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Alexander Birbrair *Editor*

Tumor Microenvironment

The Role of Interleukins – Part A

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Preface

This book's initial title was "Tumor Microenvironment." However, due to the current great interest in this topic, we were able to assemble more chapters than would fit in one book, covering tumor microenvironment biology from different perspectives. Therefore, the book was subdivided into several volumes.

This book, *Tumor Microenvironment: The Role of Interleukins – Part A*, presents contributions by expert researchers and clinicians in the multidisciplinary areas of medical and biological research. The chapters provide timely detailed overviews of recent advances in the field. This book describes major contributions of different interleukins in the tumor microenvironment during cancer development. Further insights into these mechanisms will have important implications for our understanding of cancer initiation, development, and progression. The authors focus on the modern methodologies and the leading-edge concepts in the field of cancer biology. In recent years, remarkable progress has been made in the identification and characterization of different components of the tumor microenvironment in several tissues using state-of-the-art techniques. These advantages facilitated identification of key targets and definition of the molecular basis of cancer progression within different organs. Thus, the present book is an attempt to describe the most recent developments in the area of tumor biology which is one of the emergent hot topics in the field of molecular and cellular biology today. Here, we present a selected collection of detailed chapters on what we know so far about the interleukins in the tumor microenvironment in various tissues. Eight chapters written by experts in the field summarize the present knowledge about distinct interleukins during tumor development.

Weizhou Zhang and colleagues from the University of Florida describe interleukin-1 signaling in the tumor microenvironment. Jong-Hyuk Kim from the University of Minnesota discusses interleukin-8 in the tumor immune niche. Guangwei Liu and colleagues from Beijing Normal University address the importance of interleukin-9 and Th9 cells in tumor immunity. R.M. Gorczynski from the University of Toronto compiles our understanding of interleukin-17 signaling in the tumor microenvironment. Tracy L. Putoczki and colleagues from the University of Melbourne, Australia, update us with what we know about the emerging roles for interleukin-18 in the gastrointestinal tumor microenvironment. Virginie Lafont and colleagues from the Institut de Recherche en Cancérologie de Montpellier summarize current knowledge on the interleukin-21 signaling in the tumor microenvironment.

Yi-Ching Wang and colleagues from the National Cheng Kung University focus on interleukin-33 signaling in the tumor microenvironment. Finally, Walter J. Storkus and colleagues from the University of Pittsburgh School of Medicine give an overview of interleukin-36 signaling in the tumor microenvironment.

It is hoped that the articles published in this book will become a source of reference and inspiration for future research ideas. I would like to express my deep gratitude to Veranika Ushakova, my wife, and Mr. Murugesan Tamilsevan, from Springer, who helped at every step of the execution of this project.

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IL-1 Signaling in Tumor Microenvironment

1

Weizhou Zhang, Nicholas Borcherding, and Ryan Kolb

Abstract

Interleukin 1 (IL-1) has long been known for its pleiotropic effects on inflammation that plays a complex, and sometimes contrasting, role in different stages of cancer development. As a major proinflammatory cytokine, IL-1β is mainly expressed by innate immune cells. IL-1α, however, is expressed by various cell types under physiological and pathological conditions. IL-1R1 is the main receptor for both ligands and is expressed by various cell types, including innate and adaptive immune cell types, epithelial cells, endothelial cells, adipocytes, chondrocytes, fibroblasts, etc. IL-1 and IL-1R1 receptor interaction leads to a set of common signaling pathways, mainly the NF-kB and MAP kinase pathways, as a result of complex positive and negative regulations. The variety of cell types with IL-1R1 expression dictates the role of IL-1 signaling at different stages of cancer, which under certain circumstances leads to contrasting roles in tumor development. Recent availability of IL-1R1 conditional knockout mouse model has made it possible to dissect the role of IL-1/IL-1R1 signaling transduction in different cell types within the tumor microenvironment. This chapter will focus on the role of IL-1/IL-1R1 in different cell types within the tumor microenvironment and discuss the potential of targeting this pathway in cancer therapy.

Keywords

Interleukin-1α · Interleukin-1β · IL-1R1 · IL-1RA · IL-1 signaling pathway · Tumor microenvironment · Breast cancer · Sarcoma · Melanoma · Colorectal cancer · Hepatocellular carcinoma · Mouse models · Pleiotropic effects · Cancer progression · Cancer therapy

1.1 Introduction

Starting with the identification of interleukin protein function in the early 1970s, the nomenclature of IL-1 was established in 1979 [1]. IL-1 signaling transduction is well controlled and regulated via different levels of positive and negative regulators. There are two major agonistic IL-1 ligands, IL-1α and IL-1β, and one antagonistic ligand IL-1RA (anakinra). At the receptor level, IL-1R1 is the major receptor mediating positive signaling transduction from agonistic ligands and is ubiquitously expressed across many cell types.

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The IL-1 receptor accessory protein (IL-1RaP, also referred to as IL-1R3) facilitates the positive signaling via the formation of a tertiary complex (IL-1α or IL-1β, IL-1R1, IL-1RaP) with IL-1R1 and accessory proteins, which recruits downstream signaling proteins. IL-1R2 is a decoy receptor that has no intracellular signaling domain and leads to the sequestration of agonistic ligands, thus quenching downstream signaling activation. At the downstream effector level, the tertiary complex leads to activation of two major pathways, NF-κB and MAP kinase pathways (Fig. 1.1). This intracellular level of signaling regulation is much more complicated than the level of ligand-receptor interaction due to the interaction of a number of the downstream effector proteins, including scaffolding proteins, kinases, ubiquitin/de-ubiquitin enzymes, etc. The complex protein interactions and regulations often crosstalk with other signaling pathways such as those mediated by Toll-like receptors (TLRs). The detailed signaling transduction networks have been extensively reviewed and will not be discussed here [2–6]. As a negative feedback, IL-1 signaling activation can induce the expression of the negative regulator IL-1RA by NF-κB- and AP-1-dependent transcription [7, 8].

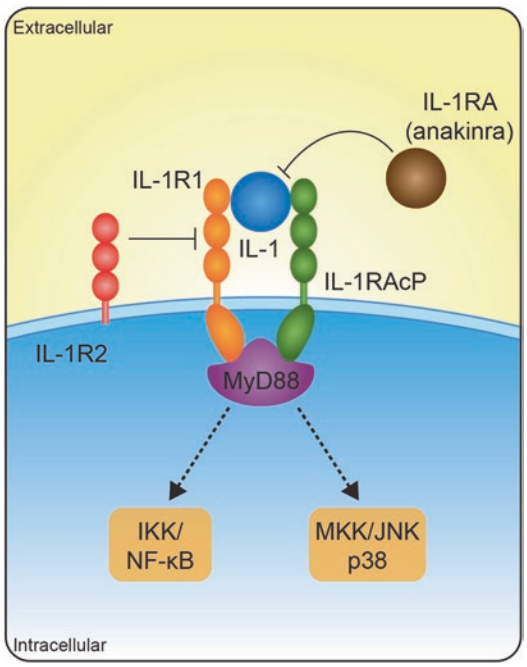

Fig. 1.1 Essential components of IL-1 signaling transduction

As the major agonistic ligands, IL-1α and IL-1β are encoded by two distinct genes with moderate shared homology. Both genes and proteins are tightly regulated at transcriptional and posttranslational levels. The transcription is generally activated by NF-κB family transcription factors. NF-κB signaling can be activated by various factors, such as pathogen infection (pathogen-associated molecular patterns, PAMP) or sterile inflammation/tissue damage (danger-associated molecular patterns, DAMP) via TLRs, NOD-like receptors (NLRs), or other cytokines/growth factors/chemokines. IL-1 signaling can propagate IL-1 production via a positive feedback loop mediated by IκB kinase (IKK)/NF-kB activation. IL-1α and IL-1β are not secreted via classic endoplasmic reticulum/Golgi pathways. IL-1α and IL-1β proteins are translated as pro-forms and secreted via distinct mechanisms.

IL-1α Biogenesis: Pro-IL-1α can be posttranslationally modified by phosphorylation, myristoylation, and acetylation, although the functional significance of these modifications is yet to be determined. Pro-IL-1α is active and binds to IL-1R1 equally compared to the cleaved IL-1α. Several proteases including calpain, granzyme B, elastase, or chymase can cleave pro-IL-1α into the mature forms at different cleavage sites. The biological function of pro-IL-1α/IL-1α comes from different locations, including the induction of IL-1R1 canonical signaling transduction via released or membrane-bound pro-IL-1α/IL-1α or nuclear pro-IL-1α/IL-1α. Current theory involves the release of pro-IL-1α through necrosis-mediated passive release as an "alarmin." The alarmin function of IL-1α notifies adjacent immune cells of potential damage to tissues and stimulates regeneration. The release of mature or cleaved IL-1α is less understood, likely via a similar mechanism as mature IL-1β release.

IL-1β Biogenesis: Even though pro-IL-1β is similarly translated, it has no bioactivity to induce IL-1R1-mediated signaling pathways in the pro-form. Protease-mediated cleavage, primarily

via caspase-1 (Casp-1), leads to the release of mature IL-1β from the cytosol to extracellular space, via either pyroptosis (inflammatory cell death) or some other not-well-defined mechanism. The key enzyme Casp-1 is activated via a controlled mechanism during infection or sterile inflammation during injury, stress, and metabolic alterations. Innate immune cells, such as macrophages, can sense different molecular signatures, PAMPs or DAMPs, through sensing molecules in the NLR family, including NLRP1, NLRP3, and NLRC4, or pyrin family protein AIM-2. Via the adaptor protein apoptosis-associated speck-like containing CARD (ASC), the sensors, ASC, and pro-Casp-1 form an activating complex referred to as an inflammasome. Upon sensing various molecular signatures, the inflammasome complex forms, leading to the auto-cleavage of Casp-1 from pro-Casp-1 into p20/p10 tetramers with protease activity. The protease activity of the tetramers results in the cleavage of pro-IL-1β and pro-IL-18 into their mature forms. It is critical to understand how these different inflammasomes are activated in a tumor microenvironment in order to understand the cellular sources of IL-1β production and the downstream events. Most inflammasome-relevant studies have focused on macrophages in tumor microenvironment. Others and we have extensively reviewed the role of different inflammasomes in different cancers that will not be discussed in detail in this chapter [9–13]. There are other mechanisms that can lead to IL-1β processing and activation in neutrophils via neutrophil-specific proteases upon certain bacterial infections [14]. Neutrophil-based IL-1ββ activation may be dependent on cathepsin G in lung cancer, which is critical to mediate cancer resistance to IKK/NF-κB inhibition [15]. There are emerging reports of the pathogenic role of neutrophils in cancer [16–18], and neutrophil-produced IL-1β likely plays an important role for cancer inflammation, progression, and metastasis.

The ubiquitous expression of IL-1R1 complicates clear elucidation of the role of IL-1 signaling due to the various, and sometimes opposing, roles in different effector cells. For example, reports have shown opposing effects of IL-1R1-mediated signaling transduction in myeloid cells versus T-cells in mouse model of colon cancer [18]. In addition to cancer cells, the tumor microenvironment contains most immune cell types, fibroblasts, endothelial cells, adipocytes, and tissue-specific cell types, many of which express IL-1R1. Therefore, the overall effect of the IL-1 signaling pathway in certain cancer types needs to be carefully dissected. The chapter will focus on the role of IL-1 signaling transduction in various cell types under physiological and pathological conditions, including several types of cancer (Table 1.1).

1.2 IL-1 Signaling Pathway in Different Cancers

The IL-1/IL-1R1 signaling axis primarily induces pro-survival and pro-proliferative MAP kinase signaling, which generally promotes cancer progression. The pro-tumorigenic function of IL-1 has been an accepted concept, especially in regard to IL-1β, based on in vitro data and in vivo tumorigenic models. However, there are contradictory results, indicating more complex signaling transduction/crosstalk between IL-1 and other signaling pathways. Here we will focus on the cancer types with strong support from genetically modified mouse models (GEM) related to core IL-1 signaling, including IL-1α, IL-1β, IL-1R1, and IL-1Ra. The inflammasome mouse models have been extensively discussed in outstanding reviews [9–13]. The downstream effectors are always shared with other pathways that may complicate experimental interpretation due to the signaling crosstalk. An important point of clarification before continuing is that most studies rely on whole-body knockout or cancer-cell-line injection models in wild-type or immunocompromised mice. The cancer phenotypes are a result of combinatory impacts of the IL-1 signaling on different cell types within tumor microenvironment.

Table 1.1 Cancer phenotypes related to IL-1 core signaling components

Molecules	Major functions	References
Breast cancer		
IL-1β	Inhibits ER-positive breast cancer cell growth but promotes an aggressive invasive mesenchymal phenotype	[19, 20, 22, 23]
	Promotes migration and EMT in triple-negative breast cancer cells	[26, 27]
	Cancer cell intrinsic expression is correlated with relapse	[30]
	Promotes metastasis in lobular carcinoma mouse model	[17]
	Macrophage-produced IL-1β promotes angiogenesis and progression under obese conditions	[50, 51]
	Promotes an imbalance between tumor-infiltrating macrophages and dendritic cells leading to decreased CD8 T-cell activation	[49]
	IL-1 deficiency reduces tumorigenesis in a PyMT spontaneous breast cancer mouse model	[56]
IL-1α	Suppresses ER-positive breast cancer cell growth in vitro	[34]
	Promotes ER-positive breast cancer tumor growth in vivo	[35]
	Expression in ER cells is correlated with a more malignant phenotype and cancer progression	[36–40]
	4T1 cancer cell-derived IL-1α promotes cell survival and metastasis via inductions of TSLP from neutrophils	[16]
Sarcoma		
IL-1β	Polymorphisms are associated with risk of osteosarcoma	[71]
	Expression in fibrosarcoma cells induces a more aggressive phenotype and increased angiogenesis	[72]
	Promotes tumorigenesis and invasiveness in 3-MCA-induced fibrosarcoma model	[75]
IL-1α	Induces genes associated with survival, cell cycle, inflammation, and ECM remodeling in sarcoma cell lines	[60–62]
	Expression in fibrosarcoma cells induces antitumor immunity	[72]
	Involved in escape form immunosurveillance	[76]
Liver cancer		
IL-1β	Polymorphisms are associated with increased risk of hepatocellular carcinoma	[77–79]
	Promotes tumorigenesis in DMBA plus obesity-induced liver cancer model	[83]
	Activation downstream of NLRP3 inflammasome activation likely plays a role in HCV-related liver cancer	[84, 85]
	Macrophage-produced IL-1β acts synergistically with EGF to induce IL-6 in macrophages, an important tumor-promoting cytokine in HCC	[87]
IL-1α	Released from ROS-damaged hepatocytes and promotes carcinogenesis in a carcinogen-induced liver cancer model	[80]
Melanoma		
IL-1β	Promotes lung metastasis and adhesion to endothelial cells	[92, 93]
	Macrophage-produced IL-1β signals through fibroblasts and endothelial cells to promote angiogenesis and the upregulation of tumor-promoting factors	[88]
	Promotes invasiveness, metastasis, and angiogenesis in B16 mouse models	[100, 101]
IL-1α	Promotes lung metastasis and adhesion to endothelial cells	[92, 93]
	Critical for oncogenic RAS-induced keratinocyte transformation	[106]
	Inhibits carcinoma formation when overexpressed in keratinocytes	[107]
Colon cancer		
IL-1β	Promotes VEGF expression, EMT, invasion, and growth of human colon cancer cells	[112–115]
	Polymorphisms are associated with recurrence	[117]
IL-1R1	Promotes tumorigenesis and early progression in colon epithelial cells	[18, 123]
	Promotes tumor-elicited inflammation via IL-17 and IL-22 induction in CD4 T-cells and possibly IL-C3	[18]
	Loss in neutrophils increases bacteria-induced inflammation and increased tumor load	[18]

1.2.1 Breast Cancer

1.2.1.1 Human Cancers

Early studies supported a growth-inhibitory role of IL-1β in estrogen receptor (ER)-positive MCF-7 cells, whereas IL-1β has minimal effect on other breast cancer cell lines without ER expression [19, 20]. Interestingly, the inhibitory function of IL-1β in MCF-7 cells is likely due to the crosstalk with downstream signaling mediated by insulin-like growth factor 1 (IGF1) and insulin receptor substrate 1 (IRS-1), leading to the inhibition of phosphatidylinositol 3-kinase/Akt signaling pathway (PI3K/AKT) [21]. Later research using the same MCF-7 cells suggested that IL-1β promotes an aggressive phenotype of MCF-7, i.e., the migration/invasion and a mesenchymal phenotype [22, 23]. This invasive phenotype may be a result of the activation of Src homology (SH) 2-containing phosphotyrosine phosphatase (Shp-2) leading to the expression of matrix metalloproteinase 9 (MMP-9) [22] and the synergistic induction of Erk1/2 activation with epidermal growth factor (EGF) [24]. In a separate study using MCF-7 cells, IL-1β induced a kinase cascade involving NF-κB-inducing kinase (NIK), IKKα, and the consequent activation of NF-κB in a reactive oxygen species (ROS)-dependent manner [25], a possible parallel mechanism for IL-1β-induced aggressive phenotype of MCF-7 cells. Similar promotion of a migratory phenotype by IL-1β was seen in triple-negative breast cancer cells, MDA-MB-231, where IL-1β induces the expression of hypoxic-inducible factor α (HIF-1α) and the CXCR1 chemotaxis pathway [26]. A non-canonical activation of IL-1β-mediated β-catenin signaling was also reported [27] that leads to the onset of epithelial-mesenchymal transition (EMT). The significant induction of EMT by IL-1β also links to another important feature of breast cancer, maintaining the tumor-initiating cells via an NF-κB-dependent mechanism [28]. Interestingly, IL-1β inflammatory response phenotypically locks metastasis-initiating cancer cells (MICs) at a *ZEB1*-positive mesenchymal stage that cannot reverse for the subsequent epithelial colonization process [29]. The dormancy-locked MIC cells can undergo epithelial transition by inhibition of IL-1β pathway to establish macrometastasis [29]. The source of IL-1β is thought to be derived from innate immune cells under inflammatory conditions; however, breast cancer cells can turn on IL-1β expression [30]. The role of IL-1β in breast cancer metastasis is strongly supported by clinical data showing that cancer-cell-intrinsic expression of IL-1β protein significantly correlates with relapse in bone and other sites in a large patient cohort (greater than 1300 patients) with stage II/III breast cancer [30]. IL-1β/IL-1R1 inhibition by anakinra (IL-1RA) or canakinumab (human-specific IL-1β antibody) reduced bone metastasis, likely via a cancer-cell-intrinsic autocrine pathway [30, 31]. In a recent pilot clinical trial involving HER2-negative metastatic patients, anakinra (IL-1RA) treatment modulated transcriptional signature in blood leukocytes leading to decreased IL-1 signaling, NF-κB signaling, and innate immunity but increased genes involved in NK- and T-cell-mediated cytotoxicity. This anakinra-modulated signature, i.e., the IL-1β /IL-1R1-induced gene signature, can faithfully predict breast cancer patients with poor prognosis [32].

The cancer-cell-intrinsic function of IL-1α is much less understood in breast cancer. Limited literature supports that IL-1α may promote human breast cancer progression. Similarly to IL-1β, IL-1α was initially identified as a suppressor for estrogen-induced growth of ER+ MCF-7 cells and downregulated ER protein either alone [33] or in addition to IL-6 [34]. This in vitro inhibitory effect of IL-1α conflicts with an in vivo tumorigenic study where MCF-7 tumors with IL-1α overexpression grew faster than control cells [35], suggesting that IL-1α plays a dominant role in tumor microenvironment in the MCF-7 xenografts. In ER-negative breast cancer cells, IL-1α is preferentially produced by cancer cells with a greater basal or stem cell phenotype [36] and induces downstream activation of NF-κB and IL-6 production to promote cancer progression [37] and other metastatic genes [38]. In agreement, IL-1α protein secretion is correlated with a more malignant phenotype [39] and ER negativity [40]. This may underscore a potential local paracrine and

autocrine role of IL-1α in the maintenance of a more malignant phenotype.

There exist a number of publications regarding the polymorphisms of genes encoding for IL-1 family cytokines and their association with breast cancer risk, but the conclusions are unequivocal. Based on a meta-analysis of total 1277 breast cancer cases and 1431 control cases, there is no significant correlation between three IL-1β polymorphisms with breast cancer risk [41, 42]. This lack of correlation between IL-1β polymorphisms with breast cancer is supported by other studies including one in Korean women [43] and another in Caucasian women [44]. Meanwhile, several reports indicate IL-1RN (encoding IL-1R antagonist IL-1RA) polymorphisms are marginally associated with breast cancer risks [44, 45]. Similarly, IL-1α gene polymorphism at the C-terminal untranslated region (rs3783553, TTCA insertion genotype) is significantly associated with a decreased risk of breast cancer [46] due to the differential regulation by miR-122 and reduction in IL-1α expression [47]. An additional IL-1α polymorphism was correlated with increased breast cancer risk based on a multiplex genotyping of 1107 SNPs from 232 candidate genes [48].

1.2.1.2 Mouse Models

In the 4 T1 syngeneic transplant model, tumor-intrinsic IL-1α led to the recruitment of neutrophils and subsequent thymic stromal lymphopoietin (TSLP) production, which in turn promotes tumor cell survival and metastasis [16]. IL-1β can also promote metastasis in *K14cre;Cdh1F/F;Trp53F/F* (KEP), a lobular breast cancer model in an IL-1R1-dependent manner. IL-1 signaling leads to IL-17 production from γδ T-cells, which in turn leads to G-CSF expression and enhanced production and recruitment of metastasis-promoting neutrophils [17]. These neutrophils are critical in suppressing the cytotoxic and antimetastatic activity of CD8 T-cells [17]. In a more recent report, IL-1β was shown to balance the tumor-infiltrating macrophages versus CD11b + dendritic cells (DCs), with IL-1β deficiency leading to increased IL-12-producing CD11b + DCs and prevailing CD8 T-cell-mediated antitumor immunity [49]. Inhibition of IL-1β and an "immune checkpoint" (programmed cell death-1, PD-1) synergistically suppresses breast cancer growth [49]. In obesity, which generally promotes breast cancer in humans and animal models, our group identified that IL-1β is a causal effector molecule that drives obesity-induced breast cancer progression as a downstream effector of the NLRC4 inflammasome activation [50, 51]. Obesity induces an increase in tumor-infiltrating macrophages that produce IL-1β and activate subsequent expression of VEGFA and ANGPTL4 within adipocytes to induce angiogenesis [50, 51]. Genetic and pharmacological inhibition of IL-1β/IL-1R1 signaling suppresses obesity-driven cancer growth and angiogenesis [50, 51]. The NLRP3 inflammasome/IL-1β can also activate within tumor-associated macrophages, leading to angiogenesis and cancer progression [52, 53]. The exact mechanism for NLRC4 inflammasome activation in obesity-driven breast cancer remains not fully understood. Since NLRC4 is only known to sense bacterial products such as flagellin or type III secretion system, it is likely that NLRC4 senses obesity-associated bacterial products either via circulation or from microbiota detected within adipose tissues [54].

All syngeneic models described above focus on the role of IL-1/IL-1R1 signaling in tumor immune microenvironment. In a recent report using the polyoma middle T-antigen mammary carcinoma model (MMTV-PyMT), IL-1α/IL-1R1 signaling pathway was shown to be clearly tumor-suppressive as IL-1R1$^{-/-}$ and IL-1α$^{-/-}$ mice showed significantly elevated tumorigenesis and lung metastasis relative to wild-type and IL-1β$^{-/-}$ mice [55]. The authors did not identify any significant change in major tumor-infiltrating immune cell subtypes, albeit a trend toward increased macrophages in IL-1R1$^{-/-}$ tumors. This tumor-suppressive phenotype is likely due to a direct impact on tumor cells. These findings are further supported by an earlier report that found that tumors in MMTV-PyMT mice do not rely on adaptive immune cells for primary tumor growth but require them for lung metastasis [56]. As PyMT breast cancer

model is clearly defined as luminal breast cancer [57], it is conceivable that IL-1α/IL-1R1-mediated inhibitory effect is via the interaction with ER signaling during early initiation and progression stages, similarly as reported in human ER+ MCF-7 cells [19, 20, 33]. As PyMT tumors lose ER expression in late stages and metastasis, how IL-1α/IL-1R1 signaling suppresses metastasis is yet to be explained. The discrepancy between PyMT model and IL-1α polymorphisms in humans underscores the complex role of IL-1α in breast cancer. There could be a delicate balance between IL-1α-mediated immunosurveillance and IL-1α-induced cancer cell survival and proliferation.

1.2.2 Sarcoma

1.2.2.1 Human Cancers

Human data related to IL-1α and IL-1β is relatively sparse. The expression of both IL-1 ligands has been reported in human sarcoma cells [58, 59]. Using the osteosarcoma line MG-63, signaling through IL-1α induces a panel of genes involving protein synthesis (*S6K*, increased 22-fold), signaling proteins (*PP2A*), antiapoptotic gene (cIAP1 or *BIRC2*, increased 20-fold), cell cycle (*CDC42BPB*, increased 16-fold), and inflammation (MIP2β or *CXCL3*, increased sevenfold) [60]. Collectively, the IL-1α-induced expression changes indicate various potential functions in cell cycle, viability, and inflammation. IL-1α also induces matrix metalloproteinase 3 (MMP-3), IL-6, BMP-2, and Cox2 production in the SW1353 chondrosarcoma line [61, 62], indicating possible functions in tissue remodeling, inflammation, and invasion. A similar set of genes were also regulated by IL-1β in sarcoma cell lines [63–67], in addition to its regulation on microRNAs [68, 69]. At the mechanistic level, IL-1 induces classic NF-kB and MAP kinase activation to regulate downstream gene expression pattern [64, 65, 70]. There is only a single report using 120 patients finding that two IL-1β polymorphisms are associated with risk of osteosarcoma [71].

1.2.2.2 Mouse Models

Using mouse fibrosarcoma cell lines, IL-1α and IL-1β have very distinct roles in sarcoma progression. IL-1α overexpression in fibrosarcoma is located at the plasma membrane and transduces an antitumor immunity from cell surface to effector immune cells, evidenced by increased mononuclear immune cells in the tumor sites, as well as increased CD8 T-cell and IFNγ production [72]. IL-1β production in the same cells led to a more aggressive tumor growth with increased angiogenesis [72]. Fibrosarcomas with IL-1α deficiency grew more aggressive tumors, whereas those with IL-1β deficiency grew smaller tumors when using immunodeficient Nu/Nu mice [73] and immunocompetent mice [74]. The above cancer-cell-intrinsic IL-1α and IL-1β production is limited to tumor and its microenvironment. Using whole-body knockout mice, Krelin et al. used the 3-methylcholanthrene (3-MCA)-induced fibrosarcoma model in IL-1β$^{-/-}$, IL-1α$^{-/-}$, IL-1α/β$^{-/-}$ (double knockout), and IL-1Rα $^{-/-}$ mice with the Balb/C genetic background and found IL-1β, but not IL-1α, was able to promote tumorigenesis and invasiveness [75]. There is an observation of strong inflammatory response related to IL-1β-induced tumorigenesis that can be blocked by anakinra, suggesting a proinflammatory microenvironment is essential for tumorigenesis and progression in this model [75]. Though IL-1α did not exhibit a role in the MCA-induced primary fibrosarcomagenesis, it is critically involved in immunoediting of cancer cells that prevents the cancer cells from T-cell- and, to a lesser extent, NK-cell-dependent immunosurveillance [76]. These data suggest that both cancer-cell-intrinsic and host productions of IL-1 β play a role in the promotion of sarcoma, whereas IL-1α has a more complicated function depending on the location.

1.2.3 Liver Cancer

1.2.3.1 Human Hepatocellular Carcinoma (HCC)

A recent epidemiological study from South Korea identified IL-1β polymorphisms are significantly associated with HCC, with two polymorphisms

associated with decreased risk and one with increased HCC risk [77]. In the same study, no IL-1α or IL-1RA polymorphisms were associated with HCC risk [77]. A similar result was shown in HCC with preexisting HCV infection in a Japanese cohort [78] and an Egyptian cohort [79], with IL-1β polymorphisms associated with HCC risk but not in IL-1RA or TNFα genes. A separate study using two large cohorts of Chinese HCC patients identified an insertion/deletion polymorphism at the miRNA-122 binding site of IL-1α 3′ untranslated region increased the risk of HCC development. The relevant polymorphism disrupts the binding of miR-122 to mRNA, resulting in an increase in IL-1α expression and HCC risk [47]. The same polymorphism is also associated with a risk of breast cancer [46], indicating a general tumor-promoting function of IL-1α in various cancer types. These data suggest IL-1/IL-1R signaling transduction may play a critical role during human HCC development.

1.2.3.2 Mouse Models

In a procarcinogen diethylnitrosamine (DEN)-induced liver cancer model [80], DEN induces HCC via the induction of massive hepatocyte killing, recruitment of myeloid cells, and the production of proinflammatory cytokines that stimulate compensatory proliferation of remaining mutated hepatocytes [81, 82]. IL-1α is one of the cytokines that is released by hepatocyte damage upon the accumulation of excessive ROS, which in turn promotes hepatocyte proliferation and survival via IL-1R1- and Myd88-mediated signal transduction [80]. This process does not involve IL-1β, as deficiency in IL-1β or its major activator Casp-1 did not promote DEN-induced HCC development [80]. Interestingly, under certain conditions, such as 7,12-dimethylbenzathracene (DMBA) plus obesity-induced liver cancer in neonates, IL-1β is important for liver cancer development via regulating a senescence-related secretion phenotype (SASP) [83]. This suggests that obesity-associated chronic inflammation, similarly to what we have seen in breast cancer [50], relies on IL-1β to transmit tumor-promoting inflammation. Chronic infection of hepatitis C virus (HCV), one of the major epidemiological factors for human HCC, also activates NLRP3 inflammasome and IL-1β within hepatic macrophages to induce chronic inflammation and likely contributes to HCV-related HCC in humans [84, 85]. One of the mechanisms of liver-macrophage-produced IL-1β is the synergy with EGFR-mediated IL-6 production [86], one of the critical proinflammatory cytokines that promote HCC [87] and may account for sex differences of HCC patients [82].

1.2.4 Melanoma/Skin Cancer

1.2.4.1 Human Melanomas

IL-1R1 is mainly expressed by tumor-associated endothelial cells and fibroblasts, whereas IL-1β is mainly expressed by tumor-associated macrophages [88]. There are inconsistent reports whether human melanoma cell lines express IL-1β using various reagents, but several reports conclude that melanoma cell lines are not the major source of IL-1β protein due to the lack of inflammasome components [88, 89]. IL-1α is expressed uniformly in nevi, primary tumors, and metastases [90, 91]. Early studies have shown that IL-1α/β induces experimental lung metastasis in A375 xenografts [92] and promotes tumor cell adhesion to endothelial cells [93]. The IL-1β/IL-1R1 signaling cascade seems to be initiated by cancer cells, via an unknown mediator, to induce IL-1β transcription and processing in macrophages [88, 89]. Fibroblasts and endothelial cells propagate the signal from macrophage-produced IL-1β, leading to cancer-promoting factors and angiogenesis [88]. The autocrine IL-1β/IL-1R1 signaling cascade within macrophages is also important to promote DNA methylcytosine dioxygenase Tet2, which sustains immunosuppressive function and promotes melanoma progression [94]. Although IL-1R1 is generally expressed below detectable levels in human melanoma, IL-1 can induce downstream signaling activation using human melanoma cell lines [95] and invasiveness via upregulation of adhesive molecules from both cancer cells and endothelial cells in human xenografts [96, 97]. Limited genetic information indicates that IL-1β

polymorphism is marginally associated with invasive phenotype [98] and IL-1RA genotype is associated with patient survival [99].

1.2.4.2 Mouse Models

Most IL-1-related mouse models of melanoma are from syngeneic transplantation of B16 mouse melanoma cell line. This model provides excellent resources for host IL-1 signaling in cancer immunity, inflammation, and angiogenesis. Early studies defined the role of IL-1β as required for invasiveness, metastasis, and angiogenesis via the induction of VEGFA and lymphotoxin [100, 101]; IL-1α has a similar function with a weaker phenotype [100]. IL-1β has also been shown to promote hepatic metastasis of melanoma via upregulation of vascular cell adhesion molecule-1 (VCAM-1) [102], presumably via retention of cancer cells to endothelial cells. Treatment of B16 melanoma with IL-1RA reduces tumor growth and lung metastasis [103], suggesting that IL-1 signaling may be a viable target for melanoma therapy.

Conflicting results have been reported in the two-stage 7,12-dimethylbenzanthracene/12-O-tetradecanoylphorbol-13-acetate (DMBA/TPA)-induced skin cancer model. The DMBA/TPA model has limitations but currently is the only model used for studying IL-1 signaling in carcinogen-induced skin cancer model. Drexler et al. reported the critical role of IL-1R1 and Casp-1 in tumor progression, with whole-body deletion of IL-1R1 or Casp-1 leading to less tumorigenesis relative to the wild-type controls [104]. Similar result was also seen in a separate study using genetic knockout of IL-1R1 and MyD88 [105]. The involvement of Casp-1 strongly indicates the involvement of IL-1β, not IL-1α, in the DMBA/TPA-induced skin cancer. However, the involvement of IL-1α is vital in the mediation of the oncogenic RAS-induced keratinocyte transformation via IL-1R1- and MyD88-mediated signaling transduction [106]. Interestingly, the transgenic expression of IL-1α under the keratin-14 promoter, which drives IL-1α expression from keratinocytes, completely inhibits papilloma and carcinoma formation, suggesting a tumor-suppressive/immunosurveillance phenotype of IL-1α overexpression [107].

1.2.5 Colon Cancer

1.2.5.1 Human Colorectal Cancer (CRC)

Expressions of IL-1α and IL-1β are detectable in colonic epithelial cells, with Casp-1 and IL-1β largely diminished within colon cancer cells [108]; however, both Casp-1 and IL-1β are elevated in CRC tumors versus normal tissues likely due to tumor-infiltrating immune cells. IL-1α is maintained in colon cancers and can be induced further by proinflammatory stimuli, like prostaglandin E2, to boost inflammation and likely carcinogenesis [109] or to induce angiogenesis and IL-8 production in endothelial cells [110, 111]. IL-1β, presumably mainly from myeloid cells, directly works on human colon cancer cells to promote VEGF expression for angiogenesis [112, 113], Zeb1 for EMT, stemness and invasion [114], Wnt signaling for cancer growth [115], and COX2 for inflammation [116]. This is largely in agreement with the role of IL-1/IL-1R1 signaling in promoting CRC development and progression. Polymorphisms of IL-1β and IL-1RA have been shown to be associated with tumor recurrence in stage II colon cancer [117], and IL-1RA genotype is associated with colorectal cancer risk [118].

1.2.5.2 Mouse CRC-Colitis-Associated Colorectal Cancer (CAC) by AOM/DSS and CRC by CPC-APC

The impact of IL-1/IL-1R1 signaling transduction is very perplexing in colon cancer mouse models, as shown initially in a lack of discernable phenotype in IL-1R1$^{-/-}$ mice with azoxymethane/dextran sodium sulfate (AOM/DSS)-induced early colitis and CAC [119], as well as in *CDX2Cre-Apcf/wt* (CPC-APC) mouse model of conditional monoallelic APC loss in the colon to induce CRC [18]. IL-1 signaling has been shown to be important in stimulating IL-17 production and Th17 differentiation, two key events that are

known to promote CRC [120, 121]. Upon the availability of the recently made IL-1R1 conditional knockout mice [122], the Grivennikov group performed elegant work to dissect the roles of IL-1R1 on colonic epithelial cells, T-cells, and myeloid cells. In epithelial cells, IL-1R1 promotes initial tumor outgrowth and early progression [18], likely due to the antiapoptotic role of the major downstream IKKβ-mediated NF-kB activation [123]. In CD4 T-cells, IL-1R1-mediated signaling transduction is critical for eliciting IL-17 and IL-22 production [18] and maintaining tumor-elicited inflammation, hence driving CRC progression particularly via activation of STAT3 [124–126]. In contrast, IL-1R1 in myeloid cells plays an opposite role in tumor-elicited inflammation and CRC progression [18]. Neutrophil-specific IL-1R1 depletion leads to a deficit in neutrophil-mediated bacterial killing, thus increasing bacteria-induced tumor-elicited inflammation [18]. As a result, there is a larger tumor load in mice with neutrophil-specific IL-1R1 depletion. Within myeloid lineages, neutrophils are the dominant cell type to mediate IL-1R1 signaling since CX3CR1-Cre-mediated IL-1R1 deletion in the intestinal and tumor-associated macrophages did not yield any significant phenotype, while broader Il-1R ablation in myeloid populations using CD11b-Cre or LysM-cre demonstrated the same phenotype as in Ly-6G-Cre-mediated-neutrophil-specific deletion of IL-1R1. This neutrophil-mediated bacterial killing is enhanced by IL-1β treatment in vitro [18], indicating an anti-inflammatory role of IL-1β/IL-1R1 when encountering microbes in colon. IL-1α and IL-1β are not created equal in controlling colonic inflammation prior to carcinogenesis. In DSS-induced colitis, IL-1α is released by DSS-induced necrosis of intestinal epithelial cells as an alarmin to initiate limited colon inflammation and repair; IL-1β, on the other hand, plays a major role in colon repair [127], likely via an indirect neutrophil activation, microbial control, and/or direct pro-survival pathways in colonic epithelial cells to maintain the integrity of the barrier. Absence of IL-1β leads to severe colitis, a similar phenotype as IL-1R1$^{-/-}$ mice [127], indicating IL-1β-mediated myeloid activation and bacterial killing play a predominant role in preventing colitis in this DSS-induced mouse colitis model.

1.2.6 Other Cancers

Data from IL-1-related GEM models is lacking in most other cancer types. Research based on cancer cell lines or genetic data suggests that IL-1 signaling is critical for other cancers as well, including association of polymorphisms with risks [128–134], the promotion of aggressiveness in cancer by working on either cancer cells or microenvironment, and angiogenesis. For example, neutrophils can produce IL-1β in an inflammasome-independent manner, which is critical to mediate lung cancer resistance to IKK/NF-κB inhibition [15]. The same process is likely important for driving oncogenic KRAS-NF-κB addiction in malignant pleural effusion, a critical process for metastasis in lung cancer and other solid cancers [135]. In castration-resistant prostate cancer, IL-1RA upregulation by a combined immune checkpoint blockade and myeloid-derived suppressor cell (MDSC)-targeted therapy is critical to reduce MDSC infiltration [136]. Treatment with anakinra, the IL-R1 antagonist, provides an immune-permissive microenvironment that sensitizes castration-resistant prostate cancer to immune checkpoint blockade [136]. Based on all the information related to mouse and human data, the prevailing function of IL-1/IL-1R1-mediated signaling transduction in cancer is to promote cancer progression, and targeting IL-1/IL-1R1 signaling pathway could potentially benefit a large cancer patient population.

1.3 IL-1 Signaling Pathway in Different Cell Types Within Tumor Microenvironment: A Brief Summary

1.3.1 Cancer Cells

Cancer cells can be the primary target for IL-1 signaling that mostly transmits from IL-1/IL-1R1/IL-1RAcP to downstream IKK/NF-κB or

MAP kinase (JNK/p38). Those pathways are critical for the pro-survival and pro-invasive function of carcinoma cells. In addition, IL-1 can work on cancer cells to produce other factors such as VEGF for angiogenesis, IL-6 and TNF for tumor-induced inflammation, and other chemokines, cytokines, and growth factors to promote cancer progression. JNK and p38 activation, however, can initiate apoptosis under certain conditions, without the counteraction from IKK/NF-κB pro-survival function. The net signaling outcome can be suppressive for cancer cell growth. As cancer cells are not the primary focus of this chapter, we will not get into details here.

1.3.2 Fibroblasts

Fibroblasts can produce IL-1 and propagate IL-1 signaling. It has been shown that herpes simplex virus 1 (HSV-1) infection induces activation of NLRP3 inflammasome and consequent IL-1β activation in fibroblasts [137]. G-protein-coupled estrogen receptor (GPER) can induce IL-1β transcription and activation (presumably via NLRP3 activation) in cancer-associated fibroblasts (CAFs) [138]. Fibroblasts have been shown to relay macrophage-derived IL-1β signaling to induce cancer-promoting factors and angiogenesis [88]. In CAF cells, IL-1α has been shown to induce leukemia inhibitor factor (LIF) via a non-canonical JAK/STAT pathway, which contributes to the generation of inflammatory CAF and shapes CAF heterogeneity in pancreatic cancer [139]. IL-1 has been shown to induce PD-L1 and COX-2 in melanoma-associated fibroblasts, which is critical to induce immunosuppression in oncogenic BRAF melanoma [91]. Cellular senescence of fibroblasts has been shown not only to inhibit tumorigenesis early in life but to promote cancer in aged organisms [140]. Membrane-bound IL-1α serves as a critical upstream regulator of senescence-associated secretory phenotype (SASP) in senescent fibroblasts [141], where IL-1α mRNA is induced by NF-κB-mediated transcription and its protein is translated by mTOR-mediated mechanism [142].

1.3.3 Adipocytes

Adipocytes are integral components among several cancer types such as pancreatic and breast cancers. Cancer-associated adipocytes are known to produce IL-1β that can interact with other cell types within tumor microenvironment [143–145]. Adipose tissue is among the top expressers for IL-1R1 based on human protein atlas, indicative of its capability to receive IL-1 signaling transduction. Unsurprisingly, we found adipocytes are the major effector cells of myeloid IL-1β in obese animals carrying breast cancer [50, 51]. IL-1β induces various angiogenic factors including VEGF and ANGPTL4 to promote cancer progression [50, 51].

1.3.4 Endothelial Cells

Endothelial cells have long been known to be a direct or indirect target of IL-1 signaling. Many cell types, including endothelial cells, within tumor microenvironment can produce VEGF upon IL-1 activation, the growth factor for endothelial cells during angiogenesis. In turn, IL-1 and VEGF synergize to promote angiogenic response, and both factors are required for angiogenesis [101]. An interesting observation from a nontumor model defines a role of IL-1β in mobilizing endothelial progenitor cells, a process that could be potentially important for IL-1β-induced angiogenesis in cancer [146]. Endothelial cells have been thought to be one cellular source for CAFs via EMT [147], which has been demonstrated in vivo to contribute to cardiac fibrosis [148]. IL-1β is an important factor to promote this process via FGF-2 or other factors [149].

1.3.5 Immune Cells

IL-1β is one of the best-studied cytokines in inflammation and has been known to be one of the major cytokines involved in innate immunity and inflammation [2–6]. Activation of IL-1β is mostly studied in macrophages under infection or

sterile inflammation [2–6]. Myeloid cells are also one of the major producers of IL-1β in tumor microenvironment. Among the tumor-associated innate immune cells, IL-1β has been shown to work on all different cell types and elicit various functions. IL-1β is known to recruit and activate neutrophils that can either suppress microbiota-induced colonic inflammation and inhibit CRC [18] or promote inducible nitric oxide synthase (iNOS) production in neutrophils to inhibit CD8 T-cells in the setting of breast cancer metastasis [17]. IL-1β has been shown to induce CCL2 expression from various cellular sources and recruit tumor-associated macrophages and other myeloid lineages such as monocytic and granulocytic MDSCs [150]. In turn, these immune cells can be the cellular targets of IL-1 signaling to initiate immunosuppressive and pro-angiogenic signaling during cancer progression. IL-1β is also produced by the NLRP3 inflammasome in a subset of DCs in the presence of necrotic cancer cells during cancer progression and/or therapy [151], which is important for DC-mediated T-cell activation and cancer clearance by certain chemotherapy. DC-derived IL-1α, in a nontumor setting, promotes the proliferation of CD8 T-cells [152]. Interestingly, the DC-derived IL-1β activation requires a feed-forward mechanism from CD8 T-cells, in a specific antigen-dependent manner [153–155]. Likely depending on different cytokine milieu or DC subtypes, IL-1β has been shown to balance the presence of tumor-associated macrophages or $CD11b^+$ DC [49]. Tumors from wild-type animals favor IL-10 producing immunosuppressive tumor-associated macrophages, whereas tumors from the IL-1β-deficient host have increased $CD11b^+$ DC that can mount an antitumor Th1 and CD8 responses [150]. Among the innate lymphoid cells (ILCs), the role of IL-1 in cancer-associated NK-cells (group 1 ILCs) is largely unknown, and earlier studies indicate a role of IL-1 in promoting NK-cell activity toward tumoricidal effects [156]. Further literature supports this notion and found that co-treatment of IL-1β and IL-12 enhances the production of IFNγ and GM-CSF from a subset of human NK-cells [157]. On the other hand, IL-1β is critical to promote the development and maintenance of ILC3 cells [158, 159], and IL-1β is able to inhibit NK-cells from acquiring IFN-γ production and degranulation [158], indicative of an IL-1β-dependent suppression of NK effector function. IL-1β can also stimulate the activation of ILC3 cells that initiates antigen-specific CD4 T-cell responses [160] but also can induce production of often pro-tumorigenic cytokines such as IL-17A and IL-22. Distinct function of IL-1 signaling on ILC is yet to be established under different cancer contexts.

Among the adaptive immune cells, IL-1 has been shown to work on both αβ T-cells and γδ T-cells during cancer progression. In the latter, γδ T-cells in lung metastasis can be activated by IL-1β to express IL-17, a key cytokine that can induce G-CSF. G-CSF and IL-1β collectively lead to the successful neutrophil recruitment and polarization, which results in the production of immunosuppressive iNOS to inhibit CD8 T-cells [17]. The direct impact of IL-1 on CD4 T-cells was recently established in a CRC model where IL-1 signaling promotes a Th17 phenotype and potentiates tumor-elicited inflammation and progression [18]. This result is in agreement with the discovery that IL-1β promotes Th17 lineages when combined with IL-6 [161] or with IL-23 [162–164]. The IL-1β-dependent Th17 commitment could also be due to the alteration of plastic Tregs into Th17 [165–167]. In addition, IL-1β directly acts on a mixed memory CD4 T-cell population to induce IL-22 production [18, 168]. Both IL-17 and IL-22 have been reported to promote cancer progression in different cancers [18, 168]. IL-1β and IL-4 have been shown to promote the differentiation of Th9 cells in the absence of TGFβ, resulting in a superior antitumor CD4 Th9 population that is less exhausted with cytotoxic gene signatures [169]. In nontumor setting, there are many outstanding cases in which IL-1β can enhance antigen-specific CD4 and CD8 T-cell proliferation and activation, as well as memory responses of these T-cells, mostly likely via direct IL-1β/IL-1R1 signaling [170–172].

The various effector cell types dictate a careful evaluation of tumor microenvironment, which defines the dominant role of IL-1 signaling within

the tumor microenvironment. Treating cancers with IL-1 antagonists should be carefully evaluated using a comprehensive approach. For example, lung cancers or metastasis may benefit from IL-1 inhibition due to the role of IL-1 in recruiting cancer-promoting neutrophils and subsequent CD8 suppression. In CRC, however, neutrophils are critical to eliminate cancer-penetrating bacteria and thus control the tumor-elicited inflammation and tumor progression. Another concern is how to best use the antitumorigenic role of IL-1 signaling in DC activation and antigen-specific CD8 T-cell priming and activation, a process that requires DC to sense ATP release from necrotic cancer cells and NLRP3 activation. Thus, it would likely be detrimental to use IL-1 inhibitors under these conditions.

1.4 Clinical Implications and Perspectives

1.4.1 Clinical Studies

Due to the complex role of IL-1 signaling in immunity and physiology, clinical development of IL-1-targeted therapy is mostly related to autoimmune diseases such as the development of anakinra in rheumatoid arthritis and canakinumab (IL-1β-specific antibody) for several rare inflammatory diseases. Recently, the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS), initially designed to prevent heart attack, stroke, or cardiovascular death in patients with elevated C-reactive protein using different doses of canakinumab, identified a significant reduction in lung cancer mortality and risk in the cohort receiving canakinumab relative to placebo controls (> 50% risk reduction) [173]. This was extremely exciting in the field of cancer therapy, and Novartis immediately followed up with several trials to combine canakinumab with chemotherapy and immune checkpoint inhibitor in lung cancers, including a phase III CANOPY-2 study evaluating the efficacy and safety of canakinumab in combination with docetaxel in non-small cell lung cancers (Clinicaltrials.gov Identifier: NCT03626545).

In breast cancer, a pilot trial in HER2-negative metastatic breast cancer patients defines an anakinra-regulated gene signature from the leukocyte transcripts, mostly related to IL-1 family (IL-1B, IL-1R1, IL-1R2, IL-1RAP, IL-1RN, IL-6, IL-6R), NF-KB signaling (NF-KB2, NF-KBIZ), and innate immune sensing (TLR1, TLR2, TLR4, TLR5, TLR8, NOD2) molecules. The anakinra-regulated signature from blood leukocytes can faithfully predict patient outcome. In particular, the gene signature is enriched in the aggressive basal-like breast cancer subtype that could potentially benefit from IL-1/IL-1R1-targeted therapy [32]. In addition to blocking the role of IL-1 in cancer-associated inflammation, anakinra administration in this pilot trial led to elevated cytotoxic signatures from NK- or CD8 T-cells, indicative of activation. This phenomenon was recently explained using animal models where IL-1 blockade leads to increased DC-mediated antigen presentation and CD8 T-cell activation [49], which provides the rationale to combine immunotherapy in patients with TNBC with canakinumab (Clinicaltrials.gov Identifier: NCT03742349).

Bermekimab (MABp1, an IL-1α-specific monoclonal antibody) was recently used in a phase III trial to treat metastatic CRC with predicted poor outcomes. This study used defined primary endpoints including lean body mass (stable or increased body weight) and criteria QLQ-C30 (fatigue, pain, and anorexia; at least two of these are improved) from European Organisation for Research and Treatment of Cancer (EORTC). The bermekimab treatment significantly increased percentage of patients reaching the primary endpoints (33% versus 19%) but did not increase adverse events relative to placebo controls (23% versus 33%) [174]. In a similar setting, when pretreatment levels of serum IL-1RA were taken into account, patients with lower baseline IL-1RA levels responded better to bermekimab treatment with increased response rate [175]. There was no overall survival benefit from this trial, suggesting that inhibition of IL-1α should be intended for improving patient quality of life, like cancer-associated cachexia [174, 175]. One of the advanced phase III trials using

bermekimab to treat metastatic CRC was terminated due to inability of the study to reach futility boundary of the primary endpoint (Clinicaltrials.gov Identifier: NCT01767857).

In addition to these advances in clinical studies, outstanding literature provides a strong rationale to target this pathway for cancer therapy. We have shown that anakinra or IL-1R1 antibody can inhibit obesity-associated angiogenesis in breast cancer [50]. Chimeric antigen receptor (CAR) T-cell therapy targeting CD19 has been an outstanding approach for refractory B cell malignancies but commonly associated with severe cytokine storms. Anakinra can ameliorate the severe adverse effect [176] and is worthwhile to explore its role in CAR T-cell-treated patients. IL-1β has been shown to induce antigen presentation from DC and induce cancer-antigen-specific priming of CD8 T-cells, which indicates a role of IL-1β in chronic activation of CD8 T-cells in tumor microenvironment and provides a rationale to combine IL-1 inhibition with immune checkpoint blockage during cancer therapy [49].

1.4.2 Consideration for Clinical Studies

It is important to use caution for human clinical studies. For example, IL-1 signaling has been shown to inhibit mammary tumor growth and metastasis in luminal type of breast cancer [55] associated with "cold" immune microenvironment that is unresponsive to immune checkpoint blockage [177, 178]. IL-1α inhibits the conversion of papilloma to carcinoma in skin cancer [107]. There are also occasional reports that IL-1 treatment can inhibit cancer cell growth in vitro from various cancer types [19, 20, 33, 34, 179, 180] that may not be the best cancer types to target IL-1 signaling pathway for therapy. In addition, there are some controversial results related to the role of IL-1β in DC activation/T-cell priming. Earlier reports have shown that DC can receive ATP from dying tumor cells via the P2X7 purinergic receptors, leading to the activation of NLRP3 inflammasome and downstream IL-1β processing and secretion. IL-1β, in turn, propagates the signal from DCs to prime $CD8^+$ T-cells and induces IFNγ for cancer cell killing. This process is critical to mediate chemotherapy-induced cancer cell killing in an adaptive immune-dependent manner. In breast cancer patients with loss-of-function mutations in P2X7, that is, unable to activate NLRP3 inflammasome, doxorubicin treatment leads to faster metastasis and resistance to treatment [151]. This is further supported by another study showing that IL-1β is critical to mediate the efficacy of doxorubicin treatment in an adaptive immune-dependent manner [181]. The disagreement on the role of IL-1β in DC activation and CD8 T-cell priming [49, 151, 181] warrants careful evaluation of DC subtypes within tumor microenvironment and draining lymph nodes. IL-1RA, produced by CD11b + Gr-1+ myeloid cells, can antagonize senescence in cancer and promote PTEN-loss-mediated cancer initiation [182]. Many anticancer drugs that induce senescence of cancer cells also increase IL-1 production as summarized in a recent outstanding review article [183]. The decision to combine IL-1 inhibition and other therapy requires further understanding of the tumor immune microenvironment including the major immune cell subtypes, role of IL-1 on these immune cells, and the nature of therapeutic agents to be used.

1.4.3 Using Publicly Available Genetic Information for Assessing the Role of IL-1 Signaling in Cancer Progression

Among the Cancer Genome Atlas (TCGA) Pan-Cancer data across 30 cancer types, we analyzed the correlation between mRNA expression of IL-1α, IL-1β, IL-1R1, and IL-1RA and overall survival. Due to the inefficiency of IL-1 signaling in eradicating tumors from most preclinical research, targeting IL-1 pathways should only be considered to facilitate other established therapeutics. We found some very interesting information and summarized here (Fig. 1.2):

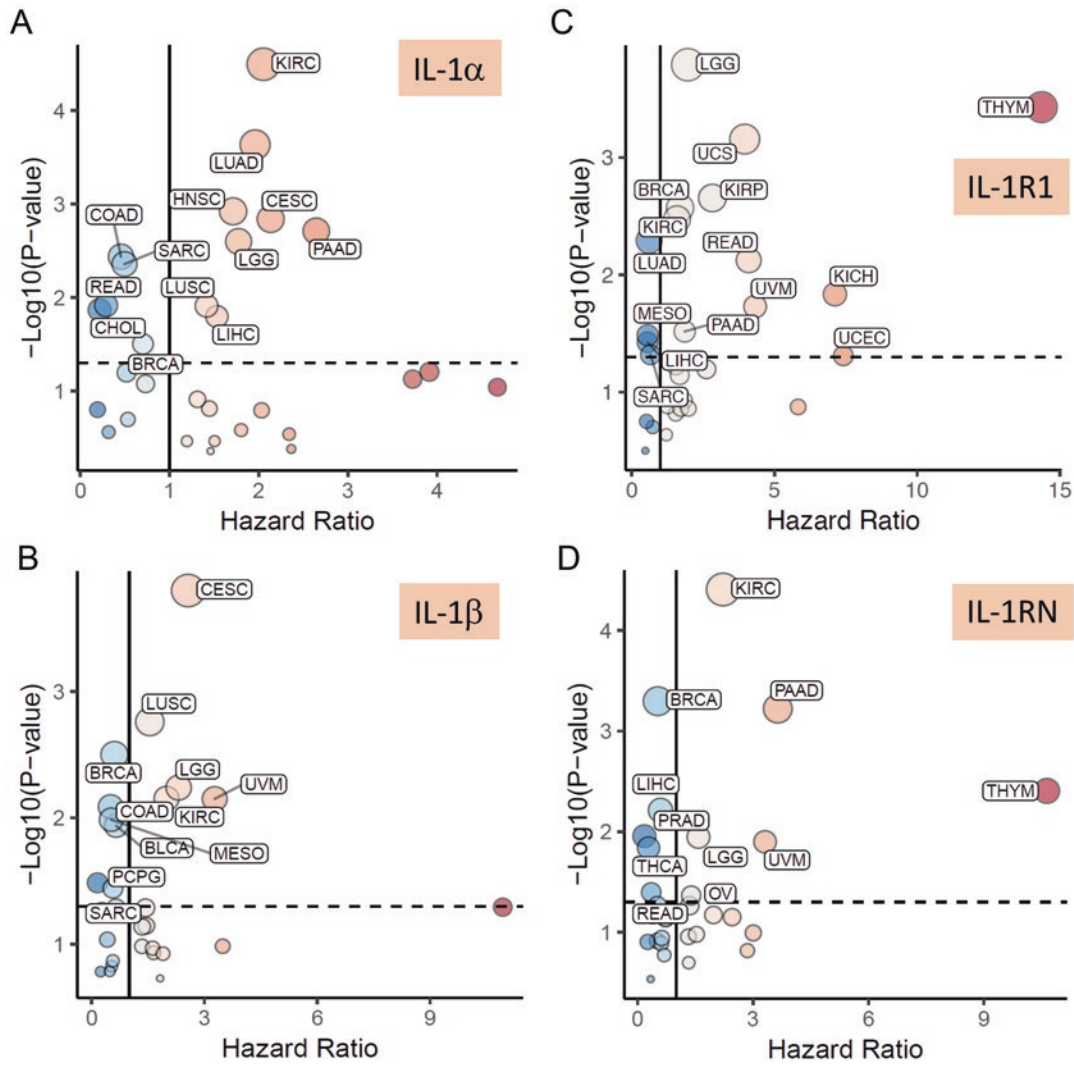

Fig. 1.2 The use of TCGA data to predict the correlation between mRNAs from IL-1 pathway and prognosis

IL-1R1, the major mediator of IL-1 signaling, is mostly correlated with poor overall prognosis (11/30 versus 4/30). Since most TCGA specimens have very restricted cancer cell content (e.g., BRCA specimens should have at least 80% carcinoma cells) [184], it is presumably that most IL-1R1 mRNA comes from cancer cells and mediates cancer progression via cancer-cell-intrinsic mechanisms.

