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## The immunological contribution of NF- $\kappa$ B within the tumor microenvironment: A potential protective role of zinc as an anti-tumor agent

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### Abstract

Over decades, cancer treatment has been mainly focused on targeting cancer cells and not much attention to host tumor microenvironment. Recent advances suggest that the tumor microenvironment requires in-depth investigation for understanding the interactions between tumor cell biology and immunobiology in order to optimize therapeutic approaches. Tumor microenvironment consists of cancer cells and tumor associated reactive fibroblasts, infiltrating non-cancer cells, secreted soluble factors or molecules, and non-cellular support materials. Tumor associated host immune cells such as Th<sub>1</sub>, Th<sub>2</sub>, Th17, regulatory cells, dendritic cells, macrophages, and myeloid-derived suppressor cells are major components of the tumor microenvironment. Accumulating evidence suggests that these tumor associated immune cells may play important roles in cancer development and progression. However, the exact functions of these cells in the tumor microenvironment are poorly understood. In the tumor microenvironment, NF- $\kappa$ B plays an important role in cancer development and progression because this is a major transcription factor which regulates immune functions within the tumor microenvironment. In this review, we will focus our discussion on the immunological contribution of NF- $\kappa$ B in tumor associated host immune cells within the tumor microenvironment. We will also discuss the potential protective role of zinc, a well-known immune response mediator, in the regulation of these immune cells and cancer cells in the tumor microenvironment especially because zinc could be useful for conditioning the tumor microenvironment toward innovative cancer therapy.

### Keywords

Tumor microenvironment; T helper cells; Tregs; MDSCs; DCs; TAMs

### 1. Introduction

Traditionally cancer therapy has been mainly focused on targeting the cancer cell itself. However, recent advances suggest that the microenvironment within or in the surrounding tissue area of the tumor, called tumor microenvironment, could play important role in cancer

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development and progression, and thus conditioning of the tumor microenvironment could be useful for designing optimal therapy to eradicate tumors. The tumor microenvironment consists of cancer cells, reactive fibroblasts and a wide range of surrounding non-cancer cells such as infiltrating immune cells, secreted soluble factors such as cytokines and cell growth signaling molecules, chemicals such as oxygen and nitrogen, and non-cellular support materials such as extracellular matrix (ECM) [1–6]. The relative contribution of each of these components varies between tumor types, between patients with the same type of tumor, and even between regions within of a tumor. The tumor microenvironment changes over the course of tumor development and progression, and changes in response to therapeutic interventions [7].

In the tumor microenvironment, the surrounding normal epithelial cells become benign tumor cells, which can be transformed to malignant cells. Furthermore, malignant cells develop into metastatic cells with invasive characteristics depending on the molecular signals between the cells and the surrounding area of tumor [8,9]. Therefore, the cancer cell behavior may depend on the microenvironment for its proliferation, progression, invasion and metastasis, suggesting that therapy targeted at the tumor microenvironment could be useful for designing innovative treatments to eradicate tumors. Such a strategy has recently been documented in an animal tumor model whereby the delivery and efficacy of gemcitabine was improved by conditioning the tumor microenvironment using hedgehog inhibitor [10]. Accumulating evidence clearly suggests that the tumor microenvironment plays a critical role in tumor initiation, progression, invasion, metastasis, as well as contributes to resistance to chemo-, radio-, and immuno-therapy. Both pro- and anti-tumor interactions between tumor cells and host surrounding cells may promote or inhibit tumor initiation and progression (Fig. 1). Therefore, targeting tumor microenvironment, in addition to tumor cells, would provide a novel therapeutic strategy for tumor treatment.

In this review, we focus our discussion on the role of host immune cells in the tumor microenvironment. Furthermore, our focus is centered on the role of NF- $\kappa$ B in the host immune cells within the tumor microenvironment and the role of zinc, a well-known immune mediator, for conditioning the tumor microenvironment.

## 2. Th<sub>1</sub> and Th<sub>2</sub> cells and its cytokines

Differentiation of functionally distinct subsets Th<sub>1</sub> and Th<sub>2</sub> of T helper cells plays an important role in tumor microenvironment, which could impact tumor initiation and progression. Differentiation of naïve CD4<sup>+</sup> T cells into Th<sub>1</sub> or Th<sub>2</sub> cells is an important and critical part of the immune response [11]. Th<sub>1</sub> cells produce IFN- $\gamma$  and IL-2, which activate CD8<sup>+</sup> T cells and natural killer (NK) cells and promote cellular immunity whereas Th<sub>2</sub> cells produce IL-4, IL-5, IL-10 and IL-13, and promote humoral immunity.

T helper cell differentiation is regulated at many levels. Interactions of peptide antigen with the T cell receptor (TCR), cytokine signaling, actions of co-stimulatory molecules and induction of key transcription factors are major players in determining the type of T helper differentiation. The classic cytokine used for *in vitro* differentiation of Th1 effector cells from naïve T cell precursors is IL-12 [12–14]. IL-12 also induces IFN- $\gamma$  production in NK and T cells. IL-12 has been reported to enhance the activity of NK and T cells. IL-12 with IFN- $\gamma$  antagonizes Th<sub>2</sub> differentiation and inhibits the production of IL-4, IL-5, and IL-13 [15–17]. The action of IL-12 is mediated through its receptor and IL-12 receptor (IL-12R), which consists of two  $\alpha$ -receptor subunits,  $\alpha_1$  and  $\alpha_2$ . Resting T cells do not express  $\alpha_1$  and  $\alpha_2$ . These receptors are induced upon T cell differentiation. Maintenance of the expression of IL-12R  $\alpha_2$  is required for normal Th<sub>1</sub> differentiation as well. Thus, the expression of IL-12R  $\alpha_2$  can be considered as a marker for Th<sub>1</sub> differentiation [18–20]. The major sources

of IL-12 are antigen-presenting cells (APCs) such as dendritic cells and macrophages, thereby establishing an important link between innate and adaptive immune responses.

Another key cytokine that regulates Th<sub>1</sub> differentiation is IFN- $\gamma$ . It is the “signature” cytokine of Th<sub>1</sub> effector cells and is important for the stabilization of the Th<sub>1</sub> phenotype itself. Differentiated Th<sub>1</sub> cells normally do not produce Th<sub>2</sub> cytokines. However, Th<sub>1</sub> cells from IFN- $\gamma$  deficient mice retain the ability to produce IL-4 if re-stimulated under Th<sub>2</sub> polarizing conditions [21]. IFN- $\gamma$  induces the transcription factor T-box protein expressed by T cells (T-bet) [22,23]. T-bet, in turn, promotes the expression of IL-12R $\beta$ 2 and IFN- $\gamma$  in CD4<sup>+</sup> T cells, thereby establishing a potential feedback loop. Transcription factor STAT4 is also involved in Th<sub>1</sub> polarization through regulation in the expression of IL-12R $\beta$ 2 and IFN- $\gamma$  gene in CD4<sup>+</sup> T cells. T-bet is now considered to be a major candidate as the Th<sub>1</sub> master gene. CD4<sup>+</sup> T cells from T-bet<sup>-/-</sup> mice have a severe defect in Th<sub>1</sub>-polarized responses [17,24], suggesting that T-bet is essential for Th<sub>1</sub> polarization.

