

Chapter 2

Biological Response Modifier in Cancer Immunotherapy

Ronghua Liu, Feifei Luo, Xiaoming Liu, Luman Wang, Jiao Yang, Yuting Deng, Enyu Huang, Jiawen Qian, Zhou Lu, Xuechao Jiang, Dan Zhang, and Yiwei Chu

Abstract Biological response modifiers (BRMs) emerge as a lay of new compounds or approaches used in improving cancer immunotherapy. Evidences highlight that cytokines, Toll-like receptor (TLR) signaling, and noncoding RNAs are of crucial roles in modulating antitumor immune response and cancer-related chronic inflammation, and BRMs based on them have been explored. In particular, besides some cytokines like IFN- α and IL-2, several Toll-like receptor (TLR) agonists like BCG, MPL, and imiquimod are also licensed to be used in patients with several malignancies nowadays, and the first artificial small noncoding RNA (microRNA) mimic, MXR34, has entered phase I clinical study against liver cancer, implying their potential application in cancer therapy. According to amounts of original data, this chapter will review the regulatory roles of TLR signaling, some noncoding RNAs, and several key cytokines in cancer and cancer-related immune response, as well as the clinical cases in cancer therapy based on them.

R. Liu • L. Wang • J. Yang • Y. Deng • E. Huang • J. Qian • Z. Lu • X. Jiang • D. Zhang • Y. Chu (✉)

Department of Immunology, Key Laboratory of Medical Molecular Virology of MOE/MOH, School of Basic Medical Sciences, Fudan University, No.138, Yi Xue Yuan Rd., mail box 226, Shanghai 200032, People's Republic of China

Biotherapy Research Center, Fudan University, Shanghai 200032, China
e-mail: yiweichu@fudan.edu.cn

F. Luo

Biotherapy Research Center, Fudan University, Shanghai 200032, China

Department of Digestive Diseases of Huashan Hospital, Fudan University, Shanghai, China

X. Liu

Department of Immunology, Key Laboratory of Medical Molecular Virology of MOE/MOH, School of Basic Medical Sciences, Fudan University, No.138, Yi Xue Yuan Rd., mail box 226, Shanghai 200032, People's Republic of China

Department of Dermatology, Shenzhen Hospital, Peking University, Shenzhen, Guangdong 518036, China

Keywords Biological response modifiers • Toll-like receptor • Cytokines
• Noncoding RNAs • Cancer immunotherapy

1 Introduction

Biological response modifiers (BRMs) are compounds that modify immune response, which can be used in immunotherapy to enhance the activity of the immune system to increase the body's natural defense against disease including cancer and also used to reduce side effects caused by some treatments against diseases. Substances that modulate the immune system by stimulating or replacing the function of one or more of the system's components can be called BRMs, and the term BRM is often used synonymously with the terms immunomodulator and immunostimulant (Kuroki et al. 2012). BRMs include various cytokines, monoclonal antibodies, chemical synthetic drugs, and some molecules with potential ability in regulating immune responses, and they can usually be categorized into two groups, specific BRMs like monoclonal antibodies that provide antigen-specific immune response or activity and nonspecific BRMs like cytokines that augment or stimulate the immune system without antigenic specificity. In addition, using hybridoma and genetic engineering technologies, large amounts of BRMs can be produced for use in the treatment of cancer, as well as of other diseases (Kuroki et al. 2012; Smit et al. 2009).

Cytokines are one of classical nonspecific BRMs, and numerous studies have demonstrated that they can function as major mediators of antitumor immunity, and some cytokines like Interleukin (IL)-2 and Interferon (IFN)-gamma (γ) have been approved for cancer treatment by the FDA; cytokines including IL-7, IL-12, IL-15, IL-18, and IL-21 have entered clinical trials. However, recent evidences also show that cytokines play a pleiotropic role in tumor pathogenesis, development, and prognosis (Cutler and Brombacher 2005). They can directly stimulate immune effector cells and enhance the killing effects and can also promote tumor growth and facilitate invasion and metastasis of cancer. Therefore, a deeper understanding of the interactions between cytokines and tumor will provide new approaches for improving cytokine-based cancer immunotherapy.

Apart from cytokines, accumulating evidences indicate that Toll like receptor (TLR) signaling and non-coding RNAs also act as crucial regulators in immune response against cancer and are of potential application in cancer immunotherapy. TLR signaling can be initiated by pathogen-associated molecular patterns (PAMPs) or some similar agonists that bind TLR and trigger downstream signaling, and thereby induce immune responses to eliminate pathogens (Song and Lee 2012), while non-coding RNAs like microRNAs and long non-coding RNAs, which are often dysregulated in cancer, can control the development, differentiation or activation of the infiltrated leucocytes in cancer (Lindsay 2008). Due to their key functions in initiating or modulating innate immune and inflammatory responses, targeting of TLR signaling or using noncoding mimics is emerging as an attractive

therapeutic approach for human cancers, and some clinical trials are dedicated to exploit TLR agonists in tumor treatment so as to transform the suppressive immunity toward antitumor immunity (Deng et al. 2014), and some artificial microRNA mimics have been used to alter the function of immune cells including cytotoxicity of tumor-specific cytotoxic T lymphocytes (CTLs) (Okada et al. 2010).

In this chapter, we will introduce and review the regulatory roles of TLR signaling, the noncoding RNAs, and several key cytokines in cancer and cancer-related immune response, and the clinical cases in cancer therapy based on them will be discussed in detail, which imply us that more BRMs based on these key regulators may be developed and used in cancer immunotherapy.

2 Toll-Like Receptor Signaling and Agonists

2.1 *Toll-Like Receptors (TLRs)*

Toll-like receptors (TLRs) are classified as type I transmembrane proteins and obtained the name due to their structural similarity to *Drosophila* Toll protein (Kawai and Akira 2010). Dr. Nomura first reported the human TLR in 1994, and for now, ten members of TLRs, named TLR1 to TLR10, have been identified in human, while mice express TLR1 to TLR13 except TLR10 (Kawai and Akira 2010). Each TLR can recognize a collection of molecules called pathogen-associated molecular patterns (PAMPs), a series of evolutionary conserved molecules derived from microbes, to signal the invasion of microbes and initiate immune response to eliminate pathogens (Song and Lee 2012) (Table 2.1). TLRs comprise three parts, an ectodomain, a transmembrane region, and Toll-IL-1 receptor (TIR) domains (Jin and Lee 2008). The leucine-rich repeats containing ectodomain is the essential part in PAMP recognition, and cytosolic TIR domains initiate downstream signaling cascades via the adaptor molecules MyD88 and TRIF, ultimately leading to the activation of transcriptional factors and expression of inflammatory cytokines (Jin and Lee 2008). Antigen-presenting cells (APCs), such as macrophages and dendritic cells, have high-level expression of TLRs that activation of TLRs causes M1 polarization of macrophages and transcription of IFN-stimulated genes (Kawai and Akira 2011). Such effects have already attracted attentions that clinical trials are dedicated to exploit TLR agonists in tumor treatment so as to transform the suppressive immunity toward antitumor immunity.

2.2 *TLR Agonists and Downstream Signaling*

Each TLR recognizes corresponding PAMPs that are derived from viruses, bacteria, fungi, mycobacteria, and parasites (Song and Lee 2012). TLRs located on the

Table 2.1 Classification and expression of TLRs and TLR agonists in human

TLRs	Cellular expression	Intracellular location	Natural ligand	Agent under investigation
TLR1	Monocyte, mDC, B cell, NK, neutrophil, basophil	Cell membrane	Triacyl lipopeptide	
TLR2	Monocyte, mastocytes, mDC	Cell membrane	Triacyl lipopeptide, lipoteichoic acid, zymosan, porins, macrophage-activating lipopeptide, bacterial peptidoglycan, lipoarabinomannan	BCG AS15
TLR3	mDC	Endosomal membrane	dsDNA	Poly-ICLC
TLR4	Monocytes, macrophages, mDC, mastocytes, basophil	Cell membrane	LPS, mannan, phospholipids, envelope proteins	AS15 BCG
TLR5	mDC, monocyte, NK and T cell	Cell membrane	Flagellin	CBLB502
TLR6	Monocyte, mastocytes, mDC	Cell membrane	Triacyl lipopeptide, lipoteichoic acid, zymosan, porins, macrophage-activating lipopeptide, bacterial peptidoglycan, lipoarabinomannan	
TLR7	pDC, eosinophil	Endosomal membrane	ssRNA (viral)	Imiquimod Resiquimod
TLR8	mDC, T and B cells, eosinophils, monocytes	Endosomal membrane	ssRNA (viral)	Resiquimod VTX-2337
TLR9	pDC, B cells, basophil, eosinophil	Endosomal membrane	DNA (bacterial/viral)	SD-101 AS15 VTX-2337 GNKG168 MGN1703
TLR10	pDC, neutrophil, B cell, basophil	Cell membrane	Unknown	

cell surface membrane or intracellular vesicles, therefore, can be broadly activated by extracellular components like lipid and protein ligands from bacteria and intracellular non-autologous nucleic acids from viruses. Generally, TLR agonists include lipoproteins recognized by TLR1, TLR2, and TLR6, double-stranded RNA recognized by TLR3, lipopolysaccharide recognized by TLR4, flagellin recognized

by TLR5, single-stranded RNA recognized by TLR7 and TLR8, and DNA recognized by TLR9 (Kawai and Akira 2010).

TLRs are considered to function as homodimers or heterodimers and also relied on other co-receptors for receptor sensitivity, such as MD-2, CD14, and LBP that are indispensable in LPS recognition by TLR4 (Kawai and Akira 2011). Upon recognition of PAMP, the ectodomains of TLRs induce the dimerization of the TIR domains that then act as binding sites for downstream adaptor molecules (Kang and Lee 2011). These adaptors also have TIR domains and bind specifically with the TLR dimers through TIR–TIR interactions (Kang and Lee 2011). The engagement of the adaptors then promotes the assembly of myeloid differentiation primary response protein 88 (MyD88) or TIR domain-containing adaptor-inducing IFN- β (TRIF) (Kawai and Akira 2010). Thus, TLR signaling pathways can be divided into two different parts, the MyD88-dependent and the TRIF-dependent pathways (Fig. 2.1).

2.2.1 MyD88-Dependent Pathway

Almost every TLR can activate the MyD88-dependent pathway besides TLR3. Consequently, the mitogen-activated protein kinase (MAPK) and the NF- κ B pathway could be activated via MyD88-dependent pathway (Kawai and Akira 2011). The TLR signaling initiates from the agonist binding to the TLR; such binding leads to receptor structure change, and then the adaptor molecule MyD88 could be recruited to the receptor site (Medzhitov et al. 1998). MyD88 further recruits other proteins, such as IRAK1, IRAK2 (Kawagoe et al. 2008), and IRAK4 (Kawai and Akira 2010). Next, the IRAK molecules activate the ubiquitination activity of TRAF6 molecule by phosphorylation (Kawai and Akira 2010). TRAF6 in addition polyubiquitinates the TAK1 leading to TAK1 activation. Then, the IKK- β could be phosphorylated by TAK1, which results in the phosphorylation of I κ B, triggering I κ B degradation and facilitating NF- κ B movement into nucleus, thus activating the transcription function of NF- κ B and resulting in inflammatory cytokine and chemokine expression (Kawai and Akira 2010).

2.2.2 TRIF-Dependent Pathway

For now, only TLR3 and TLR4 agonists have been discovered that could trigger the TRIF-dependent pathway. For TLR3 signaling pathway, the agonists like virus-derived dsRNA could be sensed by the receptor, leading to conformational changes of the receptor (Kang and Lee 2011). Then, the adaptor molecule TRIF is recruited to the receptor site to further recruit and activate the kinases such as TBK1 and RIPK1, which results in a division of the signaling pathway (Kawai and Akira 2010). The IRF3 phosphorylation caused by TRIF/TBK1 complex promotes IRF3 translocation into the nucleus and type I interferon expression (Fitzgerald et al. 2003). On the other hand, activation of RIPK1 causes the polyubiquitination

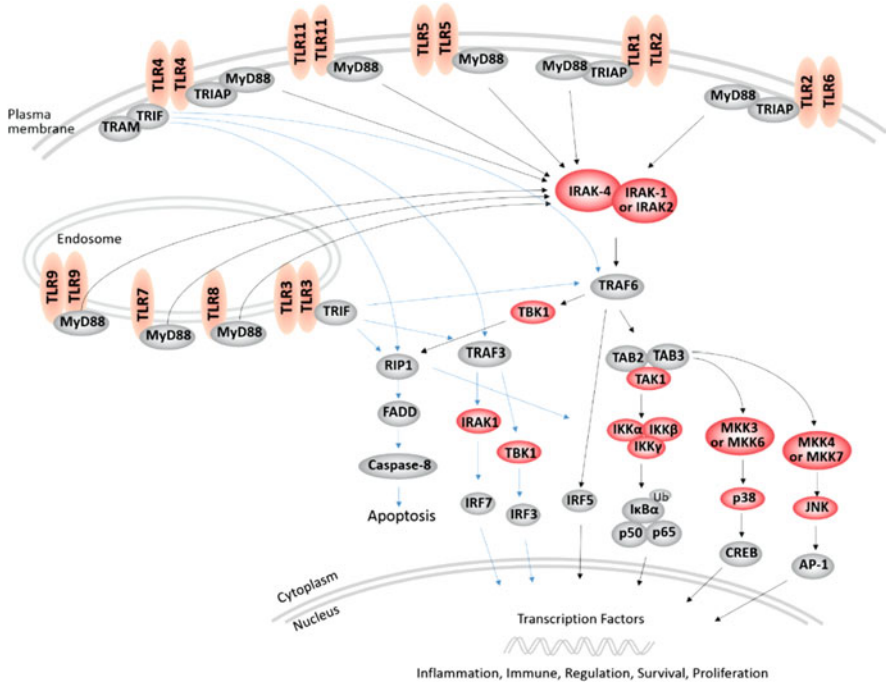


Fig. 2.1 Overview of TLR signaling pathway. Upon stimulation, TLRs activate two types of pathway that involve myeloid differentiation primary response protein 88 (MyD88) (*black line*) and/or TIR domain-containing adaptor-inducing IFN- β (TRIF) (*blue line*). *AP-1* activator protein 1, *CREB* cAMP-responsive element-binding protein, *I κ B α* inhibitor of NF- κ B α , *IKK* inhibitor of NF- κ B kinase, *IRAK* interleukin-1 receptor-associated kinase, *IRF* IFN regulatory factor, *NK* JUN N-terminal kinase, *MKK* mitogen-activated protein kinase kinase, *NEMO* NF- κ B essential modulator, *NF- κ B* nuclear factor- κ B, *RIP1* receptor-interacting protein 1, *TAB* TAK1-binding protein, *TAK1* TGF- β -activated kinase 1 (also known as MAP3K7), *TBK1* TANK-binding kinase 1, *TRAF* tumor necrosis factor receptor-associated factor, *TRAM* TRIF-related adaptor molecule

of TAK1 and NF- κ B translocation similar to the MyD88-dependent pathway (Kawai and Akira 2010).

The gene expression repertoire regulated by TLR signaling pathway orchestrates the precise immune responses. In cancer, the effects caused by TLR signaling pathway activation still remain unclear, but for immune cells, the TLR agonists have already shown biased antitumor effects, thus leading to efforts to utilize TLR agonists to alter the tolerated immunity to efficient antitumor immunity in nowadays tumor immunotherapy.

2.3 *The Role of TLR Agonists in Cancer*

It has been widely accepted that TLR agonists perform a double-edged sword in cancer: on one hand, they could enhance host antitumor immunity or display the direct tumoricidal activity to inhibit tumor growth and migration, while on the other hand, it could promote cancer progression through multiple mechanisms.

2.3.1 **The Antitumor Effect of TLR Agonists in Cancer**

Immune cells and tumor cells can both respond to stimulations by TLR agonists; the affections are dependent on the target cell itself and the characteristics of TLR agonists. Increasing evidence suggests that activation of distinct TLRs may induce different immune responses. The effect of TLR activation in antigen-presenting cells (APCs) makes TLR agonists as ideal immunologic adjuvants in cancer immunotherapy, especially dendritic cell (DC)-based immunotherapy. Such effects relied on their ability to promote maturation of APC, further leading to induction of antitumor effector cells. TLR3 agonist dsRNA induces the expression of type I IFN by DCs that produces tumor cell apoptosis and tumor cytotoxicity (Jelinek et al. 2011). The TLR4 expressed on the surface of monocyte-derived DCs could increase IL-12 and IL-18 expression via the MyD88-dependent pathway, which facilitates naive T cells to develop into T helper 1 (Th1) cells (Fang et al. 2014; Krummen et al. 2010). TLR4 agonists could also upregulate co-stimulatory proteins via the TRIF-dependent pathway, which act as a key factor in T-cell differentiation (Shen et al. 2008). TLR5 signaling was also shown to induce Th1 response, however, it has been reported that flagellin, a TLR5 agonist, could activate DC to promote Th2-biased responses (Didierlaurent et al. 2004). The TLR7 and TLR9 that are expressed on the endosome of plasmacytoid DCs utilize the MyD88-dependent pathway to yield interleukins for the development of naive T cells to Th1 (Nierkens et al. 2011; Spranger et al. 2010). Imiquimod that binds to TLR7 could induce innate and adaptive immunity, reverse local immunosuppression, and initiate tumor cell-specific apoptosis (Prins et al. 2006). Agonists of TLR7, TLR8, and TLR9 also enhance the cross-presentation and CTL activation. For instance, resiquimod, a TLR7/8 agonist, has shown potential value because of its capability to enhance the level of IL-12 and TNF- α and inhibit regulatory T cells (Treg) function (Prins et al. 2006).

TLRs have also been shown to express on T cells, and recent studies revealed that TLR agonists have direct effect on T cells. Bacterial lipoprotein, a TLR1/TLR2 agonist, inhibited tumor growth via reciprocal enhancement of the cytotoxicity of tumor-specific CTL and supersession of Treg function (Zhang et al. 2011b). TLR5 agonist induced human T-cell cytokine secretion (Tremblay et al. 2014). Targeting of TLR8 might also be viable to interrupt the dominion of Tregs, which greatly implicated in detrimental immune suppression (Peng et al. 2005). Moreover, latest studies revealed that certain TLR agonists could induce tumor cell death directly.

Synthetic dsRNA, a TLR3 agonist, could induce apoptosis of human breast cancer cells, which involves the TRIF-dependent pathway and type I IFN autocrine signaling (Salaun et al. 2006). A bacterial DNA analog, CpG, recognized by TLR9, due to its immunostimulatory effects and capability to directly induce apoptosis in several kinds of tumors, thus is under evaluation as an agent in several clinical trials (Arunkumar et al. 2013; Zhang et al. 2014a).

The effects induced by TLR agonists, including expression of type I IFN, chemokines, and pro-inflammatory cytokines, could be elicited in cancer immunotherapy. However, the anti-inflammatory effects, like IL-10 and TGF- β production, should also be under consideration, especially in clinical application for cancer (Saraiva and O'Garra 2010). Otherwise, such immunosuppressive cytokines induced by TLR agonists may act protumor roles in tumor progression. Fortunately, it is avoidable to produce anti-inflammatory cytokines after TLR agonists' stimulation by blocking MAPK or PI3K pathways in murine DCs, since the secretion of anti-inflammatory cytokines, such as IL-10 and TGF- β , is relying on the activation of MAPK or PI3K signaling pathways (Marshall et al. 2012). Also, a study revealed that blocking of mTOR signaling downregulates IL-10 expression in human DC (Weichhart et al. 2011). Thus, remodeling target cell signaling pathways to make the TLR agonists elicit antitumor-biased effects is the ideal solution for utilizing TLR agonists in cancer immunotherapy.

2.3.2 The Protumor Effect of TLR Agonists in Cancer

Toll-like receptor agonists engage TLRs expressed in tumor cells to promote tumor invasion, survival, progression, and metastasis, involving cytokine and chemokine production directly or indirectly. In the tumor immune microenvironment, TLR agonists through TLRs on tumor cells could upregulate the NF- κ B signaling cascade and produce pro-inflammatory cytokines, chemokines, and anti-apoptotic proteins that contribute to tumorigenesis and tumor cell proliferation directly (Huang et al. 2007). At the same time, TLR agonists through TLRs on tumor cells or immune cells could recruit more immune cells at the tumor sites to enhance immunity, which further induce the release of proangiogenic factors and growth factors by tumor cells to resist the cytotoxic lymphocyte attack, thereby leading to immune evasion (Basith et al. 2012).

In the last decades, the protumor effects of TLR agonists have been widely reported. Huang et al. have reported that the intratumoral injection of TLR2 agonist, *Listeria monocytogenes*, accelerates hepatocellular tumor growth (Huang et al. 2007). It has been shown in hepatocellular carcinoma (HCC) that TLR3 was expressed in both membranous and cytoplasmic HCC cells, and cell surface stimulation of TLR3 with agonist (poly(I:C)) did activate NF- κ B levels, while cytoplasmic stimulation with transfected poly(I:C) significantly induced apoptosis (Yoneda et al. 2008). Recent study has reported convincingly the functional evidence that TLR3 agonists induce cell survivability and metastasis through cell surface in breast tumor cell lines (Gonzalez-Reyes et al. 2010). Wang

et al. have shown that LPS ligation to TLR4 on tumor cells directly can increase NF- κ B-mediated, β 1 integrin-dependent tumor endothelial cell adhesion, tumor extracellular matrix adhesion, and invasion. As a result, the enhancement of tumor metastasis was observed in a murine tumor-bearing model (Wang et al. 2003). And the activation of TLR4 on tumor cells by agonists also has been found to promote tumor growth, apoptosis resistance, and chemoresistance in epithelial ovarian cancer cells, prostate cancer, and colorectal cancer (Hua et al. 2009; Kelly et al. 2006; Killeen et al. 2009). In addition, the triggering of TLR4 with LPS in tumor cell lines produces a number of factors, such as pro-inflammatory factors, inhibitory cytokines, and other small molecules, including nitric oxide, GM-CSF, VEGF, IL-6, IL-8, and IL-12. These factors mimic the inflammatory and suppressive microenvironment, resulting in the resistance of tumor cell lines to cytotoxic T lymphocytes and NK cell attack and then evasion from immune surveillance (Huang et al. 2005; Szczepanski et al. 2009). Recent study found that the activation of TLR signaling by TLR4 or TLR3 agonists could induce autophagy to increase a variety of cytokine productions via promoting TRAF6 (TNF receptor-associated factor 6, E3 ubiquitin protein ligase) ubiquitination and consequently facilitate migration and invasion of lung cancer cells (Zhan et al. 2014). Furthermore, human lung cancer cells express both TLR7 and TLR8; Cherfils-Vicini et al. reported that when TLR7 or TLR8 was triggered by agonists, NF- κ B would be activated to upregulate a kind of anti-apoptotic protein Bcl-2, leading to increased tumor cell survival and chemoresistance (Cherfils-Vicini et al. 2010). In myeloma cells, triggering of TLR7 and TLR9 by agonists would induce tumor cells to secrete IL-6, which could be taken advantage by tumor cells to spread out and escape the usual therapies (Jego et al. 2006). Additionally, TLR9 agonist suppresses apoptosis through TNF in several types of cancer. In both breast cancer and prostate cancer, TLR9 agonist, CpG ODN, dramatically increased MMP13 activity without affecting MMP8 to enhance tumor cell invasion (Ilvesaro et al. 2007).

In addition, TLR agonists also promote tumor growth by acting on host cells. In LPS-induced tumor model, LPS through TLR4 signaling in host cells increases the circulating levels of TNF, which leads to the upregulation of NF- κ B-regulated anti-apoptotic factors, such as cIAP1, cIAP2, and Bcl-xL, in the tumor cells (Luo et al. 2004). Kundu et al. found that LPS and CpG ODN, TLR4 and TLR9 agonists, stimulated immortalized prostate epithelial cells to enhance tumor proliferation due to lower susceptibility of tumor cells to TNF- α -induced apoptosis (Kundu et al. 2008).

Besides tumor cells and host cells, TLR agonists engage immune cells, not only innate immune cells but also adaptive cells. Recently, several groups have reported that TLR2 agonists promote the proliferation and suppressor function of Tregs, thus impairing the antitumor immunity (Kabelitz 2007; Liu et al. 2006; Suttmüller et al. 2006). In addition, TLR agonist stimulation might induce subpopulations of splenic DCs to produce indoleamine 2,3-dioxygenase, which has been reported to degrade tryptophan required by effector T cells. As a result, antitumor immunity

might be downregulated and even tumor growth might be promoted (Mellor et al. 2005; Wingender et al. 2006).

2.4 TLR Agonists Enhance Immunotherapy

2.4.1 The Enhancing Immunotherapy of TLR Agonist Combined with Vaccine in Glioma

Gliomas are the most ordinary tumors that originated from the central nervous system (CNS) and are considered as one of the most aggressive malignancies in human (Preusser et al. 2011). WHO classification grade IV glioma, glioblastoma multiforme (GBM), is the most antagonistic form of gliomas, and the median survival of GBM patients remains nearly 12 months despite administration of therapeutic interventions (Tanaka et al. 2013). TLR agonists have the powerful ability to promote adaptive immunity via pro-inflammatory cytokine secretion, and TLRs are abundantly expressed by myeloid-derived immune cells which are the participants of CNS immune surveillance, thus making TLR agonists as promising candidates in glioma immunotherapy.

The CNS was once regarded as immunologically privileged region. The entrance of immune cells into the CNS was in veil for decades, let alone the presence of the blood–brain barrier (BBB) (Ousman and Kubes 2012). What's more is the low MHC molecule expression level in the CNS tissue, the immunosuppressive micro-environment, and lack of local APCs that are able to migrate back to the circulation after antigen capture (Ousman and Kubes 2012). All these features lead to previous misunderstanding of CNS immunity. Current studies have revealed unique mechanisms of the CNS immune system, for example, the BBB function is compromised under the situation of inflammation and tumor progression (Hawkins and Davis 2005), the existence of CNS lymphatic drainage system have been reported recently (Louveau et al. 2015), and CNS resident microglia could be activated by TLR ligands and become effective APCs in pathologic condition like inflammation (Olson and Miller 2004). In addition, macrophages, although lacking in the brain parenchyma, are abundant in subarachnoid space, choroid plexus epithelium, and perivascular cavities as efficient APCs that could monitor the abnormalities via capture of antigens in the cerebrospinal fluid (Ousman and Kubes 2012). The points mentioned above comprise the biological foundation that makes administration of TLR agonists as a viable strategy in glioma immunotherapy.

After glioma-associated antigen is captured and presented by APCs, glioma-specific effector T cells are induced in peripheral lymphatic organs (Lampron et al. 2013). Glioma-specific T-cell level increases subsequently in the tumor site; however, the antagonistic microenvironment disarmed the infiltration of T cells (Lowther and Hafler 2012). Gliomas are able to escape from immune surveillance partially due to their ability to secrete immunosuppressive cytokines like TGF- β 2 (Eisele et al. 2006). Thus, various strategies, including utilizing TLR agonists in

tumor therapy, have been developed that aimed at converting the suppressive microenvironment and inducing antitumor immunity. TLR agonists have achieved observable changes in immune cell subset distribution or cytokine/chemokine levels in various studies of murine glioma models, which resulted in elimination of the tumor or inhibition of tumor progression. The major cell subsets that participate in anti-glioma immunity are CD8⁺ T cells. However, in certain conditions, the effector cells are not limited to CD8⁺ T cells; NK cells also take part in the process of tumor eradication, which depends on the type of agonist and the manner of administration. Cytokine/chemokine changes caused by TLR agonist administration, under certain conditions, could also be associated with immune suppression and thus must be taken into consideration.