IL-1RA, the antagonist for IL-1 ligands, is correlated with poor prognosis in six cancers, among which five are shared with IL-1R1 group. This suggests that IL-1 signaling activation often induces a negative feedback by turning on IL-1RA [7, 8].

Two cancer types (kidney renal clear cell carcinoma, KIRC, and low-grade glioma, LGG) exhibit a correlation between poor prognosis and mRNAs of all four factors, suggesting that IL-1 signaling may play a critical role in disease progression and could be targeted in these cancers. In particular, KIRC is known to have relatively low mutational burden but responds to immune checkpoint therapy [185]. A combination of anakinra and immune checkpoint blockage to inhibit both IL-1α and

IL-1β may be a better choice to treat KIRC patients by alteration of immunosuppressive microenvironment as well as inhibition of cancer growth [186, 187].

Two cancer types (uveal melanoma, UVM, and thymoma, THYM) exhibit a correlation between poor prognosis and mRNAs of IL-1β, IL-1R1, and IL-1RA, not IL-1α. Using IL-1β-specific antibody such as canakinumab could be a choice to boost immunotherapy and leave IL-1α-mediated immune surveillance arm intact.

Pancreatic adenocarcinoma (PAAD), a known immune-cold cancer type, is a difficult cancer to treat and shows elevated mRNA levels of IL-1α, IL-1R1, and IL-1RA, not IL-1β, among the patient specimens with poor prognosis. Literature has shown that cancer cell-derived IL-1α is critical for cancer cell adhesion [188], growth [189], and Treg cell infiltration [190]. These patients may benefit from IL-1α-specific treatment such as using bermekimab (MABp1) in combination with other therapies.

Both IL-1α and IL-1β are correlated with poor prognosis in cervical squamous cell carcinoma/endocervical adenocarcinoma (CESC) and lung squamous cell carcinoma (LUSC), supporting a role of common IL-1R1 signaling in squamous cell carcinomas that may benefit from anakinra co-treatment. The CANTO trial did not have enough non-small cell lung squamous cell carcinoma (LUSC) patients to determine if canakinumab reduces the risk of LUSC [173].

IL-1α, but not IL-1β, is correlated with poor prognosis in lung adenocarcinoma (LUAD), suggesting the role of IL-1α in promoting cancer progression in lung adenocarcinoma. Considering the role of canakinumab (IL-1β) in reducing LUAD risk and prolonging patient survival, the clinical trial mentioned above (Clinicaltrials.gov Identifier: NCT03626545) may benefit from comparing anakinra and canakinumab in non-small cell lung cancer patients.

Please note the above data are based on mRNA expression and the information provided above is by no means the guide on how to design clinical research and how to choose target patient populations. All known literature should be comprehensively analyzed to justify the most rational design for human studies. There are also many other drugs targeting IL-1 signaling transduction, including FDA-approved rilonacept (a soluble decoy receptor for neutralizing both IL-1 ligands) and others in development such as gevokizumab and LY2189102 (anti-IL-1β), AMG 108 (anti-IL-1R1), and AX-765 (long-lasting Casp-1 inhibitor). These agents are potential drugs to be repurposed for cancer therapy [191].

References

1. (1979) Revised nomenclature for antigen-nonspecific T cell proliferation and helper factors. J Immunol 123(6):2928–2929
2. Gabay C, Lamacchia C, Palmer G (2010) IL-1 pathways in inflammation and human diseases. Nat Rev Rheumatol 6(4):232–241
3. Sims JE, Smith DE (2010) The IL-1 family: regulators of immunity. Nat Rev Immunol 10(2):89–102
4. Mantovani A, Dinarello CA, Molgora M et al (2019) Interleukin-1 and related cytokines in the regulation of inflammation and immunity. Immunity 50(4):778–795
5. Voronov E, Dinarello CA, Apte RN (2018) Interleukin-1alpha as an intracellular alarmin in cancer biology. Semin Immunol 38:3–14
6. Garlanda C, Dinarello CA, Mantovani A (2013) The interleukin-1 family: back to the future. Immunity 39(6):1003–1018
7. La E, Rundhaug JE, Pavone A et al (2002) Regulation of transcription of the intracellular interleukin-1 receptor antagonist gene by AP-1 in mouse carcinoma cells. Mol Carcinog 33(4):237–243
8. La E, Fischer SM (2001) Transcriptional regulation of intracellular IL-1 receptor antagonist gene by IL-1 alpha in primary mouse keratinocytes. J Immunol 166(10):6149–6155
9. Swanson KV, Deng M, Ting JP (2019) The NLRP3 inflammasome: molecular activation and regulation to therapeutics. Nat Rev Immunol 19:477–489. https://doi.org/10.1038/s41577-019-0165-0
10. Karki R, Kanneganti TD (2019) Diverging inflammasome signals in tumorigenesis and potential targeting. Nat Rev Cancer 19(4):197–214
11. Afonina IS, Zhong Z, Karin M et al (2017) Limiting inflammation-the negative regulation of NF-kappaB and the NLRP3 inflammasome. Nat Immunol 18(8):861–869

12. Vance RE (2015) The NAIP/NLRC4 inflammasomes. Curr Opin Immunol 32:84–89
13. Kolb R, Liu GH, Janowski AM et al (2014) Inflammasomes in cancer: a double-edged sword. Protein Cell 5(1):12–20
14. Cassel SL, Janczy JR, Bing X et al (2014) Inflammasome-independent IL-1beta mediates autoinflammatory disease in Pstpip2-deficient mice. Proc Natl Acad Sci U S A 111(3):1072–1077
15. McLoed AG, Sherrill TP, Cheng DS et al (2016) Neutrophil-derived IL-1beta impairs the efficacy of NF-kappaB inhibitors against lung cancer. Cell Rep 16(1):120–132
16. Kuan EL, Ziegler SF (2018) A tumor-myeloid cell axis, mediated via the cytokines IL-1alpha and TSLP, promotes the progression of breast cancer. Nat Immunol 19(4):366–374
17. Coffelt SB, Kersten K, Doornebal CW et al (2015) IL-17-producing gammadelta T cells and neutrophils conspire to promote breast cancer metastasis. Nature 522(7556):345–348
18. Dmitrieva-Posocco O, Dzutsev A, Posocco DF et al (2019) Cell-type-specific responses to interleukin-1 Control microbial invasion and tumor-elicited inflammation in colorectal cancer. Immunity 50(1):166–180 e7
19. Liu L, Gudas LJ (2002) Retinoic acid induces expression of the interleukin-1beta gene in cultured normal human mammary epithelial cells and in human breast carcinoma lines. J Cell Physiol 193(2):244–252
20. Hughes R, Timmermans P, Schrey MP (1996) Regulation of arachidonic acid metabolism, aromatase activity and growth in human breast cancer cells by interleukin-1beta and phorbol ester: dissociation of a mediatory role for prostaglandin E2 in the autocrine control of cell function. Int J Cancer 67(5):684–689
21. Shen WH, Jackson ST, Broussard SR et al (2004) IL-1beta suppresses prolonged Akt activation and expression of E2F-1 and cyclin A in breast cancer cells. J Immunol 172(12):7272–7281
22. Wang FM, Liu HQ, Liu SR et al (2005) SHP-2 promoting migration and metastasis of MCF-7 with loss of E-cadherin, dephosphorylation of FAK and secretion of MMP-9 induced by IL-1beta in vivo and in vitro. Breast Cancer Res Treat 89(1):5–14
23. Franco-Barraza J, Valdivia-Silva JE, Zamudio-Meza H et al (2010) Actin cytoskeleton participation in the onset of IL-1beta induction of an invasive mesenchymal-like phenotype in epithelial MCF-7 cells. Arch Med Res 41(3):170–181
24. Ma L, Lan F, Zheng Z et al (2012) Epidermal growth factor (EGF) and interleukin (IL)-1beta synergistically promote ERK1/2-mediated invasive breast ductal cancer cell migration and invasion. Mol Cancer 11:79
25. Li Q, Engelhardt JF (2006) Interleukin-1beta induction of NFkappaB is partially regulated by H2O2-mediated activation of NFkappaB-inducing kinase. J Biol Chem 281(3):1495–1505
26. Naldini A, Filippi I, Miglietta D et al (2010) Interleukin-1beta regulates the migratory potential of MDAMB231 breast cancer cells through the hypoxia-inducible factor-1alpha. Eur J Cancer 46(18):3400–3408
27. Perez-Yepez EA, Ayala-Sumuano JT, Lezama R et al (2014) A novel beta-catenin signaling pathway activated by IL-1beta leads to the onset of epithelial-mesenchymal transition in breast cancer cells. Cancer Lett 354(1):164–171
28. Kendellen MF, Bradford JW, Lawrence CL et al (2014) Canonical and non-canonical NF-kappaB signaling promotes breast cancer tumor-initiating cells. Oncogene 33(10):1297–1305
29. Castano Z, San Juan BP, Spiegel A et al (2018) IL-1beta inflammatory response driven by primary breast cancer prevents metastasis-initiating cell colonization. Nat Cell Biol 20(9):1084–1097
30. Tulotta C, Lefley DV, Freeman K et al (2019) Endogenous production of IL1B by breast cancer cells drives metastasis and colonization of the bone microenvironment. Clin Cancer Res 25(9):2769–2782
31. Holen I, Lefley DV, Francis SE et al (2016) IL-1 drives breast cancer growth and bone metastasis in vivo. Oncotarget 7(46):75571–75584
32. Wu TC, Xu K, Martinek J et al (2018) IL1 receptor antagonist controls transcriptional signature of inflammation in patients with metastatic breast cancer. Cancer Res 78(18):5243–5258
33. Danforth DN Jr, Sgagias MK (1991) Interleukin 1 alpha blocks estradiol-stimulated growth and downregulates the estrogen receptor in MCF-7 breast cancer cells in vitro. Cancer Res 51(5):1488–1493
34. Danforth DN Jr, Sgagias MK (1993) Interleukin-1 alpha and interleukin-6 act additively to inhibit growth of MCF-7 breast cancer cells in vitro. Cancer Res 53(7):1538–1545
35. Kumar S, Kishimoto H, Chua HL et al (2003) Interleukin-1 alpha promotes tumor growth and cachexia in MCF-7 xenograft model of breast cancer. Am J Pathol 163(6):2531–2541
36. Sheridan C, Kishimoto H, Fuchs RK et al (2006) CD44+/CD24- breast cancer cells exhibit enhanced invasive properties: an early step necessary for metastasis. Breast Cancer Res 8(5):R59
37. Bhat-Nakshatri P, Newton TR, Goulet R Jr et al (1998) NF-kappaB activation and interleukin 6 production in fibroblasts by estrogen receptor-negative breast cancer cell-derived interleukin 1alpha. Proc Natl Acad Sci U S A 95(12):6971–6976
38. Nozaki S, Sledge GW Jr, Nakshatri H (2000) Cancer cell-derived interleukin 1alpha contributes to autocrine and paracrine induction of pro-metastatic genes in breast cancer. Biochem Biophys Res Commun 275(1):60–62
39. Singer CF, Kronsteiner N, Hudelist G et al (2003) Interleukin 1 system and sex steroid receptor

expression in human breast cancer: interleukin 1alpha protein secretion is correlated with malignant phenotype. Clin Cancer Res 9(13):4877–4883
40. Singer CF, Hudelist G, Gschwantler-Kaulich D et al (2006) Interleukin-1alpha protein secretion in breast cancer is associated with poor differentiation and estrogen receptor alpha negativity. Int J Gynecol Cancer 16(Suppl 2):556–559
41. Wang Y, Wang Y, Li L (2016) Note of clarification regarding data about the association between the interleukin-1beta -31T>C polymorphism and breast cancer risk. Breast Cancer Res Treat 155(3):415–417
42. Liu X, Wang Z, Yu J et al (2010) Three polymorphisms in interleukin-1beta gene and risk for breast cancer: a meta-analysis. Breast Cancer Res Treat 124(3):821–825
43. Lee KM, Park SK, Hamajima N et al (2006) Genetic polymorphisms of interleukin-1 beta (IL-1B) and IL-1 receptor antagonist (IL-1RN) and breast cancer risk in Korean women. Breast Cancer Res Treat 96(2):197–202
44. Hefler LA, Grimm C, Lantzsch T et al (2005) Interleukin-1 and interleukin-6 gene polymorphisms and the risk of breast cancer in caucasian women. Clin Cancer Res 11(16):5718–5721
45. Jin T, Cao W, Zuo X et al (2017) IL-1RN gene polymorphisms are associated with breast cancer risk in a Chinese Han population. J Gene Med 19(12):e2996
46. Huang X, Yang Y, Cui ZW et al (2016) A functional insertion/deletion polymorphism in the IL1A gene is associated with decreased risk of breast cancer. Genet Mol Res 15(1):4238
47. Gao Y, He Y, Ding J et al (2009) An insertion/deletion polymorphism at miRNA-122-binding site in the interleukin-1alpha 3′ untranslated region confers risk for hepatocellular carcinoma. Carcinogenesis 30(12):2064–2069
48. Han W, Kang SY, Kang D et al (2010) Multiplex genotyping of 1107 SNPs from 232 candidate genes identified an association between IL1A polymorphism and breast cancer risk. Oncol Rep 23(3):763–769
49. Kaplanov I, Carmi Y, Kornetsky R et al (2019) Blocking IL-1beta reverses the immunosuppression in mouse breast cancer and synergizes with anti-PD-1 for tumor abrogation. Proc Natl Acad Sci U S A 116(4):1361–1369
50. Kolb R, Phan L, Borcherding N et al (2016) Obesity-associated NLRC4 inflammasome activation drives breast cancer progression. Nat Commun 7:13007
51. Kolb R, Kluz P, Tan ZW et al (2019) Obesity-associated inflammation promotes angiogenesis and breast cancer via angiopoietin-like 4. Oncogene 38(13):2351–2363
52. Weichand B, Popp R, Dziumbla S et al (2017) S1PR1 on tumor-associated macrophages promotes lymphangiogenesis and metastasis via NLRP3/IL-1beta. J Exp Med 214(9):2695–2713
53. Guo B, Fu S, Zhang J et al (2016) Targeting inflammasome/IL-1 pathways for cancer immunotherapy. Sci Rep 6:36107
54. Grosman H, Fabre B, Lopez M et al (2016) Complex relationship between sex hormones, insulin resistance and leptin in men with and without prostatic disease. Aging Male 19(1):40–45
55. Dagenais M, Dupaul-Chicoine J, Douglas T et al (2017) The interleukin (IL)-1R1 pathway is a critical negative regulator of PyMT-mediated mammary tumorigenesis and pulmonary metastasis. Oncoimmunology 6(3):e1287247
56. DeNardo DG, Barreto JB, Andreu P et al (2009) CD4(+) T cells regulate pulmonary metastasis of mammary carcinomas by enhancing protumor properties of macrophages. Cancer Cell 16(2):91–102
57. Pfefferle AD, Herschkowitz JI, Usary J et al (2013) Transcriptomic classification of genetically engineered mouse models of breast cancer identifies human subtype counterparts. Genome Biol 14(11):R125
58. Melendez JA, Davies KJ (1996) Manganese superoxide dismutase modulates interleukin-1alpha levels in HT-1080 fibrosarcoma cells. J Biol Chem 271(31):18898–18903
59. Gigant-Huselstein C, Dumas D, Payan E et al (2002) In vitro study of intracellular IL-1beta production and beta1 integrins expression in stimulated chondrocytes—effect of rhein. Biorheology 39(1–2):277–285
60. Ju J, Huang C, Minskoff SA et al (2003) Simultaneous gene expression analysis of steady-state and actively translated mRNA populations from osteosarcoma MG-63 cells in response to IL-1alpha via an open expression analysis platform. Nucleic Acids Res 31(17):5157–5166
61. Shi J, Schmitt-Talbot E, DiMattia DA et al (2004) The differential effects of IL-1 and TNF-alpha on proinflammatory cytokine and matrix metalloproteinase expression in human chondrosarcoma cells. Inflamm Res 53(8):377–389
62. Chang DM, Liu SH, Lee HS et al (2007) Activin A suppresses interleukin-1-induced matrix metalloproteinase 3 secretion in human chondrosarcoma cells. Rheumatol Int 27(11):1049–1055
63. Ha WY, Li XJ, Yue PY et al (2006) Gene expression profiling of human synovial sarcoma cell line (Hs701.T) in response to IL-1beta stimulation. Inflamm Res 55(7):293–299
64. Choi EM, Lee YS (2010) Luteolin suppresses IL-1beta-induced cytokines and MMPs production via p38 MAPK, JNK, NF-kappaB and AP-1 activation in human synovial sarcoma cell line, SW982. Food Chem Toxicol 48(10):2607–2611
65. Lim H, Kim HP (2011) Matrix metalloproteinase-13 expression in IL-1beta-treated chondrocytes by activation of the p38 MAPK/c-Fos/AP-1 and JAK/STAT pathways. Arch Pharm Res 34(1):109–117
66. Lanier SM, Homcy CJ, Patenaude C et al (1988) Identification of structurally distinct alpha 2-adrenergic receptors. J Biol Chem 263(28):14491–14496
67. Kalinski T, Krueger S, Sel S et al (2006) Differential expression of VEGF-A and angiopoietins in car-

tilage tumors and regulation by interleukin-1beta. Cancer 106(9):2028–2038
68. Liu B, Zhou Y, Chen X et al (2017) IL-1beta-mediated NF-kappaB signaling augments the osteosarcoma cell growth through modulating miR-376c/TGFA axis. Pharmazie 72(7):419–424
69. Hu M, Yuan X, Liu Y et al (2017) IL-1beta-induced NF-kappaB activation down-regulates miR-506 expression to promotes osteosarcoma cell growth through JAG1. Biomed Pharmacother 95:1147–1155
70. Zeng L, Rong XF, Li RH et al (2017) Icariin inhibits MMP1, MMP3 and MMP13 expression through MAPK pathways in IL1betastimulated SW1353 chondrosarcoma cells. Mol Med Rep 15(5):2853–2858
71. He Y, Liang X, Meng C et al (2014) Genetic polymorphisms of interleukin-1 beta and osteosarcoma risk. Int Orthop 38(8):1671–1676
72. Song X, Voronov E, Dvorkin T et al (2003) Differential effects of IL-1 alpha and IL-1 beta on tumorigenicity patterns and invasiveness. J Immunol 171(12):6448–6456
73. Nazarenko I, Marhaba R, Reich E et al (2008) Tumorigenicity of IL-1alpha- and IL-1beta-deficient fibrosarcoma cells. Neoplasia 10(6):549–562
74. Marhaba R, Nazarenko I, Knofler D et al (2008) Opposing effects of fibrosarcoma cell-derived IL-1 alpha and IL-1 beta on immune response induction. Int J Cancer 123(1):134–145
75. Krelin Y, Voronov E, Dotan S et al (2007) Interleukin-1beta-driven inflammation promotes the development and invasiveness of chemical carcinogen-induced tumors. Cancer Res 67(3):1062–1071
76. Elkabets M, Krelin Y, Dotan S et al (2009) Host-derived interleukin-1alpha is important in determining the immunogenicity of 3-methylcholantrene tumor cells. J Immunol 182(8):4874–4881
77. Tak KH, Yu GI, Lee MY et al (2018) Association between polymorphisms of interleukin 1 family genes and hepatocellular carcinoma. Med Sci Monit 24:3488–3495
78. Wang Y, Kato N, Hoshida Y et al (2003) Interleukin-1beta gene polymorphisms associated with hepatocellular carcinoma in hepatitis C virus infection. Hepatology 37(1):65–71
79. ElSheshtawy NM, Nour MS, Hefny Z et al (2017) Gene polymorphisms of interleukin 1? And metalloproteinase 3 in hepatitis C infected patients and hepatocellular carcinoma patients. Egypt J Immunol 24(1):1–8
80. Sakurai T, He G, Matsuzawa A et al (2008) Hepatocyte necrosis induced by oxidative stress and IL-1 alpha release mediate carcinogen-induced compensatory proliferation and liver tumorigenesis. Cancer Cell 14(2):156–165
81. Maeda S, Kamata H, Luo JL et al (2005) IKKbeta couples hepatocyte death to cytokine-driven compensatory proliferation that promotes chemical hepatocarcinogenesis. Cell 121(7):977–990
82. Naugler WE, Sakurai T, Kim S et al (2007) Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. Science 317(5834):121–124
83. Yoshimoto S, Loo TM, Atarashi K et al (2013) Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. Nature 499(7456):97–101
84. Shrivastava S, Mukherjee A, Ray R et al (2013) Hepatitis C virus induces interleukin-1beta (IL-1beta)/IL-18 in circulatory and resident liver macrophages. J Virol 87(22):12284–12290
85. Negash AA, Ramos HJ, Crochet N et al (2013) IL-1beta production through the NLRP3 inflammasome by hepatic macrophages links hepatitis C virus infection with liver inflammation and disease. PLoS Pathog 9(4):e1003330
86. Lanaya H, Natarajan A, Komposch K et al (2014) EGFR has a tumour-promoting role in liver macrophages during hepatocellular carcinoma formation. Nat Cell Biol 16(10):972–977
87. He G, Dhar D, Nakagawa H et al (2013) Identification of liver cancer progenitors whose malignant progression depends on autocrine IL-6 signaling. Cell 155(2):384–396
88. Young HL, Rowling EJ, Bugatti M et al (2017) An adaptive signaling network in melanoma inflammatory niches confers tolerance to MAPK signaling inhibition. J Exp Med 214(6):1691–1710
89. Gehrke S, Otsuka A, Huber R et al (2014) Metastatic melanoma cell lines do not secrete IL-1beta but promote IL-1beta production from macrophages. J Dermatol Sci 74(2):167–169
90. Qin Y, Ekmekcioglu S, Liu P et al (2011) Constitutive aberrant endogenous interleukin-1 facilitates inflammation and growth in human melanoma. Mol Cancer Res 9(11):1537–1550
91. Khalili JS, Liu S, Rodriguez-Cruz TG et al (2012) Oncogenic BRAF(V600E) promotes stromal cell-mediated immunosuppression via induction of interleukin-1 in melanoma. Clin Cancer Res 18(19):5329–5340
92. Giavazzi R, Garofalo A, Bani MR et al (1990) Interleukin 1-induced augmentation of experimental metastases from a human melanoma in nude mice. Cancer Res 50(15):4771–4775
93. Lauri D, Bertomeu MC, Orr FW et al (1990) Interleukin-1 increases tumor cell adhesion to endothelial cells through an RGD dependent mechanism: in vitro and in vivo studies. Clin Exp Metastasis 8(1):27–32
94. Pan W, Zhu S, Qu K et al (2017) The DNA methylcytosine dioxygenase Tet2 sustains immunosuppressive function of tumor-infiltrating myeloid cells to promote melanoma progression. Immunity 47(2):284–297 e5
95. Qin Y, Milton DR, Oba J et al (2015) Inflammatory IL-1beta-driven JNK activation in stage III melanoma. Pigment Cell Melanoma Res 28(2):236–239

96. Hoffmann W, Hauser F (1993) The P-domain or trefoil motif: a role in renewal and pathology of mucous epithelia? Trends Biochem Sci 18(7):239–243
97. Chirivi RG, Chiodoni C, Musiani P et al (1996) IL-1alpha gene-transfected human melanoma cells increase tumor-cell adhesion to endothelial cells and their retention in the lung of nude mice. Int J Cancer 67(6):856–863
98. Howell WM, Turner SJ, Theaker JM et al (2003) Cytokine gene single nucleotide polymorphisms and susceptibility to and prognosis in cutaneous malignant melanoma. Eur J Immunogenet 30(6):409–414
99. Broer PN, Aung T, Heidekrueger PI et al (2017) Divisive influence of interleukin-1 receptor antagonist polymorphisms in melanoma patients. Clin Hemorheol Microcirc 67(3–4):319–326
100. Voronov E, Shouval DS, Krelin Y et al (2003) IL-1 is required for tumor invasiveness and angiogenesis. Proc Natl Acad Sci U S A 100(5):2645–2650
101. Carmi Y, Dotan S, Rider P et al (2013) The role of IL-1beta in the early tumor cell-induced angiogenic response. J Immunol 190(7):3500–3509
102. Vidal-Vanaclocha F, Fantuzzi G, Mendoza L et al (2000) IL-18 regulates IL-1beta-dependent hepatic melanoma metastasis via vascular cell adhesion molecule-1. Proc Natl Acad Sci U S A 97(2):734–739
103. Lavi G, Voronov E, Dinarello CA et al (2007) Sustained delivery of IL-1 Ra from biodegradable microspheres reduces the number of murine B16 melanoma lung metastases. J Control Release 123(2):123–130
104. Thorslund SE, Ermert D, Fahlgren A et al (2013) Role of YopK in Yersinia pseudotuberculosis resistance against polymorphonuclear leukocyte defense. Infect Immun 81(1):11–22
105. Coste I, Le Corf K, Kfoury A et al (2010) Dual function of MyD88 in RAS signaling and inflammation, leading to mouse and human cell transformation. J Clin Invest 120(10):3663–3667
106. Thoua NM, Derrett-Smith EC, Khan K et al (2012) Gut fibrosis with altered colonic contractility in a mouse model of scleroderma. Rheumatology (Oxford) 51(11):1989–1998
107. Murphy JE, Morales RE, Scott J et al (2003) IL-1 alpha, innate immunity, and skin carcinogenesis: the effect of constitutive expression of IL-1 alpha in epidermis on chemical carcinogenesis. J Immunol 170(11):5697–5703
108. Jarry A, Vallette G, Cassagnau E et al (1999) Interleukin 1 and interleukin 1beta converting enzyme (caspase 1) expression in the human colonic epithelial barrier. Caspase 1 downregulation in colon cancer. Gut 45(2):246–251
109. Shao J, Sheng H (2007) Prostaglandin E2 induces the expression of IL-1alpha in colon cancer cells. J Immunol 178(7):4097–4103
110. Wright NT, Margolis JW, Margolis FL et al (2005) Refinement of the solution structure of rat olfactory marker protein (OMP). J Biomol NMR 33(1):63–68
111. Matsuo Y, Sawai H, Ma J et al (2009) IL-1alpha secreted by colon cancer cells enhances angiogenesis: the relationship between IL-1alpha release and tumor cells' potential for liver metastasis. J Surg Oncol 99(6):361–367
112. Akagi Y, Liu W, Xie K et al (1999) Regulation of vascular endothelial growth factor expression in human colon cancer by interleukin-1beta. Br J Cancer 80(10):1506–1511
113. Konishi N, Miki C, Yoshida T et al (2005) Interleukin-1 receptor antagonist inhibits the expression of vascular endothelial growth factor in colorectal carcinoma. Oncology 68(2–3):138–145
114. Li Y, Wang L, Pappan L et al (2012) IL-1beta promotes stemness and invasiveness of colon cancer cells through Zeb1 activation. Mol Cancer 11:87
115. Kaler P, Augenlicht L, Klampfer L (2009) Macrophage-derived IL-1beta stimulates Wnt signaling and growth of colon cancer cells: a crosstalk interrupted by vitamin D3. Oncogene 28(44):3892–3902
116. Duque J, Diaz-Munoz MD, Fresno M et al (2006) Up-regulation of cyclooxygenase-2 by interleukin-1beta in colon carcinoma cells. Cell Signal 18(8):1262–1269
117. Lurje G, Hendifar AE, Schultheis AM et al (2009) Polymorphisms in interleukin 1 beta and interleukin 1 receptor antagonist associated with tumor recurrence in stage II colon cancer. Pharmacogenet Genomics 19(2):95–102
118. Viet HT, Wagsater D, Hugander A et al (2005) Interleukin-1 receptor antagonist gene polymorphism in human colorectal cancer. Oncol Rep 14(4):915–918
119. Salcedo R, Worschech A, Cardone M et al (2010) MyD88-mediated signaling prevents development of adenocarcinomas of the colon: role of interleukin 18. J Exp Med 207(8):1625–1636
120. Chung Y, Chang SH, Martinez GJ et al (2009) Critical regulation of early Th17 cell differentiation by interleukin-1 signaling. Immunity 30(4):576–587
121. Coccia M, Harrison OJ, Schiering C et al (2012) IL-1beta mediates chronic intestinal inflammation by promoting the accumulation of IL-17A secreting innate lymphoid cells and CD4(+) Th17 cells. J Exp Med 209(9):1595–1609
122. Bruttger J, Karram K, Wortge S et al (2015) Genetic cell ablation reveals clusters of local self-renewing microglia in the mammalian central nervous system. Immunity 43(1):92–106
123. Greten FR, Eckmann L, Greten TF et al (2004) IKKbeta links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. Cell 118(3):285–296
124. Wang K, Kim MK, Di Caro G et al (2014) Interleukin-17 receptor a signaling in transformed enterocytes promotes early colorectal tumorigenesis. Immunity 41(6):1052–1063
125. Grivennikov S, Karin E, Terzic J et al (2009) IL-6 and Stat3 are required for survival of intestinal epi-

thelial cells and development of colitis-associated cancer. Cancer Cell 15(2):103–113
126. Grivennikov SI, Wang K, Mucida D et al (2012) Adenoma-linked barrier defects and microbial products drive IL-23/IL-17-mediated tumour growth. Nature 491(7423):254–258
127. Bersudsky M, Luski L, Fishman D et al (2014) Non-redundant properties of IL-1alpha and IL-1beta during acute colon inflammation in mice. Gut 63(4):598–609
128. Wang T, Feng Y, Zhao Z et al (2019) IL1RN polymorphisms are associated with a decreased risk of esophageal cancer susceptibility in a Chinese population. Chemotherapy. https://doi.org/10.1159/000496400:1-8
129. Wu J, Zhang W, Cai J et al (2019) Influence of IL-1R2 polymorphisms on endometrial cancer susceptibility in the Chinese Han population. Mol Genet Genomic Med 7(5):e650
130. Wang C, Zhang C, Xu J et al (2019) Association between IL-1R2 polymorphisms and lung cancer risk in the Chinese Han population: a case-control study. Mol Genet Genomic Med 7(5):e644
131. Niu F, Wang T, Li J et al (2019) The impact of genetic variants in IL1R2 on cervical cancer risk among Uygur females from China: a case-control study. Mol Genet Genomic Med 7(1):e00516
132. Chen B, Luo MX, Zhou X et al (2016) Correlation between interleukin-1beta-511 C/T polymorphism and gastric cancer in Chinese populations: a meta-analysis. Med Sci Monit 22:1742–1750
133. Lee YH, Song GG (2014) A meta-analysis of the association between CTLA-4 +49 A/G, −318 C/T, and IL-1 polymorphisms and susceptibility to cervical cancer. Neoplasma 61(4):481–490
134. Xu H, Ding Q, Jiang HW (2014) Genetic polymorphism of interleukin-1A (IL-1A), IL-1B, and IL-1 receptor antagonist (IL-1RN) and prostate cancer risk. Asian Pac J Cancer Prev 15(20):8741–8747
135. Marazioti A, Lilis I, Vreka M et al (2018) Myeloid-derived interleukin-1beta drives oncogenic KRAS-NF-kappaBeta addiction in malignant pleural effusion. Nat Commun 9(1):672
136. Lu X, Horner JW, Paul E et al (2017) Effective combinatorial immunotherapy for castration-resistant prostate cancer. Nature 543(7647):728–732
137. Johnson KE, Chikoti L, Chandran B (2013) Herpes simplex virus 1 infection induces activation and subsequent inhibition of the IFI16 and NLRP3 inflammasomes. J Virol 87(9):5005–5018
138. De Marco P, Lappano R, De Francesco EM et al (2016) GPER signalling in both cancer-associated fibroblasts and breast cancer cells mediates a feed-forward IL1beta/IL1R1 response. Sci Rep 6:24354
139. Biffi G, Oni TE, Spielman B et al (2019) IL1-induced JAK/STAT signaling is antagonized by TGFbeta to shape CAF heterogeneity in pancreatic ductal adenocarcinoma. Cancer Discov 9(2):282–301
140. Krtolica A, Parrinello S, Lockett S et al (2001) Senescent fibroblasts promote epithelial cell growth and tumorigenesis: a link between cancer and aging. Proc Natl Acad Sci U S A 98(21):12072–12077
141. Orjalo AV, Bhaumik D, Gengler BK et al (2009) Cell surface-bound IL-1alpha is an upstream regulator of the senescence-associated IL-6/IL-8 cytokine network. Proc Natl Acad Sci U S A 106(40):17031–17036
142. Laberge RM, Sun Y, Orjalo AV et al (2015) MTOR regulates the pro-tumorigenic senescence-associated secretory phenotype by promoting IL1A translation. Nat Cell Biol 17(8):1049–1061
143. Perrier S, Caldefie-Chezet F, Vasson MP (2009) IL-1 family in breast cancer: potential interplay with leptin and other adipocytokines. FEBS Lett 583(2):259–265
144. Dirat B, Bochet L, Dabek M et al (2011) Cancer-associated adipocytes exhibit an activated phenotype and contribute to breast cancer invasion. Cancer Res 71(7):2455–2465
145. Incio J, Liu H, Suboj P et al (2016) Obesity-induced inflammation and desmoplasia promote pancreatic cancer progression and resistance to chemotherapy. Cancer Discov 6(8):852–869
146. Thacker SG, Berthier CC, Mattinzoli D et al (2010) The detrimental effects of IFN-alpha on vasculogenesis in lupus are mediated by repression of IL-1 pathways: potential role in atherogenesis and renal vascular rarefaction. J Immunol 185(7):4457–4469
147. Zeisberg EM, Potenta S, Xie L et al (2007) Discovery of endothelial to mesenchymal transition as a source for carcinoma-associated fibroblasts. Cancer Res 67(21):10123–10128
148. Zeisberg EM, Tarnavski O, Zeisberg M et al (2007) Endothelial-to-mesenchymal transition contributes to cardiac fibrosis. Nat Med 13(8):952–961
149. Lee JG, Ko MK, Kay EP (2012) Endothelial mesenchymal transformation mediated by IL-1beta-induced FGF-2 in corneal endothelial cells. Exp Eye Res 95(1):35–39
150. Song X, Krelin Y, Dvorkin T et al (2005) CD11b+/Gr-1+ immature myeloid cells mediate suppression of T cells in mice bearing tumors of IL-1beta-secreting cells. J Immunol 175(12):8200–8208
151. Ghiringhelli F, Apetoh L, Tesniere A et al (2009) Activation of the NLRP3 inflammasome in dendritic cells induces IL-1beta-dependent adaptive immunity against tumors. Nat Med 15(10):1170–1178
152. Hope JC, Sopp P, Collins RA et al (2001) Differences in the induction of CD8+ T cell responses by subpopulations of dendritic cells from afferent lymph are related to IL-1 alpha secretion. J Leukoc Biol 69(2):271–279
153. Yao Y, Chen S, Cao M et al (2017) Antigen-specific CD8(+) T cell feedback activates NLRP3 inflammasome in antigen-presenting cells through perforin. Nat Commun 8:15402
154. Novais FO, Carvalho AM, Clark ML et al (2017) CD8+ T cell cytotoxicity mediates pathology in the skin by inflammasome activation and IL-1beta production. PLoS Pathog 13(2):e1006196

155. Gardella S, Andrei C, Lotti LV et al (2001) CD8(+) T lymphocytes induce polarized exocytosis of secretory lysosomes by dendritic cells with release of interleukin-1beta and cathepsin D. Blood 98(7):2152–2159
156. Dinarello CA, Conti P, Mier JW (1986) Effects of human interleukin-1 on natural killer cell activity: is fever a host defense mechanism for tumor killing? Yale J Biol Med 59(2):97–106
157. Cooper MA, Fehniger TA, Turner SC et al (2001) Human natural killer cells: a unique innate immunoregulatory role for the CD56(bright) subset. Blood 97(10):3146–3151
158. Hughes T, Becknell B, Freud AG et al (2010) Interleukin-1beta selectively expands and sustains interleukin-22+ immature human natural killer cells in secondary lymphoid tissue. Immunity 32(6):803–814
159. Hughes T, Briercheck EL, Freud AG et al (2014) The transcription factor AHR prevents the differentiation of a stage 3 innate lymphoid cell subset to natural killer cells. Cell Rep 8(1):150–162
160. von Burg N, Chappaz S, Baerenwaldt A et al (2014) Activated group 3 innate lymphoid cells promote T-cell-mediated immune responses. Proc Natl Acad Sci U S A 111(35):12835–12840
161. Acosta-Rodriguez EV, Napolitani G, Lanzavecchia A et al (2007) Interleukins 1beta and 6 but not transforming growth factor-beta are essential for the differentiation of interleukin 17-producing human T helper cells. Nat Immunol 8(9):942–949
162. Sutton C, Brereton C, Keogh B et al (2006) A crucial role for interleukin (IL)-1 in the induction of IL-17-producing T cells that mediate autoimmune encephalomyelitis. J Exp Med 203(7):1685–1691
163. Revu S, Wu J, Henkel M et al (2018) IL-23 and IL-1beta drive human Th17 cell differentiation and metabolic reprogramming in absence of CD28 costimulation. Cell Rep 22(10):2642–2653
164. Miyahara Y, Odunsi K, Chen W et al (2008) Generation and regulation of human CD4+ IL-17-producing T cells in ovarian cancer. Proc Natl Acad Sci U S A 105(40):15505–15510
165. Gu J, Ni X, Pan X et al (2017) Human CD39(hi) regulatory T cells present stronger stability and function under inflammatory conditions. Cell Mol Immunol 14(6):521–528
166. Li L, Kim J, Boussiotis VA (2010) IL-1beta-mediated signals preferentially drive conversion of regulatory T cells but not conventional T cells into IL-17-producing cells. J Immunol 185(7):4148–4153
167. Voo KS, Wang YH, Santori FR et al (2009) Identification of IL-17-producing FOXP3+ regulatory T cells in humans. Proc Natl Acad Sci U S A 106(12):4793–4798
168. Voigt C, May P, Gottschlich A et al (2017) Cancer cells induce interleukin-22 production from memory CD4(+) T cells via interleukin-1 to promote tumor growth. Proc Natl Acad Sci U S A 114(49):12994–12999
169. Xue G, Jin G, Fang J et al (2019) IL-4 together with IL-1beta induces antitumor Th9 cell differentiation in the absence of TGF-beta signaling. Nat Commun 10(1):1376
170. Ben-Sasson SZ, Hogg A, Hu-Li J et al (2013) IL-1 enhances expansion, effector function, tissue localization, and memory response of antigen-specific CD8 T cells. J Exp Med 210(3):491–502
171. Ben-Sasson SZ, Wang K, Cohen J et al (2013) IL-1beta strikingly enhances antigen-driven CD4 and CD8 T-cell responses. Cold Spring Harb Symp Quant Biol 78:117–124
172. Ben-Sasson SZ, Hu-Li J, Quiel J et al (2009) IL-1 acts directly on CD4 T cells to enhance their antigen-driven expansion and differentiation. Proc Natl Acad Sci U S A 106(17):7119–7124
173. Ridker PM, MacFadyen JG, Thuren T et al (2017) Effect of interleukin-1beta inhibition with canakinumab on incident lung cancer in patients with atherosclerosis: exploratory results from a randomised, double-blind, placebo-controlled trial. Lancet 390(10105):1833–1842
174. Hickish T, Andre T, Wyrwicz L et al (2017) MABp1 as a novel antibody treatment for advanced colorectal cancer: a randomised, double-blind, placebo-controlled, phase 3 study. Lancet Oncol 18(2):192–201
175. Kurzrock R, Hickish T, Wyrwicz L et al (2019) Interleukin-1 receptor antagonist levels predict favorable outcome after bermekimab, a first-in-class true human interleukin-1alpha antibody, in a phase III randomized study of advanced colorectal cancer. Oncoimmunology 8(3):1551651
176. Giavridis T, van der Stegen SJC, Eyquem J et al (2018) CAR T cell-induced cytokine release syndrome is mediated by macrophages and abated by IL-1 blockade. Nat Med 24(6):731–738
177. Neville ME, Pezzella KM, Schmidt K et al (1990) In vivo inhibition of tumor growth of B16 melanoma by recombinant interleukin 1 beta. II. Mechanism of inhibition: the role of polymorphonuclear leukocytes. Cytokine 2(6):456–463
178. Pezzella KM, Neville ME, Huang JJ (1990) In vivo inhibition of tumor growth of B16 melanoma by recombinant interleukin 1 beta. I. Tumor inhibition parallels lymphocyte-activating factor activity of interleukin 1 beta proteins. Cytokine 2(5):357–362
179. Yang D, Hayashi H, Takii T et al (1997) Interleukin-1-induced growth inhibition of human melanoma cells. Interleukin-1-induced antizyme expression is responsible for ornithine decarboxylase activity down-regulation. J Biol Chem 272(6):3376–3383
180. Oelmann E, Kraemer A, Serve H et al (1997) Autocrine interleukin-1 receptor antagonist can support malignant growth of glioblastoma by blocking growth-inhibiting autocrine loop of interleukin-1. Int J Cancer 71(6):1066–1076
181. Mattarollo SR, Loi S, Duret H et al (2011) Pivotal role of innate and adaptive immunity in anthracycline chemotherapy of established tumors. Cancer Res 71(14):4809–4820

182. Di Mitri D, Toso A, Chen JJ et al (2014) Tumour-infiltrating gr-1+ myeloid cells antagonize senescence in cancer. Nature 515(7525):134–137
183. Bent R, Moll L, Grabbe S et al (2018) Interleukin-1 Beta-a friend or foe in malignancies? Int J Mol Sci 19(8):2155
184. Cancer Genome Atlas N (2012) Comprehensive molecular portraits of human breast tumours. Nature 490(7418):61–70
185. Drake CG, Stein MN (2018) The immunobiology of kidney cancer. J Clin Oncol. https://doi.org/10.1200/JCO.2018.79.2648:JCO2018792648
186. Najjar YG, Rayman P, Jia X et al (2017) Myeloid-derived suppressor cell subset accumulation in renal cell carcinoma parenchyma is associated with Intratumoral expression of IL1beta, IL8, CXCL5, and Mip-1alpha. Clin Cancer Res 23(9):2346–2355
187. Koch I, Depenbrock H, Danhauser-Riedl S et al (1995) Interleukin 1 modulates growth of human renal carcinoma cells in vitro. Br J Cancer 71(4):794–800
188. Tjomsland V, Sandnes D, Pomianowska E et al (2016) The TGFbeta-SMAD3 pathway inhibits IL-1alpha induced interactions between human pancreatic stellate cells and pancreatic carcinoma cells and restricts cancer cell migration. J Exp Clin Cancer Res 35(1):122
189. Zhuang Z, Ju HQ, Aguilar M et al (2016) IL1 receptor antagonist inhibits pancreatic Cancer growth by abrogating NF-kappaB activation. Clin Cancer Res 22(6):1432–1444
190. Wiedemann GM, Knott MM, Vetter VK et al (2016) Cancer cell-derived IL-1alpha induces CCL22 and the recruitment of regulatory T cells. Oncoimmunology 5(9):e1175794
191. Kaneko N, Kurata M, Yamamoto T et al (2019) The role of interleukin-1 in general pathology. Inflamm Regen 39:12

2 Interleukin-8 in the Tumor Immune Niche: Lessons from Comparative Oncology

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Abstract

Interleukin (IL)-8 is a chemokine that is essential for inflammation and angiogenesis. IL-8 expression is elevated in tumor cell lines and tissues, as well as in peripheral blood obtained from cancer patients. Primary works have attempted to determine the biological effect of IL-8 on tumor cells, including cell proliferation, survival, and migration. More recently, IL-8 has acquired considerable attention as an immune modulator in the context of certain tumor microenvironments (TME); specifically, it can support a niche that favors tumor progression and metastasis. Tumor-derived IL-8 stimulates inflammation by interacting with the microenvironmental constituents, including fibroblasts, endothelial cells, and immune cells. However, the tumor immune system is complex, and mechanisms that construct the immune phenotype remain incompletely characterized. Herein, we will (1) address a potential role of IL-8 in regulating gene expression to establish immune landscape in tumor. Then, we will (2) review IL-8 signaling in the maintenance of stem cells and regulation of hematopoietic progenitors. Finally, (3) IL-8 functions will be discussed in naturally occurring animal cancers that offer a clinically realistic model for translational research. This chapter will provide a new insight into the tumor immune niche and help us develop immunotherapies for cancers.

Keywords

Angiogenesis · Canine · Comparative oncology · Cytokine · Hemangiosarcoma · Hematopoiesis · Immune landscape · Immunity · Inflammation · Interleukin-8 · Microenvironment · Niche · Stem cells · Tumor

2.1 Introduction

Cancer is a complex tissue mixture that consists of cellular and noncellular components. Cytokines are bioactive signaling molecules that are produced by a wide range of cells, including immune cells, endothelial cells, and fibroblasts. These molecules are essential for immune balance and activation of immune cells. The fact that cancer cells secrete cytokines implies that the host immune response is interrupted and partly damaged in local tissue and facilitates systemic

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immune imbalance [1]. Interleukin-8 (IL-8) is a well-known pro-inflammatory and pro-angiogenic chemokine that is prominently expressed in immune, endothelial, and tumor cells [2–4]. IL-8 activates intracellular signaling pathways via G-protein-coupled receptors, termed CXCR1 and CXCR2; their roles primarily include neutrophil recruitment and macrophage degranulation [5]. Genetic copy changes and IL-8 gene polymorphisms are observed in tumors, and they may play a role in transformation of normal cells [6, 7]. IL-8 signaling pathways promote cell proliferation and survival of transformed cells, which are fundamental actions that contribute to tumor development and progression [2]. In addition to the intrinsic capacity of IL-8 in tumor cells, the effect of IL-8 signaling has received considerable attention as a key modulator in the context of the tumor microenvironment (TME) [3]. Recent biotechnology advancements, such as next-generation sequencing, high-resolution image analysis, tissue engineering, and genome engineering, have spurred studies to understand the complex system of the TME and unveil sophisticated molecular mechanisms of cancers. In this chapter, we will first address the role of IL-8 in the tumor niche, particularly its role in regulating the gene expression landscape. We will also discuss the potential of IL-8 to influence the stemness and lineage commitment of hematopoietic progenitors. Finally, IL-8 will be reviewed in cancers that occur naturally in companion animals, and these cancers offer a clinically realistic model for translational research. This approach will provide novel insights into the mechanisms of how IL-8 contributes to create the tumor immune niche, and it will help us develop potential immunotherapies.

2.2 Effect of IL-8 on the Tumor Immune Landscape

IL-8, which can be overexpressed in tumor cells, accelerates the biological effects of signaling pathways via autocrine and paracrine signaling [2]. In addition to the regulation of cellular behavior such as proliferation and survival of cancer cells, the functional consequences of IL-8 signaling contribute to transcriptional programs that establish gene expression profiles in tumors. Specifically, the promoter region of the IL-8 gene involves transcription factor binding sites [8], and IL-8 signaling activation promotes the transcriptional activity of nuclear factor-kappa B pathways that regulate expression of inflammatory genes in cancer cells [2, 9, 10]. IL-8 signaling can also alter protein expression through regulating the phosphorylation activity of kinase pathways, including AKT, extracellular signal-regulated kinase (ERK), and protein kinase C (PKC) signaling [11]. This process is most likely to occur in surrounding stromal cells that express the IL-8 receptors in response to IL-8 secreted by tumor cells, and it could at least partly establish the intratumoral heterogeneity. The stromal cells include endothelial cells and pericytes that could be another source of IL-8 production in the tumor tissue. It is well documented that endothelial cells robustly produce IL-8 to form blood vessels and maintain vascular functions. Recently, pericytes appear to acquire ability to secrete IL-8 under inflammatory conditions that induces chemoattraction of neutrophils [12, 13]. In addition, pericytes have shown angiogenic potential [14–16]: specifically, type 2 pericytes contribute to tumor angiogenesis [16], whereas type 1 pericytes are fibrogenic [17]. Thus, it is plausible that a highly inflamed niche in the tumor enables pericytes to secrete IL-8 that promotes angiogenesis and subsequent immune response. This process involves a variety of cell types that orchestrate the TME; at the same time, it accelerates the tumor heterogeneity. Recent advances in sequencing technology and bioinformatics revolutionized studies of immuno-oncology. These findings establish the immune landscape of cancer and aid in understanding the tumor heterogeneity in the TME [18–21]. Specifically, transcriptomic data using high-throughput RNA-sequencing (RNA-seq) technology can estimate the abundance of distinct subsets of immune infiltrate in the tumor tissues as well as examine the features of the inflammatory response [18, 19, 21]. The tumor tissue immune landscape appears to be associated with an adverse prognosis in cancer patients

using TCGA gene expression data [22], suggesting that the immune signature influences clinical outcomes. Intratumoral immune states are subclassified as six immune subtypes: wound healing, interferon gamma (IFN-γ) dominant, inflammatory, lymphocyte depleted, immunologically quiet, and transforming growth factor beta (TGF-β) dominant [21]. The distinct immune subtypes represent the diversity of cytokine signaling networks that are differentially regulated in the TME [21]. Thus, it is crucial to define the mechanism of cytokine regulation to better understand the relationship between tumor progression and clinical outcomes in the complex nature of tumor immunity.

2.3 Stemness Properties of Cancer Cells and Hematopoietic Stem Cells

Cancer stem cells (CSCs), or tumor-initiating cells, are thought to drive tumor growth and disease progression. CSCs are suggested to be targets that evoke an antitumor immune response [23]. Essential roles of IL-8 and its receptors include maintenance of CSCs. For instance, the IL-8R (CXCR1) is highly expressed on breast CSCs, and recombinant IL-8 increases their self-renewal and tumor growth [24]. IL-8 signaling is also a key pathway for colorectal CSCs [19] and hepatocellular carcinoma-initiating stem-like cells [25]. Activation of the Snail family is one pathway that promotes IL-8 production by tumor cells [26], and IL-8 maintains the self-renewing phenotype of CSCs through a self-amplifying loop that involves activation of transcription factors and a consequent perpetuation of elevated IL-8 production [26]. Such a mechanism can be mediated through transcription factors associated with the epithelial-to-mesenchymal transition (EMT), such as SNAIL and SLUG, TWIST1, and Zeb1/2 [27–30]. The Snail family is also a key participant in stem cell generation, including embryonic mesoderm and neural crest cell formation [31]. For example, SLUG is essential to maintain melanocyte stem cells, hematopoietic stem cells, and germ cells [32], and it regulates the activation of a self-renewal program by upregulating functional proteins that also serve as stem cell markers [33]. Furthermore, SLUG deregulation can lead to mesenchymal tumors (i.e., sarcomas), a finding that demonstrates its oncogenic potential [34].

Importantly, the potential of IL-8 to regulate stem cell properties, including self-renewal, quiescence, and mobilization, must be addressed in the context of stem cell niche [35]. Chemokines, and the signaling pathways through cooperation with their binding receptors, contribute to the maintenance of hematopoietic stem cells (HSCs) and functional regulation in the bone marrow niche. Notably, chemokine C-X-C motif ligand 12 (CXCL12) and the C-X-C chemokine receptor type 4 (CXCR4) are expressed in hematopoietic stem and progenitor cells (HSPCs) and bone marrow niche cells. They activate CXCL12-CXCR4 signaling pathways that are essential for hematopoietic cell functions (including myelopoiesis) [36, 37]. IL-8 production can be stimulated by CXCL12-CXCR4 interaction [38], and it enhances HSPC mobilization and supports the maintenance and proliferation of stem cells [39, 40]. HSCs leverage the ability to differentiate into lineage-committed progenitors under stress or pathogenic conditions to replenish mature cells [41, 42]. This finding suggests that fate decision mechanisms are influenced by different molecular programs. The molecular traits may include actions of cytokines that modulate lineage commitment and differentiation of HSCs [43–45]. Higher IL-8 levels in peripheral blood acquired from cancer patients are significantly associated with the number of circulating bone-marrow-derived progenitor cells [46]. Thus, the elevated IL-8 under this detrimental condition is postulated to modulate the function of bone-marrow-derived progenitor cells. Specifically, a recent study showed that tumor-derived IL-8 attracts myeloid-derived suppressor cells (MDSCs), which are a heterogeneous group of immature immune cells that include macrophages, granulocytes, and other myeloid precursors [47]. This finding suggests a potential role for IL-8 to create an immunosuppressive

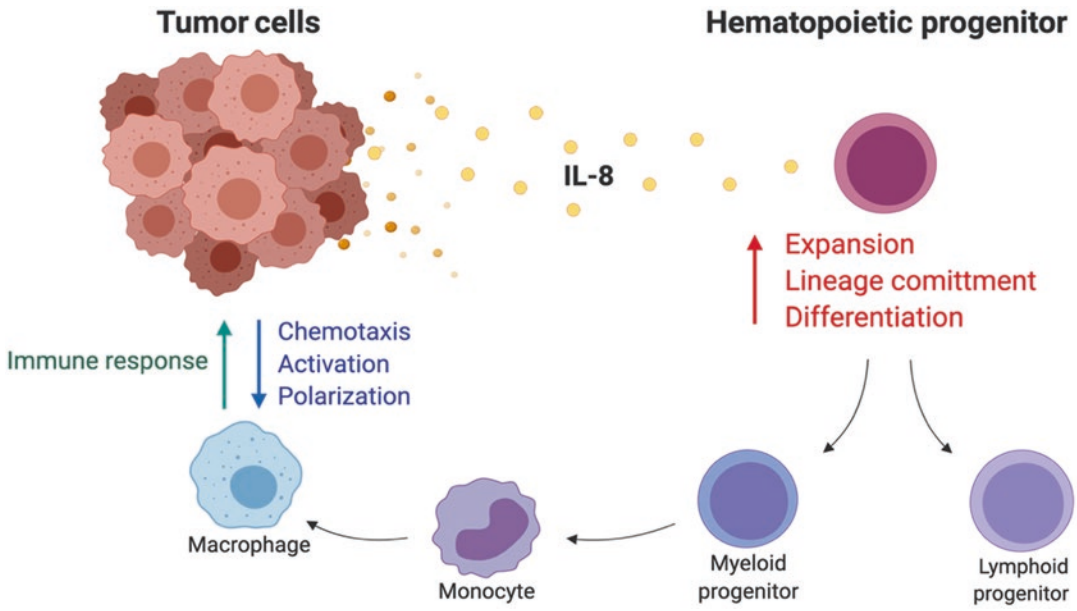

Fig. 2.1 Hypothetical model for potential role of IL-8 in the regulation of hematopoietic cells. The schematic model depicts the biological effect of IL-8 that supports expansion, lineage commitment, and differentiation of hematopoietic progenitor cells in the TME (created with Biorender.com)

milieu [3, 48, 49]. The high IL-8 concentrations appear to desensitize IL-8 receptors and modulate their chemotactic migration [5, 50, 51]. Thus, this mechanism favors systemic IL-8 circulation and its subsequent exposure to cells in the whole body. This phenomenon may disrupt the cytokine gradients that guide chemotactic function and determine differentiation of hematopoietic progenitors [48–50]. Mechanisms that establish the antitumor immune milieu in the TME include the regulation of tumor-associated macrophages by supporting functions of HSCs (Fig. 2.1). This hypothetical model illustrates a potential role of IL-8 in the maintenance of CSCs and the regulation of HSCs. IL-8 may contribute to the cell fate decisions of immune precursors presumably in early differential stages of immune cells. Yet, little is known about the precise mechanism of how IL-8 signaling influences fate decisions of HSCs to produce myeloid progenitors and macrophages. To better understand that mechanism and design additional relevant studies, it is important to expand the current knowledge by reviewing recent findings on the tumor immune niche from comparative oncology research.

2.4 IL-8 and Tumor Immunity in the Microenvironment: Comparative Perspectives

The immune system protects the host body from exogenous and endogenous pathogens, typically including foreign organisms such as bacteria, viruses, fungi, and parasites, among others [52]. Genetic mutations are a main cause of oncogenesis, and a vast majority of cancer stochastically develop independently of noninfectious or noninflammatory agents [53, 54], with a few exceptions, such as oncogenic viruses and bacteria that induce chronic inflammation (e.g., *Helicobacter pylori*) [55]. The transformed cells (like other pathogens) may be recognized and eradicated by the immune system through the host defense mechanism. However, the tumor cells are also capable of escaping from the immune surveillance. Indeed, evading the immune system is one of the "hallmarks of cancer" [56].

Tumor immunity is complicated by both the innate and adaptive immune responses, and the *cancer immunoediting* theory conceptualizes the complex immune system in tumor development

and progression by illustrating three phases of *elimination*, *equilibrium*, and *escape* [57, 58]. In the *elimination* phase, the immune cells, including cytotoxic T cells and natural killer (NK) cells, detect transformed cells and destroy them at the early stage of tumor initiation. In this step, tumor cells express tumor-specific antigens in the context of major histocompatibility complex (MHC) class I molecules and Fas and TRAIL receptors by which the immune cells attack them and induce antitumor response. The *equilibrium* phase manifests tumor cell variants that can avoid the immune recognition and antitumor activity, potentially by genetic and epigenetic changes that reduce immunogenicity of the tumor cells. The tumor cells secrete cytokines by themselves and educate the immune cells to maintain a balance between immunosuppressive (i.e., tumor-promoting) cytokines, including IL-10 and IL-23, and antitumor cytokines, such as IFN-γ and IL-12, and thereby induce immune-mediated tumor dormancy. Subsequently, tumor cells may become proliferative and outweigh antitumor immune activity in an immunocompetent host, entering the *escape* phase. In this stage, tumor cells foster a tissue niche that is favorable for tumor growth, where regulatory T cells, MDSCs, and M2 macrophages are predominantly involved. This niche is enriched with immunosuppressive cytokines such as IL-10, TGF-β, indoleamine 2,3-dioxygenase (IDO), and programmed death-ligand 1 (PD-L1), as well as vascular endothelial growth factor (VEGF), TGF-β, IL-6, and IL-8 that promote angiogenesis.

The concept of cancer immunoediting provides a simplified overview of the complex immune response against tumors; it is only based on limited studies of mice or humans [59]. Accumulating data from canine studies provide insights into the tumor immunity, particularly for the *escape* phase in a realistic preclinical setting [60]. Tumor-infiltrating immune cells are identified in multiple types of canine tumors, including mammary, testicular, melanoma, brain, and osteosarcoma, and they are suggested to play important roles in tumor progression [61–66]. For instance, an increased CD4+/CD8+ T cell ratio is correlated with decreased survival of dogs affected with mammary carcinomas [67], and enrichment of foxp3+ regulatory T cells localized within intratumoral area is associated with tumor progression [62, 63]. Intratumoral administration of exogenous Fas ligand appears to alter the immune phenotype of osteosarcomas in dogs by targeting Fas-expressing tumor cells and promoting local tissue inflammation [66].

Genetic and epigenetic traits of tumor cells may influence the immune functions in the TME [68, 69]. Considerable efforts have advanced our understanding of the molecular mechanisms that regulate the tumor immune response via comprehensive genomic analysis. Immune cell transcripts are quantified in osteosarcoma tissues from mice, dogs, and humans via a multi-species approach, an endeavor that offers a new tool for prognosis prediction in tumor patients [70]. Another novel, recently developed bioinformatics technique facilitates the identification of species-specific transcripts from xenograft tumors. It revolutionizes the way to isolate the source of transcripts between tumor and stromal cells using an in vivo platform [71]. Additionally, RNA-seq transcriptomic analysis has identified three distinct molecular subtypes of spontaneous hemangiosarcomas in companion dogs, and a subset of dogs with the tumor-enriching immune gene signatures reveals a better survival outcome [72]. Histologically, hemangiosarcoma is a tumor with extensive vascular networks where cells form abnormal, distorted blood vessels, capillaries, and sinusoids [73]. Hemangiosarcoma cells appear to enrich angiogenic and inflammatory gene signatures [74, 75], as the inherent nature of hemangiosarcoma implies greater propensity of the vascular malignant cells to promote inflammation and angiogenesis. The IL-8 gene is significantly upregulated in canine hemangiosarcoma cells compared to nonmalignant endothelial cells, and molecular networks activated by IL-8 are key canonical pathways that control both angiogenesis and inflammation and contribute to the pathogenesis of the disease [74, 75]. Furthermore, an in vivo xenograft model of canine hemangiosarcoma demonstrated that the tumor cells secrete IL-8 and create a reactive microenvironment that supports tumor growth [7, 76]. Accordingly, the

xenotransplantation of canine hemangiosarcoma cells into immunodeficient mice is a useful platform that provides a comparative view to explore IL-8 functions. IL-8 is rarely conserved among animal species, and there is no murine IL-8 gene. Murine IL-8 homologues include keratinocyte chemoattractant (KC) CXCL1, macrophage inflammatory protein-2 (MIP-2), and lipopolysaccharide-induced CXC chemokine (LIX), all of which promote neutrophil chemotaxis and angiogenesis [77–79]. Yet, a functional role for these molecules in cancer and xenograft models remains to be formally documented. Multiple groups have reported that xenografts of canine hemangiosarcoma are achievable in mice [7, 76, 80–83], and IL-8 contributes to the survival and engraftment of canine tumor cells in xenogenic conditions [76]. There is no homology between canine IL-8 and any of the murine homologues, a fact that makes it unlikely that the anti-canine IL-8 antibody interferes with stromal-derived chemokines. Thus, the only IL-8 available in the system would be most likely derived from the canine tumor xenografts, and antibody depletion is feasible.

Our recent work revealed that the self-renewal capacity of tumor cells is associated with immune regulation and the cytokine expression pattern in canine tumor models that represent three ontogenetically distinct tumors: brain tumor, osteosarcoma, and hemangiosarcoma [84]. We also found that canine hemangiosarcoma cells support expansion, proliferation, and differentiation of HSCs. Thus, we suggest that IL-8 produced by hemangiosarcoma cells is a key molecule that contributes to the stemness properties of hematopoietic cells to establish a tumor niche.

2.5 Summary

IL-8 is an essential signaling cytokine for maintaining autonomous functions of tumor cells and for creating the tumor immune niche. We especially discussed the potential role of IL-8 to support the hematopoietic progenitors that produce immune cells to foster a unique immune phenotype in the TME. This chapter provides a novel concept to elucidate the mechanism of how tumors establish their niche environments via IL-8 and the associated signaling network. Ultimately, the pathways could be effective immunotherapeutic targets. Furthermore, canine tumors are natural animal models in a realistic preclinical setting [85] and offer an opportunity to determine outstanding translational approaches to help us develop novel immunotherapies.

References

1. Lippitz BE (2013) Cytokine patterns in patients with cancer: a systematic review. Lancet Oncol 14(6):e218–e228
2. Waugh DJ, Wilson C (2008) The interleukin-8 pathway in cancer. Clin Cancer Res 14(21):6735–6741
3. Alfaro C, Sanmamed MF, Rodriguez-Ruiz ME, Teijeira A, Onate C, Gonzalez A, Ponz M, Schalper KA, Perez-Gracia JL, Melero I (2017) Interleukin-8 in cancer pathogenesis, treatment and follow-up. Cancer Treat Rev 60:24–31
4. Harada A, Sekido N, Akahoshi T, Wada T, Mukaida N, Matsushima K (1994) Essential involvement of interleukin-8 (IL-8) in acute inflammation. J Leukoc Biol 56(5):559–564
5. Stillie R, Farooq SM, Gordon JR, Stadnyk AW (2009) The functional significance behind expressing two IL-8 receptor types on PMN. J Leukoc Biol 86(3):529–543
6. Wang N, Zhou R, Wang C, Guo X, Chen Z, Yang S, Li Y (2012) -251 T/A polymorphism of the interleukin-8 gene and cancer risk: a HuGE review and meta-analysis based on 42 case-control studies. Mol Biol Rep 39(3):2831–2841
7. Kim JH, Graef AJ, Dickerson EB, Modiano JF (2015) Pathobiology of hemangiosarcoma in dogs: research advances and future perspectives. Vet Sci 2(4):388–405
8. Elliott CL, Allport VC, Loudon JA, Wu GD, Bennett PR (2001) Nuclear factor-kappa B is essential for up-regulation of interleukin-8 expression in human amnion and cervical epithelial cells. Mol Hum Reprod 7(8):787–790
9. Wang D, Richmond A (2001) Nuclear factor-kappa B activation by the CXC chemokine melanoma growth-stimulatory activity/growth-regulated protein involves the MEKK1/p38 mitogen-activated protein kinase pathway. J Biol Chem 276(5):3650–3659
10. Wilson C, Purcell C, Seaton A, Oladipo O, Maxwell PJ, O'Sullivan JM, Wilson RH, Johnston PG, Waugh DJ (2008) Chemotherapy-induced CXC-chemokine/CXC-chemokine receptor signaling in metastatic prostate cancer cells confers resistance to oxaliplatin

through potentiation of nuclear factor-kappaB transcription and evasion of apoptosis. J Pharmacol Exp Ther 327(3):746–759
11. MacManus CF, Pettigrew J, Seaton A, Wilson C, Maxwell PJ, Berlingeri S, Purcell C, McGurk M, Johnston PG, Waugh DJ (2007) Interleukin-8 signaling promotes translational regulation of cyclin D in androgen-independent prostate cancer cells. Mol Cancer Res 5(7):737–748
12. Pieper C, Pieloch P, Galla HJ (2013) Pericytes support neutrophil transmigration via interleukin-8 across a porcine co-culture model of the blood-brain barrier. Brain Res 1524:1–11
13. Smyth LCD, Rustenhoven J, Park TI, Schweder P, Jansson D, Heppner PA, O'Carroll SJ, Mee EW, Faull RLM, Curtis M et al (2018) Unique and shared inflammatory profiles of human brain endothelia and pericytes. J Neuroinflammation 15(1):138
14. Dar A, Domev H, Ben-Yosef O, Tzukerman M, Zeevi-Levin N, Novak A, Germanguz I, Amit M, Itskovitz-Eldor J (2012) Multipotent vasculogenic pericytes from human pluripotent stem cells promote recovery of murine ischemic limb. Circulation 125(1):87–99
15. Stratman AN, Schwindt AE, Malotte KM, Davis GE (2010) Endothelial-derived PDGF-BB and HB-EGF coordinately regulate pericyte recruitment during vasculogenic tube assembly and stabilization. Blood 116(22):4720–4730
16. Birbrair A, Zhang T, Wang ZM, Messi ML, Olson JD, Mintz A, Delbono O (2014) Type-2 pericytes participate in normal and tumoral angiogenesis. Am J Physiol Cell Physiol 307(1):C25–C38
17. Birbrair A, Zhang T, Wang ZM, Messi ML, Mintz A, Delbono O (2013) Type-1 pericytes participate in fibrous tissue deposition in aged skeletal muscle. Am J Physiol Cell Physiol 305(11):C1098–C1113
18. Newman AM, Liu CL, Green MR, Gentles AJ, Feng W, Xu Y, Hoang CD, Diehn M, Alizadeh AA (2015) Robust enumeration of cell subsets from tissue expression profiles. Nat Methods 12(5):453–457
19. Aran D, Hu Z, Butte AJ (2017) xCell: digitally portraying the tissue cellular heterogeneity landscape. Genome Biol 18(1):220
20. Binnewies M, Roberts EW, Kersten K, Chan V, Fearon DF, Merad M, Coussens LM, Gabrilovich DI, Ostrand-Rosenberg S, Hedrick CC et al (2018) Understanding the tumor immune microenvironment (TIME) for effective therapy. Nat Med 24(5):541–550
21. Thorsson V, Gibbs DL, Brown SD, Wolf D, Bortone DS, Ou Yang TH, Porta-Pardo E, Gao GF, Plaisier CL, Eddy JA et al (2018) The immune landscape of cancer. Immunity 48(4):812–830.e814
22. Gentles AJ, Newman AM, Liu CL, Bratman SV, Feng W, Kim D, Nair VS, Xu Y, Khuong A, Hoang CD et al (2015) The prognostic landscape of genes and infiltrating immune cells across human cancers. Nat Med 21(8):938–945
23. Wefers C, Schreibelt G, Massuger L, de Vries IJM, Torensma R (2018) Immune curbing of cancer stem cells by CTLs directed to NANOG. Front Immunol 9:1412
24. Ginestier C, Liu S, Diebel ME, Korkaya H, Luo M, Brown M, Wicinski J, Cabaud O, Charafe-Jauffret E, Birnbaum D et al (2010) CXCR1 blockade selectively targets human breast cancer stem cells in vitro and in xenografts. J Clin Invest 120(2):485–497
25. Tang KH, Ma S, Lee TK, Chan YP, Kwan PS, Tong CM, Ng IO, Man K, To KF, Lai PB et al (2012) CD133(+) liver tumor-initiating cells promote tumor angiogenesis, growth, and self-renewal through neurotensin/interleukin-8/CXCL1 signaling. Hepatology 55(3):807–820
26. Hwang WL, Yang MH, Tsai ML, Lan HY, Su SH, Chang SC, Teng HW, Yang SH, Lan YT, Chiou SH et al (2011) SNAIL regulates interleukin-8 expression, stem cell-like activity, and tumorigenicity of human colorectal carcinoma cells. Gastroenterology 141(1):279–291, 291 e271–275
27. Sannino G, Marchetto A, Kirchner T, Grunewald TGP (2017) Epithelial-to-mesenchymal and mesenchymal-to-epithelial transition in mesenchymal tumors: a paradox in sarcomas? Cancer Res 77(17):4556–4561
28. Li Y, Laterra J (2012) Cancer stem cells: distinct entities or dynamically regulated phenotypes? Cancer Res 72(3):576–580
29. Zhou H, Neelakantan D, Ford HL (2017) Clonal cooperativity in heterogenous cancers. Semin Cell Dev Biol 64:79–89
30. Bhat-Nakshatri P, Appaiah H, Ballas C, Pick-Franke P, Goulet R Jr, Badve S, Srour EF, Nakshatri H (2010) SLUG/SNAI2 and tumor necrosis factor generate breast cells with CD44+/CD24- phenotype. BMC Cancer 10:411
31. Savagner P, Karavanova I, Perantoni A, Thiery JP, Yamada KM (1998) Slug mRNA is expressed by specific mesodermal derivatives during rodent organogenesis. Dev Dyn 213(2):182–187
32. Perez-Losada J, Sanchez-Martin M, Rodriguez-Garcia A, Sanchez ML, Orfao A, Flores T, Sanchez-Garcia I (2002) Zinc-finger transcription factor Slug contributes to the function of the stem cell factor c-kit signaling pathway. Blood 100(4):1274–1286
33. Wu Y, Zhou BP (2010) Snail: more than EMT. Cell Adhes Migr 4(2):199–203
34. Perez-Mancera PA, Gonzalez-Herrero I, Perez-Caro M, Gutierrez-Cianca N, Flores T, Gutierrez-Adan A, Pintado B, Sanchez-Martin M, Sanchez-Garcia I (2005) SLUG in cancer development. Oncogene 24(19):3073–3082
35. Lilly AJ, Johnson WE, Bunce CM (2011) The haematopoietic stem cell niche: new insights into the mechanisms regulating haematopoietic stem cell behaviour. Stem Cells Int 2011:274564
36. Nagasawa T, Hirota S, Tachibana K, Takakura N, Nishikawa S, Kitamura Y, Yoshida N, Kikutani H, Kishimoto T (1996) Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. Nature 382(6592):635–638

37. Zou YR, Kottmann AH, Kuroda M, Taniuchi I, Littman DR (1998) Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. Nature 393(6685):595–599
38. Li KC, Huang YH, Ho CY, Chu CY, Cha ST, Tsai HH, Ko JY, Chang CC, Tan CT (2012) The role of IL-8 in the SDF-1alpha/CXCR4-induced angiogenesis of laryngeal and hypopharyngeal squamous cell carcinoma. Oral Oncol 48(6):507–515
39. Mendt M, Cardier JE (2015) Role of SDF-1 (CXCL12) in regulating hematopoietic stem and progenitor cells traffic into the liver during extramedullary hematopoiesis induced by G-CSF, AMD3100 and PHZ. Cytokine 76(2):214–221
40. Jung JH, Lee SJ, Kim J, Lee S, Sung HJ, An J, Park Y, Kim BS (2015) CXCR2 and its related ligands play a novel role in supporting the pluripotency and proliferation of human pluripotent stem cells. Stem Cells Dev 24(8):948–961
41. Yamamoto R, Morita Y, Ooehara J, Hamanaka S, Onodera M, Rudolph KL, Ema H, Nakauchi H (2013) Clonal analysis unveils self-renewing lineage-restricted progenitors generated directly from hematopoietic stem cells. Cell 154(5):1112–1126
42. Ito K, Bonora M, Ito K (2019) Metabolism as master of hematopoietic stem cell fate. Int J Hematol 109(1):18–27
43. Kondo M, Scherer DC, Miyamoto T, King AG, Akashi K, Sugamura K, Weissman IL (2000) Cell-fate conversion of lymphoid-committed progenitors by instructive actions of cytokines. Nature 407(6802):383–386
44. Rieger MA, Hoppe PS, Smejkal BM, Eitelhuber AC, Schroeder T (2009) Hematopoietic cytokines can instruct lineage choice. Science 325(5937):217–218
45. Sarrazin S, Mossadegh-Keller N, Fukao T, Aziz A, Mourcin F, Vanhille L, Kelly Modis L, Kastner P, Chan S, Duprez E et al (2009) MafB restricts M-CSF-dependent myeloid commitment divisions of hematopoietic stem cells. Cell 138(2):300–313
46. Blogowski W, Deskur A, Budkowska M, Salata D, Madej-Michniewicz A, Dabkowski K, Dolegowska B, Starzynska T (2014) Selected cytokines in patients with pancreatic cancer: a preliminary report. PLoS One 9(5):e97613
47. Sendo S, Saegusa J, Morinobu A (2018) Myeloid-derived suppressor cells in non-neoplastic inflamed organs. Inflamm Regen 38:19
48. Alfaro C, Teijeira A, Onate C, Perez G, Sanmamed MF, Andueza MP, Alignani D, Labiano S, Azpilikueta A, Rodriguez-Paulete A et al (2016) Tumor-produced interleukin-8 attracts human myeloid-derived suppressor cells and elicits extrusion of neutrophil extracellular traps (NETs). Clin Cancer Res 22(15):3924–3936
49. Gonzalez-Aparicio M, Alfaro C (2019) Influence of interleukin-8 and neutrophil extracellular trap (NET) formation in the tumor microenvironment: is there a pathogenic role? J Immunol Res 2019:6252138
50. Alfaro C, Suarez N, Martinez-Forero I, Palazon A, Rouzaut A, Solano S, Feijoo E, Gurpide A, Bolanos E, Erro L et al (2011) Carcinoma-derived interleukin-8 disorients dendritic cell migration without impairing T-cell stimulation. PLoS One 6(3):e17922
51. Dowling MF (2018) Understanding the role of interleukin-8 (IL-8) in canine osteosarcoma metastasis. University of Illinois at Urbana-Champaign. http://hdl.handle.net/2142/101656
52. Casadevall A, Pirofski LA (2014) Microbiology: ditch the term pathogen. Nature 516(7530):165–166
53. Tomasetti C, Vogelstein B (2015) Cancer etiology. Variation in cancer risk among tissues can be explained by the number of stem cell divisions. Science 347(6217):78–81
54. Tomasetti C, Li L, Vogelstein B (2017) Stem cell divisions, somatic mutations, cancer etiology, and cancer prevention. Science 355(6331):1330–1334
55. Grivennikov SI, Greten FR, Karin M (2010) Immunity, inflammation, and cancer. Cell 140(6):883–899
56. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144(5):646–674
57. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD (2002) Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol 3(11):991–998
58. Dunn GP, Old LJ, Schreiber RD (2004) The three Es of cancer immunoediting. Annu Rev Immunol 22:329–360
59. Mittal D, Gubin MM, Schreiber RD, Smyth MJ (2014) New insights into cancer immunoediting and its three component phases – elimination, equilibrium and escape. Curr Opin Immunol 27:16–25
60. Overgaard NH, Fan TM, Schachtschneider KM, Principe DR, Schook LB, Jungersen G (2018) Of mice, dogs, pigs, and men: choosing the appropriate model for immuno-oncology research. ILAR J 59:247
61. Kim JH, Yu CH, Yhee JY, Im KS, Sur JH (2010) Lymphocyte infiltration, expression of interleukin (IL) -1, IL-6 and expression of mutated breast cancer susceptibility gene-1 correlate with malignancy of canine mammary tumours. J Comp Pathol 142(2–3):177–186
62. Kim JH, Chon SK, Im KS, Kim NH, Cho KW, Sur JH (2013) Infiltrating Foxp3+ regulatory T cells and histopathological features in canine classical and spermatocytic seminomas. Reprod Domest Anim 48(2):218–222
63. Kim JH, Hur JH, Lee SM, Im KS, Kim NH, Sur JH (2012) Correlation of Foxp3 positive regulatory T cells with prognostic factors in canine mammary carcinomas. Vet J 193(1):222–227
64. Porcellato I, Brachelente C, De Paolis L, Menchetti L, Silvestri S, Sforna M, Vichi G, Iussich S, Mechelli L (2019) FoxP3 and IDO in canine melanocytic tumors. Vet Pathol 56(2):189–199
65. Filley A, Henriquez M, Bhowmik T, Tewari BN, Rao X, Wan J, Miller MA, Liu Y, Bentley RT, Dey M (2018) Immunologic and gene expression profiles of spontaneous canine oligodendrogliomas. J Neuro-Oncol 137(3):469–479
66. Modiano JF, Bellgrau D, Cutter GR, Lana SE, Ehrhart NP, Ehrhart E, Wilke VL, Charles JB, Munson S, Scott MC et al (2012) Inflammation, apoptosis, and necrosis induced by neoadjuvant fas ligand gene ther-

apy improves survival of dogs with spontaneous bone cancer. Mol Ther 20(12):2234–2243
67. Estrela-Lima A, Araujo MS, Costa-Neto JM, Teixeira-Carvalho A, Barrouin-Melo SM, Cardoso SV, Martins-Filho OA, Serakides R, Cassali GD (2010) Immunophenotypic features of tumor infiltrating lymphocytes from mammary carcinomas in female dogs associated with prognostic factors and survival rates. BMC Cancer 10:256
68. Davoli T, Uno H, Wooten EC, Elledge SJ (2017) Tumor aneuploidy correlates with markers of immune evasion and with reduced response to immunotherapy. Science 355(6322):eaaf8399
69. Bonavita E, Gentile S, Rubino M, Maina V, Papait R, Kunderfranco P, Greco C, Feruglio F, Molgora M, Laface I et al (2015) PTX3 is an extrinsic oncosuppressor regulating complement-dependent inflammation in cancer. Cell 160(4):700–714
70. Scott MC, Temiz NA, Sarver AE, LaRue RS, Rathe SK, Varshney J, Wolf NK, Moriarity BS, O'Brien TD, Spector LG et al (2018) Comparative transcriptome analysis quantifies immune cell transcript levels, metastatic progression, and survival in osteosarcoma. Cancer Res 78(2):326–337
71. Scott MC, Tomiyasu H, Garbe JR, Cornax I, Amaya C, O'Sullivan MG, Subramanian S, Bryan BA, Modiano JF (2016) Heterotypic mouse models of canine osteosarcoma recapitulate tumor heterogeneity and biological behavior. Dis Model Mech 9(12):1435–1444
72. Kim JH, Megquier K, Sarver AL, Thomas R, Wang C, Elvers I, Karlsson E, Breen M, Lindblad-Toh K, Modiano JF (2018) Mutational and transcriptomic profiling identify distinct angiogenic and inflammatory subtypes of angiosarcoma. In: AACR annual meeting. American Association for Cancer Research, Chicago, p 2018
73. Fosmire SP, Dickerson EB, Scott AM, Bianco SR, Pettengill MJ, Meylemans H, Padilla M, Frazer-Abel AA, Akhtar N, Getzy DM et al (2004) Canine malignant hemangiosarcoma as a model of primitive angiogenic endothelium. Lab Investig 84(5):562–572
74. Tamburini BA, Phang TL, Fosmire SP, Scott MC, Trapp SC, Duckett MM, Robinson SR, Slansky JE, Sharkey LC, Cutter GR et al (2010) Gene expression profiling identifies inflammation and angiogenesis as distinguishing features of canine hemangiosarcoma. BMC Cancer 10:619
75. Tamburini BA, Trapp S, Phang TL, Schappa JT, Hunter LE, Modiano JF (2009) Gene expression profiles of sporadic canine hemangiosarcoma are uniquely associated with breed. PLoS One 4(5):e5549
76. Kim JH, Frantz AM, Anderson KL, Graef AJ, Scott MC, Robinson S, Sharkey LC, O'Brien TD, Dickerson EB, Modiano JF (2014) Interleukin-8 promotes canine hemangiosarcoma growth by regulating the tumor microenvironment. Exp Cell Res 323(1):155–164
77. Rovai LE, Herschman HR, Smith JB (1998) The murine neutrophil-chemoattractant chemokines LIX, KC, and MIP-2 have distinct induction kinetics, tissue distributions, and tissue-specific sensitivities to glucocorticoid regulation in endotoxemia. J Leukoc Biol 64(4):494–502
78. Asfaha S, Dubeykovskiy AN, Tomita H, Yang X, Stokes S, Shibata W, Friedman RA, Ariyama H, Dubeykovskaya ZA, Muthupalani S et al (2013) Mice that express human interleukin-8 have increased mobilization of immature myeloid cells, which exacerbates inflammation and accelerates colon carcinogenesis. Gastroenterology 144(1):155–166
79. Moldobaeva A, Baek A, Eldridge L, Wagner EM (2010) Differential activity of pro-angiogenic CXC chemokines. Microvasc Res 80(1):18–22
80. Akhtar N, Padilla ML, Dickerson EB, Steinberg H, Breen M, Auerbach R, Helfand SC (2004) Interleukin-12 inhibits tumor growth in a novel angiogenesis canine hemangiosarcoma xenograft model. Neoplasia 6(2):106–116
81. Andersen NJ, Nickoloff BJ, Dykema KJ, Boguslawski EA, Krivochenitser RI, Froman RE, Dawes MJ, Baker LH, Thomas DG, Kamstock DA et al (2013) Pharmacologic inhibition of MEK signaling prevents growth of canine hemangiosarcoma. Mol Cancer Ther 12(9):1701–1714
82. Murai A, Asa SA, Kodama A, Hirata A, Yanai T, Sakai H (2012) Constitutive phosphorylation of the mTORC2/Akt/4E-BP1 pathway in newly derived canine hemangiosarcoma cell lines. BMC Vet Res 8:128
83. Kodama A, Sakai H, Matsuura S, Murakami M, Murai A, Mori T, Maruo K, Kimura T, Masegi T, Yanai T (2009) Establishment of canine hemangiosarcoma xenograft models expressing endothelial growth factors, their receptors, and angiogenesis-associated homeobox genes. BMC Cancer 9:363
84. Kim JH, Frantz AM, Sarver AL, Gorden Klukas BH, Lewellen M, O'Brien TD, Dickerson EB, Modiano JF (2018) Modulation of fatty acid metabolism and immune suppression are features of in vitro tumour sphere formation in ontogenetically distinct dog cancers. Vet Comp Oncol 16(1):E176–E184
85. Borgatti A, Koopmeiners JS, Sarver AL, Winter AL, Stuebner K, Todhunter D, Rizzardi AE, Henriksen JC, Schmechel S, Forster CL et al (2017) Safe and effective sarcoma therapy through bispecific targeting of EGFR and uPAR. Mol Cancer Ther 16(5):956–965

IL-9 and Th9 Cells in Tumor Immunity

3

Ying He, Lin Dong, Yejin Cao, Yujing Bi, and Guangwei Liu

Abstract

T cells can be categorized into functionally diverse subpopulations, which include Th1, Th2, Th9, Th17, Th22, and Tfh cells and Foxp3$^+$ Tregs, based on their role in maintaining normal immune homeostasis and affecting pathological immune-associated diseases. Among these subpopulations, Th9 cells are relatively new, and less is known about their signaling and effects on tumor immunity. Recently, some studies have focused on regulation of the IL-9/IL-9R signaling pathway and Th9 cell differentiation and their roles in tumor environments. Herein, we summarize recent progress in understanding the regulatory signaling of IL-9 and Th9 cells and their critical roles and mechanisms in antitumor immunity.

Keywords

IL-9 · Th9 cells · Th9 · PU.1 · Antitumor · Tumor immunity · Cancer · Tumor immunotherapy · T cell function · T cell differentiation · CTL · T cell activity · Tc9 · HIF1α · SIRT1

Abbreviation

AKT	Protein kinase B
ALCL	Anaplastic large cell lymphoma
Bcl6	B cell lymphoma 6
CTL	Cytotoxic lymphocyte
DCs	Dendritic cells
EAE	Experimental autoimmune encephalitis
FOXO1	Forkhead box protein O1
GATA3	GATA binding protein 3
GITR	TNF receptor-related protein
GrzB	Granzyme B
HAT	Histone acetyltransferase
HIF1α	Hypoxia-inducible factor-1α
HTLV-1	Human cell leukemia virus 1
IFNγ	Interferon-γ
IL-9	Interleukin 9
IL-9R	IL-9 receptor
MAPK	Mitogen-activated protein kinase
mTOR	Mechanistic target of rapamycin
NFAT	Nuclear factor of activated T cells
NF-kB	Nuclear factor-kappa B
NK cells	Natural killer cells

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NKT cells	Natural killer T cells
PI-3K	Phosphatidylinositol 3′-kinase
rIL-9	Recombinant IL-9 protein
STAT6	Signal transducer and activator of transcription 6
TAK1	Transforming growth factor-activated kinase-1
T-bet	T-box transcription factor
Tc9	IL-9-producing $CD8^+$ T cells
Tfh cells	T follicular helper cells
TGFβ1	Transforming growth factor-β1
Th	T helper cells
Th1	IFNγ-producing $CD4^+$ T cells
Th9	IL-9-producing $CD4^+$ T cells
TNFα	Tumor necrosis factor-α
Treg cells	Regulatory T cells
TSLP	Thymic stromal lymphopoietin

3.1 Introduction

IL-9 was first purified and characterized as a T cell and mast cell growth factor and termed p40 based on its molecular weight and mast cell growth-enhancing activity [1–3]. IL-9 is also a pleiotropic cytokine that is secreted mainly by Th9, Th2, and mast cells [4]. Some other cells also secrete IL-9. These cells include Th17 cells, Tregs, $CD8^+$ T cells, innate lymphoid cells, NKT cells, and dendritic cells [5] (DCs). IL-9 signaling occurs when it constitutively binds to the IL-9 receptor, which includes two subunits, IL-9Rα and IL-9Rγ [6]. IL-9Rα is specific for IL-9 signaling, and IL-9Rγ is a common γ-chain in IL-2, IL-4, IL-7, IL-15, and IL-21 receptors [7]. IL-9 specifically binds to IL-9Rα but cannot mediate any further effects without binding to IL-9Rγ. Upon IL-9 receptor ligation with IL-9, there is activation of downstream targets, including the phosphorylation of JAK and the STAT1, STAT3, and STAT5 transcription factors [8–10]. The phosphorylated STAT molecules dimerize and translocate into the nucleus, where they can direct IL-9 gene expression and production to link IL-9 to diverse biological functions [11]. Additionally, IL-9/IL-9R signaling activates the MAPK [12], forkhead [13, 14], and PI3K-AKT [15] pathways, although the physiological requirement for these pathways in primary cells has not been well addressed (Fig. 3.1).

IL-9 has pleiotropic effects on hematopoietic progenitor cells, immune cells (T cells, B cells, and mast cells), and epithelial cells and has roles in all kinds of immune-associated conditions, such as allergic inflammation, parasitic infection, autoimmune diseases, and transplant rejection [5, 16, 17] (Fig. 3.2). Interestingly, recently, some studies have indicated roles of IL-9 in tumorigenesis. Herein, we summarize the latest advances regarding the role of IL-9 and Th9 cells in the tumor environment.

3.2 IL-9 and Th9 Cells

Although IL-9 was originally thought to be produced primarily by Th2 cells, naïve $CD4^+$ T cells differentiate into a distinct IL-9-producing T cell subset known as Th9 cells in the presence of IL-4 and TGFβ1 [5]. Th9 cells secrete IL-9 and IL-10 in mice but do not produce cytokines characteristic of other T helper cell subsets (Fig. 3.3). Since the precise role of Th9 cells in the pathogenesis of allergic inflammation and other human diseases is not currently well understood, growing interest in this area will help to better define the effects of IL-9 signaling. Schimid [18] was the first to link IL-9 with Th9 cells in 1994 and defined the IL-9-producing Th9 cells as a new cell type. That study showed that naïve $CD4^+$ T cells could be differentiated into IL-9-producing $CD4^+$ T cells (Th9 cells) in the presence of IL-4 and TGFβ1. Although IL-4 alone was insufficient to induce the production of IL-9, when combined with TGFβ1, it significantly enhanced the IL-9 production. Thereafter, serial studies showed that IL-4 inhibited Foxp3 expression, and in combination with TGFβ1, IL-4 induced naïve $CD4^+$ T cells to differentiate into Th9 cells. TGFβ1 is a potent inhibitor of Th2 cells [5, 16, 19–22]. IL-4

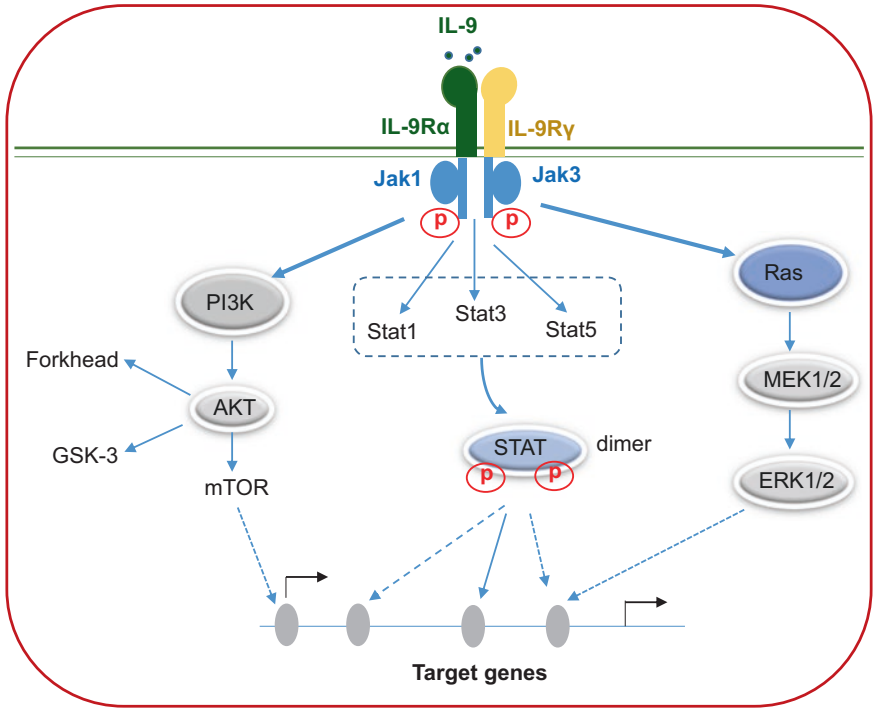

Fig. 3.1 IL-9 signaling pathway overview. IL-9 signals through a receptor complex consisting of IL-9Rα and IL-9Rγ (the common gamma-chain/IL-2 R gamma subunit)

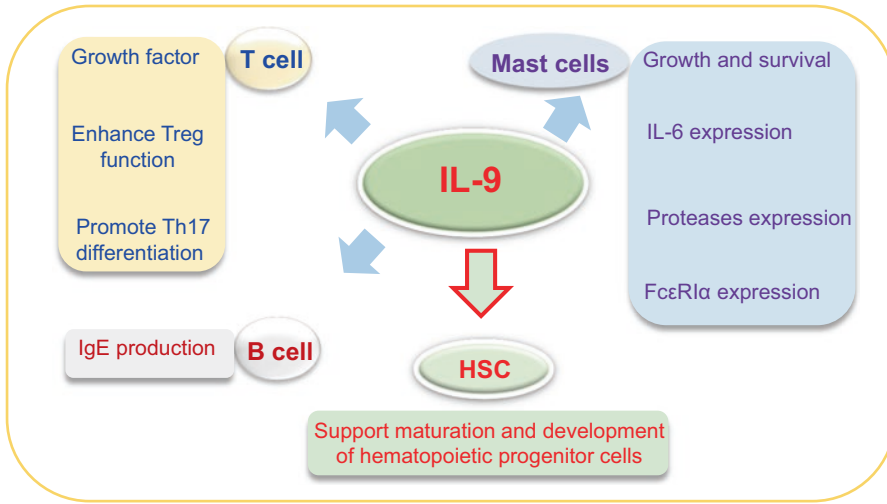

Fig. 3.2 IL-9 effects on immune cells. IL-9 has direct and indirect effects on T cells, B cells, mast cells, and hematopoietic progenitor cells

supports Th2 cell differentiation without TGFβ1, but it supports Th9 cell differentiation with TGFβ1 [23]. Thus, IL-4 and TGFβ1 are required for Th9 cell differentiation. These studies provide a basis for establishing Th9 cells as a new subtype (Fig. 3.3).

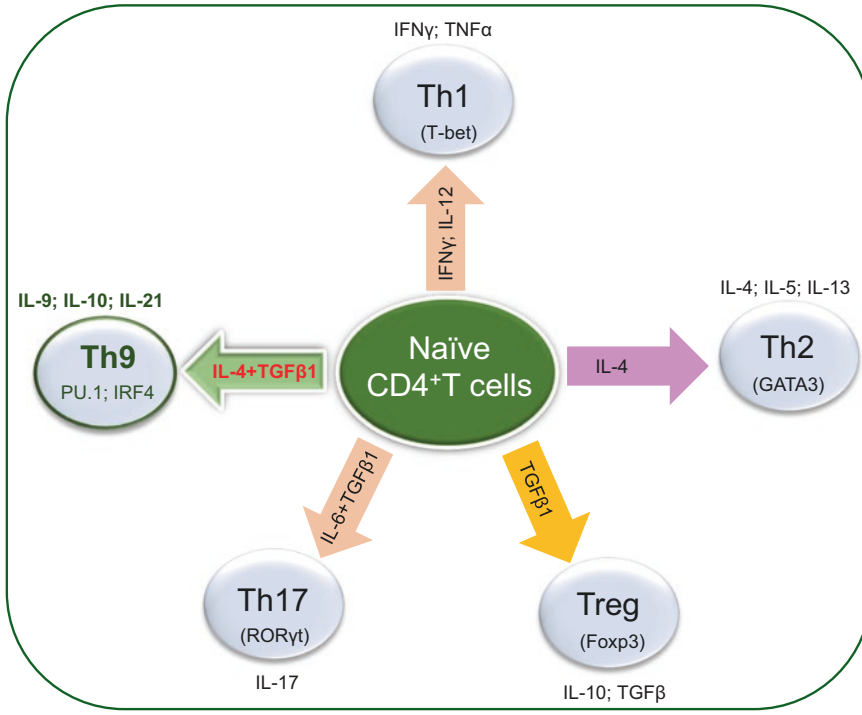

Fig. 3.3 Differentiation of the T cell lineage. Naïve CD4+ T cells are activated by T cell receptor (TCR) signaling and differentiate into various T cell lineages depending on the cytokine environment. Prototypical differentiation cytokine sets, corresponding specific transcriptional factors, and functional cytokine effects that regulate T helper cell fate and functions (including those of Th9 cells) are shown

3.3 Th9 Cells and Other Cell Types

Th9 cells are closely related to other T cell subsets, especially Th2 cells (Fig. 3.3). However, Th9 cells are different from Th2 cells. Although IL-4 and the transcription factors GATA3 and STAT6 are all involved in the induction of Th2 and Th9 cells, Th9 cells are still different from Th2 cells in having some unique cell features [3, 24, 25]. In regard to the cytokine milieu needed for induction, TGFβ1 is critical for Th9 cells, but it inhibits Th2 cells [26]. Additionally, in vivo studies have shown that if Th2 cells are removed, the effect of Th9 cells will be more obvious [27]. Th9 cell generation could occur in the absence of TGFβ1. When TGFβ1 was replaced by IL-1β, the combination of IL-1β and IL-4 efficiently promoted Th9 cell differentiation [28].

Transcription factors are critical for the development of T helper cell lineages. One specific T cell lineage transcription factor often inhibits effector cytokine production by other T helper cell lineages. Therefore, T-bet and Runx3, which are Th1 cell-specific transcription factors, significantly inhibit the production of IL-9 in Th9 cells [29, 30]. Additionally, Foxp3, a Treg-specific transcription factor, significantly decreases IL-9 production in Th9 cells [26, 31]. Expression of both T-bet and Foxp3 significantly inhibited the generation of Th9 cells [32]. The Th17 cell-specific transcriptional factor RORγt showed similar effects. RORγt-deficient mice display significantly enhanced differentiation of Th9 cells [14, 32].

Effector molecules of diverse T helper cell lineages also show similar relationships. The Th1 cell effector factor IFNγ inhibits IL-9 production in Th9 cells [3, 33]. The Th17 cell effector factor IL-17A significantly inhibits IL-9 expression and secretion in Th9 cells [34, 35]. Our previous studies analyzed different T helper cell lineages in an in vitro induction system, including Th1, Th2, and Th17 cells, which all showed significantly inhibited IL-9 production and expression levels [23].

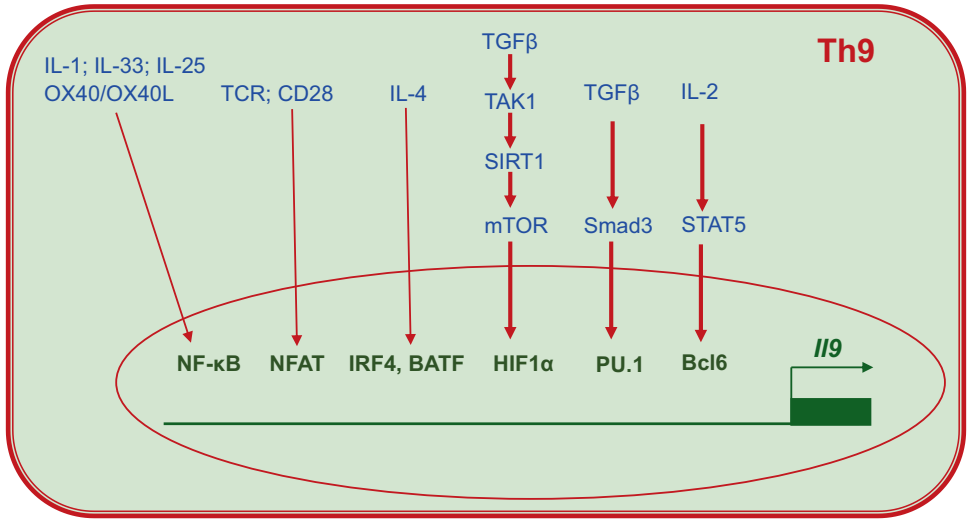

Fig. 3.4 Transcriptional regulation of Th9 cell differentiation. IL-4, TGFβ1, IL-2, TCR, and other stimuli induce the expression of downstream transcriptional factors and interact with the IL-9 promoter to induce IL-9 production and secretion from Th9 cells

3.4 Regulation of Th9 Cell Differentiation

As we recently reviewed [16], a variety of cytokines are known to contribute to the differentiation and generation of Th9 cells. In addition to IL-4 and TGFβ1, IL-1 [36], IL-2 [20, 37], IL-6 [35], IL-10 [26], IL-21 [37], IL-23 [38], IL-25 [39], IL-33 [40], IFNα/β [41], and thymic stromal lymphopoietin (TSLP) [42] all contribute to the induction of Th9 cells, while IFNγ [43] and IL-27 [43] inhibit the differentiation of Th9 cells (Fig. 3.4). Certain transcriptional factors, including STAT6 [25], PU.1 [19, 44], IRF1 [45], IRF4 [20], NF-κB [46], Bcl-6 [37, 47], and the Smad/Notch complex [48], directly interact with the *Il9* gene promoter to increase IL-9 production (Fig. 3.4). The TGFβ-activated kinase TAK1, a mediator of the Smad-independent TGFβ pathway, plays a pivotal role in directing Th9 cell differentiation [49]. Our recent results [23] showed that TAK1 inhibition released SIRT1downregulation during Th9 cell differentiation. SIRT1 deficiencies promote Th9 cell differentiation, while SIRT1 overexpression inhibits Th9 cell differentiation in mouse and human T cells. Interestingly, the mTOR-hypoxia-inducible factor-1α (HIF1α)-dependent glycolysis pathway is responsible for the SIRT1-deficiency-induced increase in Th9 cell differentiation. More importantly, the transcription factor HIF1α directly interacts with *Il9* and can bind with the *Il9* promoter region to regulate *Il9* promoter activity during Th9 cell differentiation. Thus, the TAK1-SIRT1-mTOR-HIF1α glycolytic pathways are essential for the differentiation of Th9 cells (Fig. 3.4).

3.5 Effects of Th9 Cells in Tumors

The antitumor and protumor effects of IL-9 signaling are still controversial, and some results have been obtained in different cancer cell lines or clinical disease-related studies (Table 3.1). IL-9 is involved in the pathogenesis of lung cancer [50], leukemia [51, 52], breast cancer [24–26, 53], thyroid cancer [54], colon cancer [55], lymphoma [56–58], and melanoma [23, 49, 59–63]. Therefore, the effect of Th9 cells may be related to the type of tumor cells.

Table 3.1 Effects of Th9 cells on different kinds of cancer

Cancer	Model system	Tumor effects	Mechanisms	References
Lung cancer	Cell lines	Protumor	IL-9 pathway direct effects on cancer cell proliferation	[50]
	Patients	Protumor		[50]
Leukemia	Primary cells	Protumor	IL-9 direct effects on cancer cell proliferation and antiapoptosis	[51, 52]
	Patients	Protumor	IL-9 expression in pathogenesis of chronic lymphoid leukemia	[88]
Breast cancer	Cell lines	Protumor	Wnt-IL-9 signaling in mast cells; IL-9 enhances Treg activities	[53, 68, 80, 89]
Colon cancer	Cell lines	Protumor	IL-9 inhibits Treg differentiation	[55]
Thyroid cancer	Patients	Protumor	Th9-JAK/STAT signaling	[54]
Lymphoma	Cell lines	Protumor	IL-9-JAK3-STAT3 signaling	[56, 57]
	Patients	Protumor	IL-9 enhances immune inhibition by Tregs and mast cells	[90, 91]
Melanoma	Cell lines	Anticancer (direct)	IL-9 inhibits p21 and enhances the antiapoptotic molecule TRAIL	[72]
	Cell lines	Anticancer (indirect)	IL-9- or IL-21-dependent mechanisms	[23, 49, 59–63]

IL-9 is involved in the pathogenesis of some types of cancer. IL-9 promotes cell proliferation in the A549 and SK-MES-1 lung cancer cell lines [50]. IL-9 produced by Th9 cells phosphorylates STAT3 on serine 727 and activates the downstream JAK/STAT pathway and eventually regulates cancer cell proliferation and protects cancer cells from apoptosis. IL-9 also plays an important role in leukemia. IL-9 was necessary for human T cell leukemia virus (HTLV-1)-infected T cell proliferation [51, 52]. The IL-9 gene also contains sequences that are required for the expression of HTLV-1. There are some relationships between IL-9 expression and the pathogenesis of chronic lymphoid leukemia. Breast cancer cell lines could not grow in IL-9$^{-/-}$ mice, but IL-9R expression was not related to these effects. Some mechanism studies have shown that IL-9 is negatively regulated by Wnt signaling and plays a role in tumor cell growth, invasion, and migration and inhibits tumor cell apoptosis [24–26, 53]. IL-9 is also required for growth of the colon cancer cell line CT26 [55]. In IL-9$^{-/-}$ mice, CT26 growth is significantly delayed compared with that in wild-type mice. Moreover, IL-9 is also involved in the pathogenesis of Hodgkin lymphoma, B cell non-Hodgkin lymphoma, anaplastic large cell lymphoma (ALCL), and NK/T cell lymphoma [56, 57].

Currently, the antitumor properties of Th9 cells are mainly derived from studies in mouse melanoma models [23, 49, 59–64]. Th9 cells exist in metastatic pleural effusions, and in mouse xenograft models of melanoma, infiltrating lymphocytes and Th9 cells display antitumor properties [60, 61]. Adoptive transfer of Th9 cells into mice bearing B16 melanoma or lung carcinoma or treatment of these mice with recombinant IL-9 protein (rIL-9) [50] significantly delayed tumor cell growth. Exogenous IL-9 also restricted the growth of B16F10 melanoma and LLC1 cells but not in EL-4 cells, while a neutralizing anti-IL-9 antibody abolished the beneficial effect of adoptively transferred Th9 cells in vivo [65]. These data show that IL-9 is critical for suppressing tumor growth. IL-9R deficiency significantly promotes melanoma tumor growth [66, 67]. This suggests that IL-9 may play an antitumor role by interacting with IL-9R. Additionally, Treg [68, 69], Th17 [69], and Th2 [27, 70] cells secrete low levels of IL-9. A considerable portion of Th9 cells acquire a Th1 cell phenotype and produce IFNγ in vivo [29, 71].

Our studies [23] also indicate that Th9 cells are critical for antitumor immunity. Naïve T cells isolated from WT or SIRT1$^{-/-}$ mice were differentiated under Th9 cell-inducing conditions and transferred into Rag1$^{-/-}$ mice, a T cell- and B cell-deficient mouse model, which were then subcutaneously injected with B16 melanoma cells. The mice that received SIRT1$^{-/-}$ CD4^{+} T cells developed smaller tumors than wild-type controls. Tumor-infiltrating CD4^{+} T cells isolated from the SIRT1$^{-/-}$ group had a higher IL-9^{+} ratio than these cells in the wild-type group. Importantly, administration of an anti-IL-9 antibody reversed the changes induced by SIRT1$^{-/-}$ Th9 cell transfer. Therefore, these data show that SIRT1 is necessary for the suppression of Th9 cell differentiation and Th9-mediated antitumor immunity in melanoma.

3.6 Th9 Cell Mechanisms in Tumor Immunity

IL-9 produced by Th9 cells elicits protumor or antitumor immune responses through both direct and indirect regulatory mechanisms (Fig. 3.5).

Several studies have shown direct regulatory mechanisms of Th9 cells in tumor immunity. IL-9 signaling is directly associated with JAK/STAT3 signaling and gives rise to lung cancer cell growth in A549 and SK-MES-1 lung cancer cell lines [50]. In addition to the direct pro-proliferation effects of IL-9-JAK/STAT3, STAT3 can be recruited upon binding of IL-9 to its receptor via its SH2 domain and is crucial for inhibiting apoptosis [4, 5]. Additionally, IL-9 can directly enhance the functions of p21 and the antiapoptotic molecule TRAIL and can suppress the growth of HTB-72 melanoma cells [72]. IL-9 significantly suppresses the proliferation of breast cancer cell lines [53, 73]. Further results show that IL-9 is downregulated by the Wnt signaling pathway [73]. One hypothesis demonstrates that tumor cells produce significant amounts of IL-9 to polarize the cytokine environment toward one that favors Th9 cells. Thus, IL-9 contributes to the proliferation, invasion, and migration of tumor cells.

Moreover, T cell plasticity also plays a pivotal role in directly regulating tumor cell growth. Human memory Th9 cells from blood and tissue are often coexpressed with TNFα and granzyme B, suggesting that they play a proinflammatory role [74, 75]. Several recent studies [59, 76, 77] have also shown that glucocorticoid-induced TNF receptor-related protein (GITR) ligation directs the differentiation of Tregs into Th9 cells and mediates antitumor immunity. These data

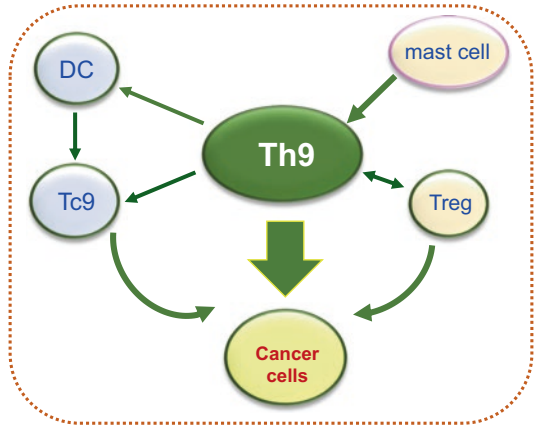

Fig. 3.5 Regulatory mechanism of Th9-cell-mediated tumor immunity. IL-9 production by Th9 cells exerts tumor immunity through direct and indirect mechanisms. IL-9 promotes tumor cell proliferation and migration through IL-9R-JAK/STAT signaling or reciprocal differentiation between Th9 cells and other T cell lineages. Th9 cells elicit tumor immunity indirectly though mast cells, dendritic cells (DCs), Tc9 cells, or Tregs

show that the plasticity of Th9 cells in tumor microenvironment is essential for the tumor immune effects of Th9 cells, but the clear mechanisms still need to be explored in the future.

Indirect regulatory mechanisms of Th9 cells are likely also critical for protumor or antitumor immunity. IL-9$^{-/-}$ mice delay breast cancer cell growth, but these cells have negligible IL-9R expression [37, 50, 51]. This suggests that IL-9 does not directly have protumor effects. Further studies show that CD4$^+$ T cells are necessary for the protumor effects of breast cancer cells in IL-9$^{-/-}$ mice [59]. This suggests that Th9 cells probably have an indirect regulatory role. Mast cells and dendritic cells (DCs) and CD8$^+$CTLs are critically involved in the indirect regulation of tumor growth.

Mast cells are critical for Th9 cell-mediated tumor immunity [65, 78–80]. Administration of recombinant IL-9 in mast-cell-deficient mice might not have any antitumor or protumor effects. Moreover, blocking the activities of mast cells or depleting mast cells in vivo significantly abrogates the tumor immune effects of Th9 cells in tumor-bearing mice. These data collectively indicate the essential role of mast cells in Th9-cell-mediated tumor immunity.

The survival and function of myeloid DCs are also required for Th9-mediated tumor immunity [81]. IL-9 production from Th9 cells can recruit DCs to local tumor tissues by enhancing the expression of CCL20 and CCR6 and promoting host CD8$^+$CTL activity and antitumor immunity [60, 82]. Moreover, IL-9-producing CD8$^+$ T cells (Tc9) primed by Th9-cell-inducing conditions play a pivotal role in antitumor immunity, and Tc9 cells exhibit much stronger antitumor effects than CD8$^+$ T cells in OT-I/B16-OVA and Pmel-1/B16 mouse models of melanoma [63, 83], which indicates that the antitumor activities of Th9 cells are partially due to their effects on Tc9 cells.

Additionally, Th9 cells secrete IL-21 to elicit antitumor effects. IL-1β can promote the secretion of IL-21 from Th9 cells, and IL-1β-induced Th9 cells exhibit enhanced IL-21 but not IL-9 levels in Th9-cell-dependent antitumor immunity [84, 85]. IL-21 can have effects on antitumor immunity by promoting IFNγ production and enhancing the activities of NK cells and CD8$^+$CTLs [86, 87].

3.7 Future Perspective

Although we already know that Th9 cells are a new cell type and play an important role in tumor immunity, our knowledge of Th9 cells is still limited. For example, we remain poorly informed about how Th9 cells are induced and sustained, especially under in vivo conditions. Other T cell subsets are associated with specific transcription factors; we still do not know the specific transcription factors associated with Th9 cells. Known Th9 cell transcription factors often play a role in the differentiation of other T cell subsets. This suggests that Th9 cell differentiation may involve a combination of multiple transcription factors and signaling pathways. We are also still uncertain about the tumor immune effect of Th9 cells. At present, a large number of research results are still limited to certain types of cancer cells; specifically, the mechanism of Th9-cell-associated tumor immunity is still unclear. In addition to the production of IL-9 by Th9 cells, many other cells also produce IL-9. The relationships between Th9 cells and their specific mechanisms in tumor immunity are still unclear. These clarifications will help in understanding the immunological theory of Th9 cells and IL-9 and their effect on tumor immunity and will provide the basis for developing new antitumor immunotherapies.

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References

1. Van Snick J, Goethals A, Renauld JC, Van Roost E, Uyttenhove C, Rubira MR et al (1989) Cloning and characterization of a cDNA for a new mouse T cell growth factor (P40). J Exp Med 169(1):363–368
2. Mock BA, Krall M, Kozak CA, Nesbitt MN, McBride OW, Renauld JC et al (1990) IL9 maps to mouse chromosome 13 and human chromosome 5. Immunogenetics 31(4):265–270
3. Kaplan MH, Hufford MM, Olson MR (2015) The development and in vivo function of T helper 9 cells. Nat Rev Immunol 15(5):295–307
4. Goswami R, Kaplan MH (2011) A brief history of IL-9. J Immunol 186(6):3283–3288
5. Rojas-Zuleta WG, Vasquez G (2016) Th9 lymphocytes: a recent history from IL-9 to its potential role in rheumatic diseases. Autoimmun Rev 15(7):649–655
6. Demoulin JB, Uyttenhove C, Van Roost E, DeLestre B, Donckers D, Van Snick J et al (1996) A single tyrosine of the interleukin-9 (IL-9) receptor is required for STAT activation, antiapoptotic activity, and growth regulation by IL-9. Mol Cell Biol 16(9):4710–4716
7. Vermeesch JR, Petit P, Kermouni A, Renauld JC, Van Den Berghe H, Marynen P (1997) The IL-9 receptor gene, located in the Xq/Yq pseudoautosomal region, has an autosomal origin, escapes X inactivation and is expressed from the Y. Hum Mol Genet 6(1):1–8
8. Knoops L, Renauld JC (2004) IL-9 and its receptor: from signal transduction to tumorigenesis. Growth Factors 22(4):207–215
9. Fawaz LM, Sharif-Askari E, Hajoui O, Soussi-Gounni A, Hamid Q, Mazer BD (2007) Expression of IL-9 receptor alpha chain on human germinal center B cells modulates IgE secretion. J Allergy Clin Immunol 120(5):1208–1215
10. Nalleweg N, Chiriac MT, Podstawa E, Lehmann C, Rau TT, Atreya R et al (2015) IL-9 and its receptor are predominantly involved in the pathogenesis of UC. Gut 64(5):743–755
11. Fontaine RH, Cases O, Lelievre V, Mesples B, Renauld JC, Loron G et al (2008) IL-9/IL-9 receptor signaling selectively protects cortical neurons against developmental apoptosis. Cell Death Differ 15(10):1542–1552
12. Hong CH, Chang KL, Wang HJ, Yu HS, Lee CH (2015) IL-9 induces IL-8 production via STIM1 activation and ERK phosphorylation in epidermal keratinocytes: a plausible mechanism of IL-9R in atopic dermatitis. J Dermatol Sci 78(3):206–214
13. Malik S, Sadhu S, Elesela S, Pandey RP, Chawla AS, Sharma D et al (2017) Transcription factor Foxo1 is essential for IL-9 induction in T helper cells. Nat Commun 8(1):815
14. Bi E, Ma X, Lu Y, Yang M, Wang Q, Xue G et al (2017) Foxo1 and Foxp1 play opposing roles in regulating the differentiation and antitumor activity of TH9 cells programmed by IL-7. Sci Signal 10(500)
15. Martelli AM, Tabellini G, Ricci F, Evangelisti C, Chiarini F, Bortul R et al (2012) PI3K/AKT/mTORC1 and MEK/ERK signaling in T-cell acute lymphoblastic leukemia: new options for targeted therapy. Adv Biol Regul 52(1):214–227
16. Li Y, Yu Q, Zhang Z, Wang J, Li S, Zhang J et al (2016) TH9 cell differentiation, transcriptional control and function in inflammation, autoimmune diseases and cancer. Oncotarget 7(43):71001–71012
17. Gorczynski R, Yu K, Chen Z (2014) Anti-CD200R2, anti-IL-9, anti-IL-35, or anti-TGF-beta abolishes increased graft survival and Treg induction induced in cromolyn-treated CD200R1KO.CD200tg mice. Transplantation 97(1):39–46
18. Schmitt E, Germann T, Goedert S, Hoehn P, Huels C, Koelsch S et al (1994) IL-9 production of naive CD4+ T cells depends on IL-2, is synergistically enhanced by a combination of TGF-beta and IL-4, and is inhibited by IFN-gamma. J Immunol 153(9):3989–3996
19. Gerlach K, Hwang Y, Nikolaev A, Atreya R, Dornhoff H, Steiner S et al (2014) TH9 cells that express the transcription factor PU.1 drive T cell-mediated colitis via IL-9 receptor signaling in intestinal epithelial cells. Nat Immunol 15(7):676–686
20. Gomez-Rodriguez J, Meylan F, Handon R, Hayes ET, Anderson SM, Kirby MR et al (2016) Itk is required for Th9 differentiation via TCR-mediated induction of IL-2 and IRF4. Nat Commun 7:10857
21. Veldhoen M, Uyttenhove C, van Snick J, Helmby H, Westendorf A, Buer J et al (2008) Transforming growth factor-beta 'reprograms' the differentiation of T helper 2 cells and promotes an interleukin 9-producing subset. Nat Immunol 9(12):1341–1346
22. Jabeen R, Goswami R, Awe O, Kulkarni A, Nguyen ET, Attenasio A et al (2013) Th9 cell development requires a BATF-regulated transcriptional network. J Clin Invest 123(11):4641–4653
23. Wang Y, Bi Y, Chen X, Li C, Li Y, Zhang Z et al (2016) Histone deacetylase SIRT1 negatively regulates the differentiation of interleukin-9-producing CD4(+) T cells. Immunity 44(6):1337–1349
24. Micosse C, von Meyenn L, Steck O, Kipfer E, Adam C, Simillion C et al (2019) Human "T_H9" cells are a subpopulation of PPAR-gamma(+) T_H2 cells. Sci Immunol 4(31)
25. Goswami R, Jabeen R, Yagi R, Pham D, Zhu J, Goenka S et al (2012) STAT6-dependent regulation of Th9 development. J Immunol 188(3):968–975
26. Dardalhon V, Awasthi A, Kwon H, Galileos G, Gao W, Sobel RA*, et al.* IL-4 inhibits TGF-beta-induced Foxp3+ T cells and, together with TGF-beta, generates IL-9+ IL-10+ Foxp3(-) effector T cells. Nat Immunol 2008, **9**(12)**:** 1347–1355
27. Fallon PG, Jolin HE, Smith P, Emson CL, Townsend MJ, Fallon R et al (2002) IL-4 induces characteristic Th2 responses even in the combined absence of IL-5, IL-9, and IL-13. Immunity 17(1):7–17
28. Xue G, Jin G, Fang J, Lu Y (2019) IL-4 together with IL-1beta induces antitumor Th9 cell differentiation

in the absence of TGF-beta signaling. Nat Commun 10(1):1376
29. Wu B, Huang C, Kato-Maeda M, Hopewell PC, Daley CL, Krensky AM et al (2008) IL-9 is associated with an impaired Th1 immune response in patients with tuberculosis. Clin Immunol 126(2):202–210
30. Matsuki A, Takatori H, Makita S, Yokota M, Tamachi T, Suto A et al (2017) T-bet inhibits innate lymphoid cell-mediated eosinophilic airway inflammation by suppressing IL-9 production. J Allergy Clin Immunol 139(4):1355–1367. e1356
31. Smith SE, Hoelzinger DB, Dominguez AL, Van Snick J, Lustgarten J (2011) Signals through 4-1BB inhibit T regulatory cells by blocking IL-9 production enhancing antitumor responses. Cancer Immunol Immunother 60(12):1775–1787
32. Kaplan MH (2013) Th9 cells: differentiation and disease. Immunol Rev 252(1):104–115
33. Jager A, Dardalhon V, Sobel RA, Bettelli E, Kuchroo VK (2009) Th1, Th17, and Th9 effector cells induce experimental autoimmune encephalomyelitis with different pathological phenotypes. J Immunol 183(11):7169–7177
34. Lu Y, Lin H, Zhai K, Wang X, Zhou Q, Shi H (2016) Interleukin-17 inhibits development of malignant pleural effusion via interleukin-9-dependent mechanism. Sci China Life Sci 59(12):1297–1304
35. Schutze N, Trojandt S, Kuhn S, Tomm JM, von Bergen M, Simon JC et al (2016) Allergen-induced IL-6 regulates IL-9/IL-17A balance in CD4+ T cells in allergic airway inflammation. J Immunol 197(7):2653–2664
36. Anuradha R, George PJ, Hanna LE, Chandrasekaran V, Kumaran P, Nutman TB et al (2013) IL-4-, TGF-beta-, and IL-1-dependent expansion of parasite antigen-specific Th9 cells is associated with clinical pathology in human lymphatic filariasis. J Immunol 191(5):2466–2473
37. Liao W, Spolski R, Li P, Du N, West EE, Ren M et al (2014) Opposing actions of IL-2 and IL-21 on Th9 differentiation correlate with their differential regulation of BCL6 expression. Proc Natl Acad Sci U S A 111(9):3508–3513
38. Beriou G, Bradshaw EM, Lozano E, Costantino CM, Hastings WD, Orban T et al (2010) TGF-beta induces IL-9 production from human Th17 cells. J Immunol 185(1):46–54
39. Angkasekwinai P, Sodthawon W, Jeerawattanawart S, Hansakon A, Pattanapanyasat K, Wang YH (2017) ILC2s activated by IL-25 promote antigen-specific Th2 and Th9 functions that contribute to the control of Trichinella spiralis infection. PLoS One 12(9):e0184684
40. Verma M, Liu S, Michalec L, Sripada A, Gorska MM, Alam R (2018) Experimental asthma persists in IL-33 receptor knockout mice because of the emergence of thymic stromal lymphopoietin-driven IL-9(+) and IL-13(+) type 2 innate lymphoid cell subpopulations. J Allergy Clin Immunol 142(3):793–803. e798
41. Pritchard AL, Carroll ML, Burel JG, White OJ, Phipps S, Upham JW (2012) Innate IFNs and plasmacytoid dendritic cells constrain Th2 cytokine responses to rhinovirus: a regulatory mechanism with relevance to asthma. J Immunol 188(12):5898–5905
42. Siracusa MC, Saenz SA, Hill DA, Kim BS, Headley MB, Doering TA et al (2011) TSLP promotes interleukin-3-independent basophil haematopoiesis and type 2 inflammation. Nature 477(7363):229–233
43. Murugaiyan G, Beynon V, Pires Da Cunha A, Joller N, Weiner HL (2012) IFN-gamma limits Th9-mediated autoimmune inflammation through dendritic cell modulation of IL-27. J Immunol 189(11):5277–5283
44. Rivera Vargas T, Cai Z, Shen Y, Dosset M, Benoit-Lizon I, Martin T et al (2017) Selective degradation of PU.1 during autophagy represses the differentiation and antitumour activity of T_H9 cells. Nat Commun 8(1):559
45. Vegran F, Berger H, Boidot R, Mignot G, Bruchard M, Dosset M et al (2014) The transcription factor IRF1 dictates the IL-21-dependent anticancer functions of TH9 cells. Nat Immunol 15(8):758–766
46. Maier E, Werner D, Duschl A, Bohle B, Horejs-Hoeck J (2014) Human Th2 but not Th9 cells release IL-31 in a STAT6/NF-kappaB-dependent way. J Immunol 193(2):645–654
47. Bassil R, Orent W, Olah M, Kurdi AT, Frangieh M, Buttrick T et al (2014) BCL6 controls Th9 cell development by repressing Il9 transcription. J Immunol 193(1):198–207
48. Elyaman W, Bassil R, Bradshaw EM, Orent W, Lahoud Y, Zhu B et al (2012) Notch receptors and Smad3 signaling cooperate in the induction of interleukin-9-producing T cells. Immunity 36(4):623–634
49. Nakatsukasa H, Zhang D, Maruyama T, Chen H, Cui K, Ishikawa M et al (2015) The DNA-binding inhibitor Id3 regulates IL-9 production in CD4(+) T cells. Nat Immunol 16(10):1077–1084
50. Ye ZJ, Zhou Q, Yin W, Yuan ML, Yang WB, Xiong XZ et al (2012) Differentiation and immune regulation of IL-9-producing CD4+ T cells in malignant pleural effusion. Am J Respir Crit Care Med 186(11):1168–1179
51. Kelleher K, Bean K, Clark SC, Leung WY, Yang-Feng TL, Chen JW et al (1991) Human interleukin-9: genomic sequence, chromosomal location, and sequences essential for its expression in human T-cell leukemia virus (HTLV)-I-transformed human T cells. Blood 77(7):1436–1441
52. Lavorgna A, Matsuoka M, Harhaj EW (2014) A critical role for IL-17RB signaling in HTLV-1 tax-induced NF-kappaB activation and T-cell transformation. PLoS Pathog 10(10):e1004418
53. Hsieh TH, Hsu CY, Tsai CF, Chiu CC, Liang SS, Wang TN et al (2016) A novel cell-penetrating peptide suppresses breast tumorigenesis by inhibiting beta-catenin/LEF-1 signaling. Sci Rep 6:19156
54. Zivancevic-Simonovic S, Mihaljevic O, Majstorovic I, Popovic S, Markovic S, Milosevic-Djordjevic O et al (2015) Cytokine production in patients with papillary thyroid cancer and associated autoimmune Hashimoto thyroiditis. Cancer Immunol Immunother 64(8):1011–1019

55. Hoelzinger DB, Dominguez AL, Cohen PA, Gendler SJ (2014) Inhibition of adaptive immunity by IL9 can be disrupted to achieve rapid T-cell sensitization and rejection of progressive tumor challenges. Cancer Res 74(23):6845–6855
56. Qiu L, Lai R, Lin Q, Lau E, Thomazy DM, Calame D et al (2006) Autocrine release of interleukin-9 promotes Jak3-dependent survival of ALK+ anaplastic large-cell lymphoma cells. Blood 108(7):2407–2415
57. Koo GC, Tan SY, Tang T, Poon SL, Allen GE, Tan L et al (2012) Janus kinase 3-activating mutations identified in natural killer/T-cell lymphoma. Cancer Discov 2(7):591–597
58. Lv X, Wang X (2013) The role of interleukin-9 in lymphoma. Leuk Lymphoma 54(7):1367–1372
59. Kim IK, Kim BS, Koh CH, Seok JW, Park JS, Shin KS et al (2015) Glucocorticoid-induced tumor necrosis factor receptor-related protein co-stimulation facilitates tumor regression by inducing IL-9-producing helper T cells. Nat Med 21(9):1010–1017
60. Lu Y, Hong S, Li H, Park J, Hong B, Wang L et al (2012) Th9 cells promote antitumor immune responses in vivo. J Clin Invest 122(11):4160–4171
61. You FP, Zhang J, Cui T, Zhu R, Lv CQ, Tang HT et al (2017) Th9 cells promote antitumor immunity via IL-9 and IL-21 and demonstrate atypical cytokine expression in breast cancer. Int Immunopharmacol 52:163–167
62. Lu Y, Wang Q, Yi Q (2014) Anticancer Tc9 cells: long-lived tumor-killing T cells for adoptive therapy. Oncoimmunology 3:e28542
63. Lu Y, Hong B, Li H, Zheng Y, Zhang M, Wang S et al (2014) Tumor-specific IL-9-producing CD8+ Tc9 cells are superior effector than type-I cytotoxic Tc1 cells for adoptive immunotherapy of cancers. Proc Natl Acad Sci U S A 111(6):2265–2270
64. Quezada SA, Peggs KS (2014) An antitumor boost to TH9 cells. Nat Immunol 15(8):703–705
65. Purwar R, Schlapbach C, Xiao S, Kang HS, Elyaman W, Jiang X et al (2012) Robust tumor immunity to melanoma mediated by interleukin-9-producing T cells. Nat Med 18(8):1248–1253
66. Shang Y, Kakinuma S, Nishimura M, Kobayashi Y, Nagata K, Shimada Y (2012) Interleukin-9 receptor gene is transcriptionally regulated by nucleolin in T-cell lymphoma cells. Mol Carcinog 51(8):619–627
67. Li HJ, Sun QM, Liu LZ, Zhang J, Huang J, Wang CH et al (2015) High expression of IL-9R promotes the progression of human hepatocellular carcinoma and indicates a poor clinical outcome. Oncol Rep 34(2):795–802
68. Eller K, Wolf D, Huber JM, Metz M, Mayer G, McKenzie AN et al (2011) IL-9 production by regulatory T cells recruits mast cells that are essential for regulatory T cell-induced immune suppression. J Immunol 186(1):83–91
69. Elyaman W, Bradshaw EM, Uyttenhove C, Dardalhon V, Awasthi A, Imitola J et al (2009) IL-9 induces differentiation of TH17 cells and enhances function of FoxP3+ natural regulatory T cells. Proc Natl Acad Sci U S A 106(31):12885–12890
70. Hoyle GW, Brody AR (2001) IL-9 and lung fibrosis: a Th2 good guy? Am J Respir Cell Mol Biol 24(4):365–367
71. Liu J, Harberts E, Tammaro A, Girardi N, Filler RB, Fishelevich R et al (2014) IL-9 regulates allergen-specific Th1 responses in allergic contact dermatitis. J Invest Dermatol 134(7):1903–1911
72. Fang Y, Chen X, Bai Q, Qin C, Mohamud AO, Zhu Z et al (2015) IL-9 inhibits HTB-72 melanoma cell growth through upregulation of p21 and TRAIL. J Surg Oncol 111(8):969–974
73. Lendeckel U, Arndt M, Frank K, Spiess A, Reinhold D, Ansorge S (2000) Modulation of WNT-5A expression by actinonin: linkage of APN to the WNT-pathway? Adv Exp Med Biol 477:35–41
74. Clark RA, Schlapbach C (2017) TH9 cells in skin disorders. Semin Immunopathol 39(1):47–54
75. Schlapbach C, Gehad A, Yang C, Watanabe R, Guenova E, Teague JE et al (2014) Human TH9 cells are skin-tropic and have autocrine and paracrine proinflammatory capacity. Sci Transl Med 6(219):219ra218
76. Kim IK, Chung Y, Kang CY (2016) GITR drives TH9-mediated antitumor immunity. Oncoimmunology 5(5):e1122862
77. Xiao X, Shi X, Fan Y, Zhang X, Wu M, Lan P et al (2015) GITR subverts Foxp3(+) Tregs to boost Th9 immunity through regulation of histone acetylation. Nat Commun 6:8266
78. Abdul-Wahid A, Cydzik M, Prodeus A, Alwash M, Stanojcic M, Thompson M et al (2016) Induction of antigen-specific TH 9 immunity accompanied by mast cell activation blocks tumor cell engraftment. Int J Cancer 139(4):841–853
79. Vegran F, Apetoh L, Ghiringhelli F (2015) Th9 cells: a novel CD4 T-cell subset in the immune war against cancer. Cancer Res 75(3):475–479
80. Lu LF, Lind EF, Gondek DC, Bennett KA, Gleeson MW, Pino-Lagos K et al (2006) Mast cells are essential intermediaries in regulatory T-cell tolerance. Nature 442(7106):997–1002
81. Park J, Li H, Zhang M, Lu Y, Hong B, Zheng Y et al (2014) Murine Th9 cells promote the survival of myeloid dendritic cells in cancer immunotherapy. Cancer Immunol Immunother 63(8):835–845
82. Jiang Y, Chen J, Bi E, Zhao Y, Qin T, Wang Y et al (2019) TNF-alpha enhances Th9 cell differentiation and antitumor immunity via TNFR2-dependent pathways. J Immunother Cancer 7(1):28
83. Ma X, Bi E, Huang C, Lu Y, Xue G, Guo X et al (2018) Cholesterol negatively regulates IL-9-producing CD8(+) T cell differentiation and antitumor activity. J Exp Med 215(6):1555–1569
84. Petrella TM, Tozer R, Belanger K, Savage KJ, Wong R, Smylie M et al (2012) Interleukin-21 has activity in patients with metastatic melanoma: a phase II study. J Clin Oncol Off J Am Soc Clin Oncol 30(27):3396–3401

85. Zeng R, Spolski R, Finkelstein SE, Oh S, Kovanen PE, Hinrichs CS et al (2005) Synergy of IL-21 and IL-15 in regulating CD8+ T cell expansion and function. J Exp Med 201(1):139–148
86. Strengell M, Matikainen S, Siren J, Lehtonen A, Foster D, Julkunen I et al (2003) IL-21 in synergy with IL-15 or IL-18 enhances IFN-gamma production in human NK and T cells. J Immunol 170(11):5464–5469
87. Kasaian MT, Whitters MJ, Carter LL, Lowe LD, Jussif JM, Deng B et al (2002) IL-21 limits NK cell responses and promotes antigen-specific T cell activation: a mediator of the transition from innate to adaptive immunity. Immunity 16(4):559–569
88. Chen N, Lv X, Li P, Lu K, Wang X (2014) Role of high expression of IL-9 in prognosis of CLL. Int J Clin Exp Pathol 7(2):716–721
89. Yang Z, Zhang B, Li D, Lv M, Huang C, Shen GX et al (2010) Mast cells mobilize myeloid-derived suppressor cells and Treg cells in tumor microenvironment via IL-17 pathway in murine hepatocarcinoma model. PLoS One 5(1):e8922
90. Rawstron AC, Bottcher S, Letestu R, Villamor N, Fazi C, Kartsios H et al (2013) Improving efficiency and sensitivity: European Research Initiative in CLL (ERIC) update on the international harmonised approach for flow cytometric residual disease monitoring in CLL. Leukemia 27(1):142–149
91. Nagato T, Kobayashi H, Kishibe K, Takahara M, Ogino T, Ishii H et al (2005) Expression of interleukin-9 in nasal natural killer/T-cell lymphoma cell lines and patients. Clin Cancer Res 11(23):8250–8257

4 IL-17 Signaling in the Tumor Microenvironment

R. M. Gorczynski

Abstract

Inflammation is recognized as representing a double-edged sword in terms of tumor growth, in some instances contributing to attenuation of growth and in others to enhanced progression and metastasis. Extracellular signals, released by cells within the tumor microenvironment (TME), including cancer cells themselves, as well as infiltrating immune cells, stromal cells, and other components of the extracellular matrix, all can contribute to reshaping the tumor microenvironment (TME) and tumor growth/survival. Most recently, attention has centered on contributions in the TME made by the pro-inflammatory interleukin 17 (IL-17) and the T cells (Th17) and non-T cells which produce this cytokine, as well as the target cells (IL-17 receptor positive, IL-17R$^+$) signaled by IL-17. The IL-17 family itself comprises at least six members, IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (also called IL-25), and IL-17F, all of which are known to be secreted as disulfide-linked homo- or heterodimers. These in turn bind to IL-17R, a type I cell surface receptor, of which at least five variants have been described to date, IL-17RA to IL-17RE. The discussion below focuses on what we know to date about the role of IL-17/IL-17R interactions in the tumor microenvironment in regulation of tumor growth and metastasis and highlights recent ideas concerning the possible utility of this knowledge in the clinic.

Keywords

IL-17 isoforms · Il-17R isoforms · IL-6 · Immunoregulation · Cytokines · Chronic lymphocytic leukemia (CLL) · Cancer · Tumor microenvironment (TME) · Tumor growth/survival · Tumor metastasis/invasion · Checkpoint blockade · Inflammation · Extracellular signaling · Regulatory T cells (Tregs) · Immunotherapy

4.1 Introduction

4.1.1 Tumor Microenvironment and IL-17

Tumor cells have enhanced capacities of proliferation, neo-angiogenesis development, and distance seeding under the form of metastases [1, 2]. The tumor microenvironment, which comprises malignant and nonmalignant cells distinguished by specific markers and interacting in a dynamic fashion, is an important aspect of cancer biology that contributes to tumor initiation, tumor progression, and responses to therapy [3]. Cells and

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molecules of the immune system are a fundamental component of the TME. While important for antitumor responses, cells of the immune system including macrophages, neutrophils, mast cells, dendritic cells (DCs), and lymphocytes can also promote the development and progression of almost every solid tumor [4–6], often producing cytokines and mediators which modify the TME such that it becomes more favorable to tumor development and progression [7, 8].

Fundamental to our understanding of the regulation of the TME has been a wealth of newer data on a subset of lymphocytes secreting pro-inflammatory IL-17 cytokines, Th17 cells [9]. IL-17 has been shown to play a crucial role in the proliferation of LN and splenic stromal cells, particularly fibroblastic reticular cells [10]. Since inflammation is correlated with cancer development [1], it was not altogether surprising to discover an important role for Th17 and other cytokines in cancer development and progression [11–14]. As discussed in greater depth below, understanding and targeting the IL-17/IL-17R axis seems now to be a key focal point in cancer immunotherapy.

4.1.2 The IL-17/IL-17R Axis

Tumor-infiltrating lymphocytes (TIL), a subset of which is Th17 cells, can be detected in the microenvironment of tumors of multiple histological types, with their numbers often correlated with disease stage [3]. Th17 cells are now well characterized in terms of their cytokine secretion profile, transcription regulation, and immune functions [4]. Their development is regulated by RORγt, STAT3, and IFN regulatory factor-4 transcription factors, along with several cytokines [15]. In mouse, these include TGF-β and IL-6/IL-21 [16] and IL-23 [17], while in humans it seems that IL-1 is a major controlling cytokine, with an important role for IL-23, IL-6, and TGF-β also now well documented [18].

Phenotypically, tumor-infiltrating Th17 cells express $CD45RA^{-}CD45RO^{+}$ $CD49^{+}$ but not Foxp3 and PD-1 (unlike Tregs [19]), and they express other surface receptors controlling their trafficking to peripheral tissues including CXCR4, CCR6, and C-type lectin CD161 [20]. Lacking CCR2, CCR5, and CCR7, Th17 cells have limited traffic to lymph nodes [21]. Within the TME are high levels of CCL20 (which Th17 also produce) and CXCL12, likely responsible for maintenance of Th17 within in the TME [22].

Th17 cells, as well as γδT cells, natural killer T (NKT) cells, neutrophils, and eosinophils [23], produce IL-17 within the TME. IL-17 family comprises six members, from IL-17A to IL-17F, with IL-17A and IL-17F closest in homology and most well characterized in terms of function, with the secreted homodimer of IL-17A being the most potent (Fig. 4.1) [24]. The IL-17R is a transmembrane protein with a large extracellular domain (293aa), a 21-aa transmembrane domain, and a large cytoplasmic tail (525 aa) [25]. Five members of the IL-17R family are described, IL-17RA-E, with IL-17RB-E each representing a subunit which associates with IL-17RA to form a functional receptor (Fig. 4.1) [26].

The IL-17RC subunit is thought to be an obligate co-receptor for IL-17RA to mediate IL-17A, IL-17F, and IL-17A/F signaling through the phosphorylation of mitogen-activated protein kinases (MAPK) and nuclear factor-κB (NF-κB) protein Act1, via TNF receptor-associated factor-6 (TRAF6) [27, 28]. Following Act1 and TRAF6 activation, Iκβ kinase (IKK)phosphorylates p105, releasing tumor progression locus 2 (TPL2), which in turn phosphorylates MEK1, leading to activation of ERK1 and ERK2, transcription factor phosphorylation, and modulation of gene expression [29] (see [8]).

4.1.3 IL-17/IL-17R Interactions in Malignancy

4.1.3.1 Effects of Th17 vs IL-17

While Th17 cells are known to play important roles in inflammation and autoimmune diseases, their roles in tumor immunity remain more controversial [30, 31]. However, Th17 cells have been reported to be implicated in disease outcome in multiple tumor types, including ovarian cancer, [32], non-Hodgkin's lymphoma [33], and HER2-positive breast cancer [34]. It has been

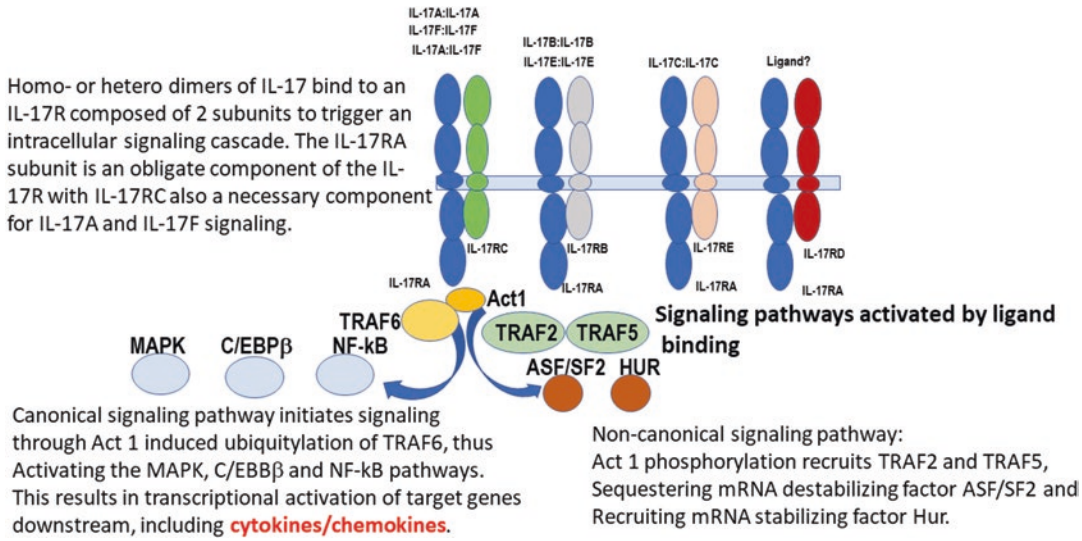

Fig. 4.1 IL-17 and IL-17R interactions leading to intracellular signaling

suggested that Th17 cells may contribute to protective immunity through recruitment of other cells to the tumor microenvironment [35] or may even themselves evolve into such effector, inflammation-inducing cells (IFNγ-producing cells), which may be beneficial in tumor resistance [36, 37]. As but one example, in a neuroblastoma mouse model, investigating the delivery of IFNγ as an antitumor therapeutic agent using mesenchymal stromal cell (MSC) therapy, it was observed that the MSCs polarized murine macrophages to an M1 phenotype. In vivo delivery of the MSCs in nude mice did indeed lead to a reduced tumor growth rate and increased survival [38]. It has been reported that other tumor-associated inflammatory cytokines including IL-6 and TNFα regulate levels and activity of Th17 cells in the tumor microenvironment in a murine ovarian cancer model [39].

In a mouse colon cancer model, an antitumor effect of IL-17 gene transfection was thought to be associated with a change in the distribution of different subsets of spleen lymphocytes in mice, altered lymphocyte infiltration into tumor tissues, and increased expression of IFNγ in tumor tissue, with reduction in expression of IL-10 and IL-13, all combining to produce an antitumor effect [40]. Note, however, that in a recent review of studies of serum, formalin-fixed, paraffin-embedded (FFPE) tissue, and peripheral blood samples, while high IL-17 quantities were correlated with poor prognosis, high Th17 cell frequencies were correlated with improved prognosis [41]. These authors argued that since Th17 cells are a subpopulation of IL-17$^+$ cells with a different correlation with prognosis than total IL-17, a distinction must be made between Th17 and other IL-17$^+$ cells [41]. Consistent with this notion, IL-17 levels were reported strongly associated with increased tumor growth, not protection from tumor growth, in irradiated stroma beds [42]. In addition, in a recent study in a mouse melanoma model [43] in which the authors investigated the therapeutic potential of blocking the IL-17/IL-17RA pathway on melanoma tumor growth, while recombinant IL-17 was seen to increase proliferation of mouse B16F10 cells and human A375 and A2058 cells, silencing IL-17RA by small hairpin RNA (shRNA) in B16F10 cells reduced proliferation, migration, and invasion, along with reducing vascular endothelial growth factor and matrix metalloproteinase production. A role for IL-17 in attenuating the effect of inhibition of VEGF on tumor growth and metastasis was reported by others to result from IL-17 modulation of stromal cell activities including endothelial cells, tumor-associated macrophages, and cancer-associated fibroblasts [44]. The

knockdown of IL-17RA led to a decreased capability of B16F10 cells to form tumors in vivo analogous to that seen in IL-17-deficient mice and simultaneously increased antitumor immunity through augmentation of IFNγ-Th cells, not through Treg [43].

4.1.3.2 Regulatory T Cells and Th17

$CD4^+CD25^+$regulatory T cells (Treg) are another important tumor-infiltrating population controlling growth and metastasis in vivo, in part at least through downregulation of the (protective) functions of $CD4^+$, $CD8^+$, NK, and NKT cells [44–46]. Importantly, despite the disparate functions of Treg and Th17 subsets, the two subsets show strong interrelation [47]. Both are dependent on TGFβ for their differentiation which is regulated further by IL-6, addition of which to TGFβ enhances differentiation of Treg cells into Th17 cells [48]. TGFβ in the absence of inflammatory cytokines induces $Foxp3^+$ Treg cell differentiation, while inflammatory cytokines which promote Th17 responses counteract activation and function of Tregs [48–50]. More recently, it was reported that Th17 cells themselves can be a source of tumor-induced $Foxp3^+$ cells [51] (Fig. 4.2).

IL-17 may also impact on other regulatory cells within the tumor microenvironment, as suggested by a recent report indicating that suppression of IL-17A at tumor sites eliminated myeloid-derived suppressor cells and regulatory T cells at tumor sites and indirectly augmented cytotoxic activity at tumor sites [52]. Other cells in the tumor environment are also crucial to T cell development. Recently, in a pancreatic tumor model, a unique subset of tumor-infiltrating dendritic cells ($CD11b^+CD103^-$ DCs), expressing high IL-23 and TGF-β, was shown to promote tumor growth by inducing $FoxP3^{neg}$ tumor-promoting $IL\text{-}10^+IL\text{-}17^+IFN\gamma^+$ regulatory $CD4^+$ T cells. This differentiation was further modulated by DC expression of retinoic acid, and the Th signature seen mimicked closely that seen in human pancreatic carcinomas again associated with immune depression and poor survival [53].

4.1.3.3 Documented Roles for IL-17 in Different Malignancies

(i) Cervical Cancer

Cervical cancer cells cultured with IL-17 showed increased production of both IL-6 and IL-8 at both mRNA and protein levels, and tumors transfected with IL-17 in nude mice grew to a larger size compared with non-transfected tumors [54]. In patient samples, high numbers of

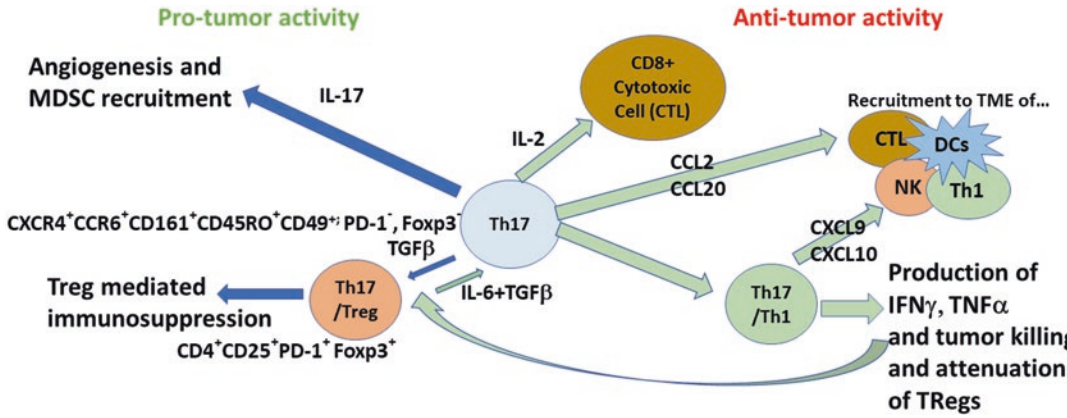

Fig. 4.2 Thl7 cells exhibit both pro- and anti-tumoral activity. IL-17 production contributes to angiogenesis and MDSC recruitment to the TME. Moreover, TGF-β might induce immunosuppression in Thl7 cells by inducing ectonucleotidases expression. Inhibition of tumor growth can occur through recruitment/induction of immune effector cells within the tumor and by activation of tumor-specific cytotoxic $CD8^+$ T cells (CTL). There is evidence that Thl7 cells can convert toward a Treg cell phenotype in the TME in the (relative) absence of IL-6, while Thl7 cell conversion to a Th1 cell phenotype results in IFN-γ and TNF-α production, tumor killing, and attenuation of Treg function

neutrophils expressing IL-17 were correlated with a poorer survival [55].

(ii) Prostate Cancer

An IL-17R-like receptor has been described in prostate cells [56], with elevation in malignancy and prostatic hypertrophy [56], while expression of IL-17R is quite ubiquitous [57]. Most recently, murine studies have suggested that IL-17-mediated prostate cancer promotion occurs through epithelial to mesenchymal transition via MMP7 enhancement with subsequent digestion of the extracellular matrix and basement membrane and ongoing invasion [58].

(iii) Colon Cancer

Evaluation of the expression of IL-17A in the tumor microenvironment during the transformative colorectal adenoma–carcinoma sequence indicated a progressive increase of IL-17 mRNA level throughout the sequence which was correlated with dysplasia severity [59]. A similar gradation of change was noted in expression of Th17-stimulating factors including IL-1β, IL-6, IL-23, and TGF-β by real-time PCR, again thought to reflect activation of Th17 cells in the same sequence.

(iv) Skin Cancer

Studies in melanoma (B16) and bladder cancer (MB49) cell lines (B16) showed reduced growth in IL-17−/− mice and enhanced growth in IFNγ−/− mice, with corresponding changes in intra-tumor IL-17 levels [60]. In both cell lines, IL-6 production was stimulated by signal transducer and activator of transcription (STAT3), with similar effects seen in tumor-associated stromal cells including fibroblasts and endothelial cells. Blockade of IL-6 attenuated tumor growth, implying a protumor effect of IL-17 mediated by IL-6 in a STAT3-dependent pathway [61].

(v) Brain Tumors

Blood levels of IL-17 were compared in 80 brain tumor patients versus 26 healthy patients and found to be elevated only in 30% of gliomas, 4% of meningioma, and 5.5% of schwannomas, compared with control group [62]. A functional IL-17R has been detected in glioma stem cells [63]. In vivo studies confirmed the presence of Th17 cells and IL-17A mRNA in mouse and human glioma samples [64]. Interestingly, mouse recipients of glioma cells also receiving splenic Th17 from glioma donors vs naïve mice showed decreased survival which the authors speculated was associated most strongly with increased angiogenesis promoted by the Th17 cell population [65].

(vi) CLL

We have published analysis of the role of IL-17 in a mouse NOD/SCID model of human CLL growth in vivo [66]. We concluded there was an important role for the IL-17/IL-6 axis in CLL patients and in an animal model of CLL, when BM-derived mesenchymal cells (BMMSCs), a critical component of the tumor microenvironment (TME), were used to support CLL survival in vitro and/or in vivo. BMMSCs have been reported by other groups to contribute to tumor growth in both solid tumors and hematologic malignancies [67–70], and in CLL, BMMSCs are thought to provide a supportive niche for CLL cells and other cells [71, 72]. We observed that while direct cell–cell contact between CLL cells and BMMSCs was not critical to CLL survival, IL-6 production was an essential factor to enhance CLL growth, and yet human recombinant IL-6 alone showed no effect on the survival of CLL cells in vitro using working concentrations equivalent with those in BMMSC supernatant [66]. Since BMMSCs induced CLL cells to produce IL-6 which was critical to CLL survival in vitro, we explored whether other factors contributed to survival and observed a key

role for IL-17, a known inducer of IL-6 in various cells in BMMSCs and CLL production of IL-6. When CLL cells, BMMSCs, or co-cultures of the two were treated with human recombinant IL-17A, we observed higher levels of IL-6 mRNA and protein in both CLL cells and BMMSCs. Given the effect of IL-6 on CLL survival shown above, we suggested that IL-17 may be a contributing factor to CLL growth/ survival. This possibility was supported by further animal experiments which showed that the IL-6R antagonist, tocilizumab, attenuated the effects of IL-17. Interestingly, we observed elevated and linearly correlated levels of IL-6 and IL-17 in CLL patients compared to healthy controls, consistent with reports that higher levels of both IL-6 and IL-17 were associated with poorer outcome of CLL patients, although this issue remains controversial [73, 74]. A novel mAb to human IL-17 was recently reported to inhibit multiple myeloma cell growth in the presence and the absence of BM stromal cells [75], while other groups have discussed the possibility of targeting the IL-6/ IL-6R axis for cancer therapy [76, 77].

(vii) Breast Cancer

Initial reports in humans suggested that IL-17, IL-6, and GCS-F levels were elevated in breast cancer patients compared to controls [78] though in these preliminary studies no attempt was made to correlate levels with prognosis. In an in vitro study using multiple breast cancer cell lines in a Matrigel invasion assay, IL-17 and TNFα increased invasion for two cell lines, with the IL-17 effect attenuated by addition of matrix metalloproteinase (MMP) inhibitors to the cultures [79]. In contrast, other groups have argued that tumor promotion by IL-17 was dependent on the angiogenesis-promoting effects of IL-17 as assessed by microvascular density measurements [80]. An alternative hypothesis focused on the potential role of IL-17 in modulation of the TME, following reports that human breast cancer cell lines expressed IL-17RA and IL-17RC. These studies showed that stimulation with IL-17A activated the MAPK pathway following upregulation of phosphorylated ERK1/2, which in turn enhanced migration, invasion, and resistance to chemotherapy, and was abolished by anti-IL-17A [81, 82] (Fig. 4.3).

In mouse breast cancer models, my laboratory explored the growth and tumor metastasis of either a 4THM breast tumor or the slower growing, more immunogenic, EMT6 breast cancer in female BALB/c mice with tumor cells implanted in the mammary fat pad [83]. We examined in depth the contribution to tumor regulation of activation of CD200/CD200R interactions in regulation inflammation and host resistance, given previous evidence that overexpression of an immunosuppressive signal, CD200, was associated with increased tumor growth, with signaling of suppression of host immunity dependent on an intact CD200R1 in the host [83]. EMT6 tumor cells overexpressing CD200 metastasized at higher frequency, while both CD200KO and CD200R1KO mice were more resistant both to primary and metastatic growth of tumor. In contrast, suppression of inflammation (in CD200 transgenic mice) attenuated growth of 4THM tumors [84], suggesting that inflammatory responses were permissive for growth of this tumor. Further studies exploring the mechanisms(s) responsible for these effects showed that serum or serum-derived exosomes from 4THM tumor-bearing CD200R1KO mice augmented EMT6 tumor invasion in wild-type mice, an effect abolished by infusion of both anti-IL-6 and anti-IL-17 [85]. A recent report [86] investigating how exosomes from heat-stressed tumor cells containing heat shock protein hsp70 induced antitumor immune responses also concluded the effect was likely mediated through hsp70-induced IL-6 promoting IL-17 expression and causing rejection of established (prostate) tumors. The effects were in turn thought to depend on conversion of Tregs to Th17 cells with high efficiency in an IL-6 dependent process.

Subsequently, using an in vitro model system to explore other cytokine factors which might control breast cancer cell invasion, we found that serum from 4THM tumor mice showed elevated levels of TNFα, IL-6, IL-8, and IL-17 compared with similar sera from EMT6 tumor immune mice, consistent with studies implicating these cytokines in regulation of tumor invasion [87–91]. Infiltration of tumor cells into a

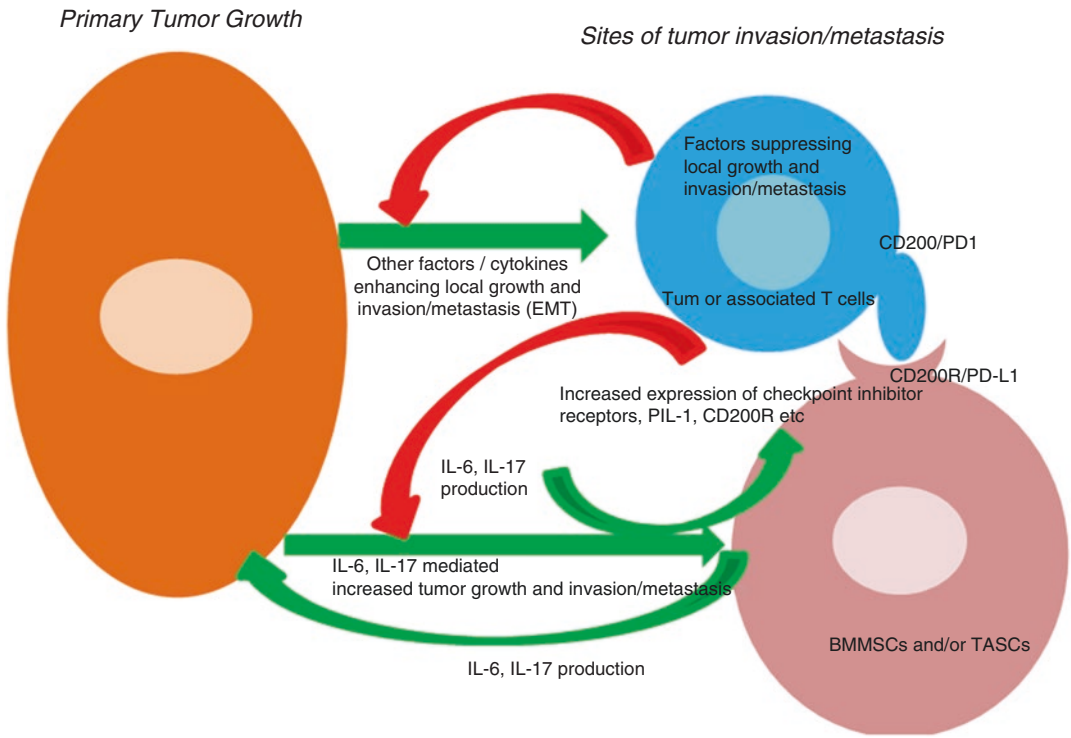

Fig. 4.3 Tumor cells (including CLL and breast cancer cells) release multiple factors influencing local growth and metastasis, e.g., IL-6, IL-17, TNFα, etc. BMMSCs and other TACs also produce IL-6 and IL-17, and influence tumor cell production. IL-17 has been reported to augment expression of checkpoint inhibitor receptors (PD-L1, CD200R) which, following interaction with the corresponding ligands, can modulate stromal cell cytokine production and/or tumor-associated T-cell-mediated tumor immunity

3D collagen matrix over 7d in culture was promoted by the presence of stromal cells in the culture matrix, but while tumor metastasis in vivo differed for 4THM and EMT6 tumors in WT, CD200KO, and CD200R1KO mice, we observed that all stromal cells were equally efficacious in promoting tumor invasion of tumor cells into the matrix. We concluded that there was a qualitative role for stromal cells in tumor invasion which was independent of the CD200/CD200R axis [92]. Further exploration of the potential mechanism(s) involved in regulation of tumor invasion suggested that augmented IL-6 and IL-17, seen in 4THM tumor sera, was associated with the increased tumor invasion both in vivo and in vitro, an effect abolished by anti-IL-6/IL-17 antibodies (which also decreased metastasis of EMT6 in vivo [85]). Our comparison of in vitro/in vivo model studies suggested that IL-6/IL-17 were primary mediators of tumor invasion for breast tumor cells and that an independent lymphocyte-dependent function (acting through CD200/CD200R checkpoint blockade [93]) could cause attenuation of tumor invasion. Other studies have also suggested a role for IL-17 itself in regulation of breast cancer growth through targeting of other checkpoint inhibitors, PD-1/PD-L1 [94]. This study showed that IL-17A promoted PDL1 expression in both human and mouse cells and that in mice targeting of IL-17A led to decreased PDL1 expression in the tumor microenvironment, Treg tumor-infiltrating cells, and augmented secretion of IFNγ from both CD4+ and CD8+ T cells. Combined anti-IL-17A and anti-PDL1 antibodies enhanced tumor clearance. Note that all such models must also consider the relevance of EMT transition in draining lymph nodes (and sera) in individuals with increased metastasis and evidence that IL-17 may act independently or in concert with other factors to augment EMT [95, 96].

4.2 Conclusions

It is becoming increasingly apparent that IL-17 and Th17 cells are present in the tumor bed of most solid tumors and indeed in hematopoietic malignancies. Multiple different studies have shown, both in experimental animals and even in humans, that their presence is consistent with a role in the observed changes in cell proliferation, tumor growth and progression, and treatment resistance seen. These effects are likely occurring through cell IL-17R signaling activation and further downstream activation of MAPK pathways, though it is clear that they do not occur in isolation but in association with signaling through other pathways mediated by other inflammatory and anti-inflammatory cytokines, as well as other receptors including EGFR or IGFR, all of which can contribute to modulation of the TME. While there are reports of antitumor activity mediated by IL-17/IL-17R interactions, in the main analysis of cancer patient, blood or tissue samples mostly suggest that high values of IL-17 and IL-17-secreting cells favor tumor growth and metastasis. It is also now evident that there is a close relationship between regulatory T lymphocytes and Th17 cells and that IL-17 (secreted by Th17 and other cells in the TME), along with other cytokines present in the microenvironment, can modulate the balance between Treg and Th17 and thus tumor host resistance. These studies highlight the IL-17/L-17R axis as a potential novel immunotherapeutic target. Further studies may lead to the development of new strategies with clinical utility.