Evidences suggest that Th<sub>1</sub> polarized response plays an important role in tumor surveillance. IFN- $\gamma$  has been shown to have an antitumor response in tumor immuno-surveillance [25]. Our recent study demonstrates that both human breast cancer cells SK-BR-3 (Her2/neu with epitheloid morphology) and MDA-MB-231 (a triple negative and highly invasive cell line) cultured in the presence of IFN- $\gamma$  and IL-2 cytokines exhibited smaller tumorspheres in 3D culture condition compared to larger tumorspheres in regular medium without IFN- $\gamma$  and IL-2, suggesting that Th<sub>1</sub> cytokines enriched microenvironment may inhibit tumor growth [26].

On the other hand, Th<sub>2</sub> differentiation is central to the promotion of humoral immunity, allergic reactions to environmental antigens, and resistance to parasite infections. Th<sub>2</sub> cells produce IL-4, IL-5, IL-10, and IL-13, which act with toxic mediators of innate immune cells to establish environments that are inhospitable to parasites and other organisms. IL-4 is a known major determinant that regulates Th<sub>2</sub> differentiation. Naive CD4<sup>+</sup> T cells stimulated through the TCR in the presence of IL-4, develop into Th<sub>2</sub> effector cells capable of producing IL-4, IL-5 and the related cytokine, IL-13, another important component of Th<sub>2</sub> responses. IL-4 also suppresses the production of IFN- $\gamma$  from Th<sub>1</sub> cells, resulting in the switch of from Th<sub>1</sub> to Th<sub>2</sub> polarization [27].

The transcription factors STAT6 and GATA-3 play central roles in modulating Th<sub>2</sub> differentiation. Upon binding, IL-4 recruits STAT6 to its receptor, where it is activated. GATA-3 expression is STAT6 activation dependent and is up-regulated during Th<sub>2</sub> differentiation and down-regulated during Th<sub>1</sub> differentiation. Activation of GATA-3 also strongly inhibits the production of IFN- $\gamma$  and down regulates IL-12R $\beta$ 2 expression in an IL-4 independent manner [11]. The role of Th<sub>2</sub> in tumor microenvironment has not been fully elucidated. Several studies showed that in Hodgkin's lymphoma, most abundant infiltrating CD4<sup>+</sup> T lymphocytes display a phenotype of Th<sub>2</sub> and regulatory T cells, a group of immunosuppressive cells [28–30]. The evidence from mouse tumor model studies suggests that Th<sub>2</sub> cells and Th<sub>2</sub> cytokines are involved in tumor development and progression through various mechanisms such as the activation of macrophages and myeloid-derived suppressor cells (MDSCs) [31–33]. The balance between the Th<sub>1</sub> and Th<sub>2</sub> polarizations is important for immune response in tumor microenvironment. Therefore, further studies are required to explore the function of Th<sub>2</sub> polarization in tumor microenvironment.

### 3. Regulatory T cells

Another type of tumor infiltrating immune cells is the regulatory T cells (Tregs). In general Tregs are CD4<sup>+</sup>CD25<sup>+</sup>FOXP3<sup>+</sup> T cells defined as inhibitors of the T effector cell activity by

suppressing the secretion of IFN- $\gamma$  and IL-2 while increasing the production of IL-10 and TGF- $\beta$ . In the presence of Tregs, the cytolytic functions of CD8 $^{+}$  and natural killer (NK) cell activity are suppressed [34,35]. The regulation mediated by Tregs is desirable in transplantation patients since they suppress immune responses to alloantigens. Excess accumulation of Tregs has been described as a cause of fatal course in infections and malignancies [34,36,37], which was related to the suppressive action of Tregs. The ranges of immune cells that can be suppressed by Tregs are quite wide and include CD4 $^{+}$ CD25 $^{-}$  T cells, natural killer (NK) cells, dendritic cells, monocytes and CD8 $^{+}$  T cells. An increased frequency and/or altered function of Tregs may contribute to the documented immunosuppressed states such as aging and cancer. Non-antigen specific Tregs are anergic to mitogenic stimuli; they fail to proliferate after stimulation via its T-cell receptor (TCR) in a cytokine-independent but cell contact-dependent manner [37].

In prostate cancer tissues, tumor infiltrating T cells are skewed towards Treg (FOXP3 $^{+}$ ) and Th17 cells [38]. The number of Tregs in peripheral blood is increased in men with prostate cancer compared to normal healthy individuals [38]. Furthermore, studies revealed that tumor infiltrating Tregs, defined as CD8 $^{+}$ FOXP3 $^{+}$  cells, suppressed naive T-cell proliferation mainly through a cell contact-dependent mechanism [39]. Interestingly, Tregs isolated from patients with prostate cancer exert a significantly greater suppressive activity than Tregs isolated from healthy donors [40]. These results suggest that Tregs may play potential pro-tumor role in tumor microenvironment; however, the exact molecular mechanism of Tregs action on the suppression of T cell activation in tumor immunology is unknown and requires further investigation.

#### 4. Th17 cells and IL-17 cytokine

Th17 positive cells play an important role in tumor microenvironment and may impact cancer biology and therapeutics. Th17 cells are defined as CD4 $^{+}$  T helper effector cells that produce extensive amounts of IL-17, a pro-inflammatory cytokine that attracts and activates granulocytes and monocytes [41]. Development of Th17 cells is regulated by multiple cytokines [35,42,43] and the transcription factor retinoic acid receptor-related orphan receptor T (ROR T) [42,43].

Accumulating evidence suggests that Th17 cells exert an antitumor effect within the tumor microenvironment. In ovarian cancer patients, ascites level of IL-17, solely produced from Th17 cells was found to be positively associated with disease-free survival. Even after controlling for surgical therapy and other parameters, tumor-associated IL-17 had death hazard ratio that was negative. Average survival of cancer patients with greater than 220 pg/ml of IL-17 in ascitic fluid was 78 months, whereas death hazard ratio of patients with less IL-17 in their ascitic fluid was 27 months. In the tumor microenvironment, IL-17 synergized with IFN- $\gamma$  to induce the production of CXCL9 and CXCL10, the Th $_{1}$ -type chemokines that recruit T cell effector populations within the tumor itself. Furthermore, ascites levels of CXCL9 and CXCL10 was correlated directly with the presence of tumor-infiltrating natural killer and CD8 $^{+}$  T cells [44]. Another recent study examined the role of tumor-infiltrating Th17 cells in 30 patients with lung adenocarcinoma or squamous cell carcinoma [45]. The results showed that malignant pleural effusion from these patients was chemotactic for Th17 cells, and this activity was partially abrogated by CCL20 and/or CCL22 blockade. Interestingly, higher accumulation of Th17 cells in malignant pleural effusion was positively associated with improved patient survival [45]. Several animal studies have also confirmed that Th17 cells has better anti-tumor efficacy, and especially synergized with IFN- $\gamma$  or Th $_{1}$  cells or activated CD8 $^{+}$  T cells [46–48].