The critical change caused by TLR agonists is shifting of immune response pattern toward CD8⁺ T-cell-biased mode, and the frequency of Tregs is decreased locally or systemically. In murine GL261 glioma model, a strategy combining TLR1/TLR2 agonist bacterial lipoprotein administration with tumor-specific T-cell adoptive transfer achieved long-term survival of the model mice, and the established efficient immune memory was confirmed by rechallenge experiment (Zhang et al. 2014b). The agonist promotes the CD8⁺ T cells to assemble in the tumor site by improving the survival and facilitating CD8⁺ T-cell infiltration into the tumor site; moreover, the function of infiltrated T cells is enhanced due to the altered glioma microenvironment (Zhang et al. 2014b). A strategy combining glioma-specific antigen vaccine with poly-ICLC, a TLR3 agonist, can effectively promote the efficacy of vaccine strategy against CNS tumors (Zhu et al. 2007). Such combined strategy promoted systemic induction of glioma-specific CTLs and upregulated the expressing level of very late activation (VLA) antigen-4, which referred to the CNS homing ability of T cells (Zhu et al. 2007). Furthermore, the combination strategy enhanced expression of IFN- γ by tumor-infiltrating CTLs and prolonged the survival of glioma mice. Topical administration of TLR7/TLR8 agonist imiquimod was also evaluated in a murine glioma model and actually eliminated the intracranial tumor (Xiong and Ohlfest 2011). The CD4⁺ and CD8⁺ T-cell amounts of peripheral blood circulation were decreased in imiquimod-treated mice; however, the frequencies of these cells were elevated in CLNs and the brain. What's more is that administration of imiquimod reduced the frequency of Tregs in the tumor site significantly. All these effects led to activation of brain infiltrating lymphocytes and intracranial tumor growth inhibition. Notably, anti-glioma immune memory had established in imiquimod-treated mice. The other TLR7/TLR8 agonists have also shown effectiveness in glioma (Scheel et al. 2006). Stabilized synthetic RNA oligonucleotides and protected messenger RNA were lately discovered to own an immunostimulatory capability in a TLR7-/TLR8-dependent manner. Intratumor injections of protamine-stabilized mRNA could promote tumor regression and establish long-term anti-glioma immunity. Besides, residual RNA-injected tumors showed the increase of CD8⁺ T-cell infiltration (Scheel et al. 2006). TLR9 agonist CpG treatment combined with tumor lysate vaccine and effector T-cell transfer has been confirmed to inhibit murine intracranial glioma growth efficiently (Wu et al. 2007). The ratio of CD8⁺ effector T cells to

Tregs at the tumor site was elevated in CpG-treated group. In peripheral immune organs like CLNs, the CD4⁺ and CD8⁺ T-cell activation induced by tumor antigen was observed after CpG treatment. The increased frequency of glioma-infiltrating IFN- γ producing CD4⁺ and CD8⁺ T cells is another key sign to evaluate the effectiveness of TLR agonist therapy. In TLR1/TLR2, TLR7/TLR8, and TLR9 agonists, preclinical studies have observed increased tumor reactivity of glioma-infiltrating CD8⁺ T cells as demonstrated by degranulation and IFN- γ secretion in a tumor-dependent manner (Xiong and Ohlfest 2011; Zhang et al. 2014a, b).

TLR1/TLR2 agonist bacterial lipoprotein could decrease immunosuppressive myeloid-derived suppressor cells (MDSCs) homing to the tumor site, thus adjusting the glioma microenvironment that facilitated antitumor functions of effector immune cells (Zhang et al. 2014b). In addition, TLR agonist-modified DCs play an essential role in initiating antitumor immune response in the glioma cell-dominated immunosuppressive environment, for example, glioma cell-derived TLR2 agonist HMGB1 activated DCs and enhanced the effect of comprehensive therapy (Curtin et al. 2009). By using this strategy, about half of the intracranial glioma model mice acquired long-term survival and established a state of immunological memory. Studies showed that TLR2 agonists could boost T-cell clonal expansion by inducing the co-stimulator signaling in T cells (Zhang et al. 2011b). In the circumstance of glioma, both bacterial lipoprotein and HMGB1 could induce glioma antigen-specific T-cell expansion in the glioma model mice, with a considerably high frequency of T cells secreting IFN- γ in response to tumor antigen (Curtin et al. 2009; Zhang et al. 2014b). In clinical trials, patients with GBM received three injections of tumor lysate-pulsed DCs, once every 2 weeks, combined with imiquimod or poly-ICLC as adjuvant (Prins et al. 2011). Combination strategy could improve tumor antigen presentation by DCs and, therefore, initiate antitumor response. One study reported that the DCs stimulated with the TLR agonists poly(I:C) or R848-containing cocktail resulted in higher MHC class II and IL-12 expression level of DCs. Moreover, poly(I:C)- or R848-containing cocktail-stimulated DCs showed the ability of resistance to TGF- β 2 stimulation (Grauer et al. 2007). These data suggest that the characteristics of TLR agonist-induced mature DCs, such as preserving their ability to secrete IL-12 and resist to TGF- β 2, are promising to offer benefits in immunotherapy-based clinical applications for glioma, especially DC-based therapies. TLR9 agonist CpG administration in murine intracranial model showed extended survival in tumor-bearing mice (Alizadeh et al. 2010). Notably, a single injection of high-dose CpG ODN leads to significant increase of MDSC infiltration in tumor site. Such outcome limits the CpG ODN application in glioma treatment. However, research revealed that multiple low-dose injections of CpG could boost antitumor immune response without increased MDSC infiltration in tumor site (Alizadeh et al. 2010). Additionally, the CpG ODN administration seems to improve the antigen-presenting function of microglia, a type of resident APC in CNS tissue (Ravindran et al. 2010). Moreover, TLR agonists are reported to have direct effects on glioma cells. TLR2, TLR3, and TLR4 agonists could enhance MHC class I expression and induce IL-6 secretion of glioma cells in vitro (Grauer et al. 2008). Soluble imiquimod could directly inhibit

the proliferation of GL261 cells in a TLR7-independent manner (Xiong and Ohlfest 2011). And TLR9 agonist CpG could also induce glioma cell apoptosis in vitro and in vivo (El Andaloussi et al. 2006).

Current TLR agonist-related clinical trials for glioma are focusing on application of TLR agonists to modify immunosuppressive tumor environment and induce antitumor immunity and then inhibit tumor progression. Numerous trials have been designed to use TLR agonists for such purpose, many of which combined TLR agonist treatment with classic chemotherapies and radiotherapies and more combined TLR agonists with other immunotherapies for glioma, mainly DC-based vaccines and tumor antigen vaccines. Thus, TLR agonists are added into procedures to elicit the therapeutic effect. TLR agonists for TLR3, TLR7, and TLR9 are the most common agents in clinical trials for glioma, and studies on these TLR agonists not only exploit the potential effective manner of these agents for glioma treatment but also underline the importance of improving their efficacy generally, such as decreasing toxicity while maintaining antitumor activity, in a clinical situation.

2.4.2 TLR Agonists Alone Enhance Immunotherapy

Enhancement of T-cell immunity has been recently attributed to the effect of TLR agonists on various T-cell subsets, which have been considered as a promising strategy to enhance the efficacy of cancer immunotherapies. Naïve T cells express low levels of TLRs, while they can upregulate TLRs in both mRNA and protein levels upon activation by TLR agonists (Sobek et al. 2004). The co-stimulatory effects of TLRs on T cells are mostly dependent on concomitant TCR stimulation, since TLR agonists alone have minimal effects on naïve T cells (Asprodites et al. 2008). Almost all TLR agonists have been found to stimulate T-cell activation to facilitate cancer immunotherapy.

TLR2 agonists have been considered as the most effective reagents, which trigger powerful antitumor immunity by stimulating CD8⁺ CTLs and CD4⁺ Th cells and regulating Foxp3⁺ Tregs. TLR1/TLR2 agonist enhances CTLs to produce IFN- γ and TNF- α and secrete IL-2 (Cottalorda et al. 2006; Lu et al. 2011; McCarron and Reen 2009). Also TLR2 agonist can increase expression of granzyme B and perforin, which are the major molecules secreted by CTLs to mediate cytotoxicity against tumor cells (Geng et al. 2010). T-cell tolerance represents most obstacles of hindering T-cell effects. Of which, regulatory T cells mediate the strongest immunosuppression by secretion of IL-10 and TGF- α . Interestingly, TLR2 agonist stimulation on Tregs directly reduces the suppressive function of Treg, as a result, to promote CTL and Th cell proliferation and function (Rahman et al. 2009).

Synthetic bacterial lipoprotein (BLP), a TLR1/TLR2 agonist, has a general tumor therapeutic property and is thought as an effective example. In our previous study (Zhang et al. 2011b), Lewis lung cancer 3LL-bearing mice were treated intraperitoneally with BLP, from day 5 every 5 days for a total of four times. A significant tumor regression and prolonged survival were observed, and the “cured” mice could resist the rechallenge of parent tumor cells, but not other tumor cells.

Antitumor effect of BLP is universal, since we also detected the similar results in melanoma F10 and leukemia FBL3 mice models. The mechanisms of antitumor effect of BLP mainly rely on the impact of BLP on CTLs and Tregs, but not tumor cells. On one hand, BLP enhances cytokine secretion, proliferation, and cytotoxicity of CTLs directly. On the other hand, BLP decreases Foxp3 expression of Tregs and blocks the proliferative suppression of Tregs to further enhance CTL activity both in vitro and in vivo, where TLR2 expression on Tregs is required.

The effect of TLR3 agonist on CD4⁺ T cells has been shown to enhance NF- κ B-dependent cell survival and high expression of anti-apoptotic molecule Bcl-xL (Gelman et al. 2004). Similar study also demonstrates that TLR3 agonist poly(I:C) induces CD8⁺ T-cell proliferation and function (Seki et al. 2002). In addition, TLR3 agonist promotes generation of memory T cells, as a result of prolonging T-cell survival (Hervas-Stubbs et al. 2007). These results highlight the application of TLR3 agonists for cancer immunotherapy.

TLR7 and TLR8 agonists have been demonstrated to induce high levels of IFN- α and IFN- β , which could reverse the immunosuppressive activity of Tregs, which results in the proliferation of effector CD4⁺ T cells (Peng et al. 2005). Moreover, administration of poly-G10, a TLR8 agonist, results in enhanced antitumor activity by downregulating immunosuppression (Peng et al. 2005). The activation of TLR7 and TLR8 with the agonist R848 increases IFN- γ , IL-2, and IL-10 secretion and also enhances proliferation of Th cells directly (Caron et al. 2005).

Several studies have shown effects of TLR9 agonists on T-cell survival and proliferation. Further studies have found that the mechanism is dependent on NF- κ B signaling and is related with upregulated expression of the anti-apoptotic protein Bcl-xL (Gelman et al. 2004). Interestingly, the engagement of TLR9 on CD4⁺ T cells with the agonist CpG ODN enhances their proliferation independently of APCs. During the proliferation, both CD4⁺CD25⁻ cells (effector T cells, T_{eff}) and CD4⁺CD25⁺ cells (regulatory T cells, T_{reg}) expand, but the suppression of T_{reg} is attenuated. As a result, the immunosuppressive effects mediated by Tregs are rejected (Chiffolleau et al. 2007). In addition, TLR9 agonists increase CD4⁺ and CD8⁺ T-cell frequency by enhancing IL-2 production and CD25 expression. More importantly, T-cell activation by TLR9 agonists also happens in the absence of CD28, which highlights the potential of TLR9 as co-stimulatory signal (Bendigs et al. 1999). These results emphasize the application of TLR9 agonists for cancer immunotherapy.

It is known to all that a variety of tumor cells express TLRs, and the triggering of these TLRs on cancer cells by agonists could impact tumor growth by various mechanisms, including the induction of apoptosis and the increase of sensitivity to chemotherapy.

Although some groups including ours demonstrate that there is no detectable change in the effect of TLR2 agonists on cancer cells (Seif et al. 2009; Zhang et al. 2011b), several reports suggest that TLR2 agonists directly inhibit bladder cancer cells to play antitumor effect. PGN induces IL-8 production in bladder cancer cell lines, T24 and 5637. BCG aids host to secrete inflammatory cytokines, such as IL-1 β and IL-6, and also induces tumor cell death and apoptosis to regress

tumors. Water extract of *C. nuda* (WE-CN) includes the nuclear translocation of NF- κ B and activation of JNK and also enhances MHC II and co-stimulatory molecule expression for DC maturation (Adams 2009; Chen et al. 2013; Shimizu et al. 2004).

Various types of cancers express TLR3, such as breast cancer, colon cancer, lung cancer, pharynx cancer, bladder cancer, and head/neck cancer. In breast, colon, pharynx, and lung cancer, TLR3 agonist induces tumor cell apoptosis involving caspase pathway (Salaun et al. 2006). In bladder cancer, TLR3 stimulation by poly(I:C) results in the production of CXCL10 and other CTL-attracting factors to enhance CTL entry in tumor environment (Muthuswamy et al. 2015). The triggering of TLR3 by poly(I:C) in head and neck carcinoma cells leads to downregulated migration of cancer cells before the beginning of tumor migration (Rydberg et al. 2009).

TLR5 agonists have been shown to inhibit tumor cell growth in breast cancer, head and neck cancer, and colon cancer. In breast cancer, TLR5 agonist, flagellin, can inhibit tumor cell proliferation and is mechanistically linked to the regulation of autophagy protein MAP1S (Shi et al. 2014). In head and neck cancer, flagellin also decreased viability and increased apoptosis (Rydberg et al. 2009). Similarly, in colon cancer, TLR5 agonist suppresses tumor growth due to tumor necrosis (Rhee et al. 2008).

In glioma, TLR9 agonist through NF- κ B and NO induces cell cycle arrest to decrease tumor cell proliferation along with irradiation. Furthermore, CpG ODN stimulation of TLR9 on neuroblastoma cells decreases cell proliferation and increases caspase-dependent apoptosis, resulting in prolonged survival of tumor-bearing mice (Deng et al. 2014).

2.5 Clinical Trials of TLR Agonists in Cancer Immunotherapy

2.5.1 TLR3 Agonist: Poly-ICLC

As dsRNA analogs, polyriboinosinic–polyribocytidylic acid (poly(I:C)) is a ligand of endosomal TLR3. Poly-ICLC, a synthetic complex of poly(I:C) stabilized with poly-L-lysine and carboxymethyl cellulose, was named one of the immunotherapeutic agents with the highest potential to improve cancer immunotherapy by the National Cancer Institute (Cheever 2008).

Poly-ICLC has been extensively evaluated in patients with malignant gliomas as a single therapeutic agent, proving clinical safety and feasibility. The safety and efficacy of poly-ICLC has been demonstrated when combined with chemotherapy (temozolomide) and radiotherapy in adults with newly diagnosed glioma in a phase II study (Rosenfeld et al. 2010). Patients received external beam radiation with concurrent temozolomide followed by adjuvant temozolomide and intramuscular poly-ICLC, and median overall survival (OS) for subjects 18–70 years old was

18.3 months (95 % CI: 15.9–19.8 months), as compared with 14.6 (95 % CI: 13.2–16.8) reported before. The results suggested that poly-ICLC might elevate the efficacy of chemoradiation without additional significant toxicities. Forty-seven children diagnosed with brain tumor with various kinds of histopathologic subtypes were treated with poly-ICLC in a pediatric phase II trial (Hartman et al. 2014), four children with progressive low-grade glioma experienced stable disease for 18–24 months, and overall five of ten LGG patients had responded. Basing on the promising response and low toxicity, poly-ICLC is believed to be effective for childhood low-grade glioma treatment as a single agent or adjuvant.

Various strategies are being investigated to maximize the antitumor immune stimulation of poly(I:C) and poly-ICLC in cancer vaccine currently. Prins and colleagues (Prins et al. 2011) reported the potential therapeutic effect of poly-ICLC in glioblastoma multiforme patients and found that combination of poly-ICLC with DC vaccine significantly improved the overall survival compared to sole DC vaccination. In this study, median OS from the time of initial surgical diagnosis was 31.4 months for all glioblastoma patients ($n = 23$), while OS was reported as 21.4 months in DC-based vaccine trial (Wheeler et al. 2008). Sabbatini and his colleagues (Sabbatini et al. 2012) also described the efficiency of poly-ICLC in ovarian cancer. In this study, a significant increased time to disease progression was observed in NY-ESO-1-positive ovarian cancer patients after administration of NY-ESO-1 overlapping long peptides combined with poly-ICLC. Larger-scale clinical trials are currently under way to confirm the safety for poly-ICLC in single-agent trials and combinational vaccine trials.

Poly(I:C) and poly-ICLC effectively promote antitumor responses of host as immunostimulatory elements and are used as vaccine adjuvant in preclinical and clinical trials in patients with lymphoma, melanoma, and other tumors. Combining poly(I:C)/poly-ICLC with compounds that block immunosuppressive signal pathways might result in further improvement of therapeutic prognosis. Although the long-term function has not been described, immunological activity exhibited in cancer patients in clinical trials make poly-ICLC a good candidate for cancer vaccine adjuvant.

2.5.2 TLR7 Agonist: Imiquimod

Imiquimod (Aldara® topical cream), a synthetic small molecule that preferentially activates TLR7 with weak activation of TLR8, is originally approved as a treatment for external genital and perianal warts. Recently, the FDA has approved its use for the treatment of skin cancer superficial basal cell carcinoma (sBCC).

Topical application results in complete clearance of external genital warts in up to 70 % of cases, actinic keratosis in 57 %, and basal cell carcinoma in 90 % (Gollnick et al. 2008). A multicenter, double-blind phase III clinical study showed that imiquimod 5 % cream administration is a safe and effective strategy for sBCC. Composite clearance rate of imiquimod group (77 %) was much higher than vehicle group (6 %), as well as histological clearance rates (Schulze et al. 2005). Chun-

Guang and colleagues (2014) recently reported two cases of giant (>5 cm) BCC successfully treated with topical imiquimod 5 % cream. Both of the two giant tumors ($6 \times 8 \text{ cm}^2$, $5.2 \times 4.2 \text{ cm}^2$) were cured according to the clinical and pathological data, with 6-year and 3.5-year follow-up, respectively. A multicenter, controlled randomized comparative trial in 2013 showed that the cure rate for imiquimod (83.4 % (78.2–88.9)) is higher than fluorouracil (80.1 % (74.7–85.9)) and MAL-PDT (72.8 % (66.8–79.4)); the difference between imiquimod and MAL-PDT was significant (95 % CI 1.5–19.5; $p=0.021$); therefore, imiquimod could be considered as the preferred treatment of sBCC (Arits et al. 2013). With regard to imiquimod 5 %, five applications per week is the recommended regimen because it provides the best compromise between clinical efficacy and safety with minimal side effects (Micali et al. 2014).

The exact mechanism of function of imiquimod is unknown; however, it has been commonly hypothesized that imiquimod enhance the antitumor immune response by activating DCs, natural killer cells, macrophages, and B lymphocytes and releasing inflammatory cytokines (IFN- α , IL-6, and TNF- α) and chemokines triggered by TLR signal pathways. In addition to immune stimulation, imiquimod has anti-angiogenic properties, and it also can stimulate intrinsic apoptosis (Smith et al. 2007). Imiquimod has been shown useful in the treatment of actinic keratosis and skin metastasis from breast cancer and malignant melanoma (Hesling et al. 2004). In a phase II trial (Adams et al. 2012), response rate of 20 % was observed in patients with refractory breast cancer skin metastases after being treated with topical imiquimod. Responders showed histologic tumor regression with evidence of an immune-mediated response, including activation of Th1 and Tc1 T-cell responses and decreased concentrations of IL-6 and IL-10 in tumor supernatants posttreatment, suggesting that imiquimod can promote a proimmunogenic tumor microenvironment in breast cancer. Henriques et al. (2014) reported that a woman with breast cancer skin metastasis was successfully cured by imiquimod, while radiotherapy and chemotherapy had no effect. Application of imiquimod resulted in significant regression of the skin metastases and great reduction in pain from the skin metastasis. Clinical trials are ongoing to investigate the combinations of imiquimod with other treatments.

2.5.3 TLR7/TLR8 Agonist: Resiquimod

Resiquimod (R848, or S28463, or VML600) (1-(2-methylpropyl)-1H-imidazo [4,5-c]quinolin-4 amine), a small molecule that belongs to the group of immune response modifiers, stimulates immune responses by TLR7 and TLR8 activation and possesses antiviral and antitumor activity. Resiquimod is chemically related to imiquimod, but was shown to more potently induce cytokine expression (IFN- α , IL-6, IP-10, and IL-1Ra) and secretion in peripheral blood mononuclear cells (Kwissa et al. 2012). It was developed by 3M Pharmaceuticals (St Paul, MN, USA) in the early 1980s during attempts to identify nucleoside analog structures inhibiting HSV 2 infection. But associated studies were terminated because of

lacking efficacy in clinical trials. In 2010 a license agreement conferred rights relative to resiquimod to Spirig Pharma AG (Egerkingen, Switzerland) for further development in the therapy of sun-damaged skin.

Resiquimod is a compound with low molecular weight (314.4 Da) and is able to penetrate into the upper layers of the epidermis. Topical application of resiquimod leads to cytokine production and is largely confined to the skin with virtually no systemic exposure to the drug and serum cytokine response (Meyer et al. 2013). In a placebo-controlled study of healthy subjects receiving topical resiquimod 0.25, 0.05, or 0.01 %, detectable levels of resiquimod or its metabolite S28371 in serum (0.23 ng/mL, 8 h after the last dose) were only found in one of eight subjects applying the highest concentration (0.25 % resiquimod applied for 8 h two times a week over 3 weeks) (Sauder et al. 2003).

The efficiency of resiquimod in the treatment of skin lesions (Szeimies et al. 2008), such as those caused by the herpes simplex virus (Fife et al. 2008), has been demonstrated in plenty of clinical trials. In addition, resiquimod has been evaluated in numerous clinical studies for cancer because of their immune modulatory activity, namely, the ability to activate innate immunity associated with type I interferon production and improve the effect of vaccines (Tomai et al. 2007). The safety and immunostimulatory profiles of resiquimod are being assessed in cutaneous T-cell lymphoma and nBCC patients, with resiquimod topically used as a therapeutic intervention, and also in patients with recurrent or advanced melanoma, treated with resiquimod as an adjuvant to vaccine (Vacchelli et al. 2013). In Sabado's study (Sabado et al. 2015), resiquimod was proved to be a strong immunologic adjuvant of vaccines in melanoma patients. NY-ESO-1 vaccine formulated in Montanide was intradermally injected, and 0.2 % resiquimod gel was applied to the vaccine site in surgically resected melanoma patients. NY-ESO-1 protein in combination with topical resiquimod was proved to be safe and induced both antibody and CD4⁺ T-cell responses in most patients. Patients with TLR7 SNP rs179008 had a greater likelihood of developing NY-ESO-1-specific CD8⁺ T-cell responses. Similar investigator-initiated cancer vaccine studies for resiquimod in melanoma and glioma patients are currently in progress.

Further improvements can be expected by conjugating TLR agonists with antigens or by combining resiquimod with other TLR agonists or co-stimulatory factors to induce synergistic activation of DC. In view of these clinical data, resiquimod remains an interesting candidate as a potential vaccine adjuvant, and its efficiency remains to be further determined.

2.5.4 TLR8 Agonist: Motolimod

Motolimod (VTX-2337) is a synthetic small molecule based on a 2-aminobenzazepine core. Motolimod is a selective TLR8 agonist and has no clinically relevant activity on any other TLR family members, and due to its non-nucleotide structure, motolimod does not interfere with purine catabolism or interact with purinergic receptors.

TLR8 is a potent activator of innate immunity, and in human TLR8 is expressed in the endosomal membrane of monocytes and the mDC. This is a clear distinction between human TLR7 and TLR9 in the endosome of plasmacytoid dendritic cells – with very different phenotypes and biology with mDC. Activation of the innate immune system using motolimod is different from TLR7 (imiquimod) and TLR9 agonist (CpG ODN2006), which have been widely investigated in various types of cancer. Motolimod directly activates mDCs, monocytes, and NK cells, increasing the production of adaptive antitumor response mediators IL-12, TNF- α , and IFN- γ . Motolimod activates mDCs and other APCs to more effectively present tumor-expressed antigens to T cells. Motolimod-induced activation of NK cells also enhances antibody-dependent cell-mediated cytotoxicity of tumor cells by the production of mAbs and IFN- γ in the treatment of some cancers.

Recently, Gregory N. Dietsch (Dietsch et al. 2015) demonstrated that late-stage cancer patients are highly sensitive to TLR8 activation by motolimod. In late-stage cancer patients, plasma levels of IL-6, G-CSF, MCP-1, and MIP1- β were increased with increasing motolimod dose, which closely aligned with the response seen in preclinical studies, demonstrating that advanced cancer patients remained responsive to TLR8 activation. Activation of TLR8 in late-stage cancer patients is expected to enhance tumor-directed immune responses. A clinical trial has been performed by Northfelt to evaluate the pharmacokinetics, pharmacodynamic responses, safety, and tolerability of motolimod in late-stage oncology patients (Northfelt et al. 2014). The data from this study in patients with advanced cancer demonstrate dose-dependent pharmacology and predictable, transient adverse events associated with systemic immune activation. Pro-inflammatory cytokines and chemokines were induced by motolimod, provide a reliable set of biomarkers for TLR8 activation, and identified biologically active doses suitable for further evaluation. Motolimod highlights the possibility of modulating innate immune responses as a means to induce productive immunity in patients with cancer. Clinical development of motolimod is being advanced in combination with various anticancer agents in multiple solid tumor indications.

2.5.5 TLR9 Agonist: MGN1703

TLR9, an intracellular receptor, is constitutively expressed in APCs and activated by the unmethylated CpG islands and bacterial and viral DNA. TLR9 agonists activate DCs and B lymphocyte and initiate secondary effects, including cytokine and chemokine production, activation of NK cells, and antigen presentation. Therefore, TLR9 has become a target of investigation for various malignancies.

To date, PF-3512676 has been the most widely studied CpG ODN TLR9 agonist. PF-3512676 is a synthetic oligodeoxynucleotide (ODN) and mimics the structure of unmethylated CpG single-strand DNA. Mixed results were found in the investigations of the efficiency of PF-3512676 monotherapy in a variety of cancers (Kim et al. 2010; Pashenkov et al. 2006). Safety and efficacy of PF-3512676 in advanced RCC has been demonstrated (Thompson et al. 2009). However, no response was

reported in a phase II trial involving 41 patients with chronic lymphocytic leukemia following PF-3512676 given as an intravenous (i.v.) infusion (Zent et al. 2012) (1.05 mg/kg) or subcutaneously (0.45 mg/kg). In patients with advanced non-small cell lung cancer, PF-3512676 combined with standard chemotherapy regimens didn't show any improvement (Manegold et al. 2012; Wittig et al. 2015). Thus, it's not surprising that, at the present time, no CpG ODN has been approved in oncology indication.

MGN1703, a small DNA molecule containing 116 nucleotides, is a novel and alternative TLR9 agonist for cancer therapy. MGN1703 has been investigated at very low doses as an adjuvant to vaccination. A phase I/II study investigated MGN1703 in combination with vaccination and chemotherapy in 17 patients with metastatic CRC (Weihrauch et al. 2005). In this study, five patients (29 %) achieved a CR (four receiving an MGN1703 vaccine), one (6 %) achieved a PR, five (29 %) had SD (four receiving an MGN1703 vaccine), and six (36 %) showed PD (one receiving an MGN1703 vaccine). In addition, this kind of vaccination was generally well tolerated; only transient side effects such as short-term body temperature increasing or local skin reactions were found. Another double-blind phase II trial was performed to evaluate MGN1703 in the maintenance setting for patients who had achieved disease control of metastatic CRC after standard first-line induction therapy. A total of 59 patients were randomized in a 2:1 ratio to receive subcutaneous MGN1703 (60 mg) placebo, both given twice per week until disease progression (Schmoll et al. 2014). PFS was analyzed from the beginning of induction therapy, statistically improved with MGN1703 (2.8 months, 2.8–4.1) compared with placebo (2.6 months, 2.5–2.8) following both independent and investigator assessment. The findings from this phase II study are encouraging and indicate that maintenance therapy with MGN1703 may improve PFS compared with placebo in advanced CRC. MGN1703 was also found to be well tolerated in this study, suggesting that the tolerability profile of this agent is more in line with that of therapeutic vaccines rather than the single-stranded TLR agonists. The absence of significant systemic toxicity of MGN1703 may potentially be due its composition of only natural (i.e., non-modified) DNA. The development of TLR9 agonists in cancer immunotherapy is ongoing with research mainly being directed at a variety of ODN.

2.6 Summary and Discussion

Due to the key functions of TLRs in initiating innate immune and inflammatory responses as mentioned above, targeting of TLRs has become an attractive therapeutic approach for human cancers. However, so far, only a few TLR agonists have been approved by international regulatory agencies to treat human diseases, including BCG, MPL, imiquimod (approved by the US FDA), and Picibanil (approved by the Japanese Ministry of Health, Labour and Welfare). Meanwhile, clinical trials aiming to evaluate the safety and therapeutic profiles of TLR agonists in cancers are

fewer than last decade and are continuously reducing. This phenomenon has surely been caused by the limited availability and efficiency of TLR agonists, urging scientific researchers to pay more attention to alternative compounds. Furthermore, TLR agonists activate a complex group of signal transduction pathways that involve not only immune effector cells but also malignant cells since TLRs are widely expressed in human cells. TLR agonists might only induce efficient antitumor responses in specific subsets of patients, which still need to be formally demonstrated. Thus, the future of TLR agonists might not only focus on the precise signaling pathways that they trigger but also on the predictors of the propensity of individual cancer patients in order to obtain a clinical benefit from TLR agonists.

3 Noncoding RNAs in Cancer Immunotherapy

3.1 Introduction of Noncoding RNAs

Noncoding RNAs (ncRNAs) refer to transcripts that do not encode protein. These RNAs were initially considered as junk RNAs. However, researchers discovered later that ncRNAs could act as important regulators of protein-coding genes, through mechanisms such as chromosome modification, transcription factor recruitment, blocking the transcription through steric hindrance, blocking the transportation of transcriptional factors into the nucleus, blocking the translation of mRNA, and leading to the degradation of the target RNA (with the help of other factors). Noncoding RNAs can be divided into housekeeping ncRNAs and regulatory ncRNAs. The former include ribosomal RNAs (rRNAs), transfer RNAs (tRNAs), small nuclear RNAs (snRNAs), and small nucleolar RNAs (snoRNAs), which are expressed constitutively in all kinds of cells. The latter can be further categorized according to their length; the shorter ones include microRNAs (miRs), small interfering RNAs (siRNAs), and Piwi-interacting RNAs (piRNAs); the longer ones are called long noncoding RNAs (lncRNAs) collectively. A more comprehensive classification can be found in a review by Zhou et al. (2010). In this chapter, we will focus on regulatory ncRNAs, mainly on the small noncoding RNAs (microRNAs, miRs) and the long noncoding RNAs (lncRNAs).

3.1.1 MicroRNAs

MicroRNAs (miRs) are ~22 nt-long noncoding RNAs. Most of the time, their genes locate quite distant from other genes, indicating they're regulated and expressed as separate units. Many miRs are located in the introns of other genes, for example, miR-126 locates in the intron of a coding gene *EGFL7* and is regulated by its promoter (Liu et al. 2015). Some miRs form clusters and are regulated by the same promoter, for example, the miR-15a/miR-16-1 cluster locates in the intron of the

gene of noncoding RNA *Dleu2* and is regulated by the promoter of that “host gene” (Lerner et al. 2009). In closely related species, most miRs are conserved in sequences; some miRs also have conserved relationships with their host genes.

miRs regulate their target genes at the posttranscriptional level. Mature miR duplexes will incorporate into a protein complex called RNA-induced silencing complex (RISC). Some subunits of the RISC may have helicase activities and can unwind the duplexes, leaving only one strand in the RISC while the other peeled away and degraded. The strand that remained usually binds to the 3'UTR of the target mRNA and leads to translational repression (which is often the case in mammals) or cleavage of the mRNA. It should be noted that the residues 2–8 of the miR are of importance because this area should highly complement to the target mRNA for effective miR-mediated repression. Any alterations at the site of mRNA recognized by this area can greatly compromise the repressing effect, as demonstrated by several experiments which deliberately introduce mutations on the 3'UTR to validate the targeting relationship of certain miRs to this 3'UTR. This special area in the miR and the site recognized by this area in mRNA are conserved across species. Thus the residues 2–8 in miRs are given the name “seed sequence.”

It takes a few steps to get mature miRs after transcription by RNA polymerase II (Fig. 2.2). The original transcripts of miR genes are called pri-miRs. Pri-miRs are long RNAs containing hairpin-like pre-miR precursors. After transcription, they will be cleaved by an RNase III endonuclease named Drosha, which liberates stem-loop intermediates. These intermediates are called pre-miRs and will be transported out of the nucleus by Ran-GTPase and exportin-5. In the cytoplasm, pre-miRs will meet another RNase III endonuclease named Dicer. Dicer can cleave away the loop and a few base pairs from the terminal, forming a double-stranded RNA with 2 nt overhang on the 3' terminal on both strands. These duplexes are mature miRs, which will be incorporated into DISC and repress the expression of their target genes.

3.1.2 Long Noncoding RNAs

Long noncoding RNAs (lncRNAs) are usually more than 200 nucleotides long. If their encoding genes overlap that of another transcript, according to which strand is the lncRNA to be transcribed, they can be called “sense” (on the same stand) or “antisense” (on the opposite strands). In terms of the linear location of a lncRNA gene relating to another gene along the chromosome, a lncRNA can be “bidirectional” (have close initiation points, transcribed on opposite strands), “intronic” (located in the intron of the other gene), or “intergenic” (located in the interval between two genes) (Ponting et al. 2009).

Long noncoding RNAs are usually transcribed near the promoters, exons, or introns of other genes, so as to act as cis-regulators. Unlike miRs, they're processed similarly as mRNAs (Peschansky and Wahlestedt 2014).

Like miRs, they regulate the expression of their target genes; however, unlike miRs, which interfere the translation level, lncRNAs regulate the transcription of

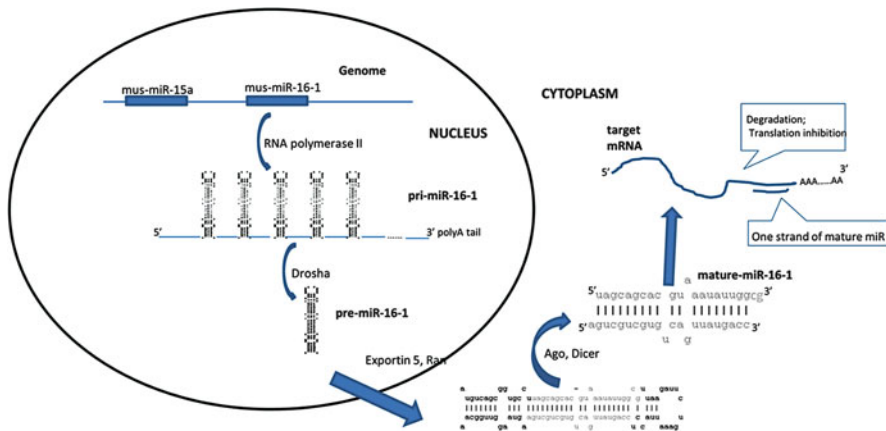


Fig. 2.2 Biogenesis of microRNAs. The maturation of microRNAs takes a few steps after transcription by RNA polymerase II. The original transcripts of miR genes are called pri-miRs. Pri-miRs are long RNAs containing hairpin-like pre-miR precursors. After transcription, they will be cleaved by an RNase III endonuclease named Drosha, which liberates stem-loop intermediates. These intermediates are called pre-miRs and will be transported out of the nucleus by Ran-GTPase and exportin-5. In the cytoplasm, pre-miRs will meet another RNase III endonuclease named Dicer. Dicer can cleave away the loop and a few base pairs from the terminal, forming a double-stranded RNA with 2nt overhang on the 3' terminal on both strands. These duplexes are mature miRs, which will be incorporated into DISC and repress the expression of their target genes

their target genes. Also unlike miRs that always act as suppressors, lncRNAs can act as promoters or suppressors. Many mechanisms through which lncRNAs exert their functions have been discovered. As reviewed by Ponting et al. (2009), there are at least nine mechanisms:

1. The promoter of the lncRNA is adjacent to that of its target gene, and the lncRNA is transcribed through the target gene, blocking the transcription of the target gene.
2. The promoter of lncRNA is adjacent to that of its target gene, but instead of interfering the transcription of the target gene, transcription of the lncRNA remodels the chromatin structure and allows easier access of transcription machinery to the promoter of the target gene.
3. The lncRNA binds to the promoter of the target gene and the transcription factors, leading to the dissociation of the preinitiation complex. In this case, the lncRNA regulates its target gene *in cis* because the lncRNA is transcribed from a minor promoter upstream of the target gene.
4. The lncRNA binds to an RNA-binding protein, leading to conformation alteration of the RNA-binding protein. The RNA-binding protein then interacts with histone acetyltransferases and represses the expression of target gene.
5. The lncRNA associates with other factors, forming a ribonucleoprotein complex and activating the enhancer of the target gene.

6. The lncRNA assists the oligomerization of heat shock factors and further forms a complex with translation factors to induce target gene expression.
7. The lncRNA blocks the translocation of transcription factors into the nucleus by binding to the importin protein.
8. A set of ncRNAs can wrap gene clusters, establish a nuclear domain, and also attract epigenetic modifiers to repress the expression of this gene cluster.
9. The lncRNA interacts with other factors and results in the methylation and repression of a gene cluster; this time the lncRNA regulates *in trans*.

3.2 *Dysregulation of Noncoding RNAs in Cancer*

Most of previous studies focused on protein-coding RNAs; however, the recent processes of the development of noncoding RNAs have been challenging our ingrained theory and innate imagination in biology constantly. More and more evidence suggests that noncoding RNAs are important regulatory molecules *in vivo* (Huarte and Rinn 2010). A growing body of studies have found that a wide range of noncoding RNAs, especially miRs and lncRNAs, are involved in biological processes including cell growth, proliferation, and differentiation. Very few of noncoding RNAs have been characterized for the molecular mechanisms of their regulation, but an increasing number of researches suggest that dysregulations of them play important roles in many kinds of cancers. Abnormal expression of noncoding RNAs leads to the initiation and progression of different cancers (Hansen et al. 2013; Lee and Dutta 2009). Therefore, it heralded a new era of cancer treatment with noncoding RNAs. The dysregulations of noncoding RNAs, especially miRs and lncRNAs in several kinds of cancers recently, are summarized (Table 2.2).

3.2.1 **Cancer-Suppressive or Cancer-Promoting Role of Noncoding RNAs**

According to their function in tumorigenesis, noncoding RNAs can be classified into the oncogene-like ncRNAs and tumor suppressor-like ncRNAs, which mainly depends on the function of their major targets (Deng and Sui 2013). Publications showed noncoding RNAs are involved in almost all processes of tumor progressions, such as angiogenesis, invasion/metastasis, and poor survival. Noncoding RNA sequence analysis also shows cancer-promoting role or cancer-suppressive role of noncoding RNAs (Deng and Sui 2013; Finoux and Chartrand 2008; Volinia et al. 2006).

A noncoding RNA acts as a tumor suppressor if loss of its function can lead to malignant transformation of a normal cell. The first support for those microRNAs that are involved in cancer development came from the identification that microRNA-15a/microRNA-16-1 was deleted or downregulated in most of chronic

Table 2.2 Noncoding RNAs are dysregulated in tumors

Tumor types	Dysregulated ncRNAs in tumors		
	Upregulated		Downregulated
CRC	miR-21	miR-25	LET
	miR-31	miR-19	GAS5
	miR-181b		miR-23b
	HULC		miR-133b
	HOTAIR		
	miR-135b		
	miR-92		miR-145
	miR-106a		
CLL	miR-128a		miR-15a
	miR-146		miR-16-1
	miR-155		miR-223
	miR-195		miR-29
	miR-21		miR-150
HCC	MALAT1	MVIH	MEG3
	HOTAIR	miR-21	hDreh
	HULC	miR-221	LET
	TUC338	miR-224	let-7a
	H19		miR-199a
	HEIH		miR-122a
Breast cancer	miR-155		let-7(a, b, c, d)
	miR-17 ~ 92		miR-29a, b, c
	miR-21		GAS5(83)
	HOTAIR		miR-125a
			miR-125b
Lung cancer	miR-155		let-7(a, b, c, d)
	MALAT1		miR-29a, b, c
	miR-191		Linc-P21
	miR-19a		
	miR-21		
	MVIH		
Prostate cancer	ANRIL		miR-15a/miR-16
	PRNCR1		miR-143
	PCAT-1		miR-145
	miR-21		
Glioblastoma	miR-221		miR-128
	miR-10b		
			miR-181b
			miR-181a

lymphoblastic leukemia patients (Calin et al. 2002). miR-15a/miR-16 acts as a tumor suppressor by regulating the target gene Bcl-2. Recently, with the further studies, a growing number of miRs have been proven to be the tumor suppressors. For example, let-7 family of miRs were downregulated in lung and breast cancer (Hansen et al. 2013); miR-34a was downregulated in non-small cell lung cancer and so on (Yanaihara et al. 2006). A lot of microRNA expression profiling analysis also confirmed that many miRs are tumor suppressors. In esophageal squamous cell carcinoma patients, expression of miR-126 was significantly reduced. miR-126 regulates esophageal squamous cell development and differentiation by targeting ADAM-EGFR-AKT signaling pathway (Liu et al. 2015). Besides, lncRNAs are also key members of the tumor suppressors. Deletion or downregulation of BM742401 will augment gastric cancer. LincRNA-P21 acts as a tumor suppressor by regulating p53 signal pathway. Many other lncRNAs are believed to act as tumor suppressors, such as MEG3, GAS5, and so on (Zhang et al. 2010).

The number of noncoding RNAs acting as oncogenes was less than the tumor suppressors, but the evidences of their role are very conclusive. miR-17-92 located on human chromosome 13q31.3 is the first discovered tumor-promoting miR (Danielson et al. 2015). Transgenic mice overexpressing the c-myc have a high risk of B-cell lymphoma due to high expression of miR-17-92. Abnormal high expression of miR-17-92 was detected in a variety of tumors. There are two mechanisms for upregulation of miR-17-92 in cancers). miR-155 is also one of the earliest discovered tumor-promoting microRNAs. miR-155 is located on chromosome 21q23 and embedded in a host noncoding RNA named the B-cell integration cluster (BIC). miR-155 has high expression in almost all kinds of B-cell malignancies (Metzler et al. 2004). miR-21 is activated in interleukin-6 signaling pathway by STAT3 and upregulated in all kinds of cancers (Slaby et al. 2007). PCGEM1 is identified as a long noncoding RNA which was reported that it has association with prostate cancer. Recent studies have reported that HOTAIR has high expression in CRC patients, and prognosis of these patients was poor (Ifere and Ananaba 2009).

However, definition of ncRNAs as oncogene-like ncRNAs and tumor-suppressive ncRNAs is not absolute, several noncoding RNAs play both roles in tumor-promoting and tumor-suppressive activities. Take microRNA-15a/microRNA-16 for example; they act as tumor suppressors in CLL, but they also function as negative regulators of angiogenesis in critical limb ischemia (CLI) patients. The role of noncoding RNAs may be dependent on the tissues and tumor contexts (Spinetti et al. 2013).

3.2.2 Noncoding RNAs Function as Cancer Hallmarks

Noncoding RNAs can be used as diagnostic or prognostic tool. The expressions of noncoding RNA spectrum reflect the origin of the tumor processes and other pathological features. Since the expression of noncoding RNAs is stable and not degraded by RNA enzymes easily, noncoding RNAs are suitable for cancer

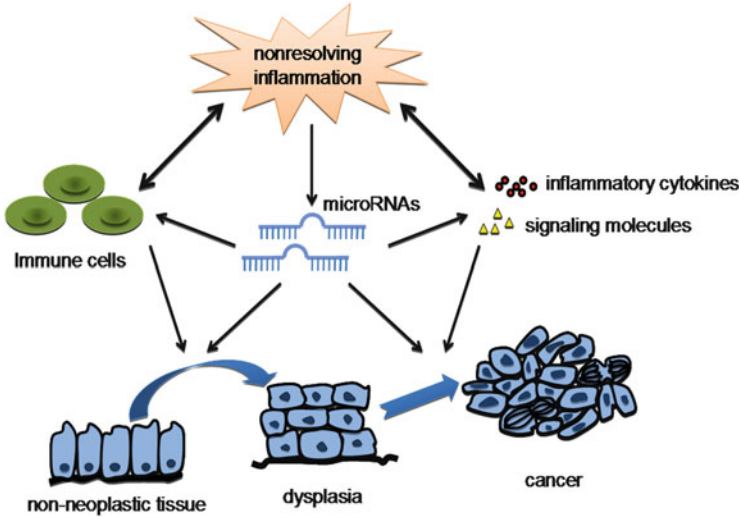


Fig. 2.3 Regulatory network of microRNAs in inflammatory bowel disease-associated neoplastic transformation. MicroRNA (miR) profiles display dysregulated upon stimulation of nonresolving inflammatory factors in inflammatory bowel disease. On one hand, the abnormal miRs may directly regulate intestinal epithelia cell growth to induce epithelium neoplasia by targeting oncogenes or tumor-suppressive gene expression; on the other hand, they may affect the neoplastic transformation via controlling inflammatory response, involving the regulation on development, differentiation, and activation of immune cells accumulated in neoplastic bowel tissue, or production of some key inflammatory cytokine and activation of their downstream signaling

hallmarks. Expression of microRNAs can be detected in formalin-fixed paraffin-embedded specimens, which are reported in recent studies. Many gene expression profiling studies confirmed that change of miR profiles plays a significant role in colitis-associated cancer (CAC) (Peters et al. 2013). The research showed the considerable part of miRs in CAC development. In the early stage of CAC, miR-21 was upregulated, while let-7 and miR-143 were downregulated. In the last stage of adenoma, miR-34a-c was downregulated. miR-192 can be considered as a diagnostic marker of CAC which is downregulated in CAC patients (Zarate et al. 2012). miR-15a/miR-16 cluster controls the progression of prostate cancer. miR-15a/miR-16 cluster is usually deleted in advanced prostate cancer and, in some cases, even in early stages which was reported by De'sire'e Bonci et al. (Spinetti et al. 2013). While the researches about noncoding RNAs have gained more attention, there are more and more the researches about long noncoding RNAs acting as new tumor markers. Expression of lncRNAs is different in different stages of tumor progression. SRA and KRASP1 are tumor markers of cell proliferation signals; lincRNA-P21 and ANRIL are tumor markers of immune escape; HOTAIR, HULC, ncR-upAR, and so on are tumor markers of invasion and metastasis; tie-1AS and ncR-uPAR are tumor markers of angiogenesis; PINC and PANDA are tumor markers of tumor cell immortalization (Gutschner and Diederichs 2012). There are an increasing number of researches on noncoding RNA as a tumor

marker. Suitable and specific noncoding RNAs can be used as a tumor marker that benefit for diagnosis and prognosis of cancer.

3.3 *Noncoding RNAs in Immune Regulation*

Besides, accumulating reports highlight that the two ncRNAs, miRs and lncRNAs, are essential in regulating the expression of genes that are critically involved in both innate and adaptive immune responses and thereby modulate the local immune microenvironment in carcinogenesis, which may be useful in developing cancer immunotherapy (Okada et al. 2010; Raisch et al. 2013). miRs are not only identified in regulating cell differentiation, development, and activation of tumor-associated macrophages (Squadraro et al. 2013), MDSCs (Chen et al. 2015), and effector T cells (Amado et al. 2015) but also identified as modulators in tumor-associated inflammation response and chronic induced tumor formation (Fernandes et al. 2015). Moreover, some miRs function as novel signature of DC vaccine for cancer immunotherapy and modulate its antitumor effect (Holmstrom et al. 2010), while some lncRNAs have been demonstrated to be involved in regulation of immune cell responses (Fitzgerald and Caffrey 2014; Heward and Lindsay 2014). Immune cells represent a highly attractive target for ncRNA-based cancer therapy, as these cells can be isolated, treated, and then reintroduced into the patient. Here, we will introduce recent discoveries on the roles of miRs (Table 2.3) and lncRNAs (Table 2.4) in immune regulation, which will advance the field of cancer immunology and immunotherapy.

3.3.1 *MicroRNAs in TAM Polarization and Activation*

Tumor-associated macrophages (TAMs) accumulated in neoplastic tissue are the major component of the inflammatory microenvironment in cancer and affect various aspects of cancer, including angiogenesis, invasion, and metastasis, which may be useful in developing the innovative diagnostics and therapeutic strategies in cancer (Qian and Pollard 2010; Squadraro and De Palma 2011). Based on original data from mouse, TAMs can be divided to two subtypes, including the classically activated (M1) and alternatively activated (M2) macrophages (Sica and Bronte 2007). To our knowledge, M1 and M2 cells in cancer, respectively, exert antitumoral and protumoral functions. Besides signaling molecules and transcription factors, miRs have been demonstrated to be essential regulators underlying macrophage activation and polarization (Mantovani and Locati 2013). The significance of miR expression in monocytes/macrophages has been identified in recent reports, and distinct miR profiles are specifically enriched in macrophages exposed to different polarizing agents (Cobos Jimenez et al. 2014). As well as, miR can modulate macrophage responses to environmental signals and regulate their polarization-associated gene profile, in which some are rapidly changed in

Table 2.3 miRNAs in immune cell development and antitumor immune response

Cells	microRNAs	Functions	Targets
TAM	miR-155	Sustaining M1 phenotypes, promoting inflammatory cytokine productions	SCOS1, Ship1
	miR-511-3p	Suppressing alternative activation of macrophages	IRF4
	miR-146a	Reducing TLR signaling, attenuating pro-inflammatory cytokine production	RAK1, TRAF6
	miR-223	Suppressor in LPS-induced pro-inflammatory cytokines IL-1 β , IL-6 and TNF- α	Pknox1
	miR-187	Promoting LPS-induced pro-inflammatory cytokines IL-6 and IL-12p40	NFKBIZ
	miR-125b	Essential for pro-inflammatory M1 cell activation and suppressing M2 cell activation	IRF4
	miR-378-3p	Inhibiting IL-4-induced activation of M2 cells	CYP2E1
	miR-21	Limiting excessive inflammation, sustaining IL-4-induced alternative activation	PDCD4
MDSC	miR-494	Enhancing MDSC infiltrating into tumor tissues, thereby facilitating tumor invasion and metastasis	PTEN
	miR-155	Promoting STAT3 activation in MDSC	SHIP-1
	miR-21	Promoting STAT3 activation in MDSC	PTEN
	miR-223	Suppressing differentiation of MDSCs from BM	MEF2C
	miR-101	Promoting MDSC-induced sphere formation, thereby increasing tumor incidence and liver metastasis	CtBP2
	miR-17-5p	Regulating the STAT3-mediated suppressive function of MDSCs	STAT3
	miR-690	Contributing to functional MDSC expansion, maintaining the immature immunosuppressive state of MDSCs	C/EBP α
T cell	miR-23a	Correlating with impaired antitumor potential of patient CTLs; its knockdown enhances granzyme B expression	BLMP-1
	MiRNA-15b	Inhibiting the activation of CD8 ⁺ T cells, causing these T cells to lose responses to tumors	DEDD
	miR-222 and miR-339	Regulating the susceptibility of cancer cells to CTLs	ICAM-1
	miR-29	Promising in immune escape by controlling B7-H3 protein expression	B7-H3
	miR-21	Leading to the tumor mass for escaping immune surveillance and promoting tumor proliferation	FasL
	miR-181a	Augmenting the sensitivity of TCR-mediated T-cell responses to peptide antigens	PTPN22, DUSP5, DUSP6

expression in activated macrophages, including miR-155, miR-125a/b, miR-146a, miR-21, and let-7e, and others target key regulatory molecules involved in M1 macrophage activation, while other miRs, such as miR-378-3p and miR-511-3p, are induced upon M2 macrophage activation (Holmstrom et al. 2010).

Table 2.4 Long noncoding RNAs in immune cell differentiation and immune response

LncRNAs	Model system
Multiple	Many kinds of lncRNAs regulate chromatin remodeling associated with variable, diversity, and joining (V(D)J) recombination required to produce antigen receptors
LincRNA-Cox2	LincRNA-Cox2 mediates the gene expression in mouse macrophages
PACER	PACER mediates Cox-2 expression in human monocytes
HOTAIRM1	HOTAIRM1 is specifically expressed in myeloid cells
Lnc-DC	Lnc-DC regulates differentiation of human monocytes into dendritic cells
IL-1 β -RBT46 and IL-1 β -eRNA	IL-1 β -RBT46 and IL-1 β -eRNA regulate LPS-induced IL-1 β and CXCL8 expression
Multiple	100 kinds of lncRNAs showed differential expression during CD8 ⁺ T-cell activation and following differentiation into CD8 ⁺ memory and effector T cells
NeST/Tmevpg1	NeST/Tmevpg1 induces IFN- γ expression in T cells
LincR-Ccr2-5'AS	LincR-Ccr2-5'AS modulates migration of Th2 cells to the lung
NRON	NRON represses nuclear translocation of NFAT in resting T cells
AK020764	AK020764 expresses in effector CD8 ⁺ cells which is a possible functional link with miR-142
	Lef1 as expressed in naïve CD8 ⁺ T cells plays a possible role in suppression

miR-155 sustains M1 type, while miR-511-3p modulates M2 type. Our previous study found miR-155 could be upregulated by NF- κ B in LPS-stimulated macrophages, and overexpression of miR-155 in macrophages enhances the production of pro-inflammatory cytokines via downregulating inhibitors of the inflammatory response, such as the suppressor of cytokine signaling-1 (SOCS1) (Jiang et al. 2012; Zheng et al. 2012). Besides, recent evidence showed that the more miR-155 delivery could reprogram the activated M2 macrophage toward the more pro-inflammatory phenotype, presenting increased TNF- α production and decreased alternative activation gene arginase-1 (Arg1) (Bala et al. 2011; He et al. 2009). Consistently, studies from Huffaker TB on the miR-based regulation of antitumor immune responses showed that miR-155 promoted IFN- γ secretion through a mechanism involving repression of Ship1 and thereby reduced solid tumor growth in vivo. The tumor growth was enhanced in miR-155^{-/-} mice; therefore, miR-155 mainly appears to primarily enhance activation of macrophage toward M1 phenotype, which was bad for the tumor growth (Huffaker et al. 2012). Different with miR-155, miR-511-3p, located in and co-regulated by its host gene, MRC1, both in mouse and human, is upregulated in macrophages upon alternative activation (Huffaker et al. 2012). As a result, the upregulated miR-511-3p suppresses alternative activation-associated gene expression in TAMs and thereby induces inhibition of tumor growth. Furthermore, exogenous miR-511-3p also directly targets IRF4, a transcription factor that promotes alternative activation of macrophages, and therefore attenuates the protumoral functions of alternatively

activated TAMs (Squadraro et al. 2012). Other miRs such as miR-223, miR-146a, miR-21, and miR-378-3p are also involved in regulating activation of TAMs. miR-378-3p is upregulated in macrophages upon alternative activation stimuli such as IL-4, and miR-378-3p inhibits IL-4-induced expression of Arg1 by negatively regulating AKT1 signaling and thereby limits the alternative activation of macrophages (Ruckerl et al. 2012). In activated macrophages, miR-21 is upregulated and suppresses programmed cell death protein-4 (PDCD4) expression, which facilitates the production of IL-10 and sustains IL-4-induced alternative activation (Sheedy et al. 2010). miR-125b is overexpressed in BM-derived macrophages (BMDMs) and essential for pro-inflammatory cell activation by targeting M2 cell activation-associated molecule, IRF4 (Chaudhuri et al. 2011). BMDMs with miR-223 deficiency express higher levels of the LPS-induced pro-inflammatory cytokines, such as IL-1 β , IL-6, and TNF- α (Zhuang et al. 2012), and NF- κ B activation-induced miR-146 in turn targets IRAK1 and TRAF6 and thereby reduces TLR signaling and attenuated pro-inflammatory cytokine production (Taganov et al. 2006).

Besides the regulation in TAM activation and polarization, recent evidences show that miRs can transfer and connect communication within TAMs and cancer cells based on the miR-containing microvesicles (MVs) or exosomes. TAM-produced MV-shuttled miRs can be conveyed to acceptor cancer cells and fuse with their plasma membrane, which may regulate tumor-associated gene expression in acceptor cells (Holmstrom et al. 2010). One in vitro study suggests that alternatively activated macrophages can influence the invasive properties of breast cancer cells via MV-mediated transfer of miR-223 to downregulate MEF2C expression in cancer cells, leading to increased nuclear localization of β -catenin and increased cancer cell invasion (Holmstrom et al. 2010). While other initial studies suggest that prometastatic functions of TAMs can also be modulated by miRs in MVs derived from cancer cells. miR-21 and miR-29b containing MVs that are produced by cancer cells can transfer into TAMs and bind to intracellular TLRs, and thereby activate a pro-inflammatory and prometastatic response of TAMs (Fabbri et al. 2012; Pucci and Pittet 2013).

Currently, interfering with miR may reprogram the cell activation state by targeting critical genes that control pro- and antitumoral macrophage functions. A notable example is that LV-based miR-511-3p has already been used to modulate TAMs (Squadraro et al. 2012).

3.3.2 MicroRNAs in MDSC Expansion and MDSC-Modulating Cancer Stemness

MDSCs play critical roles in prompting of tumor invasion and metastasis. MDSCs negatively regulate immune responses against cancer while suppressing host immune surveillance, facilitating tumor cell invasion, and participating in the formation of blood vessels (Serafini et al. 2006). MDSCs represent a heterogeneous population of myeloid cells in early differential stages that can be identified by

expression of CD11b and Gr-1 in mice and are defined as $\text{Lin}^- \text{HLA}^- \text{DR}^- \text{CD33}^+$ or $\text{CD11b}^+ \text{CD14}^- \text{CD33}^+$ populations in humans. MDSCs are divided into two subsets including granulocytic MDSCs ($\text{CD11b}^+ \text{Ly6C}^{\text{low}} \text{Ly6G}^{\text{high}}$) and monocytic MDSCs ($\text{CD11b}^+ \text{Ly6G}^{\text{low}} \text{Ly6C}^{\text{high}}$) (Gabrilovich and Nagaraj 2009). The immunosuppressive activity of MDSCs is through mechanisms involving the depletion of nutrients required by lymphocytes via producing Arg1, leading to proliferative arrest of antigen-activated T cell; the generation of oxidative stress caused by the production of ROS and reactive nitrogen species, driving the loss of TCR ζ -chain expression and interference with IL-2 receptor signaling; the interference in T-cell accumulation and recirculation via expressing disintegrin and metalloproteinase domain-containing protein 17 (ADAM17) and galectin 9; and the activation and expansion of Tregs via CD40–CD40L interactions or secretion of IFN- γ , IL-10, and TGF- β . Various factors derived from tumor cells, stromal cells, and other activated immune cells will promote expansion of MDSCs by triggering various signaling pathways in MDSCs, including STAT signaling (Gabrilovich and Nagaraj 2009; Serafini et al. 2006). Therefore, understanding the molecular networks controlling the accumulation and immunosuppressive function of MDSCs is essential in developing MDSCs as the potential therapeutic targets for cancer intervention. Current studies have demonstrated the involvement of miRs in governing the expansion and functions of MDSCs, including miR-17-5p, miR-20a, miR-223, miR-21, miR-155, miR-494, miR-690, and miR-101 (Chen et al. 2015).

The expression of miR-494 in MDSCs is at a high level, and the increased miR-494 enhances the ability of MDSCs to infiltrate into tumor tissue and thereby facilitates tumor invasion and metastasis via activating the PI3K/AKT signaling by targeting of phosphatase and tensin homolog (PTEN), with the upregulation of MMPs. Moreover, the primary tumor growth and metastasis *in vivo* is suppressed after blockage of miR-494, with decreased MDSC accumulation (Liu et al. 2012). STAT3 is a key regulator in MDSC activation and function. MDSCs isolated from tumor-bearing mice have high levels of miR-155 and miR-21, and they can promote STAT3 activation by targeting SHIP-1 and PTEN, respectively (Li et al. 2014a). Both miR-17-5p and miR-20a repressed the ability of MDSCs in suppressing antigen-specific T-cell response by targeting STAT3 (Zhang et al. 2011a). Therefore, by modulating STAT3 signal, miRs may serve as important immune regulators and might be an option for helping overcome the immune tolerance mediated by MDSCs and improving current immunotherapy strategies. Interestingly, miRs in MDSCs are regulated by the factors derived from tumor cells.

Clinical studies in breast, colorectal, pancreatic, esophageal, and gastric cancer patients indicate that high level of MDSCs in cancer tissues may be an independent prognostic factor. Notably, one new report from Cui TX showed that tumor-associated MDSCs promoted human ovarian cancer cell stemness (bCui et al. 2013). Mechanistically, they found that miR-101 expression was increased in ovarian cancer cells cultured with MDSCs, and the increased miR-101 promoted cancer cell stemness by targeting co-repressor CtBP2 (bCui et al. 2013). Significantly, this study indicates that MDSCs function as an extrinsic signal and directly target cancer cells to shape tumor phenotype via a single miR, miR-101.

In addition, high levels of miR-101 were associated with reduced ovarian cancer overall survival. Inhibition of miR-101 blocked MDSC-induced cancer sphere formation, while overexpression of miR-101 stimulated the formation and thereby increased tumor incidence and liver metastasis (bCui et al. 2013), which suggests that targeting miR-101 could block the cross talk between host MDSCs and cancer (stem) cells to augment therapeutic efficacy and reduce therapy resistance.

3.3.3 MicroRNAs in T-Cell-Related Cancer Immunosurveillance

CTLs have potent antitumor activity and therefore are of potential application in tumor immunotherapy. The application of CTLs for immunotherapy has been limited by susceptibility of the ex vivo-expanded CTLs to become dysfunctional in immunosuppressive microenvironments (Franks et al. 2012). Report indicates that miR-based modulation may be useful in augmenting CTL cytotoxicity and immunocompetence. For instance, miR-23a that was upregulated in tumor-infiltrating CTLs in lung cancer patients was correlated with impaired antitumor ability of patient CTLs (Lin et al. 2014). Functional blocking of miR-23a in human CTLs enhanced granzyme B expression, and in mice bearing tumors, immunotherapy with just a small number of tumor-specific but miR-23a low-expressing CTLs robustly hindered tumor progression, in which the transcription factor BLMP-1 as a target of miR-23a is involved (Lin et al. 2014). Different with miR-23a, miR-15b can inhibit the activation of CD8⁺ T cells via repressing the production of IL-2 and IFN- γ and expression of CD69, although its expression is also higher in CD8⁺ T cells from Lewis lung carcinoma than those from healthy ones (Zhong et al. 2013).

Enhanced tumor growth is always correlated to the inefficient immunosurveillance, and tumor cells always display the lower immunogenicity or induce effector immune cell apoptosis to reduce cytotoxicity. In this process, miRs also act as a key regulator. For instance, miR-222 and miR-339 in cancer cells downregulated the expression of intercellular cell adhesion molecule (ICAM-1) and thereby modulated the susceptibility of cancer cells to CTLs, which was one of the first reports to demonstrate the role of miR in cancer immunosurveillance (Ueda et al. 2009). miR-29a was discovered to directly target B7-H3, a surface immunomodulatory glycoprotein which was preferentially expressed in tumor tissues and inhibited T cells (Ueda et al. 2009). Regulation of miR-29 in B7-H3 expression is also of promising in promoting both cell-mediated immunotherapy and B7-H3-specific mAb 8H9-based targeted strategies. Besides, overexpression of Fas ligand (FasL) on tumor cell surface can induce the apoptosis of specific activated tumor-infiltrating CTLs via the Fas/FasL pathway, leading to the tumor mass for escaping immune surveillance and promoting tumor proliferation, invasion, and metastasis. FasL is identified as a target gene of miR-21 in human breast carcinoma cell (Wu et al. 2014). After upregulation of miR-21 in MCF-7 cells, Fas/FasL-mediated Jurkat T-lymphocyte apoptosis was decreased (Wu et al. 2014), which will imply a new approach to enhance antitumor immunity.

3.3.4 MicroRNAs Link Inflammation and Cancer

Early in the nineteenth century, it was perceived that cancer is linked to inflammation. Currently, accumulating reports focus on and highlight the inflammation–cancer connection based on numerous experiments *in vitro* and *in vivo*. During chronic inflammation process, various inflammatory cells and mediators create a favorable microenvironment for tumorigenesis, which involves the regulation of miRs (Raisch et al. 2013).

In hepatocellular oncogenesis, an HNF4 α –miRNA inflammatory feedback circuit regulates hepatocellular oncogenesis (Hatzia Apostolou et al. 2011). HNF4 α is indispensable for development and maintenance of the hepatic epithelium and also related to inflammation because its gene is identified as a susceptibility locus for ulcerative colitis (UC). Report from Hatzia Apostolou M showed that inhibition of HNF4 α initiated hepatocellular transformation through a microRNA inflammatory feedback loop circuit consisting of miR-124, IL-6R, STAT3, miR-24, and miR-629 (Hatzia Apostolou et al. 2011). The miRs, miR-21, miR-125, and miR-155, were the most frequently expressed during infection and therefore had a potential role in carcinogenesis induced by infectious agents. It has been shown that overexpression of miR-21 and miR-182 is associated with carcinogenesis associated with HPV (Hatzia Apostolou et al. 2011).

Chronic inflammation also plays a critical role in gastrointestinal (GI) tract cancers, for which the two major etiological factors are tissue injuries and altered microbiota. STAT3, activated by IL-6, directly activates miR-181b-1 and miR-21. miR-181b-1 and miR-21 inhibit tumor suppressors, CYLD and PTEN, respectively, leading to activation of NF- κ B (Iliopoulos et al. 2010). These STAT3-mediated regulatory circuits are essential for the malignant transformation and tumor growth in xenografts, and their transcriptional signatures are observed in colon adenocarcinomas. Other evidences show that miR-124 is downregulated specifically in colon tissues from patients with UC and directly targeted STAT3 mRNA (Koukos et al. 2013). Moreover, higher levels of miR-214 were detected in colon tissues from patients with active UC or colitis-associated cancer (CAC) than patients with other disorders or controls and correlated with disease progression. Interleukin-6 induced STAT3-mediated transcription of miR-214 (Koukos et al. 2013). A miR-214 chemical inhibitor reduced the severity of dextran sulfate sodium (DSS) salt-induced colitis in mice, as well as the number and size of tumors that formed in mice given azoxymethane (AOM) and DSS (Koukos et al. 2013). In fresh colonic biopsies from patients with active UC, the miR-214 inhibitor reduced inflammation by increasing levels of PDLIM2 and PTEN. IL-6 upregulates STAT3-mediated transcription of miR-214 in colon tissues, which leads to the decrease of PDLIM2 and PTEN. The activity of this circuit correlates with disease activity in patients with UC and progression to colorectal cancer (Koukos et al. 2013).

In our current study, we found that miRNAs were often dysregulated in DSS-induced colitis and the associated neoplastic transformation, and among them, miR-15/miR-16 cluster (Huang et al. 2015) was downregulated during the

transformation. Deletion of miR-15/miR-16 promotes colitis-associated cancer (CAC) progression, with increased percentage of Tregs and B cells and decreased CD8⁺ T cells in colon tissues. miR-15/miR-16 has been reported that it can promote CD5⁺ B-cell proliferation and protect B cell from apoptosis by targeting Bcl-2. Recent evidence indicates that B cells with IgA-positive expression have immunosuppressive function and impede T-cell-dependent immunogenic chemotherapy in human prostate cancer, and elimination of these cells may allow CTL-dependent eradication of oxaliplatin-treated prostate cancer cells (Shalapour et al. 2015).

3.3.5 LncRNAs Regulate Immune Cell Differentiation and Immune Response

Like microRNAs, long noncoding RNAs (lncRNAs) play different roles in a range of biological processes via a variety of mechanisms. Immune response has an important and protective effect on the organism from infections. Dysregulation of immune response will result in a number of diseases, including tumors. Tumor immunotherapy has gained more and more attention in recent years. Since microRNAs are well studied, the researches about that long noncoding RNAs contribute to modulate innate responses, and adaptive responses are on the rise (Carpenter et al. 2013).

So far, a majority of researches about long noncoding RNAs focus on the relationship between the long noncoding RNAs and cancer (Deng and Sui 2013; Gutschner and Diederichs 2012). However, the long noncoding RNAs have great effects on regulation of differentiation process of immune cells. Chips and RNA sequencing have demonstrated that the expression of long noncoding RNAs in activation process of monocytes, macrophages, dendritic cells, and T cells is different. CD11c+ dendritic cells express distinctive long noncoding RNAs when they are stimulated with LPS (Guttman et al. 2009). A variety of long noncoding RNAs have high expression in mice infected with acute respiratory virus. These long noncoding RNAs are expressed by CD4⁺ T cells. CD8⁺ T cells express hundreds of long noncoding RNAs which are connected with the regulation and activation of lymphocytes (Kirigin et al. 2012). NeST/Tmevpg1 induces expression of IFN- γ in T cells (Gomez et al. 2013). LincR-Ccr2-5'AS is involved in transfer of Th2 cells into the lung in mouse. LincRNA-Cox2 activates bone marrow-derived macrophages by interacting with the nucleoprotein A/B (Krawczyk and Emerson 2014). Differentiation of human monocytes is dependent on lnc-DC regulates. Differentiation of human monocytes is dependent on lnc-DC (Wang et al. 2014).

The first evidence of long noncoding RNAs involved in innate response was reported by Guttman et al. (2009). They found that the mice bone marrow-derived dendritic cells stimulated with LPS can induce the production of 20 kinds of long noncoding RNAs. LincRNA-Cox2 regulates gene expression in mice macrophages. LincRNA-Cox2 suppresses expression of 787 genes and induces expression of 713 genes in bone marrow-derived macrophages of mice. These genes contribute to expression of CCL5 and IL-6 (Krawczyk and Emerson 2014). They are also

involved in many other immune processes. Mechanisms of it remain unclear, but that lincRNA-Cox2 plays a fundamental role by interacting with nuclear protein A/B is generally accepted. THRIL also regulates secretion of TNF- α by the interaction with the nuclear protein in human monocytes (Li et al. 2014b). That LOC645638 is involved in differentiation of monocytes into dendritic cells has been proven by gene expression profiling. Due to the role LOC645638 plays in dendritic cells, it was renamed lnc-DC. Lnc-DC knockdown had effect on the antigen presentation of DCs and generation of CD4⁺ T cells as reported. Lnc-DC mainly regulated cell differentiation by activating STAT3 (Wang et al. 2014).

Recently, researches have confirmed that a class of lncRNAs is expressed in mammalian naive CD8⁺ T lymphocytes and memory T lymphocytes preferentially (Pang et al. 2009). Over 1000 lncRNAs have been detected in humans and mice. Expression of these noncoding RNAs is in a cell-specific or stage-specific manner. Particularly, it has been proven that there are 96 kinds of long noncoding RNAs which are expressed specifically in lymphocytes. Interestingly, 29 of these transcripts were specific for CD8⁺ T lymphocytes; 21 kinds of long noncoding RNAs contribute to differentiation of T cell; and activation of T cell is related to 81 kinds of long noncoding RNAs which have high expression (Pang et al. 2009). Several lncRNAs are expressed in Th2 and Th17 selectively, and the expression of lncRNAs depends on the Th1-specific transcription factors such as STAT4 and T-bet (Pagani et al. 2013). NeST is the first lncRNA which is involved in the regulation of immune system and has been confirmed to regulate the expression of master cytokines, such as IFN- α . NeST regulates RNA expression and protein expression of IFN- γ by binding to WDR5 (Gomez et al. 2013). In Th2 cells, lincR-Ccr2-5'AS suppresses expression of CCR1, CCR2, CCR3, and CCR5 which are involved in metastasis of lung cancer. A variety of lymphoid tissues express NRON, such as lymph nodes and thymus. NRON interferes with the function of NFAT by interacting with several kinds of proteins, such as karyopherin importin-b1 (KPMB1), IQ motif-containing GTPase-activating protein (IQGAP), and so on (Zhang et al. 2013a), and mechanistic studies discovered that transcriptional activity of NFAT was suppressed because of interaction between NRON and KPMB1 which blocked the nucleocytoplasmic transport (Willingham et al. 2005). A synergetic function between NRON and IQGAP1 on blocking NFAT dephosphorylation also was reported which plays a significant role in induction of IL-2 (Sharma et al. 2011). In addition, there are a few long noncoding RNA genes that host in their sequence small RNAs and produce function via these smaller RNAs. miR-142-5p and miR-142-3p are located in the first intron of lncRNA (AK020764) and involved in regulating expression of Foxp3 in Tregs (Pang et al. 2009). In conclusion, lncRNAs have great effects on T-cell activation, differentiation, and development. The researches about the roles that lncRNAs play in regulation of B cells are much less. There are multiple long noncoding RNAs that are related to loop of VH regions in close proximity with the DJH region during recombination in pro-B cells (Bolland et al. 2004). We summarize the regulation of long noncoding RNAs in innate immune responses and adaptive immune responses in Table 2.4.

To gain the proper function of the immune system, it needs steps of development and differentiation that must be regulated at the right time and in the appropriate effector cells, strictly. Disorders of the immune system are great risk of tumors. More and more evidences suggest that lncRNAs have significant roles on important regulation of immune responses like miRs. It is possible that many immune-related lncRNAs will be discovered. Researchers will devote to the specific mechanisms of long noncoding RNAs involved in immune responses in the future. Future studies need to investigate aberrant expression of lncRNAs in a variety of tumors, autoimmune diseases, and so on by means of RNA sequencing. There are more and more reports about dysregulated expression of lncRNAs in inflammatory diseases and some kinds of tumors. Overall, the roles of lncRNAs in immune regulation have far-reaching significance for immunotherapy.

3.4 Targeting Noncoding RNAs in Cancer Therapy

3.4.1 Methods of siRNA, MicroRNA, or Anti-microRNA Delivery In Vivo and Case Studies

Regarding the critical role of miRs in many types of disease, researchers seek to manually regulate their level in vivo in order to improve the effects of ordinary therapeutics. The agents used can be miR mimics or miR inhibitors. If the signal pathways involved are known, siRNAs targeting specific mRNAs can also be used. Therapies concerning miRs can be miR inhibition therapies, which block the suppressing effects of overexpressed miRs, or miR replacement therapies, which increase the level of miR when miR is decreased in the disease. According to the routes through which to deliver the oligonucleotides, the delivery can be intratumoral or intravenous. The former requires less complex delivery systems and has less side effects that are due to nonspecific distribution to other organs. The latter requires more complex systems in order to avoid stimulation of immune response, reduce toxicity, and reduce accumulation to other tissues. Because RNAs have short half-lives in the blood, mainly due to the abundance of RNase in the serum, they have to be chemically modified to increase their resistance to degradation. Because naked RNAs are negatively charged and have high molecular weight, they're hard to get across the cell membrane. To increase the transfection efficiency and resistance to RNase cleavage, also to avoid triggering immune responses, the oligonucleotides need special carriers. Many delivery materials have been devised and their characteristics vary (Scomparin et al. 2015).

The oligonucleotide to be transfected depends on its inhibition therapy or replacement therapy. In an inhibitory therapy, anti-miR oligonucleotides (AMOs), miR sponges, or miR masks are used. Making use of the RNAi mechanism in vivo, AMOs inhibit the interaction of miR and RISC or RISC and the target mRNA. miR sponges are plasmids which transcribe transcripts with many "target sites" of the miR, thus occupying the RISC, and protect the real target mRNAs. miR

masks are oligonucleotides designed to bind to and protect the binding sites of the target mRNA. Comparing to the two methods just mentioned, which target the miR itself, miR masks specifically rescue one signaling pathway, leaving other pathways intact. In a replacement therapy, the most widely used are miR mimics which resemble mature miRs, but are chemically modified to increase stability. Comparing to single-stranded RNAs, double-stranded RNAs composed of a guide strand, which incorporates into RISC, and a passenger strand, which complements the guide strand, are more effective (Bader et al. 2011).

In a review by Zhang et al. (2013b), chemically modified oligonucleotides include phosphorothioate-containing oligonucleotides, 2-O-methyl-(2-O-Me) or 2-O-methoxyethyl oligonucleotides (2'-O-MOE), locked nucleic acid (LNA) oligonucleotides, peptide nucleic acids (PNAs), fluorine derivatives (FANA and 2'-F), and others. In phosphorothioate-containing oligonucleotides, one of the oxygen in the phosphate group at the terminus was replaced by sulfur. This modification increases RNase resistance of the oligonucleotide and elicits RNase H activity to degrade target mRNA and also promote binding of the oligonucleotide to serum or cellular proteins for uptake. But it has deficiencies including short half-life, low affinity to mRNA, and inhibitory to cell growth. Adding a 2-O-Meto group or a 2'-O-MOE group, the ribose moiety improves half-life and reduces the inhibitory effect. In locked nucleic acids (LNAs), the 2'-oxygen and the 4'-carbon of each ribose moiety are connected. This conformation confers LNA-RNA pair with greater thermal stability and improved mismatch discrimination (Kurreck et al. 2002). An LNA-based therapeutic, miravirsin or SPC3649, entered phase II clinical trial to treat patients with chronic HCV genotype 1 infection. Miravirsin is an anti-miR oligonucleotide which sequesters miR-122. miR-122 is important for the stability and propagation of HCV. PNAs are oligonucleotide analogs with their backbone replaced by a peptide-like strand. PNAs are not charged, facilitating their entry into the cell without the help of transfection agents. PNAs are specific and stable, without generating unwanted toxicity (Fabani et al. 2010).

Many delivery materials have been invented to improve the efficiency of transfection. The main types include liposomes and polymer-based delivery systems. Although liposomes are widely used in *in vitro* experiments for oligonucleotide transfection, they may not suit *in vivo* treatments due to the toxicity, nonspecific uptake, and the potential to elicit immune response (Lv et al. 2006; Zhang et al. 2013b). Some researchers seek to modify the liposomes for better effects and have made some success in mouse models. For example, liposome-polycation-hyaluronic acid (LPH) with tumor-targeting single-chain antibody fragment (scFv) was intravenously administered into mice with lung metastasis of B16F10 melanoma, delivering miR-34a and several siRNAs targeting a variety of oncogenes. The tumor load was effectively reduced and tumor cells undergone apoptosis. Several oncogenes were downregulated. miR-34a and siRNA had synergic effects (Chen et al. 2010). Because the toxicity of liposomes owe to their positive charge, researchers also tried to use neutral liposomes. Trang et al. (2011) used neutral lipid emulsion (NLE) as carrier for miR-34a and let-7 mimics and administered through tail vein into Kras-activated autochthonous mouse model of non-small cell lung

cancer. The treatment upregulated miR-34a and let-7 in the lung and reduced tumor burden significantly. After removal of peripheral blood from different organs with PBS, it was found that the miR delivered using NLE specifically accumulated in the lung. This characteristic of NLE is advantageous to NSCLC treatment but may be bad for its use in other diseases.

Researchers have also devised many types of polymeric delivery systems. According to Anna Scomparin et al. (2015) and Y. Zhang et al. (2013b), they at least include poly(lactide-co-glycolide) (PLGA), polyethylenimine (PEI), dendrimers, chitosan, polyethylene glycol (PEG), polyamino acid, and polyacrylates. We list the characteristic of applications of some below:

1. PEI is formed by repeated units of two carbon spacers and one amino group and can be linear or branched. Due to its cytotoxicity, PEI is usually modified to reduce the toxicity. For instance, polyurethane-short branch polyethylenimine (PU-PEI) was used to deliver miR-145 to treat cancer stem cell derived from lung adenocarcinoma and glioblastoma tumors (Chiou et al. 2012; Yang et al. 2012). PU-PEI-miR alone can reduce tumor growth, while better effects could be achieved by combination with radiation and chemical therapy. When connected to a peptide derived from RVG (rabies virus glycoprotein), and in combination with mannitol, PEI-RVG can transport across the blood-brain barrier and specifically deliver miR to neurons (Hwang do et al. 2011; Maiorano and Mallamaci 2009).
2. Dendrimers have highly branched structures so they have high ratio of surface moieties to molecular volume. But they have not yet reported to be used in vivo. Some in vitro reports show that dendrimers may be promising delivery systems in vivo (Ren et al. 2010a, b).
3. Chitosan is a kind of polysaccharide derived from chitin (Ballarin-Gonzalez et al. 2014). Electrostatic interactions between the oligonucleotides and the protonated glucosamine subunits of the primary amine (native chitosan) or secondary/tertiary amine of certain types of functionalized chitosan help to form the complex for delivery, while positively charged backbone of chitosan facilitates the chitosan-oligonucleotide complex to adhere and get across mucosal tissues, indicating their potential to be administered orally or intranasally (Kim et al. 2007). Chitosan-based delivery systems have been used to treat tumors in vivo through intratumoral administration or intravenous administration. For example, chitosan-based hydrogel was applied to deliver anti-TG2 siRNA to tumor (melanoma and breast cancer)-bearing mice intratumorally. Tumor growth was reduced significantly, and TG2 in tumors was downregulated comparing with non-siRNA chitosan hydrogel control. Marimprey et al. (2008) used chitosan-coated poly(isobutylcyanoacrylate) nanoparticles to deliver ret/PTC1-targeting siRNAs in mice inoculated with ret/PHC1-engineered fibroblasts through intratumoral administration. Rearrangements of ret and H4 gene (ret/PTC1) often happens in papillary thyroid carcinomas. This treatment reduced ret/PHC1 level significantly in tumors and inhibited the growth of tumors. Chitosan-based system is also compatible to systematic delivery.

Chitosan-coated poly(isohexylcyanoacrylate) (PIHCA) nanoparticles were used to deliver RhoA-targeting siRNAs intravenously in xenografted aggressive breast cancer (MDA-MB-231)-bearing mice (Pille et al. 2006). The treatment caused a 90 % reduction of tumor volume and body weight; biomarkers of hepatic, renal functions were normal, indicating its low toxicity. Chitosan-coated poly(isobutylcyanoacrylate) and poly(isohexylcyanoacrylate) nanoparticles containing ret/PTC1-targeting siRNAs were used to treat ret/PTC1-engineered RPI cell-inoculated mice (de Martimprey et al. 2010). Treatment led to almost a stop of tumor growth, while naked siRNA treatment was far less effective. Chitosan-based system has not yet been used to deliver miR mimics, but its low toxicity and high efficiency make it a promising choice.

4. With specific modifications (Blum and Saltzman 2008), PLGA can have high-encapsulation efficiency, potential of long-time release, and high-loading capacity. Babar et al. generated a transgenic mice with miR-155 overexpressed in the spleen and bone marrow. These mice developed pre-B-cell lymphoma. Then, the mice were intravenously administered with anti-miR-155 PNAs encapsulated in PLGA polymer nanoparticle. For the sake of increasing transfecting efficiency, a cell-penetrating peptide, penetratin, was attached to the surface of the PLGA nanoparticle via a PEG linker. The PEG linker can increase the circulation time of PLGA-anti-miR-155. It was shown that the nanoparticle could decrease tumor growth significantly while requiring far less anti-miR PNA than treating with naked anti-miR (Babar et al. 2012).

3.4.2 Clinical Trials: Anti-miR-122 LNA and MRX34

In September 2010, a phase IIa study was conducted to test the antiviral activity of miravirsin, a 15nt locked nucleic acid-modified antisense oligonucleotide, against HCV (Janssen et al. 2013). It's been shown that HCV can make use of the abundance of miR-122 in the liver to protect itself. miR-155 can bind to the 5'UTR of the genome of HCV and protect it from being degraded by nuclease and eliminated by innate immune response. Miravirsin can sequester miR-155 through binding to its 5' region. Thirty-six patients were recruited and were grouped. Each group included nine patients, and they were treated with different doses (0 mg (placebo), 3 mg, 5 mg, or 7 mg per kg of body weight). Patients were miravirsin treated subcutaneously, once a week, and totally for 5 weeks. After the administration period, patients returned for follow-up visits till week 18.

Results showed that miravirsin could decrease HCV RNA levels in patients in a dose-dependent and long-lasting manner. The mean of maximum reduction in HCV RNA levels (log₁₀ IU/ml) from baseline was 1.2 for 3 mg/kg group, 2.9 for 5 mg/kg group, and 3.0 for 7 mg/kg group, comparing with a decline of 0.4 in the placebo group. Patients treated with miravirsin had low HCV RNA till week 18. Comparing to placebo group, the miravirsin groups did not have significant higher adverse event rates. In addition, the miR-122 binding site in the 5'UTR of HCV genome was shown to be highly conserved, and escape mutations were not observed in HCV

genomes in primates or humans treated with miravirsen (Li et al. 2011). Unlike the currently used protease inhibitors, miravirsen is not a substrate for cytochrome P450, so drug–drug interactions may not be significant. These indicate miravirsen can stay effective during the treatment and can combine with other antiviral therapeutics to have better effects. A phase II study using a 12-week regimen is ongoing, in hope of keeping the burden of virus at a very low level and preventing rebound.

Another miR, miR-34a, also entered phase I study to treat patients with unresectable primary liver cancer or advanced or metastatic cancer with liver involvement or hematologic malignancies. miR-34 has been proven to target many oncogenes and was found to be downregulated in human epithelial ovarian cancer, neuroblastoma, chronic lymphocytic leukemia, and other types of cancer (Misso et al. 2014). In vitro and in vivo experiments indicate the antitumor potential of miR-34a. It can sensitize cancer cells to chemotherapy, reduce tumorigenesis, suppress tumor growth, and prevent epithelial–mesenchymal transition (EMT). This study was started in April 2013, and patients are treated with MRX34, which are miR-34a mimics contained in liposomes, intravenously, twice a week for 3 weeks with a week off, or consecutive for 5 days with 2 weeks off. The maximum tolerated dose, peak blood concentration, and the percentage of patients responding to MRX34 will be found out.

3.4.3 MicroRNAs in Modulating Dendritic Cell Vaccines

Since the discovery of DCs by Steinman and Cohn (1973) in 1973, DCs have been shown to be important in immune responses against infection and cancer. DCs have been used as a vaccine against cancer in many clinical trials, and this DC-based immunotherapy usually involves the obtainment of peripheral blood monocytes or CD34⁺ precursor cells from patients and differentiation and maturation into DCs ex vivo using cytokines, loading tumor antigens (tumor lysates or known tumor antigens) onto the DCs, and infusion back into the patient. However, the effectiveness of the DC vaccine is influenced by many factors, for example, the specificity of the antigen and the activation status of the DC in vivo. miRs play an important role in the latter.

As reviewed by Francesco de Rosa et al. (2014), miRs impact the DC plasticity, usually by negative regulation. For example, miR-155 targets the antigen-processing machinery which is crucial for antigen presentation after DC maturation. miR-148 may target HLA-C to regulate antigen presentation. miR-146a and miR-29a downregulate the co-stimulators CD40L and B7-H3, respectively, decreasing their ability to elicit immune response. miRs can also regulate the response of DCs toward stimulation signal. Stimulation of TLR4 can upregulate miR-155, miR-223, miR-146, and miR-21. They decrease the production of pro-inflammatory cytokines and promote the expression of anti-inflammatory cytokines like IL-10.

miRs in DCs can also be regulated through the cross talk between tumors and DCs. As reported by Siping Min et al. (2013), miR-16-1, miR-21, miR-22, miR-142, miR-146, miR-155, and miR-503 were all upregulated in DCs when cocultured with cancer cell lines or in lymph nodes of tumor-bearing mice. This accompanied increased apoptosis of DCs. Further investigation confirmed their effect to influence the life-span of DCs, and their inhibitor could reverse the pro-apoptotic effect of tumor cells on DCs. At least in CT-26 colon cancer cells and 1D8 ovarian cancer cells, the pro-apoptotic effect of these miRs mainly by targeting YWHAZ and Bcl-2, the former is required for activation of pathways downstream of growth factors, and the latter is an inhibitor of mitochondria-associated apoptotic pathway.

Level of miRs in DCs may be interfered to increase the effectiveness of DC vaccines. Depending on the function of the miR in DC activation, it can be upregulated or inhibited. Also, even if we don't regulate the miR in DC vaccines, finding miR marker that can reveal the activation status of the DCs is also valuable. Holmstrom et al. conducted a study on human DCs (Holmstrom et al. 2010), peripheral blood monocytes from healthy donors were differentiated into immature DCs using IL-4 and GM-CSF, and then these immature DCs were treated with or without IL-1, IL-6, TNF- α , and PEG2 for maturation. They found that hsa-miR-155 level in DCs could reliably indicate the maturation status of the DCs and was positively correlated to the expression level of CCR7, IL-23, and IL-12p70. Cubillos-Ruiz et al. (2012) transfected DCs from mice bearing advanced orthotopic ID8-Defb29/Vegf-A tumors with pre-miR-155 mimics and found that after CD40L stimulation, pre-miR-155 mimic-transfected DCs could promote T-cell proliferation and activate T-cell response and TNF- α , IL-12, IFN- γ , CCL5 secretion more effectively. This optimized DC vaccine did not show obvious toxicity or cause secondary tumor growth in distant organs. The median life-span was raised from 52 days to around 60 days. In mice challenged by parental ID8 tumor, the overall survival was raised to about 35 % from zero. The effect of this optimized vaccine endured; even the T cells were sorted and transferred to another tumor-bearing mice. Those T cells sorted from splenocytes of ID8-Defb29/Vegf-A tumor-bearing mice treated with CD40 agonistic Abs (or PBS as control) and pre-miR-155 mimics combined were transferred to mice challenged with the same tumor on either flank. Anti-CD40 Ab plus pre-miR-155 mimics could reduce the tumor volume from 175 to 75 mm³ on average. Using miRs to improve DC-based immunotherapy remains largely unexplored; how miRs control the activation of DCs should be further studied to provide knowledge for clinical application.

3.5 Summary and Discussion

With accumulating evidences demonstrating the importance of miRs and long noncoding RNAs in cancer biology and cancer-related immune response, ncRNA-based cancer therapy emerges as a hot issue and also a big challenge in

the current ncRNA research. To be excited, MRX34, as the first miR-based therapy for cancer, is entering the phase I trial (Bouchie 2013). Data from our recent study showed that the exogenous miR-126 mimics suppressed esophageal cancer cell growth (Liu et al. 2015), which implies its potential therapeutic significance in esophageal cancer using miRs' "replacement" strategy. However, ncRNA-based therapy for cancer in practice still needs to be further explored, considering some challenges, especially technical limitations, such as how to improve its accumulation in the target tissues and balance the safety and efficiency.

4 Cytokines in Cancer Immunotherapy

4.1 *Introduction of Cytokines*

The description of "cytokine" can be traced back to the 1970s as a group of cellular messenger molecules: "cyto" means cell and "kinos" means movement. Cytokine, which exists in peptide, protein, and glycoprotein forms, encompasses a wide range of low molecular weight proteins including interleukins, interferons, mesenchymal growth factors, chemokine family, tumor necrosis factor family, and adipokines. The effects of cytokines depend on several factors, including cytokine concentration, cytokine receptor expression, and the signaling pathways in responding cells. Cytokines allow cells to communicate with each other and stimulate the movement of immunocytes toward sites of inflammation, trauma, infection, and tumor (Akdis et al. 2011; Arango Duque and Descoteaux 2014; Sahoo and Im 2010). In cancer microenvironment, cytokines play a pleiotropic role in tumor pathogenesis, development, and prognosis. Cytokines can directly stimulate immune effector cells and enhance the killing effects. Cytokines can also promote tumor growth and facilitate invasion and metastasis of cancer. Numerous studies have demonstrated that cytokines can function as major mediators of antitumor immunity (Christian and Hunter 2012; Smyth et al. 2004). Cytokines like IL-2 and IFN- γ have been approved for cancer treatment by the FDA; lots of cytokines including IL-7, IL-12, IL-15, IL-18, and IL-21 have entered clinical trials. Preclinical works indicate that neutralization of suppressive cytokines like IL-10 and TGF- β can promote antitumor immunity (Ardolino et al. 2015; Ngiow et al. 2013; Smyth et al. 2004; Yeung et al. 2013). A deeper understanding of the interactions between cytokine and tumor will provide new approaches for improving cancer immunotherapy.

4.2 *Cytokines in Carcinogenesis*

4.2.1 Cytokines and Tumor Microenvironment

Tumor microenvironment includes cytokines produced by all kinds of cells besides the tumor that support the proliferation and differentiation of the cancer cells. Recent studies indicated that interleukins combined with chemokine have crucial role in tumor progression (Drexler and Yazdi 2013; Voronov et al. 2014; Zarogoulidis et al. 2014). Th17 cell-related cytokines are expressed at a high level in tumor tissue (Bailey et al. 2014). Qian et al. demonstrated that IL-23, which is essential to maintain Th17 differentiation highly expressed in tumor tissue, and tumor-secreted prostaglandin E2 (PGE2) induced the IL-23 secretion of tumor cells, leading to Th17 cell expansion (Qian et al. 2013). In another study, Th17 infiltrated in the tumor tissue, promoting high levels of CD154, granulocyte colony-stimulating factor (G-CSF), CXCL1, IL-6, IL-8, and macrophage inhibitory factor (MIF) in tumor tissue cultures (Li et al. 2012a). Furthermore, Th1- and Th2-produced cytokines present in the tumor tissue are also involved in the carcinogenesis. IL-12 combined with hepatitis B virus X protein (HBx) prevents the progress of hepatocellular carcinoma (HCC) by inducing apoptosis of HCC cells and expanding of CD8⁺ T leukocytes, macrophages, and dendritic cells within tumors tissue and reducing angiogenic properties (He et al. 2012). Besides, interleukin (IL)-4 and IL-13, the Th2 cytokines, are also functionally related. They regulate the immune microenvironment via binding to their receptors, IL-4R α and IL-13R α 1 chains. Both cytokines and the receptors mediate tumor proliferation, cell survival, cell adhesion, and metastasis. Thus, clinical studies tend to design the receptors targeting (Suzuki et al. 2015). In addition, follicular T helper cell (Tfh)-related IL-21 contributes to proliferation of malignant cells in Waldenstrom macroglobulinemia. In the bone marrow environment, IL-21 combined with IL-21 receptors expressed on B cells and promoted IgM production via JAK-STAT3 signal pathway (Hodge et al. 2012).

4.2.2 Cytokines and Cancer Stem Cells

Cytokines mediated the differentiation and survival of cancer stem cells (CSC). Th17 cells play a complex and controversial role in tumor immunity, and the cytokines such as IL-1 β , IL-6, IL-17, and IL-22 play an essential role in promoting the growth of CSC. IL-1 β contributes to the colon cancer stem cell (CSC) development, leading to promote colon tumor growth and invasion. IL-1 β treated in vitro led to epithelial–mesenchymal transition (EMT) of colon cancer cells with loss of E-cadherin and by upregulating of Zeb1 (Li et al. 2012b). IL-6 also induced progress of human CSC. In vitro experiments demonstrated that endothelial cell-derived IL-6 enhanced orosphere formation, p-STAT3 activation, survival, and self-renewal of human CSC, which present in human head and neck squamous

cell carcinomas (HNSCC); Xie et al. provided evidence that IL-6 is capable of generating CD44⁺ cells with stemlike properties through induction of the EMT in the epithelial-like T47D breast cancer cells (Krishnamurthy et al. 2014; Xie et al. 2012). Xiang et al. demonstrated the role of IL-17 in promoting the self-renewal of ovarian CD133⁺ cancer stemlike cells (CSLCs); the stimulation function of IL-17 on self-renewal of ovarian CD133⁺ CSLCs might be mediated by the nuclear factor NF- κ B and p38 mitogen-activated protein kinase (MAPK) signaling pathway (Xiang et al. 2015). In colon carcinomas, IL-22 promoted activation of the transcription factor STAT3 and expression of the histone 3 lysine 79 (H3K79) methyltransferase DOT1L. The DOT1L complex induced the core stem cell genes NANOG, SOX2, and Pou5F1, resulting in increased cancer stemness and tumorigenic potential. Thus, IL-22⁺ cells promote colon cancer stemness via regulation of stemness genes that negatively affects patient outcome (Kryczek et al. 2014).

4.2.3 Cytokines and MicroRNAs

miRs regulate about various protein-coding mRNAs including the expression of tumor related cytokines. IL-1 β , for instance, induced the upregulation of miR-425, which negatively regulates phosphatase and tensin homolog expression by targeting its 3'UTR, promoting gastric cancer cell proliferation (Kryczek et al. 2014); miR-127-5p suppressed IL-1 β -induced MMP13 production as well as the activity of a reporter construct containing the 3'UTR of human MMP13 mRNA. In addition, mutation of the miR-127-5p binding site in the 3'UTR of MMP13 mRNA abolished miR-127-5p-mediated repression of reporter activity. Conversely, treatment with anti-miR-127-5p remarkably increased reporter activity and MMP13 production in human chondrocytes and may contribute to the development of osteoarthritis (OA) (Park et al. 2013). Likewise, miR-205 targeted the IL-24 promoter and directly suppressed the growth of KB oral cancer cells and prostate cancer cells (Kim et al. 2013; Majid et al. 2010). Regulatory cytokines, such as IL-1 β and IL-11, targeted by miR-204, miR-211, and miR-379 by binding to their 3'UTR (Majid et al. 2010).

4.2.4 Cytokines and Epithelial–Mesenchymal Transition

The epithelial–mesenchymal transition (EMT) is a process during which epithelial cells lose their cell polarity and cell–cell adhesion and gain mesenchymal characteristics, such as cell migration and invasion; cytokines influence the EMT environment (Steinestel et al. 2014). During lung cancer, autocrine IL-8 and VEGF mediate epithelial–mesenchymal transition via p38/JNK-ATF-2 axis. These changes were accompanied by enhanced tumor cell invasion (Desai et al. 2013). IL-6 has also been shown as an EMT inducer. Miao showed that IL-6 receptor and STAT3 were highly expressed in human cervical squamous cell carcinoma (CSCC) tissues, which markedly promoted cell growth and altered cell morphology (Miao

et al. 2014). In addition, Th2-/Th17-polarized inflammation induces bronchial EMT; IL-4 and IL-17A synergized with TGF- β 1 induce epithelial cells reentering cell cycle by the regulation of ERK1/ERK2 activity (Ji et al. 2013).

4.2.5 Cytokines and Autophagy

Autophagy is the natural mechanism that disassembles unnecessary or dysfunctional cellular components, with orderly degradation and recycling of cellular components through a regulated process. Cytokines play a dual role during tumorigenesis by inhibiting or promoting autophagy. IL-2 itself can both induce autophagic process and inhibit autophagic flux during tumor progression (Buchser et al. 2012). In most tumor environment, cancer cells maintained the same level of autophagy through increasing IL-2 treatment. In contrast, in patients with melanoma and renal cell carcinoma, administration of IL-2 inhibited autophagic flux (Liang et al. 2012). In addition, treatment of pancreatic cells AR42J with IL-1 β can induce trypsin activation via intracellular calcium changes (Xu et al. 2014).

4.2.6 Cytokines and DNA Methylation

Recent studies indicated that DNA methylation status of genes related to tumorigenesis. For instance, Tekpli reveals that DNA methylation frequently occurs at promoter regions of IL-1 β , IL-6, and IL-8 in non-small cell lung cancer (NSCLC). Tumor cells have significantly more DNA methylation levels than normal tissue cells. Lung cancer cells or tissues had significantly different DNA methylation and mRNA levels than normal human bronchial epithelial cells or adjacent non-tumorous tissues, respectively. High DNA methylation of promoter in lung cancer cells or tissues was associated with low mRNA levels of cytokines (Tekpli et al. 2013). Moreover, during colorectal cancer (CRC), IL-6 induces CYP1B1 and CYP2E1 gene expression in HCT116 and SW480 cells. IL-6 downregulates CYP1B1-targeting miR-27b through a mechanism involving DNA methylation. This leads to increasing activation of carcinogens and DNA damage, thus promoting colorectal carcinogenesis (Patel et al. 2014). In oral cancer cells, IL-6 induced global hypomethylation of long interspersed nuclear element-1 (LINE-1) sequences, and hypermethylation promoted tumorigenesis (Gasche et al. 2011). In contrast, IL-10 family member IL-20 significantly elevated in NSCLC, and its receptors IL-20Rb and IL-22R1 were significantly increased too. IL-20 and its receptors were found to be epigenetically regulated through histone posttranslational modifications and DNA CpG residue methylation. Treatment with recombinant IL-20 resulted in decreased expression of the VEGF family members at the mRNA level (Baird et al. 2011).

4.3 Cytokines in Cancer Immunotherapy

4.3.1 The Interferons (IFNs)

Interferons (IFNs) are a family of molecular messengers synthesized and secreted mainly by natural killer cells, and T cells replied to the presence of antigen such as bacteria, viruses, parasites, or tumor cells. There are three types of IFNs named type I, type II, and type III. They are classified by their specific receptor binding ability (De Andrea et al. 2002).

The most encouraging IFNs for the clinical cancer immunotherapy are type I IFNs which comprised of IFN- α and IFN- β . Type I IFNs can induce tumor cells to express major histocompatibility complex (MHC) class I molecules and participate in the dendritic cell (DC) maturation process. They can also promote activation of antigen-presenting cells and natural killer (NK) cells as well as cytotoxic T lymphocytes (CTLs) (Jewett and Bonavida 1995; Siegal et al. 1999). The type I IFNs can reduce tumor cell growth and accelerate tumor cell apoptosis as well as antitumor angiogenesis despite their immunologic effects.

As a cytokine approved by the FDA, IFN- α was applied for the treatment of melanoma patients and some hematologic malignancies as well as AIDS-related Kaposi's sarcoma in advanced renal cancer therapy; it also acts as an ingredient in an anti-angiogenesis combination project with bevacizumab. IFN- α -2b has been regarded as an effective way of immunotherapy for chronic myelogenous leukemia (CML) and hairy cell leukemia (HCL). For a 1-year treatment of HCL, a well-tolerated low dose of 2 million units/m² subcutaneously three times a week generated an overall response rate of 77 %. Retreatment affords remissions in major patients while recurrences are common in HCL after IFN- α therapy (Lee and Margolin 2011).

The side effect profile of IFN- α is dose dependent, including fatigue, fever, headaches, gastrointestinal symptoms, and myalgias. This toxicity profile is quite common and occurring in 80 % or more of patients. The neuropsychiatric issues including depression (45 %), confusion (10 %), and mania are more serious questions.

It has been demonstrated in lots of preclinical models that IFN- β has therapeutic effects in immune modulatory strategies for alleviation of autoimmune reactivity as well as for the malignancies treatment. In preclinical animal cancer models, IFN- β is more powerful than IFN- α in inhibiting cell growth. But it has the limitation in the clinical cancer immunotherapy because of the sustaining side effects such as fever and the low bioavailability (Lee and Margolin 2011).

IFN- γ is the only member of type II class IFNs which can enhance the expression of MHC classes and co-stimulatory molecules on APCs. IFN- γ is an important modulator of antitumor immune response that has moderate antitumor angiogenesis and is cytotoxic to some cancer cells. It may also play an important role in the antitumor activity triggered by other factors such as other cytokines, for example, IL-12 and IL-2. In addition, it has been demonstrated that IFN- γ has very limited

clinical potential in cancer immunotherapy partially because of inducing expression of an enzyme, indoleamine 2,3-dioxygenase (IDO), which is responsible for tryptophan catabolism (Taylor and Feng 1991). Tryptophan exhaustion can enhance the suppressive activity of myeloid-derived suppressor cells.

4.3.2 Interleukin-2

Discovery of interleukin-2 (IL-2) has significant importance because it opens a new chapter for the immunotherapy and basic immunology research not only for cancer but also for many other diseases. IL-2 was regarded as “T-cell growth factor” (TCGF) secreted mainly by the CD4⁺ Th1 subset in a paracrine and autocrine manner when it was discovered in 1976. The primary physiologic function of IL-2 is to regulate the survival, proliferation, and differentiation of activated T cells and NK cells. The key transcription factor STAT5 is responsible for the activation of IL-2 signaling pathway. This is an important discovery and enables immunologists the first time to expand T cells and NK cells in vitro long-term culture.

The FDA has approved IL-2 as one of the cytokines to treat the patients with metastatic renal cell carcinoma and melanoma. This significant discovery made modulation of immune system a powerful method for cancer immunotherapy. The objective response rate in advanced melanoma patients is 5–27 % for high-dose IL-2 ranged from 10,000 to 72,000 IU/kg combined with LAK or IFN. Monotherapy and the durable complete responses are 5–7 % in these patients (Petrella et al. 2007). Metastatic renal cell carcinoma patients who received high-dose intravenous IL-2 got the similar response rate and the durable complete responses to the melanoma patients (Yang et al. 1994).

Because of its significant role in activating NK and T cells, IL-2 was applied in the combination therapy with immune effector cells like lymphokine-activated killer (LAK). The NCI has conducted lots of phase II clinical trials to combine the adoptive transfer of TILs propagated in vitro and high-dose IL-2. Metastatic melanoma patients treated with IL-2 expanded TILs received very promising results. The clinical response rate of the TIL and IL-2 combination-treated patients is close to 50 %. After high-dose IL-2 and TIL therapy for almost 8 years, some patients have even still maintained disease-free (Sim and Radvanyi 2014).

Although IL-2-based TIL therapy has received very promising results, IL-2 can influence the differentiated phenotype of TILs and their long-term survival in vivo. In addition, IL-2 has been used in the generation and maintenance of regulatory T cells (de la Rosa et al. 2004). These disadvantages may neutralize the clinical advantages of this therapy.

The side effect assembly of IL-2 is mainly correlated to the capillary leak syndrome, which is featured by hypotension resulting in a leakage of fluid from the circulation system to the interstitial space. Moreover, IL-2 can induce gastrointestinal disorders such as vomiting and diarrhea. It can also induce the flu-like symptoms such as chill, fatigue, and fever. Despite of hypotension, IL-2 may also cause cardiovascular disease such as cardiac arrhythmias and myocarditis. There

are also other symptoms such as pulmonary edema, pruritus, electrolyte abnormalities, reversible renal and hepatic dysfunction, thrombocytopenia, coagulopathy, and anemia. Rarely side effects such as disorientation, confusion, or visual hallucinations may also occur. Lots of modifications such as alterations of dose, schedule, and route are implicated in order to reduce the IL-2 therapy-related side effects. There are also efforts to alter the molecular structure of IL-2. Other methods such as adding toxic regulators such as drugs with anti-angiogenic agents or anti-inflammatory properties have also been tested. However, these modifications all failed to demonstrate an improved therapeutic index. Efforts to improve the therapeutic potential of IL-2 immunotherapy are still ongoing (Sim and Radvanyi 2014).

4.3.3 IL-2-Related Cytokines (IL-7, IL-15, IL-21)

IL-2 receptor (IL-2R) has three subunits α , β , and γ . Research and identification of the IL-2 signaling pathway including the common γ chain (CD132) enabled the discovery of other major common γ -chain cytokines. Cytokines such as IL-7, IL-15, and IL-21 share the γ chain with IL-2 to regulate the immune response in a distinct or synergistic way.

IL-7 is produced mainly by nonhematopoietic cells such as stromal cells and epithelial cells derived from the thymus and bone marrow although it is a hematopoietic growth factor. Normal lymphocytes are not producing IL-7. The signaling pathway of IL-7 plays important role in the differentiation and homeostasis of T cell. Recombinant IL-7 has been safely administered to melanoma and metastatic sarcoma patients in several phase I and phase II clinical trials. Patients with refractory nonhematologic malignancy got mild to moderate constitutional symptoms, such as reversible spleen and lymph node enlargement and marked increase of lymphocytes in a dose-dependent manner from 3 to 60 $\mu\text{g/kg/dose}$ after receiving rhIL-7 subcutaneously every other day for 2 weeks (Sportes et al. 2010; Capitini et al. 2009).

IL-15 is mainly synthesized and secreted by mononuclear phagocytes and has been considered as a potent candidate for cancer immunotherapy because of its ability to maintain the survival of NK and T cells and prevent their apoptosis as well as to facilitate IFN- γ production of CD8⁺ T cells. The combination of IL-15 and other agents such as rituximab and cetuximab can also boost the antitumor response by reinforcing the antibody-dependent cellular cytotoxicity of T cell and NK cell (Croce et al. 2012).

IL-21 is another cytokine of IL-2-related family that has great clinical effects because of its promising ability to regulate both innate and adaptive immune response. It is now under exploitation for its potential in cancer immunotherapy. The combination therapies of IL-21 and IL-15 lead to tumor regression in melanoma patients with a maximum tolerated dose (MTD) of 30 $\mu\text{g/kg}$ for daily i.v. infusions. IL-21 can also enhance the proliferation and aggregation of IFN- γ producing CD8⁺ T cells (Croce et al. 2015).

4.3.4 Additional Cytokines (IL-12, IL-18, GM-CSF)

IL-12 is an important cytokine for the regulation of Th1-type immune response. The major source of IL-12 is the activating antigen-presenting cells. It has been regarded as a T-cell-stimulating factor playing a critical role in the proliferation and function of T cells. Stimulation by IL-12 could lead to the upregulation of tumor necrosis factor-alpha (TNF- α) and IFN- γ produced by T cells and natural killer (NK) cells. IL-12 has been regarded as a potential effective adjuvant for cancer therapy vaccines. Results of preclinical models indicate that IL-12 could be a potent therapeutic cytokine against cancer. However, the adverse effects such as muscle necrosis observed in the clinical trials of IL-12 raise the necessity to modify the project without reducing the antitumor effects of IL-12 (Lasek et al. 2014).

IL-18 is a cytokine which belongs to the IL-1 superfamily. The predominant source of IL-18 is macrophage and dendritic cell. Both innate and adaptive immune responses are regulated by IL-18 through its influence on innate immune cells as well as adaptive immune cells. Safety and efficacy of IL-18 are confirmed by a phase II study carried out in metastatic melanoma patients. But the efficacy turned out to be limited in this setting. The combined therapy with other agents such as alpha-galactosylceramide or monoclonal antibodies such as rituximab could improve the efficacy of IL-18 (Palma et al. 2013; Robertson et al. 2013).

The full name of GM-CSF is granulocyte-macrophage colony-stimulating factor; it is also known as colony-stimulating factor 2 (CSF2). This cytokine is widely used in cancer immunotherapy as a potent adjuvant. GM-CSF can recruit and activate the macrophage and dendritic cell to eliminate tumor. The ability to mature dendritic cell makes it a powerful tool to elicit an antitumor immune response. The FDA approved the use of recombinant GM-CSF in the lymphocyte recovery treatment after chemotherapy in acute myelogenous leukemia patients (Thorne 2013). The cancer vaccine Provenge approved by the FDA to treat prostate cancer also comprises GM-CSF as its adjuvant. Cancer vaccines basing GM-CSF also raised much attention.

4.4 *Combination Cancer Immunotherapy*

Cancer immunotherapy has obtained a positive clinical outcome during the past decades. However, due to tumor heterogeneity and the discrepancy immune response of tumor, the efficacies of cancer immunotherapy are quite restricted. For this reason, combined immunotherapy may be an ideal solution to improve the anticancer efficacy of cancer immunotherapy. And in combination cancer immunotherapy, cytokines are not only of crucial importance for generating vaccines and adoptive cells in vitro but also important to enhance the potency and duration of antitumor response of immunotherapy in vivo (Lee and Margolin 2011). Dendritic cell vaccine is a promising strategy in clinic, IL-4 and GM-CSF are used to elicit

DC from peripheral blood, and IL-1 is used to promote DC maturation in vitro (Ridgway 2003; Trepiaakas et al. 2009). In patients with renal or breast cancer, dendritic cell vaccines combined with IL-2 could decrease TGF- β and CD4⁺CD25⁺ T-cell levels and increase IL-12 levels, thereby enhancing antigen-specific immune response (Baek et al. 2011). In addition, cytokines such as IL-2, IL-7, IL-21, and IL-15 are used in the activation and expansion of T cells in vitro. And after T-cell infusion, low-dose IL-2 is used to maintain the viability of the adoptively transferred T cells (Yee et al. 2002). Furthermore, therapy with IL-15 and mTOR inhibitor everolimus could increase the proportion of CD4⁺ T and NK cells and inhibit breast cancer metastasis (Zhao et al. 2013). In addition, the efficacy of combination therapy using IL-12, human tyrosinase (hTyr) DNA vaccination, and metronomic cyclophosphamide (CPX) on B16F10 mouse with melanoma is also promising (Denies et al. 2014). These successful trials on combinatorial therapies indicated potential directions for future use of cytokines in cancer immunotherapy.

However, cytokines can sometimes mediate opposing effects during combination cancer immunotherapy. For example, IL-2 is a potent activator of the effector T cells as well as the regulatory T cells which may suppress immune response and allows tumors to escape immunologic surveillance (Sakaguchi 2000). Furthermore, cytokines can also exacerbate the toxic effects of chemotherapy and affect drug metabolism. High levels of pro-inflammatory cytokines have been demonstrated to decrease the activity of CYP enzyme cytochrome P450 and other coenzymes in the liver (Harvey and Morgan 2014). In addition, the organ toxicity of chemotherapy is closely related to cytokine levels. For example, cisplatin causes kidney damage by increasing TNF- α levels, and bleomycin increases pulmonary toxicity by increasing levels of TGF- β 1, IL-1, IL-6, and TNF- α (Della Latta et al. 2015; Miller et al. 2010; Wargo et al. 2015). Thus, the biological activities and toxicity of these combined elements must be considered before implementing cytokine-combined cancer therapies.

4.5 Summary and Discussion

Cytokines that existed in tumor microenvironment are secreted by all kinds of cells besides the cancer cells. As an important component of tumor microenvironment, cytokines are closely related to tumor stem cells, microRNA expression, epithelial–mesenchymal transition, autophagy, and DNA methylation of tumor cells. Cytokines play a critical role in antitumor strategies, and many interleukin-based approaches in cancer immunotherapy have been demonstrated as effective both in laboratory and clinical treatment. The pleiotropic function of cytokine indicated that cytokine-based therapy may be in combination regimens. The future of cytokine-based immunotherapy is to amplify the antitumor response, suppress the regulatory pathways, and minimize the toxicities. A deep understanding of the molecular signaling pathways of various cytokines in tumor will be critical in developing effective cytokine-based immunotherapy. Undoubtedly, cytokines will

continue to play a major role in the ongoing development of cancer immunotherapy.

5 Conclusion and Prospects

Evidences show that potential BRMs including TLRs, cytokines, and miRs are crucial in initiating or regulating innate and adaptive immune responses against cancer and also function in modulating chronic inflammation-associated cancers. Currently, clinical trials based on these BRMs for cancer therapy are under way. Besides the classical BRMs, cytokines, such as IFN- α and IL-2, have been approved by the FDA for the treatment of patients with melanoma, some hematologic malignancies, and AIDS-related Kaposi's sarcoma or patients with metastatic melanoma and renal cell carcinoma, respectively; some TLR agonists are nowadays licensed by international regulatory agencies for use in cancer patients, including BCG, MPL, imiquimod, and Picibanil. Also in 2012, one artificial miR-34a mimic, MXR34, also entered phase I study to treat patients with unresectable primary liver cancer or advanced or metastatic cancer with liver involvement or hematologic malignancies.

Although there are some abstracts, especially technical limitations, in front of the BRM-based cancer immunotherapy, including how to reduce therapy-induced side effect in patients, improve its accumulation in the target tissues, and balance the safety and efficiency, in general, further understanding of the mechanisms underlying the regulation of these potential BRMs in modulating antitumor immune response and archiving breakthrough in techniques will facilitate the translation of fundamental research of BRM-based cancer to clinical applications.

References

- Adams, S. (2009). Toll-like receptor agonists in cancer therapy. *Immunotherapy*, 1, 949–964.
- Adams, S., Kozhaya, L., Martiniuk, F., Meng, T. C., Chiriboga, L., Liebes, L., Hochman, T., Shuman, N., Axelrod, D., Speyer, J., et al. (2012). Topical TLR7 agonist imiquimod can induce immune-mediated rejection of skin metastases in patients with breast cancer. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*, 18, 6748–6757.
- Akdis, M., Burgler, S., Cramer, R., Eiwegger, T., Fujita, H., Gomez, E., Klunker, S., Meyer, N., O'Mahony, L., Palomares, O., et al. (2011). Interleukins, from 1 to 37, and interferon-gamma: Receptors, functions, and roles in diseases. *The Journal of Allergy and Clinical Immunology*, 127, 701–721.e701–770.
- Alizadeh, D., Zhang, L., Brown, C. E., Farrukh, O., Jensen, M. C., & Badie, B. (2010). Induction of anti-glioma natural killer cell response following multiple low-dose intracerebral CpG therapy. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*, 16, 3399–3408.

- Amado, T., Schmolka, N., Metwally, H., Silva-Santos, B., & Gomes, A. Q. (2015). Cross-regulation between cytokine and microRNA pathways in T cells. *European Journal of Immunology*, 45, 1584–1595.
- Arango Duque, G., & Descoteaux, A. (2014). Macrophage cytokines: Involvement in immunity and infectious diseases. *Frontiers in Immunology*, 5, 491.
- Ardolino, M., Hsu, J., & Raut, D. H. (2015). Cytokine treatment in cancer immunotherapy. *Oncotarget*, 6, 19346–19347.
- Arits, A. H., Mosterd, K., Essers, B. A., Spoorenberg, E., Sommer, A., De Rooij, M. J., van Pelt, H. P., Quaedvlieg, P. J., Krekels, G. A., van Neer, P. A., et al. (2013). Photodynamic therapy versus topical imiquimod versus topical fluorouracil for treatment of superficial basal-cell carcinoma: A single blind, non-inferiority, randomised controlled trial. *The Lancet Oncology*, 14, 647–654.
- Arunkumar, N., Liu, C., Hang, H., & Song, W. (2013). Toll-like receptor agonists induce apoptosis in mouse B-cell lymphoma cells by altering NF-kappaB activation. *Cellular & Molecular Immunology*, 10, 360–372.
- Asprodites, N., Zheng, L., Geng, D., Velasco-Gonzalez, C., Sanchez-Perez, L., & Davila, E. (2008). Engagement of Toll-like receptor-2 on cytotoxic T-lymphocytes occurs in vivo and augments antitumor activity. *FASEB Journal: Official Publication Federation American Societies for Experimental Biology*, 22, 3628–3637.
- Babar, I. A., Cheng, C. J., Booth, C. J., Liang, X., Weidhaas, J. B., Saltzman, W. M., & Slack, F. J. (2012). Nanoparticle-based therapy in an in vivo microRNA-155 (miR-155)-dependent mouse model of lymphoma. *Proceedings of the National Academy of Sciences of the United States of America*, 109, E1695–E1704.
- Bader, A. G., Brown, D., Stoudemire, J., & Lammers, P. (2011). Developing therapeutic microRNAs for cancer. *Gene Therapy*, 18, 1121–1126.
- Baek, S., Kim, C. S., Kim, S. B., Kim, Y. M., Kwon, S. W., Kim, Y., Kim, H., & Lee, H. (2011). Combination therapy of renal cell carcinoma or breast cancer patients with dendritic cell vaccine and IL-2: Results from a phase I/II trial. *Journal of Translational Medicine*, 9, 178.
- Bailey, S. R., Nelson, M. H., Himes, R. A., Li, Z., Mehrotra, S., & Paulos, C. M. (2014). Th17 cells in cancer: The ultimate identity crisis. *Frontiers in Immunology*, 5, 276.
- Baird, A. M., Gray, S. G., & O'Byrne, K. J. (2011). IL-20 is epigenetically regulated in NSCLC and down regulates the expression of VEGF. *European Journal of Cancer*, 47, 1908–1918.
- Bala, S., Marcos, M., Kodys, K., Csak, T., Catalano, D., Mandrekar, P., & Szabo, G. (2011). Up-regulation of microRNA-155 in macrophages contributes to increased tumor necrosis factor {alpha} (TNF{alpha}) production via increased mRNA half-life in alcoholic liver disease. *Journal of Biological Chemistry*, 286, 1436–1444.
- Ballarin-Gonzalez, B., Ebbesen, M. F., & Howard, K. A. (2014). Polycation-based nanoparticles for RNAi-mediated cancer treatment. *Cancer Letters*, 352, 66–80.
- Basith, S., Manavalan, B., Yoo, T. H., Kim, S. G., & Choi, S. (2012). Roles of toll-like receptors in cancer: A double-edged sword for defense and offense. *Archives of Pharmacological Research*, 35, 1297–1316.
- bCui, T. X., Kryczek, I., Zhao, L., Zhao, E., Kuick, R., Roh, M. H., Vatan, L., Szeliga, W., Mao, Y., Thomas, D. G., et al. (2013). Myeloid-derived suppressor cells enhance stemness of cancer cells by inducing microRNA101 and suppressing the corepressor CtBP2. *Immunity*, 39, 611–621.
- Bendigs, S., Salzer, U., Lipford, G. B., Wagner, H., & Heeg, K. (1999). CpG-oligodeoxynucleotides co-stimulate primary T cells in the absence of antigen-presenting cells. *European Journal of Immunology*, 29, 1209–1218.
- Blum, J. S., & Saltzman, W. M. (2008). High loading efficiency and tunable release of plasmid DNA encapsulated in submicron particles fabricated from PLGA conjugated with poly-L-lysine. *Journal of Controlled Release: Official Journal of the Controlled Release Society*, 129, 66–72.

- Bolland, D. J., Wood, A. L., Johnston, C. M., Bunting, S. F., Morgan, G., Chakalova, L., Fraser, P. J., & Corcoran, A. E. (2004). Antisense intergenic transcription in V(D)J recombination. *Nature Immunology*, 5, 630–637.
- Bouchie, A. (2013). First microRNA mimic enters clinic. *Nature Biotechnology*, 31, 577.
- Buchser, W. J., Laskow, T. C., Pavlik, P. J., Lin, H. M., & Lotze, M. T. (2012). Cell-mediated autophagy promotes cancer cell survival. *Cancer Research*, 72, 2970–2979.
- Calin, G. A., Dumitru, C. D., Shimizu, M., Bichi, R., Zupo, S., Noch, E., Aldler, H., Rattan, S., Keating, M., Rai, K., et al. (2002). Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proceedings of the National Academy of Sciences of the United States of America*, 99, 15524–15529.
- Capitini, C. M., Chisti, A. A., & Mackall, C. L. (2009). Modulating T-cell homeostasis with IL-7: Preclinical and clinical studies. *Journal of Internal Medicine*, 266, 141–153.
- Caron, G., Duluc, D., Fremaux, I., Jeannin, P., David, C., Gascan, H., & Delneste, Y. (2005). Direct stimulation of human T cells via TLR5 and TLR7/8: Flagellin and R-848 up-regulate proliferation and IFN-gamma production by memory CD4+ T cells. *Journal of Immunology (Baltimore, Md.: 1950)*, 175, 1551–1557.
- Carpenter, S., Aiello, D., Atianand, M. K., Ricci, E. P., Gandhi, P., Hall, L. L., Byron, M., Monks, B., Henry-Bezy, M., Lawrence, J. B., et al. (2013). A long noncoding RNA mediates both activation and repression of immune response genes. *Science (New York, NY)*, 341, 789–792.
- Chaudhuri, A. A., So, A. Y., Sinha, N., Gibson, W. S., Taganov, K. D., O'Connell, R. M., & Baltimore, D. (2011). MicroRNA-125b potentiates macrophage activation. *Journal of Immunology*, 187, 5062–5068.
- Cheever, M. A. (2008). Twelve immunotherapy drugs that could cure cancers. *Immunological Reviews*, 222, 357–368.
- Chen, Y., Zhu, X., Zhang, X., Liu, B., & Huang, L. (2010). Nanoparticles modified with tumor-targeting scFv deliver siRNA and miRNA for cancer therapy. *Molecular Therapy: The Journal of the American Society of Gene Therapy*, 18, 1650–1656.
- Chen, M. H., Li, W. S., Lue, Y. S., Chu, C. L., Pan, I. H., Ko, C. H., Chen, D. Y., Lin, C. H., Lin, S. H., Chang, C. P., et al. (2013). Clitocybe nuda activates dendritic cells and acts as a DNA vaccine adjuvant. *Evidence-Based Complementary Alternative Medicine: eCAM*, 2013, 761454.
- Chen, S., Zhang, Y., Kuzel, T. M., & Zhang, B. (2015). Regulating tumor myeloid-derived suppressor cells by MicroRNAs. *Cancer Cell Microenvironment* 2, 761454.
- Cherfils-Vicini, J., Platonova, S., Gillard, M., Laurans, L., Validire, P., Caliendo, R., Magdeleinat, P., Mami-Chouaib, F., Dieu-Nosjean, M. C., Fridman, W. H., et al. (2010). Triggering of TLR7 and TLR8 expressed by human lung cancer cells induces cell survival and chemoresistance. *The Journal of Clinical Investigation*, 120, 1285–1297.
- Chiffolleau, E., Heslan, J. M., Heslan, M., Louvet, C., Condamine, T., & Cuturi, M. C. (2007). TLR9 ligand enhances proliferation of rat CD4+ T cell and modulates suppressive activity mediated by CD4+ CD25+ T cell. *International Immunology*, 19, 193–201.
- Chiou, G. Y., Cherg, J. Y., Hsu, H. S., Wang, M. L., Tsai, C. M., Lu, K. H., Chien, Y., Hung, S. C., Chen, Y. W., Wong, C. I., et al. (2012). Cationic polyurethanes-short branch PEI-mediated delivery of Mir145 inhibited epithelial-mesenchymal transdifferentiation and cancer stem-like properties and in lung adenocarcinoma. *Journal of Controlled Release: Official Journal of the Controlled Release Society*, 159, 240–250.
- Christian, D. A., & Hunter, C. A. (2012). Particle-mediated delivery of cytokines for immunotherapy. *Immunotherapy*, 4, 425–441.
- Chun-Guang, M., Qi-Man, L., Yu-Yun, Z., Li-Hua, C., Cheng, T., & Jian-De, H. (2014). Successful treatment of giant basal cell carcinoma with topical imiquimod 5% cream with long term follow-up. *Indian Journal of Dermatology*, 59, 575–578.
- Cobos Jimenez, V., Bradley, E. J., Willemsen, A. M., van Kampen, A. H., Baas, F., & Kootstra, N. A. (2014). Next-generation sequencing of microRNAs uncovers expression signatures in polarized macrophages. *Physiological Genomics*, 46, 91–103.

- Cottalorda, A., Verschelde, C., Marçais, A., Tomkowiak, M., Musette, P., Uematsu, S., Akira, S., Marvel, J., & Bonnefoy-Berard, N. (2006). TLR2 engagement on CD8 T cells lowers the threshold for optimal antigen-induced T cell activation. *European Journal of Immunology*, 36, 1684–1693.
- Croce, M., Orengo, A. M., Azzarone, B., & Ferrini, S. (2012). Immunotherapeutic applications of IL-15. *Immunotherapy*, 4, 957–969.
- Croce, M., Rigo, V., & Ferrini, S. (2015). IL-21: A pleiotropic cytokine with potential applications in oncology. *Journal of Immunology Research*, 2015, 696578.
- Cubillos-Ruiz, J. R., Baird, J. R., Tesone, A. J., Rutkowski, M. R., Scarlett, U. K., Camposeco-Jacobs, A. L., Anadon-Arillas, J., Harwood, N. M., Korc, M., Fiering, S. N., et al. (2012). Reprogramming tumor-associated dendritic cells in vivo using miRNA mimetics triggers protective immunity against ovarian cancer. *Cancer Research*, 72, 1683–1693.
- Curtin, J. F., Liu, N., Candolfi, M., Xiong, W., Assi, H., Yagiz, K., Edwards, M. R., Michelsen, K. S., Kroeger, K. M., Liu, C., et al. (2009). HMGB1 mediates endogenous TLR2 activation and brain tumor regression. *PLoS Medicine*, 6, e10.
- Cutler, A., & Brombacher, F. (2005). Cytokine therapy. *Annals of the New York Academy of Sciences*, 1056, 16–29.
- Danielson, L. S., Reavie, L., Coussens, M., Davalos, V., Castillo-Martin, M., Guijarro, M. V., Coffre, M., Cordon-Cardo, C., Aifantis, I., Ibrahim, S., et al. (2015). Limited miR-17-92 overexpression drives hematologic malignancies. *Leukemia Research*, 39, 335–341.
- De Andrea, M., Ravera, R., Gioia, D., Gariglio, M., & Landolfo, S. (2002). The interferon system: An overview. *European Journal of Paediatric Neurology: EJPN: Official Journal European Paediatric Neurology Society*, 6(Suppl A), A41–A46; discussion A55–48.
- de la Rosa, M., Rutz, S., Dorninger, H., & Scheffold, A. (2004). Interleukin-2 is essential for CD4+CD25+ regulatory T cell function. *European Journal of Immunology*, 34, 2480–2488.
- de Martimprey, H., Bertrand, J. R., Fusco, A., Santoro, M., Couvreur, P., Vauthier, C., & Malvy, C. (2008). siRNA nanoformulation against the ret/PTC1 junction oncogene is efficient in an in vivo model of papillary thyroid carcinoma. *Nucleic Acids Research*, 36, e2.
- de Martimprey, H., Bertrand, J. R., Malvy, C., Couvreur, P., & Vauthier, C. (2010). New core-shell nanoparticles for the intravenous delivery of siRNA to experimental thyroid papillary carcinoma. *Pharmaceutical Research*, 27, 498–509.
- de Rosa, F., Fanini, F., Guidoboni, M., Vannini, I., Amadori, D., Ridolfi, R., Ridolfi, L., & Fabbri, M. (2014). MicroRNAs and dendritic cell-based vaccination in melanoma patients. *Melanoma Research*, 24, 181–189.
- Della Latta, V., Cecchetti, A., Del Ry, S., & Morales, M. A. (2015). Bleomycin in the setting of lung fibrosis induction: From biological mechanisms to counteractions. *Pharmacological Research*, 97, 122–130.
- Deng, G., & Sui, G. (2013). Noncoding RNA in oncogenesis: A new era of identifying key players. *International Journal of Molecular Sciences*, 14, 18319–18349.
- Deng, S., Zhu, S., Qiao, Y., Liu, Y. J., Chen, W., Zhao, G., & Chen, J. (2014). Recent advances in the role of toll-like receptors and TLR agonists in immunotherapy for human glioma. *Protein & Cell*, 5, 899–911.
- Denies, S., Cicchelero, L., Van Audenhove, I., & Sanders, N. N. (2014). Combination of interleukin-12 gene therapy, metronomic cyclophosphamide and DNA cancer vaccination directs all arms of the immune system towards tumor eradication. *Journal of Controlled Release: Official Journal of the Controlled Release Society*, 187, 175–182.
- Desai, S., Laskar, S., & Pandey, B. N. (2013). Autocrine IL-8 and VEGF mediate epithelial-mesenchymal transition and invasiveness via p38/JNK-ATF-2 signalling in A549 lung cancer cells. *Cellular Signalling*, 25, 1780–1791.
- Didierlaurent, A., Ferrero, I., Otten, L. A., Dubois, B., Reinhardt, M., Carlsen, H., Blomhoff, R., Akira, S., Kraehenbuhl, J. P., & Sirard, J. C. (2004). Flagellin promotes myeloid differentiation factor 88-dependent development of Th2-type response. *Journal of Immunology*, 172, 6922–6930.

- Dietsch, G. N., Randall, T. D., Gottardo, R., Northfelt, D. W., Ramanathan, R. K., Cohen, P. A., Manjarrez, K. L., Newkirk, M., Bryan, J. K., & Hershberg, R. M. (2015). Late stage cancer patients remain highly responsive to immune activation by the selective TLR8 agonist motolimod (VTX-2337). *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*, 21, 5445–5452.
- Drexler, S. K., & Yazdi, A. S. (2013). Complex roles of inflammasomes in carcinogenesis. *Cancer Journal*, 19, 468–472.
- Eisele, G., Wischhusen, J., Mittelbronn, M., Meyermann, R., Waldhauer, I., Steinle, A., Weller, M., & Friese, M. A. (2006). TGF-beta and metalloproteinases differentially suppress NKG2D ligand surface expression on malignant glioma cells. *Brain: A Journal of Neurology*, 129, 2416–2425.
- El Andaloussi, A., Sonabend, A. M., Han, Y., & Lesniak, M. S. (2006). Stimulation of TLR9 with CpG ODN enhances apoptosis of glioma and prolongs the survival of mice with experimental brain tumors. *Glia*, 54, 526–535.
- Fabani, M. M., Abreu-Goodger, C., Williams, D., Lyons, P. A., Torres, A. G., Smith, K. G., Enright, A. J., Gait, M. J., & Vigorito, E. (2010). Efficient inhibition of miR-155 function in vivo by peptide nucleic acids. *Nucleic Acids Research*, 38, 4466–4475.
- Fabbri, M., Paone, A., Calore, F., Galli, R., Gaudio, E., Santhanam, R., Lovat, F., Fadda, P., Mao, C., Nuovo, G. J., et al. (2012). MicroRNAs bind to Toll-like receptors to induce prometastatic inflammatory response. *Proceedings of the National Academy of Sciences of the United States of America*, 109, E2110–E2116.
- Fang, H., Ang, B., Xu, X., Huang, X., Wu, Y., Sun, Y., Wang, W., Li, N., Cao, X., & Wan, T. (2014). TLR4 is essential for dendritic cell activation and anti-tumor T-cell response enhancement by DAMPs released from chemically stressed cancer cells. *Cellular & Molecular Immunology*, 11, 150–159.
- Fernandes, J. V., Cobucci, R. N., Jatoba, C. A., Fernandes, T. A., de Azevedo, J. W., & de Araujo, J. M. (2015). The role of the mediators of inflammation in cancer development. *Pathology and Oncology Research*, 21, 527–534.
- Fife, K. H., Meng, T. C., Ferris, D. G., & Liu, P. (2008). Effect of resiquimod 0.01 % gel on lesion healing and viral shedding when applied to genital herpes lesions. *Antimicrobial Agents and Chemotherapy*, 52, 477–482.
- Finoux, A. L., & Chartrand, P. (2008). Oncogenic and tumour suppressor microRNAs. *Medicine Sciences: M/S*, 24, 1049–1054.
- Fitzgerald, K. A., & Caffrey, D. R. (2014). Long noncoding RNAs in innate and adaptive immunity. *Current Opinion in Immunology*, 26, 140–146.
- Fitzgerald, K. A., McWhirter, S. M., Faia, K. L., Rowe, D. C., Latz, E., Golenbock, D. T., Coyle, A. J., Liao, S. M., & Maniatis, T. (2003). IKKepsilon and TBK1 are essential components of the IRF3 signaling pathway. *Nature Immunology*, 4, 491–496.
- Franks, H. A., Wang, Q., & Patel, P. M. (2012). New anticancer immunotherapies. *Anticancer Research*, 32, 2439–2453.
- Gabrilovich, D. I., & Nagaraj, S. (2009). Myeloid-derived suppressor cells as regulators of the immune system. *Nature Reviews Immunology*, 9, 162–174.
- Gasche, J. A., Hoffmann, J., Boland, C. R., & Goel, A. (2011). Interleukin-6 promotes tumorigenesis by altering DNA methylation in oral cancer cells. *International Journal of Cancer*, 129, 1053–1063.
- Gelman, A. E., Zhang, J., Choi, Y., & Turka, L. A. (2004). Toll-like receptor ligands directly promote activated CD4+ T cell survival. *Journal of Immunology (Baltimore, Md.: 1950)*, 172, 6065–6073.
- Geng, D., Zheng, L., Srivastava, R., Asprodites, N., Velasco-Gonzalez, C., & Davila, E. (2010). When toll-like receptor and T-cell receptor signals collide: A mechanism for enhanced CD8 T-cell effector function. *Blood*, 116, 3494–3504.
- Gollnick, H., Barona, C. G., Frank, R. G., Ruzicka, T., Megahed, M., Maus, J., & Munzel, U. (2008). Recurrence rate of superficial basal cell carcinoma following treatment with

- imiquimod 5% cream: Conclusion of a 5-year long-term follow-up study in Europe. *European Journal of Dermatology: EJD*, 18, 677–682.
- Gomez, J. A., Wapinski, O. L., Yang, Y. W., Bureau, J. F., Gopinath, S., Monack, D. M., Chang, H. Y., Brahic, M., & Kirkegaard, K. (2013). The NeST long ncRNA controls microbial susceptibility and epigenetic activation of the interferon-gamma locus. *Cell*, 152, 743–754.
- Gonzalez-Reyes, S., Marin, L., Gonzalez, L., Gonzalez, L. O., del Casar, J. M., Lamelas, M. L., Gonzalez-Quintana, J. M., & Vizoso, F. J. (2010). Study of TLR3, TLR4 and TLR9 in breast carcinomas and their association with metastasis. *BMC Cancer*, 10, 665.
- Grauer, O., Poschl, P., Lohmeier, A., Adema, G. J., & Bogdahn, U. (2007). Toll-like receptor triggered dendritic cell maturation and IL-12 secretion are necessary to overcome T-cell inhibition by glioma-associated TGF-beta2. *Journal of Neuro-Oncology*, 82, 151–161.
- Grauer, O. M., Molling, J. W., Bennink, E., Toonen, L. W., Suttmuller, R. P., Nierkens, S., & Adema, G. J. (2008). TLR ligands in the local treatment of established intracerebral murine gliomas. *Journal of Immunology*, 181, 6720–6729.
- Gutschner, T., & Diederichs, S. (2012). The hallmarks of cancer: A long non-coding RNA point of view. *RNA Biology*, 9, 703–719.
- Guttman, M., Amit, I., Garber, M., French, C., Lin, M. F., Feldser, D., Huarte, M., Zuk, O., Carey, B. W., Cassady, J. P., et al. (2009). Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. *Nature*, 458, 223–227.
- Hansen, T. B., Kjems, J., & Damgaard, C. K. (2013). Circular RNA and miR-7 in cancer. *Cancer Research*, 73, 5609–5612.
- Hartman, L. L., Crawford, J. R., Makale, M. T., Milburn, M., Joshi, S., Salazar, A. M., Hasenauer, B., VandenBerg, S. R., MacDonald, T. J., & Durden, D. L. (2014). Pediatric phase II trials of poly-ICLC in the management of newly diagnosed and recurrent brain tumors. *Journal of Pediatric Hematology/Oncology*, 36, 451–457.
- Harvey, R. D., & Morgan, E. T. (2014). Cancer, inflammation, and therapy: Effects on cytochrome p450-mediated drug metabolism and implications for novel immunotherapeutic agents. *Clinical Pharmacology and Therapeutics*, 96, 449–457.
- Hatziaepostolou, M., Polytarchou, C., Aggelidou, E., Drakaki, A., Poultsides, G. A., Jaeger, S. A., Ogata, H., Karin, M., Struhl, K., Hadzopoulou-Cladaras, M., et al. (2011). An HNF4alpha-miRNA inflammatory feedback circuit regulates hepatocellular oncogenesis. *Cell*, 147, 1233–1247.
- Hawkins, B. T., & Davis, T. P. (2005). The blood-brain barrier/neurovascular unit in health and disease. *Pharmacological Reviews*, 57, 173–185.
- He, M., Xu, Z., Ding, T., Kuang, D. M., & Zheng, L. (2009). MicroRNA-155 regulates inflammatory cytokine production in tumor-associated macrophages via targeting C/EBPbeta. *Cellular & Molecular Immunology*, 6, 343–352.
- He, H., Fan, P., Yin, T., Chen, Q., Shi, H., Liu, S., Li, H., Jing, Q., Yan, Y., Zhang, H., et al. (2012). Local delivery of recombinant adenovirus expressing hepatitis B virus X protein and interleukin-12 results in antitumor effects via inhibition of hepatoma cell growth and intervention of tumor microenvironment. *International Journal of Molecular Medicine*, 30, 599–605.
- Henriques, L., Palumbo, M., Guay, M. P., Bahoric, B., Basik, M., Kavan, P., & Batist, G. (2014). Imiquimod in the treatment of breast cancer skin metastasis. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*, 32, e22–e25.
- Hervas-Stubbs, S., Olivier, A., Boisgerault, F., Thieblemont, N., & Leclerc, C. (2007). TLR3 ligand stimulates fully functional memory CD8+ T cells in the absence of CD4+ T-cell help. *Blood*, 109, 5318–5326.
- Hesling, C., D'Incan, M., Mansard, S., Franck, F., Corbin-Duval, A., Chevenet, C., Dechelotte, P., Madelmont, J. C., Veyre, A., Souteyrand, P., et al. (2004). In vivo and in situ modulation of the expression of genes involved in metastasis and angiogenesis in a patient treated with topical imiquimod for melanoma skin metastases. *The British Journal of Dermatology*, 150, 761–767.

- Heward, J. A., & Lindsay, M. A. (2014). Long non-coding RNAs in the regulation of the immune response. *Trends in Immunology*, 35, 408–419.
- Hodge, L. S., Ziesmer, S. C., Yang, Z. Z., Secreto, F. J., Gertz, M. A., Novak, A. J., & Ansell, S. M. (2012). IL-21 in the bone marrow microenvironment contributes to IgM secretion and proliferation of malignant cells in Waldenstrom macroglobulinemia. *Blood*, 120, 3774–3782.
- Holmstrom, K., Pedersen, A. W., Claesson, M. H., Zocca, M. B., & Jensen, S. S. (2010). Identification of a microRNA signature in dendritic cell vaccines for cancer immunotherapy. *Human Immunology*, 71, 67–73.
- Hua, D., Liu, M. Y., Cheng, Z. D., Qin, X. J., Zhang, H. M., Chen, Y., Qin, G. J., Liang, G., Li, J. N., Han, X. F., et al. (2009). Small interfering RNA-directed targeting of Toll-like receptor 4 inhibits human prostate cancer cell invasion, survival, and tumorigenicity. *Molecular Immunology*, 46, 2876–2884.
- Huang, B., Zhao, J., Li, H., He, K. L., Chen, Y., Chen, S. H., Mayer, L., Unkeless, J. C., & Xiong, H. (2005). Toll-like receptors on tumor cells facilitate evasion of immune surveillance. *Cancer Research*, 65, 5009–5014.
- Huang, B., Zhao, J., Shen, S., Li, H., He, K. L., Shen, G. X., Mayer, L., Unkeless, J., Li, D., Yuan, Y., et al. (2007). *Listeria monocytogenes* promotes tumor growth via tumor cell toll-like receptor 2 signaling. *Cancer Research*, 67, 4346–4352.
- Huang, E., Liu, R., & Chu, Y. (2015). miRNA-15a/16: As tumor suppressors and more. *Future Oncology*, 11, 2351–2363.
- Huarte, M., & Rinn, J. L. (2010). Large non-coding RNAs: Missing links in cancer? *Human Molecular Genetics*, 19, R152–R161.
- Huffaker, T. B., Hu, R., Runtsch, M. C., Bake, E., Chen, X., Zhao, J., Round, J. L., Baltimore, D., & O'Connell, R. M. (2012). Epistasis between microRNAs 155 and 146a during T cell-mediated antitumor immunity. *Cell Reports*, 2, 1697–1709.
- Hwang do, W., Son, S., Jang, J., Youn, H., Lee, S., Lee, D., Lee, Y. S., Jeong, J. M., Kim, W. J., & Lee, D. S. (2011). A brain-targeted rabies virus glycoprotein-disulfide linked PEI nanocarrier for delivery of neurogenic microRNA. *Biomaterials*, 32, 4968–4975.
- Ifere, G. O., & Ananaba, G. A. (2009). Prostate cancer gene expression marker 1 (PCGEM1): A patented prostate-specific non-coding gene and regulator of prostate cancer progression. *Recent Patents on DNA & Gene Sequences*, 3, 151–163.
- Iliopoulos, D., Jaeger, S. A., Hirsch, H. A., Bulyk, M. L., & Struhl, K. (2010). STAT3 activation of miR-21 and miR-181b-1 via PTEN and CYLD are part of the epigenetic switch linking inflammation to cancer. *Molecular Cell*, 39, 493–506.
- Ilvesaro, J. M., Merrell, M. A., Swain, T. M., Davidson, J., Zayzafoon, M., Harris, K. W., & Selander, K. S. (2007). Toll like receptor-9 agonists stimulate prostate cancer invasion in vitro. *The Prostate*, 67, 774–781.
- Janssen, H. L., Reesink, H. W., Lawitz, E. J., Zeuzem, S., Rodriguez-Torres, M., Patel, K., van der Meer, A. J., Patick, A. K., Chen, A., Zhou, Y., et al. (2013). Treatment of HCV infection by targeting microRNA. *The New England Journal of Medicine*, 368, 1685–1694.
- Jego, G., Bataille, R., Geffroy-Luseau, A., Descamps, G., & Pellat-Deceunynck, C. (2006). Pathogen-associated molecular patterns are growth and survival factors for human myeloma cells through Toll-like receptors. *Leukemia*, 20, 1130–1137.
- Jelinek, I., Leonard, J. N., Price, G. E., Brown, K. N., Meyer-Manlapat, A., Goldsmith, P. K., Wang, Y., Venzon, D., Epstein, S. L., & Segal, D. M. (2011). TLR3-specific double-stranded RNA oligonucleotide adjuvants induce dendritic cell cross-presentation, CTL responses, and antiviral protection. *Journal of Immunology*, 186, 2422–2429.
- Jewett, A., & Bonavida, B. (1995). Interferon-alpha activates cytotoxic function but inhibits interleukin-2-mediated proliferation and tumor necrosis factor-alpha secretion by immature human natural killer cells. *Journal of Clinical Immunology*, 15, 35–44.
- Ji, X., Li, J., Xu, L., Wang, W., Luo, M., Luo, S., Ma, L., Li, K., Gong, S., He, L., et al. (2013). IL4 and IL-17A provide a Th2/Th17-polarized inflammatory milieu in favor of TGF-beta1 to

- induce bronchial epithelial-mesenchymal transition (EMT). *International Journal of Clinical and Experimental Pathology*, 6, 1481–1492.
- Jiang, P., Liu, R., Zheng, Y., Liu, X., Chang, L., Xiong, S., & Chu, Y. (2012). MiR-34a inhibits lipopolysaccharide-induced inflammatory response through targeting Notch1 in murine macrophages. *Experimental Cell Research*, 318, 1175–1184.
- Jin, M. S., & Lee, J. O. (2008). Structures of the toll-like receptor family and its ligand complexes. *Immunity*, 29, 182–191.
- Kabelitz, D. (2007). Expression and function of Toll-like receptors in T lymphocytes. *Current Opinion in Immunology*, 19, 39–45.
- Kang, J. Y., & Lee, J. O. (2011). Structural biology of the Toll-like receptor family. *Annual Review of Biochemistry*, 80, 917–941.
- Kawagoe, T., Sato, S., Matsushita, K., Kato, H., Matsui, K., Kumagai, Y., Saitoh, T., Kawai, T., Takeuchi, O., & Akira, S. (2008). Sequential control of Toll-like receptor-dependent responses by IRAK1 and IRAK2. *Nature Immunology*, 9, 684–691.
- Kawai, T., & Akira, S. (2010). The role of pattern-recognition receptors in innate immunity: Update on toll-like receptors. *Nature Immunology*, 11, 373–384.
- Kawai, T., & Akira, S. (2011). Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. *Immunity*, 34, 637–650.
- Kelly, M. G., Alvero, A. B., Chen, R., Silasi, D. A., Abrahams, V. M., Chan, S., Visintin, I., Rutherford, T., & Mor, G. (2006). TLR-4 signaling promotes tumor growth and paclitaxel chemoresistance in ovarian cancer. *Cancer Research*, 66, 3859–3868.
- Killeen, S. D., Wang, J. H., Andrews, E. J., & Redmond, H. P. (2009). Bacterial endotoxin enhances colorectal cancer cell adhesion and invasion through TLR-4 and NF-kappaB-dependent activation of the urokinase plasminogen activator system. *British Journal of Cancer*, 100, 1589–1602.
- Kim, T.-H., Jiang, H.-L., Jere, D., Park, I.-K., Cho, M.-H., Nah, J.-W., Choi, Y.-J., Akaike, T., & Cho, C.-S. (2007). Chemical modification of chitosan as a gene carrier in vitro and in vivo. *Progress in Polymer Science*, 32, 726–753.
- Kim, Y. H., Girardi, M., Duvic, M., Kuzel, T., Link, B. K., Pinter-Brown, L., & Rook, A. H. (2010). Phase I trial of a Toll-like receptor 9 agonist, PF-3512676 (CPG 7909), in patients with treatment-refractory, cutaneous T-cell lymphoma. *Journal of the American Academy of Dermatology*, 63, 975–983.
- Kim, J. S., Yu, S. K., Lee, M. H., Park, M. G., Park, E., Kim, S. G., Lee, S. Y., Kim, C. S., Kim, H. J., Chun, H. S., et al. (2013). MicroRNA-205 directly regulates the tumor suppressor, interleukin-24, in human KB oral cancer cells. *Molecules and Cells*, 35, 17–24.
- Kirigin, F. F., Lindstedt, K., Sellars, M., Ciofani, M., Low, S. L., Jones, L., Bell, F., Pauli, F., Bonneau, R., Myers, R. M., et al. (2012). Dynamic microRNA gene transcription and processing during T cell development. *Journal of Immunology (Baltimore, Md.: 1950)*, 188, 3257–3267.
- Koukos, G., Polytarchou, C., Kaplan, J. L., Morley-Fletcher, A., Gras-Miralles, B., Kokkotou, E., Baril-Dore, M., Pothoulakis, C., Winter, H. S., & Iliopoulos, D. (2013). MicroRNA-124 regulates STAT3 expression and is down-regulated in colon tissues of pediatric patients with ulcerative colitis. *Gastroenterology*, 145, 842–852.e842.
- Krawczyk, M., & Emerson, B. M. (2014). p50-associated COX-2 extragenic RNA (PACER) activates COX-2 gene expression by occluding repressive NF-kappaB complexes. *eLife*, 3, e01776.
- Krishnamurthy, S., Warner, K. A., Dong, Z., Imai, A., Nor, C., Ward, B. B., Helman, J. I., Taichman, R. S., Bellile, E. L., McCauley, L. K., et al. (2014). Endothelial interleukin-6 defines the tumorigenic potential of primary human cancer stem cells. *Stem Cells*, 32, 2845–2857.
- Krummen, M., Balkow, S., Shen, L., Heinz, S., Loquai, C., Probst, H. C., & Grabbe, S. (2010). Release of IL-12 by dendritic cells activated by TLR ligation is dependent on MyD88

- signaling, whereas TRIF signaling is indispensable for TLR synergy. *Journal of Leukocyte Biology*, 88, 189–199.
- Kryczek, I., Lin, Y., Nagarsheth, N., Peng, D., Zhao, L., Zhao, E., Vatan, L., Szeliga, W., Dou, Y., Owens, S., et al. (2014). IL-22(+)CD4(+) T cells promote colorectal cancer stemness via STAT3 transcription factor activation and induction of the methyltransferase DOT1L. *Immunity*, 40, 772–784.
- Kundu, S. D., Lee, C., Billips, B. K., Habermacher, G. M., Zhang, Q., Liu, V., Wong, L. Y., Klumpp, D. J., & Thumbikat, P. (2008). The toll-like receptor pathway: A novel mechanism of infection-induced carcinogenesis of prostate epithelial cells. *The Prostate*, 68, 223–229.
- Kuroki, M., Miyamoto, S., Morisaki, T., Yotsumoto, F., Shirasu, N., Taniguchi, Y., & Soma, G. (2012). Biological response modifiers used in cancer biotherapy. *Anticancer Research*, 32, 2229–2233.
- Kurreck, J., Wyszko, E., Gillen, C., & Erdmann, V. A. (2002). Design of antisense oligonucleotides stabilized by locked nucleic acids. *Nucleic Acids Research*, 30, 1911–1918.
- Kwissa, M., Nakaya, H. I., Oluoch, H., & Pulendran, B. (2012). Distinct TLR adjuvants differentially stimulate systemic and local innate immune responses in nonhuman primates. *Blood*, 119, 2044–2055.
- Lampron, A., Elali, A., & Rivest, S. (2013). Innate immunity in the CNS: Redefining the relationship between the CNS and its environment. *Neuron*, 78, 214–232.
- Lasek, W., Zagodzón, R., & Jakobsiak, M. (2014). Interleukin 12: Still a promising candidate for tumor immunotherapy? *Cancer Immunology, Immunotherapy: CII*, 63, 419–435.
- Lee, Y. S., & Dutta, A. (2009). MicroRNAs in cancer. *Annual Review of Pathology*, 4, 199–227.
- Lee, S., & Margolin, K. (2011). Cytokines in cancer immunotherapy. *Cancers*, 3, 3856–3893.
- Lerner, M., Harada, M., Loven, J., Castro, J., Davis, Z., Oscier, D., Henriksson, M., Sangfelt, O., Grandér, D., & Corcoran, M. M. (2009). DLEU2, frequently deleted in malignancy, functions as a critical host gene of the cell cycle inhibitory microRNAs miR-15a and miR-16-1. *Experimental Cell Research*, 315, 2941–2952.
- Li, Y. P., Gottwein, J. M., Scheel, T. K., Jensen, T. B., & Bukh, J. (2011). MicroRNA-122 antagonism against hepatitis C virus genotypes 1-6 and reduced efficacy by host RNA insertion or mutations in the HCV 5' UTR. *Proceedings of the National Academy of Sciences of the United States of America*, 108, 4991–4996.
- Li, J., Mo, H. Y., Xiong, G., Zhang, L., He, J., Huang, Z. F., Liu, Z. W., Chen, Q. Y., Du, Z. M., Zheng, L. M., et al. (2012a). Tumor microenvironment macrophage inhibitory factor directs the accumulation of interleukin-17-producing tumor-infiltrating lymphocytes and predicts favorable survival in nasopharyngeal carcinoma patients. *Journal of Biological Chemistry*, 287, 35484–35495.
- Li, Y., Wang, L., Pappan, L., Galliher-Beckley, A., & Shi, J. (2012b). IL-1 β promotes stemness and invasiveness of colon cancer cells through Zeb1 activation. *Molecular Cancer*, 11, 87.
- Li, L., Zhang, J., Diao, W., Wang, D., Wei, Y., Zhang, C. Y., & Zen, K. (2014a). MicroRNA-155 and MicroRNA-21 promote the expansion of functional myeloid-derived suppressor cells. *Journal of Immunology (Baltimore, Md.: 1950)*, 192, 1034–1043.
- Li, Z., Chao, T. C., Chang, K. Y., Lin, N., Patil, V. S., Shimizu, C., Head, S. R., Burns, J. C., & Rana, T. M. (2014b). The long noncoding RNA THRIL regulates TNF α expression through its interaction with hnRNPL. *Proceedings of the National Academy of Sciences of the United States of America*, 111, 1002–1007.
- Liang, X., De Vera, M. E., Buchser, W. J., Romo de Vivar Chavez, A., Loughran, P., Beer Stolz, D., Basse, P., Wang, T., Van Houten, B., Zeh, H. J., 3rd, et al. (2012). Inhibiting systemic autophagy during interleukin 2 immunotherapy promotes long-term tumor regression. *Cancer Research*, 72, 2791–2801.
- Lin, R., Chen, L., Chen, G., Hu, C., Jiang, S., Sevilla, J., Wan, Y., Sampson, J. H., Zhu, B., & Li, Q. J. (2014). Targeting miR-23a in CD8 $^{+}$ cytotoxic T lymphocytes prevents tumor-dependent immunosuppression. *Journal of Clinical Investigation*, 124, 5352–5367.

- Lindsay, M. A. (2008). microRNAs and the immune response. *Trends in Immunology*, 29, 343–351.
- Liu, H., Komai-Koma, M., Xu, D., & Liew, F. Y. (2006). Toll-like receptor 2 signaling modulates the functions of CD4+ CD25+ regulatory T cells. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 7048–7053.
- Liu, Y., Lai, L., Chen, Q., Song, Y., Xu, S., Ma, F., Wang, X., Wang, J., Yu, H., Cao, X., et al. (2012). MicroRNA-494 is required for the accumulation and functions of tumor-expanded myeloid-derived suppressor cells via targeting of PTEN. *Journal of Immunology (Baltimore, Md.: 1950)*, 188, 5500–5510.
- Liu, R., Gu, J., Jiang, P., Zheng, Y., Liu, X., Jiang, X., Huang, E., Xiong, S., Xu, F., Liu, G., et al. (2015). DNMT1-microRNA126 epigenetic circuit contributes to esophageal squamous cell carcinoma growth via ADAM9-EGFR-AKT signaling. *Clinical Cancer Research: Official Journal of the American Association for Cancer Research*, 21, 854–863.
- Louveau, A., Smirnov, I., Keyes, T. J., Eccles, J. D., Rouhani, S. J., Peske, J. D., Derecki, N. C., Castle, D., Mandell, J. W., Lee, K. S., et al. (2015). Structural and functional features of central nervous system lymphatic vessels. *Nature*, 523, 337–341.
- Lowther, D. E., & Hafler, D. A. (2012). Regulatory T cells in the central nervous system. *Immunological Reviews*, 248, 156–169.
- Lu, H., Yang, Y., Gad, E., Wenner, C. A., Chang, A., Larson, E. R., Dang, Y., Martzen, M., Standish, L. J., & Disis, M. L. (2011). Polysaccharide krestin is a novel TLR2 agonist that mediates inhibition of tumor growth via stimulation of CD8 T cells and NK cells. *Clinical Cancer Research: Official Journal of the American Association for Cancer Research*, 17, 67–76.
- Luo, J. L., Maeda, S., Hsu, L. C., Yagita, H., & Karin, M. (2004). Inhibition of NF-kappaB in cancer cells converts inflammation- induced tumor growth mediated by TNFalpha to TRAIL-mediated tumor regression. *Cancer Cell*, 6, 297–305.
- Lv, H., Zhang, S., Wang, B., Cui, S., & Yan, J. (2006). Toxicity of cationic lipids and cationic polymers in gene delivery. *Journal of Controlled Release: Official Journal of the Controlled Release Society*, 114, 100–109.
- Maiorano, N. A., & Mallamaci, A. (2009). Promotion of embryonic cortico-cerebral neuronogenesis by miR-124. *Neural Development*, 4, 40.
- Majid, S., Dar, A. A., Saini, S., Yamamura, S., Hirata, H., Tanaka, Y., Deng, G., & Dahiya, R. (2010). MicroRNA-205-directed transcriptional activation of tumor suppressor genes in prostate cancer. *Cancer*, 116, 5637–5649.
- Manegold, C., van Zandwijk, N., Szczesna, A., Zatloukal, P., Au, J. S., Blasinska-Morawiec, M., Serwatowski, P., Krzakowski, M., Jassem, J., Tan, E. H., et al. (2012). A phase III randomized study of gemcitabine and cisplatin with or without PF-3512676 (TLR9 agonist) as first-line treatment of advanced non-small-cell lung cancer. *Annals of Oncology: Official Journal European Society Medical Oncology/ESMO*, 23, 72–77.
- Mantovani, A., & Locati, M. (2013). Tumor-associated macrophages as a paradigm of macrophage plasticity, diversity, and polarization: Lessons and open questions. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 33, 1478–1483.
- Marshall, N. A., Galvin, K. C., Corcoran, A. M., Boon, L., Higgs, R., & Mills, K. H. (2012). Immunotherapy with PI3K inhibitor and Toll-like receptor agonist induces IFN-gamma+IL-17 + polyfunctional T cells that mediate rejection of murine tumors. *Cancer Research*, 72, 581–591.
- McCarron, M., & Reen, D. J. (2009). Activated human neonatal CD8+ T cells are subject to immunomodulation by direct TLR2 or TLR5 stimulation. *Journal of Immunology (Baltimore, Md.: 1950)*, 182, 55–62.
- Medzhitov, R., Preston-Hurlburt, P., Kopp, E., Stadlen, A., Chen, C., Ghosh, S., & Janeway, C. A., Jr. (1998). MyD88 is an adaptor protein in the hToll/IL-1 receptor family signaling pathways. *Molecular Cell*, 2, 253–258.

- Mellor, A. L., Baban, B., Chandler, P. R., Manlapat, A., Kahler, D. J., & Munn, D. H. (2005). Cutting edge: CpG oligonucleotides induce splenic CD19+ dendritic cells to acquire potent indoleamine 2,3-dioxygenase-dependent T cell regulatory functions via IFN Type 1 signaling. *Journal of Immunology (Baltimore, Md.: 1950)*, 175, 5601–5605.
- Metzler, M., Wilda, M., Busch, K., Viehmann, S., & Borkhardt, A. (2004). High expression of precursor microRNA-155/BIC RNA in children with Burkitt lymphoma. *Genes, Chromosomes & Cancer*, 39, 167–169.
- Meyer, T., Surber, C., French, L. E., & Stockfleth, E. (2013). Resiquimod, a topical drug for viral skin lesions and skin cancer. *Expert Opinion on Investigational Drugs*, 22, 149–159.
- Miao, J. W., Liu, L. J., & Huang, J. (2014). Interleukin-6-induced epithelial-mesenchymal transition through signal transducer and activator of transcription 3 in human cervical carcinoma. *International Journal of Oncology*, 45, 165–176.
- Micali, G., Lacarrubba, F., Nasca, M. R., Ferraro, S., & Schwartz, R. A. (2014). Topical pharmacotherapy for skin cancer: Part II. Clinical applications. *Journal of the American Academy of Dermatology*, 70, 979. e971-912; quiz 9912; quiz 9912.
- Miller, R. P., Tadagavadi, R. K., Ramesh, G., & Reeves, W. B. (2010). Mechanisms of cisplatin nephrotoxicity. *Toxins*, 2, 2490–2518.
- Min, S., Liang, X., Zhang, M., Zhang, Y., Mei, S., Liu, J., Liu, J., Su, X., Cao, S., Zhong, X., et al. (2013). Multiple tumor-associated microRNAs modulate the survival and longevity of dendritic cells by targeting YWHAZ and Bcl2 signaling pathways. *Journal of Immunology*, 190, 2437–2446.
- Misso, G., Di Martino, M. T., De Rosa, G., Farooqi, A. A., Lombardi, A., Campani, V., Zarone, M. R., Gulla, A., Tagliaferri, P., Tassone, P., et al. (2014). Mir-34: A new weapon against cancer? *Molecular Therapy Nucleic Acids*, 3, e194.
- Muthuswamy, R., Wang, L., Pitteroff, J., Gingrich, J. R., & Kalinski, P. (2015). Combination of IFN α and poly-I:C reprograms bladder cancer microenvironment for enhanced CTL attraction. *Journal for Immunotherapy of Cancer*, 3, 6.
- Ngiow, S. F., Teng, M. W., & Smyth, M. J. (2013). A balance of interleukin-12 and -23 in cancer. *Trends in Immunology*, 34, 548–555.
- Nierkens, S., den Brok, M. H., Garcia, Z., Togher, S., Wagenaars, J., Wassink, M., Boon, L., Ruers, T. J., Figdor, C. G., Schoenberger, S. P., et al. (2011). Immune adjuvant efficacy of CpG oligonucleotide in cancer treatment is founded specifically upon TLR9 function in plasmacytoid dendritic cells. *Cancer Research*, 71, 6428–6437.
- Northfelt, D. W., Ramanathan, R. K., Cohen, P. A., Von Hoff, D. D., Weiss, G. J., Dietsch, G. N., Manjarrez, K. L., Randall, T. D., & Hershberg, R. M. (2014). A phase I dose-finding study of the novel toll-like receptor 8 agonist VTX-2337 in adult subjects with advanced solid tumors or lymphoma. *Clinical Cancer Research: Official Journal of the American Association for Cancer Research*, 20, 3683–3691.
- Okada, H., Kohanbash, G., & Lotze, M. T. (2010). MicroRNAs in immune regulation – opportunities for cancer immunotherapy. *International Journal of Biochemistry & Cell Biology*, 42, 1256–1261.
- Olson, J. K., & Miller, S. D. (2004). Microglia initiate central nervous system innate and adaptive immune responses through multiple TLRs. *Journal of Immunology*, 173, 3916–3924.
- Ousman, S. S., & Kubes, P. (2012). Immune surveillance in the central nervous system. *Nature Neuroscience*, 15, 1096–1101.
- Pagani, M., Rossetti, G., Panzeri, I., de Candia, P., Bonnal, R. J., Rossi, R. L., Geginat, J., & Abbrignani, S. (2013). Role of microRNAs and long-non-coding RNAs in CD4(+) T-cell differentiation. *Immunological Reviews*, 253, 82–96.
- Palma, G., Barbieri, A., Bimonte, S., Palla, M., Zappavigna, S., Caraglia, M., Ascierto, P. A., Ciliberto, G., & Arra, C. (2013). Interleukin 18: Friend or foe in cancer. *Biochimica et Biophysica Acta*, 1836, 296–303.

- Pang, K. C., Dinger, M. E., Mercer, T. R., Malquori, L., Grimmond, S. M., Chen, W., & Mattick, J. S. (2009). Genome-wide identification of long noncoding RNAs in CD8+ T cells. *Journal of Immunology (Baltimore, Md.: 1950)*, 182, 7738–7748.
- Park, S. J., Cheon, E. J., Lee, M. H., & Kim, H. A. (2013). MicroRNA-127-5p regulates matrix metalloproteinase 13 expression and interleukin-1beta-induced catabolic effects in human chondrocytes. *Arthritis and Rheumatism*, 65, 3141–3152.
- Pashenkov, M., Goess, G., Wagner, C., Hormann, M., Jandl, T., Moser, A., Britten, C. M., Smolle, J., Koller, S., Mauch, C., et al. (2006). Phase II trial of a toll-like receptor 9-activating oligonucleotide in patients with metastatic melanoma. *Journal of Controlled Release: Official Journal of the Controlled Release Society*, 24, 5716–5724.
- Patel, S. A., Bhambra, U., Charalambous, M. P., David, R. M., Edwards, R. J., Lightfoot, T., Boobis, A. R., & Gooderham, N. J. (2014). Interleukin-6 mediated upregulation of CYP1B1 and CYP2E1 in colorectal cancer involves DNA methylation, miR27b and STAT3. *British Journal of Cancer*, 111, 2287–2296.
- Peng, G., Guo, Z., Kiniwa, Y., Voo, K. S., Peng, W., Fu, T., Wang, D. Y., Li, Y., Wang, H. Y., & Wang, R. F. (2005). Toll-like receptor 8-mediated reversal of CD4+ regulatory T cell function. *Science (New York, NY)*, 309, 1380–1384.
- Peschansky, V. J., & Wahlestedt, C. (2014). Non-coding RNAs as direct and indirect modulators of epigenetic regulation. *Epigenetics: Official Journal of the DNA Methylation Society*, 9, 3–12.
- Peters, U., Jiao, S., Schumacher, F. R., Hutter, C. M., Aragaki, A. K., Baron, J. A., Berndt, S. I., Bezieau, S., Brenner, H., Butterbach, K., et al. (2013). Identification of genetic susceptibility loci for colorectal tumors in a genome-wide meta-analysis. *Gastroenterology*, 144, 799–807. e724.
- Petrella, T., Quirt, I., Verma, S., Haynes, A. E., Charette, M., Bak, K., & Melanoma Disease Site Group of Cancer Care Ontario's Program in Evidence-based, C. (2007). Single-agent interleukin-2 in the treatment of metastatic melanoma: A systematic review. *Cancer Treatment Reviews*, 33, 484–496.
- Pille, J. Y., Li, H., Blot, E., Bertrand, J. R., Pritchard, L. L., Opolon, P., Maksimenko, A., Lu, H., Vannier, J. P., Soria, J., et al. (2006). Intravenous delivery of anti-RhoA small interfering RNA loaded in nanoparticles of chitosan in mice: Safety and efficacy in xenografted aggressive breast cancer. *Human Gene Therapy*, 17, 1019–1026.
- Ponting, C. P., Oliver, P. L., & Reik, W. (2009). Evolution and functions of long noncoding RNAs. *Cell*, 136, 629–641.
- Preusser, M., de Ribaupierre, S., Wohrer, A., Erridge, S. C., Hegi, M., Weller, M., & Stupp, R. (2011). Current concepts and management of glioblastoma. *Annals of Neurology*, 70, 9–21.
- Prins, R. M., Craft, N., Bruhn, K. W., Khan-Farooqi, H., Koya, R. C., Stripecke, R., Miller, J. F., & Liao, L. M. (2006). The TLR-7 agonist, imiquimod, enhances dendritic cell survival and promotes tumor antigen-specific T cell priming: Relation to central nervous system antitumor immunity. *Journal of Immunology*, 176, 157–164.
- Prins, R. M., Soto, H., Konkankit, V., Odesa, S. K., Eskin, A., Yong, W. H., Nelson, S. F., & Liao, L. M. (2011). Gene expression profile correlates with T-cell infiltration and relative survival in glioblastoma patients vaccinated with dendritic cell immunotherapy. *Clinical Cancer Research: Official Journal of the American Association for Cancer Research*, 17, 1603–1615.
- Pucci, F., & Pittet, M. J. (2013). Molecular pathways: Tumor-derived microvesicles and their interactions with immune cells in vivo. *Clinical Cancer Research*, 19, 2598–2604.
- Qian, B. Z., & Pollard, J. W. (2010). Macrophage diversity enhances tumor progression and metastasis. *Cell*, 141, 39–51.
- Qian, X., Gu, L., Ning, H., Zhang, Y., Hsueh, E. C., Fu, M., Hu, X., Wei, L., Hoft, D. F., & Liu, J. (2013). Increased Th17 cells in the tumor microenvironment is mediated by IL-23 via tumor-secreted prostaglandin E2. *Journal of Immunology*, 190, 5894–5902.
- Rahman, A. H., Taylor, D. K., & Turka, L. A. (2009). The contribution of direct TLR signaling to T cell responses. *Immunologic Research*, 45, 25–36.

- Raisch, J., Darfeuille-Michaud, A., & Nguyen, H. T. (2013). Role of microRNAs in the immune system, inflammation and cancer. *World Journal of Gastroenterology*, *19*, 2985–2996.
- Ravindran, C., Cheng, Y. C., & Liang, S. M. (2010). CpG-ODNs induces up-regulated expression of chemokine CCL9 in mouse macrophages and microglia. *Cellular Immunology*, *260*, 113–118.
- Ren, Y., Kang, C. S., Yuan, X. B., Zhou, X., Xu, P., Han, L., Wang, G. X., Jia, Z., Zhong, Y., Yu, S., et al. (2010a). Co-delivery of as-miR-21 and 5-FU by poly(amidoamine) dendrimer attenuates human glioma cell growth in vitro. *Journal of Biomaterials Science Polymer Edition*, *21*, 303–314.
- Ren, Y., Zhou, X., Mei, M., Yuan, X. B., Han, L., Wang, G. X., Jia, Z. F., Xu, P., Pu, P. Y., & Kang, C. S. (2010b). MicroRNA-21 inhibitor sensitizes human glioblastoma cells U251 (PTEN-mutant) and LN229 (PTEN-wild type) to taxol. *BMC Cancer*, *10*, 27.
- Rhee, S. H., Im, E., & Pothoulakis, C. (2008). Toll-like receptor 5 engagement modulates tumor development and growth in a mouse xenograft model of human colon cancer. *Gastroenterology*, *135*, 518–528.
- Ridgway, D. (2003). The first 1000 dendritic cell vaccinees. *Cancer Investigation*, *21*, 873–886.
- Robertson, M. J., Kline, J., Struemper, H., Koch, K. M., Bauman, J. W., Gardner, O. S., Murray, S. C., Germaschewski, F., Weisenbach, J., Jonak, Z., et al. (2013). A dose-escalation study of recombinant human interleukin-18 in combination with rituximab in patients with non-Hodgkin lymphoma. *Journal of Immunotherapy (Hagerstown, Md: 1997)*, *36*, 331–341.
- Rosenfeld, M. R., Chamberlain, M. C., Grossman, S. A., Peereboom, D. M., Lesser, G. J., Batchelor, T. T., Desideri, S., Salazar, A. M., & Ye, X. (2010). A multi-institution phase II study of poly-ICLC and radiotherapy with concurrent and adjuvant temozolomide in adults with newly diagnosed glioblastoma. *Neuro-Oncology*, *12*, 1071–1077.
- Ruckerl, D., Jenkins, S. J., Laqtom, N. N., Gallagher, I. J., Sutherland, T. E., Duncan, S., Buck, A. H., & Allen, J. E. (2012). Induction of IL-4R α -dependent microRNAs identifies PI3K/Akt signaling as essential for IL-4-driven murine macrophage proliferation in vivo. *Blood*, *120*, 2307–2316.
- Rydberg, C., Mansson, A., Uddman, R., Riesbeck, K., & Cardell, L. O. (2009). Toll-like receptor agonists induce inflammation and cell death in a model of head and neck squamous cell carcinomas. *Immunology*, *128*, e600–e611.
- Sabado, R. L., Pavlick, A., Gnjatich, S., Cruz, C. M., Vengco, I., Hasan, F., Spadaccia, M., Darvishian, F., Chiriboga, L., Holman, R. M., et al. (2015). Resiquimod as an immunologic adjuvant for NY-ESO-1 protein vaccination in patients with high-risk melanoma. *Cancer Immunology Research*, *3*, 278–287.
- Sabbatini, P., Tsuji, T., Ferran, L., Ritter, E., Sedrak, C., Tuballes, K., Jungbluth, A. A., Ritter, G., Aghajanian, C., Bell-McGuinn, K., et al. (2012). Phase I trial of overlapping long peptides from a tumor self-antigen and poly-ICLC shows rapid induction of integrated immune response in ovarian cancer patients. *Clinical Cancer Research: Official Journal of the American Association for Cancer Research*, *18*, 6497–6508.
- Sahoo, A., & Im, S. H. (2010). Interleukin and interleukin receptor diversity: Role of alternative splicing. *International Reviews of Immunology*, *29*, 77–109.
- Sakaguchi, S. (2000). Regulatory T cells: Key controllers of immunologic self-tolerance. *Cell*, *101*, 455–458.
- Salaun, B., Coste, I., Rissoan, M. C., Lebecque, S. J., & Renno, T. (2006). TLR3 can directly trigger apoptosis in human cancer cells. *Journal of Immunology (Baltimore, Md.: 1950)*, *176*, 4894–4901.
- Saraiva, M., & O'Garra, A. (2010). The regulation of IL-10 production by immune cells. *Nature Reviews Immunology*, *10*, 170–181.
- Sauder, D. N., Smith, M. H., Senta-McMillan, T., Soria, I., & Meng, T. C. (2003). Randomized, single-blind, placebo-controlled study of topical application of the immune response modulator resiquimod in healthy adults. *Antimicrobial Agents and Chemotherapy*, *47*, 3846–3852.

- Scheel, B., Aulwurm, S., Probst, J., Stitz, L., Hoerr, I., Rammensee, H. G., Weller, M., & Pascolo, S. (2006). Therapeutic anti-tumor immunity triggered by injections of immunostimulating single-stranded RNA. *European Journal of Immunology*, 36, 2807–2816.
- Schmoll, H. J., Wittig, B., Arnold, D., Riera-Knorrenschild, J., Nitsche, D., Kroening, H., Mayer, F., Andel, J., Ziehermayr, R., & Scheithauer, W. (2014). Maintenance treatment with the immunomodulator MGN1703, a toll-like receptor 9 (TLR9) agonist, in patients with metastatic colorectal carcinoma and disease control after chemotherapy: A randomised, double-blind, placebo-controlled trial. *Journal of Cancer Research and Clinical Oncology*, 140, 1615–1624.
- Schulze, H. J., Cribier, B., Requena, L., Reifemberger, J., Ferrandiz, C., Garcia Diez, A., Tebbs, V., & McRae, S. (2005). Imiquimod 5% cream for the treatment of superficial basal cell carcinoma: Results from a randomized vehicle-controlled phase III study in Europe. *The British Journal of Dermatology*, 152, 939–947.
- Scomparin, A., Polyak, D., Krivitsky, A., & Satchi-Fainaro, R. (2015). Achieving successful delivery of oligonucleotides – From physico-chemical characterization to in vivo evaluation. *Biotechnology Advances*, 33, 1294–1309.
- Seif, A. E., Barrett, D. M., Milone, M., Brown, V. I., Grupp, S. A., & Reid, G. S. (2009). Long-term protection from syngeneic acute lymphoblastic leukemia by CpG ODN-mediated stimulation of innate and adaptive immune responses. *Blood*, 114, 2459–2466.
- Seki, E., Tsutsui, H., Tsuji, N. M., Hayashi, N., Adachi, K., Nakano, H., Futatsugi-Yumikura, S., Takeuchi, O., Hoshino, K., Akira, S., et al. (2002). Critical roles of myeloid differentiation factor 88-dependent proinflammatory cytokine release in early phase clearance of *Listeria monocytogenes* in mice. *Journal of Immunology (Baltimore, Md.: 1950)*, 169, 3863–3868.
- Serafini, P., Borrello, I., & Bronte, V. (2006). Myeloid suppressor cells in cancer: Recruitment, phenotype, properties, and mechanisms of immune suppression. *Seminars in Cancer Biology*, 16, 53–65.
- Shalapour, S., Font-Burgada, J., Di Caro, G., Zhong, Z., Sanchez-Lopez, E., Dhar, D., Willmsky, G., Ammirante, M., Strasner, S., Hansel, D. E., et al. (2015). Immunosuppressive plasma cells impede T-cell-dependent immunogenic chemotherapy. *Nature*, 521, 94–98.
- Sharma, S., Findlay, G. M., Bandukwala, H. S., Oberdoerffer, S., Baust, B., Li, Z., Schmidt, V., Hogan, P. G., Sacks, D. B., & Rao, A. (2011). Dephosphorylation of the nuclear factor of activated T cells (NFAT) transcription factor is regulated by an RNA-protein scaffold complex. *Proceedings of the National Academy of Sciences of the United States of America*, 108, 11381–11386.
- Sheedy, F. J., Palsson-McDermott, E., Hennessy, E. J., Martin, C., O’Leary, J. J., Ruan, Q., Johnson, D. S., Chen, Y., & O’Neill, L. A. (2010). Negative regulation of TLR4 via targeting of the proinflammatory tumor suppressor PDCD4 by the microRNA miR-21. *Nature Immunology*, 11, 141–147.
- Shen, H., Tesar, B. M., Walker, W. E., & Goldstein, D. R. (2008). Dual signaling of MyD88 and TRIF is critical for maximal TLR4-induced dendritic cell maturation. *Journal of Immunology*, 181, 1849–1858.
- Shi, M., Yao, Y., Han, F., Li, Y., & Li, Y. (2014). MAP1S controls breast cancer cell TLR5 signaling pathway and promotes TLR5 signaling-based tumor suppression. *PLoS One*, 9, e86839.
- Shimizu, T., Yokota, S., Takahashi, S., Kunishima, Y., Takeyama, K., Masumori, N., Takahashi, A., Matsukawa, M., Itoh, N., Tsukamoto, T., et al. (2004). Membrane-anchored CD14 is important for induction of interleukin-8 by lipopolysaccharide and peptidoglycan in uroepithelial cells. *Clinical and Diagnostic Laboratory Immunology*, 11, 969–976.
- Sica, A., & Bronte, V. (2007). Altered macrophage differentiation and immune dysfunction in tumor development. *Journal of Clinical Investigation*, 117, 1155–1166.
- Siegel, F. P., Kadowaki, N., Shodell, M., Fitzgerald-Bocarsly, P. A., Shah, K., Ho, S., Antonenko, S., & Liu, Y. J. (1999). The nature of the principal type 1 interferon-producing cells in human blood. *Science (New York, NY)*, 284, 1835–1837.

- Sim, G. C., & Radvanyi, L. (2014). The IL-2 cytokine family in cancer immunotherapy. *Cytokine & Growth Factor Reviews*, 25, 377–390.
- Slaby, O., Svoboda, M., Fabian, P., Smerdova, T., Knoflickova, D., Bednarikova, M., Nenutil, R., & Vyzula, R. (2007). Altered expression of miR-21, miR-31, miR-143 and miR-145 is related to clinicopathologic features of colorectal cancer. *Oncology*, 72, 397–402.
- Smit, E., Oberholzer, H. M., & Pretorius, E. (2009). A review of immunomodulators with reference to Canova. *Homeopathy*, 98, 169–176.
- Smith, E. B., Schwartz, M., Kawamoto, H., You, X., Hwang, D., Liu, H., & Scherr, D. S. (2007). Antitumor effects of imidazoquinolines in urothelial cell carcinoma of the bladder. *The Journal of Urology*, 177, 2347–2351.
- Smyth, M. J., Cretney, E., Kershaw, M. H., & Hayakawa, Y. (2004). Cytokines in cancer immunity and immunotherapy. *Immunological Reviews*, 202, 275–293.
- Sobek, V., Birkner, N., Falk, I., Wurch, A., Kirschning, C. J., Wagner, H., Wallich, R., Lamers, M. C., & Simon, M. M. (2004). Direct Toll-like receptor 2 mediated co-stimulation of T cells in the mouse system as a basis for chronic inflammatory joint disease. *Arthritis Research & Therapy*, 6, R433–R446.
- Song, D. H., & Lee, J. O. (2012). Sensing of microbial molecular patterns by Toll-like receptors. *Immunological Reviews*, 250, 216–229.
- Spinetti, G., Fortunato, O., Caporali, A., Shantikumar, S., Marchetti, M., Meloni, M., Descamps, B., Floris, I., Sangalli, E., Vono, R., et al. (2013). MicroRNA-15a and microRNA-16 impair human circulating proangiogenic cell functions and are increased in the proangiogenic cells and serum of patients with critical limb ischemia. *Circulation Research*, 112, 335–346.
- Sportes, C., Babb, R. R., Krumlauf, M. C., Hakim, F. T., Steinberg, S. M., Chow, C. K., Brown, M. R., Fleisher, T. A., Noel, P., Maric, I., et al. (2010). Phase I study of recombinant human interleukin-7 administration in subjects with refractory malignancy. *Clinical Cancer Research: Official Journal of the American Association for Cancer Research*, 16, 727–735.
- Spranger, S., Javorovic, M., Burdek, M., Wilde, S., Mosetter, B., Tippmer, S., Bigalke, I., Geiger, C., Schendel, D. J., & Frankenberger, B. (2010). Generation of Th1-polarizing dendritic cells using the TLR7/8 agonist CL075. *Journal of Immunology*, 185, 738–747.
- Squadruto, M. L., & De Palma, M. (2011). Macrophage regulation of tumor angiogenesis: Implications for cancer therapy. *Molecular Aspects of Medicine*, 32, 123–145.
- Squadruto, M. L., Pucci, F., Magri, L., Moi, D., Gilfillan, G. D., Ranghetti, A., Casazza, A., Mazzone, M., Lyle, R., Naldini, L., et al. (2012). miR-511-3p modulates genetic programs of tumor-associated macrophages. *Cell Reports*, 1, 141–154.
- Squadruto, M. L., Etzrodt, M., De Palma, M., & Pittet, M. J. (2013). MicroRNA-mediated control of macrophages and its implications for cancer. *Trends in Immunology*, 34, 350–359.
- Steinestel, K., Eder, S., Schrader, A. J., & Steinestel, J. (2014). Clinical significance of epithelial-mesenchymal transition. *Clinical and Translational Medicine*, 3, 17.
- Steinman, R. M., & Cohn, Z. A. (1973). Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. *The Journal of Experimental Medicine*, 137, 1142–1162.
- Sutmoller, R. P., den Brok, M. H., Kramer, M., Bennink, E. J., Toonen, L. W., Kullberg, B. J., Joosten, L. A., Akira, S., Netea, M. G., & Adema, G. J. (2006). Toll-like receptor 2 controls expansion and function of regulatory T cells. *The Journal of Clinical Investigation*, 116, 485–494.
- Suzuki, A., Leland, P., Joshi, B. H., & Puri, R. K. (2015). Targeting of IL-4 and IL-13 receptors for cancer therapy. *Cytokine*, 75, 79–88.
- Szczepanski, M. J., Czystowska, M., Szajnik, M., Harasymczuk, M., Boyiadzis, M., Kruk-Zagajewska, A., Szyfter, W., Zeromski, J., & Whiteside, T. L. (2009). Triggering of Toll-like receptor 4 expressed on human head and neck squamous cell carcinoma promotes tumor development and protects the tumor from immune attack. *Cancer Research*, 69, 3105–3113.

- Szeimies, R. M., Bichel, J., Ortonne, J. P., Stockfleth, E., Lee, J., & Meng, T. C. (2008). A phase II dose-ranging study of topical resiquimod to treat actinic keratosis. *The British Journal of Dermatology*, 159, 205–210.
- Taganov, K. D., Boldin, M. P., Chang, K. J., & Baltimore, D. (2006). NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 12481–12486.
- Tanaka, S., Louis, D. N., Curry, W. T., Batchelor, T. T., & Dietrich, J. (2013). Diagnostic and therapeutic avenues for glioblastoma: No longer a dead end? *Nature Reviews. Clinical Oncology*, 10, 14–26.
- Taylor, M. W., & Feng, G. S. (1991). Relationship between interferon-gamma, indoleamine 2,3-dioxygenase, and tryptophan catabolism. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology*, 5, 2516–2522.
- Tekpli, X., Landvik, N. E., Anmarkud, K. H., Skaug, V., Haugen, A., & Zienoldind, S. (2013). DNA methylation at promoter regions of interleukin 1B, interleukin 6, and interleukin 8 in non-small cell lung cancer. *Cancer Immunology, Immunotherapy*, 62, 337–345.
- Thompson, J. A., Kuzel, T., Drucker, B. J., Urba, W. J., & Bukowski, R. M. (2009). Safety and efficacy of PF-3512676 for the treatment of stage IV renal cell carcinoma: An open-label, multicenter phase I/II study. *Clinical Genitourinary Cancer*, 7, E58–E65.
- Thorne, S. H. (2013). The role of GM-CSF in enhancing immunotherapy of cancer. *Immunotherapy*, 5, 817–819.
- Tomai, M. A., Miller, R. L., Lipson, K. E., Kieper, W. C., Zarraga, I. E., & Vasilakos, J. P. (2007). Resiquimod and other immune response modifiers as vaccine adjuvants. *Expert Review of Vaccines*, 6, 835–847.
- Trang, P., Wiggins, J. F., Daige, C. L., Cho, C., Omotola, M., Brown, D., Weidhaas, J. B., Bader, A. G., & Slack, F. J. (2011). Systemic delivery of tumor suppressor microRNA mimics using a neutral lipid emulsion inhibits lung tumors in mice. *Molecular Therapy: The Journal of the American Society of Gene Therapy*, 19, 1116–1122.
- Tremblay, M. M., Bilal, M. Y., & Houtman, J. C. (2014). Prior TLR5 induction in human T cells results in a transient potentiation of subsequent TCR-induced cytokine production. *Molecular Immunology*, 57, 161–170.
- Trepiakas, R., Pedersen, A. E., Met, O., & Svane, I. M. (2009). Addition of interferon-alpha to a standard maturation cocktail induces CD38 up-regulation and increases dendritic cell function. *Vaccine*, 27, 2213–2219.
- Ueda, R., Kohanbash, G., Sasaki, K., Fujita, M., Zhu, X., Kastenhuber, E. R., McDonald, H. A., Potter, D. M., Hamilton, R. L., Lotze, M. T., et al. (2009). Dicer-regulated microRNAs 222 and 339 promote resistance of cancer cells to cytotoxic T-lymphocytes by down-regulation of ICAM-1. *Proceedings of the National Academy of Sciences of the United States of America*, 106, 10746–10751.
- Vacchelli, E., Eggermont, A., Sautes-Fridman, C., Galon, J., Zitvogel, L., Kroemer, G., & Galluzzi, L. (2013). Trial watch: Toll-like receptor agonists for cancer therapy. *Oncoimmunology*, 2, e25238.
- Volinia, S., Calin, G. A., Liu, C. G., Ambs, S., Cimmino, A., Petrocca, F., Visone, R., Iorio, M., Roldo, C., Ferracin, M., et al. (2006). A microRNA expression signature of human solid tumors defines cancer gene targets. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 2257–2261.
- Voronov, E., Carmi, Y., & Apte, R. N. (2014). The role IL-1 in tumor-mediated angiogenesis. *Frontiers in Physiology*, 5, 114.
- Wang, J. H., Manning, B. J., Wu, Q. D., Blankson, S., Bouchier-Hayes, D., & Redmond, H. P. (2003). Endotoxin/lipopolysaccharide activates NF-kappa B and enhances tumor cell adhesion and invasion through a beta 1 integrin-dependent mechanism. *Journal of Immunology (Baltimore, Md.: 1950)*, 170, 795–804.

- Wang, P., Xue, Y., Han, Y., Lin, L., Wu, C., Xu, S., Jiang, Z., Xu, J., Liu, Q., & Cao, X. (2014). The STAT3-binding long noncoding RNA lnc-DC controls human dendritic cell differentiation. *Science (New York, NY)*, *344*, 310–313.
- Wargo, J. A., Reuben, A., Cooper, Z. A., Oh, K. S., & Sullivan, R. J. (2015). Immune effects of chemotherapy, radiation, and targeted therapy and opportunities for combination with immunotherapy. *Seminars in Oncology*, *42*, 601–616.
- Weichhart, T., Haidinger, M., Katholnig, K., Kopecky, C., Poglitsch, M., Lassnig, C., Rosner, M., Zlabinger, G. J., Hengstschlager, M., Muller, M., et al. (2011). Inhibition of mTOR blocks the anti-inflammatory effects of glucocorticoids in myeloid immune cells. *Blood*, *117*, 4273–4283.
- Weihrauch, M. R., Ansen, S., Jurkiewicz, E., Geisen, C., Xia, Z., Anderson, K. S., Gracien, E., Schmidt, M., Wittig, B., Diehl, V., et al. (2005). Phase I/II combined chemoimmunotherapy with carcinoembryonic antigen-derived HLA-A2-restricted CAP-1 peptide and irinotecan, 5-fluorouracil, and leucovorin in patients with primary metastatic colorectal cancer. *Clinical Cancer Research: Official Journal of the American Association for Cancer Research*, *11*, 5993–6001.
- Wheeler, C. J., Black, K. L., Liu, G., Mazer, M., Zhang, X. X., Pepkowitz, S., Goldfinger, D., Ng, H., Irvin, D., & Yu, J. S. (2008). Vaccination elicits correlated immune and clinical responses in glioblastoma multiforme patients. *Cancer Research*, *68*, 5955–5964.
- Willingham, A. T., Orth, A. P., Batalov, S., Peters, E. C., Wen, B. G., Aza-Blanc, P., Hogenesch, J. B., & Schultz, P. G. (2005). A strategy for probing the function of noncoding RNAs finds a repressor of NFAT. *Science (New York, NY)*, *309*, 1570–1573.
- Wingender, G., Garbi, N., Schumak, B., Jungerkes, F., Endl, E., von Bubnoff, D., Steitz, J., Striegler, J., Moldenhauer, G., Tuting, T., et al. (2006). Systemic application of CpG-rich DNA suppresses adaptive T cell immunity via induction of IDO. *European Journal of Immunology*, *36*, 12–20.
- Wittig, B., Schmidt, M., Scheithauer, W., & Schmoll, H. J. (2015). MGN1703, an immunomodulator and toll-like receptor 9 (TLR-9) agonist: From bench to bedside. *Critical Reviews in Oncology/Hematology*, *94*, 31–44.
- Wu, A., Oh, S., Gharagozlou, S., VEDI, R. N., Ericson, K., Low, W. C., Chen, W., & Ohlfest, J. R. (2007). In vivo vaccination with tumor cell lysate plus CpG oligodeoxynucleotides eradicates murine glioblastoma. *Journal of Immunotherapy*, *30*, 789–797.
- Wu, M. F., Yang, J., Xiang, T., Shi, Y. Y., & Liu, L. J. (2014). miR-21 targets Fas ligand-mediated apoptosis in breast cancer cell line MCF-7. *Journal of Huazhong University of Science and Technology. Medical Sciences*, *34*, 190–194.
- Xiang, T., Long, H., He, L., Han, X., Lin, K., Liang, Z., Zhuo, W., Xie, R., & Zhu, B. (2015). Interleukin-17 produced by tumor microenvironment promotes self-renewal of CD133+ cancer stem-like cells in ovarian cancer. *Oncogene*, *34*, 165–176.
- Xie, G., Yao, Q., Liu, Y., Du, S., Liu, A., Guo, Z., Sun, A., Ruan, J., Chen, L., Ye, C., et al. (2012). IL-6-induced epithelial-mesenchymal transition promotes the generation of breast cancer stem-like cells analogous to mammosphere cultures. *International Journal of Oncology*, *40*, 1171–1179.
- Xiong, Z., & Ohlfest, J. R. (2011). Topical imiquimod has therapeutic and immunomodulatory effects against intracranial tumors. *Journal of Immunotherapy*, *34*, 264–269.
- Xu, B., Bai, B., Sha, S., Yu, P., An, Y., Wang, S., Kong, X., Liu, C., Wei, N., Feng, Q., et al. (2014). Interleukin-1 β induces autophagy by affecting calcium homeostasis and trypsinogen activation in pancreatic acinar cells. *International Journal of Clinical and Experimental Pathology*, *7*, 3620–3631.
- Yanaihara, N., Caplen, N., Bowman, E., Seike, M., Kumamoto, K., Yi, M., Stephens, R. M., Okamoto, A., Yokota, J., Tanaka, T., et al. (2006). Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. *Cancer Cell*, *9*, 189–198.
- Yang, J. C., Topalian, S. L., Parkinson, D., Schwartzentruber, D. J., Weber, J. S., Ettinghausen, S. E., White, D. E., Steinberg, S. M., Cole, D. J., Kim, H. I., et al. (1994). Randomized comparison of high-dose and low-dose intravenous interleukin-2 for the therapy of metastatic

- renal cell carcinoma: An interim report. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*, 12, 1572–1576.
- Yang, Y. P., Chien, Y., Chiou, G. Y., Cherng, J. Y., Wang, M. L., Lo, W. L., Chang, Y. L., Huang, P. I., Chen, Y. W., Shih, Y. H., et al. (2012). Inhibition of cancer stem cell-like properties and reduced chemoradioresistance of glioblastoma using microRNA145 with cationic polyurethane-short branch PEI. *Biomaterials*, 33, 1462–1476.
- Yee, C., Thompson, J. A., Byrd, D., Riddell, S. R., Roche, P., Celis, E., & Greenberg, P. D. (2002). Adoptive T cell therapy using antigen-specific CD8+ T cell clones for the treatment of patients with metastatic melanoma: In vivo persistence, migration, and antitumor effect of transferred T cells. *Proceedings of the National Academy of Sciences of the United States of America*, 99, 16168–16173.
- Yeung, Y. T., McDonald, K. L., Grewal, T., & Munoz, L. (2013). Interleukins in glioblastoma pathophysiology: Implications for therapy. *British Journal of Pharmacology*, 168, 591–606.
- Yoneda, K., Sugimoto, K., Shiraki, K., Tanaka, J., Beppu, T., Fuke, H., Yamamoto, N., Masuya, M., Horie, R., Uchida, K., et al. (2008). Dual topology of functional toll-like receptor 3 expression in human hepatocellular carcinoma: Differential signaling mechanisms of TLR3-induced NF-kappaB activation and apoptosis. *International Journal of Oncology*, 33, 929–936.
- Zarate, R., Boni, V., Bandres, E., & Garcia-Foncillas, J. (2012). MiRNAs and LincRNAs: Could they be considered as biomarkers in colorectal cancer? *International Journal of Molecular Sciences*, 13, 840–865.
- Zarogoulidis, P., Lampaki, S., Yarmus, L., Kioumis, I., Pitsiou, G., Katsikogiannis, N., Hohenforst-Schmidt, W., Li, Q., Huang, H., Sakkas, A., et al. (2014). Interleukin-7 and interleukin-15 for cancer. *Journal of Cancer*, 5, 765–773.
- Zent, C. S., Smith, B. J., Ballas, Z. K., Wooldridge, J. E., Link, B. K., Call, T. G., Shanafelt, T. D., Bowen, D. A., Kay, N. E., Witzig, T. E., et al. (2012). Phase I clinical trial of CpG oligonucleotide 7909 (PF-03512676) in patients with previously treated chronic lymphocytic leukemia. *Leukemia & Lymphoma*, 53, 211–217.
- Zhan, Z., Xie, X., Cao, H., Zhou, X., Zhang, X. D., Fan, H., & Liu, Z. (2014). Autophagy facilitates TLR4- and TLR3-triggered migration and invasion of lung cancer cells through the promotion of TRAF6 ubiquitination. *Autophagy*, 10, 257–268.
- Zhang, X., Gejman, R., Mahta, A., Zhong, Y., Rice, K. A., Zhou, Y., Cheunsuchon, P., Louis, D. N., & Klibanski, A. (2010). Maternally expressed gene 3, an imprinted noncoding RNA gene, is associated with meningioma pathogenesis and progression. *Cancer Research*, 70, 2350–2358.
- Zhang, M., Liu, Q., Mi, S., Liang, X., Zhang, Z., Su, X., Liu, J., Chen, Y., Wang, M., Zhang, Y., et al. (2011a). Both miR-17-5p and miR-20a alleviate suppressive potential of myeloid-derived suppressor cells by modulating STAT3 expression. *Journal of Immunology*, 186, 4716–4724.
- Zhang, Y., Luo, F., Cai, Y., Liu, N., Wang, L., Xu, D., & Chu, Y. (2011b). TLR1/TLR2 agonist induces tumor regression by reciprocal modulation of effector and regulatory T cells. *Journal of Immunology (Baltimore, Md.: 1950)*, 186, 1963–1969.
- Zhang, C., Bai, D. S., Huang, X. Y., Shi, G. M., Ke, A. W., Yang, L. X., Yang, X. R., Zhou, J., & Fan, J. (2013a). Prognostic significance of capn4 overexpression in intrahepatic cholangiocarcinoma. *PloS One*, 8, e54619.
- Zhang, Y., Wang, Z., & Gemeinhart, R. A. (2013b). Progress in microRNA delivery. *Journal of Controlled Release: Official Journal of the Controlled Release Society*, 172, 962–974.
- Zhang, Y., Lin, A., Sui, Q., Zhang, C., Tian, Z., & Zhang, J. (2014a). Phosphorothioate modification of the TLR9 ligand CpG ODN inhibits poly(I:C)-induced apoptosis of hepatocellular carcinoma by entry blockade. *Cancer Letters*, 355, 76–84.
- Zhang, Y., Luo, F., Li, A., Qian, J., Yao, Z., Feng, X., & Chu, Y. (2014b). Systemic injection of TLR1/2 agonist improves adoptive antigen-specific T cell therapy in glioma-bearing mice. *Clinical Immunology*, 154, 26–36.

- Zhao, N., Li, X., He, X., Qiu, Y., Zhu, L., & Qi, F. (2013). Interleukin-15 gene therapy and the mammalian target of rapamycin inhibitor everolimus inhibit the growth of metastatic breast cancer. *The Journal of Gene Medicine*, 15, 366–374.
- Zheng, Y., Xiong, S., Jiang, P., Liu, R., Liu, X., Qian, J., Zheng, X., & Chu, Y. (2012). Glucocorticoids inhibit lipopolysaccharide-mediated inflammatory response by downregulating microRNA-155: A novel anti-inflammation mechanism. *Free Radical Biology & Medicine*, 52, 1307–1317.
- Zhong, G., Cheng, X., Long, H., He, L., Qi, W., Xiang, T., Zhao, Z., & Zhu, B. (2013). Dynamically expressed microRNA-15b modulates the activities of CD8+ T lymphocytes in mice with Lewis lung carcinoma. *Journal of Translational Medicine*, 11, 71.
- Zhou, H., Hu, H., & Lai, M. (2010). Non-coding RNAs and their epigenetic regulatory mechanisms. *Biology of the Cell/Under the Auspices of the European Cell Biology Organization*, 102, 645–655.
- Zhu, X., Nishimura, F., Sasaki, K., Fujita, M., Dusak, J. E., Eguchi, J., Fellows-Mayle, W., Storkus, W. J., Walker, P. R., Salazar, A. M., et al. (2007). Toll like receptor-3 ligand poly-ICLC promotes the efficacy of peripheral vaccinations with tumor antigen-derived peptide epitopes in murine CNS tumor models. *Journal of Translational Medicine*, 5, 10.
- Zhuang, G., Meng, C., Guo, X., Cheruku, P. S., Shi, L., Xu, H., Li, H., Wang, G., Evans, A. R., Safe, S., et al. (2012). A novel regulator of macrophage activation: miR-223 in obesity-associated adipose tissue inflammation. *Circulation*, 125, 2892–2903.

Progress in Cancer Immunotherapy

Zhang, S. (Ed.)

2016, XII, 283 p. 15 illus., 14 illus. in color., Hardcover

ISBN: 978-94-017-7553-3