References

1. Joyce JA, Fearon DT (2015) T cell exclusion, immune privilege, and the tumor microenvironment. Science 348:74–80
2. He D, Li H, Yusuf N, Elmets CA, Li J, Mountz JD, Xu H (2010) IL-17 promotes tumor development through the induction of tumor promoting microenvironments at tumor sites and myeloid-derived suppressor cells. J Immunol 184:2281–2288
3. Quail DF, Joyce JA (2013) Microenvironmental regulation of tumor progression and metastasis. Nat Med 19:1423–1437
4. Bowman RL, Joyce JA (2014) Therapeutic targeting of tumor-associated macrophages and microglia in glioblastoma. Immunotherapy 6:663–666
5. Beyer M, Schultze JL (2006) Regulatory T cells in cancer. Blood 108:804–811
6. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD (2002) Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol 3:991–998
7. Mantovani A, Romero P, Palucka AK, Marincola FM (2008) Tumor immunity: effector response to tumor and role of the microenvironment. Lancet 371:771–783
8. Fabre J, Giustiniani J, Garbar C, Antonicelli F, Merrouche Y, Bensussan A, Bagot M, Al DR (2016) Targeting the tumor microenvironment: the protumor effects of IL-17 related to cancer type. Int J Mol Sci 17:1433–1440
9. Yao Z, Painter SL, Fanslow WC, Ulrich D, Macduff BM, Spriggs MK, Armitage RJ (1995) Human IL-17: a novel cytokine derived from T cells. J Immunol 155:5483–5486
10. Majumder S, Amatya N, Revu S, Jawale CV, Wu D, Rittenhouse N, Menk A, Kupul S, Du F, Raphael I, Bhattacharjee A, Siebenlist U, Hand TW, Delgoffe GM, Poholek AC, Gaffen SL, Biswas PS, McGeachy MJ (2019) IL-17 metabolically reprograms activated fibroblastic reticular cells for proliferation and survival. Nat Immunol. https://doi.org/10.1038/s41590-019-0367-4. [Epub ahead of print]
11. Maniati E, Soper R, Hagemann T (2010) Up for mischief? IL-17/Th17 in the tumor microenvironment. Oncogene 29:5653–5662
12. Su X, Ye J, Hsueh EC, Zhang Y, Hoft DF, Peng G (2010) Tumor microenvironments direct the recruitment and expansion of human Th17 cells. J Immunol 184:1630–1641
13. Miyashita M, Sasano H, Tamaki K, Hirakawa H, Takahashi Y, Nakagawa S, Watanabe G, Tada H, Suzuki A, Ohuchi N et al (2015) Prognostic significance of tumor-infiltrating CD8+ and FOXP3+ lymphocytes in residual tumors and alterations in these parameters after neoadjuvant chemotherapy in triple-negative breast cancer: a retrospective multicenter study. Breast Cancer Res 17:124–137
14. Gu C, Wu L, Li X (2013) IL-17 family: cytokines, receptors and signaling. Cytokine 64:477–485
15. Yang XO, Pappu BP, Nurieva R, Akimzhanov A, Kang HS, Chung Y, Ma L, Shah B, Panopoulos AD, Schluns KS et al (2008) T helper 17 lineage differentiation is programmed by orphan nuclear receptors RORα and RORγ. Immunity 28:29–39
16. Zhou L, Ivanov II, Spolski R, Min R, Shenderov K, Egawa T, Levy DE, Leonard WJ, Littman DR (2007) IL-6 programs TH-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. Nat Immunol 8:967–974
17. Cua DJ, Sherlock J, Chen Y, Murphy CA, Joyce B, Seymour B, Lucian L, To W, Kwan S, Churakova T et al (2003) Interleukin-23 rather than interleukin-12

is the critical cytokine for autoimmune inflammation of the brain. Nature 421:744–748
18. Wilson NJ, Boniface K, Chan JR, McKenzie BS, Blumenschein WM, Mattson JD, Basham B, Smith K, Chen T, Morel F et al (2007) Development, cytokine profile and function of human interleukin 17-producing helper T cells. Nat Immunol 8:950–957
19. Kryczek I, Liu R, Wang G, Wu K, Shu X, Szeliga W, Vatan L, Finlayson E, Huang E, Simeone D et al (2009) FOXP3 defines regulatory T cells in human tumor and autoimmune disease. Cancer Res 69:3995–4000
20. Kryczek I, Wei S, Gong W, Shu X, Szeliga W, Vatan L, Chen L, Wang G, Zou W (2008) Cutting edge: IFNγ enables APC to promote memory Th17 and abate Th1 cell development. J Immunol 181:5842–5846
21. Martin-Orozco N, Muranski P, Chung Y, Yang XO, Yamazaki T, Lu S, Hwu P, Restifo NP, Overwijk WW, Dong C (2009) T helper 17 cells promote cytotoxic T cell activation in tumor immunity. Immunity 31:787–798
22. Kryczek I, Banerjee M, Cheng P, Vatan L, Szeliga W, Wei S, Huang E, Finlayson E, Simeone D, Welling TH et al (2009) Phenotype, distribution, generation, and functional and clinical relevance of Th17 cells in the human tumor environments. Blood 114:1141–1149
23. Song X, Qian Y (2013) The activation and regulation of IL-17 receptor mediated signaling. Cytokine 62:175–182. https://doi.org/10.1016/j.cyto.2013.03.014. [PubMed] [CrossRef] [Google Scholar]
24. Xu S, Cao X (2010) Interleukin-17 and its expanding biological functions. Cell Mol Immunol 7:164–174
25. Chen Z, O'Shea JJ (2008) Regulation of IL-17 production in human lymphocytes. Cytokine 41:71–78
26. Kirkham BW, Kavanaugh A, Reich K (2014) Interleukin-17A: a unique pathway in immune-mediated diseases: psoriasis, psoriatic arthritis and rheumatoid arthritis. Immunology 141:133–142
27. Toy D, Kugler D, Wolfson M, Vanden BT, Gurgel J, Derry J, Tocker J, Peschon J (2006) Cutting edge: interleukin 17 signals through a heteromeric receptor complex. J Immunol 177:36–39
28. Shalom-Barak T, Quach J, Lotz M (1998) Interleukin-17-induced gene expression in articular chondrocytes is associated with activation of mitogen-activated protein kinases and NF-κB. J Biol Chem 273:27467–27473
29. Sun F, Qu Z, Xiao Y, Zhou J, Burns TF, Stabile LP, Siegfried JM, Xiao G (2016) NF-κB1 p105 suppresses lung tumorigenesis through the Tpl2 kinase but independently of its NF-κB function. Oncogene 35:2299–2310. https://doi.org/10.1038/onc.2015.299. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
30. Ji Y, Zhang W (2010) Th17 cells: positive or negative role in tumor? Cancer Immunol Immunother 59:979–987
31. Bronte V (2008) Th17 and cancer: friends or foes? Blood 112:214
32. Kryczek I, Banerjee M, Cheng P, Vatan L, Szeliga W, Wei S, Huang E, Finlayson E, Simeone D, Welling TH, Chang A, Coukos G, Liu R, Zou W (2009) Phenotype, distribution, generation, and functional and clinical relevance of Th17 cells in the human tumor environments. Blood 114:1141–1149
33. Yang ZZ, Novak AJ, Ziesmer SC, Witzig TE, Ansell SM (2009) Malignant B cells skew the balance of regulatory T cells and TH17 cells in B-cell non-Hodgkin's lymphoma. Cancer Res 69:5522–5530
34. Horlock C, Stott B, Dyson PJ, Morishita M, Coombes RC, Savage P, Stebbing J (2009) The effects of trastuzumab on the CD4+CD25+FoxP3+ and CD4+IL17A+ T-cell axis in patients with breast cancer. Br J Cancer 100:1061–1067
35. Muranski P, Boni A, Antony PA, Cassard L, Irvine KR, Kaiser A, Paulos CM, Palmer DC, Touloukian CE, Ptak K, Gattinoni L, Wrzesinski C, Hinrichs CS, Kerstann KW, Feigenbaum L, Chan CC, Restifo NP (2008) Tumor-specific Th17polarized cells eradicate large established melanoma. Blood 112:362–373
36. Dougan M, Dranoff G (2008) Inciting inflammation: the RAGE about tumor promotion. J Exp Med 205:267–270
37. Colotta F, Allavena P, Sica A, Garlanda C, Mantovani A (2009) Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. Carcinogenesis 30:1073–1081
38. Relation T, Yi T, Guess AJ, La Perle K, Otsuru S, Hasgur S, Dominici M, Breuer C, Horwitz EM (2018) Intratumoral delivery of interferonγ-secreting mesenchymal stromal cells repolarizes tumor-associated macrophages and suppresses neuroblastoma proliferation in vivo. Stem Cells 36(6):915–924. https://doi.org/10.1002/stem.2801. Epub 2018 Feb 27
39. Charles KA, Kulbe H, Soper R, Lawrence T, Schultheis A, Chakravarty P, Thompson RG, Kollias G, Smyth JF, Balkwill FR, Hagemann T (2009) The tumor promoting actions of TNF-alpha involve TNFR1 and IL-17 in ovarian cancer in mice and humans. J Clin Invest 119:3011–3023
40. Yang L, Liu H, Zhang L, Hu J, Chen H, Wang L, Yin X, Li Q, Qi Y (2016) Effect of IL-17 in the development of colon cancer in mice. Oncol Lett 12(6):4929–4936. https://doi.org/10.3892/ol.2016.5329. Epub 2016 Oct 31
41. Punt S, Langenhoff JM, Putter H, Fleuren GJ, Gorter A, Jordanova ES (2015) The correlations between IL-17 vs. Th17 cells and cancer patient survival: a systematic review. Oncoimmunology 4(2):e984547. eCollection 2015 Feb
42. Lee EJ, Park HJ, Lee IJ, Kim WW, Ha SJ, Suh YG, Seong J (2014) Inhibition of IL-17A suppresses enhanced-tumor growth in low dose pre-irradiated tumor beds. PLoS One 9(9):e106423. https://doi.org/10.1371/journal.pone.0106423. eCollection 2014
43. Chen YS, Huang TH, Liu CL, Chen HS, Lee MH, Chen HW, Shen CR (2019) Locally targeting the IL-17/IL-17RA axis reduced tumor growth in a

murine B16F10 melanoma model. Hum Gene Ther 30(3):273–285. https://doi.org/10.1089/hum.2018.104. Epub 2018 Oct 3
44. Darvishi B, Majidzadeh-A K, Ghadirian R, Mosayebzadeh M, Farahmand L (2019) Recruited bone marrow derived cells, local stromal cells and IL-17 at the front line of resistance development to anti-VEGF targeted therapies. Life Sci 217:34–40. https://doi.org/10.1016/j.lfs.2018.11.033. Epub 2018 Nov 22
45. Darrasse-Jeze G, Bergot A, Cordier C, Ngo-Abdalla S, Klatzmann D, Azogui O (2007) Regulatory T cells prevent CD8 T cell maturation by inhibiting CD4 Th cells at tumor sites. J Immunol 179:4969–4978
46. Nishikawa H, Kato T, Tawara I, Takemitsu T, Saito K, Wang L, Ikarashi Y, Wakasugi H, Nakayama T, Taniguchi M, Kuribayashi K, Old LJ, Shiku H (2005) Accelerated chemically induced tumor development mediated by CD4 +CD25+ regulatory T cells in wild-type hosts. Proc Natl Acad Sci U S A 102:9253–9257
47. Kryczek I, Wei S, Zou L, Zhu G, Mottram P, Xu H, Chen L, Zou W (2006) Cutting edge: induction of B7-H4 on APCs through IL-10: novel suppressive mode for regulatory T cells. J Immunol 177:40–44
48. Najafi S, Mirshafiey A (2019) The role of T helper 17 and regulatory T cells in tumor microenvironment. Immunopharmacol Immunotoxicol:1–9. https://doi.org/10.1080/08923973.2019.1566925. [Epub ahead of print]
49. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, Weiner HL, Kuchroo VK (2006) Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature 441:235–238
50. Korn T, Bettelli E, Gao W, Awasthi A, Jäger A, Strom TB, Oukka M, Kuchroo VK (2007) IL-21 initiates an alternative pathway to induce proinflammatory TH17 cells. Nature 448:484–487
51. Zhou L, Lopes JE, Chong MM, Ivanov II, Min R, Victora GD, Shen Y, Du J, Rubtsov YP, Rudensky AY, Ziegler SF, Littman DR (2008) TGF-β-induced Foxp3 inhibits TH17 cell differentiation by antagonizing RORγt function. Nature 453:236–240
52. Downs-Canner S, Berkey S, Delgoffe GM, Edwards RP, Curiel T, Odunsi K, Bartlett DL, Obermajer N (2017) Suppressive IL-17A^{+}Foxp3^{+} and ex-Th17 IL-17A^{neg}Foxp3^{+} T_{reg} cells are a source of tumour-associated T_{reg} cells. Nat Commun 8:14649. https://doi.org/10.1038/ncomms14649
53. Barilla RM, Diskin B, Caso RC, Lee KB, Mohan N, Buttar C, Adam S, Sekendiz Z, Wang J, Salas RD, Cassini MF, Karlen J, Sundberg B, Akbar H, Levchenko D, Gakhal I, Gutierrez J, Wang W, Hundeyin M, Torres-Hernandez A, Leinwand J, Kurz E, Rossi JAK, Mishra A, Liria M, Sanchez G, Panta J, Loke P, Aykut B, Miller G (2019) Specialized dendritic cells induce tumor-promoting IL-10^{+}IL-17^{+} FoxP3^{neg} regulatory CD4^{+} T cells in pancreatic carcinoma. Nat Commun 10(1):1424. https://doi.org/10.1038/s41467-019-09416-2
54. Tartour E, Fossiez F, Joyeux I, Galinha A, Gey A, Claret E, Sastre-Garau X, Couturier J, Mosseri V, Vives V et al (1999) Interleukin 17, a T-cell-derived cytokine, promotes tumorigenicity of human cervical tumors in nude mice. Cancer Res 59:3698–3704. [PubMed] [Google Scholar]
55. Punt S, Fleuren GJ, Kritikou E, Lubberts E, Trimbos JB, Jordanova ES, Gorter A (2015) Angels and demons: Th17 cells represent a beneficial response, while neutrophil IL-17 is associated with poor prognosis in squamous cervical cancer. Oncoimmunology 4:e984539. https://doi.org/10.4161/2162402X.2014.984539. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
56. Haudenschild D, Moseley T, Rose L, Reddi AH (2002) Soluble and transmembrane isoforms of novel interleukin-17 receptor-like protein by RNA splicing and expression in prostate cancer. J Biol Chem 277:4309–4316. https://doi.org/10.1074/jbc.M109372200. [PubMed] [CrossRef] [Google Scholar]
57. Steiner GE, Newman ME, Paikl D, Stix U, Memaran-Dagda N, Lee C, Marberger MJ (2003) Expression and function of pro-inflammatory interleukin IL-17 and IL-17 receptor in normal, benign hyperplastic, and malignant prostate. Prostate 56:171–182. https://doi.org/10.1002/pros.10238. [PubMed] [CrossRef] [Google Scholar]
58. Zhang Q, Liu S, Parajuli KR, Zhang W, Zhang K, Mo Z, Liu J, Chen Z, Yang S, Wang AR et al (2016) Interleukin-17 promotes prostate cancer via MMP7-induced epithelial-to-mesenchymal transition. Oncogene. https://doi.org/10.1038/onc.2016.240. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
59. Li B, Shi XY, Liao DX, Cao BR, Luo CH, Cheng SJ (2015) Advanced colorectal adenoma related gene expression signature may predict prognostic for colorectal cancer patients with adenoma-carcinoma sequence. Int J Clin Exp Med 8:4883–4898. [PMC free article] [PubMed] [Google Scholar]
60. He D, Li H, Yusuf N, Elmets CA, Athar M, Katiyar SK, Xu H (2012) IL-17 mediated inflammation promotes tumor growth and progression in the skin. PLoS One 7:1433. https://doi.org/10.1371/journal.pone.0032126. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
61. Wang L, Yi T, Kortylewski M, Pardoll DM, Zeng D, Yu H (2009) IL-17 can promote tumor growth through an IL-6-STAT3 signaling pathway. J Exp Med 206:1457–1464. https://doi.org/10.1084/jem.20090207. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
62. Doroudchi M, Pishe ZG, Malekzadeh M, Golmoghaddam H, Taghipour M, Ghaderi A (2013) Elevated serum IL-17A but not IL-6 in glioma versus meningioma and schwannoma. Asian Pac J Cancer Prev 14:5225–5230. https://doi.org/10.7314/APJCP.2013.14.9.5225. [PubMed] [CrossRef] [Google Scholar]
63. Parajuli P, Anand R, Mandalaparty C, Suryadevara R, Sriranga PU, Michelhaugh SK, Cazacu S, Finniss

S, Thakur A, Lum LG et al (2016) Preferential expression of functional IL-17R in glioma stem cells: potential role in self-renewal. Oncotarget 7:6121–6135. [PMC free article] [PubMed] [Google Scholar]
64. Wainwright DA, Sengupta S, Han Y, Ulasov IV, Lesniak MS (2010) The presence of IL-17A and T helper 17 cells in experimental mouse brain tumors and human glioma. PLoS One 5:1433. https://doi.org/10.1371/journal.pone.0015390. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
65. Hu J, Ye H, Zhang D, Liu W, Li M, Mao Y, Lu Y (2013) U87MG glioma cells overexpressing IL-17 accelerate early-stage growth in vivo and cause a higher level of CD31 mRNA expression in tumor tissues. Oncol Lett 6:993–999. [PMC free article] [PubMed] [Google Scholar]
66. Zhu F, McCaw L, Spaner DE, Gorczynski RM (2018) Targeting the IL-17/IL-6 axis can alter growth of Chronic Lymphocytic Leukemia in vivo/in vitro. Leuk Res 66:28–38. https://doi.org/10.1016/j.leukres.2018.01.006. Epub 2018 Jan 17
67. Ono M, Kosaka N, Tominaga N, Yoshioka Y, Takeshita F et al (2014) Exosomes from bone marrow mesenchymal stem cells contain a microRNA that promotes dormancy in metastatic breast cancer cells. Sci Signal 7:ra63
68. Karnoub AE, Dash AB, Vo AP, Sullivan A, Brooks MW et al (2007) Mesenchymal stem cells within tumor stroma promote breast cancer metastasis. Nature 449:557–563
69. Suzuki K, Sun R, Origuchi M, Kanehira M, Takahata T et al (2011) Mesenchymal stromal cells promote tumor growth through the enhancement of neovascularization. Mol Med 17:579–587
70. Shinagawa K, Kitadai Y, Tanaka M, Sumida T, Kodama M et al (2010) Mesenchymal stem cells enhance growth and metastasis of colon cancer. Int J Cancer 127:2323–2333
71. Burgess M, Cheung C, Chambers L, Ravindranath K, Minhas G et al (2012) CCL2 and CXCL2 enhance survival of primary chronic lymphocytic leukemia cells in vitro. Leuk Lymphoma 53:1988–1998
72. Wilson A, Trumpp A (2006) Bone-marrow hematopoietic-stem-cell niches. Nat Rev Immunol 6:93–106
73. Lai R, O'Brien S, Maushouri T, Rogers A, Kantarjian H et al (2002) Prognostic value of plasma interleukin-6 levels in patients with chronic lymphocytic leukemia. Cancer 95:1071–1075
74. Tang D, Niu Q, Jiang N, Li J, Zheng Q et al (2014) Increased frequencies of Th17 in the peripheral blood of patients with chronic lymphocytic leukemia: a one year follow up. Pak J Med Sci 30:1128–1133
75. Prabhala RH, Fulciniti M, Pelluru D, Rashid N, Nigroiu A et al (2015) Targeting IL17A in multiple myeloma: a potential novel therapeutic approach in myeloma. Leukemia 30:379–389
76. Hunter CA, Jones SA (2015) IL-6 as a keystone cytokine in health and disease. Nat Immunol 16:448–457
77. Heo T-H, Wahler J, Suh N (2016) Potential therapeutic implications of IL-6/IL-6R/ gp130-targeting agents in breast cancer. Oncotarget 7:15460–15473
78. Lyon DE, McCain NL, Walter J, Schubert C (2008) Cytokine comparisons between women with breast cancer and women with a negative breast biopsy. Nurs Res 57:51–58. https://doi.org/10.1097/01.NNR.0000280655.58266.6c. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
79. Zhu X, Mulcahy LA, Mohammed RA, Lee AH, Franks HA, Kilpatrick L, Yilmazer A, Paish EC, Ellis IO, Patel PM et al (2008) IL-17 expression by breast-cancer-associated macrophages: IL-17 promotes invasiveness of breast cancer cell lines. Breast Cancer Res 10:95–106. https://doi.org/10.1186/bcr2195. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
80. Du JW, Xu KY, Fang LY, Qi XL (2012) Interleukin-17, produced by lymphocytes, promotes tumor growth and angiogenesis in a mouse model of breast cancer. Mol Med Rep 6:1099–1102. https://doi.org/10.3892/mmr.2012.1036. [PubMed] [CrossRef] [Google Scholar]
81. Kim G, Khanal P, Lim SC, Yun HJ, Ahn SG, Ki SH, Choi HS (2013) Interleukin-17 induces AP-1 activity and cellular transformation via upregulation of tumor progression locus 2 activity. Carcinogenesis 34:341–350. https://doi.org/10.1093/carcin/bgs342. [PubMed] [CrossRef] [Google Scholar]
82. Mombelli S, Cochaud S, Merrouche Y, Garbar C, Antonicelli F, Laprevotte E, Alberici G, Bonnefoy N, Eliaou JF, Bastid J et al (2015) IL-17A and its homologs IL-25/IL-17E recruit the c-RAF/S6 kinase pathway and the generation of pro-oncogenic LMW-E in breast cancer cells. Sci Rep 5:11874–11884. https://doi.org/10.1038/srep11874. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
83. Gorczynski R, Chen Z, Erin N, Khatri I, Podnos A (2014) Comparison of immunity in mice cured of primary/metastatic growth of EMT6 or 4THM breast cancer by chemotherapy or immunotherapy. PLoS One 9(11):e113597. https://doi.org/10.1371/journal.pone.0113597
84. Erin N, Podnos A, Tanriover G, Duymus O, Cote E, Khatri I, Gorczynski RM (2015) Bidirectional effect of CD200 on breast cancer development and tumor invasion with ultimate outcome determined by tumor aggressiveness and a cancer-induced inflammatory response. Oncogene 34(29):3860–3870
85. Gorczynski RM, Erin N, Zhu F (2016) Serum-derived exosomes from mice with highly metastatic breast cancer transfer increased metastatic capacity to a poorly metastatic tumor. Cancer Med 5(2):325–336. https://doi.org/10.1002/cam4.575. Epub 2016 Jan 4
86. Guo D, Chen Y, Wang S, Yu L, Shen Y, Zhong H, Yang Y (2018) Exosomes from heat-stressed tumor cells inhibit tumor growth by converting regulatory T cells to Th17 cells via IL-6. Immunology 154(1):132–143. https://doi.org/10.1111/imm.12874. Epub 2018 Jan 2
87. Knupfer H, Preiß R (2007) Significance of interleukin-6 (IL-6) in breast cancer. Breast Cancer

Res Treat 102:129–135. https://doi.org/10.1007/s10549-006-9328-3
88. Benoy IH, Salgado R, Van Dam P, Geboers K, Van Marck E, Scharpé S, Vermeulen PB, Dirix LY (2004) Increased serum interleukin-8 in patients with early and metastatic breast cancer correlates with early dissemination and survival. Clin Cancer Res 10(21):7157–7162
89. Bar-Eli M (1999) Role of interleukin-8 in tumor growth and tumor invasion of human melanoma. Pathobiology 67:12–18. https://doi.org/10.1159/000028045
90. XingWu Zhu X-W, Mulcahy LA, Mohammed RAA, Lee AHS, Franks HA, Kilpatrick L, Yilmazer A, Paish EC, Ellis IO, Patel PM, Jackson AM (2008) IL-17 expression by breast-cancer-associated macrophages: IL-17 promotes invasiveness of breast cancer cell lines. Breast Cancer Res 10:R95. https://doi.org/10.1186/bcr2195
91. Coffelt SB, Kersten K, Doornebal CW, Weiden J, Vrijland K, Hau C-S, Verstegen NJM, Ciampricotti M, Hawinkels LJAC, Jonkers J, de Visser KE (2015) IL-17-producing γδ T cells and neutrophils conspire to promote breast cancer tumor invasion. Nature 522:345–348. https://doi.org/10.1038/nature14282
92. Gorczynski RM, Erin N, Maqbool T, Gorczynski CP, Gorczynski LY (2018) Characterization of an in vitro model system to explore control of tumor invasion of EMT6 and 4THM breast tumors by CD200:CD200R interactions. Breast Cancer 25(5):547–559. https://doi.org/10.1007/s12282-018-0851-y. Epub 2018 Mar 12
93. Gorczynski RM, Zhu F (2017) Checkpoint blockade in solid tumors and B cell malignancies with special consideration of the role of CD200. Cancer Manag Res 9:601–609
94. Ma YF, Chen C, Li D, Liu M, Lv ZW, Ji Y (2017) Targeting of interleukin (IL)-17A inhibits PDL1 expression in tumor cells and induces anticancer immunity in an estrogen receptor-negative murine model of breast cancer. Oncotarget 8(5):7614–7624. https://doi.org/10.18632/oncotarget.13819
95. Gorczynski RM, Zhu F, Chen Z, Kos O, Khatri I (2017) A comparison of serum miRNAs influencing metastatic growth of EMT6 vs 4THM tumor cells in wild-type and CD200R1KO mice. Breast Cancer Res Treat 162:255–266
96. Gu K, Li MM, Shen J, Liu F, Cao JY, Jin S, Yu Y (2015) Interleukin-17-induced EMT promotes lung cancer cell migration and invasion via NF-κB/ZEB1 signal pathway. Am J Cancer Res 5(3):1169–1179. eCollection 2015

5 Emerging Roles for Interleukin-18 in the Gastrointestinal Tumor Microenvironment

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Abstract

Interleukin (IL)-18, a member of the IL-1 family of cytokines, has emerged as a key regulator of mucosal homeostasis within the gastrointestinal tract. Like other members of this family, IL-18 is secreted as an inactive protein and is processed into its active form by caspase-1, although other contributors to precursor processing are emerging.

Numerous studies have evaluated the role of IL-18 within the gastrointestinal tract using genetic or complementary pharmacological tools and have revealed multiple roles in tumorigenesis. Most striking among these are the divergent roles for IL-18 in colon and gastric cancers. Here, we review our current understanding of IL-18 biology and how this applies to colorectal and gastric cancers.

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Keywords

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5.1 Introduction

The development of cancer is a multistep process whereby normal cells acquire characteristics that promote malignant transformation. The "hallmarks of cancer" describe a series of processes that enable a cancer cell to resist death, evade growth suppressors, induce angiogenesis, continually proliferate, avoid immune destruction, deregulate cell metabolism, and invade and metastasize to other organs [1]. Tumor-promoting inflammation is also an important cancer hallmark, with innate immune cells including macrophages, dendritic cells (DC), and natural killer (NK) cells, as well as adaptive immune cells including T and B cells present in the tumor microenvironment. These tumor-infiltrating immune cells are the primary source of cytokines that can further perpetuate tumor development [2]. In this review, we discuss the biology of IL-18, its receptor, and our current appreciation of its immunoregulatory functions in cancer. We focus on the expression and role of IL-18 in two

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gastrointestinal (GI) cancers, colorectal cancer (CRC) and gastric cancer (GC).

5.2 The Biology of IL-18

IL-18 is a pro-inflammatory and immunoregulatory cytokine that belongs to the IL-1 superfamily, which also includes IL-1α and β; IL-33; IL-36α, β, and γ; IL-1Rα; IL-36Rα; and IL-38 [3]. IL-18 was discovered in 1989 as "IFN-γ-inducing factor," following the observation that it induced production of IFN-γ in mouse spleen cells following injection with bacterial lipopolysaccharide (LPS) [4]. Subsequently, murine IL-18 was purified and cloned from liver cells of mice treated with *Propionibacterium acnes (P. acnes)* in 1995 [5].

5.2.1 IL-18 Is a Beta-Trefoil Structured Protein

Cloned murine and human IL-18 cDNA encodes proteins consisting of 192 and 193 amino acids, respectively, and shares approximately 65% sequence homology with each other [5–7]. Similar to IL-1β, IL-18 lacks the usual leader sequence necessary for secretion from the cell membrane at the N-terminus, so it is synthesized as a 24-kDa inactive precursor protein (pro-IL-18), which becomes active following cleavage by the IL-1β-converting enzyme, also known as caspase-1 [8]. The crystal structure of IL-18 highlights that it folds into a β-trefoil sheet, similar to IL-1β, although the surface residues are dissimilar [6].

5.2.2 Multiple Mechanisms of IL-18 Secretion

Using in vivo murine models, it was shown that the intracellular cysteine protease caspase-1 cleaves pro-IL-18 at an Asp35-Asn36 cleavage site, creating a bioactive protein that is secreted from the cell [8]. Subsequent experiments demonstrated that caspase-1 was essential for secretion of IL-18, as serum IL-18 was not increased in *P. acnes*-primed caspase-1-deficient mice (*caspase-1*$^{-/-}$) following LPS challenge when compared to wild-type control mice (*caspase-1*$^{+/+}$) [8]. In addition, transgenic mice overexpressing human caspase-1 showed elevated serum levels of mature IL-18, further suggesting an important role for caspase-1 in mature IL-18 production [9].

More recently, caspase-1-independent IL-18 production has been documented [10]. This is facilitated by proteinase-3 (PR3), a 29-kDa serine proteinase, that is able to induce the production of mature IL-18 after IFN-γ priming in epithelial cells [10]. In combination with a proteinase inhibitor, IFN-γ-primed cells treated with PR3 did not produce IL-18, suggesting that the extracellular enzymatic activity of PR3 contributes to pro-IL-18 cleavage [10]. Most importantly, using a caspase-1-specific inhibitor revealed that PR3-induced mature IL-18 production was independent of caspase-1 [10].

In addition, Fas signaling which triggers apoptosis and induces the production of many pro-inflammatory cytokines has been shown to lead to an increase in IL-18 [11]. *P. acnes*-primed macrophages can secrete mature IL-18 upon stimulation with Fas ligand independent of caspase-1 [11]. It has also been shown that Fas signaling activates capase-8 in macrophages and dendritic cells and leads to the maturation of IL-18 independent of inflammasome-induced capase-1 pathway [12].

Finally, upon stimulation with vascular endothelial growth factor (VEGF)-D, IL-18 is secreted in GC cell lines and is linked to a metalloprotease 33 (ADAM33) which has a metalloprotease activity [13]. VEGF-D-induced IL-18 secretion is inhibited by knocking down ADAM33 expression, suggesting ADAM33 could be a novel regulator for mature IL-18 secretion [13].

5.2.3 IL-18-Producing Cells in the Gastrointestinal Tract

Pro-IL-18 is constitutively expressed by numerous cell types within the GI tract, including endo-

thelial cells, keratinocytes, and intestinal epithelial cells [14]. Recent studies have shown that IL-22 can induce the expression of pro-IL-18 in intestinal epithelial cells and in turn provides protection against gut bacterial infections [15]. However, both hemopoietic and epithelial cell-derived mature IL-18 have been shown to mediate intestinal inflammation in a murine DSS-induced experimental colitis model [16]. Interestingly, elevated human IL-18 mRNA transcripts were found in both epithelial cells and other mucosal cells from patients with Crohn's disease (CD) and ulcerative colitis (UC) [17]. In addition, immunohistochemical staining of IL-18 was increased mainly in epithelial cells from severely inflamed UC patients compared to that of healthy control patients [17]. On the other hand, intense staining of IL-18 was found in both the colonic epithelium and inflammatory cells, including macrophages and dendritic cells, in the lamina propria of CD patients [17]. These results suggest that IL-18 may play a different role in UC and CD.

5.3 IL-18 Signaling

The receptor for IL-18, IL-18R, was first purified and characterized from a Hodgkin's disease cell line L428 in 1997 [18]. IL-18R forms a heterodimer at the cell surface, which contains an α chain (IL-18R1) responsible for extracellular binding of IL-18 and a nonbinding signal-transducing β chain (IL-18RAP) [19, 20]. As the binding of IL-18 and IL-18R1 is a low-affinity interaction, the expression of a co-receptor, IL-18RAP, is required for functional IL-18 signaling [18]. Murine IL-18R1 is 65% homologous to human IL-18R1 in overall amino acid sequence [18]. It was previously reported that the binary complex of human IL-18/IL18-R1 is identical to that of IL-1β and its receptor; however, X-ray crystallography of the human tertiary complex IL-18/IL18-R1/IL-18RAP revealed substantial differences from IL-1β and its receptor [6, 21]. For example, the second domain (D2) of the two IL-18Rs lacks one β-strand, which is conserved among other IL-1-related receptors and was shown to contribute to the inter-receptor interaction [21]. This could explain the binding specificity of IL-18 and its receptor.

5.3.1 Expression of IL-18R in the Gastrointestinal Tract

In the GI tract, IL-18R is expressed by multiple cell types, including neutrophils, T cells, NK cells, macrophages, and endothelial cells [22]. IL-18R is also expressed by colonic epithelial cells, with genetic ablation of IL-18R protecting mice from DSS-induced experimental colitis [16]. IL-18R is also expressed on the surface of T cells isolated from both UC and CD patients [23].

5.3.2 IL-18 Activates Multiple Signaling Pathways

Upon engagement of the IL-18 signaling complex (Fig. 5.1), IL-1R-associated kinase (IRAK) is recruited via the adaptor protein, myeloid differentiation primary response 88 (MyD88). IRAK then dissociates from the receptor complex and interacts with TNF-receptor-associated factor (TRAF6), which then relays the signal via NF-κB-inducing kinase (NIK) to two I-κB kinases (IKK-1 and IKK-2) leading to NF-κB activation [22]. The requirement of each component for the signaling pathway has been demonstrated using mice deficient in each individual molecule. For example, IL-18-induced IFN-γ production was abolished in MyD88-deficient mice, while IL-18-induced NF-κB and JNK activation was blocked in MyD88-deficient Th1 cells [24]. These findings suggest that MyD88 is essential for IL-18-mediated signaling and function. Most importantly, co-immunoprecipitation revealed an interaction between MyD88 and IL-18R1 and that this interaction was required to induce NF-κB and AP-1 activation [24]. Similarly, IL-18-induced IFN-γ production was impaired in IRAK-deficient mice in response to *P. acnes* and LPS stimulation [25].

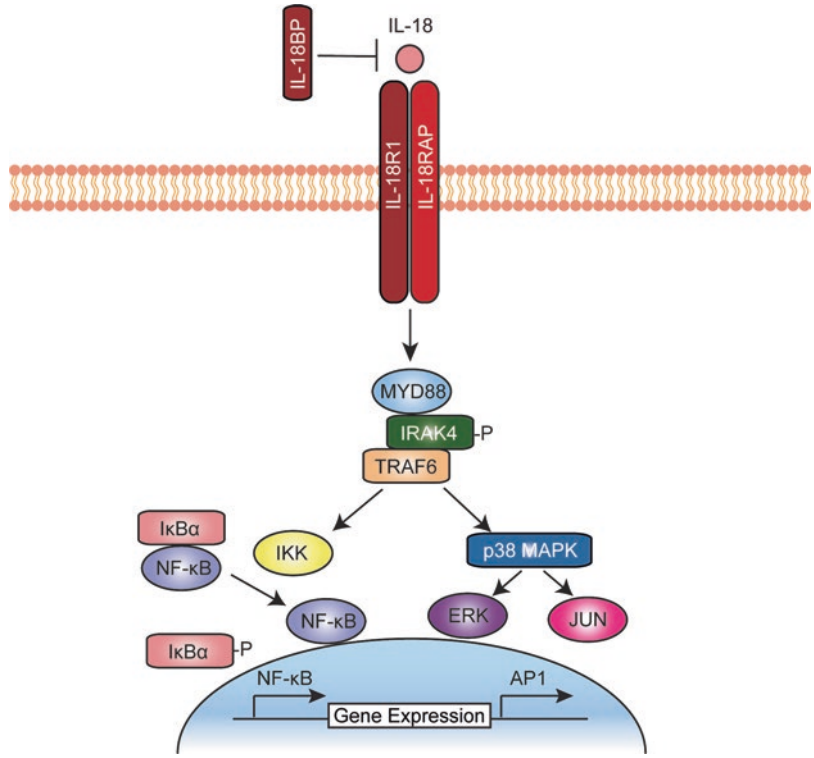

Fig. 5.1 Schematic of IL-18 signaling. IL-18 forms a signaling complex by binding to the IL-18R1 which then recruits the co-receptor, IL-18RAP, to form a high-affinity complex [14]. Upon ligand binding and the formation of the heterodimer, the Toll–IL-1-receptor (TIR) domains of the receptor trigger the binding of a signaling molecule, MyD88 [22]. The death domain of MyD88 interacts and phosphorylates IRAK4, which then associates with TRAF-6 [22]. This results in the activation of IKK and finally NFκB, which translocates to the nucleus and regulates gene expression. Alternatively, TRAF-6 can activate ERK and JUN which will translocate to the nucleus and regulate gene expression through binding to AP1. The IL-18BP is a negative regulator, present in the extracellular compartment where it binds to mature IL-18 and prevents binding to the IL-18 receptor

Moreover, IRAK-deficient Th1 and NK cells also exhibit defects in IFN-γ production, which is induced by IL-18 [25]. Additionally, IL-18-stimulated mouse lymphoma cells, EL-4, showed recruitment of IRAK to IL-18R1 and the formation of complex between IRAK and TRAF6 [26].

Increasing evidence suggests that IL-18 can activate other signaling pathways including mitogen-activated protein kinase (MAPK) p38. Stimulation of IL-18 in epithelial [27] and hippocampal cell lines [28] showed enhanced phosphorylation of MAPKp38 and STAT3. Interestingly, IL-18-induced IFN-γ production is mainly mediated through MAPK and STAT3 pathway in NK cells [29].

5.3.3 Negative Regulation: IL-18 Binding Protein

IL-18 binding protein (BP) was discovered from human urine as a soluble receptor for IL-18 [30]. It was then isolated, characterized, and cloned in 1999 [30]. IL-18BP is a constitutively expressed and secreted protein that is found at high levels in the serum of healthy humans [31]. It was found that IL-18BP binds IL-18 with an affinity significantly higher than that of IL-18R1 [30]. Most importantly, IL-18BP blocks IL-18-induced IFN-γ production in vitro and in vivo, suggesting that IL-18BP is a natural negative regulator of IL-18-mediated immune responses [30]. Indeed, stimulation of different cell lines with IFN-γ

induces the expression of IL-18BP via IFN regulatory factor 1 activation [32, 33]. These results suggest that the activity of IL-18 is modulated by a negative feedback loop which is mediated by IFN-γ-induced IL-18BP.

5.4 Immunomodulatory Role of IL-18 in the Gastrointestinal Tract

The emergence of a role for IL-18 as an immunoregulatory cytokine originated from the observation that it modulates IFN-γ production from Th1 and NK cells [34, 35]. Subsequently, other functions of IL-18 in these immune cell types, as well as other T cell subsets, have been explored.

5.4.1 Synergistic Action of IL-18 and IL-12 in IFN-γ-Producing Th1 Cells

IL-18 itself is not able to induce Th1 differentiation since naïve CD4+ T cells do not express IL-18R; however, IL-18 synergizes with IL-12 to promote IFN-γ production in murine T cells in vitro [35]. This was then confirmed in IL-18-deficient (*Il18*$^{-/-}$) mice which exhibited reduced IFN-γ production by Th1 cells [34]. Furthermore, IL-18- and IL-12-double-deficient (*Il12*$^{-/-}$;*Il18*$^{-/-}$) mice showed a severely defective Th1 response when compared to the single cytokine-deficient mice [34]. This synergistic effect is mediated by the induction of IL-18R on the surface of Th1, but not Th2, cells by IL-12 [36, 37]. Moreover, it is believed that IFN-γ production by IL-18 and IL-12 is mediated at the transcriptional level [38]. IL-12-dependent IFN-γ activation requires both AP-1 and STAT4 binding sites in the IFN-γ promoter, while IL-18 activates the IFN-γ promoter through AP-1 and NFκB binding sites [22, 38].

Similarly, human T cells also require both IL-18 and IL-12 for optimal production of IFN-γ [39]. Additionally, IL-12 can induce IL-18R expression in human CD4+ T cells and triggers dose-dependent production of IFN-γ following IL-18 stimulation [39].

5.4.2 IL-18 Upregulates NK Cell Cytotoxic Activity

IL-18 enhances NK cell cytotoxic activity in vitro, and this activity is further augmented when co-stimulated with IL-12 [39]. Although *Il18*$^{-/-}$ mice have similar numbers of NK cells compared to wild-type mice, *Il18*$^{-/-}$ mice showed reduced NK cell activity, and *Il12*$^{-/-}$;*Il18*$^{-/-}$ mice showed a marked reduction in NK cell activity [39]. This observation suggested that the cooperative action of both IL-12 and IL-18 is essential for cytolytic function of NK cells. However, unlike the IFN-γ production activity, IL-18 can facilitate NK cell activity in the absence of IL-12, demonstrated by the lack of change in NK cell activity following IL-18 stimulation in IL-12-deficient mice [40]. This can be explained by the expression of both IL-18R and IL-12R on NK cells [40]. Consistent with murine IL-18, human IL-18 can also induce the cytotoxic activity of NK cells [41]. Instead of IL-12, IL-18 synergizes with IL-2 to enhance the IFN-γ-production, cytotoxic activity, and expansion of NK cells in human blood [41]. Interestingly, IL-2 receptor was not expressed on NK cells, but its expression was substantially upregulated when isolated human NK cells were cultured with both IL-18 and IL-2 [42].

5.4.3 Effect of IL-18 on Other Immune Cell Populations

The role for IL-18 in the behavior of CD8+ T cells is controversial. Several studies have shown that IL-18 and IL-12 mediated cytotoxic activity, including IFN-γ and granzyme production, of CD8+ T cells using murine spleen cell cultures [43]. Moreover, proliferation of CD8+ T cells was impaired in *Il18*$^{-/-}$ mice, and blocking IL-18 signaling in wild-type CD8+ T cells showed reduced live cells, suggesting that IL-18 promotes survival of CD8+ T cells [44]. Flow cytometry analysis demonstrated the upregulation of IL-18RAP on anti-CD3-activated CD8+ T cells when compared to naïve CD8+ T cells [44]. However, in vivo studies showed that CD8+ T

cell numbers were not affected in IL-18R-deficient (*Il18r*$^{-/-}$) mice following infection with the parasite *Trypanosoma cruzi* [45].

It has also been reported that there is an increase in frequency of Th17 cells in the lamina propria of *Il18*$^{-/-}$ and *Il18r*$^{-/-}$ mice, suggesting that IL-18 limits Rorγt+ Th17 cell differentiation [46]. Moreover, IL-18 is not required for Foxp3+ Treg cell differentiation but promotes optimal Treg cell function by enhancing the expression of key effector molecules, including *Furin*, *Bcl11b*, *Tnfrsf4,* and *Stat3* in the colonic lamina propria [46]. These molecules have been shown to play a crucial role in suppressing T cell-mediated colitis by Treg cells [46]. However, a recent study suggested that NLRP1/IL-18 signaling regulates the gut microbiome rather than immune response in a mouse colitis model [47].

5.5 The Role of IL18 in the Gastrointestinal Microenvironment: From Homeostasis to Tumorigenesis

5.5.1 Microbial Colonization

In order to maintain a homeostatic state in the gut, several mechanisms are required to limit the interaction with microbiota in the lumen. These mechanisms include a physical barrier provided by epithelial cells, the production of antimicrobial proteins, and a mucus layer produced by goblet cells. The gut microbiota has been shown to induce activation of the inflammasome, as germ-free mice showed abolished caspase-1 cleavage and a subsequent reduction in IL-18 production [48]. Interestingly, at steady state, the colonic tissue from *Il18*$^{-/-}$ mice showed reduced expression of antimicrobial peptides (AMPs), in particular angiogenin-4 (Ang4), when compared to WT mice [48]. These results suggest that IL-18 helps maintain gut homeostasis through induction of AMPs. Moreover, it has also been shown that IL-18-mediated AMP production is downstream of the NLRP6 inflammasome, rather than the NLRP3 inflammasome [48]. Most importantly, specific metabolites from the microbiome activate an NLRP6-IL-18-AMP axis which, in turn, optimize commensal colonization and persistence [49]. Nlrp6-deficient mice had concomitant dysbiosis, characterized by an expansion of bacterial phyla *Bacteroidetes* and TM7 [48]. Interestingly, *nlrp1*-deficient mice (*nlrp1*$^{-/-}$) are less susceptible to a murine model of acute colitis, although these mice have no difference in immune cell infiltration, in particular Th1 and Th17, in the colon when compared to their control [47]. Instead, *nlrp1*$^{-/-}$-deficient mice have increased butyrate-producing *Clostridiales* in the gut, suggesting that the NLRP1/IL-18 axis promotes IBD through limiting butyrate-producing microbiome [47]. Taken together, these results suggest that IL-18 is a crucial regulator of mucosal homeostasis.

5.5.2 Inflammatory Bowel Disease

Colitis is one of the predisposing factors for tumorigenesis. IL-18 is highly expressed in both the colonic epithelium and immune cells within the lamina propria of inflammatory bowel disease (IBD) patients [17], suggesting that IL-18 could play a role in contributing to the pathogenesis of these diseases. CD is an IBD associated with a Th1 cytokine profile and accumulation of IL-12 [50]. One animal model of CD is induced by 2,4,6-trinitrobenzene sulfonic acid (TNBS), which disrupts the mucosal epithelial barrier and modifies cell surface proteins [51]. In this model, administration of a neutralizing antibody against IL-18 resulted in less weight-loss and decreased histological scores and inflammation compared to control mice [51]. Similarly, TNBS immunization in *Il18*$^{-/-}$ mice showed a reduction in colonic thickening and immune cell infiltration in the colon when compared to *Il18*$^{+/+}$ mice [51].

In contrast to CD, UC is an IBD associated with a Th2 cytokine profile. A commonly used murine UC model is induced by dextran sulfate sodium (DSS), which damages the epithelial barrier defenses against luminal bacterial products. In this model, administration of DSS to wild-type mice together with a neutralizing antibody

against IL-18 [52], and the negative regulator, IL-18BP [53], showed reduced disease severity including decreased weight-loss, clinical scores, rectal bleeding, and inflammation when compared to controls [52, 53]. These results suggest that IL-18 causes detrimental effects in this model.

As mentioned previously, mature IL-18 production requires caspase-1; therefore, studies have also investigated in the role of caspase-1 in IBD using caspase-1-deficient (*caspase1*$^{-/-}$) mice. In one study, it was shown that *caspase1*$^{-/-}$ mice are protected from DSS-induced colitis, which is consistent with DSS mice treated with an antibody blocking IL-18 signaling [52, 54]. However, conflicting results have been reported whereby *caspase1*$^{-/-}$ mice had increased disease in the DSS-induced colitis model [55]. The disparity in these results may be attributed to differences in the intestinal microflora between animal facilities. In *caspase1*$^{-/-}$ mice, IL-18 was barely detected in serum, suggesting that the caspase-1 protective effect is due to IL-18 production [55]. To confirm this, recombinant IL-18 was administered to *caspase1*$^{-/-}$ mice, which reversed the phenotype, suggesting that the caspase-1/IL-18 axis is protective [55]. Furthermore, inflammasomes such as NLRP3 [56] and NLRP6 [57], which are required for mature IL-18 production, also showed worsening of disease following DSS treatment. Similarly, *Il18*$^{-/-}$ and *Il18r*$^{-/-}$ mice developed severe colitis associated with high lethality and increased histopathological abnormalities compared with control mice [58], suggesting that IL-18 is required for gut barrier protection.

In order to resolve the conflicting role of IL-18 in DSS-induced colitis, IL-18- and IL-18R-deficient tissue-specific mice were developed to delineate the involvement of IL-18 in epithelial and hematopoietic cells [16]. Mice with deletion of IL-18R in epithelial cells showed protection against DSS-induced colitis, suggesting that IL-18 signaling may disrupt the epithelial cell barrier [16]. Most importantly, mice deficient for IL-18BP showed severe colitis and progressive loss of mature goblet cells, which could be reversed by deleting the epithelial IL-18R in these mice [16]. It was also shown that IL-18 could disrupt the maturation of goblet cells through transcriptional regulation of goblet cell differentiation factors [16]. These results suggest that IL-18 is the key regulator for goblet cell dysfunction, which may be a key pathological event in human UC [59].

5.5.3 Anti-tumorigenic Role for IL-18 in Colon Cancer

Humans heterozygous for the IL-18 A607C polymorphism exhibit increased risk of CRC development [60]. Since IL-18 has a strong immunoregulatory role associated with the production of IFN-γ by Th1 cells and enhances the cytolytic activity of CD8+ cells and NK cells, it has been suggested that IL-18 may play an important role in anti-tumor immunity (Fig. 5.2).

The role of IL-18 in CRC has mainly been explored using the azoxymethane (AOM) DSS model, in which cyclic administration of DSS in the drinking water following the administration of AOM results in the formation of colonic tumors [61]. DSS-induced damage of epithelial cells leads to the activation of inflammasome-induced inflammation through NLRP3, NLRP6, caspase-1, and IL-18 [56]. In this model, *Il18*$^{-/-}$ mice showed increased tumor number and burden, which was associated with severe colitis histopathology compared with wild-type mice [56]. The increase in tumorigenesis was attributed to the lack of IFN-γ production in this model. In addition, mice deficient in *Nlrp3*, *Nlrp6*, and *caspase-1* also developed more tumors and had defective IL-18 production, suggesting that the inflammasome provides protection against tumor formation via IL-18 [56]. Similarly, myeloid cell-restricted Card9-deficient mice have been used to demonstrate the crucial role of inflammation-induced IL-18 production by myeloid cells in colon cancer development [62]. In this model, the commensal gut fungi recognition signaling pathway, the caspase recruitment domain 9/spleen tyrosine kinase (Card9-Syk) axis, was abrogated.

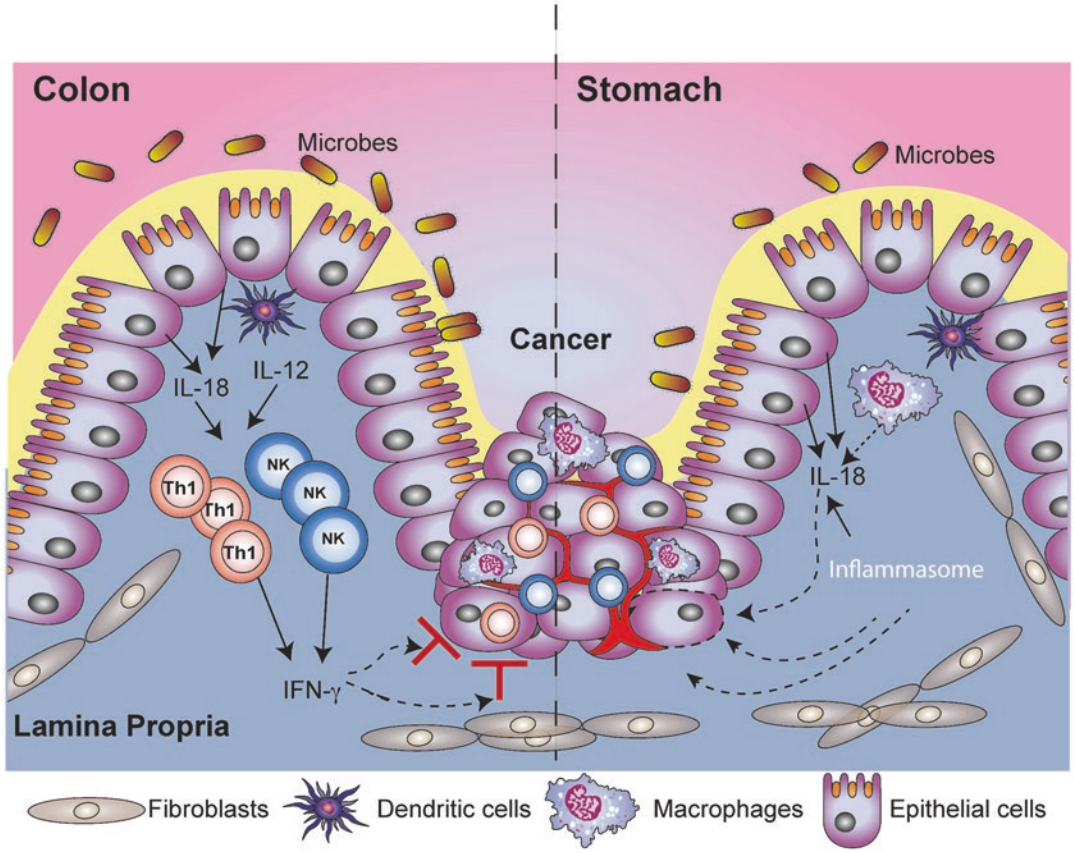

Fig. 5.2 Schematic of IL-18 signaling in GI tract tumorigenesis. In both colon and gastric cancers, IL-18 is mainly produced by epithelial cells. (Left) In colon cancer, IL-18 has an anti-tumorigenic role through cooperation with IL-12 resulting in IFN-γ production by Th1 cells and NK cells. Moreover, IL-18 acts on intestinal epithelial cells to trigger the release of antimicrobial peptides (AMPs) regulating the microbiota and maintaining gut barrier homeostasis [48]. (Right) In contrast, IL-18 has a pro-tumorigenic role in gastric cancer, which includes activation of the inflammasome [69]. However, the role of microbes/IL-18/inflammasome in gastric cancer has not yet been explored

Approximately 40% of CRC patients progress to fatal metastasis (80% being confined to the liver); therefore, the role of the inflammasome and IL-18 in CRC metastasis has also recently been explored [63]. In order to mimic liver metastasis in CRC, a mouse model of intrasplenic injection of transplantable tumor cells derived from a murine primary colon carcinoma (MC38) is often used. In this model, mice deficient in *Nlrp3* showed reduced IL-18 production and increased liver metastasis [63]. Moreover, *Il18*$^{-/-}$ and *Il18r*$^{-/-}$ mice also developed an increase in liver metastasis. This was attributed to IL-18-mediated immune surveillance being independent to T and B cells but dependent on hepatic NK cell maturation and priming Fas-mediated cytotoxicity [63]. In line with this, treatment of NK cells with recombinant IL-18 enhances NK cell-mediated death and eliminates tumor cells in a co-culture system [64]. More recently, IL-18 has been shown to promote the anti-tumor properties of eosinophils against colon cell lines by enhancing cell-cell contact between the two cell types [65].

5.5.4 Pro-tumorigenic Role for IL-18 in Gastric Cancer

The expression of IL-18 is elevated in the sera of patients with GC and CRC [66, 67]. IL-18 is also expressed in both GC and CRC biopsies, but in

CRC its expression does not correlate with survival [68]. However, in contrast to CRC, a number of studies examining IL-18 in GC have suggested a pro-tumorigenic role for this cytokine [69, 70] (Fig. 5.2). Initial studies demonstrated that administration of recombinant IL-18 increased cell proliferation in four GC cell lines (MKN28, MKN45, NUGC3, and KATOIII) through the activation of NF-κB [70]. This was consistent with observations made in xenograft experiments, where transplantation of GC cell lines into immunocompromised mice followed by administration of IL-18 resulted in a reduction in survival compared to control mice [70].

More recently, in addition to IL-18, components of the inflammasome upstream have also been demonstrated to have a pro-tumorigenic role in murine models of GC. Using the *Gp130*$^{F/F}$ model of GC, where mice develop spontaneous gastric adenomas from 4 weeks of age, it has been shown that mice deficient in IL-18 (*Gp130*$^{F/F}$:*Il18*$^{-/-}$) had reduced tumor burden, which was also observed in Asc-deficient mice (*Gp130*$^{F/F}$:*Asc*$^{-/-}$), signifying an important role of inflammasome activation in tumor formation in this model [69]. The reduction in tumor burden was found to not be due to a change in cell proliferation, as previous studies have suggested, but rather was a result of increased apoptosis of tumor cells due to increased expression of caspase 8, as well as a reduction in NF-κB activation [69]. However, unlike previous findings in vitro that demonstrated a role for immune cell responses in the tumor, no significant differences were found in immune subsets between *Gp130*$^{F/F}$ and *Gp130*$^{F/F}$:*Asc*$^{-/-}$ mice, suggesting a dispensable role for inflammation in this model [69]. While these studies demonstrate an apparent pro-tumorigenic role for IL-18 in GC, it remains to be seen whether the same inflammasomes that are activated in CRC, such as NLRP3 and NLRP6, also have a role in GC.

IL-18 has also been implicated in metastasis in GC. In vitro and xenograft experiments have shown that IL-18 induces the expression of CD44, COX2, and VEGF, which were involved in metastasis of GC cell xenografts and angiogenesis [67]. Additionally, neutralization of IL-18 in these models through siRNA knockdown or administration of IL-18BP was found to significantly reduce tumor burden while promoting expression of CD70, which has been suggested to increase susceptibility of cancer cells to NK cell-mediated anti-tumor responses [67].

5.6 Therapeutic Activation or Inhibit IL-18 Signaling

Several studies have suggested that recombinant IL-18 may be a useful therapeutic modality for cancer. This stems from the observation that recombinant IL-18 administration to mice before or after inoculation of CL8-1, a mouse melanoma cell line, showed decreased tumor development associated with elevated serum IFN-γ levels [71]. A similar observation was obtained in mice inoculated with the Meth A sarcoma cell line, where all mice administered recombinant IL-18 survived 3 weeks longer than controls [72]. The anti-tumor effect in this model was abrogated when treating mice with a neutralizing antibody against NK cells, suggesting that IL-18 induced NK cell anti-tumor activity [72]. Other more recent studies have shown that IL-18 promoted the expansion of NK cells from lung cancer patients and enhanced the anti-tumor phenotype of NK cells [41]. In line with our understanding of the role of capase-1 in CRC, caspase-1 mice that received recombinant IL-18 in the AOM/DSS model showed mild disease with significantly less weight loss and less inflammation, ulceration, and hyperplasia on histological colon sections [56]. The combined use of IL-18 with a low dose of IL-2 showed a synergistic anti-tumor effect in mice subcutaneously inoculated with a sarcoma cell line, MCA205 [73]. This effect was also largely dependent on NK cell-mediated IFN-γ production [73]. Moreover, combined treatment of IL-18 and IL-12 also demonstrated anti-tumor efficacy in MCA205 tumor-bearing mice [71]. However, it was found that administration of both IL-18 and IL-12 resulted in a dose-dependent systemic inflammatory response in mice, with elevated serum levels of IFN-γ and other cytokines resulting in acute multiorgan pathology, particularly in the liver [74]. More recently, the combination of recombinant IL-18

and immune checkpoint inhibitors resulted in synergistic inhibition tumor growth without adverse events in animal models of peritoneal dissemination of CT-26 colon carcinoma, which was associated with the accumulation of NK cells [75].

5.6.1 Therapeutics in Clinical Development

Currently, there are a number of therapeutic agents in clinical trials that target the IL-18 signaling pathway. These include recombinant IL-18 (SB-485232) and IL-18BP (Tadekinig alfa) and a neutralizing IL-18 monoclonal antibody (GSK1070806).

SB-485232 is a recombinant IL-18 protein developed by GlaxoSmithKline (GSK), which has been trailed in patients with metastatic melanoma and non-Hodgkin's lymphoma [76, 77]. In a separate phase II trial, stage IV metastatic melanoma patients were administered single agent SB-485232 at doses from 0.01 to 1.0 mg/kg each day for five consecutive days, followed by a 23-day rest period [77]. While the treatment was generally well tolerated, complete response was not observed in any of the patients, while only two patients achieved unconfirmed partial responses, suggesting a limited role for single-agent recombinant IL-18 in this disease. Combinatorial therapies involving IL-18 may yield more promising results, such as in a phase I clinical trial combining SB-485232 with the monoclonal antibody rituximab, a standard-of-care treatment, in patients with non-Hodgkin's lymphoma [76]. In this context, responses were observed in approximately 25% of patients, with two patients achieving a complete response; however, further clinical trials would be required to determine proper efficacy of this treatment regime [76]. It is important to note that administration of recombinant IL-18 has been associated with adverse events. These included chills, fever, fatigue, and nausea, while more serious adverse events included pleural effusion, increased lipase, deep vein thrombosis, and pulmonary embolism [77]. Other reported complications include deep vein thrombosis and abnormal EKG; however, this was not attributed to the study drug.

The pharmaceutical company AB2 Bio has developed a human recombinant IL-18BP, called Tadekinig alfa, and has demonstrated that it was safe and well tolerated in phase I/Ib clinical trials and a phase II trial, with injection site reactions, upper airway infections, and arthralgia the most common adverse events reported [78]. And, recently, they have conducted a phase III clinical trial in patients with single-point mutations in the NLRC4 gene. These patients have gain-of-function mutations and give rise to severe, life-threatening systemic inflammation associated with extremely high levels of IL-18 (NCT03113760). However, trials in patients with IBD or cancer have not been investigated.

Recently, a phase II trial of a neutralizing IL-18 monoclonal antibody, GSK1070806, was registered for patients with moderate to severe CD after a phase I trial demonstrated safety in healthy and obese subjects [79]; however, results have yet to be reported. Additionally, this therapeutic will also be investigated in a phase II trial in Behcet's disease, an inflammatory disease affecting the blood vessels, highlighting the potential for targeting the IL-18 pathway in other inflammatory conditions (NCT03522662).

5.6.2 Emerging Opportunities to Alter IL18 Signaling

As an alternative to systemic administration of recombinant protein, gene transfer technology has also been developed. Gene therapy works by inserting the gene of interest into a cell aiming to increase the amount of a specific gene in a targeted cell/tissue rather than systemically [80]. Interestingly, intravenous injection of plasmid encoding both IL-18 and IL-12 showed antitumor efficacy with diminished toxicity in mice [81]. Moreover, a cancer vaccine that delivered IL-18 also showed promising results in eradicating tumor cells in mice inoculated with murine colon cancer cell lines. This was associated with increased in CD8 and CD4 T cell infiltration in the tumor [82].

5.7 Concluding Remarks

IL-18 is clearly associated with a number of physiological functions throughout the GI tract. As further studies elucidate the cell-, disease-, and context-specific role of IL-18 signaling, the evolutionary significance of the divergent role of IL-18 in CRC and GC will become more apparent.

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Conflict of Interest The authors declare no conflicts.

References

1. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144:646–674. https://doi.org/10.1016/j.cell.2011.02.013
2. Nguyen PM, Putoczki TL (2018) Could the inhibition of IL-17 or IL-18 be a potential therapeutic opportunity for gastric cancer? Cytokine 118:8. https://doi.org/10.1016/j.cyto.2018.01.008
3. Garlanda C, Dinarello CA, Mantovani A (2013) The interleukin-1 family: back to the future. Immunity 39:1003–1018. https://doi.org/10.1016/j.immuni.2013.11.010
4. Nakamura K, Okamura H, Wada M, Nagata K, Tamura T (1989) Endotoxin-induced serum factor that stimulates gamma interferon production. Infect Immun 57:590–595
5. Okamura H et al (1995) Cloning of a new cytokine that induces IFN-gamma production by T cells. Nature 378:88–91. https://doi.org/10.1038/378088a0
6. Kato Z et al (2003) The structure and binding mode of interleukin-18. Nat Struct Biol 10:966–971. https://doi.org/10.1038/nsb993
7. Ushio S et al (1996) Cloning of the cDNA for human IFN-gamma-inducing factor, expression in Escherichia coli, and studies on the biologic activities of the protein. J Immunol 156:4274–4279
8. Ghayur T et al (1997) Caspase-1 processes IFN-gamma-inducing factor and regulates LPS-induced IFN-gamma production. Nature 386:619–623. https://doi.org/10.1038/386619a0
9. Yamanaka K et al (2000) Skin-specific caspase-1-transgenic mice show cutaneous apoptosis and pre-endotoxin shock condition with a high serum level of IL-18. J Immunol 165:997–1003. https://doi.org/10.4049/jimmunol.165.2.997
10. Sugawara S et al (2001) Neutrophil proteinase 3-mediated induction of bioactive IL-18 secretion by human oral epithelial cells. J Immunol 167:6568–6575. https://doi.org/10.4049/jimmunol.167.11.6568
11. Tsutsui H et al (1999) Caspase-1-independent, Fas/Fas ligand-mediated IL-18 secretion from macrophages causes acute liver injury in mice. Immunity 11:359–367
12. Bossaller L et al (2012) Cutting edge: FAS (CD95) mediates noncanonical IL-1beta and IL-18 maturation via caspase-8 in an RIP3-independent manner. J Immunol 189:5508–5512. https://doi.org/10.4049/jimmunol.1202121
13. Kim KE et al (2009) Expression of ADAM33 is a novel regulatory mechanism in IL-18-secreted process in gastric cancer. J Immunol 182:3548–3555. https://doi.org/10.4049/jimmunol.0801695
14. Dinarello CA, Novick D, Kim S, Kaplanski G (2013) Interleukin-18 and IL-18 binding protein. Front Immunol 4:289. https://doi.org/10.3389/fimmu.2013.00289
15. Munoz M et al (2015) Interleukin-22 induces interleukin-18 expression from epithelial cells during intestinal infection. Immunity 42:321–331. https://doi.org/10.1016/j.immuni.2015.01.011
16. Nowarski R et al (2015) Epithelial IL-18 equilibrium controls barrier function in colitis. Cell 163:1444–1456. https://doi.org/10.1016/j.cell.2015.10.072
17. Pizarro TT et al (1999) IL-18, a novel immunoregulatory cytokine, is up-regulated in Crohn's disease: expression and localization in intestinal mucosal cells. J Immunol 162:6829–6835
18. Torigoe K et al (1997) Purification and characterization of the human interleukin-18 receptor. J Biol Chem 272:25737–25742. https://doi.org/10.1074/jbc.272.41.25737
19. Born TL, Thomassen E, Bird TA, Sims JE (1998) Cloning of a novel receptor subunit, AcPL, required for interleukin-18 signaling. J Biol Chem 273:29445–29450. https://doi.org/10.1074/jbc.273.45.29445
20. Hoshino K et al (1999) Cutting edge: generation of IL-18 receptor-deficient mice: evidence for IL-1 receptor-related protein as an essential IL-18 binding receptor. J Immunol 162:5041–5044
21. Tsutsumi N et al (2014) The structural basis for receptor recognition of human interleukin-18. Nat Commun 5:5340. https://doi.org/10.1038/ncomms6340
22. Akira S (2000) The role of IL-18 in innate immunity. Curr Opin Immunol 12:59–63
23. Kanai T et al (2000) Interleukin 18 is a potent proliferative factor for intestinal mucosal lymphocytes in Crohn's disease. Gastroenterology 119:1514–1523
24. Adachi O et al (1998) Targeted disruption of the MyD88 gene results in loss of IL-1- and IL-18-mediated function. Immunity 9:143–150

25. Kanakaraj P et al (1999) Defective interleukin (IL)-18-mediated natural killer and T helper cell type 1 responses in IL-1 receptor-associated kinase (IRAK)-deficient mice. J Exp Med 189:1129–1138. https://doi.org/10.1084/jem.189.7.1129
26. Kojima H et al (1998) Interleukin-18 activates the IRAK-TRAF6 pathway in mouse EL-4 cells. Biochem Biophys Res Commun 244:183–186. https://doi.org/10.1006/bbrc.1998.8236
27. Lee JK et al (2004) Differences in signaling pathways by IL-1beta and IL-18. Proc Natl Acad Sci U S A 101:8815–8820. https://doi.org/10.1073/pnas.0402800101
28. Alboni S et al (2014) Interleukin 18 activates MAPKs and STAT3 but not NF-kappaB in hippocampal HT-22 cells. Brain Behav Immun 40:85–94. https://doi.org/10.1016/j.bbi.2014.02.015
29. Kalina U et al (2000) IL-18 activates STAT3 in the natural killer cell line 92, augments cytotoxic activity, and mediates IFN-gamma production by the stress kinase p38 and by the extracellular regulated kinases p44erk-1 and p42erk-21. J Immunol 165:1307–1313. https://doi.org/10.4049/jimmunol.165.3.1307
30. Novick D et al (1999) Interleukin-18 binding protein: a novel modulator of the Th1 cytokine response. Immunity 10:127–136
31. Kim SH et al (2000) Structural requirements of six naturally occurring isoforms of the IL-18 binding protein to inhibit IL-18. Proc Natl Acad Sci U S A 97:1190–1195. https://doi.org/10.1073/pnas.97.3.1190
32. Hurgin V, Novick D, Rubinstein M (2002) The promoter of IL-18 binding protein: activation by an IFN-gamma -induced complex of IFN regulatory factor 1 and CCAAT/enhancer binding protein beta. Proc Natl Acad Sci U S A 99:16957–16962. https://doi.org/10.1073/pnas.262663399
33. Muhl H et al (2000) Interferon-gamma mediates gene expression of IL-18 binding protein in nonleukocytic cells. Biochem Biophys Res Commun 267:960–963. https://doi.org/10.1006/bbrc.1999.2064
34. Takeda K et al (1998) Defective NK cell activity and Th1 response in IL-18-deficient mice. Immunity 8:383–390
35. Robinson D et al (1997) IGIF does not drive Th1 development but synergizes with IL-12 for interferon-gamma production and activates IRAK and NFkappaB. Immunity 7:571–581
36. Xu D et al (1998) Selective expression and functions of interleukin 18 receptor on T helper (Th) type 1 but not Th2 cells. J Exp Med 188:1485–1492. https://doi.org/10.1084/jem.188.8.1485
37. Yoshimoto T et al (1998) IL-12 up-regulates IL-18 receptor expression on T cells, Th1 cells, and B cells: synergism with IL-18 for IFN-gamma production. J Immunol 161:3400–3407
38. Barbulescu K et al (1998) IL-12 and IL-18 differentially regulate the transcriptional activity of the human IFN-gamma promoter in primary CD4+ T lymphocytes. J Immunol 160:3642–3647
39. Tominaga K et al (2000) IL-12 synergizes with IL-18 or IL-1beta for IFN-gamma production from human T cells. Int Immunol 12:151–160. https://doi.org/10.1093/intimm/12.2.151
40. Hyodo Y et al (1999) IL-18 up-regulates perforin-mediated NK activity without increasing perforin messenger RNA expression by binding to constitutively expressed IL-18 receptor. J Immunol 162:1662–1668
41. Senju H et al (2018) Effect of IL-18 on the expansion and phenotype of human natural killer cells: application to cancer immunotherapy. Int J Biol Sci 14:331–340. https://doi.org/10.7150/ijbs.22809
42. Son YI et al (2001) Interleukin-18 (IL-18) synergizes with IL-2 to enhance cytotoxicity, interferon-gamma production, and expansion of natural killer cells. Cancer Res 61:884–888
43. Okamoto I, Kohno K, Tanimoto T, Ikegami H, Kurimoto M (1999) Development of CD8+ effector T cells is differentially regulated by IL-18 and IL-12. J Immunol 162:3202–3211
44. Li W, Kashiwamura S, Ueda H, Sekiyama A, Okamura H (2007) Protection of CD8+ T cells from activation-induced cell death by IL-18. J Leukoc Biol 82:142–151. https://doi.org/10.1189/jlb.0706431
45. Oliveira AC et al (2017) Crucial role for T cell-intrinsic IL-18R-MyD88 signaling in cognate immune response to intracellular parasite infection. Elife 6. https://doi.org/10.7554/eLife.30883
46. Harrison OJ et al (2015) Epithelial-derived IL-18 regulates Th17 cell differentiation and Foxp3(+) Treg cell function in the intestine. Mucosal Immunol 8:1226–1236. https://doi.org/10.1038/mi.2015.13
47. Tye H et al (2018) NLRP1 restricts butyrate producing commensals to exacerbate inflammatory bowel disease. Nat Commun 9:3728. https://doi.org/10.1038/s41467-018-06125-0
48. Levy M et al (2015) Microbiota-modulated metabolites shape the intestinal microenvironment by regulating NLRP6 inflammasome signaling. Cell 163:1428–1443. https://doi.org/10.1016/j.cell.2015.10.048
49. Hand TW (2015) Interleukin-18: the bouncer at the mucosal bar. Cell 163:1310–1312. https://doi.org/10.1016/j.cell.2015.11.041
50. Peluso I, Pallone F, Monteleone G (2006) Interleukin-12 and Th1 immune response in Crohn's disease: pathogenetic relevance and therapeutic implication. World J Gastroenterol 12:5606–5610. https://doi.org/10.3748/wjg.v12.i35.5606
51. Kanai T et al (2001) Macrophage-derived IL-18-mediated intestinal inflammation in the murine model of Crohn's disease. Gastroenterology 121:875–888. https://doi.org/10.1053/gast.2001.28021
52. Siegmund B et al (2001) Neutralization of interleukin-18 reduces severity in murine colitis and intestinal IFN-gamma and TNF-alpha production. Am J Physiol Regul Integr Comp Physiol 281:R1264–R1273. https://doi.org/10.1152/ajpregu.2001.281.4.R1264

53. Sivakumar PV et al (2002) Interleukin 18 is a primary mediator of the inflammation associated with dextran sulphate sodium induced colitis: blocking interleukin 18 attenuates intestinal damage. Gut 50:812–820. https://doi.org/10.1136/gut.50.6.812
54. Siegmund B, Lehr HA, Fantuzzi G, Dinarello CA (2001) IL-1 beta -converting enzyme (caspase-1) in intestinal inflammation. Proc Natl Acad Sci U S A 98:13249–13254. https://doi.org/10.1073/pnas.231473998
55. Dupaul-Chicoine J et al (2010) Control of intestinal homeostasis, colitis, and colitis-associated colorectal cancer by the inflammatory caspases. Immunity 32:367–378. https://doi.org/10.1016/j.immuni.2010.02.012
56. Zaki MH et al (2010) The NLRP3 inflammasome protects against loss of epithelial integrity and mortality during experimental colitis. Immunity 32:379–391. https://doi.org/10.1016/j.immuni.2010.03.003
57. Elinav E et al (2011) NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. Cell 145:745–757. https://doi.org/10.1016/j.cell.2011.04.022
58. Takagi H et al (2003) Contrasting action of IL-12 and IL-18 in the development of dextran sodium sulphate colitis in mice. Scand J Gastroenterol 38:837–844
59. Gersemann M et al (2009) Differences in goblet cell differentiation between Crohn's disease and ulcerative colitis. Differentiation 77:84–94. https://doi.org/10.1016/j.diff.2008.09.008
60. Nikiteas N, Yannopoulos A, Chatzitheofylaktou A, Tsigris C (2007) Heterozygosity for interleukin-18 -607 A/C polymorphism is associated with risk for colorectal cancer. Anticancer Res 27:3849–3853
61. Fung KY, Putoczki T (2018) In vivo models of inflammatory bowel disease and colitis-associated cancer. Methods Mol Biol 1725:3–13. https://doi.org/10.1007/978-1-4939-7568-6_1
62. Malik A et al (2018) SYK-CARD9 signaling axis promotes gut fungi-mediated inflammasome activation to restrict colitis and colon cancer. Immunity 49:515–530 e515. https://doi.org/10.1016/j.immuni.2018.08.024
63. Dupaul-Chicoine J et al (2015) The Nlrp3 inflammasome suppresses colorectal cancer metastatic growth in the liver by promoting natural killer cell tumoricidal activity. Immunity 43:751–763. https://doi.org/10.1016/j.immuni.2015.08.013
64. Tanaka F et al (2000) Rapid generation of potent and tumor-specific cytotoxic T lymphocytes by interleukin 18 using dendritic cells and natural killer cells. Cancer Res 60:4838–4844
65. Gatault S et al (2015) IL-18 is involved in eosinophil-mediated tumoricidal activity against a colon carcinoma cell line by upregulating LFA-1 and ICAM-1. J Immunol 195:2483–2492. https://doi.org/10.4049/jimmunol.1402914
66. Haghshenas MR et al (2009) IL-18 serum level and IL-18 promoter gene polymorphism in Iranian patients with gastrointestinal cancers. J Gastroenterol Hepatol 24:1119–1122. https://doi.org/10.1111/j.1440-1746.2009.05791.x
67. Kang JS et al (2009) Interleukin-18 increases metastasis and immune escape of stomach cancer via the downregulation of CD70 and maintenance of CD44. Carcinogenesis 30:1987–1996. https://doi.org/10.1093/carcin/bgp158
68. Uhlen M et al (2015) Proteomics. Tissue-based map of the human proteome. Science 347:1260419. https://doi.org/10.1126/science.1260419
69. Deswaerte V et al (2018) Inflammasome adaptor ASC suppresses apoptosis of gastric cancer cells by an IL18-mediated inflammation-independent mechanism. Cancer Res 78:1293–1307. https://doi.org/10.1158/0008-5472.Can-17-1887
70. Majima T et al (2006) Exploitation of interleukin-18 by gastric cancers for their growth and evasion of host immunity. Int J Cancer 118:388–395. https://doi.org/10.1002/ijc.21334
71. Osaki T et al (1998) IFN-gamma-inducing factor/IL-18 administration mediates IFN-gamma- and IL-12-independent antitumor effects. J Immunol 160:1742–1749
72. Micallef MJ et al (1997) In vivo antitumor effects of murine interferon-gamma-inducing factor/interleukin-18 in mice bearing syngeneic Meth A sarcoma malignant ascites. Cancer Immunol Immunother 43:361–367
73. Son YI, Dallal RM, Lotze MT (2003) Combined treatment with interleukin-18 and low-dose interleukin-2 induced regression of a murine sarcoma and memory response. J Immunother 26:234–240
74. Carson WE et al (2000) Coadministration of interleukin-18 and interleukin-12 induces a fatal inflammatory response in mice: critical role of natural killer cell interferon-gamma production and STAT-mediated signal transduction. Blood 96:1465–1473
75. Ma Z et al (2016) Augmentation of immune checkpoint cancer immunotherapy with IL18. Clin Cancer Res 22:2969–2980. https://doi.org/10.1158/1078-0432.CCR-15-1655
76. Robertson MJ et al (2013) A dose-escalation study of recombinant human interleukin-18 in combination with rituximab in patients with non-Hodgkin lymphoma. J Immunother 36:331–341. https://doi.org/10.1097/CJI.0b013e31829d7e2e
77. Tarhini AA et al (2009) A phase 2, randomized study of SB-485232, rhIL-18, in patients with previously untreated metastatic melanoma. Cancer 115:859–868. https://doi.org/10.1002/cncr.24100
78. Gabay C et al (2018) Open-label, multicentre, dose-escalating phase II clinical trial on the safety and efficacy of tadekinig alfa (IL-18BP) in adult-onset Still's disease. Ann Rheum Dis 77:840–847. https://doi.org/10.1136/annrheumdis-2017-212608
79. Mistry P et al (2014) Safety, tolerability, pharmacokinetics, and pharmacodynamics of single-dose antiinterleukin- 18 mAb GSK1070806 in healthy and obese subjects. Int J Clin Pharmacol Ther 52:867–879. https://doi.org/10.5414/CP202087

80. O'Reilly M et al (2014) Gene therapy: charting a future course--summary of a National Institutes of Health Workshop, April 12, 2013. Hum Gene Ther 25:488–497. https://doi.org/10.1089/hum.2014.045
81. Rodriguez-Galan MC et al (2009) Coexpression of IL-18 strongly attenuates IL-12-induced systemic toxicity through a rapid induction of IL-10 without affecting its antitumor capacity. J Immunol 183:740–748. https://doi.org/10.4049/jimmunol.0804166
82. Higashi K et al (2014) A novel cancer vaccine strategy with combined IL-18 and HSV-TK gene therapy driven by the hTERT promoter in a murine colorectal cancer model. Int J Oncol 45:1412–1420. https://doi.org/10.3892/ijo.2014.2557

6 IL-21 Signaling in the Tumor Microenvironment

Ghita Chabab, Nathalie Bonnefoy, and Virginie Lafont

Abstract

IL-21 is an immunomodulatory cytokine produced by natural killer (NK) cells and T cells that has pleiotropic roles in immune and non-immune cells. IL-21 can modulate innate and specific immunity activities. It is a potent stimulator of T and natural killer cell-mediated antitumor immunity but also has pro-inflammatory functions in many tissues and is involved in oncogenesis. It is important to understand IL-21 biology in these different situations to ensure the maximal benefit of therapeutic strategies targeting this cytokine. This chapter summarizes IL-21 characteristics and signaling, its role in immune system components, and its use in cancer immunotherapies.

Keywords

Cytokine · Immune cells · Lymphocytes · Myeloid cells · Tumor microenvironment · Antitumor activity · Pro-tumoral activity · Signaling pathways · γc Cytokine family · JAK-STAT pathways · PI3-kinase pathway · ERK pathway · Transcription factors · Regulation

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6.1 Introduction

For a long time, cancer was thought to originate from a single cell that started to proliferate in an uncontrolled manner due to chronic stress or a gene mutation. Later, thanks to the progress in sequencing methodologies, the notion of tumor heteroclonality emerged and could be demonstrated. Genetic heterogeneity during tumor development/progression challenges cancer therapies because it increases the likelihood of drug resistance, treatment failure, and relapse. Therefore, new therapeutic strategies have been investigated in parallel with the unraveling of cancer complexity. A tumor is now considered as a heterogeneous ecosystem composed of cancer cells and other cell types (i.e., cancer-associated fibroblasts, endothelial cells, and immune cells). These cells are referred to as tumor-infiltrating cells and constitute the tumor microenvironment (TME). It has been demonstrated that TME cell composition directly influences tumor development. Among these cells, immune cells infiltrate the tumor and establish a permanent cross talk with cancer cells. Analysis of the tumor immune microenvironment (TIME) revealed that interactions between immune and cancer cells can be

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resumed in three phases, called the three E's. In the first phase (elimination phase), immune cells detect and eliminate tumor cells. In the second phase (equilibrium phase), tumor cell lysis and death and tumor cell proliferation are equivalent. In the third and final phase (escape phase), tumor cells escape the immune surveillance, thus leading to tumor growth and progression. During the escape phase, the immune system is no longer able to eliminate tumor cells due to the appearance of resistance mechanisms that cooperate to thwart the antitumor immunity. It has been shown that the escape phase relies on the ability of cancer cells to shape and control the immune system. For instance, cancer cells express the immune checkpoint programmed cell death ligand-1 (PDL-1), whereas effector T lymphocytes, especially cytotoxic T lymphocytes (CTL), express its receptor programmed cell death protein 1 (PD1). Upon binding to its receptor, PDL-1 inhibits CTL activity, thus impairing tumor cell lysis. As TME's, particularly TIME, importance became clear, new therapeutic concepts were developed, tested, and adopted in the clinic for the treatments of some cancer types. For instance, tumor-infiltrating immune cells can be targeted to promote, stimulate, or restore an effective antitumor immune response. The development of antibodies against immune checkpoints, such as PDL-1, PD1, and cytotoxic T lymphocyte protein 4 (CTLA4), brought huge advances in cancer therapies with fantastic results in some cancers (melanoma, lung, kidney, and bladder), when used alone or in combination with conventional therapies (chemotherapy, radiotherapy). Besides immune checkpoint inhibitors, there are other approaches to potentiate the immune system, such as using vaccines and cytokines to boost the immune antitumor response. For example, interleukin-2 (IL-2) is a key cytokine with pleiotropic roles in the immune system, such as supporting T cell survival and inducing the proliferation of CTL and natural killer (NK) cells. IL-2-based therapy has been approved for metastatic renal cancer and melanoma [1]. However, IL-2 therapeutic potential in cancer therapies has been limited by its severe side effects (hypotension, heart toxicity, vascular leak syndrome, etc.). Another limitation is IL-2 ability to amplify and activate regulatory T (Treg) cells that promote tumor growth through the impairment of CTL functions. The ability of other cytokines to control, directly or not, the antitumor immune responses is still not well known and remains a major research axis. Recent studies highlighted an interesting role of IL-21. IL-21 is a member of the common gamma chain cytokine family (γc) and displays a dual role in tumor development that needs to be clarified [2]. Indeed, IL-21 overexpression in the gut mucosa of patients with ulcerative colitis (UC) exacerbates inflammation that has a pro-tumoral effect and favors colon cancer development. However, IL-21 also has an antitumor effect in skin and kidney cancer [3]. Many studies have been performed to understand IL-21 role and signaling in the microenvironment of various tumors. In this chapter, we first summarize IL-21 signaling and roles in the immune system, and then we discuss its contribution to cancer therapies.

6.2 IL-21 Signaling (Fig. 6.1)

IL-21 is an immunomodulatory cytokine with pleiotropic actions in many immune and nonimmune cell types. In its mature form, IL-21 is a protein of 162 amino acids (aa) that form a four-helix bundle of 14 kDa. Human IL-21 shares 63% sequence identity with mouse IL-21. IL-21 binds to the IL-21 receptor (IL-21R), a heterodimeric complex composed of the common gamma chain (CD132 or γc) and the specific IL-21 receptor chain (IL-21Rα or CD360). IL-21Rα is a transmembrane glycoprotein of 75 kDa with six tyrosine residues on the intracytoplasmic domain that are essential for IL-21 signaling. CD132 is a 347-aa-long protein, with a molecular weight of 40 kDa. This receptor is used also by other cytokines: IL-2, IL-4, IL-7, IL-9, and IL-15 [4]. Together, they form the common gamma chain family of cytokines [5]. IL-21 is produced mainly by CD4+ T cells and NK T cells (NKT). IL-21 signals via three different pathways: the Janus kinase-signal transducers and activator of transcription (JAK-STAT)

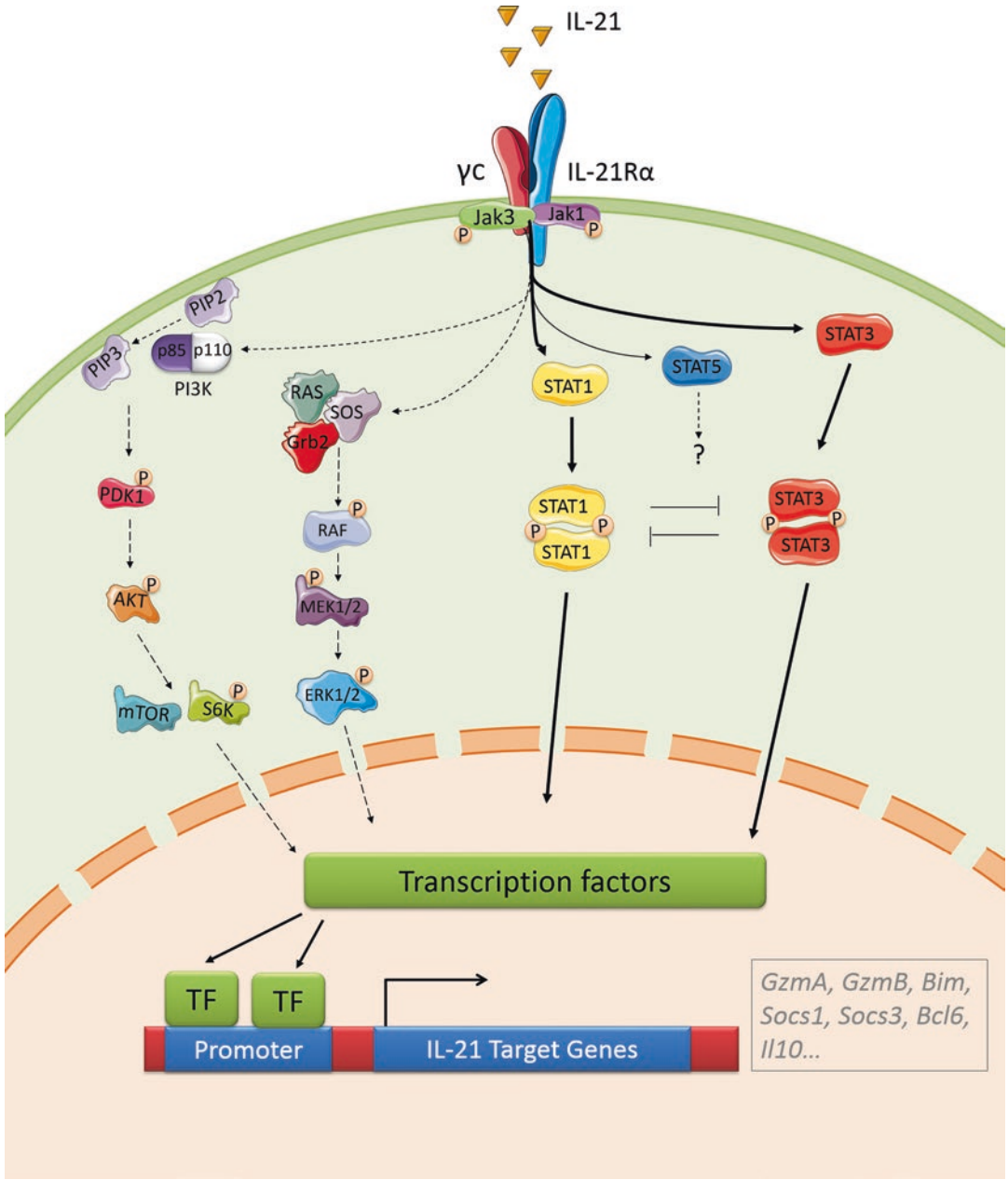

Fig. 6.1 IL-21 signaling pathways. IL-21 activates the JAK-STAT, PI3K-AKT, and MAPK pathways. STAT3 is the main contributor to IL-21 biological effects. STAT1 also participates in IL-21 signaling and co-regulates with STAT3 IL-21 target gene expression. STAT5 role is not well understood yet. The PI3K and MAPK pathways contribute to the activation of transcription factors and cofactors, such as cJun and cFos. The PI3K and MAPK pathways contribute to IL-21 proliferative effects

pathway, the phosphoinositide 3-kinase (PI3K)-serine/threonine kinase (AKT) pathway, and the mitogen-activated protein kinase (MAPK) pathway. The PI3K-AKT and MAPK signaling pathways are implicated in IL-21-dependent effects on cell survival and proliferation [6]. Upon IL-21 binding, the receptor complex (IL-21Rα and CD132) dimerizes allowing the transphos-

phorylation and activation of JAK1 and JAK3, two kinases that are associated with IL-21Rα and CD132 intracytoplasmic parts, respectively. Then, activated JAKs phosphorylate STAT1, STAT3, and, to a lesser extent, STAT5. Phosphorylated STAT3 proteins form homodimers that translocate to the nucleus and bind to interferon (IFN) γ-activated sequences (GAS) and modulate the expression of IL-21-responsive genes, such as *BCL2* and *BAD* (two antiapoptotic genes), activation-induced cytidine deaminase (*AICDA* or *AID*; a gene encoding the AID protein involved in isotypic class switching in B cells), *IFNG*, suppressor of cytokine signaling 3 and 1 (*SOCS3* and *SOCS1*, two negative regulators of the JAK-STAT pathway via the inhibition of JAK kinase activity), and also *IL21* [6, 7]. IL-21 can also signal through STAT1 and STAT5. Although little is known about STAT5's role in IL-21 signaling, it has been shown that STAT1 and STAT3 work together to potentiate the expression of IL-21 target genes. They also have partially opposite roles in signaling pathway activation and cross-inhibition [7]. Specifically, Wan et al. observed in murine models that IL-21-mediated STAT1 phosphorylation is increased in STAT3-deficient (*Stat3*$^{-/-}$)/CD4^{+} T cells, suggesting that STAT3 downregulates STAT1 phosphorylation. They also showed that *Stat3*$^{-/-}$/CD4^{+} T cells express lower levels of SOCS1 and SOCS3, leading to an increase of STAT1 phosphorylation. Specifically, 10% of IL-21 target genes are STAT1-dependent, but 50% of these genes are also co-regulated by STAT3. For example, Th1 signature genes (i.e., *Tbx21* and *Ifng*) are not induced by IL-21 in *Stat1*$^{-/-}$/CD4^{+} T cells but are increased in *Stat3*$^{-/-}$/CD4^{+} T cells, suggesting that STAT1 induces while STAT3 represses their expression. Moreover, STAT1 and STAT3 homodimers have different binding sites (ChIP-seq analysis): STAT1, but not STAT3, binds to the *Il21* promoter site, while both STAT1 and STAT3 bind to the *Tbx21* and *Ifng* promoters. This explains the differential gene regulation observed by the authors. IL-21R is expressed by various immune cell types, such as T cells, B cells, NK cells, NKT cells, dendritic cells, and also nonimmune cells (gut epithelial cells). Consequently, IL-21 may impact their activity and phenotype in the TME.

6.3 IL-21 in Immunity (Fig. 6.2)

In this part, we discuss IL-21 roles in different immune cell types that express IL-21R.

Natural killer cells Several studies showed that IL-21 enhances NK cell effector activities, in different species, through the JAK/STAT and PI3K/MAPK pathways [8]. In murine models, IL-21 inhibits NK cell proliferation but also induces their activation and maturation as well as the production of cytolytic molecules (perforin and granzyme), thus strengthening their cytotoxic functions [9]. In humans, IL-21 also potentiates the cytotoxic activity of NK cells. In vitro experiments showed that culture in the presence of IL-21 promotes survival and activation of NK cells [10]. Interestingly, like in murine models, IL-21 can act in synergy with other cytokines, such as IL-15, to potentiate NK cell cytotoxic activity, leading to increased expression of perforin and granzyme and production of IFNγ [10, 11]. NK cells constitutively express the low-affinity Fcγ receptor (FcγRIIIa or CD16) that is essential for antibody-dependent cellular cytotoxicity (ADCC), an important mechanism exploited in cancer therapy with antitumor antibodies [12–14]. Roda et al. showed that the ADCC-dependent antitumor effect of NK cells is enhanced in the presence of IL-21. Specifically, when HER2^{+} human breast cancer cell lines coated with trastuzumab (an anti-HER2/Neu monoclonal antibody) are cocultured with human NK cells in the presence of IL-21, NK cell production of IFNγ, TNFα, and chemokines, such as IL-8 and MIP-1α, is increased as well as their cytolytic activity. Experiments using *Stat1*-deficient mouse NK cells and antibody-coated murine tumor cells demonstrated that IFNγ production is STAT1-dependent [14]. Other studies in human and murine models showed that IL-21 also modifies the expression of proteins involved in NK cell activation or the modulation of their

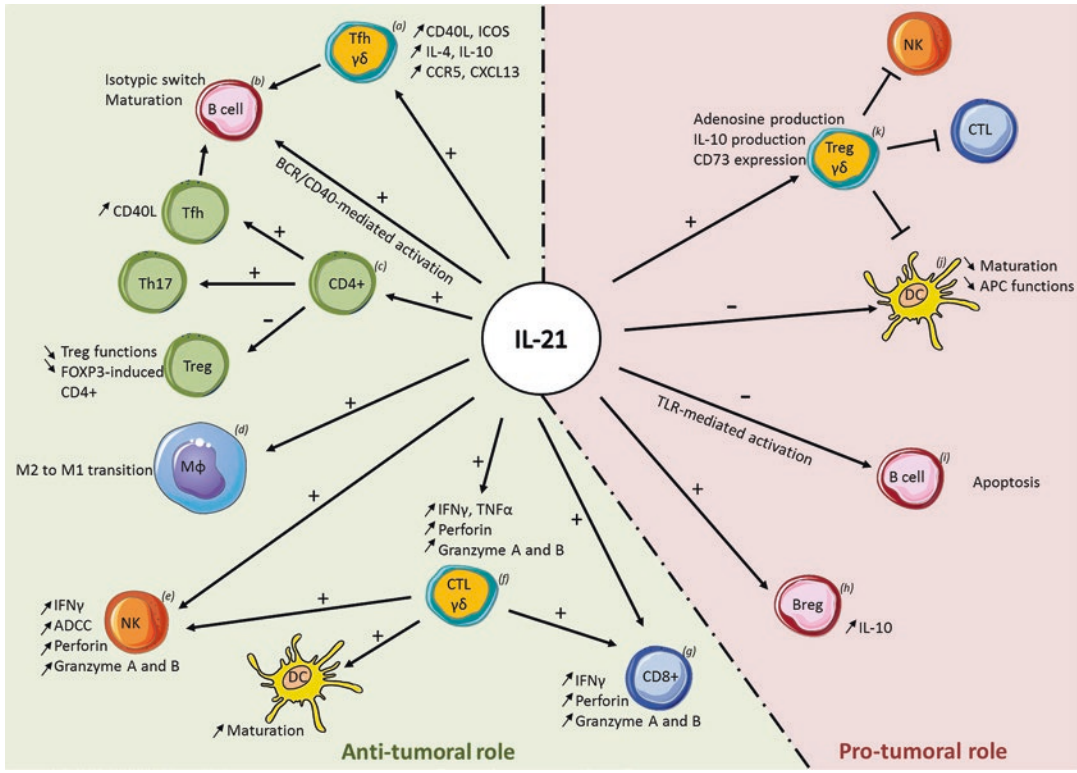

Fig. 6.2 IL-21 impact on immune cells. IL-21 has a broad range of effects on immune cells, leading to antitumor or pro-tumoral effects. IL-21 induces the differentiation of γδ T cells into follicular helper T cells *(a)* that can activate B cells *(b)* and induce their maturation. IL-21 can also act directly on B cells and induces their maturation and differentiation into Ig-producing cells when activated by B cell receptor (BCR) and/or CD40 stimulation *(b)* or triggers their apoptosis when activated by Toll-like receptor (TLR) recruitment *(i)*. IL-21 induces $CD4^+$ T cell activation and proliferation *(c)* and their differentiation into follicular helper T cells (Tfh) or Th17 cells. Conversely, IL-21 inhibits T regulatory (Treg) cell differentiation and functions, thus increasing the antitumor functions of the other cells (e.g., cytotoxic T lymphocytes, CTL). IL-21 also induces M2 to M1 transition in macrophages *(d)*, restoring their antitumor functions. IL-21 can enhance cytotoxic effects of natural killer (NK) cells*(e)*, CTL γδ T cells *(f)*, and $CD8^+$ T cells *(g)* by increasing perforin and granzyme production and cytokine production and promoting antibody-dependent cellular cytotoxicity mechanisms (for NK cells). IL-21 indirectly induces $CD8^+$ T cell and NK cell activation through its effect on CTL γδ T cells *(f)*. CTL γδ T cell activation promotes dendritic cell (DC) maturation, improving the presentation of tumor antigens to T cells and the activation of the immune response. Conversely, IL-21 also favors the differentiation or activity of various regulatory cell types, such as regulatory B cells *(h)* and regulatory γδ T cells *(k)*. Regulatory γδ T cells inhibit NK cell and CTL functions and also DC maturation and functions. Both regulatory B cells and γδ T cells produce IL-10, an immunosuppressive cytokine. IL-21 also directly inhibits DC *(j)* maturation and APC functions, thus limiting the immune system activation

activity. For instance, NKG2A and CD25 are increased [9, 10], while NKG2D is decreased [10, 15]. These and many other studies demonstrated the stimulating effect of IL-21 on NK cell cytotoxic activity and cytokine production.

B cells IL-21 has various effects on B cells depending on the type of activation (CD40-, B cell receptor- or Toll-like receptor-mediated) and the cell maturation state. Indeed, IL-21R expression is different in naive, memory, and plasma cells [16, 17]. Diehl et al. showed that in the presence of IL-21, B cell activation through B cell receptor and/or CD40 recruitment enhances their proliferation and differentiation into immunoglobulin (Ig)-producing plasma cells [18, 19]. This mechanism is STAT3-dependent [18, 20]. Conversely, when activated through the Toll-like

receptor pathway, IL-21 triggers B cell apoptosis [19]. Another study demonstrated that IL-21 affects Ig isotypic class-switch. Indeed, when cultured in the presence of both IL-21 and an anti-CD40 antibody, naive B cells are activated and differentiate into IgG1- and IgG3-producing cells through STAT3-dependent induction of AID expression [21]. Moreover, on regulatory B cells also called B10 cells due to their ability to produce the immunosuppressive IL-10 cytokine, IL-21 increases proliferation and IL-10 production [22, 23]. All these studies showed that IL-21 has pleiotropic effects in B cells, depending on the recruited signaling pathway(s) and the B cell subset. This must be taken into account when developing IL-21-based therapeutics for B cell malignancies, as discussed below.

Helper and cytotoxic T cells IL-21 modulates T cell responses in different manners. First, IL-21 induces survival and proliferation of $CD4^+$ T cell by rendering them resistant to Treg-mediated suppression. In vitro experiments on $CD4^+$ T cell proliferation showed that when cocultured with Treg cells, CD3-stimulated $CD4^+$ T cells do not proliferate. However, addition of IL-21 restores their proliferation [24]. Second, IL-21 produced by Th17 cells favors their differentiation through an autocrine mechanism [25]. Third, IL-21 in synergy with IL-15 induces survival and expansion of naive and memory $CD8^+$ T cells [26]. It also enhances the cytotoxic activities of effector and memory $CD8^+$ T cells through increased production of perforin and IFNγ [27–29]. Finally, IL-21 induces T follicular helper cell proliferation and maturation (i.e., expression of CD40L), allowing the maturation of B cells present in germinal centers through CD40 activation.

Regulatory T cells IL-21 inhibits Treg cell activation, differentiation, and survival and thus limits their immunoregulatory effects toward effector cells [24]. Interestingly, using IL-21R-deficient conventional and/or regulatory T cells, Attridge et al. showed that functional IL-21R signaling in conventional T cells is required to block Treg-mediated immunosuppression through reduction of IL-2 production by T cells. IL-2 is a key activation and survival factor for Treg cells. By decreasing IL-2 secretion by conventional T cells, IL-21 indirectly inhibits Treg cell immunosuppressive activity [24, 30, 31]. IL-21 also inhibits cancer-mediated FOXP3 induction in naive human CD4 T cells, thus directly blocking their differentiation into regulatory cells [32, 33].

γδ T cells Vγ9Vδ2 T cells are the main subpopulation of peripheral γδ T cells in human blood. They are involved in antitumor responses through their capacity to recognize and react against a broad range of tumors [34]. Like in B cells, IL-21 has pleiotropic effects in Vγ9Vδ2 T cells. Several studies showed that IL-21, in combination with IL-2, induces Vγ9Vδ2 T cell differentiation into Tfh-type γδ T cells [35, 36]. Upon TCR-mediated activation, a subset of Vγ9Vδ2 T cells cultured in the presence of IL-21 expresses CXCR5, the receptor of the chemokine CXCL13 that is involved in the migration of Tfh and B cells to lymph node follicles. They also express ICOS and CD40L, and consequently they can activate B cells through CD40/CD40L interactions. They also produce IL-10 and IL-4, two cytokines involved in antibody production by B cells, thus enhancing the humoral antitumor response [35, 36]. IL-21 also directly modulates antitumor responses mediated by γδ T cells. Ex vivo TCR-stimulated Vγ9Vδ2 T cells show Th1-like differentiation with higher expression of lytic granules (perforin, granzymes A and B) and increased production of IFNγ in the presence of IL-2 and IL-21 compared with IL-2 alone, suggesting that these cells have a more potent antitumor activity [34]. Conversely, more recent studies showed that IL-21 promotes the development of a regulatory subtype of Vγ9Vδ2 T cells that produces inhibitory molecules, such as adenosine and IL-10 [37]. These regulatory cells express the ectonucleotidase CD73 that is involved in adenosine production. The authors also observed that the degranulation capacity of $CD73^+$ Vγ9Vδ2 T cells was reduced compared with $CD73^-$ Vγ9Vδ2 T cells, suggesting a reduced cytotoxic activity of these cells against tumor cells [37]. Once again,

IL-21 signaling in antitumor activities varies depending on the activation conditions and the considered cell subset.

Dendritic cells Dendritic Cells (DC) are professional antigen-presenting cells (APC) involved in the initiation of T cell–mediated immune responses. As DCs express IL-21R at their surface, they are regulated by IL-21 signaling. When cultured in vitro with IL-21, DC maturation is impaired as indicated by the decreased expression of CCR7, a marker of mature DCs, and of MHCII, leading to reduced antigen presentation [38]. Furthermore, DCs cultured in the presence of IL-21 cannot stimulate $CD8^+$ T cell proliferation. Thus, IL-21 has a negative role on DC maturation and APC functions.

Altogether, the different IL-21 effects in immune cells confirm the pleiotropic role of this cytokine and emphasize the difficulty of considering IL-21 for cancer therapies. Therefore, many groups tried to determine in which cancer types IL-21-based therapies would be beneficial. These studies will be discussed in the next part of this chapter.

6.4 IL-21 in Cancer and IL-21-Based Therapies

As discussed before, IL-21 effects in immune cells depend on many parameters (e.g., cell type, maturation status, activation stimuli). Similarly, IL-21 can promote or inhibit tumor development in function of the tumor type and stage.

Colorectal cancer (CRC) was one the first cancers where IL-21 was shown to be involved in cancer initiation. IL-21 is a key player in CRC tumorigenesis, by establishing a pro-inflammatory environment and thus contributing to the installation of chronic inflammation, a driving force in CRC initiation and progression [39]. Indeed, CRC risk is higher in patients with chronic inflammatory diseases, such as UC and Crohn's disease, due to the maintenance of a more or less severe inflammation in the intestine [39, 40]. Stolfi et al. showed by immunostaining that IL-21 is overexpressed in intestinal tissue sections from patients with UC and with UC-associated CRC compared with healthy controls and that IL-21 overexpression is correlated with increased $CD3^+$ cell infiltration. Similarly, IL-21 expression is increased in murine models of colitis (wild-type mice treated with azoxymethane) and colitis-associated colon cancer (CAC; wild-type mice treated with azoxymethane and dextran sulfate sodium) compared with control non-treated mice. Furthermore, comparison of colon tumors and paired healthy tissues from CAC mice indicated that IL-21 expression is significantly increased in tumors, suggesting an important role of IL-21 in tumor formation and progression. Experiments in *Il21*$^{-/-}$ mice confirmed this hypothesis. Indeed, tumor size and number following azoxymethane and dextran sulfate sodium treatment were reduced in *Il21*$^{-/-}$ mice compared with wild-type mice. Additional experiments suggested that tumor promotion by IL-21 is mainly due to its effect on immune cells, because IL-21R is not expressed by tumor epithelial cells but by cells in the TME. Moreover, IL-21 inhibits the development of Treg cells (fewer $FOXP3^+$ $CD4^+$ T cells). This leads to an increase of effector T cell activity and of inflammatory signals through production of IFNγ and IL-17A in the TME, thus sustaining an inflammatory TME and promoting tumor initiation and progression [39, 40].

On the other hand, murine melanoma, fibrosarcoma, and murine mammary adenocarcinoma that secrete functional IL-21 are rejected by 90% of mice compared with control tumor cells that do not produce IL-21 [41]. Tumor rejection is associated with a higher production of IFNγ by $CD8^+$ T cells and enhanced cytotoxic activity of NK and $CD8^+$ T cells (perforin and granzyme expression) [41, 42]. Expression of IL-21R by B cells is interesting in the context of B cell malignancy therapies. Malignant cells isolated from patients with chronic lymphocytic leukemia (CLL) express IL-21R, and in vitro experiments showed IL-21R upregulation after CD40 stimula-

tion. IL-21 inhibits CLL B cell proliferation and induces their apoptosis through caspase activation and BID cleavage [43]. Gelebart et al. also showed IL-21-induced cancer cell apoptosis in mantle cell lymphoma, a B cell lymphoma that is resistant to chemotherapies, which gives more relevance to IL-21 use in antitumor therapies [44]. IL-21 pro-apoptotic activity in B cell malignancies supports its development as a potential therapeutic molecule. A clinical trial using recombinant IL-21 in combination with rituximab was performed in patients with CLL, small lymphocytic lymphoma, follicular lymphoma, and marginal zone lymphoma. IL-21 improved rituximab efficacy because tumor burden was decreased in 84% of patients, and complete and/or partial response was observed in 42% of patients. IL-21 potentiated rituximab effects through its positive effect on NK cell activity [45].

IL-21 efficacy as an immunotherapeutic cytokine in non-hematologic cancers has been assessed in phase I and II clinical trials [46, 47]. In metastatic melanoma, 62.5% of patients displayed a partial response or stable disease upon treatment with IL-21. Response to treatment was independent of the tumor mutational status[47]. In metastatic renal cell carcinoma, IL-21 combined with sorafenib, a tyrosine kinase inhibitor, showed poor response rate (21%) but good disease control rate (82%) [46]. In this clinical trial, the response to IL-21 treatment was dose-dependent. Nevertheless, therapies based on IL-21 need to be improved, particularly concerning the adverse events observed in some patients.

6.5 Future Directions

In this chapter, we presented an overview of IL-21 role, its signaling, and its effects on tumor development and in TME cells. The potential use of IL-21 as a therapeutic target in many cancers is currently investigated, with variable results depending on the tumor type, the used dose, and the TME immune contexture. Specifically, in CRC, IL-21 promotes the early phase of tumor development through its inflammatory properties. On the other hand, in various solid and hematological tumors, IL-21 improves effector T cell and NK cell cytotoxic activity.

It is clear now that the choice of the best cancer treatment for a patient cannot be based on one single indication, for instance, the tumor mutational status, but requires many different analyses to capture the tumor complexity, interactivity, and dynamics. This might allow predicting which therapy could be more effective for a patient and could limit the adverse effects. This is called personalized medicine, a method that uses different prediction, prevention, and diagnosis approaches to find the best way to treat patients, depending on the information given by a combination of analyses.

References

1. Rosenberg SA (2014) IL-2: the first effective immunotherapy for human cancer. J Immunol 192:5451. https://doi.org/10.4049/jimmunol.1490019
2. Spolski R, Leonard WJ (2014) Interleukin-21: a double-edged sword with therapeutic potential. Nat Rev Drug Discov 13:379. https://doi.org/10.1038/nrd4296
3. Søndergaard H, Skak K (2009) IL-21: roles in immunopathology and cancer therapy. Tissue Antigens 74:467–479
4. Habib T, Senadheera S, Weinberg K, Kaushansky K (2002) The common γ chain (γc) is a required signaling component of the IL-21 receptor and supports IL-21-induced cell proliferation via JAK3. Biochemistry 41:8725. https://doi.org/10.1021/bi0202023
5. Parrish-Novak J et al (2000) Interleukin 21 and its receptor are involved in NK cell expansion and regulation of lymphocyte function. Nature 408:57. https://doi.org/10.1038/35040504
6. Zeng R et al (2007) The molecular basis of IL-21-mediated proliferation. Blood 109:4135. https://doi.org/10.1182/blood-2006-10-054973
7. Wan C-K et al (2015) Opposing roles of STAT1 and STAT3 in IL-21 function in CD4 + T cells. Proc Natl Acad Sci 112:9394–9399
8. Konjević GM, Vuletić AM, Mirjačić Martinović KM, Larsen AK, Jurišić VB (2019) The role of cytokines in the regulation of NK cells in the tumor environment. Cytokine 117:30–40
9. Brady J, Hayakawa Y, Smyth MJ, Nutt SL (2014) IL-21 induces the functional maturation of murine NK cells. J Immunol 172:2048–2058
10. Skak K, Frederiksen KS, Lundsgaard D (2008) Interleukin-21 activates human natural killer cells

and modulates their surface receptor expression. Immunology 123:575–583
11. Strengell M et al (2003) IL-21 in synergy with IL-15 or IL-18 enhances IFN- production in human NK and T cells. J Immunol 170:5464–5469
12. Park YK et al (2012) Interleukin-21 increases direct cytotoxicity and IFN-γ production of ex vivo expanded nk cells towards breast cancer cells. Anticancer Res 32:839–846
13. McMichael EL et al (2017) Activation of the FcgammaReceptorIIIa on human natural killer cells leads to increased expression of functional interleukin-21 receptor. Oncoimmunology 6:1–10
14. Roda JM et al (2014) Interleukin-21 enhances NK cell activation in response to antibody-coated targets. J Immunol 177:120–129
15. Burgess SJ, Marusina AI, Pathmanathan I, Borrego F, Coligan JE (2014) IL-21 Down-regulates NKG2D/DAP10 expression on human NK and CD8+ T cells. J Immunol 176:1490–1497
16. Good KL, Bryant VL, Tangye SG (2014) Kinetics of human B cell behavior and amplification of proliferative responses following stimulation with IL-21. J Immunol 177:5236–5247
17. Wang SP et al (2015) Amplification of IL-21 signalling pathway through bruton's tyrosine kinase in human B cell activation. Rheumatology (Oxford) 54:1488. https://doi.org/10.1093/rheumatology/keu532
18. Diehl SA et al (2008) STAT3-mediated up-regulation of BLIMP1 is coordinated with BCL6 down-regulation to control human plasma cell differentiation. J Immunol 180:4805–4815
19. Jin H, Carrio R, Yu A, Malek TR (2014) Distinct activation signals determine whether IL-21 induces B cell costimulation, growth arrest, or Bim-dependent apoptosis. J Immunol 173:657–665
20. Ding BB, Bi E, Chen H, Yu JJ, Ye BH (2013) IL-21 and CD40L synergistically promote plasma cell differentiation through upregulation of Blimp-1 in human B cells. J Immunol 190:1827. https://doi.org/10.4049/jimmunol.1201678
21. Pene J et al (2014) Cutting edge: IL-21 is a switch factor for the production of IgG1 and IgG3 by human B cells. J Immunol 172:5154–5157
22. Iwata Y et al (2011) Characterization of a rare IL-10-competent B-cell subset in humans that parallels mouse regulatory B10 cells. Blood 117:530. https://doi.org/10.1182/blood-2010-07-294249
23. Yoshizaki A et al (2012) Regulatory B cells control T-cell autoimmunity through IL-21-dependent cognate interactions. Nature 491:264. https://doi.org/10.1038/nature11501
24. Peluso I et al (2014) IL-21 counteracts the regulatory T cell-mediated suppression of human CD4+ T lymphocytes. J Immunol 178:732–739
25. Wei L, Laurence A, Elias KM, O'Shea JJ (2007) IL-21 is produced by Th17 cells and drives IL-17 production in a STAT3-dependent manner. J Biol Chem 282:34605. https://doi.org/10.1074/jbc.M705100200
26. Zeng R et al (2005) Synergy of IL-21 and IL-15 in regulating CD8 + T cell expansion and function. J Exp Med 201:139. https://doi.org/10.1084/jem.20041057
27. Sutherland APR et al (2013) IL-21 promotes CD8+ CTL activity via the transcription factor T-bet. J Immunol 190:3977. https://doi.org/10.4049/jimmunol.1201730
28. Alves NL, Arosa FA, van Lier RAW (2014) IL-21 sustains CD28 expression on IL-15-activated human naive CD8+ T cells. J Immunol 175:755–762
29. Casey KA, Mescher MF (2007) IL-21 promotes differentiation of naive CD8 T cells to a unique effector phenotype. J Immunol 178:7640–7648
30. Attridge K et al (2012) IL-21 inhibits T cell IL-2 production and impairs Treg homeostasis. Blood 119:4656. https://doi.org/10.1182/blood-2011-10-388546
31. Gowda A et al (2010) Differential effects of IL-2 and IL-21 on expansion of the CD4 +CD25+Foxp3+ T regulatory cells with redundant roles in natural killer cell mediated antibody dependent cellular cytotoxicity in chronic lymphocytic leukemia. MAbs 2:35–41
32. Kim-Schulze S, Kim HS, Fan Q, Kim DW, Kaufman HL (2009) Local IL-21 promotes the therapeutic activity of effector T cells by decreasing regulatory T cells within the tumor microenvironment. Mol Ther 17:380–388
33. Kannappan V et al (2017) Interleukin 21 inhibits cancer-mediated FOXP3 induction in naïve human CD4 T cells. Cancer Immunol Immunother 66:637. https://doi.org/10.1007/s00262-017-1970-6
34. Thedrez A et al (2009) IL-21-mediated potentiation of antitumor cytolytic and proinflammatory responses of human V 9V 2 T cells for adoptive immunotherapy. J Immunol 182:3423–3431
35. Bansal RR, Mackay CR, Moser B, Eberl M (2012) IL-21 enhances the potential of human γδ T cells to provide B-cell help. Eur J Immunol 42:110. https://doi.org/10.1002/eji.201142017
36. Caccamo N et al (2012) IL-21 regulates the differentiation of a human γδ T cell subset equipped with B cell helper activity. PLoS One 7:e41940. https://doi.org/10.1371/journal.pone.0041940
37. Barjon C et al (2017) IL-21 promotes the development of a CD73-positive Vγ9Vδ2 T cell regulatory population. Oncoimmunology 7:1–11
38. Brandt K, Bulfone-Paus S, Foster DC, Rückert R (2003) Interleukin-21 inhibits dendritic cell activation and maturation. Blood 102:4090. https://doi.org/10.1182/blood-2003-03-0669
39. Stolfi C et al (2011) Involvement of interleukin-21 in the regulation of colitis-associated colon cancer. J Exp Med 208:2279–2290
40. De Simone V et al (2015) Interleukin-21 sustains inflammatory signals that contribute to sporadic colon tumorigenesis. Oncotarget 6:9908
41. Ma H-L et al (2003) IL-21 activates both innate and adaptive immunity to generate potent antitumor

responses that require Perforin but are independent of IFN-gamma. J Immunol 171:608–615
42. Di Carlo E et al (2014) IL-21 induces tumor rejection by specific CTL and IFN- -dependent CXC chemokines in syngeneic mice. J Immunol 172:1540–1547
43. De Totero D et al (2006) Interleukin-21 receptor (IL-21R) is up-regulated by CD40 triggering and mediates proapoptotic signals in chronic lymphocytic leukemia B cells. Blood 107:3708–3715
44. Gelebart P et al (2009) Interleukin-21 effectively induces apoptosis in mantle cell lymphoma through a STAT1-dependent mechanism. Leukemia 23:1836–1846
45. Timmerman JM et al (2012) A phase I dose-finding trial of recombinant interleukin-21 and rituximab in relapsed and refractory low grade B-cell lymphoproliferative disorders. Clin Cancer Res 18:5752. https://doi.org/10.1158/1078-0432.CCR-12-0456
46. Bhatia S et al (2014) Recombinant interleukin-21 plus sorafenib for metastatic renal cell carcinoma: a phase 1/2 study. J Immunother Cancer 2:1–11
47. Petrella TM et al (2012) Interleukin-21 has activity in patients with metastatic melanoma: a phase II study. J Clin Oncol 30:3396. https://doi.org/10.1200/JCO.2011.40.0655

ST2 Signaling in the Tumor Microenvironment

7

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Abstract

Suppression of tumorigenicity 2 (ST2), also known as interleukin-1 receptor-like 1 (IL1RL1), is one of the natural receptors of IL-33. Three major isoforms, ST2L (transmembrane form), sST2 (soluble form), and ST2V, are generated by alternative splicing. Damage to stromal cells induces necrosis and release of IL-33, which binds to heterodimeric ST2L/IL-1RAcP complex on the membrane of a variety of immune cells. This IL-33/ST2L signal induces transcription of the downstream inflammatory and anti-inflammatory genes by activating diverse intracellular kinases and factors to mount an adequate immune response, even in tumor microenvironment. For example, activation of IL-33/ST2L signal may trigger Th2-dependent M2 macrophage polarization to facilitate tumor progression. Notably, sST2 is a soluble form of ST2 that lacks a transmembrane domain but preserves an extracellular domain similar to ST2L, which acts as a "decoy" receptor for IL-33. sST2 has been shown to involve in the inflammatory tumor microenvironment and the progression of colorectal cancer, non-small cell lung cancer, and gastric cancer. Therefore, targeting the IL-33/ST2 axis becomes a promising new immunotherapy for treatment of many cancers. This chapter reviews the recent findings on IL-33/ST2L signaling in tumor microenvironment, the trafficking mode of sST2, and the pharmacological strategies to target IL-33/ST2 axis for cancer treatment.

Keywords

ST2 · ST2L · sST2 · IL-33 · Alternative splicing · Vesicle trafficking · Inflammatory cytokines/chemokines · Inflammatory gene transcription · Th2 lymphocytes · Macrophage polarization · Tumor microenvironment · Anti-IL33 neutralizing antibody · ST2 neutralizing antibody · sST2 recombinant protein · Immunotherapy

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Abbreviations

IL-33	Interleukin-33
NF-kB	Nuclear factor kappa-B
NSCLC	Non-small cell lung cancer
sST2	Soluble ST2
ST2	Suppression of tumorigenicity 2/ interleukin-1 receptor-like 1
ST2L	Transmembrane form of ST2
TAM	Tumor-associated macrophages
TLRs	Toll-like receptors
TME	Tumor microenvironment
Treg	Regulatory T cells

7.1 Introduction

7.1.1 Gene Structure and Variants of Suppression of Tumorigenicity 2 (*ST2*) Gene

7.1.1.1 Mouse *ST2* Gene

Suppression of tumorigenicity 2 (ST2) protein is a member of the interleukin-1 receptor-like 1 (IL1RL1) family. The *ST2* gene (Mus musculus) is located at chromosome 1 B|1 19.19 cM and has three transcript variants [41]. The variant 1 is the longest transcript and encodes the longer isoform representing the transmembrane form of ST2 (ST2L). The variant 1 coding region (1704 nt) encodes a polypeptide that is 567 aa in length. The variant 2 is the soluble form of ST2 (sST2) which has a distinct C-terminus and is shorter than the variant 1. The variant 2 coding region (1014 nt) encodes a polypeptide that is 337 aa in length. The variant 2 lacks several exons, and its 3′ terminal exon extends past a splice site used in variant 1, which results in a novel 3′ coding region and 3′ UTR compared to variant 1 [41]. The variant 3 contains an alternate 5′ terminal exon, and its 3′ terminal exon extends past a splice site that is used in variant 1. The coding region of the variant 3 gene is the same as variant 2. Therefore, variants 2 and 3 encode the same isoform (Fig. 7.1a).

7.1.1.2 Human *ST2* Gene

The *ST2* gene (*Homo sapiens*) is located at chromosome 2q12.1 which contains three transcript variants [30]. The variant 1 is the longest transcript and encodes the longer isoform representing the transmembrane form of ST2, ST2L. The variant 1 coding region (1671 nt) encodes a polypeptide that is 556 aa in length. The coding region (987 nt) of the variant 2 encodes a polypeptide that is 328 aa in length. The variant 2 possesses a distinct C-terminus. The variant 2 has an alternate 5′ UTR exon and lacks several 3′ terminal exons, and its transcription extends past a splice site that is used in variant 1, resulting in a novel 3′ coding region and 3′ UTR. The variant 3 has multiple distinct 5′ and 3′ UTRs, leading to translation initiation at a downstream start codon and translation termination at a different stop codon. As a result, the variant 3 has a shorter N-terminus and a shorter and distinct C-terminus. The variant 3 coding region (636 nt) encodes a polypeptide that is 211 aa in length, making it the shortest one. The variants 2 and 3 with overlapping coding region both produce the soluble form of ST2, sST2 (Fig. 7.1b).

In humans, there is an additional variant named ST2V which is produced by alternative splicing [45]. ST2V is characterized by the absence of an immunoglobulin-like motif and alternative splicing of the C-terminal portion of *ST2* inserting a new exon [31]. The only difference in the nucleotide sequences between the new clone ST2V and the original human ST2 clone is the presence of 170 bp in the *ST2V* cDNA that was inserted at the end of the *ST2* cDNA. Schematic representation of the structure of ST2 and ST2V protein and the shaded area indicates the distinct tail of the ST2V (Fig. 7.1c).

Interestingly, in the course of cloning the ST2 and ST2L cDNAs, Iwahana and associates identified a novel chicken variant cDNA named ST2LV, which lacked the transmembrane domain of ST2L. The chicken *ST2* gene products have signal peptides at the N-terminal ends but lack the hydrophobic region corresponding to the transmembrane domain. As a result, ST2LV is a

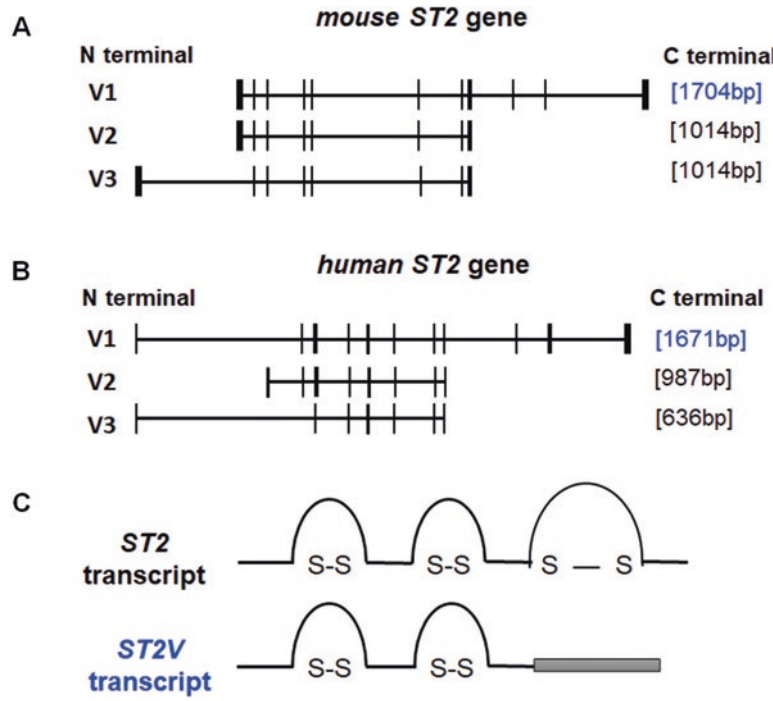

Fig. 7.1 The *ST2* gene map of three transcript variants. (**a**) The *ST2* gene (Mus musculus) contains three transcript variants. The variant 1 represents the transmembrane form of the *ST2* gene and encodes the longer isoform known as ST2L. The variants 2 and 3 encode the soluble form of ST2 protein also named sST2. (**b**) The *ST2* gene (*Homo sapiens*) contains three transcript variants. The variant 1 encodes the longer isoform with the transmembrane domain known as ST2L. The variants 2 and 3 encode the soluble form of ST2 protein also named sST2. The left of the figure is the N-terminal, the right is the C-terminal, and the vertical bars represent the exons. The figures are adapted and modified from NCBI. (**c**) Schematic representation of the structure of ST2 and ST2V protein. The shaded area indicates the distinct tail of the ST2V. (The figures are adapted and modified from Tominaga et al. [31])

new secreted soluble and N-glycosylated variant of the ST2 gene product [15].

7.1.2 Transmembrane ST2L Signaling in Tumor Microenvironment

7.1.2.1 ST2L Ligand: Interleukin-33 (IL-33)

ST2L is a membrane-bound receptor, and interleukin 33 (IL-33) is the functional ligand for ST2L. IL-33 is a member of the IL-1 family of cytokines that is expressed in multiple organs and cell types in humans and mice [18, 24]. IL-33 is predominantly a pro-inflammatory cytokine and is induced in response to various stimuli in epithelial cells, myofibroblasts, adipocytes, endothelial cells, smooth muscle cells, and macrophages [12, 23]. IL-33 is a 30-kDa protein that has a dual role; it functions as a transcription factor and a cytokine. Its N-terminus contains a nuclear localization signal, a DNA-binding homeodomain-like helix-turn-helix motif, and a chromatin binding domain, while the C-terminus contains an IL-1-like cytokine domain [26]. IL-33 is quickly released from the nucleus of necrotic cells and secreted into extracellular space in response to cellular damage, tissue injury, or viral infection. It can bind to the membrane-bound ST2L receptor through its cytokine domain and trigger the downstream IL-33/ST2L signaling [4, 44]. On the other hand, sST2 negatively regulates IL-33/ST2 signaling by acting as a decoy receptor and sequestering IL-33 to block its interaction with ST2L [13], which is described in detail in the next section.

7.1.2.2 IL-33/ST2L Signaling in Promoting an Adequate Immune Response

IL-33/ST2L signaling often leads to an inflammatory gene transcription and ultimately to the production of inflammatory cytokines/chemokines and an adequate immune response [24]. Damage to stromal cells induces necrosis and release of full-length IL-33 (active IL-33) which activates the heterodimeric ST2L/IL-1RAcP complex on the membrane of a variety of immune cells. Upon activation of the ST2L/IL-1RAcP complex, signaling through the Toll-IL-1 receptor (TIR) domain of IL-1RAcP is induced. By activating diverse intracellular kinases and factors, including MyD88, IRAK-1, IRAK-4, and TRAF-6, IL-33/ST2L induces transcription of the downstream genes via NF-kB, p-38, JNK, and ERK [9]. This leads to an inflammatory gene transcription and ultimately to the production of inflammatory cytokines/chemokines and mounting of an adequate immune response. Notably, the IL-33/ST2L signaling and immune response can be neutralized by sST2, which acts as a "decoy" receptor for IL-33 (Fig. 7.2).

7.1.2.3 IL-33/ST2L Signaling in Promoting M2 Macrophage Polarization

Naive monocytes can differentiate into M1 and M2 macrophages in response to various stimuli [39]. Generally, tumor-associated macrophages (TAMs) acquire an M2-polarized phenotype, promote angiogenesis and metastasis, and suppress adaptive immunity through production of cytokines, chemokines, growth factors, and matrix metalloproteases [7, 19]. IL-33 has been proposed to promote M2 but not M1 macrophage polarization through inducing Th2 responses [14, 29, 40]. IL-33 enhances the production of both pro- and anti-inflammatory cytokines in ST2L-expressing inflammatory cells, including Th2

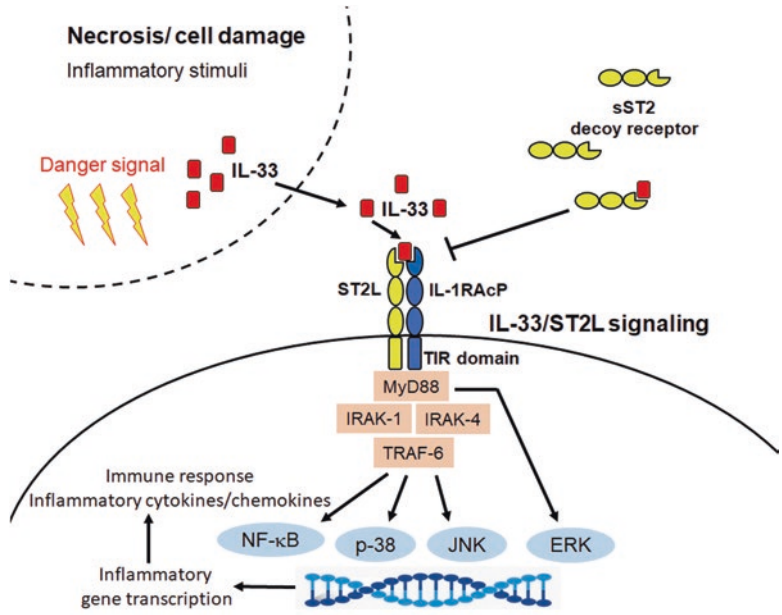

Fig. 7.2 Interleukin-33/ST2L signaling. Damage to stromal cells can induce necrosis and release of full-length IL-33 (active IL-33) which activates the heterodimeric ST2L/IL-1RAcP complex on a variety of immune cells or can be neutralized by sST2, which acts as a "decoy" receptor for IL-33. Activation of the ST2L/IL-1RAcP complex leads to the induction of Toll-IL-1 receptor domain (TIR) signaling which in turn activates several intracellular kinases and factors resulting in inflammatory gene transcription and subsequently the production of inflammatory cytokines/chemokines and mounting of an adequate immune response. (The figure is adapted and modified from Miller (2011) [48])

lymphocytes and mast cells, primarily via NF-kB signaling [5, 21]. Evidences have shown that IL-33 can promote the shift of M1 macrophages to M2 lineage or amplify the development of M2 macrophages which play a part in type 2 immune response during tissue wound healing and airway inflammation [3, 17]. Furthermore, most data available indicate that macrophages can respond to IL-33 by upregulating the expression of IL-13 and arginase, creating an autocrine M2 polarization loop and increasing Th2 polarization, which ultimately leads to an anti-inflammatory response [27, 42] (Fig. 7.3a).

While the direct interaction of IL-33 and ST2L on macrophages in promoting M2 macrophage polarization in tumor microenvironment (TME) remains to be explored, we have learned from studies on the central nervous system that IL-33 is released rapidly after injury by cells of the central nervous system and contributes to the activation of immune responses in lesion areas [10]. IL-33 promotes microglia ST2 expression, which suppresses neuronal damage by promoting anti-inflammatory cytokine IL-10 expression after inducing microglial polarization toward the M2 phenotype, which is a beneficial macrophage to protect neuronal cell death [43]. Notably, downregulation of ST2 by interference RNA promotes M1 microglia polarization even in the presence of high level of IL-33, suggesting that ST2 expression is required for IL-33-induced M2 microglia polarization [43].

Based on these reports, the IL-33/ST2L signaling and the Th2-related cytokine expression may cooperatively induce macrophage M2 polarization. Therefore, IL-33 can enhance and activate the Th2 cell differentiation and further increase the expression of Th2-associated cytokines such as IL-4, IL-10, and IL-13, subsequently triggering the macrophage M2 polarization in TME. It remains unclear whether IL-33 activates polarization of M2 macrophages by directly binding to the membrane-attached heterodimeric ST2L/IL-1RAcP complex [28], and the mechanism needs to be elucidated (Fig. 7.3a).

7.1.3 Role of Soluble sST2 and Its Downstream Effectors in Cancer Cells

7.1.3.1 Trafficking and Secretion Mode of sST2 in Cancer Cells

sST2 is a soluble form of ST2 that is secreted as a glycosylated protein. It lacks the transmembrane domain but contains an extracellular domain similar to ST2L and an additional nine amino acids at the C-terminal tail [18]. Although sST2 has long been known as a secreted protein, its trafficking mode has never been defined.

Our previous study has shown that Rab37 mediates intracellular vesicle trafficking from *trans*-Golgi network to plasma membrane [32]. Rab37 belongs to the Ras superfamily of small GTPases which have been recognized as crucial regulators of membrane trafficking including endocytosis and exocytosis [46, 47]. Rabs function as small GTPases that switch between active (GTP-bound form) and inactive (GDP-bound form) to turn on and off distinct trafficking process. Rab37 mediates the secretion of several antitumor cargoes in a GTP-dependent manner to inhibit tumor progression. We found that Rab37 regulates the exocytosis of tissue inhibitor of metalloproteinase 1 (TIMP1), which inhibits the activity of MMP9, to suppress migration, invasion, and metastasis of lung cancer cells [32]. Moreover, thrombospondin-1 (TSP1), which inhibits angiogenesis of stromal endothelial cells in the tumor microenvironment, is also identified as a cargo protein of Rab37-mediated exocytosis [33]. In addition, we found that Rab37-mediated secreted frizzled-related protein 1 (SFRP1) suppresses cancer stemness properties. Low Rab37, low SFRP1, and high Oct4 stemness protein expression can predict poor prognosis in lung cancer patients [6]. However, the role of Rab37 in immune cells still remains unclear, and a limited number of studies investigated the effect of Rab37 in the tumor microenvironment. Thus, it is important to explore more Rab37-regulated trafficking mechanism in immune cells that affect tumor progression.

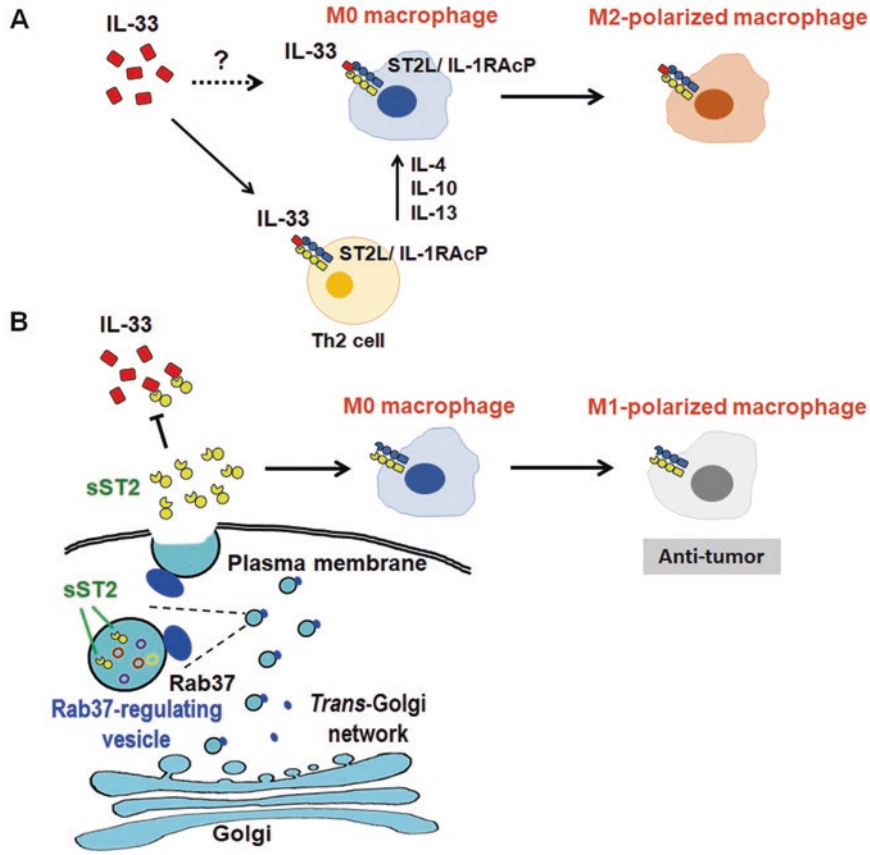

Fig. 7.3 Rab37 mediates the exocytosis of sST2 to inhibit M2-specific macrophage polarization in tumor microenvironment. (**a**) IL-33 has been proposed to promote M2 polarization through inducing Th2 responses. The expression of Th2-associated cytokines such as IL-4, IL-10, and IL-13 is increased by IL-33 to polarize macrophage toward M2 phenotype in the tumor microenvironment. It remains to be elucidated whether IL-33 activates polarization of M2 macrophages through direct binding to the membrane-attached heterodimeric ST2L/IL-1RAcP complex on macrophages. (**b**) GTP-bound Rab37 mediates exocytosis of sST2 to extracellular compartment to act as a decoy receptor to interrupt the IL-33/ST2L signaling. Downregulation of IL-33 signal on microenvironmental macrophages suppresses M2-specific polarization and elevates the population of M1-polarized macrophages, thus shaping an antitumor microenvironment

We have shown that Rab37 mediates the crosstalk between cancer cells and their surrounding immune cells such as macrophages via exocytosis of sST2 [34]. To investigate whether Rab37 regulates macrophage infiltration and polarization through exocytosis of cargo protein secretion to educate macrophages, we collected conditioned media (CM) from Rab37-manipulated non-small cell lung cancer (NSCLC) cells and added the CM to the culture media of macrophages. Interestingly, CM from Rab37-WT NSCLC cells suppressed M2-specific gene expression including *arginase, Fizz1*, and *IL-10* in macrophages, while CM derived from Rab37-knockdown NSCLC cells skewed macrophage polarization toward M2 phenotypes. To identify the potential cargo proteins of Rab37-mediated exocytosis, we collected CM from Rab37-overexpressed (WT) and Rab37-knockdown (shRab) cancer cells to perform cytokine/chemokine array. Among 102 proteins spotted on the array membranes, the level of sST2 increased in CM from Rab37-WT cancer cells compared to that from EV control group, while sST2 expression decreased in CM from shRab cancer cells.

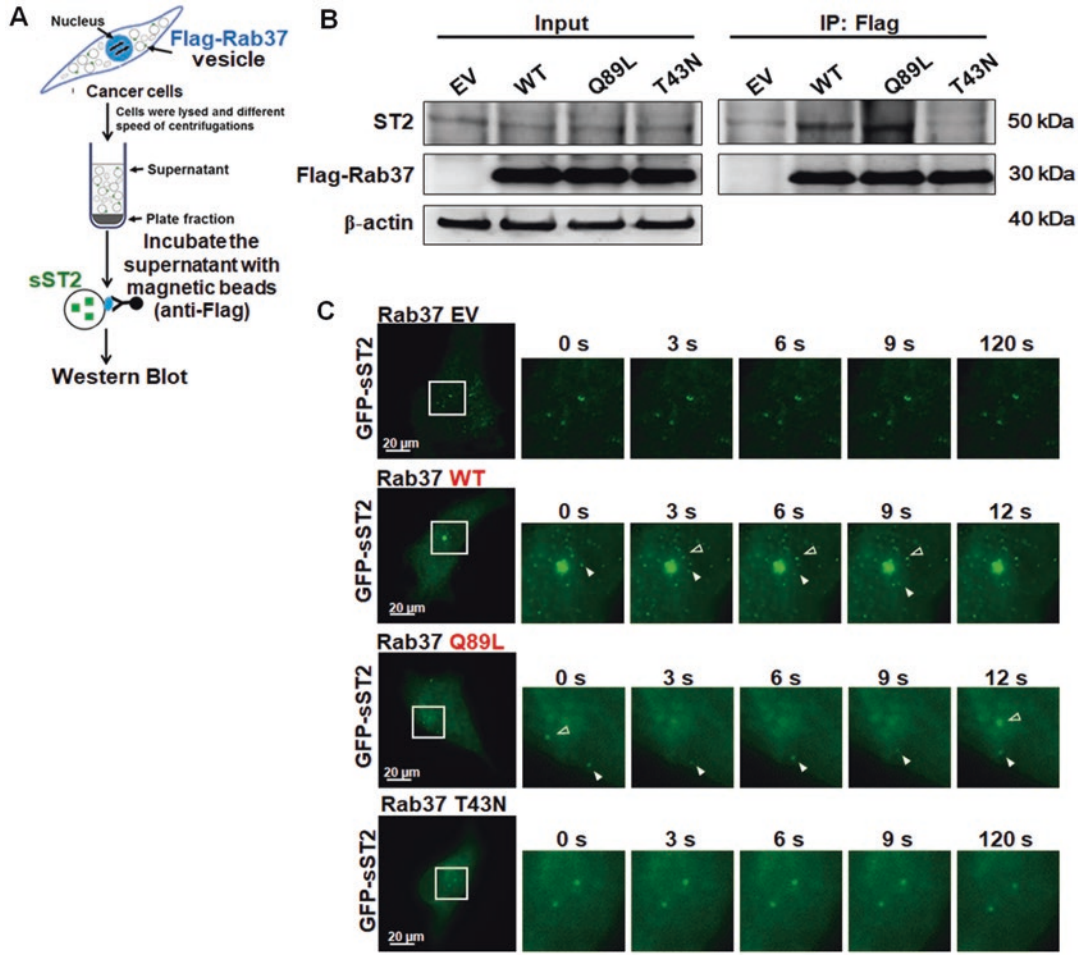

Fig. 7.4 sST2 is secreted by Rab37 at the GTPase nucleotide-dependent manner. (**a**) Vesicles of Flag-tagged Rab37 cancer cells were enriched by serial centrifugations. The vesicle-containing supernatants were immunoprecipitated (IP) with anti-Flag beads, and lysates were subjected to Western blotting. (**b**) Vesicles of Flag-tagged Rab37 EV (empty vector), WT (wild-type), Q89L (GTP-bound active form), and T43N (GDP-bound inactive form) PC-14 lung cancer cells were isolated and blotted for Flag-tagged Rab37 and endogenous sST2. Western blot confirmed that ST2 proteins were enriched in WT- and Q89L-specific vesicles. (**c**) Selected frames from time-lapse clips of total internal reflection fluorescence (TIRF) images of EV, WT, Q89L, and T43N PC-14 transfected with GFP-tagged ST2 to detect real-time sST2 trafficking within 100 nm below plasma membrane. Enlarged images of the boxed areas from time-lapse movies with time intervals in seconds are shown (right). Filled triangle indicates horizontal movement of GFP-ST2, and open triangle indicates vertical movement. Scale bar: 20 μm

To confirm that ST2 is a cargo protein of Rab37-mediated exocytosis, we enriched vesicles in EV, WT, GTP-bound active Rab37 (Q89L), or GTP-bound inactive Rab37 (T43N) cells with anti-Flag antibody and performed Western blotting to detect ST2 level in Rab37-speicific vesicles (Fig. 7.4a). The results show that ST2 level elevates in vesicles from WT and Q89L cells while decreases in those from T43N group (Fig. 7.4b). To detect the effects of Rab37 on sST2 transportation in real-time visualization, we observed Rab37-manipulated cancer cells with total internal reflection fluorescence (TIRF) microscopy, which provides visualization of fluorescent signal within 100 nm below plasma membrane. NSCLC cells that were transfected with GFP-tag sST2 expressed fluorescence-labeled vesicles under the field of microscope.

The fluorescence-labeled vesicles in Rab37-WT and Q89L overexpressed NSCLC cells shifted rapidly in horizontal and vertical directions, while those in T43N group barely moved (Fig. 7.4c). In this study, we reveal for the first time the trafficking mode of sST2.

7.1.3.2 sST2: A Decoy Receptor in Tumor Microenvironment

sST2 is shown to be involved in the inflammatory TME and the progression of colorectal cancer (CRC) [1]. The expression level of sST2 is inversely associated with the malignant growth of CRC. sST2 is downregulated in high-metastatic cells compared with low-metastatic human and mouse CRC cells. Knockdown of sST2 in low-metastatic cells enhances tumor growth, metastasis, and neo-angiogenesis, whereas overexpression of sST2 in high-metastatic cells suppresses these processes. Notably, sST2 suppresses IL-33-induced angiogenesis, Th1 and Th2 responses, macrophage infiltration, and macrophage M2a polarization. Therefore, the IL-33/ST2L axis may be a potential therapeutic target in CRC [1].

The role of sST2 expression in the TME is also studied in gastric cancer [2]. The protein levels of sST2 and ST2V are markedly decreased in cancer tissues compared with noncancerous tissues using Western blot analysis of 12 gastric cancer specimens and paired adjacent tissues. Interestingly, decreased ST2 expression is significantly associated with advanced tumor node metastasis stage and tumor differentiation, suggesting that ST2 protein may be a valuable biomarker of gastric cancer progression and pathogenesis [2].

Our group has reported that Rab37 small G protein regulates the exocytosis of sST2 from lung cancer epithelial cells to skew macrophages toward M1 tumor-suppressive phenotype. Loss of Rab37 or sST2 abrogates M1 polarization of macrophages and tumor growth suppression. Clinically, the expression profile of low Rab37, low ST2, and low ratio of M1/M2 macrophages correlates with poor overall survival based on immunohistochemical staining result of tumor specimens in 122 lung cancer patients. Our finding of Rab37-mediated sST2 secretion in cancer cells provides the missing link for the crosstalk between cancer epithelial cells and immune cells in TME to inhibit pro-tumor M2 macrophage polarization and suppress tumor propagation. Secreted sST2 skews macrophage polarization toward antitumoral M1-like phenotype in vitro. In addition, Rab37-mediated sST2 secretion significantly increases the ratio of M1 vs. M2 in tumor specimens from lung cancer patients and thus reduces tumor growth [34] (Fig. 7.3b). Collectively, sST2, the decoy receptor in TME, is an important target for cancer therapy and the biomarker for cancer prognosis.

7.1.4 Targeting IL-33/ST2 Axis for Cancer Therapy

IL-33/ST2 axis plays a dual role in tumor development in a context-dependent manner. This axis is able to modulate tumor-associated immunity by remodeling the TME to either enhance the recruitment of immunosuppressive cells to create a tumor-promoting environment or enrich natural killer and cytotoxic T cells to suppress tumor growth. Thus, targeting the IL-33/ST2 axis becomes a promising immunotherapy in treatment of cancers. The widely used pharmacological strategies to target IL-33/ST2 axis are anti-IL-33 and anti-ST2 blocking antibodies, recombinant IL-33, and soluble ST2.

7.1.4.1 Treatment via Enhancing an Antitumor Immunity

There are several studies demonstrating a reduction of tumor growth by administration of these blocking antibodies in mouse models. For example, one study has delineated that IL-33 blockade using either anti-IL-33 or anti-ST2 antibodies restricts tumor growth, dampens M2 polarization of TAMs, and reduces regulatory T cell (Treg) accumulation in tumor sites, suggesting a promising treatment against NSCLC [36]. In head and neck squamous cell carcinoma, the level of IL-33 is known to be dramatically upregulated in advanced tumor stage and is positively correlated with increased infiltration of Tregs in tumor tis-

sues. Blockage of IL-33/ST2 signaling by anti-ST2 monoclonal antibodies reduces the population of Tregs as well as their functionalities to suppress the antitumor immunity in the TME and tumor growth [37]. In a 4T1 breast cancer mouse model, blockage of ST2 by anti-ST2 antibodies suppresses accumulation and function of myeloid-derived suppressor cells and hence inhibits tumor growth [38].

On the other hand, IL-33/ST2 signaling also shows a tumor-suppressing characteristic in certain cancers such as melanoma. A study showed that exogenous inoculation of IL-33 induces strong antitumor immunity via activating $CD8^+$ T cells as well as myeloid dendritic cells in established cancers. Recombinant IL-33 therapy restores the tumor-suppressive function of cytotoxic T cells and increases antigen cross-presentation in the TME, suggesting a prospective use of recombinant IL-33 as a new immunotherapy choice to treat established cancers [8]. In a similar observation, increased production of IL-33 in tumor-bearing mice via a DNA vaccine setting boosts antigen-specific $CD4^+$ and $CD8^+$ T cell responses in human papilloma virus-associated cancer and hepatocellular carcinoma models [16, 35].

7.1.4.2 Treatment via Inducing an Allergic Microenvironment

The signaling of IL-33/ST2 is primarily associated with Th2-related inflammation and is involved in allergic reaction. Significant reduction of eosinophils and Th2-related cytokines is observed in IL-33 or ST2 knockout mice. Blockage of IL-33 or ST2 by neutralizing antibodies attenuates Th2-mediated allergic airway inflammation [17, 22]. This IL-33/ST2-mediated allergic reaction is also induced in response to cancer growth. One study has demonstrated that IL-33 is capable of activating eosinophils to efficiently eliminate melanoma cells, while administration of IL-33 shows a promising treatment against melanoma growth [20]. In a recent report, Perales-Puchalt A et al. revealed that direct peritoneal administration of IL-33 delays the progression of metastatic ovarian cancer. The IL-33-triggered increased recruitment of activated Th2 cells, eosinophils, and peritoneal macrophages is responsible for this IL-33-mediated anticancer activity [25].

In addition to suppression of cancer progression, blockage of IL-33/ST2 signaling may be beneficial for attenuating intestinal damage caused by cancer chemotherapeutic agent. In a CT26 colon carcinoma mouse model, Guabiraba et al. have revealed that IL-33/ST2 axis mediates the severe intestinal mucositis in mice treated with a commonly used chemotherapeutic drug, irinotecan (CPT-11). Treatment with anti-IL-33 antibodies or soluble ST2 reduces mucositis and further ameliorates the therapeutic efficacy of CPT-11 [11].

7.1.5 Conclusion and Perspectives

Collectively, these studies have shed light on the potentiality of IL-33/ST2 axis as a therapeutic target. The immunoregulatory effects of IL-33/ST2 signaling are highly context-dependent and vary in different cell targets, phases of immune response, and experimental models. Therefore, more investigations on the mechanism of targeting IL-33/ST2 axis in treating cancers are still needed.

Disclosure The authors declare no potential conflicts of interests.

References

1. Akimoto M, Maruyama R, Takamaru H, Ochiya T, Takenaga K (2016) Soluble IL-33 receptor sST2 inhibits colorectal cancer malignant growth by modifying the tumour microenvironment. Nat Commun 7:13589
2. Bai F, Ba F, You Y, Feng Y, Tao W, Wu C, Jiu M, Nie Y (2019) Decreased ST2 expression is associated with gastric cancer progression and pathogenesis. Oncol Lett 17(6):5761–5767
3. Care AS, Diener KR, Jasper MJ, Brown HM, Ingman WV, Robertson SA (2013) Macrophages regulate corpus luteum development during embryo implantation in mice. J Clin Invest 123(8):3472–3487
4. Cayrol C, Girard JP (2009) The IL-1-like cytokine IL-33 is inactivated after maturation by caspase-1. Proc Natl Acad Sci U S A 106(22):9021–9026
5. Cayrol C, Girard JP (2014) IL-33: an alarmin cytokine with crucial roles in innate immunity, inflammation and allergy. Curr Opin Immunol 31:31–37

6. Cho SH, Kuo IY, Lu PJ, Tzeng HT, Lai WW, Su WC, Wang YC (2018) Rab37 mediates exocytosis of secreted frizzled-related protein 1 to inhibit Wnt signaling and thus suppress lung cancer stemness. Cell Death Dis 9(9):868
7. Colegio OR, Chu NQ, Szabo AL, Chu T, Rhebergen AM, Jairam V, Cyrus N, Brokowski CE, Eisenbarth SC, Phillips GM, Cline GW, Phillips AJ, Medzhitov R (2014) Functional polarization of tumour-associated macrophages by tumour-derived lactic acid. Nature 513(7519):559–563
8. Dominguez D, Ye C, Geng Z, Chen S, Fan J, Qin L, Long A, Wang L, Zhang Z, Zhang Y, Fang D, Kuzel TM, Zhang B (2017) Exogenous IL-33 restores dendritic cell activation and maturation in established cancer. J Immunol 198(3):1365–1375
9. Ernst O, Vayttaden SJ, Fraser IDC (2018) Measurement of NF-κB activation in TLR-activated macrophages. Methods Mol Biol 1714:67–78
10. Gadani SP, Walsh JT, Smirnov I, Zheng J, Kipnis J (2015) The glia-derived alarmin IL-33 orchestrates the immune response and promotes recovery following CNS injury. Neuron 85(4):703–709
11. Guabiraba R, Besnard AG, Menezes GB, Secher T, Jabir MS, Amaral SS, Braun H, Lima-Junior RC, Ribeiro RA, Cunha FQ, Teixeira MM, Beyaert R, Graham GJ, Liew FY (2014) IL-33 targeting attenuates intestinal mucositis and enhances effective tumor chemotherapy in mice. Mucosal Immunol 7(5):1079–1093
12. Haraldsen G, Balogh J, Pollheimer J, Sponheim J, Küchler AM (2009) Interleukin-33 - cytokine of dual function or novel alarmin? Trends Immunol 30(5):227–233
13. Hayakawa H, Hayakawa M, Kume A, Tominaga S (2007) Soluble ST2 blocks interleukin-33 signaling in allergic airway inflammation. J Biol Chem 282(36):26369–26380
14. Italiani P, Boraschi D (2014) From monocytes to M1/M2 macrophages: phenotypical vs. functional differentiation. Front Immunol 5:514
15. Iwahana H, Hayakawa M, Kuroiwa K, Tago K, Yanagisawa K, Noji S, Tominaga S (2004) Molecular cloning of the chicken ST2 gene and a novel variant form of the ST2 gene product, ST2LV. Biochim Biophys Acta 1681(1):1–14
16. Jin Z, Lei L, Lin D, Liu Y, Song Y, Gong H, Zhu Y, Mei Y, Hu B, Wu Y, Zhang G, Liu H (2018) IL-33 released in the liver inhibits tumor growth via promotion of CD4+ and CD8+ T cell responses in hepatocellular carcinoma. J Immunol 201(12):3770–3779
17. Kurowska-Stolarska M, Stolarski B, Kewin P, Murphy G, Corrigan CJ, Ying S, Pitman N, Mirchandani A, Rana B, van Rooijen N, Shepherd M, McSharry C, McInnes IB, Xu D, Liew FY (2009) IL-33 amplifies the polarization of alternatively activated macrophages that contribute to airway inflammation. J Immunol 183(10):6469–6477
18. Larsen KM, Minaya MK, Vaish V, Peña MMO (2018) The role of IL-33/ST2 pathway in tumorigenesis. Int J Mol Sci 9(19):9
19. Liu M, Sun X, Shi S (2018) MORC2 enhances tumor growth by promoting angiogenesis and tumor-associated macrophage recruitment via Wnt/beta-catenin in lung cancer. Cell Physiol Biochem 51(4):1679–1694
20. Lucarini V, Zicchedda G, Macchia I, La Sorsa V, Peschiaroli F, Buccione C, Sistigu A, Sanchez M, Andreone S, D'Urso MT, Spada M, Macchia D, Afferni C, Mattei F, Schiavoni G (2017) IL-33 restricts tumor growth and inhibits pulmonary metastasis in melanoma-bearing mice through eosinophils. Oncoimmunology. 6(6):e1317420
21. Milovanovic M, Volarevic V, Radosavljevic G, Jovanovic I, Pejnovic N, Arsenijevic N, Lukic ML (2012) IL-33/ST2 axis in inflammation and immunopathology. Immunol Res 52(1–2):89–99
22. Morita H, Arae K, Ohno T, Kajiwara N, Oboki K, Matsuda A, Suto H, Okumura K, Sudo K, Takahashi T, Matsumoto K, Nakae S (2012) ST2 requires Th2-, but not Th17-, type airway inflammation in epicutaneously antigen- sensitized mice. Allergol Int 61(2):265–273
23. Moussion C, Ortega N, Girard JP (2008) The IL-1-like cytokine IL-33 is constitutively expressed in the nucleus of endothelial cells and epithelial cells in vivo: a novel 'alarmin'? PLoS One 3(10):e3331
24. Mueller T, Jaffe AS (2015) Soluble ST2 – analytical considerations. Am J Cardiol 115(7 Suppl):8B–21B
25. Perales-Puchalt A, Svoronos N, Villarreal DO, Zankharia U, Reuschel E, Wojtak K, Payne KK, Duperret EK, Muthumani K, Conejo-Garcia JR, Weiner DB (2019) IL-33 delays metastatic peritoneal cancer progression inducing an allergic microenvironment. Oncoimmunology 8(1):e1515058
26. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, Zurawski G, Moshrefi M, Qin J, Li X, Gorman DM, Bazan JF, Kastelein RA (2005) IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. Immunity 23(5):479–490
27. Schwartz C, O'Grady K, Lavelle EC, Fallon PG (2016) Interleukin 33: an innate alarm for adaptive responses beyond Th2 immunity-emerging roles in obesity, intestinal inflammation, and cancer. Eur J Immunol 46(5):1091–1100
28. Sheng YR, Hu WT, Wei CY, Tang LL, Liu YK, Liu YY, Qiu JP, Li DJ, Zhu XY (2018) IL-33/ST2 axis affects the polarization and efferocytosis of decidual macrophages in early pregnancy. Am J Reprod Immunol 79(6):e12836
29. Sica A, Larghi P, Mancino A, Rubino L, Porta C, Totaro MG, Rimoldi M, Biswas SK, Allavena P, Mantovani A (2008) Macrophage polarization in tumour progression. Semin Cancer Biol 18(5):349–355
30. Tominaga S, Yokota T, Yanagisawa K, Tsukamoto T, Takagi T, Tetsuka T (1992) Nucleotide sequence of a complementary DNA for human ST2. Biochim Biophys Acta 1171(2):215–218
31. Tominaga S, Kuroiwa K, Tago K, Iwahana H, Yanagisawa K, Komatsu N (1999) Presence and expression of a novel variant form of ST2 gene prod-

uct in human leukemic cell line UT-7/GM. Biochem Biophys Res Commun 264(1):14–18
32. Tsai CH, Cheng HC, Wang YS, Lin P, Jen J, Kuo IY, Chang YH, Liao PC, Chen RH, Yuan WC, Hsu HS, Yang MH, Hsu MT, Wu CY, Wang YC (2014) Small GTPase Rab37 targets tissue inhibitor of metalloproteinase 1 for exocytosis and thus suppresses tumour metastasis. Nat Commun 5:4804
33. Tzeng HT, Tsai CH, Yen YT, Cheng HC, Chen YC, Pu SW, Wang YS, Shan YS, Tseng YL, Su WC, Lai WW, Wu LW, Wang YC (2017) Dysregulation of Rab37-mediated cross-talk between cancer cells and endothelial cells via thrombospondin-1 promotes tumor neovasculature and metastasis. Clin Cancer Res 23(9):2335–2345
34. Tzeng HT, Su CC, Chang CP, Lai WW, Su WC, Wang YC (2018) Rab37 in lung cancer mediates exocytosis of soluble ST2 and thus skews macrophages toward tumor-suppressing phenotype. Int J Cancer 143:1753–1763
35. Villarreal DO, Wise MC, Walters JN, Reuschel EL, Choi MJ, Obeng-Adjei N, Yan J, Morrow MP, Weiner DB (2014) Alarmin IL-33 acts as an immunoadjuvant to enhance antigen-specific tumor immunity. Cancer Res 74(6):1789–1800
36. Wang K, Shan S, Yang Z, Gu X, Wang Y, Wang C, Ren T (2017) IL-33 blockade suppresses tumor growth of human lung cancer through direct and indirect pathways in a preclinical model. Oncotarget 8(40):68571–68582
37. Wen YH, Lin HQ, Li H, Zhao Y, Lui VWY, Chen L, Wu XM, Sun W, Wen WP (2019) Stromal interleukin-33 promotes regulatory T cell-mediated immunosuppression in head and neck squamous cell carcinoma and correlates with poor prognosis. Cancer Immunol Immunother 68(2):221–232
38. Xiao P, Wan X, Cui B, Liu Y, Qiu C, Rong J, Zheng M, Song Y, Chen L, He J, Tan Q, Wang X, Shao X, Liu Y, Cao X, Wang Q (2016) Interleukin 33 in tumor microenvironment is crucial for the accumulation and function of myeloid-derived suppressor cells. Oncoimmunology. 5(1):e1063772
39. Xu LL, McVicar DW, Ben-Baruch A, Kuhns DB, Johnston J, Oppenheim JJ, Wang JM (1995) Monocyte chemotactic protein-3 (MCP3) interacts with multiple leukocyte receptors: binding and signaling of MCP3 through shared as well as unique receptors on monocytes and neutrophils. Eur J Immunol 25(9):2612–2617
40. Xuan W, Qu Q, Zheng B, Xiong S, Fan GH (2014) The chemotaxis of M1 and M2 macrophages is regulated by different chemokines. J Leukoc Biol 97(1):61–69
41. Yanagisawa K, Takagi T, Tsukamoto T, Tetsuka T, Tominaga S (1993) Presence of a novel primary response gene ST2L, encoding a product highly similar to the interleukin 1 receptor type 1. FEBS Lett 318(1):83–87
42. Yang Z, Grinchuk V, Urban JF Jr, Bohl J, Sun R, Notari L, Yan S, Ramalingam T, Keegan AD, Wynn TA, Shea-Donohue T, Zhao A (2013) Macrophages as IL-25/IL-33-responsive cells play an important role in the induction of type 2 immunity. PLoS One 8(3):e59441
43. Yang Y, Liu H, Zhang H, Ye Q, Wang J, Yang B, Mao L, Zhu W, Leak RK, Xiao B, Lu B, Chen J, Hu X (2017) ST2/IL-33-dependent microglial response limits acute ischemic brain injury. J Neurosci 37(18):4692–4704
44. Zhao W, Hu Z (2010) The enigmatic processing and secretion of interleukin-33. Cell Mol Immunol 7(4):260–262
45. Zhao Y, De Los Santos FG, Wu Z, Liu T, Phan SH (2018) An ST2-dependent role of bone marrow-derived group 2 innate lymphoid cells in pulmonary fibrosis. J Pathol 245(4):399–409
46. Li G, Marlin MC (2015) Rab family of GTPases. Methods Mol Biol 1298:1–15
47. Stenmark H (2009) Rab GTPases as coordinators of vesicle traffic. Nat Rev Mol Cell Biol 10(8):513–525
48. Miller AM (2011) Role of IL-33 in inflammation and disease. J Inflamm (Lond) 8(1):22

8 IL-36 Signaling in the Tumor Microenvironment

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Abstract

The ability of the immune system to prevent or control the growth of tumor cells is critically dependent on inflammatory processes that lead to the activation, expansion, and recruitment of antitumor effector cells into the tumor microenvironment (TME). These processes are orchestrated by soluble cytokines produced in tissues that alarm local immune surveillance cells (such as dendritic cells, DCs) to mobilize protective antitumor immune populations (B cells, T cells). The interleukin (IL)-36 family of pro-inflammatory cytokines plays an important role in multiple disease processes, ranging from an instigator of autoimmune psoriasis to an initiator of therapeutic immune responses against tumor cells. This chapter will focus on the biologic role of immunomodulatory IL-36 family cytokines in the cancer setting and their potential utility in the design of effective interventional therapies. (127 words)

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8.1 Introduction

The immune system has evolved to quickly mobilize an army of cells that can circulate throughout the body to regulate local or systemic sites of infection or disease. Signals contributed by soluble interleukins (ILs) interacting with their corresponding receptors expressed on immune cells serve to activate, functionally condition, and direct immune cell populations in a disease-relevant manner. Cancer is a disease that progresses in concert with its ability to evade immune surveillance, and, here, interleukins can play key roles as rheostats to either enhance or extinguish protective antitumor responses. This chapter reviews one such family of interleukins, interleukin-36 or IL-36, and their effects within the evolving TME. A brief history on the discovery of IL-36 will first be discussed followed by a closer inspection of various IL-36 family members. Since IL-36 is initially synthesized as a pro-interleukin, we will then discuss recent studies regarding proteolytic activation of IL-36. The

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IL-36 receptor (IL-36R) and the downstream effects of IL-36-mediated IL-36R signaling on various cell populations within the TME and its impact on tumor growth and disease progression will then be reviewed. Finally, we will discuss the relevance of IL-36/IL-36R-targeted interventions as possible cancer immunotherapies.

8.2 IL-1: An Extended Family of Interleukins

Cytokines have been well characterized as orchestrators of inflammation over the past several decades, although the idea that inflammation can be induced by a soluble protein factor initially surprised scientists in the 1950s. Pioneering work by Eli Menkin established that the transfer of cell-free supernatants from leukocytes isolated from rabbits with sterile peritonitis was sufficient to elicit fever in healthy, naïve animals [2]. Paul Beeson further demonstrated that an endotoxin-free protein extract from supernatants of rabbit leukocytes could cause fever [5]. Together, these works laid the foundation for discovery of soluble inflammation-inducing factors called interleukins. The first interleukin to be identified was unsurprisingly designated interleukin-1 (IL-1). IL-1 is a classic pyrogen produced by cells as a response to sterile inflammation [23]. Over the years, IL-1 has been given a number of names including leukocytic pyrogen (LP), endogenous pyrogen, and leukocyte-activating factor (LAF). It was only in 1979 that this pyrogenic factor was given its consensus name of interleukin-1 [21].

The study of mechanisms through which IL-1 causes inflammation has since led to the discovery of other interleukin families, the identification of the inflammasome, the identification of MyD88 as an adaptor protein, and the characterization of the TIR domain that is central to the signaling of both the IL-1 receptor and the Toll-like receptor (TLR) families. IL-1 is produced in two isoforms, i.e., IL-1α and IL-1β. IL-1α is produced in a biologically active form primarily by epithelial cells of the lung, kidney, and liver and by endothelial cells (ECs) and serves as an alarmin. IL-1β, however, is produced primarily by the cells of the immune system in a pro-/full-length form wherein pro-IL-1β demonstrates an inability to even bind the IL-1R. However, proteolytic processing of pro-IL-1β by enzymes like NLRP3 inflammasome-activated caspase-1 enables interaction between the IL-1β and its receptor which facilitates further downstream signaling [23, 37]. Activated IL-1 was one of the earliest pleiotropic immune factors to be identified as it acts at extremely low concentrations to influence the physiology of a wide variety of cells. IL-1 binding to its cognate receptor induces fever, activates the hypothalamic-pituitary-adrenal (HPA) axis, initiates acute phase responses, increases the expression of adhesion molecules in the endothelium, and provides recruiting/survival signals to infiltrating monocytes and macrophages. In addition to mediating signals through its receptor, IL-1α possesses a nuclear localization sequence (NLS), which allows it to translocate into the producer cell nucleus where it may directly impact cellular transcriptional programming [17].

The unprecedented pleiotropic nature of IL-1 led to the identification of pro- and anti-inflammatory cytokines sharing significant sequence homology with IL-1. This set of cytokines constitutes the extended IL-1 superfamily, containing seven receptor agonists (IL-1α, IL-1β, IL-18, IL-33, IL-36α, IL-36β, IL-36γ), three receptor antagonists (IL-1Ra, IL-36Ra/IL-1F5, IL-38), and one anti-inflammatory cytokine (IL-37). Correspondingly, there are 10 different subunits that differentially dimerize to form receptors for IL-1 family cytokines (IL-1R1, IL-1R2, IL-1R3 [IL-1RAcP], IL-1R4 [ST2], IL-1R5 [IL-18Ra], IL-1R6 [IL-1Rrp2, IL-1RL2, IL-36R], IL-1R7 [IL-18Rb], IL-1R8 [TIR8, SIGIRR], IL-1R9 [TIGIRR-2], and IL-1R10 [TIGIRR-1]) [23].

Although many IL-1 family cytokines operationally participate at many levels in regulating immune responses within the setting of cancer, in this chapter, we will focus on the tumor immunobiology of extended IL-1 family members IL-36α, IL-36β, and IL-36γ.

8.3 IL-36: A Family of Cytokines

Genomic mapping showed that IL-1α, IL-1β, and IL-1Ra, the first three members of the IL-1 cytokine family to be identified, were all localized to a short region of 450 kb on human chromosome 2, where they are postulated to represent the product of gene duplication events occurring nearly 350 million years ago [10]. The IL-1 gene locus has since become the subject of many subsequent genomic analyses to identify novel IL-1 homologs. Briefly, two groups independently reported similar discoveries of IL-1 homologs found on chromosome 2. The first report detailed three novel IL-1-like cytokines, designating them IL-1-related protein 1 (IL-1RP1), IL-1RP2, and IL-1RP3 based on their order of discovery [10]. A second report by Sims and colleagues identified four novel IL-1-like proteins, designating them as family of IL-1 (FIL1) FIL1-epsilon, FIL-eta, FIL1-delta, and FIL1-zeta [51]. Through comparison, it was later concluded that indeed FIL1-zeta and IL-1RP1 and FIL1-delta and IL-1RP3 were synonymous proteins. The two studies together described five new members of the IL-1 family. These proteins have since been reclassified using current cytokine nomenclature. An overview of the properties of all IL-36 cytokine family members is presented in Table 8.1.

IL-36 cytokines share several features with other IL-1 family members. Specifically, protein modeling of IL-36α (aka IL-1F6), IL-36β (aka IL-1F8), IL-36γ (aka IL-1F9), and IL-36Ra has confirmed that like IL-1β and IL-1Ra, these molecules share an evolutionarily conserved 12-stranded β-sheet structure [51]. At the genomic level, IL-36 cytokines also contain exactly four exon segments, like other IL-1 family genes. Further, besides sharing similar secondary protein structures and having similar gene segment arrangements, all IL-36 cytokines also demonstrate a 20–35% genetic sequence homology to IL-1, a threshold commonly used to consider inclusion of proteins into larger family units [51]. Therefore, a comparison of genetic and protein sequences places IL-36α, IL-36β, IL-36γ, and IL-36Ra firmly within the IL-1 cytokine superfamily.

8.4 IL-36 Processing

In some cases, IL-36 cytokines, like IL-1α, are secreted and function as unprocessed full-length proteins. Poly(I:C)-treated keratinocytes release full-length IL-36γ following the induction of pyroptosis, a highly inflammatory form of cell death [34]. Unprocessed IL-36α is also found to be secreted from skin in a murine model of psoriasis, where it is able to signal through IL-36R to induce IL-1α expression through a feed-forward loop [36]. However, the vast majority of studies highlight the critical importance of proteolytic processing of IL-36 cytokines for optimal biologic activity.

Table 8.1 The IL-36 family of cytokines

Cytokine (size)	Alternative names	% Homology		Tissues/cells found in
		To IL-1β	To IL-1Rα	
IL-36α (18 kDa)	FIL1-epsilon, IL-1F6	27	30	Spleen, lymph node, thymus, tonsil, bone marrow, T cells, monocytes, B cells
IL-36β (20 kDa)	FIL1-eta, IL-1F8	32	30	Tonsil, bone marrow, placenta, lung, testis, colon, monocytes, B cells
IL-36γ (18 kDa)	IL-1RP2, IL-1 epsilon, IL-1F9	21.2	23.0	Keratinocytes, epithelial cells
IL-36Ra (17 kDa)	IL-1RP3, FIL1-delta, IL-1 delta, IL-1F5	24.6	49.6	Lymph node, thymus, tonsil, brain, placenta, lung, prostate, testis, monocytes, B cells, activated dendritic cells
IL-37 (24 kDa)	IL-1RP1, FIL1-zeta, IL-1F7	24	24.2	Lymph node, thymus, bone marrow, placenta, lung, NK cells, monocytes, activated B cells

One of the hallmark features of pro-inflammatory cytokines is their ability to induce immune cell recruitment/activation when present in even miniscule amounts within tissue sites. At the time of discovery of the IL-36 cytokine family, IL-1 cytokines had already been reported to have EC50 values in the nM range, and it was expected that other IL-1 family members would share this property. However, while initial studies characterizing IL-36/IL-36R signaling confirmed ligand-receptor specificity, they intriguingly suggested that IL-36 cytokines only effectively functioned at much higher concentrations, i.e., in the μM range [19]. Separately, challenges in understanding the antagonist function of IL-36Ra initially reported by the Sims group [55] led to protein sequencing analyses which revealed that the N-terminus of all IL-36 family members contained a consensus cleavage site for methionine aminopeptidases. This suggested a conserved requirement for proteolytic activation prior to IL-36 signaling [56] and that, like IL-1β, IL-36 cytokines require enzymatic cleavage prior to achieving their optimal signaling potential. Specifically, posttranslational cleavage of IL-36 cytokines at the N-terminus preceding an A-X-aspartic acid domain has shown to improve their biologic EC50s from the μM to nM range. Indeed, enzymatic cleavage has been observed to improve the specific activity of IL-36α, IL-36β, and IL-36γ by 3000-, 8000-, and 1500-fold, respectively [56]. Additionally, the truncated forms of IL-36α, IL-36β, and IL-36γ demonstrated improved binding to the IL-36R by 36,000-, 13,000-, and 980-fold, respectively [56]. This improved signaling ability is further demonstrated by the requirement of a molar excess of the antagonist IL-36Ra to inhibit IL-36R signaling when stimulated with truncated/activated IL-36 agonists in Jurkat cells [56]. While most evidence suggests that proteolytic cleavage of IL-36 family cytokines is necessary for optimal signaling efficacy, the enzyme(s) responsible for IL-36 family member activation remains unknown. Nevertheless, several candidate enzymes have been implicated to cleave IL-36 and are briefly discussed below.

8.4.1 Cathepsin S and Cathepsin G

Cathepsin S is a cysteine protease produced by numerous cell types including DCs, epithelial cells, keratinocytes, and macrophages. Cathepsin S expression is markedly increased in human psoriatic skin lesions, which represent sites of high IL-36 production/bioactivity. Among cell types found in the skin, cathepsin S has been reported to be coordinately upregulated with genes downstream of IL-36γ-mediated signaling. This positive feedback relationship is presumed to be necessary for processing existing and/or newly secreted IL-36 under pro-inflammatory conditions [1].

Another cathepsin, cathepsin G, has also been posited to cleave IL-36. Both in vitro *and* in vivo studies in mice and humans demonstrated that cathepsin G cleaves IL-36γ. In vitro, combination of full-length IL-36γ + cathepsin G led to IL-36γ cleavage, and administration of this truncated IL-36γ to a human keratinocyte cell line led to increased secretion of pro-inflammatory CXCL1 and CXCL8 compared to full-length IL-36γ. Administration of cathepsin G to the psoriatic skin of mice increased the expression of truncated IL-36γ [27], and skin eluates from human psoriasis patients produced high levels of biologically active cathepsin G that could cleave IL-36β [28]. Further evidence supporting the ability of cathepsin G to cleave the IL-36 cytokines was shown when peptide antagonists of cathepsin G inhibited the cleavage of IL-36β and IL-36γ [53].

8.4.2 Neutrophil Proteases

It was reported that in the skin an alternate class of protease that may be capable of enzymatically activating IL-36γ are neutrophil-associated protease 3 and elastase [28]. These enzymes are involved in a wide variety of functions from bacterial killing to chemokine processing but appear to play a more prominent role in the cleavage of IL-1 family cytokines, including IL-36 [14, 43]. Antagonism of elastase inhibited the cleavage of

IL-36β and IL-36γ [53]. However, the IL-36 cleavage events mediated by active neutrophil proteases give rise to biologically suboptimal forms of IL-36γ. Interestingly, neutrophil proteases show a superior capacity to cleave/activate the IL-36R antagonist, IL-36Ra [35]. Thus, a range of proteases may be involved in customizing/fine-tuning regulation of the bioactivity of IL-36 family member cytokines in peripheral tissues.

8.5 IL-36 Signaling

The IL-1 cytokine superfamily contains a plethora of ligand-receptor pairs, and the IL-36 family is no different in its use of a unique receptor chain. A novel receptor chain, IL-36R(IL-1R6), in combination with the IL-1 receptor accessory protein (IL-1RAcP), comprises the receptor for the IL-36 cytokine family. Not all cells express the IL-36R under resting state conditions but can be induced to express it upon cell activation by inflammatory stimuli, leading to coordinate responsiveness to all three IL-36 agonists. Analogous to the IL-1R signaling cascade, IL-1RAcP serves as the active signaling subunit of the receptor. Indeed, cells lose their capacity to sense IL-36 when IL-1RAcP is blocked by specific antibodies. Furthermore, the cytoplasmic tail of IL-1RAcP is critical for delivering the IL-36-mediated signal into the cell cytoplasm, with ectopic expression of an IL-1RAcP cytoplasmic tail dominant-negative mutant rendering cells incapable of responding to IL-36 ligands in vitro [55] (Fig. 8.1).

Successful activation of the IL-36R is initiated by binding to IL-36 ligands. Activation of IL-36R initiates the hetero-dimerization of the IL-36R with the IL-1RAcP which can be identified as complexes in immunoprecipitation assays. Formation of IL-36R/IL-1RAcP heterodimers activates the transcription factors NFκB and MAPKs JNK and ERK, as early as 15–30 minutes post-receptor activation [55]. The kinetics of IL-36/IL-36R signaling recapitulates that of IL-1/IL-1R signaling in transfected Jurkat cells [55] likely due to the common requirement of IL-1RAcP in facilitating the signaling cascade. This confers on cells the ability to produce acute phase cytokines IL-6 and IL-8, IFNγ, IL-23, IL-12, IL-18, IL-4, IL-17, and IL-2, among others [59, 60, 62, 63]. Like other IL-1 family cytokines including IL-1α [45] and IL-33 [38], IL-36γ also exhibits an alarmin function due to its induction of antimicrobial peptides including LL37 [33] .

The IL-36R is expressed predominantly on mammalian cells of epithelial origin especially in the skin, GI tract, ovaries, lung, kidney, and lymphoid organs [55]. In epithelial cells, IκBζ, a selective and atypical downstream mediator of NFκB, turns on the transcription of inflammatory mediators including IL-8, IL-36α, IL-36β, and many antimicrobial peptides, downstream of IL-36R signaling [39]. Several different cells of the mammalian immune system have also been reported to express the IL-36R [20, 40, 62, 63]. Given the secretory nature of the IL-36 ligands and the distribution patterns of the IL-36R, assigning an important role for IL-36 cytokines in barrier immunity appears well justified.

8.5.1 IL-36 Receptor Antagonist: IL-36Ra

IL-36Ra is a natural antagonist to IL-36R, sharing significant homology with IL-1Ra, the receptor antagonist to the IL-1R. Although IL-36Ra was initially predicted to inhibit the activating function of IL-36α [19], it has since been shown to antagonize signaling by all IL-36 agonists. IL-36Ra competitively binds the extracellular Ig domains of IL-36R and prevents the activation-induced hetero-dimerization of IL-36R with IL-1RAcP, thereby preventing downstream receptor conformational changes and recruitment of signaling proteins.

IL-36Ra is expressed by many cell types including DCs, B cells, and macrophages, where it appears to serve as a regulatory “rheostat” to control IL-36-mediated inflammation [51]. Of note, IL-36Ra expression is acutely upregulated in LPS-activated dendritic cells (DCs) where it may represent a potent negative regulator of these important antigen-presenting cells and major secretors of IL-36 cytokines [51].

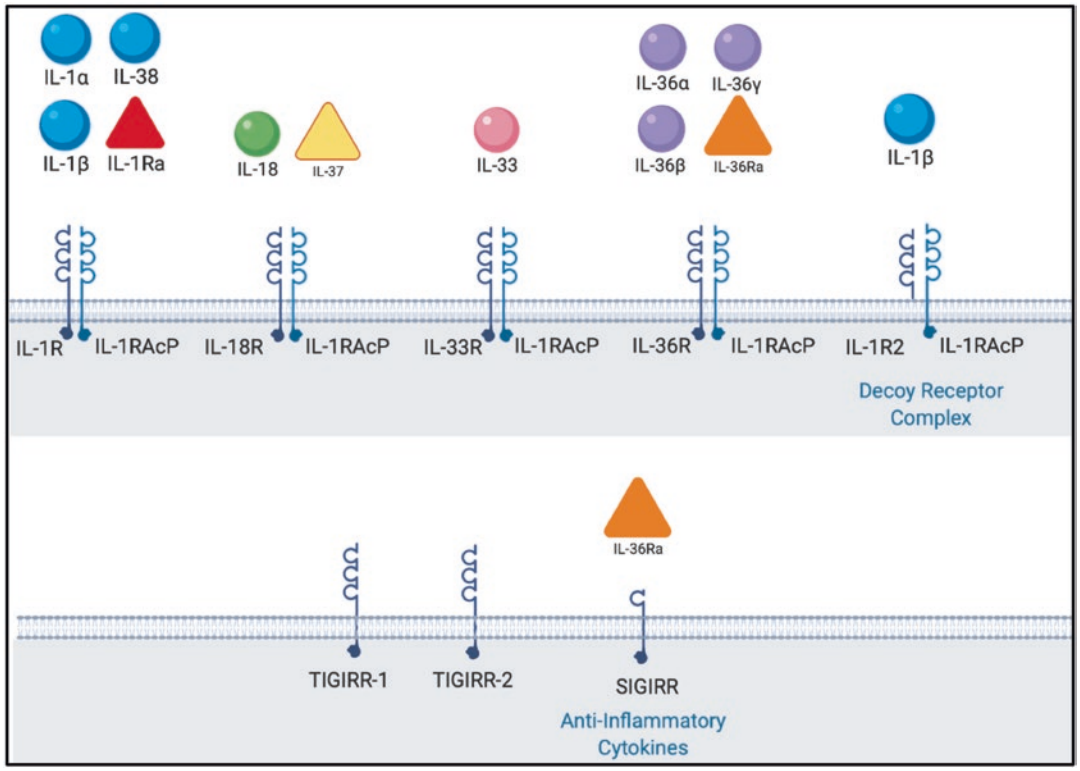

Fig. 8.1 IL-1 family cytokines and their cognate receptors: All IL-1 family receptors are heterodimers composed of a ligand-sensing monomer and a signal transduction monomer. Signal-activating ligands IL-1α, IL-1β, IL-38, IL-18, IL-33, IL-36α, IL-36β, and IL-36γ are represented with circular shapes, while signaling antagonists IL-1Ra, IL-36Ra, and IL-37 are represented with triangular shapes

IL-36Ra suppresses IL-36-mediated inflammation not only by competing for binding domains on the IL-36R but also by binding to other receptors like SIGIRR, which was previously thought to represent an orphan receptor in the IL-1R family. IL-36Ra binding to SIGIRR in brain cells in vitro and in vivo leads to the inhibition of downstream production of IL-6 and IL-8. It also prevents phosphorylation of downstream transcription factors including pJNK. Interestingly, IL-36Ra/SIGIRR binding also acts as a trigger for the production of the immunomodulatory cytokine IL-4 [15]. Curiously, this IL-36Ra-SIGIRR-IL-4 signaling event appears to be isolated to cells in the CNS and has not been observed in the case of hematopoietic cells (i.e., bone marrow-derived DCs and macrophages) [15] or other organ systems (i.e., liver) [49]. This indicates the possibility of multiple independent signaling interactions of IL-36Ra in different compartments of mammalian tissues, suggestive of the context-dependent nature of IL-36-associated biology.

The importance of IL-36Ra is readily apparent in patients with generalized pustular psoriasis (GPP), a lethal skin inflammatory condition. Patients with severe GPP express loss-of-function mutations in the IL-36Ra gene locus. Thus, given the inflammatory presence of IL-36 cytokines in epithelial (skin, gut, etc.) surfaces, a loss of function of the corresponding inflammatory regulator, IL-36Ra, causes dysregulated, severe, and even lethal dermatological conditions which speaks to the necessity for controlled IL-36/IL-36Ra balance in normal tissue homeostasis [42, 44]. In the cancer setting, expression

of IL-36Ra is positively correlated with expression of the immune checkpoint molecules PD-1, PD-L1, and CTLA-4, further supporting its role in regulating inflammation [64].

8.5.2 MyD88: An Important IL-36R Adaptor Protein

Several proteins are involved in the signaling cascade initiated by the IL-36R, but none is more important in this context than the signaling adaptor protein MyD88. RNAseq profiling of IL-36-stimulated keratinocytes revealed the coordinate upregulation/downregulation of unique sets of MyD88-dependent gene products and confirmed that signaling via IL-36 is dependent on MyD88. The profile of up- and downregulated genes revealed that the IL-36 signaling pathway controls the expression of many pro-inflammatory cytokines and chemokines (e.g., components of the interferon signaling pathway, PD-L1, CXCL10, and CXCL11) and is also self-regulatory (i.e., signaling upregulates IL-37 and IL-36Ra) [54]. CRISPR-engineered MyD88 knockout keratinocytes failed to exhibit such gene expression changes after stimulation with IL-36 [54]. This study supports earlier reports in other cell types showing a role for MyD88 in IL-36 signaling. MyD88 is required for NLRP3 inflammasome activation and the secretion of pro-inflammatory cytokines (i.e., IL-1β) downstream of IL-36α signaling in bone marrow-derived macrophages (BMDM). BMDM derived from MyD88-deficient mice are defective in NLRP3 activation or secretion of IL-1β [13]. IL-36R signaling in colonic fibroblasts also depends on MyD88. This pathway leads to the secretion of cytokines, including GM-CSF and IL-6, in support of mucosal tissue healing [48]. Together, these studies support a central role for MyD88 in IL-36R signaling in both hematopoietic and non-hematopoietic cell types. This is not surprising, as MyD88 is the canonical adaptor protein for many IL-1 family receptors. It directly associates with IL-1RAcP, a shared subunit of several IL-1 family receptors [9, 32] (Fig. 8.2).

8.6 IL-36 Biology in the TME

8.6.1 Dendritic Cells

Dendritic cells (DCs) are found in a variety of tissue niches in mammals. In their immature state, they continuously sample the tissue microenvironment for foreign signals/antigens and therefore serve as sentinels for the conditional activation of our protective immune effector cells. DCs employ a range of receptors, including TLRs and cytokine receptors, to sense danger/disruption to tissue homeostasis. An immature DC becomes mature when one or more of these receptors gets activated [18]. The hallmark features of DC maturation include an upregulation in expression of major histocompatibility complex (MHC) molecules, increased expression of co-stimulatory molecules, and increased secretion of (pro-inflammatory) cytokines that serve as "signal 3" for the activation of adaptive immune responses, the nature of which depends on the context of DC activation based on environmental cues (Fig. 8.3).

In this context, all three isoforms of IL-36 have been shown to induce maturation of many subsets of human and mouse DC directly via IL-36R stimulation. In humans, monocyte-derived dendritic cells (MDDCs) express IL-36R and, when stimulated with IL-36β or IL-36γ, undergo maturation as evidenced by increased expression of HLA-DR and CD83 and decreased CD1a expression. IL-36β also increases human MDDC expression of co-stimulatory molecules including CD40 and CD80 and promotes their production of the pro-inflammatory cytokines IL-12 and IL-18, which leads to potent induction of T cell proliferation [40]. Murine monocyte-derived DCs and myeloid DCs both express IL-36R and demonstrate more mature phenotypes following stimulation with IL-36 cytokines, based on increased secretion of cytokines IL-1β and IL-6 and increased expression of surface co-stimulatory markers CD83, HLA-DR, and CD86. IL-36α-treated monocyte-derived murine DCs also exhibit increased capacity to induce the proliferation of alloreactive murine $CD4^+$ T cells [22].

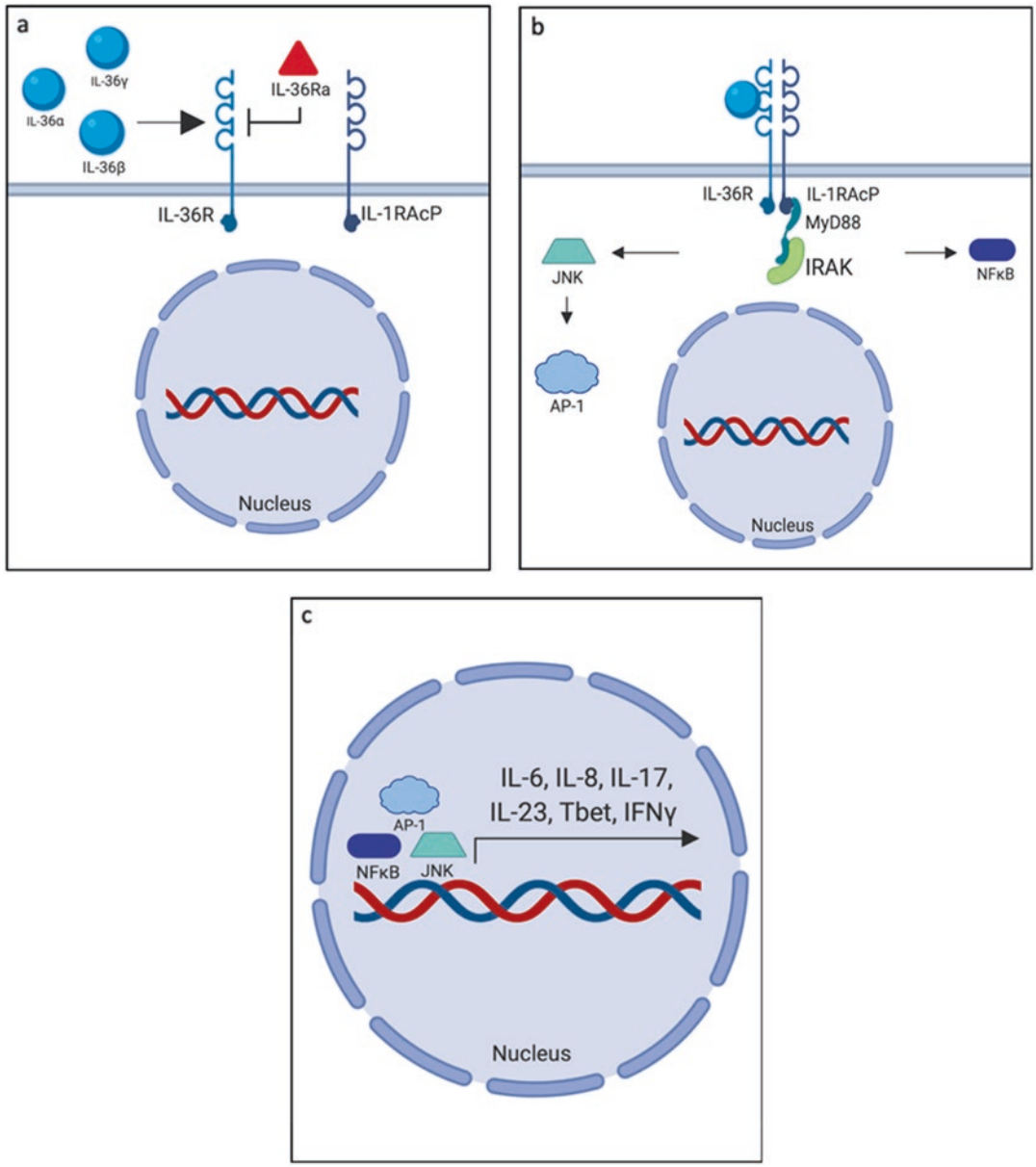

Fig. 8.2 Ligand-dependent activation of IL-36R: (**a**) The IL-36R is a heterodimer composed of a monomer of the ligand-binding IL-36R (IL-1Rrp2) and a signal-initiating IL-1RAcP subunit which do not exist as a dimer under resting state conditions. IL-36 cytokines IL-36α, IL-36β, and IL-36γ have activating roles, while IL-36Ra serves as a competitive receptor antagonist to the IL-36R subunit. (**b**) Upon successful binding of one of the IL-36 cytokines, IL-36R dimerizes with IL-1RAcP which recruits signaling mediators such as MyD88 and IRAKs. Such proximal signaling results in the activation of transcription factors AP-1 and NFkB via activation of MAPKs (JNK) and IKKs, respectively. (**c**) Ligand-induced activation of AP-1 and NFkB leads to the nuclear translocation and subsequent transcription of IL-36-dependent cytokines and transcription factors

Genome-wide association studies in murine DC demonstrated a Tbet binding site in the promoter region of *IL36G*, with Tbet representing a master regulator of type 1 (pro-inflammatory) immunity [3]. Activation of Tbet in DCs plays a central role in the upregulation of genes involved in the promotion of pro-inflammatory Th1 immune responses and coordinate suppression of

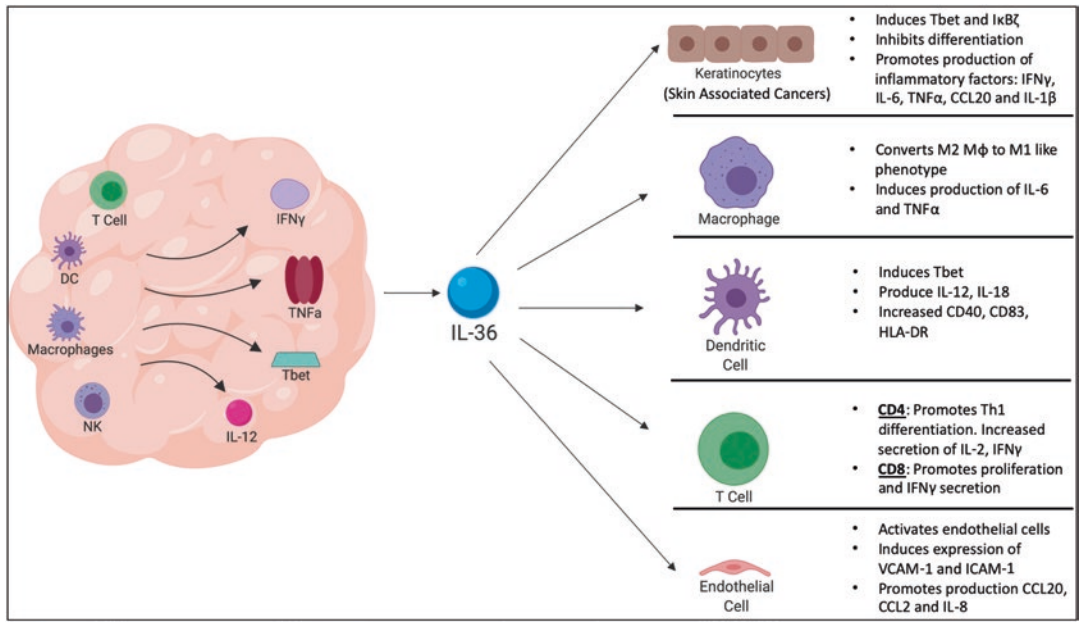

Fig. 8.3 Impact of IL-36 signaling in the TME. While IL-36 responses by immune cells and vascular endothelial cells play an important role in a number of cancer types, responses by keratinocytes [3, 61] are expected to be limited to skin-associated cancers such as in melanoma, basal cell carcinoma, and squamous cell carcinoma

genes involved in Th2 differentiation, suggesting a key role for the IL-36 cytokines in the development of polarized type 1 antitumor response [68]. IL-36R/IL-36γ signaling in DC was subsequently shown to upregulate Tbet, highlighting a novel feed-forward loop downstream of the IL-36R that potentiates type 1 immunity [63].

8.6.2 T Cells

T cells play a central role in the elimination of transformed mammalian cells. Tumor-specific $CD4^+$ T cells orchestrate global antitumor immune responses by recruiting, activating, and functionally polarizing DCs, NK cells, macrophages, and $CD8^+$ T cells. On the other hand, activated antigen-specific cytotoxic $CD8^+$ T cells migrate to the site of tumor, recognize tumor-specific antigens, and program tumor cells for apoptosis via the production of cytotoxic factors such as granzyme B, perforin, and IFNγ [6]. Hence, the development of functional type 1 T cell responses is predictive of response to immunotherapy.

8.6.3 CD4 T Cells

Naïve $CD4^+$ T cells (Th0) can differentiate to become Th1, Th2, Th17, or T regulatory cells, depending on the environment in which activation occurs and the nature/balance of conditioning cytokines. Key cytokines responsible for Th1 differentiation include IL-12 and IFNγ [66]. As previously mentioned, IL-36 can indirectly promote Th1 differentiation by inducing the production of IL-12p70, IL-18, and IFNγ by DCs. IL-36 can also directly influence Th1 $CD4^+$ T cell differentiation. Among spleen cells, Th0 $CD4^+$ T cells express the highest levels of IL-36R [60], with direct IL-36 signaling in Th0 cells inducing the production of T cell growth factor (aka IL-2), wherein IL-2 promotes the proliferation of tumor antigen-specific $CD8^+$ cytotoxic T cells [60]. Furthermore, stimulation of $CD4^+$ T cells with IL-36β promotes their production of IFNγ [60]. Thus, by licensing Th0 cells to coordinately produce IL-2 and IFNγ, IL-36 skews naïve $CD4^+$ T cells toward a Th1 functional phenotype. The pathway responsible for this cascade in $CD4^+$ T cells replicates that seen in myeloid cells.

Coordinate stimulation of CD4+ T cells with IL-36β and IL-12 leads to the expression of Tbet by these cells, which can subsequently induce the production of IL-36 by these cells [60].

8.6.4 CD8 T Cells

Both naïve and effector CD8+ T cells express IL-36R. Naïve T cells greatly benefit from IL-36 signaling during activation. CD8+ T cells show an increase in proliferation potential, size, biomass production, and IFNγ secretion when the IL-36R is stimulated by IL-36γ coordinately with TCR stimulation by anti-CD3/anti-CD28 agonist antibodies [62]. Effector CD8+ T cells, interestingly, lose sensitivity to IL-36R signaling, i.e., restimulation of effector CD8+ T cells with anti-CD3/anti-CD28 plus IL-36γ fails to increase secretion of IFNγ. Addition of IL-12 in combination with anti-CD3/anti-CD28 stimulation and IL-36γ, however, drastically improves CD8+ T cell secretion of IFNγ [62]. In the cancer setting, effector CD8+ T cells that can perform aerobic glycolysis can also be reconditioned to secrete IFNγ in response to IL-36R signaling following exposure to IL-2 [57]. These data support a synergistic relationship between TCR, IL-2R, IL-12R, and the IL-36R-mediated signaling in potentiating optimal (antitumor) CD8+ T effector cell responses. The mechanism responsible for CD8+ T cell activation downstream of IL-36β/IL-36R signaling has recently been elucidated to be dependent on mTORC1 and MyD88 in the TME [67]. A similar pathway has already been described in other T cell subsets; mTORC1 and MyD88 are both required for CD4+ Th17 [11] and CD8+ Tc17 [41] responses.

8.6.5 NK Cells

Natural killer (NK) cells play an important role in antitumor immune responses by limiting early tumor growth and metastatic seeding, allowing time for the development of specific adaptive immune responses [12]. Notably, NK cell depletion in mice prior to tumor implantation reduces overall survival. NK cells express the IL-36R, and IL-36γ stimulation of NK cells enhances their ability to produce IFNγ. Specifically, NK cells co-stimulated with IL-36γ and IL-2 produce six times more IFNγ than NK cells stimulated with IL-2 alone [62]. IL-36γ also synergizes with IL-2 to improve the survival of NK cells in vitro. In a transplantable murine melanoma model, B16.F10 cells transfected to ectopically express IL-36γ showed a significant increase in infiltration by NK cells in vivo vs. wild-type B16.F10 tumors [62]. Therefore, IL-36γ directly and indirectly influences NK cells by improving NK cell infiltration in the TME, promoting NK cell survival, and enhancing NK cell IFNγ production leading to facilitated type 1 antitumor immune responses [62].

8.6.6 Macrophages

Macrophages are commonly found in peripheral tissues and evolving tumor lesions in vivo. Macrophages are prolific phagocytes that can sample antigens in their local environment and serve as antigen-presenting cells. In addition to presenting antigens, macrophages can produce a number of pro- and anti-inflammatory factors that directly and indirectly sustain immune responses under diverse physiologic conditions. Such diversity in functionality is predicated on the segregation of macrophages into differentially polarized subsets, such as pro-inflammatory M1 macrophages and the anti-inflammatory/tissue-healing M2 macrophages. Undifferentiated (M0), M1, and M2 macrophages all express the IL-36R, but only M2 macrophages retain the ability to respond to IL-36β and produce IL-6 and TNFα, while M1 macrophages fail to respond to further stimulation by IL-36β [20].

The density of M1 macrophages in tumors serves as a positive prognostic indicator in the setting of a number of human cancers, where they are believed to assist in the initiation of a type 1 antitumor immune response [31, 65]. However, a recurring theme for macrophages in the TME is in their heavily skewed maintenance of an immunosuppressive phenotype (i.e., a specialized

M2-like phenotype referred to as tumor-associated macrophages, TAM), characterized by an abundant production of regulatory factors such as arginase I, HIF1A, TGFβ, IL-4, and IL-10 within the TME [46, 50]. IL-36 cytokines, IL-36β in particular, stimulate M2 macrophages in the TME to produce M1-like pro-inflammatory cytokines [20]. Transcriptomic analysis of human immune cell subsets revealed that M1 macrophages express high levels of *IL36G* and $CD68^+$ macrophages in tumors were shown to be a source of IL-36γ in the TME [64].

These data suggest that IL-36-based interventional therapies in the cancer setting may invigorate antitumor immune responses in the TME by targeting and converting pro-tumorigenic M2 macrophages into cells bearing an antitumor M1-like phenotype [64].

8.6.7 Endothelial Cells

Endothelial cells (ECs) express IL-36R, with IL-36γ reported to promote the phosphorylation of NFκB and MAPKs, the upregulated expression of cell adhesion molecules VCAM-1 and ICAM-1, and the production of inflammatory factors IL-8, CCL2, and CCL20 in ECs in a dose-dependent manner [7]. T cells expressing VLA-4 and LFA-1 interact with cell adhesion molecules VCAM-1 and ICAM-1, respectively, on the luminal surface of ECs, allowing for their local adherence and extravasation into the (inflamed) tissue space, while CCL2 and CCL20 serve as chemokine attractants for rolling T cells [7].

IL-36 cytokines have been strongly linked to local inflammatory diseases of the skin, most notably in psoriasis. Scaly psoriatic lesions exhibit robust infiltration of inflammatory T cells and a dense network of aberrant blood vessels (leaky, high degree of vascular complexity, limited perfusion) [4, 8, 16]. The tumor vasculature bears similarity to the vasculature found in psoriatic plaques, in being leaky and tortuous, but it commonly fails to support T cell infiltration via growth factor-dependent downregulation of vascular adhesion molecules ICAM-1 and ICAM-2 [25, 26]. In such a context, activation of the IL-36R on tumor-associated ECs with IL-36γ would be expected to promote enhanced T cell infiltration via the upregulation of adhesion molecules on ECs and the local production of T cell chemo-attractants.

8.7 Discussions on IL-36 Signaling in Cancer Therapy

8.7.1 IL-36γ Promotes the Development/Maintenance of Tertiary Lymphoid Structures (TLS) in the TME

A common challenge in cancer therapy is improving protective T cell infiltration into tumor lesions, particularly in the disseminated disease setting. Patients receiving immune checkpoint blockade have variable response rates, where failure to respond has been correlated to poor $CD8^+$ T cell infiltration in the lesion at the time of initiating the therapy [58]. Hence, many adjuvant strategies have been developed to improve "baseline" T cell infiltration in tumors prior to delivering standard of care immune checkpoint blockade antibodies. One observed effect of boosting type I inflammation in the TME is the development of compact organizations of DCs, T cells, B cells, and high endothelial venules akin to those observed in lymph nodes. These aggregates of immune and specialized endothelial cells are called tertiary lymphoid structures (TLS), which serve as sites of local DC-mediated tumor antigen sampling and T cell (cross)priming [24]. TLS, which typically form at the tumor margin (i.e., the interface between tumor and normal adjacent tissue), are thought to improve antitumor immune responses by facilitating T cell activation proximal to sites of (neo)antigen load and active disease, limiting the inefficient process associated with distal T induction and subsequent recruitment into the TME [30, 47].

IL-36γ supports TLS formation within the TME. In untreated human colorectal cancer, IL-36γ can be expressed by the tumor vasculature, and this expression pattern is correlated

with an increase in the density of $CD20^+$ B cells within TLS in tumors, indicating that local IL-36γ production may have a role in maintaining TLS [64]. In a therapeutic setting, murine DCs engineered to overexpress Tbet (i.e., DC.Tbet) were particularly effective in sponsoring TLS development upon direct injection into tumor lesions [63]. This effect was strictly dependent on production of IL-36γ by DC.Tbet cells, as both the therapeutic benefit and TLS formation were lost in IL-$36R^{-/-}$ mice receiving DC.Tbet treatment. Further experiments with DCs engineered to express IL-36γ suggested that DC. IL36γ consequently upregulate expression of Tbet, highlighting an operational positive feedback loop between IL-36γ and Tbet associated with the ability of these genetically modified DCs to instigate the formation of therapeutic TLS in a transplantable mouse model of colon cancer [63]. Several factors that appear to be intimately involved in local TLS formation, including but not limited to LTα, IFNγ, Tbet, CXCL9, and CXCL10, are likely produced via the autocrine/paracrine activation of the IL-36R expressed by immune and stromal cells found in the TME.

Therefore, IL-36γ signaling in the TME can improve innate cell activation, T/B cell and DC infiltration, and TLS formation and facilitate local T cell (cross)priming in support of robust therapeutic antitumor responses.

8.8 IL-36-Based Immunotherapies: Current and Future

The discovery of immune checkpoints has changed the course of clinical treatment of cancer. While antibodies against PD-(L)1 and CTLA-4 represent standard of care for many cancer types, response rates to checkpoint blockade is highly variable across the various cancer types. New experimental treatment approaches are shifting their focus away from simply blocking checkpoint molecules and moving toward coordinate activation of T cells via the provision of co-stimulatory and/or pro-inflammatory signals. IL-36 cytokines have been used in this context due to their ability to coordinately activate APCs and helper T cells in the TME in support of strong antitumor $CD8^+$ T cell responses. To this end, IL-36γ has been explored as a treatment option via gene (cDNA or mRNA) therapy and demonstrated to be effective against cancer types that have proven inherently refractory to immune checkpoint blockade.

In murine fibrosarcomas, direct transfection of WEHI-164 cells with mIL-36 cDNA resulted in reduced tumor growth as compared to WT fibrosarcoma cells. Resected IL-36 transfected tumor cells expressed increased levels of IFNγ, suggesting that intrinsic production of IL-36 by tumor cells may promote antitumor responses via IL-36-mediated signaling in immune cells, endothelial cells and stromal cells located within the TME [52].

Co-delivery of IL-36γ mRNA along with mRNA encoding OX40L and IL-23 into tumors also improves infiltration by antigen cross-presenting $Batf3^+$ DCs, NK cells, and T cells in HT22 hepatoma, MC38 colon carcinoma, and B16 melanoma. This increased infiltration of therapeutic immune cells corresponds with an enhanced pro-inflammatory gene signature in the TME. When further combined with immune checkpoint blockade, this treatment regimen provided superior systemic benefit against untreated distal tumors [29]. Therefore, IL-36γ appears to play an important role in supporting the effective treatment of disseminated tumors when administered in combination with conventional T cell-activating/T cell-sustaining modalities.

The study of autoimmune conditions such as inflammatory bowel disease and psoriasis strongly supports the pro-inflammatory potential of IL-36 family cytokines, with their therapeutic utility in the cancer setting being increasingly evaluated in monotherapy and combined modality approaches. IL-36 gene delivery via oncolytic viruses and the extended, timed-release of IL-36 protein via nanoparticles are also expected to expand the range of translational IL-36-based treatment strategies by facilitating the targeted and/or controlled delivery of biologically active IL-36 into (disseminated) tumor lesions in cancer patients. Given the alarmin nature of IL-36 and

its ability to strongly activate epithelial cells, the sustained release of IL-36 via dissolvable transdermal microneedle patches is also being explored for (accessible) cutaneous malignancies, such as melanoma and cutaneous squamous cell carcinoma. Additionally, systemic delivery of activating/agonist monoclonal antibodies targeting the IL-36R would be expected to initiate and/or bolster antitumor immune responses in situ in inaccessible non-cutaneous lesions. Such new developments in gene/drug delivery hold great promise for the therapeutic utility of IL-36 and IL-36R signaling agonists as cancer therapeutic agents.

References

1. Ainscough JS, Macleod T, McGonagle D, Brakefield R, Baron JM, Alase A et al (2017) Cathepsin S is the major activator of the psoriasis-associated proinflammatory cytokine IL-36gamma. Proc Natl Acad Sci U S A 114(13):E2748–E2757. https://doi.org/10.1073/pnas.1620954114
2. Atkins E (1984) Fever: the old and the new. J Infect Dis 149(3):339–348. https://doi.org/10.1093/infdis/149.3.339
3. Bachmann M, Scheiermann P, Hardle L, Pfeilschifter J, Muhl H (2012) IL-36gamma/IL-1F9, an innate T-bet target in myeloid cells. J Biol Chem 287(50):41684–41696. https://doi.org/10.1074/jbc.M112.385443
4. Barton SP, Abdullah MS, Marks R (1992) Quantification of microvascular changes in the skin in patients with psoriasis. Br J Dermatol 126(6):569–574. https://doi.org/10.1111/j.1365-2133.1992.tb00101.x
5. Beeson PB (1948) Temperature-elevating effect of a substance obtained from polymorphonuclear leucocytes. J Clin Invest 27(4):524
6. Borst J, Ahrends T, Babala N, Melief CJM, Kastenmuller W (2018) CD4(+) T cell help in cancer immunology and immunotherapy. Nat Rev Immunol 18(10):635–647. https://doi.org/10.1038/s41577-018-0044-0
7. Bridgewood C, Stacey M, Alase A, Lagos D, Graham A, Wittmann M (2017) IL-36gamma has proinflammatory effects on human endothelial cells. Exp Dermatol 26(5):402–408. https://doi.org/10.1111/exd.13228
8. Bull RH, Bates DO, Mortimer PS (1992) Intravital video-capillaroscopy for the study of the microcirculation in psoriasis. Br J Dermatol 126(5):436–445. https://doi.org/10.1111/j.1365-2133.1992.tb11815.x
9. Burns K, Martinon F, Esslinger C, Pahl H, Schneider P, Bodmer JL et al (1998) MyD88, an adapter protein involved in interleukin-1 signaling. J Biol Chem 273(20):12203–12209. https://doi.org/10.1074/jbc.273.20.12203
10. Busfield SJ, Comrack CA, Yu G, Chickering TW, Smutko JS, Zhou H et al (2000) Identification and gene organization of three novel members of the IL-1 family on human chromosome 2. Genomics 66(2):213–216. https://doi.org/10.1006/geno.2000.6184
11. Chang J, Burkett PR, Borges CM, Kuchroo VK, Turka LA, Chang CH (2013) MyD88 is essential to sustain mTOR activation necessary to promote T helper 17 cell proliferation by linking IL-1 and IL-23 signaling. Proc Natl Acad Sci U S A 110(6):2270–2275. https://doi.org/10.1073/pnas.1206048110
12. Chen L, Taylor JL, Sabins NC, Lowe DB, Qu Y, You Z, Storkus WJ (2013) Extranodal induction of therapeutic immunity in the tumor microenvironment after intratumoral delivery of Tbet gene-modified dendritic cells. Cancer Gene Ther 20(8):469–477. https://doi.org/10.1038/cgt.2013.42
13. Chi H-H, Hua K-F, Lin Y-C, Chu C-L, Hsieh C-Y, Hsu Y-J et al (2017) IL-36 signaling facilitates activation of the NLRP3 inflammasome and IL-23/IL-17 axis in renal inflammation and fibrosis. J Am Soc Nephrol 28(7):2022–2037. https://doi.org/10.1681/asn.2016080840
14. Clancy DM, Sullivan GP, Moran HBT, Henry CM, Reeves EP, McElvaney NG et al (2018) Extracellular neutrophil proteases are efficient regulators of IL-1, IL-33, and IL-36 cytokine activity but poor effectors of microbial killing. Cell Rep 22(11):2937–2950. https://doi.org/10.1016/j.celrep.2018.02.062
15. Costelloe C, Watson M, Murphy A, McQuillan K, Loscher C, Armstrong ME et al (2008) IL-1F5 mediates anti-inflammatory activity in the brain through induction of IL-4 following interaction with SIGIRR/TIR8. J Neurochem 105(5):1960–1969. https://doi.org/10.1111/j.1471-4159.2008.05304.x
16. Creamer D, Allen MH, Sousa A, Poston R, Barker JN (1997) Localization of endothelial proliferation and microvascular expansion in active plaque psoriasis. Br J Dermatol 136(6):859–865
17. Curtis BM, Widmer MB, deRoos P, Qwarnstrom EE (1990) IL-1 and its receptor are translocated to the nucleus. J Immunol 144(4):1295–1303
18. Dalod M, Chelbi R, Malissen B, Lawrence T (2014) Dendritic cell maturation: functional specialization through signaling specificity and transcriptional programming. EMBO J 33(10):1104–1116. https://doi.org/10.1002/embj.201488027
19. Debets R, Timans JC, Homey B, Zurawski S, Sana TR, Lo S et al (2001) Two novel IL-1 family members, IL-1 delta and IL-1 epsilon, function as an antagonist and agonist of NF-kappa B activation through the orphan IL-1 receptor-related protein 2. J Immunol 167(3):1440–1446. https://doi.org/10.4049/jimmunol.167.3.1440
20. Dietrich D, Martin P, Flacher V, Sun Y, Jarrossay D, Brembilla N et al (2016) Interleukin-36 potently stimulates human M2 macrophages, Langerhans cells

and keratinocytes to produce pro-inflammatory cytokines. Cytokine 84:88–98. https://doi.org/10.1016/j.cyto.2016.05.012
21. Dinarello CA (2010) IL-1: discoveries, controversies and future directions. Eur J Immunol 40(3):599–606. https://doi.org/10.1002/eji.201040319
22. Foster AM, Baliwag J, Chen CS, Guzman AM, Stoll SW, Gudjonsson JE et al (2014) IL-36 promotes myeloid cell infiltration, activation, and inflammatory activity in skin. J Immunol 192(12):6053–6061. https://doi.org/10.4049/jimmunol.1301481
23. Garlanda C, Dinarello CA, Mantovani A (2013) The interleukin-1 family: back to the future. Immunity 39(6):1003–1018. https://doi.org/10.1016/j.immuni.2013.11.010
24. Goc J, Germain C, Vo-Bourgais TKD, Lupo A, Klein C, Knockaert S et al (2014) Dendritic cells in tumor-associated tertiary lymphoid structures signal a Th1 cytotoxic immune contexture and license the positive prognostic value of infiltrating CD8⁺ T cells. Cancer Res 74(3):705–715. https://doi.org/10.1158/0008-5472.Can-13-1342
25. Griffioen AW, Damen CA, Blijham GH, Groenewegen G (1996a) Tumor angiogenesis is accompanied by a decreased inflammatory response of tumor-associated endothelium. Blood 88(2):667–673
26. Griffioen AW, Damen CA, Martinotti S, Blijham GH, Groenewegen G (1996b) Endothelial intercellular adhesion molecule-1 expression is suppressed in human malignancies: the role of angiogenic factors. Cancer Res 56(5):1111–1117
27. Guo J, Tu J, Hu Y, Song G, Yin Z (2019) Cathepsin G cleaves and activates IL-36γ and promotes the inflammation of psoriasis. Drug Des Devel Ther 13:581–588. https://doi.org/10.2147/dddt.S194765
28. Henry CM, Sullivan GP, Clancy DM, Afonina IS, Kulms D, Martin SJ (2016) Neutrophil-derived proteases escalate inflammation through activation of IL-36 family cytokines. Cell Rep 14(4):708–722. https://doi.org/10.1016/j.celrep.2015.12.072
29. Hewitt SL, Bai A, Bailey D, Ichikawa K, Zielinski J, Karp R et al (2019) Durable anticancer immunity from intratumoral administration of IL-23, IL-36gamma, and OX40L mRNAs. Sci Transl Med 11(477):eaat9143. https://doi.org/10.1126/scitranslmed.aat9143
30. Hiraoka N, Ino Y, Yamazaki-Itoh R (2016) Tertiary lymphoid organs in cancer tissues. Front Immunol 7:244. https://doi.org/10.3389/fimmu.2016.00244
31. Jackute J, Zemaitis M, Pranys D, Sitkauskiene B, Miliauskas S, Vaitkiene S, Sakalauskas R (2018) Distribution of M1 and M2 macrophages in tumor islets and stroma in relation to prognosis of non-small cell lung cancer. BMC Immunol 19(1):3. https://doi.org/10.1186/s12865-018-0241-4
32. Li C, Zienkiewicz J, Hawiger J (2005) Interactive sites in the MyD88 Toll/interleukin (IL) 1 receptor domain responsible for coupling to the IL1beta signaling pathway. J Biol Chem 280(28):26152–26159. https://doi.org/10.1074/jbc.M503262200
33. Li N, Yamasaki K, Saito R, Fukushi-Takahashi S, Shimada-Omori R, Asano M, Aiba S (2014) Alarmin function of cathelicidin antimicrobial peptide LL37 through IL-36gamma induction in human epidermal keratinocytes. J Immunol 193(10):5140–5148. https://doi.org/10.4049/jimmunol.1302574
34. Lian LH, Milora KA, Manupipatpong KK, Jensen LE (2012) The double-stranded RNA analogue polyinosinic-polycytidylic acid induces keratinocyte pyroptosis and release of IL-36gamma. J Invest Dermatol 132(5):1346–1353. https://doi.org/10.1038/jid.2011.482
35. Macleod T, Doble R, McGonagle D, Wasson CW, Alase A, Stacey M, Wittmann M (2016) Neutrophil Elastase-mediated proteolysis activates the anti-inflammatory cytokine IL-36 receptor antagonist. Sci Rep 6:24880. https://doi.org/10.1038/srep24880
36. Milora KA, Fu H, Dubaz O, Jensen LE (2015) Unprocessed interleukin-36alpha regulates psoriasis-like skin inflammation in cooperation with interleukin-1. J Invest Dermatol 135(12):2992–3000. https://doi.org/10.1038/jid.2015.289
37. Mosley B, Urdal DL, Prickett KS, Larsen A, Cosman D, Conlon PJ et al (1987) The interleukin-1 receptor binds the human interleukin-1 alpha precursor but not the interleukin-1 beta precursor. J Biol Chem 262(7):2941–2944
38. Moussion C, Ortega N, Girard JP (2008) The IL-1-like cytokine IL-33 is constitutively expressed in the nucleus of endothelial cells and epithelial cells in vivo: a novel 'alarmin'. PLoS One 3(10):e3331. https://doi.org/10.1371/journal.pone.0003331
39. Müller A, Hennig A, Lorscheid S, Grondona P, Schulze-Osthoff K, Hailfinger S, Kramer D (2018) IκBζ is a key transcriptional regulator of IL-36-driven psoriasis-related gene expression in keratinocytes. Proc Natl Acad Sci U S A 115(40):10088–10093. https://doi.org/10.1073/pnas.1801377115
40. Mutamba S, Allison A, Mahida Y, Barrow P, Foster N (2012) Expression of IL-1Rrp2 by human myelomonocytic cells is unique to DCs and facilitates DC maturation by IL-1F8 and IL-1F9. Eur J Immunol 42(3):607–617. https://doi.org/10.1002/eji.201142035
41. Nanjappa SG, Hernandez-Santos N, Galles K, Wuthrich M, Suresh M, Klein BS (2015) Intrinsic MyD88-Akt1-mTOR signaling coordinates disparate Tc17 and Tc1 responses during vaccine immunity against fungal pneumonia. PLoS Pathog 11(9):e1005161. https://doi.org/10.1371/journal.ppat.1005161
42. Onoufriadis A, Simpson MA, Pink AE, Di Meglio P, Smith CH, Pullabhatla V et al (2011) Mutations in IL36RN/IL1F5 are associated with the severe episodic inflammatory skin disease known as generalized pustular psoriasis. Am J Hum Genet 89(3):432–437. https://doi.org/10.1016/j.ajhg.2011.07.022
43. Pham CT (2006) Neutrophil serine proteases: specific regulators of inflammation. Nat Rev Immunol 6(7):541–550. https://doi.org/10.1038/nri1841

44. Renert-Yuval Y, Horev L, Babay S, Tams S, Ramot Y, Zlotogorski A, Molho-Pessach V (2014) IL36RN mutation causing generalized pustular psoriasis in a Palestinian patient. Int J Dermatol 53(7):866–868. https://doi.org/10.1111/ijd.12525
45. Rider P, Kaplanov I, Romzova M, Bernardis L, Braiman A, Voronov E, Apte RN (2012) The transcription of the alarmin cytokine interleukin-1 alpha is controlled by hypoxia inducible factors 1 and 2 alpha in hypoxic cells. Front Immunol 3:290. https://doi.org/10.3389/fimmu.2012.00290
46. Rodriguez PC, Quiceno DG, Zabaleta J, Ortiz B, Zea AH, Piazuelo MB et al (2004) Arginase I production in the tumor microenvironment by mature myeloid cells inhibits T-cell receptor expression and antigen-specific T-cell responses. Cancer Res 64(16):5839–5849. https://doi.org/10.1158/0008-5472.Can-04-0465
47. Sautes-Fridman C, Petitprez F, Calderaro J, Fridman WH (2019) Tertiary lymphoid structures in the era of cancer immunotherapy. Nat Rev Cancer 19(6):307–325. https://doi.org/10.1038/s41568-019-0144-6
48. Scheibe K, Backert I, Wirtz S, Hueber A, Schett G, Vieth M et al (2017) IL-36R signalling activates intestinal epithelial cells and fibroblasts and promotes mucosal healing in vivo. Gut 66(5):823–838. https://doi.org/10.1136/gutjnl-2015-310374
49. Scheiermann P, Bachmann M, Härdle L, Pleli T, Piiper A, Zwissler B et al (2015) Application of IL-36 receptor antagonist weakens CCL20 expression and impairs recovery in the late phase of murine acetaminophen-induced liver injury. Sci Rep 5:8521. https://doi.org/10.1038/srep08521
50. Schmid MC, Varner JA (2010) Myeloid cells in the tumor microenvironment: modulation of tumor angiogenesis and tumor inflammation. J Oncol 2010:201026. https://doi.org/10.1155/2010/201026
51. Smith DE, Renshaw BR, Ketchem RR, Kubin M, Garka KE, Sims JE (2000) Four new members expand the interleukin-1 superfamily. J Biol Chem 275(2):1169–1175. https://doi.org/10.1074/jbc.275.2.1169
52. Solahaye-Kahnamouii S, Farhadi F, Rahkare-Farshi M, Pakdel F, Kashefimehr A, Pouralibaba F et al (2014) The effect of interleukin 36 gene therapy in the regression of tumor. Iran J Cancer Prev 7(4):197–203
53. Sullivan GP, Henry CM, Clancy DM, Mametnabiev T, Belotcerkovskaya E, Davidovich P et al (2018) Suppressing IL-36-driven inflammation using peptide pseudosubstrates for neutrophil proteases. Cell Death Dis 9(3):378. https://doi.org/10.1038/s41419-018-0385-4
54. Swindell WR, Beamer MA, Sarkar MK, Loftus S, Fullmer J, Xing X et al (2018) RNA-Seq analysis of IL-1B and IL-36 responses in epidermal keratinocytes identifies a shared MyD88-dependent gene signature. Front Immunol 9:80. https://doi.org/10.3389/fimmu.2018.00080
55. Towne JE, Garka KE, Renshaw BR, Virca GD, Sims JE (2004) Interleukin (IL)-1F6, IL-1F8, and IL-1F9 signal through IL-1Rrp2 and IL-1RAcP to activate the pathway leading to NF-kappaB and MAPKs. J Biol Chem 279(14):13677–13688. https://doi.org/10.1074/jbc.M400117200
56. Towne JE, Renshaw BR, Douangpanya J, Lipsky BP, Shen M, Gabel CA, Sims JE (2011) Interleukin-36 (IL-36) ligands require processing for full agonist (IL-36alpha, IL-36beta, and IL-36gamma) or antagonist (IL-36Ra) activity. J Biol Chem 286(49):42594–42602. https://doi.org/10.1074/jbc.M111.267922
57. Tsurutani N, Mittal P, St Rose MC, Ngoi SM, Svedova J, Menoret A et al (2016) Costimulation endows immunotherapeutic CD8 T cells with IL-36 responsiveness during aerobic glycolysis. J Immunol 196(1):124–134. https://doi.org/10.4049/jimmunol.1501217
58. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L et al (2014) PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature 515(7528):568–571. https://doi.org/10.1038/nature13954
59. Vigne S, Palmer G, Lamacchia C, Martin P, Talabot-Ayer D, Rodriguez E et al (2011) IL-36R ligands are potent regulators of dendritic and T cells. Blood 118(22):5813–5823. https://doi.org/10.1182/blood-2011-05-356873
60. Vigne S, Palmer G, Martin P, Lamacchia C, Strebel D, Rodriguez E et al (2012) IL-36 signaling amplifies Th1 responses by enhancing proliferation and Th1 polarization of naive CD4+ T cells. Blood 120(17):3478–3487. https://doi.org/10.1182/blood-2012-06-439026
61. Wang W, Yu X, Wu C, Jin H (2017) IL-36γ inhibits differentiation and induces inflammation of keratinocyte via Wnt signaling pathway in psoriasis. Int J Med Sci 14(10):1002–1007. https://doi.org/10.7150/ijms.20809
62. Wang X, Zhao X, Feng C, Weinstein A, Xia R, Wen W et al (2015) IL-36gamma transforms the tumor microenvironment and promotes type 1 lymphocyte-mediated antitumor immune responses. Cancer Cell 28(3):296–306. https://doi.org/10.1016/j.ccell.2015.07.014
63. Weinstein AM, Chen L, Brzana EA, Patil PR, Taylor JL, Fabian KL et al (2017) Tbet and IL-36gamma cooperate in therapeutic DC-mediated promotion of ectopic lymphoid organogenesis in the tumor microenvironment. Oncoimmunology 6(6):e1322238. https://doi.org/10.1080/2162402x.2017.1322238
64. Weinstein AM, Giraldo NA, Petitprez F, Julie C, Lacroix L, Peschaud F et al (2019) Association of IL-36γ with tertiary lymphoid structures and inflammatory immune infiltrates in human colorectal cancer. Cancer Immunol Immunother 68(1):109–120. https://doi.org/10.1007/s00262-018-2259-0
65. Zhang M, He Y, Sun X, Li Q, Wang W, Zhao A, Di W (2014a) A high M1/M2 ratio of tumor-associated macrophages is associated with extended survival in ovarian cancer patients. J Ovarian Res 7:19. https://doi.org/10.1186/1757-2215-7-19

66. Zhang Y, Zhang Y, Gu W, Sun B (2014b) TH1/TH2 cell differentiation and molecular signals. Adv Exp Med Biol 841:15–44. https://doi.org/10.1007/978-94-017-9487-9_2
67. Zhao X, Chen X, Shen X, Tang P, Chen C, Zhu Q et al (2019) IL-36β promotes CD8+ T cell activation and antitumor immune responses by activating mTORC1. Front Immunol 10(1803). https://doi.org/10.3389/fimmu.2019.01803
68. Zhu J, Jankovic D, Oler AJ, Wei G, Sharma S, Hu G et al (2012) The transcription factor T-bet is induced by multiple pathways and prevents an endogenous Th2 cell program during Th1 cell responses. Immunity 37(4):660–673. https://doi.org/10.1016/j.immuni.2012.09.007

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