However, other evidence suggests that Th17 cells may exert a pro-tumor effect within the tumor microenvironment. One clinical study demonstrated an inverse correlation between pretreatment circulating levels of Th17 cells and time to disease progression in prostate cancer patients [49]. Another study with 20 prostate cancer patients undergoing radical prostatectomy found an inverse correlation between the differentiation stage of Th17 cells in prostate glands of cancer patients and its association with tumor progression [38]. Furthermore, those prostate cancer patients responsive to immunotherapy with significant reductions in PSA level showed a Th17 profile similar to healthy male controls, but a significant difference from the patients who did not respond [49].

These controversial data regarding the role of Th17 cells in cancer may arise from the studies examining the function of cytokine IL-17 itself. IL-17 is a pro-inflammatory cytokine, which can induce the production of other pro-inflammatory cytokines, chemokines and prostaglandins. Currently, it has been identified that six family members of IL-17 (A–F) are expressed by a variety of innate and adaptive immune cell types, including mast cells, epithelial cells, smooth muscle cells, invariant natural killer T cells, natural killer cells, Paneth cells, lymphoid-tissue inducer-like cells, neutrophils, and  $\gamma\delta$  and  $\alpha\beta$  T cells [43]. Although the role of IL-17 remains controversial, it is clear that IL-17 plays an important role in tumor microenvironment. For example, one clinical observation was that higher levels of IL-17 are positively associated with cancer patient survival [50]. However, other studies showed that high levels of IL-17 are associated with tumor microvessel density, poor prognosis, and short disease survival [51,52]. Further studies are needed to elucidate the function of Th17 cells and IL-17 in the tumor microenvironment.

## 5. Dendritic cells

Dendritic cells (DCs) are one group of antigen-presenting cells (APCs) that possess the ability to transport antigen from peripheral sites of infection into draining lymph nodes via activation of tolllike receptors (TLRs) [2]. Immature DCs effectively capture and trim exogenous antigen within the peripheral tissue where DCs initiate maturation [53]. After antigen uptake, the immature DCs migrate to the draining lymph nodes where immature DCs differentiate into phenotypically and functionally mature DCs [54]. Mature DCs express high levels of surface molecules such as CD80, CD86, MHC class I and MHC class II and activate naïve T cells, thereby inducing their differentiation toward Th<sub>1</sub>, Th<sub>2</sub> or regulatory T cells [55]. Furthermore, mature DCs can produce various pro-inflammatory cytokines and activate innate immune cells [56], and act as a link between innate and adaptive immune responses. Mature DCs are the major source of pro-inflammatory cytokine IL-12 [2], which can be responsible for the activation of T cells to differentiate Th<sub>1</sub> cells, suggesting a role of mature DCs in the activation of Th<sub>1</sub> immune response.

DCs play a dual role in tumor biology, showing either anti-tumor activity or tumor promoting activity, depending on the cytokine milieu encountered within the tumor microenvironment, and the degree of maturation. A decreased population of mature DCs in tissue is shown to impair the ability of immune system to induce and maintain an effective anti-tumor immune response [57,58]. Accumulating evidence suggests that DCs play important roles in the tumor microenvironment; however, the exact mechanism(s) of DC function in tumor microenvironment is not fully understood.

## 6. Myeloid-derived suppressor cells

Myeloid-derived suppressor cells (MDSCs) are a group of myeloid cells comprising of immature macrophages, granulocytes, DCs, and other myeloid cells at earlier stages of differentiation. MDSCs can suppress the proliferation and function of both CD4<sup>+</sup> and CD8<sup>+</sup> T cells [59,60], and are responsible for significant immune dysfunctions. The murine cell-

surface phenotype of MDSCs was identified as CD11b<sup>+</sup>CD11c<sup>+</sup>Gr-1<sup>+</sup>IL-4R<sup>+</sup>, indicating that these cells belong to one class of inflammatory monocytes [61]. In addition, a subset of the bone marrow-derived MDSC population that matures at the tumor site can differentiate into tumor-infiltrating macrophages [59,62]. Prior to exerting effector functions, MDSCs are first activated by cytokines such as IL-4, IL-13, or IFN- $\gamma$ , which are generated by tumor-infiltrating lymphocytes or by MDSCs themselves in an autocrine feedback pathway [63].

MDSCs can promote tumor growth and development by secretion of various molecules and factors such as inflammatory cytokines essential for tumor growth and angiogenesis and also exert a profound inhibitory activity on both tumor specific and non-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells [63–65]. The functional plasticity of MDSC has been reported to be regulated by T helper cytokines. Particularly, the suppressive activity of MDSCs can be enhanced by the addition of Th<sub>2</sub> cytokines such as IL-4 and IL-10 to the cultures. However, the co-culture of MDSCs with Th<sub>1</sub> cytokines can enhance antigen specific T cell cyto-toxicity. Our recent study reports a reduced percentage of granulocytic CD33<sup>+</sup>/CD11b<sup>+</sup>/CD14<sup>-</sup>/HLA-DR<sup>-</sup> and monocytic CD33<sup>+</sup>/CD11b<sup>+</sup>/CD14<sup>-</sup>/HLA-DR<sup>+</sup> MDSC populations in the presence of Th<sub>1</sub> cytokines for human breast cancer SK-BR-3 and MDA-MB-231 cells, compared to co-culture without Th<sub>1</sub> cytokines. Low levels of IFN- $\gamma$ , CXCL9 and CXCL10 were associated with increased numbers of MDSC. Increased levels of IFN- $\gamma$ , CXCL9 and CXCL10 corroborate with reduced number of MDSC, suggesting that Th<sub>1</sub> cytokine enriched microenvironment inhibits the development of MDSC [26].

## 7. Tumor-associated macrophages

Macrophages play critical roles in the regulation of inflammatory responses, wound healing, tissue repair and tissue remodeling. Due to its antigen-presenting activity, macrophages are critically involved in cellular immunity and can destroy tumor cells via cytotoxic mechanisms [66,67], which can then impact tumor progression. Usually, tumor cell damage and hypoxia attract macrophages within or in the surrounding tissue area of tumors, namely tumor-associated macrophages (TAM). TAMs are usually abundant in the tumor microenvironment and are known to play an essential role in tumor progression. TAMs have varying functions, depending on the microenvironment of tumor tissues. Although some studies have reported that an increased number of TAMs represses tumor growth and progression [68,69], most clinical and experimental data demonstrate that an increased population of TAMs is correlated with reduced disease-free and overall survival of cancer patients.

