

# The paradoxical role of tumor-infiltrating immune cells in lung cancer

Xiaodan Zheng<sup>1</sup>, Yuhai Hu<sup>1</sup>, Chengfang Yao<sup>2,\*</sup>

<sup>1</sup> Clinical Laboratory, Wuhan Hankou Hospital, Wuhan, China;

<sup>2</sup> Key Laboratory for Tumor Immunology and Traditional Chinese Medicine Immunology, Institute of Basic Medicine, Shandong Academy of Medical Sciences, Ji'nan, China.

**Summary** Lung cancer remains one of the leading causes of death worldwide, and lung cancers have often already metastasized when diagnosed. Numerous studies have noted the infiltration of immune cells in the lung cancer microenvironment, but these cells play a dualistic role, *i.e.* they suppress and/or promote tumor development and growth based on tumor progression and different cytokines in the microenvironment. These tumor-infiltrating immune cells create different microenvironments depending on their type and interaction. Chemokines act as a bridge in this process by recruiting immune cells to the tumor site and they regulate the phenotypes and functions of those cells. The current review summarizes current knowledge about the tumor-infiltrating immune cells in lung cancer as well as the mechanisms involved in suppression and promotion of tumor development and growth.

**Keywords:** Lung cancer, tumor microenvironment, infiltrating immune cells, chemokine

## 1. Introduction

Lung carcinoma is a major cause of death throughout the world; more than 1.82 million people are diagnosed with lung carcinoma annually (13% of all cancers) and lung carcinoma causes 1.59 million deaths (19.4% of all cancers) (1,2). By the time the tumor is diagnosed, it has often already metastasized (3). Despite advances, treatments such as surgery, radiotherapy, chemotherapy, and multimodal therapies can rarely control metastatic disease and they are seldom curative. Patients with lung cancer have a limited long-term survival (4) because treated lung tumors will ultimately relapse since a number of tumor clones or "cancer-initiating cells" have escaped the initial treatment. These escaped cells will be more resistant to therapeutic modalities. Therefore, an adjuvant therapy that could effectively destroy these remaining tumor cells would have a substantial impact on tumor treatment.

Immunotherapy has a demonstrable efficacy in patients with cancer (5). These cells or cytokines can infiltrate the tumor site by activating the host immune cells, thus inducing a specific antitumor immune response. Therefore, the effect of the treatment depends, to a great extent, on the tumor microenvironment. Tumor-infiltrating immune cells play critical roles in the tumor microenvironment by promoting or suppressing tumor development and progression depending on their type and functional interactions (6).

There is an obvious infiltration of different types of immune cells in lung cancer. These immune cells typically include natural killer (NK) cells, T lymphocytes, macrophages, dendritic cells (DC), myeloid-derived suppressor cells (MDSCs), and B cells (7). These cells serve different functions and combine or cancel out each other, thus creating the microenvironment for lung cancer. Cancer progression and survival are significantly associated with these cell types and functions and their localization in tissue.

## 2. Diagnostic subtypes of lung cancer

Lung carcinoma is a solid tumor with low antigenicity and a heterogenic phenotype that evades the host's immune defense. Lung cancers usually arise from basal epithelial cells and have two main histological

Released online in J-STAGE as advance publication November 15, 2017.

\*Address correspondence to:

Dr. Chengfang Yao, Institute of Basic Medicine, Shandong Academy of Medical Sciences, No. 18877 Jingshi Road, Ji'nan 250062, Shandong, China.

E-mail: xyz27315@sina.com



in response to IL-4 and IL-6. Activated Th2 cells, which produce IL-4, IL-5, IL-6, IL-10, and IL-13, are key to allergic responses and protection against infection by helminthic parasites (20,21). It is widely believed that cytokines that are secreted by Th1 cells can facilitate CTLs generation, whereas a Th2 cytokine profile might favor antibody response and be detrimental to the induction of CTLs (22,23). A study (24) examined the expression of Th1 and Th2 cells in lung cancer and found that the proportion of Th2 cells was significantly lower than that of Th1 cells, indicating that Th1 cells are dominant tumor infiltrators.

Th17 cells become activated through the transcription factor retinoid-related orphan receptor gamma t (ROR $\gamma$ T) when stimulated with transforming growth factor  $\beta$  (TGF- $\beta$ ), IL-6, IL-23, IL-1 $\beta$  (in humans), and prostaglandin E (PGE) (25,26). Th17 cells have been found to play a contradictory role in tumorigenesis. Numerous studies have identified the tumor-promoting effects of Th17 cells. However, IL-17A<sup>+</sup>IFN- $\gamma$ <sup>+</sup> Th17 cells have tumor-protective characteristics in tumor immunity (27). The varying functions of the Th17 subset in tumor immunity are largely due to the cytokines they secrete, such as IL-17A, IL-17F, IL-21, IL-22, IFN- $\gamma$ , and granulocyte-macrophage colony-stimulating factor (GM-CSF) (28). In addition, the antitumor functions of Th17 cells are related to effector lymphocytes, including Th1, Tc1, and NK cells (29).

Treg cells are formed as a result of the transcription factor forkhead box P3 (Foxp3), which is the dominant transcription factor of Treg cells. Developmental pathways of Th17 and Treg cells are closely related. TGF- $\beta$  alone induces expression of FoxP3 (30,31) and ROR $\gamma$ T (31) in TCR-stimulated naive CD4<sup>+</sup> T cells. Thus TGF- $\beta$  is a critical factor for Th17 and Treg cells and is essential for inducing both ROR $\gamma$ T and Foxp3. Despite the induction of these transcription factors, TGF- $\beta$  is unable to initiate Th17 differentiation *in vitro* unless pro-inflammatory cytokines, such as IL-6 or IL-21, are present. When these cytokines are present, the TGF- $\beta$ -induced Foxp3 expression is down-regulated and ROR $\gamma$ T expression is up-regulated (30,32). In the absence of significant inflammation, TGF- $\beta$  promotes the differentiation of Treg cells, which maintain immune tolerance. This is because of Foxp3-mediated inhibition of the activity of ROR $\gamma$ T, resulting in silencing of IL-17 and IL-23 expression (33).

Th17 and Treg cells have opposite roles in the development of autoimmune and inflammatory diseases. Th17 cells promote autoimmunity while Treg cells seem to control it and therefore play an important role in autoimmune pathogenesis by maintaining self-tolerance and by controlling the growth and activation of autoreactive CD4<sup>+</sup> effector T cells (34). Tregs inhibit the recruitment of CD8<sup>+</sup> T lymphocytes in tumors and their effector functions are critical to inhibiting lung tumor

growth (35). Tregs inhibit the immune system partly by utilizing membrane TGF- $\beta$  and are thought to be key to downregulating Th1, Th2, and Th17 cells, possibly preventing autoimmunity (36). Thus, the abundance of Tregs are within lung tumor tissue is not surprising (37).

Accordingly, the Th1/Th2/Th17/Treg paradigm is essential for maintaining immune homeostasis based on cytokine production, and the cytokine environment they create determines the fate of the host's antitumor immunity.

#### 4. The role of MDSCs in lung cancer

##### 4.1. Functional and molecular characterization of MDSCs

MDSCs were first identified as immunosuppressive CD11b<sup>+</sup> Gr-1<sup>+</sup> myeloid cells in cancer patients in the 1980s (38). MDSCs represent a heterogenic population of immature myeloid cells that consists of myeloid progenitors and precursors of macrophages, granulocytes, and DCs they are characterized by a potent ability to suppress various T cell functions (39).

In mice, MDSCs are cells that simultaneously express the two markers CD11b and Gr-1 (38). Gr-1 includes the macrophage and neutrophil markers Ly6C and Ly6G, while CD11b is characteristic of macrophages (40). More recently, MDSCs have been subdivided into different subtypes based on their expression of Ly6C and Ly6G. CD11b<sup>+</sup>Ly6G<sup>+</sup>Ly6Clo cells with a granulocytic-like morphology and multilobed nuclei are called granulocytic MDSCs, whereas CD11b<sup>+</sup>Ly6G<sup>-</sup>Ly6C<sup>hi</sup> cells with a monocytic-like morphology are referred to as monocytic MDSCs (41). In cancer patients, MDSCs are defined as cells that express the common myeloid marker CD33 but that lack expression of markers of mature myeloid and lymphoid cells (42). MDSCs are typically CD11b<sup>+</sup>CD33<sup>+</sup>CD34<sup>+</sup>CD14<sup>-</sup> cells that vary in the expression of CD15, CD124, CD66, and MHC-II, along with that of other markers (43).

##### 4.2. Cellular and molecular mechanisms of MDSC-mediated immune suppression

Lung cancer escapes the host's immune surveillance by dysregulating inflammation. Tumors and their surrounding stroma can produce growth factors, cytokines, and chemokines that recruit, expand, and activate MDSCs. Teixeira and colleagues (44) noted an increased number of MDSCs infiltrating lung cancer in mice. Another study confirmed the hypothesis that activating immune cells through disruption of MDSC-mediated immune suppression would promote antitumor immunity in a murine model of lung cancer (45). MDSCs can suppress immune responses to newly displayed tumor antigens and promote tumor progression; they may even contribute to the metastasis

of the tumor. There are several mechanisms by which MDSCs suppress antitumor responses. MDSCs restrain T cell functions in tumor tissues and draining lymph nodes by using two enzymes involved in L-arginine metabolism: arginase-1 (ARG-1), which depletes the milieu of L-arginine, and induced nitric oxide synthase 2 (iNOS2), which generates nitric oxide (NO) (46,47).

Reactive oxygen species (ROS) are physiologically produced by activated neutrophils and macrophages as mediators of innate immunity. In chronic inflammation, ROS can weaken T cell responses by affecting CD3- $\zeta$  expression. H<sub>2</sub>O<sub>2</sub> produced either by tumor-associated macrophages or by neutrophils isolated from the synovial fluid of patients with rheumatoid arthritis has been found to substantially decrease T cell proliferation *in vitro* (48,49). ROS can also affect the affinity of Ag-specific TCR, which may explain the specificity of the tolerance induced by Gr-1<sup>+</sup>CD11b<sup>+</sup> MDSCs. In addition to the effect of ARG-1 and ROS, MDSCs may affect CD8<sup>+</sup> T cells via the inhibitory molecules B7-H1 and B7-H4 (50). In addition, MDSCs can enhance immune suppression by directly inducing Tregs through the production of IL-10 and TGF- $\beta$  or ARG (51,52). Tregs actively down-regulate the activation and expansion of antitumor-reactive T cells (53) and NK cells (54). Cysteine, another essential amino acid for T-cell activation, is depleted by MDSCs (55).

### 5. The role of macrophages in lung cancer

Macrophages are among the most abundant normal cells in the tumor microenvironment and play a central role in tissue repair and remodeling during homeostasis and stress response. They are the first line of defense against pathogens. These cells originate from bone marrow precursors and extravasate into normal tissues, where they acquire distinct morphological and functional properties directed by local tissue and the immunological microenvironment (56).

Tumor-associated macrophages (TAMs) have complex dual functions in their interaction with neoplastic cells, and evidence suggests that they are part of inflammatory circuits that promote tumor progression (57). This is evidence that macrophages, rather than being tumoricidal as suggested after their activation *in vitro* (58), have a pro-tumoral phenotype *in vivo* both at the primary site and at metastases (59). Indeed, macrophages are polarized into a pro-tumoral phenotype when lung cancer develops (60).

Macrophages are exceptionally diverse in their functions, reflecting their different origins, differing local environments, and different responses to challenges (61). Given the function of macrophages in immunity, two classes of macrophages have been proposed: *i*) activated macrophages responding to IFN- $\gamma$ , TNF- $\alpha$ , and Toll-like receptor 4 (TLR4) activation are involved in Th1-type responses since Th1 cells are capable of

killing pathogens via mechanisms just like iNOS, and *ii*) alternatively activated macrophages that differentiate in response to IL-4 and IL-13 are involved in Th2-type responses, including humoral immunity and wound healing (62). Mills and colleagues called these states M1 (activated) and M2 (alternatively activated). M1 activity inhibits cell proliferation and causes tissue damage, while M2 activity promotes cell proliferation and tissue repair (63). These descriptions suggest that TAMs could either suppress (M1) or promote (M2) tumor development and progression (64). However, such definitions are limited and may not be applicable to the complex tumor microenvironment (65). In contrast to this dualistic M1/M2 definition, TAMs include several distinct populations that often share characteristics of both types but with greater overall similarity to macrophages involved in developmental processes (66,67).

### 6. The role of NK cells in lung cancer

NK cells can mediate several effector functions: *i*) direct cytotoxicity, including exocytosis of cytotoxic granules containing perforin and granzyme B, *ii*) up-regulation of death receptor ligand expression and the engagement of cognate death receptors on target cells, which can lead to apoptosis of target cells, *iii*) NK cells produce an array of immune-active cytokines, including IFN- $\gamma$ , TNF- $\alpha$ , and GM-CSF (68), which places them at the crossroads of innate and adaptive immunity. They also augment monoclonal antibody activity through antibody-mediated cellular cytotoxicity and can be transfected with chimeric antigen receptors (69), and *iv*) NK cells can release other soluble mediators, such as PGE<sub>2</sub>, that shape the responses of the immune system. A clear correlation between NK cell activity in the lungs and tumor cell clearance from the lungs has been reported in both mouse and human studies (70). Manuela and colleagues found that depletion of NK cells resulted in an increase in the number of tumor nodes in lung cancer, suggesting that NK cells are key to the control of the growth of lung cancer. A study has found that the activity of NK cells is key to the development or rejection of MHC-I-deficient lymphomas (71) while another study found that depletion of NK cells promoted the growth of fibrosarcomas (72). In addition, a recent study found that the development of lung cancer depends on the function of NK cells. Kreisel and colleagues found that depletion of NK cells promoted urethane-induced lung tumor growth in a mouse strain that is normally not susceptible to lung cancer (73).

### 7. The role of dendritic cells in lung cancer

Antigen-presenting cells can deliver TAA and prime TAA-specific T cells. DCs are professional antigen-presenting cells and can express MHC-I and MHC-II molecules and costimulatory molecules on their

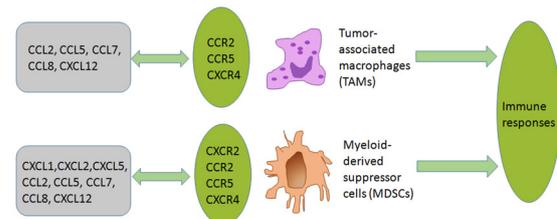
cell surface, all of which assist in T-cell activation (74). There are several cell types capable of antigen presentation in the lung, including resident DCs (75).

Evidence indicates that DCs play a significant role in induction of antitumour immunity (76). Immunotherapy with DCs co-cultured with cytokine-induced killer cells (DC-CIK) has been widely studied and might be a new strategy for treatment of NSCLC. Studies have indicated the efficacy and the safety of DC-CIK immunotherapy (77). When immature in peripheral tissue, DCs express different chemokine receptors, such as CC chemokine receptor 1 (CCR1), CCR2, CCR4, CCR5, CCR6, CCR8, and CXC chemokine receptor 4 (CXCR4) (76). When they encounter exogenous and endogenous antigens including tumor cell-derived antigens around a tumor, DCs can capture those antigens and they can mature in response to inflammatory stimuli such as toll-like receptor-mediated signals. Mature DCs start to express chemokine receptors such as CCR7 and CXCR4. Guided by chemokines, DCs enter the T-cell areas of regional lymph nodes (78).

## 8. The important role of chemokines in lung carcinoma

When considering the role of tumor-infiltrating immune cells in the lung cancer microenvironment, one cannot ignore the importance of chemokines, which recruit immune cells to the tumor and also regulate their phenotypes and functions. Relatively recent studies have revealed that chemokines regulate the movement of a wide variety of immune cells including lymphocytes, NK cells, and DCs in both physiological and pathological conditions (79). These features mean that chemokines play crucial roles in immune responses. Chemokines function biologically by linking to their corresponding receptors, which are 7-transmembrane G protein-coupled receptors (GPCR) (80). Chemokines are structurally divided into 4 subgroups: CXC, CC, CX3C, and C (81).

TAMs are derived mostly from circulating monocytes that are attracted to tumor sites by locally produced chemotactic factors, such as CCL2, CCL5, CCL7, CCL8, and CXCL12, and macrophage colony stimulating factor (M-CSF). CCL2 is presumed to play an important role in TAM recruitment (82). MDSCs can be recruited by CCL2 in several types of mouse cancers, such as Lewis lung cancer, meth A sarcoma, melanoma, and lymphoma (83). MDSCs express CXCR2 and are detected in abundance in NSCLC (84). CXCR2 ligands, including CXCL1, CXCL2, and CXCL5, are abundant in lung cancer tissue and tumorigenesis can be markedly inhibited by a deficiency in CXCR2 as a result of inhibiting infiltration of MDSCs (Figure 2). NK cells migrate to lymph nodes mainly by utilizing CXCR3, while their migration to inflamed tissues, including tumor sites, involves CCR1, CCR2, CCR5, CXCR3, and CX3CR1 (85). Thus, the ligands for these receptors



**Figure 2. Effects of chemokines on TAMs and MDSCs.** TAMs are derived mostly from circulating monocytes which are attracted to tumor sites by locally produced chemotactic factors including CCL2, CCL5, CCL7, CCL8, and CXCL12. MDSCs express CXCR2 and can be recruited to a tumor site by CXCR2 ligands like CXCL1, CXCL2, and CXCL5. MDSCs can also be recruited by CCL2, CCL5, CCL7, CCL7, CCL8, and CXCL12 in lung cancer.

can regulate the migration of NK cells and may enhance their functions. Treg cells express CCR4, and its ligand, CCL22, regulates intratumoral infiltration of Tregs in various tumors (86).

Lung stroma cells can produce abundant chemokines, such as CXCL1, CXCL2, CXCL5, CXCL9, CXCL10, and CXCL11. CXCL10 is an important chemokine responsible for the recruitment and localization of inflammatory cells to sites of tissue damage or infection. It is bound by CXCR3, a receptor shared with CXCL4. CXCL9 and CXCL11, which are expressed on CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells, NK cells, B cells, and DCs (87,88), are responsible for their recruitment and localization to the lungs.

## 9. Conclusion

Studies have indicated the importance of tumor-infiltrating immune cells in tumor development and control and studies have sought to characterize the infiltration of a tumor by those immune cells, providing insight into the effects of tumor-infiltrating immune cells on tumor behavior. Different tumors have different immune profiles, and the current review has focused on lung cancer. There is an obvious infiltration of different types of immune cells, including NK cells, T lymphocytes, macrophages, DCs, MDSCs, and B cells, in lung cancer. A distinctive lung cancer microenvironment is created by immune cells with various functions and interactions, and this microenvironment can ultimately affect tumor progression and survival. In this process, chemokines act as a bridge since they can recruit immune cells to a tumor and they can also regulate the phenotypes and functions of those cells. Although the role of lung cancer-infiltrated immune cells has been reviewed here, the paradoxical role of tumor-infiltrating cells in lung carcinoma still needs to be studied further.

## Acknowledgements

This work was supported by grants from the Natural

Science Foundation of China (No. 81573728) and the Natural Science Foundation of Shandong Province (No. ZR2015HM007).

## References

- Mendes F, Antunes C, Abrantes AM, Gonçalves AC, Nobre-Gois I, Sarmiento AB, Botelho MF, Rosa MS. Lung cancer: The immune system and radiation. *Br J Biomed Sci.* 2015; 72:78-84.
- Chapman AM, Sun KY, Ruestow P, Cowan DM, Madl AK. Lung cancer mutation profile of EGFR, ALK, and KRAS: Meta-analysis and comparison of never and ever smokers. *Lung Cancer.* 2016; 102:122-134.
- Tyl M, Domagała-Kulawik J. Lung cancer and COPD - Growing clinical problem. *Pol Merkur Lekarski.* 2017; 43:5-9.
- Skowronek J. Brachytherapy in the treatment of lung cancer - A valuable solution. *J Contemp Brachytherapy.* 2015; 7:297-311.
- Bustamante Alvarez JG, González-Cao M, Karachaliou N, Santarpia M, Viteri S, Teixidó C, Rosell R. Advances in immunotherapy for treatment of lung cancer. *Cancer Biol Med.* 2015; 12:209-222.
- Ohue Y, Kurose K, Nozawa R, Isobe M, Nishio Y, Tanaka T, Doki Y, Hori T, Fukuoka J, Oka M, Nakayama E. Survival of lung adenocarcinoma patients predicted from expression of PD-L1, galectin-9, and XAGE1 (GAGED2a) on tumor cells and tumor-infiltrating T cells. *Cancer Immunol Res.* 2016; 4:1049-1060.
- Domingues P, González-Tablas M, Otero Á, Pascual D, Miranda D, Ruiz L, Sousa P, Ciudad J, Gonçalves JM, Lopes MC, Orfao A, Tabernero MD. Tumor infiltrating immune cells in gliomas and meningiomas. *Brain Behav Immun.* 2016; 53:1-15.
- Geng Y, Shao Y, He W, Hu W, Xu Y, Chen J, Wu C, Jiang J. Prognostic role of tumor-infiltrating lymphocytes in lung cancer: A meta-analysis. *Cell Physiol Biochem.* 2015; 37:1560-1571.
- Hiraoka K, Miyamoto M, Cho Y, Suzuoki M, Oshikiri T, Nakakubo Y, Itoh T, Ohbuchi T, Kondo S, Katoh H. Concurrent infiltration by CD8+ T cells and CD4+ T cells is a favourable prognostic factor in non-small-cell lung carcinoma. *Br J Cancer.* 2006; 94:275-280.
- Naito Y, Saito K, Shiiba K, Ohuchi A, Saigenji K, Nagura H, Ohtani H. CD8+ T cells infiltrated within cancer cell nests as a prognostic factor in human colorectal cancer. *Cancer Res.* 1998; 58:3491-3494.
- Ibrahim EM, Al-Foheidi ME, Al-Mansour MM, Kazkaz GA. The prognostic value of tumor-infiltrating lymphocytes in triple-negative breast cancer: A meta-analysis. *Breast Cancer Res Treat.* 2014; 148:467-476.
- Rathore AS, Kumar S, Konwar R, Makker A, Negi MP, Goel MM. CD3+, CD4+ & CD8+ tumour infiltrating lymphocytes (TILs) are predictors of favourable survival outcome in infiltrating ductal carcinoma of breast. *Indian J Med Res.* 2014; 140:361-369.
- Rahbar M, Naraghi ZS, Mardanpour M, Mardanpour N. Tumor-infiltrating CD8+ lymphocytes effect on clinical outcome of muco-cutaneous melanoma. *Indian J Dermatol.* 2015; 60:212.
- Hu WH, Miyai K, Cajas-Monson LC, Luo L, Liu L, Ramamoorthy SL. Tumor-infiltrating CD8+ T lymphocytes associated with clinical outcome in anal squamous cell carcinoma. *J Surg Oncol.* 2015; 112:421-426.
- Oguejiofor K, Hall J, Slater C, Betts G, Hall G, Slevin N, Dovedi S, Stern PL, West CM. Stromal infiltration of CD8 T cells is associated with improved clinical outcome in HPV-positive oropharyngeal squamous carcinoma. *Br J Cancer.* 2015; 113:886-893.
- Sznurkowski JJ, Zawrocki A, Emerich J, Biernat W. Prognostic significance of CD4+ and CD8+ T cell infiltration within cancer cell nests in vulvar squamous cell carcinoma. *Int J Gynecol Cancer.* 2011; 21:717-721.
- Wang L, Song Y, Men X. Variance of TNFAIP8 expression between tumor tissues and tumor-infiltrating CD4+ and CD8+ T cells in non-small cell lung cancer. *Tumour Biol.* 2014; 35:2319-2325.
- Weaver CT, Hatton RD. Interplay between the T<sub>H</sub>17 and T<sub>Reg</sub> cell lineages: A (co-)evolutionary perspective. *Nat Rev Immunol.* 2009; 9:883-889.
- Sallusto F, Lanzavecchia A. Heterogeneity of CD4+ memory T cells: Functional modules for tailored immunity. *Eur J Immunol.* 2009; 39:2076-2082.
- Meng M, Li C, Yang F, Chen H, Li X, Yang Y, Chen D. Novel immunostimulators with a thiazolidin-4-one ring promote the immunostimulatory effect of human iNKT cells on the stimulation of Th2-like immune responsiveness via GATA3 activation *in vitro*. *Int Immunopharmacol.* 2016; 39:353-358.
- Bredo G, Storie J, Shrestha Palikhe N, Davidson C, Adams A, Vliagoftis H, Cameron L. Interleukin-25 initiates Th2 differentiation of human CD4+ T cells and influences expression of its own receptor. *Immun Inflamm Dis.* 2015; 3:455-468.
- Sadeghi S, Sanati MH, Taghizadeh M, Mansouri P, Jadali Z. Study of Th1/Th2 balance in peripheral blood mononuclear cells of patients with alopecia areata. *Acta Microbiol Immunol Hung.* 2015; 62:275-285.
- Guo L, Huang Y, Chen X, Hu-Li J, Urban JF Jr, Paul WE. Innate immunological function of TH2 cells *in vivo*. *Nat Immunol.* 2015; 16:1051-1059.
- Ito N, Nakamura H, Tanaka Y, Ohgi S. Lung carcinoma: analysis of T helper type 1 and 2 cells and T cytotoxic type 1 and 2 cells by intracellular cytokine detection with flow cytometry. *Cancer.* 1999; 85:2359-2367.
- Quintana FJ. Old dog, new tricks: IL-6 cluster signaling promotes pathogenic TH17 cell differentiation. *Nat Immunol.* 2016; 18:8-10.
- Peters A, Fowler KD, Chalmin F, Merkler D, Kuchroo VK, Pot C. IL-27 induces Th17 differentiation in the absence of STAT1 signaling. *J Immunol.* 2015; 195:4144-4153.
- Martin-Orozco N, Muranski P, Chung Y, Yang XO, Yamazaki T, Lu S, Hwu P, Restifo NP, Overwijk WW, Dong C. T helper 17 cells promote cytotoxic T cell activation in tumor immunity. *Immunity.* 2009; 31:787-798.
- Llosa NJ, Geis AL, Thiele Orberg E, Housseau F. Interleukin-17 and type 17 helper T cells in cancer management and research. *Immunotargets Ther.* 2014; 3:39-54.
- Jones SA, Sutton CE, Cua D, Mills KH. Therapeutic potential of targeting IL-17. *Nat Immunol.* 2012; 13:1022-1025.
- Li S, Fan Q, He S, Tang T, Liao Y, Xie J. MicroRNA-21 negatively regulates Treg cells through a TGF-β1/Smad-independent pathway in patients with coronary heart

- disease. *Cell Physiol Biochem*. 2015; 37:866-878.
31. Lee YJ, Hyung KE, Yoo JS, Jang YW, Kim SJ, Lee DI, Lee SJ, Park SY, Jeong JH, Hwang KW. Effects of exposure to extremely low-frequency electromagnetic fields on the differentiation of Th17 T cells and regulatory T cells. *Gen Physiol Biophys*. 2016; 35:487-495.
  32. Zhou L, Ivanov II, Spolski R, Min R, Shenderov K, Egawa T, Levy DE, Leonard WJ, Littman DR. IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. *Nat Immunol*. 2007; 8:967-974.
  33. Zhou L, Lopes JE, Chong MM, Ivanov II, Min R, Victora GD, Shen Y, Du J, Rubtsov YP, Rudensky AY, Ziegler SF, Littman DR. TGF-beta-induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing RORgamma function. *Nature*. 2008; 453:236-240.
  34. Noack M, Miossec P. Th17 and regulatory T cell balance in autoimmune and inflammatory diseases. *Autoimmun Rev*. 2014; 13:668-677.
  35. Ganesan AP, Johansson M, Ruffell B, Yagui-Beltrán A, Lau J, Jablons DM, Coussens LM. Tumor-infiltrating regulatory T cells inhibit endogenous cytotoxic T cell responses to lung adenocarcinoma. *J Immunol*. 2013; 191:2009-2017.
  36. Arellano B, Graber DJ, Sentman CL. Regulatory T cell-based therapies for autoimmunity. *Discov Med*. 2016; 22:73-80.
  37. Wei T, Zhang J, Qin Y, Wu Y, Zhu L, Lu L, Tang G, Shen Q. Increased expression of immunosuppressive molecules on intratumoral and circulating regulatory T cells in non-small-cell lung cancer patients. *Am J Cancer Res*. 2015; 5:2190-2201.
  38. Atrerkhany KN, Drutskaya MS. Myeloid-derived suppressor cells and proinflammatory cytokines as targets for cancer therapy. *Biochemistry (Mosc)*. 2016; 81:1274-1283.
  39. Ballbach M, Hall T, Brand A, Neri D, Singh A, Schaefer I, Herrmann E, Hansmann S, Handgretinger R, Kuemmerle-Deschner J, Hartl D, Rieber N. Induction of myeloid-derived suppressor cells in cryopyrin-associated periodic syndromes. *J Innate Immun*. 2016; 8:493-506.
  40. Srivastava MK, Andersson Å, Zhu L, Harris-White M, Lee JM, Dubinett S, Sharma S. Myeloid suppressor cells and immune modulation in lung cancer. *Immunotherapy*. 2012; 4:291-304.
  41. Condamine T, Gabrilovich DI. Molecular mechanisms regulating myeloid-derived suppressor cell differentiation and function. *Trends Immunol*. 2011; 32:19-25.
  42. Qu P, Wang LZ, Lin PC. Expansion and functions of myeloid-derived suppressor cells in the tumor microenvironment. *Cancer Lett*. 2016; 380:253-256.
  43. Meirou Y, Kanterman J, Baniyash M. Paving the road to tumor development and spreading: Myeloid-derived suppressor cells are ruling the fate. *Front Immunol*. 2015; 6:523.
  44. Teixeira D, Almeida JS, Visniauskas B, Gomes GN, Hirata AE, Bueno V. Myeloid-derived suppressor cells and associated events in urethane-induced lung cancer. *Clinics (Sao Paulo)*. 2013; 68:858-864.
  45. Srivastava MK, Zhu L, Harris-White M, Kar UK, Huang M, Johnson MF, Lee JM, Elashoff D, Strieter R, Dubinett S, Sharma S. Myeloid suppressor cell depletion augments antitumor activity in lung cancer. *PLoS One*. 2012; 7:e40677.
  46. Gato-Cañas M, Martínez de Morentin X, Blanco-Luquín I, Fernández-Irigoyen J, Zudaire I, Liechtenstein T, Arasanz H, Lozano T, Casares N, Chaikuad A, Knapp S, Guerrero-Setas D, Escors D, Kochan G, Santamaría E. A core of kinase-regulated interactomes defines the neoplastic MDSC lineage. *Oncotarget*. 2015; 6:27160-27175.
  47. Otsubo D, Yamashita K, Fujita M, Nishi M, Kimura Y, Hasegawa H, Suzuki S, Kakeji Y. Early-phase treatment by low-dose 5-fluorouracil or primary tumor resection inhibits MDSC-mediated lung metastasis formation. *Anticancer Res*. 2015; 35:4425-4431.
  48. Otsuji M, Kimura Y, Aoe T, Okamoto Y, Saito T. Oxidative stress by tumor-derived macrophages suppresses the expression of CD3 zeta chain of T-cell receptor complex and antigen-specific T-cell responses. *Proc Natl Acad Sci U S A*. 1996; 93:13119-13124.
  49. Liu YK, Yang HW, Wang MH, Wang W, Liu F, Yang HL. N-acetylcysteine attenuates cobalt nanoparticle-induced cytotoxic effects through inhibition of cell death, reactive oxygen species-related signaling and cytokines expression. *Orthop Surg*. 2016; 8:496-502.
  50. Chou HS, Hsieh CC, Charles R, Wang L, Wagner T, Fung JJ, Qian S, Lu LL. Myeloid-derived suppressor cells protect islet transplants by B7-H1 mediated enhancement of T regulatory cells. *Transplantation*. 2012; 93:272-282.
  51. Rastad JL, Green WR. Myeloid-derived suppressor cells in murine AIDS inhibit B-cell responses in part via soluble mediators including reactive oxygen and nitrogen species, and TGF-β. *Virology*. 2016; 499:9-22.
  52. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. *Nat Rev Immunol*. 2012; 12:253-268.
  53. Enokida T, Nishikawa H. Regulatory T cells, as a target in anticancer immunotherapy. *Immunotherapy*. 2017; 9:623-627.
  54. Fan R, Xiang Y, Yang L, Liu Y, Chen P, Wang L, Feng W, Yin K, Fu M, Xu Y, Wu J. Impaired NK cells' activity and increased numbers of CD4 + CD25+ regulatory T cells in multidrug-resistant Mycobacterium tuberculosis patients. *Tuberculosis (Edinb)*. 2016; 98:13-20.
  55. Edgington-Mitchell LE, Rautela J, Duivenvoorden HM, Jayatilake KM, van der Linden WA, Verdoes M, Bogoy M, Parker BS. Cysteine cathepsin activity suppresses osteoclastogenesis of myeloid-derived suppressor cells in breast cancer. *Oncotarget*. 2015; 6:27008-27022.
  56. Panni RZ, Linehan DC, DeNardo DG. Targeting tumor-infiltrating macrophages to combat cancer. *Immunotherapy*. 2013; 5:1075-1087.
  57. Liu Y, Cao X. The origin and function of tumor-associated macrophages. *Cell Mol Immunol*. 2015; 12:1-4.
  58. Fidler IJ. Macrophage therapy of cancer metastasis. *Ciba Found Symp*. 1988; 141:211-222.
  59. Biswas SK, Allavena P, Mantovani A. Tumor-associated macrophages: Functional diversity, clinical significance, and open questions. *Semin Immunopathol*. 2013; 35:585-600.
  60. Huang WC, Chan ML, Chen MJ, Tsai TH, Chen YJ. Modulation of macrophage polarization and lung cancer cell stemness by MUC1 and development of a related small-molecule inhibitor pterostilbene. *Oncotarget*. 2016; 7:39363-39375.
  61. Wynn TA, Chawla A, Pollard JW. Macrophage biology in development, homeostasis and disease. *Nature*. 2013; 496:445-455.
  62. Roy S, Schmeier S, Arner E, et al. Redefining the transcriptional regulatory dynamics of classically and

- alternatively activated macrophages by deepCAGE transcriptomics. *Nucleic Acids Res.* 2015; 43:6969-6982.
63. Mills CD. M1 and M2 macrophages: Oracles of health and disease. *Crit Rev Immunol.* 2012; 32:463-488.
  64. Almendros I, Gileles-Hillel A, Khalyfa A, Wang Y, Zhang SX, Carreras A, Farré R, Gozal D. Adipose tissue macrophage polarization by intermittent hypoxia in a mouse model of OSA: Effect of tumor microenvironment. *Cancer Lett.* 2015; 361:233-239.
  65. Qian BZ, Pollard JW. Macrophage diversity enhances tumor progression and metastasis. *Cell.* 2010; 141:39-51.
  66. Zhang ZM, Yang Z, Zhang Z. Distribution and characterization of tumor-associated macrophages/microglia in rat C6 glioma. *Oncol Lett.* 2015; 10:2442-2446.
  67. Zhang J, Yao H, Song G, Liao X, Xian Y, Li W. Regulation of epithelial-mesenchymal transition by tumor-associated macrophages in cancer. *Am J Transl Res.* 2015; 7:1699-1711.
  68. Li J, Dong X, Zhao L, Wang X, Wang Y, Yang X, Wang H, Zhao W. Natural killer cells regulate Th1/Treg and Th17/Treg balance in chlamydial lung infection. *J Cell Mol Med.* 2016; 20:1339-1351.
  69. Klingemann HG. Cellular therapy of cancer with natural killer cells-Where do we stand? *Cytotherapy.* 2013; 15:1185-1194.
  70. Al Omar SY, Marshall E, Middleton D, Christmas SE. Increased killer immunoglobulin-like receptor expression and functional defects in natural killer cells in lung cancer. *Immunology.* 2011; 133:94-104.
  71. Kärre K, Ljunggren HG, Piontek G, Kiessling R. Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defence strategy. *J Immunol.* 2005; 174:6566-6569.
  72. Smyth MJ, Crowe NY, Godfrey DI. NK cells and NKT cells collaborate in host protection from methylcholanthrene-induced fibrosarcoma. *Int Immunol.* 2001; 13:459-463.
  73. Kreisel D, Gelman AE, Higashikubo R, Lin X, Vikis HG, White JM, Toth KA, Deshpande C, Carreno BM, You M, Taffner SM, Yokoyama WM, Bui JD, Schreiber RD, Krupnick AS. Strain-specific variation in murine natural killer gene complex contributes to differences in immunosurveillance for urethane-induced lung cancer. *Cancer Res.* 2012; 72:4311-4317.
  74. Sabado RL, Balan S, Bhardwaj N. Dendritic cell-based immunotherapy. *Cell Res.* 2017; 27:74-95.
  75. Desch AN, Henson PM, Jakubzick CV. Pulmonary dendritic cell development and antigen acquisition. *Immunol Res.* 2013; 55:178-186.
  76. Palucka K, Banchereau J. Cancer immunotherapy via dendritic cells. *Nat Rev Cancer.* 2012; 12:265-277.
  77. Wang S, Wang Z. Efficacy and safety of dendritic cells co-cultured with cytokine-induced killer cells immunotherapy for non-small-cell lung cancer. *Int Immunopharmacol.* 2015; 28:22-28.
  78. Sozzani S. Dendritic cell trafficking: more than just chemokines. *Cytokine Growth Factor Rev.* 2005; 16:581-592.
  79. Liu J, Li F, Ping Y, Wang L, Chen X, Wang D, Cao L, Zhao S, Li B, Kalinski P, Thorne SH, Zhang B, Zhang Y. Local production of the chemokines CCL5 and CXCL10 attracts CD8<sup>+</sup> T lymphocytes into esophageal squamous cell carcinoma. *Oncotarget.* 2015; 6:24978-24989.
  80. Moser B, Wolf M, Walz A, Loetscher P. Chemokines: Multiple levels of leukocyte migration control. *Trends Immunol.* 2004; 25:75-84.
  81. Zlotnik A, Yoshie O. The chemokine superfamily revisited. *Immunity.* 2012; 36:705-716.
  82. Sica A, Allavena P, Mantovani A. Cancer related inflammation: The macrophage connection. *Cancer Lett.* 2008; 267:204-215.
  83. Lazenec G, Richmond A. Chemokines and chemokine receptors: New insights into cancer-related inflammation. *Trends Mol Med.* 2010; 16:133-144.
  84. Spaks A. Role of CXC group chemokines in lung cancer development and progression. *J Thorac Dis.* 2017; 9:S164-S171.
  85. Walzer T, Vivier E. G-protein-coupled receptors in control of natural killer cell migration. *Trends Immunol.* 2011; 32:486-492.
  86. Smyth MJ, Ngiow SF, