletal complications of malignancy. Cancer. 1997;80(8 Suppl):1588–94.
106. Paget S. The distribution of secondary growths in cancer of the breast. Lancet. 1889;29 (1):571–3.

107. Coleman RE. Clinical features of metastatic bone disease and risk of skeletal morbidity. Clin Cancer Res. 2006;12(20 Pt 2):6243s–9s.
108. Oster G, Lamerato L, Glass AG, Richert-Boe KE, Lopez A, Chung K, et al. Natural history of skeletal-related events in patients with breast, lung, or prostate cancer and metastases to bone: a 15-year study in two large US health systems. Support Care Cancer. 2013;21 (12):3279–86.
109. Gokaslan ZL, Aladag MA, Ellerhorst JA. Melanoma metastatic to the spine: a review of 133 cases. Melanoma Res. 2000;10(1):78–80.
110. Hartwich JE, Orr WS, Ng CY, Spence Y, McLaughlin JM, Furman WL, et al. Rapamycin increases neuroblastoma xenograft and host stromal derived osteoprotegerin inhibiting osteolytic bone disease in a bone metastasis model. J Pediatr Surg. 2013;48(1):47–55.
111. DiSibio G, French SW. Metastatic patterns of cancers: results from a large autopsy study. Arch Pathol Lab Med 2008;132(6):931–9.
112. Zhang M, Sun J. Bone metastasis from ovarian cancer. clinical analysis of 26 cases. Saudi Med J. 2013;34(12):1270–3.
113. Sehouli J, Olschewski J, Schotters V, Fotopoulou C, Pietzner K. Prognostic role of early versus late onset of bone metastasis in patients with carcinoma of the ovary, peritoneum and fallopian tube. Ann Oncol. 2013;24(12):3024–8.
114. Hillner BE, Ingle JN, Chlebowski RT, Gralow J, Yee GC, Janjan NA, et al. American Society of Clinical Oncology 2003 update on the role of bisphosphonates and bone health issues in women with breast cancer. J Clin Oncol. 2003;21(21):4042–57.
115. Coleman R, Body JJ, Aapro M, Hadji P, Herrstedt J. Bone health in cancer patients: ESMO Clinical Practice Guidelines. Ann Oncol. 2014;25(Suppl 3):iii124–iii137.
116. He J, Liu Z, Zheng Y, Qian J, Li H, Lu Y, et al. p38 MAPK in myeloma cells regulates osteoclast and osteoblast activity and induces bone destruction. Cancer Res. 2012;72 (24):6393–402.
117. Choi B, Lee EJ, Song DH, Yoon SC, Chung YH, Jang Y, et al. Elevated Pentraxin 3 in bone metastatic breast cancer is correlated with osteolytic function. Oncotarget. 2014;5(2):481–92.
118. Gartrell BA, Coleman R, Efstathiou E, Fizazi K, Logothetis CJ, Smith MR, et al. Metastatic prostate cancer and the bone: significance and therapeutic options. Eur Urol. 2015;68 (5):850–8.
119. Mathew A, Brufsky A. Bisphosphonates in breast cancer. Int J Cancer. 2015;137(4):753–64.
120. Granchi D, Baglio SR, Amato I, Giunti A, Baldini N. Paracrine inhibition of osteoblast differentiation induced by neuroblastoma cells. Int J Cancer. 2008;123(7):1526–35.
121. Lee JW, Chung HY, Ehrlich LA, Jelinek DF, Callander NS, Roodman GD, et al. IL-3 expression by myeloma cells increases both osteoclast formation and growth of myeloma cells. Blood. 2004;103(6):2308–15.
122. Ehrlich LA, Chung HY, Ghobrial I, Choi SJ, Morandi F, Colla S, et al. IL-3 is a potential inhibitor of osteoblast differentiation in multiple myeloma. Blood. 2005;106(4):1407–14.
123. McCoy EM, Hong H, Pruitt HC, Feng X. IL-11 produced by breast cancer cells augments osteoclastogenesis by sustaining the pool of osteoclast progenitor cells. BMC Cancer. 2013;13:16.
124. Fromigue O, Kheddoumi N, Lomri A, Marie PJ, Body JJ. Breast cancer cells release factors that induced apoptosis in human bone marrow stromal cells. J Bone Miner Res. 2001;16 (9):1600–10.
125. Ibrahim T, Flamini E, Mercatali L, Sacanna E, Serra P, Amadori D. Pathogenesis of osteoblastic bone metastases from prostate cancer. Cancer. 2010;116(6):1406–18.
126. Roudier MP, Morrissey C, True LD, Higano CS, Vessella RL, Ott SM. Histopathological assessment of prostate cancer bone osteoblastic metastases. J Urol. 2008;180(3):1154–60.
127. Seeman E. Bone quality: the material and structural basis of bone strength. J Bone Miner Metab. 2008;26(1):1–8.
128. Larson SR, Chin J, Zhang X, Brown LG, Coleman IM, Lakely B, et al. Prostate cancer derived prostatic acid phosphatase promotes an osteoblastic response in the bone microenvironment. Clin Exp Metastasis. 2014;31(2):247–56.

129. Rabbani SA, Desjardins J, Bell AW, Banville D, Mazar A, Henkin J, et al. An amino-terminal fragment of urokinase isolated from a prostate cancer cell line (PC-3) is mitogenic for osteoblast-like cells. Biochem Biophys Res Commun. 1990;173(3):1058–64.
130. Seidlitz EP, Sharma MK, Singh G. Extracellular glutamate alters mature osteoclast and osteoblast functions. Can J Physiol Pharmacol. 2010;88(9):929–36.
131. Alam AS, Gallagher A, Shankar V, Ghatei MA, Datta HK, Huang CL, et al. Endothelin inhibits osteoclastic bone resorption by a direct effect on cell motility: implications for the vascular control of bone resorption. Endocrinology. 1992;130(6):3617–24.
132. Liu F, Shen W, Qiu H, Hu X, Zhang C, Chu T. Prostate cancer cells induce osteoblastic differentiation via semaphorin 3A. Prostate. 2015;75(4):370–80.
133. Chiao JW, Moonga BS, Yang YM, Kancherla R, Mittelman A, Wu-Wong JR, et al. Endothelin-1 from prostate cancer cells is enhanced by bone contact which blocks osteoclastic bone resorption. Br J Cancer. 2000;83(3):360–5.
134. Yin JJ, Mohammad KS, Kakonen SM, Harris S, Wu-Wong JR, Wessale JL, et al. A causal role for endothelin- in the pathogenesis of osteoblastic bone metastases. Proc Natl Acad Sci USA. 2003;100(19):10954–9.
135. Udagawa N, Takahashi N, Jimi E, Matsuzaki K, Tsurukai T, Itoh K, et al. Osteoblasts/stromal cells stimulate osteoclast activation through expression of osteoclast differentiation factor/RANKL but not macrophage colony-stimulating factor: receptor activator of NF-kappa B ligand. Bone. 1999;25(5):517–23.
136. Udagawa N, Takahashi N, Yasuda H, Mizuno A, Itoh K, Ueno Y, et al. Osteoprotegerin produced by osteoblasts is an important regulator in osteoclast development and function. Endocrinology. 2000;141(9):3478–84.
137. Keller ET, Brown J. Prostate cancer bone metastases promote both osteolytic and osteoblastic activity. J Cell Biochem. 2004;91(4):718–29.
138. Bunyaratavej P, Hullinger TG, Somerman MJ. Bone morphogenetic proteins secreted by breast cancer cells upregulate bone sialoprotein expression in preosteoblast cells. Exp Cell Res. 2000;260(2):324–33.
139. Kassen D, Moore S, Percy L, Herledan G, Bounds D, Rodriguez-Justo M, et al. The bone marrow stromal compartment in multiple myeloma patients retains capability for osteogenic differentiation in vitro: defining the stromal defect in myeloma. Br J Haematol. 2014;167 (2):194–206.
140. Seidlitz EP, Sharma MK, Singh G. A by-product of glutathione production in cancer cells may cause disruption in bone metabolic processes. Can J Physiol Pharmacol. 2010;88 (3):197–203.
141. Lieben L, Carmeliet G. The delicate balance between vitamin D, calcium and bone homeostasis: lessons learned from intestinal- and osteocyte-specific VDR null mice. J Steroid Biochem Mol Biol. 2013;136:102–6.
142. Masuyama R. Role of local vitamin D signaling and cellular calcium transport system in bone homeostasis. J Bone Miner Metab. 2014;32(1):1–9.
143. Lieben L, Carmeliet G, Masuyama R. Calcemic actions of vitamin D: effects on the intestine, kidney and bone. Best Pract Res Clin Endocrinol Metab. 2011;25(4):561–72.
144. Hewer W, Stark HW. Psychiatric disorders in elderly patients caused by disturbed calcium metabolism. Fortschr Neurol Psychiatr. 2010;78(3):161–7.
145. Babar G, Alemzadeh R. A case of acute psychosis in an adolescent male. Case Rep Endocrinol. 2014;2014:937631.
146. Bergwitz C, Juppner H. Regulation of phosphate homeostasis by PTH, vitamin D, and FGF23. Annu Rev Med. 2010;61:91–104.
147. Lederer E. Regulation of serum phosphate. J Physiol. 2014;592(Pt 18):3985–95.
148. Hautmann AH, Hautmann MG, Kolbl O, Herr W, Fleck M. Tumor-induced osteomalacia: an up-to-date review. Curr Rheumatol Rep. 2015;17(6):512.
149. Dallas SL, Rosser JL, Mundy GR, Bonewald LF. Proteolysis of latent transforming growth factor-beta (TGF-beta)-binding protein-1 by osteoclasts. A cellular mechanism for release of TGF-beta from bone matrix. J Biol Chem. 2002;277(24):21352–60.

150. Pfeilschifter J, Bonewald L, Mundy GR. Characterization of the latent transforming growth factor beta complex in bone. J Bone Miner Res. 1990;5(1):49–58.
151. Meng X, Vander AA, Lee P, Hostetter G, Bhowmick NA, Matrisian LM, et al. Myeloid-specific TGF-beta signaling in bone promotes basic-FGF and breast cancer bone metastasis. Oncogene. 2015.
152. Ota K, Quint P, Ruan M, Pederson L, Westendorf JJ, Khosla S, et al. TGF-beta induces Wnt10b in osteoclasts from female mice to enhance coupling to osteoblasts. Endocrinology. 2013;154(10):3745–52.
153. Duivenvoorden WC, Popovic SV, Lhotak S, Seidlitz E, Hirte HW, Tozer RG, et al. Doxycycline decreases tumor burden in a bone metastasis model of human breast cancer. Cancer Res. 2002;62(6):1588–91.
154. Saikali Z, Singh G. Doxycycline and other tetracyclines in the treatment of bone metastasis. Anticancer Drugs. 2003;14(10):773–8.
155. Seidlitz E, Saikali Z, Singh G. Use of tetracyclines for bone metastases. In: Singh G, Rabbani S, editors. Bone metastasis: experimental and clinical therapeutics. Totowa: Humana Press Inc.; 2005. p. 295–305.
156. Casbon AJ, Reynaud D, Park C, Khuc E, Gan DD, Schepers K, et al. Invasive breast cancer reprograms early myeloid differentiation in the bone marrow to generate immunosuppressive neutrophils. Proc Natl Acad Sci USA. 2015;112(6):E566–75.
157. Schepers K, Pietras EM, Reynaud D, Flach J, Binnewies M, Garg T, et al. Myeloproliferative neoplasia remodels the endosteal bone marrow niche into a self-reinforcing leukemic niche. Cell Stem Cell. 2013;13(3):285–99.
158. Young MR, Wright MA, Coogan M, Young ME, Bagash J. Tumor-derived cytokines induce bone marrow suppressor cells that mediate immunosuppression through transforming growth factor beta. Cancer Immunol Immunother. 1992;35(1):14–8.
159. Ho ML, Tsai TN, Chang JK, Shao TS, Jeng YR, Hsu C. Down-regulation of N-methyl D-aspartate receptor in rat-modeled disuse osteopenia. Osteoporos Int. 2005;16(12):1780–8.
160. Cowan RW, Seidlitz EP, Singh G. Glutamate signalling in healthy and diseased bone. Front Endocrinol. 2012;3(89):1–7.
161. Ungard RG, Seidlitz EP, Singh G. Inhibition of breast cancer-cell glutamate release with sulfasalazine limits cancer-induced bone pain. Pain. 2014;155(1):28–36.
162. Marwaha RK, Kulkarni KP, Bansal D, Trehan A. Acute lymphoblastic leukemia masquerading as juvenile rheumatoid arthritis: diagnostic pitfall and association with survival. Ann Hematol. 2010;89(3):249–54.
163. Kaur P, Shah BS, Baja P. Multiple myeloma: a clinical and pathological profile. Gulf J Oncolog. 2014;1(16):14–20.
164. Castel LD, Abernethy AP, Li Y, Depuy V, Saville BR, Hartmann KE. Hazards for pain severity and pain interference with daily living, with exploration of brief pain inventory cutpoints, among women with metastatic breast cancer. J Pain Symptom Manage. 2007;34 (4):380–92.
165. Eaton CL, Coleman RE. Pathophysiology of bone metastases from prostate cancer and the role of bisphosphonates in treatment. Cancer Treat Rev. 2003;29(3):189–98.
166. Nagae M, Hiraga T, Yoneda T. Acidic microenvironment created by osteoclasts causes bone pain associated with tumor colonization. J Bone Miner Metab. 2007;25(2):99–104.
167. Yoneda T, Hiasa M, Nagata Y, Okui T, White F. Contribution of acidic extracellular microenvironment of cancer-colonized bone to bone pain. Biochim Biophys Acta. 2015;1848 (10 Pt B):2677–84.
168. Cleeland CS, Body JJ, Stopeck A, von MR, Fallowfield L, Mathias SD, et al. Pain outcomes in patients with advanced breast cancer and bone metastases: results from a randomized, double-blind study of denosumab and zoledronic acid. Cancer. 2013;119(4):832–8.
169. Sample SJ, Heaton CM, Behan M, Bleedorn JA, Racette MA, Hao Z, et al. Role of calcitonin gene-related peptide in functional adaptation of the skeleton. PLoS ONE. 2014;9(12): e113959.

170. Rusz O, Kahan Z. Bone homeostasis and breast cancer: implications for complex therapy and the maintenance of bone integrity. Pathol Oncol Res. 2013;19(1):1–10.
171. Mundy GR. Mechanisms of bone metastasis. Cancer. 1997;80(8 Suppl):1546–56.
172. Kakonen SM, Mundy GR. Mechanisms of osteolytic bone metastases in breast carcinoma. Cancer. 2003;97(3 Suppl):834–9.
173. Reddi AH, Roodman D, Freeman C, Mohla S. Mechanisms of tumor metastasis to the bone: challenges and opportunities. J Bone Miner Res. 2003;18(2):190–4.
174. Chirgwin JM, Mohammad KS, Guise TA. Tumor-bone cellular interactions in skeletal metastases. J Musculoskelet Neuronal Interact. 2004;4(3):308–18.
175. Fournier PG, Chirgwin JM, Guise TA. New insights into the role of T cells in the vicious cycle of bone metastases. Curr Opin Rheumatol. 2006;18(4):396–404.
176. Schell MJ. The N-methyl D-aspartate receptor glycine site and D-serine metabolism: an evolutionary perspective. Philos Trans R Soc Lond B Biol Sci. 2004;359(1446):943–64.
177. Seidlitz EP, Sharma MK, Saikali Z, Ghert M, Singh G. Cancer cell lines release glutamate into the extracellular environment. Clin Exp Metastasis. 2009;26(7):781–7.
178. Aldridge SE, Lennard TW, Williams JR, Birch MA. Vascular endothelial growth factor acts as an osteolytic factor in breast cancer metastases to bone. Br J Cancer. 2005;92(8):1531–7.
179. Ghilardi JR, Freeman KT, Jimenez-Andrade JM, Mantyh WG, Bloom AP, Kuskowski MA, et al. Administration of a tropomyosin receptor kinase inhibitor attenuates sarcoma-induced nerve sprouting, neuroma formation and bone cancer pain. Mol Pain. 2010;6(1):87.
180. Iversen PO, Wiig H. Tumor necrosis factor alpha and adiponectin in bone marrow interstitial fluid from patients with acute myeloid leukemia inhibit normal hematopoiesis. Clin Cancer Res. 2005;11(19 Pt 1):6793–9.
181. Nakamura T, Hanada K, Tamura M, Shibanushi T, Nigi H, Tagawa M, et al. Stimulation of endosteal bone formation by systemic injections of recombinant basic fibroblast growth factor in rats. Endocrinology. 1995;136(3):1276–84.
182. Huang W, Fridman Y, Bonfil RD, Ustach CV, Conley-LaComb MK, Wiesner C, et al. A novel function for platelet-derived growth factor D: induction of osteoclastic differentiation for intraosseous tumor growth. Oncogene. 2012;31(42):4527–35.
183. Mohan S, Baylink DJ. Bone growth factors. Clin Orthop Relat Res. 1991;263:30–48.
184. Thomas RJ, Guise TA, Yin JJ, Elliott J, Horwood NJ, Martin TJ, et al. Breast cancer cells interact with osteoblasts to support osteoclast formation. Endocrinology. 1999;140 (10):4451–8.
185. Soki FN, Park SI, McCauley LK. The multifaceted actions of PTHrP in skeletal metastasis. Future Oncol. 2012;8(7):803–17.
186. Guo Y, Tiedemann K, Khalil JA, Russo C, Siegel PM, Komarova SV. Osteoclast precursors acquire sensitivity to breast cancer derived factors early in differentiation. Bone. 2008;43 (2):386–93.
187. Lee Y, Schwarz E, Davies M, Jo M, Gates J, Wu J, et al. Differences in the cytokine profiles associated with prostate cancer cell induced osteoblastic and osteolytic lesions in bone. J Orthop Res. 2003;21(1):62–72.
188. Saad F, Markus R, Goessl C. Targeting the receptor activator of nuclear factor-kappaB (RANK) ligand in prostate cancer bone metastases. BJU Int. 2008;101(9):1071–5.
189. Sugatani T, Hruska KA. MicroRNA-223 is a key factor in osteoclast differentiation. J Cell Biochem. 2007;101(4):996–9.
190. Sugatani T, Hruska KA. Impaired micro-RNA pathways diminish osteoclast differentiation and function. J Biol Chem. 2009;284(7):4667–78.
191. Ell B, Mercatali L, Ibrahim T, Campbell N, Schwarzenbach H, Pantel K, et al. Tumor-induced osteoclast miRNA changes as regulators and biomarkers of osteolytic bone metastasis. Cancer Cell. 2013;24(4):542–56.
192. De Winter F, Vo T, Stam FJ, Wisman LA, Bar PR, Niclou SP, et al. The expression of the chemorepellent Semaphorin 3A is selectively induced in terminal Schwann cells of a subset of neuromuscular synapses that display limited anatomical plasticity and enhanced vulnerability in motor neuron disease. Mol Cell Neurosci. 2006;32(1–2):102–17.

193. Peng Y, Li Z, Li Z. GRP78 secreted by tumor cells stimulates differentiation of bone marrow mesenchymal stem cells to cancer-associated fibroblasts. Biochem Biophys Res Commun. 2013;440(4):558–63.
194. Smadja DM, d'Audigier C, Weiswald LB, Badoual C, Dangles-Marie V, Mauge L, et al. The Wnt antagonist Dickkopf-1 increases endothelial progenitor cell angiogenic potential. Arterioscler Thromb Vasc Biol. 2010;30(12):2544–52.
195. Xu W, Liu LZ, Loizidou M, Ahmed M, Charles IG. The role of nitric oxide in cancer. Cell Res. 2002;12(5–6):311–20.
196. Yuan FL, Wang HR, Zhao MD, Yuan W, Cao L, Duan PG, et al. Ovarian cancer G protein-coupled receptor 1 is involved in acid-induced apoptosis of endplate chondrocytes in intervertebral discs. J Bone Miner Res. 2014;29(1):67–77.
197. Walker K, Medhurst SJ, Kidd BL, Glatt M, Bowes M, Patel S, et al. Disease modifying and anti-nociceptive effects of the bisphosphonate, zoledronic acid in a model of bone cancer pain. Pain. 2002;100(3):219–29.
198. Yoneda T, Hata K, Nakanishi M, Nagae M, Nagayama T, Wakabayashi H, et al. Involvement of acidic microenvironment in the pathophysiology of cancer-associated bone pain. Bone. 2011;48(1):100–5.

Chapter 10
Conclusion

Gurmit Singh

This book explores the new concept of Oncodynamics. It conceptualizes the effects of cancer on the body. The first chapter in the book sets the stage for a disrupted steady-state which is responsible for the genesis of cancer. The subsequent four chapters in this book provide a framework for the physiological changes that occur in the presence of solid tumours. The abnormal secretions of various factors from the cancer cells are largely responsible for physiological changes such as angiogenesis, neurogenesis, inflammation, and lymphedema. Similar effects can also be exasperated by cancer treatments including chemotherapy and radiation.

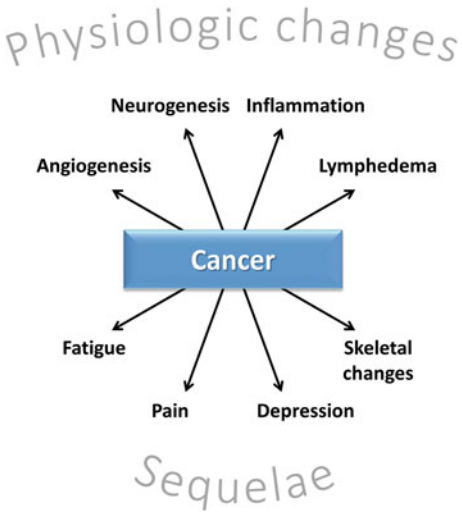
The primary purpose of this book is to dissociate the physiological effects from those caused by cancer treatment and focus purely on the responses of the body to cancer presence. A better understanding of these cancer-induced changes will provide a forum for new research in regaining physiological homeostasis in the presence of cancer. A number of investigations are already under way which examine the impact of neo-angiogenesis and the manipulation of immune surveillance to combat cancer.

The last four chapters of this book deal with the sequelae of the physiologic changes—namely fatigue, pain, depression, and skeletal responses. The sequelae of the physiological changes are complex and a result of multiple contextual factors. The complexity of the pain sequela is a result of changes not only on the neurogenesis process but also on several factors secreted by both tumour and host cells in addition to involving the immune system. Hence, multiple cell signalling molecules are likely at play.

G. Singh (✉)
Department of Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada
e-mail: singhg@mcmaster.ca

G. Singh (ed.), *Oncodynamics: Effects of Cancer Cells on the Body*,
DOI 10.1007/978-3-319-28558-0_10

In some instances it is the sequelae such as abnormal pain or depression that are responsible for the identification of tumours and a cancer diagnosis. These oncodynamic effects are largely responsible for the quality of life from a biological viewpoint.



Index

G. Singh (ed.), *Oncodynamics: Effects of Cancer Cells on the Body*,
DOI 10.1007/978-3-319-28558-0