Many tumors secrete molecules or factors that prevent macrophages from signaling other immune cells in the presence of tumor cells, resulting in the loss of capacity of the immune system to recognize the tumor cells. TAMs themselves secrete factors such as inflammatory cytokines that enhance tumor cell proliferation and invasion, and promote angiogenesis. In addition, TAMs release reactive oxygen species and other mutagenic compounds that may cause mutations in surrounding host cells. Ablation of macrophages in mouse tumor models results in the inhibition of tumor angiogenesis and metastasis [70–72]. Increased numbers of TAMs have been found to be associated with increased tumor angiogenesis, invasion and metastasis, or poor prognosis in cancer [73,74]. Therefore, TAMs play a critical role in tumor growth, progression, invasion and metastasis, suggesting that the conditioning of tumor microenvironment by targeting TAMs could be a useful strategy for the treatment of human malignancies.

### 7.1. M1 and M2 subpopulations of TAMs

TAMs constitute the majority of tumor-infiltrating leukocytes in the tumor microenvironment, and are defined as two distinct and polarized TAM sub-populations [75].

Classical, or M1, macrophages are characterized by the expression of high levels of iNOS and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and exhibit an anti-tumor response [76]. Whereas alternatively activated M2 macrophages are characterized by the high expression of arginase-1 (ARG1) and IL-10 [77,78].

Polarized TAMs differ in their expression of receptors, effector function and, cytokine and chemokine production. Differential cytokine production is a key feature of polarized macrophages. Cytokines produced by M1 macrophages such as IL-12 and TNF induce T-cell effector functions [79] while M2 macrophages largely produce immune suppressive cytokine such as IL-10 [80]. In addition to cytokine polarization in M1 and M2 macrophages, chemokine receptors and ligands are also differentially regulated. M1 macrophages produce IFN- $\gamma$ -inducible chemokines IP-10 (CXCL10) and MIG (CXCL9) that attract Th1 cells and M2 macrophages produce CCL22 that attracts Th<sub>2</sub> or Tregs [81]. At the tumor site, M2-like macrophages constitute the predominant population of TAMs, which can suppress T cell-mediated anti-tumor responses and promote tumor progression, metastasis, and angiogenesis [79,80,82].

## 8. Immunological role of NF- $\kappa$ B in the tumor microenvironment

NF- $\kappa$ B is one of the major transcription factors for the development of immune responses, and thus alterations of NF- $\kappa$ B activation are known to play critical roles in the development of chronic diseases including cancer. This transcription factor is activated by many stimuli including inflammatory cytokines, LPS, protein kinase C activators, reactive oxygen species (ROS), ultraviolet light and ionizing radiation, and other cellular stresses [83,84]. NF- $\kappa$ B activation regulates the expression of numerous genes such as inflammatory cytokines, chemokines, enzymes (iNOS and inducible cyclooxygenase), transforming growth factor 2, adhesion molecules, receptors, and other immune mediators [83–85]. NF- $\kappa$ B is a central player involved in critical innate and adaptive immune and stress responses as well as cell survival and proliferation responses (Table 1).

NF- $\kappa$ B is normally present in the cytoplasm in an inactive form bound to an inhibitory protein of NF- $\kappa$ B (I $\kappa$ B), which has several sub-units such as I $\kappa$ B- $\alpha$ , I $\kappa$ B- $\beta$ , and I $\kappa$ B- $\gamma$  [83–85]. Classically, the NF- $\kappa$ B is activated when it is dissociated from I $\kappa$ B. Cytosolic NF- $\kappa$ B becomes active and translocates to the nucleus only when I $\kappa$ B is dissociated from the NF- $\kappa$ B heterodimer p50/p65. Many stimuli activate NF- $\kappa$ B via phosphorylation of I $\kappa$ B proteins through the action of specific kinases such as I $\kappa$ B kinase (IKK). Phosphorylation of I $\kappa$ B results in the attachment of ubiquitin residues and subsequent degradation by the multifunctional proteolytic enzyme proteasome complex [83–85]. Dissociation of NF- $\kappa$ B from I $\kappa$ B results in the rapid translocation of NF- $\kappa$ B to the nucleus for activation of NF- $\kappa$ B target genes.

### 8.1. Role of NF- $\kappa$ B in tumor progression

Loss of regulation of the normally latent NF- $\kappa$ B contributes to the deregulated growth, resistance to apoptosis, and propensity to metastasize as observed in many human cancers, and thus targeted inactivation of NF- $\kappa$ B has been recognized as a promising strategy for the prevention and/or treatment of human cancers. High levels of NF- $\kappa$ B activity are associated with increased expression of NF- $\kappa$ B-regulated genes such as proinflammatory cytokines IL-1 $\alpha$ , IL-6, and IL-8, which may contribute to tumor progression and metastasis [86].

The molecular mechanisms of NF- $\kappa$ B pathway in cancer cells have been thoroughly investigated. However, the immunological contribution of NF- $\kappa$ B to the tumor microenvironment remains unclear. The immunological role of NF- $\kappa$ B in tumor growth and progression within the context of tumor microenvironment is very complex, due to its

different functions depending on different cell lineages. Limited studies have been conducted to examine the role of NF- $\kappa$ B activation in the regulation of tumor associated host immune cells, especially T helper cells, Treg cells, and DCs that comprises the tumor microenvironment.

### 8.2. Role of NF- $\kappa$ B in the development of innate immune responses

The activation and nuclear translocation of NF- $\kappa$ B have been associated with increased transcription of chemokines, cytokines and adhesion molecules, which constitute important components of the innate immune response to invading microorganisms. Induction of chemokines, cytokines and adhesion molecules can facilitate recruitment and activation of inflammatory cells at the site of NF- $\kappa$ B activation. In addition to direct NF- $\kappa$ B activation as a consequence of inflammation and infection, there are indirect pathways that lead to NF- $\kappa$ B activation. For example, the release of IL-1 can activate the NF- $\kappa$ B pathway which, in turn, leads to the production of the other cytokines and chemokines [87].

NF- $\kappa$ B can be activated by many bacterial products; however, the recognition of the Toll-like receptors (TLRs) which has been shown to be a specific pattern recognition molecules [88] leads to the activation of NF- $\kappa$ B, and this result enhanced our understanding of pathogens stimulated NF- $\kappa$ B activation. TLRs are widely distributed on macrophage, dendritic and B-cells [89]. Signaling through the TLR has been linked to increased expression of a number of genes known to be regulated by NF- $\kappa$ B, including cytokines, co-stimulatory molecules, nitric oxide, and further contributed to the susceptibility to apoptosis as well as an autocrine increase in TLR expression.

IL-12 is one of the most important cytokines involved in the activation of innate and adaptive responses; its production leads to the innate activation of NK cells and enhances their cytolytic activity and production of IFN- $\gamma$ . IL-12p40 promoter was demonstrated to have NF- $\kappa$ B binding sites that are involved in the transcriptional production of IL-12 [90,91].

RelB, an NF- $\kappa$ B family member, has been reported to be responsible for DC differentiation [92]. In DCs, the activation of NF- $\kappa$ B induced by pro-inflammatory cytokines and toll-like receptor (TLR) signal results in the induction of transcription of genes involved in DC maturation, and antigen-processing and presentation [93]. Inhibition of NF- $\kappa$ B activation could maintain DCs in an immature state promoting immune tolerance [93]. Specifically, the loss of RelB in DCs resulted in a shift of a Th<sub>1</sub>/Th17 to a Th<sub>2</sub> and FOXP3<sup>+</sup> Treg cell profile in the co-culture system [92]. These studies suggest that NF- $\kappa$ B plays a key role in maturation and function of DC, which impact cancer development and progression within the context of tumor microenvironment [93].

### 8.3. Role of NF- $\kappa$ B in adaptive immune responses

T-cell responses can be broadly divided into two Th<sub>1</sub> (characterized by IL-2 and IFN- $\gamma$  production) or Th<sub>2</sub> (characterized by IL-4 and IL-5 production) functional subsets. NF- $\kappa$ B can directly be involved in the polarization T-cell responses or via IL-12 production that is required for the generation of Th<sub>1</sub> responses [94,95].

Maintenance of long-term memory cells is the hallmark of adaptive immunity. NF- $\kappa$ B signaling has recently been implicated in the signals that allow development of memory phenotype. NF- $\kappa$ B protein families have been identified to be involved in the regulation of IL-2 and IFN- $\gamma$  gene expression in T lymphocytes with co-activation of MAPK pathway [20,24,96] and controlling the activation of T cells via TCR and CD28 ligation [97]. One study showed that the loss of NF- $\kappa$ B activation induced by its inhibitor resulted in decreased level of IFN- $\gamma$  and IL-17 in activated CD4<sup>+</sup> T cells [98]. NF- $\kappa$ B also promotes T-cell

proliferation; transgenic mice that express the degradation-deficient I $\kappa$ B transgene which acts as a global inhibitor of NF- $\kappa$ B activity showed impaired T cell proliferation [99]. Another study showed that signaling through the T-cell receptor (TCR) in mature T cells requires NF- $\kappa$ B activity [100] which may be important in TCR-mediated proliferative signals.

Involvement of NF- $\kappa$ B in the development of Th<sub>1</sub>-type responses is well known but its regulation of Th<sub>2</sub>-type responses is less clear. Studies using I $\kappa$ B mutant mice indicated that it did not alter the ability of these mice to develop Th<sub>2</sub>-type responses *in vivo* [101]. In experimental allergic encephalomyelitis [102] and pulmonary inflammation [103], NF- $\kappa$ B<sub>1</sub> is required for the development of Th<sub>2</sub> responses through the regulation of transcription factor GATA3 signaling [104], which plays an important role in differentiation of Th<sub>2</sub> cells and their production of IL-4 and IL-10 [105,106].

#### 8.4. Role of NF- $\kappa$ B in B-cell activation and effector function

Presence of constitutive NF- $\kappa$ B activity in B cells and identifying that a nuclear factor was able to bind to the B site in the immunoglobulin light chain enhancer underscore the importance of NF- $\kappa$ B in the control of B-cell functions. In fact multiple studies using mice deficient in NF- $\kappa$ B<sub>1</sub>, NF- $\kappa$ B<sub>2</sub>, RelA, RelB, c-Rel, or Bcl-3 showed compromised humoral immune responses and documented its role in immunoglobulin class switching [107,108]. These studies suggest that NF- $\kappa$ B is an important regulator of B cell survival, cell cycle progression, and their effector functions.

#### 8.5. Role of NF- $\kappa$ B in regulatory T cells, TAM and MDSC

It has been found that c-Rel/RelB but not NF- $\kappa$ B<sub>1</sub> (p50) is important for Treg cell development by up-regulation of FOXP3 expression, a major transcription factor for Tregs [109–112]. However, more studies are required to examine the role of NF- $\kappa$ B in the regulation of Tregs in the tumor microenvironment.

The role of NF- $\kappa$ B in the regulation of macrophage in cancer biology has been intensively investigated. Activation of NF- $\kappa$ B in macrophages is required for the onset of tumor development in several inflammation-induced cancer models [113–119]. Inhibition of NF- $\kappa$ B signaling pathway induces TAMs to become cytotoxic to tumor cells [71,120]. NF- $\kappa$ B is also known to regulate the expression of many important genes including tumor-promoting genes such as VEGF, IL-6, TNF- $\alpha$ , and COX2, which support its crucial role in the activation of tumor-associated macrophages in the tumor microenvironment [121,122].

#### 8.6. Potential target of NF- $\kappa$ B for cancer therapy

NF- $\kappa$ B transcription factors have a key role in many physiological processes such as innate and adaptive immune responses, cell proliferation, cell death, and inflammation. Aberrant regulation of NF- $\kappa$ B and the signaling pathways that control its activity promote cancer development, progression and resistance to therapy, which makes it a potential therapeutic target. There are many strategies that have been experimentally employed to inhibit NF- $\kappa$ B activation or function and one of the strategies to inhibit the NF- $\kappa$ B activation is by using proteasome inhibitors. Inhibitors of the 26S proteasome were shown to inhibit I $\kappa$ B degradation and NF- $\kappa$ B nuclear translocation. At least one proteasome inhibitor, bortezomib (Velcade; Millenium), has entered into clinical development for the treatment of myeloma; however, prolonged NF- $\kappa$ B inhibition can be detrimental due to its role in innate immunity. Therefore, NF- $\kappa$ B inhibition strategies that are transient and reversible would be more beneficial while avoiding long-term immuno-suppression.

## 9. The protective role of zinc in tumor microenvironment

Zinc, an essential trace mineral element in humans and animals, has been known to participate in the activation of approximately 300 enzymes and is involved in the regulation of over 2000 zinc-dependent transcription factors, which are involved in DNA synthesis, protein synthesis, cell division, and other metabolisms. Therefore, zinc plays critical roles in various biological processes in the body [123–127]. Early evidence has indicated that zinc deficiency can cause body growth retardation, delayed sexual maturation, depressed immune response, and cause abnormal cognitive functions. Zinc supplementation prevents these adverse effects.

The relationship of zinc and tumor has been intensively investigated in experimental and human studies over decades. Although one early report demonstrated that dietary zinc deficiency inhibited tumor growth in immunocompetent mice and rats [128]; however, the majority of reports have demonstrated that zinc deficiency increases the growth and/or incidence of carcinogen-induced gastrointestinal tumors in immuno-competent mice and rats [129–145], consistent with the recent findings [128,146,147]. Furthermore, zinc supplementation can inhibit the growth of human prostate cancer PC-3 cells-induced tumor in nude mice, compared to zinc deficient nude mice [148]. All these animal studies are based on carcinogen-induced and xerograft tumor models. Recently, the protective role of dietary zinc in prostate cancer has been investigated in TRAMP (transgenic adenocarcinoma of the mouse prostate) mouse model [149]. The results showed that tumor weights were significantly higher when the dietary zinc intake was either deficient or high in comparison to normal zinc intake level, suggesting that an optimal dietary zinc intake may play a protective role against prostate cancer. A large number of epidemiological and clinical studies demonstrate that zinc deficiency is associated with increased risk of some cancers, such as prostate, lung, esophageal, and oral cancers [150–153]. These data from experimental animal and human studies have strongly suggested that zinc may play a protective role in the development and progression of cancer; however, the exact mechanism of zinc action within the tumor microenvironment has not been fully understood. A large number of studies have demonstrated that zinc deficiency induces inflammatory cytokines and oxidative stress, apoptosis and cellular dysfunction, disrupts DNA-protein interaction, and depresses the function of T cell mediated immune response [153,154]. Zinc supplementation reverses these adverse effects, suggesting that the anti-tumor effect of zinc could be potentially due to its differential regulation of host immune cells and cancer cells within tumor microenvironment; however, how zinc accomplishes this task has not been investigated. Zinc has been implicated as a chemo-preventive agent, but the role of zinc in conditioning the tumor microenvironment is not known and requires more mechanistic investigation.

### 9.1. The inhibitory effect of zinc on cancer cell growth

A large number of cell culture studies have demonstrated that the physiological level of zinc could exhibit anti-tumor effects by inhibiting cell cycle, cell growth and proliferation, and cancer cell invasion by regulating oncogenic signaling pathways such as NF- $\kappa$ B, AP-1, Notch-1, and PI3K/Akt [155,156]. Moreover, zinc is also known for induction of mitochondria-mediated apoptosis, cytotoxicity, up-regulation of zinc-dependent activities of tumor suppressors (such as p21, p53, and A20) and stabilization of protein-DNA binding integrity in many different normal and cancer cell lines (such as pancreatic cancer cells, prostate cancer cells, and liver cancer cells) [157–163]. The loss of zinc could be responsible for the development and progression of human malignancies, and thus suggesting that retention of the normal levels of cellular zinc might exhibit cytotoxic effects on the malignant cells. Exposure of the human pancreatic cancer Panc1 cells to a physiological concentration of 2.5–10  $\mu$ M zinc has been reported to increase the cellular level of zinc,

which resulted in 60%–80% inhibition of cell proliferation, compared to the zinc deficient condition. This cytotoxic effect indicates that incorporation of zinc into malignant pancreatic cells could exhibit anti-tumor effects [164].

### 9.2. The effect of zinc on Th<sub>1</sub>/Th<sub>2</sub> cells and T-bet activity

One early study showed that zinc is essential for IL-2 mediated T cell activation [165]. Our previous cell culture studies revealed that zinc increases IL-2 and IFN- $\gamma$  cytokines and mRNAs in HUT-78 (Th0 malignant leukemia) and D1.1 (Th<sub>1</sub> malignant leukemia) cells after PMA/PHA and PMA/ionomycin D stimulation, compared to zinc deficient condition [166,167]. Studies in our experimental human model, where we induced a mild deficiency of zinc by dietary restriction, showed that *ex vivo* production of IL-2, and IFN- $\gamma$  in isolated peripheral blood mononuclear cells (PBMC) was decreased and was normalized following zinc supplementation. Production of IL-4, IL-5 and IL-6 was not affected in zinc deficiency [168]. Our previous clinical observation demonstrated that 50% of patients with newly diagnosed head and neck cancer had zinc deficiency. The tumor size and overall stage of the disease was correlated with baseline zinc status. These patients also had decreased production of Th<sub>1</sub> cytokines, but not Th<sub>2</sub> cytokines [169–171]. Our recent study also demonstrates that zinc-dependent IL-2 and IFN- $\gamma$  productions are associated with the up-regulation of T-bet gene expression via increased intracellular free zinc in HUT-78 cells [167]. This suggests that zinc plays an important role in Th<sub>1</sub> phenotype and function.

### 9.3. The effect of zinc on T cell subpopulations

In our experimental human studies, zinc deficiency was associated with a decrease in the CD4<sup>+</sup>/CD8<sup>+</sup> ratio, which was normalized following zinc supplementation [168]. Our studies showed that naive T cells (CD4<sup>+</sup>CD45RA<sup>+</sup>) newly exported from the thymus were affected in zinc deficiency. A significant decrease in the ratio of CD4<sup>+</sup>CD45RA<sup>+</sup>/CD4<sup>+</sup>CD45RO<sup>+</sup> cells was observed at the end of zinc depletion phase, which was normalized following zinc supplementation. Inasmuch as maturation of the T cell is dependent upon intact thymic functions, one may expect a decrease in the T cell population if thymic function is abnormal [172]. We have demonstrated that thymulin, an important thymic hormone, is zinc dependent. Zinc deficiency results in the reduction of thymulin activity. Therefore, zinc appears to play an essential role in T cell activation, which could be very important within the tumor microenvironment although such studies have not been done to-date.

### 9.4. The role of zinc on Tregs, TAM, DCs, Th17, and MDSCs

As an essential immune responsive mediator, the role of zinc in the regulation of Treg, TAM, DC, Th17, and MDSC function in tumor development and progression has not been fully elucidated. However, emerging evidence suggest that zinc is required to maintain the normal functions of macrophages and DCs. For example, zinc deficiency has been found to cause either bacterial phagocytic dysfunction in alveolar macrophages from alcohol-fed rats or DC disintegration and immaturation in mice and rats. Zinc supplementation reverses this adverse effect [173–175]. These results suggest that zinc may play a protective role in tumor development and progression although further in-depth investigation is required for understanding of its molecular roles in the tumor microenvironment.

### 9.5. The role of zinc on inflammation and oxidative stress

A large number of cell culture and animal studies have demonstrated that zinc deficiency increases oxidative stress and inflammatory responses, which are known as key contributors of chemo- and radiotherapy resistance. For example, zinc deficiency causes increased productions of ROS and inflammatory cytokines such as TNF- $\alpha$ , IL-1 $\beta$ , IL-8, VCAM, and MCP-1 in both non-malignant (such as vascular endothelial, lung, and prostate cells) and

malignant cells (such as prostate cancer cells, colon cancer cells, promyelocytic and monocytic leukemia cells) [155,173–179]. Zinc supplementation reverses these adverse effects, which suggests that zinc could be useful as an anti-oxidant and anti-inflammatory agent.

Some early cell culture and animal studies have shown that zinc could function as an anti-oxidant agent as a site-specific antioxidant in the body by several mechanisms [180–183]. First, zinc competes with iron and copper ions, which catalyze the production of OH from H<sub>2</sub>O<sub>2</sub>, for binding to cell membranes and some proteins, by displacing these redox-active metals. Secondly, it binds to sulfhydryl (SH) groups protecting them from oxidation [181]. Thirdly, zinc has been shown to increase the activities of glutathione (GSH), catalase, and superoxide dismutase (SOD, a ROS scavenger), and could decrease the activities of inducible nitric oxide synthase (iNOS) and NADPH oxidase and lipid peroxidation products [184,185]. Fourthly, zinc is known to induce the expression of metallothionein (MT) protein, which is very rich in cysteine and an excellent scavenger of •OH [186,187]. NF- $\kappa$ B mediated inflammatory response has been recognized as one of the major sources of oxidative stress. Recently, it has been considered that the inhibition of NF- $\kappa$ B-mediated inflammatory response by zinc contributes to the repression of oxidative stress.

One clinical trial of 30 mg zinc supplementation on oxidative stress was conducted in 56 patients with type 2 diabetes mellitus [183]. Following 6 months of zinc supplementation, plasma zinc increased and plasma TBARS, a lipid peroxidation marker significantly decreased, whereas the placebo group showed no such changes [183]. Increased plasma lipid peroxidation by-products and decreased erythrocyte SOD were reported to be associated with decreased zinc status in children with chronic giardiasis [182]. Our earlier studies have clearly demonstrated that following zinc supplementation, oxidative stress as assessed by the generation of lipid peroxidation and DNA oxidation by-products, inflammatory cytokines, and *ex vivo* TNF- $\alpha$  induced activation of NF- $\kappa$ B in isolated PMNC, were significantly decreased compared to placebo supplemented normal human subjects [188]. Our recent studies in collaboration with Dr. Prasad in elderly subjects and sickle cell disease (SCD) patients confirmed that zinc supplementation significantly increased plasma zinc level and decreased plasma oxidative stress bio-markers, and inflammatory cytokines along with decreased rate of infection [189]. These data strongly suggest that zinc certainly functions as an anti-oxidant and anti-inflammatory agent, which could play a protective role in the development and progression of cancers; however, further definitive clinical trial in human cancer patients or in population with high-risk for the development of cancer are warranted especially within the context of tumor microenvironment.

### 9.6. The differential role of zinc in the regulation of NF- $\kappa$ B activation

As indicated above that zinc plays an important role in the regulation of NF- $\kappa$ B activation; however, the regulation of NF- $\kappa$ B activation by zinc appears to be cell type specific. For example, zinc has been found to be required for NF- $\kappa$ B DNA binding activity either by human placenta-purified proteins, recombinant NF- $\kappa$ B p50, or human Th<sub>0</sub> cell line-derived nuclear protein extracts [190–192]. Inasmuch as NF- $\kappa$ B binds to the promoter enhancer area of IL-2 and IL-2R genes, we investigated the effect of zinc deficiency on activation of NF- $\kappa$ B and its binding to DNA in HUT-78, a Th<sub>0</sub> malignant human lymphoblastoid cell line. We showed that in low zinc HUT-78 cells, phosphorylated I $\kappa$ B, IKK, and ubiquitinated I $\kappa$ B and binding of NF- $\kappa$ B to DNA were all significantly decreased, which was consistent with decreased production of IL-2 and IL-2R, and IFN- $\gamma$ , in comparison to HUT-78 cells with normal zinc levels [191]. Zinc increased the translocation of NF- $\kappa$ B from cytosol to the nucleus. We also demonstrated that binding of recombinant NF- $\kappa$ B (p50)<sub>2</sub> to DNA in HUT-78 cells was zinc specific. Inhibition of NF- $\kappa$ B activation by anti-sense p105 (p50 precursor) mRNA abrogates the zinc-mediated IL-2 and IL-2Ra production in HUT-78 cells.

These results suggest that zinc plays an important role in the regulation of the productions of IL-2 and IL-2R via the activation of NF- $\kappa$ B in HUT-78 cells. Such results are provocative although the role of these cytokines production affected by zinc must be investigated thoroughly within the context of tumor microenvironment.

Interestingly, a number of *in vitro* studies have shown that zinc inhibits LPS-, ROS-, or TNF- $\alpha$ -induced NF- $\kappa$ B activation in endothelial cells, pancreatic cells, cancer cells, and PMNC [155,176–178, 189,193,194], consistent with decreased expression of inflammatory cytokines, oxidative stress, anti-apoptotic protein C-1AP2, and activated C-Jun NH<sub>2</sub>-terminal kinases, which promotes apoptotic pathways [195,196]. Thus, anti-inflammatory and anti-oxidant functions of zinc are associated with its inhibition of NF- $\kappa$ B activation in these non-T helper cells. There are inhibitors of NF- $\kappa$ B activation that are different from I $\kappa$ B protein. One such endogenous inhibitor of NF- $\kappa$ B activation is A20 (also known as TNF- $\alpha$  induced protein 3, TNFAIP3), a cytoplasmic zinc finger-transactivating factor that plays a key role in the negative regulation of inflammation via inhibition of IL-1 $\beta$ - and TNF- $\alpha$ -induced NF- $\kappa$ B activation [197–199]. A20 has been found to inhibit NF- $\kappa$ B activation through the down-regulation of TNF-receptor associated factor (TRAF) signaling pathway, resulting in the inactivation of IKK, a NF- $\kappa$ B activating kinase.

The inhibitory role of zinc in NF- $\kappa$ B-mediated inflammation has been considered to be associated with A20 in non-T helper cells. Our recent studies have shown that zinc increases A20 and A20-TRAF1 complex, and decreases NF- $\kappa$ B activation, IKK, and the production of inflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$ , IL-8, MCP-1, and VCAM) and oxidative stress in HL-60, TPH-1, and vascular endothelial cells, compared to zinc deficient cells [155,176,188]. Silencing of A20 by its anti-sense RNA (siRNA) increased TNF- $\alpha$  and IL-1 $\beta$  production in zinc-sufficient cells [155], suggesting that zinc down-regulates the production of inflammatory cytokines via A20 pathway, which is due to deregulation of NF- $\kappa$ B activity. Therefore, zinc differentially regulates NF- $\kappa$ B activation through a cell lineage-dependent mechanism, which may contribute to its protective role in host immune cells within the tumor microenvironment.

## 10. Perspectives and conclusions

The traditional approach such as cytotoxic chemotherapeutics or even targeted therapeutics for cancer treatment by targeting cancer cells alone may not produce optimal therapeutic outcomes. Recent advances suggest that the tumor microenvironment may play a pivotal role in tumor initiation, progression, invasion and metastasis, and thus targeting the tumor microenvironment is expected to produce results that would shift paradigms of cancer therapeutics leading to improved clinical outcomes. Thus, targeting the tumor microenvironment provides a novel strategy for cancer treatment although the specific mechanisms and functions of each component of the tumor microenvironment are not fully understood, and thus require in-depth molecular investigations. Since NF- $\kappa$ B plays an important role in cancer development and progression, further understanding of the role of NF- $\kappa$ B in the deregulation of the immunological processes within the tumor microenvironment are critical. Accumulating evidence suggests that NF- $\kappa$ B may have different regulatory effects on tumor associated immune cells such as Th<sub>1</sub>, Th<sub>2</sub>, Th17, Tregs, DCs, MDSCs, and TAMs in the tumor microenvironment. Furthermore, it is tempting to speculate that zinc could serve as an important agent for conditioning the tumor microenvironment to optimize antitumor effects of the immune system since zinc is known to differentially regulate NF- $\kappa$ B activation through a cell-specific manner and is known to play an important role in Th<sub>1</sub>/Th<sub>2</sub> polarization. Therefore, zinc could be useful in conditioning the tumor microenvironment for improving therapeutic outcome of cancer patients treated with conventional therapeutics. Moreover, any approach by which the tumor

microenvironment could be conditioned would become useful for optimizing cancer therapy using existing conventional or targeted therapy.

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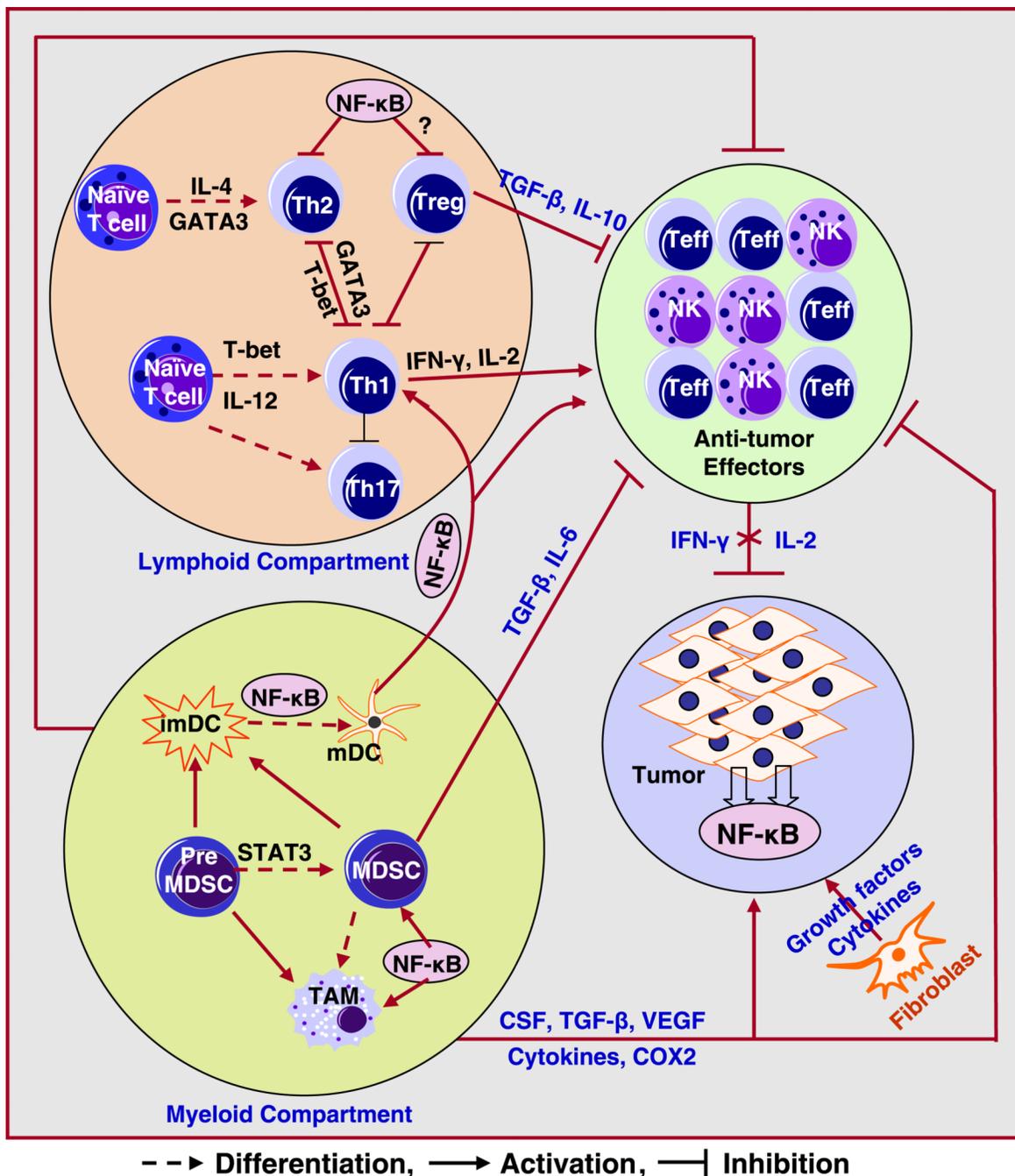
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**Fig. 1.** Schematic diagram of possible interactions between tumor cells, lymphoid, myeloid and stromal components involved in immune escape and tumor progression. Various cytokines and growth factors produced by tumors or stroma may recruit and activate several immune suppressive cells, resulting in tumor progression. (MDSC: myeloid derived suppressor; imDC: immature dendritic cells; mDC: mature dendritic cells; Treg: T regulatory cells; Teff: effector T cells; NK: natural killer cell; TAM: tumor associated macrophages).

**Table 1**NF- $\kappa$ B regulated immune modulators.

Activity	Mediators/receptors	References
<i>NF-<math>\kappa</math>B Regulation of Inflammation and adaptive immunity</i>		
NF- $\kappa$ B induction and activation	IL-1/IL-1R, TNF/TNF-R, TCR/BCR, TLRs, CD40/BAFF-R	Refs. [200–205]
NF- $\kappa$ B regulation of adaptive immunity (Defects in B- and T-cell proliferation, activation, cytokine production and B cells isotype switching)	STAT3, STAT5a, B7-1 (CD80) and B7-2 (CD86), IL-2, IL-18 and IFN- $\gamma$ , mutation or NF- $\kappa$ B <sub>1</sub> and NF- $\kappa$ B <sub>2</sub> deficiency	Refs. [206–210]
NF- $\kappa$ B regulation of inflammation	IL-1, IL-8, TNF- $\alpha$ , MIP-2, MCP-1, COX-2, ICAM-1	Refs. [211–215]
<i>NF-<math>\kappa</math>B mediated regulation of tumor promoting factors</i>		
Survival	Bcl-2, Bcl-X <sub>L</sub> , XIAP, TRAF1/2, cFLIP, cIAP, Survivin	Refs. [216–220]
Proliferation	Cyclin D, c-Myc	Refs. [221–224]
Angiogenesis	MCP-1, IL-8, IL-1, IL-6, VCAM-1	Refs. [225,226]
Epithelial-to-mesenchymal transition (EMT)	Vimentin, Cathepsin B, MMPs, Twist	Refs. [227,228]
Invasion and metastasis	MMP-2, MMP-9, VCAM-1, ICAM-1, uPA	Ref [221]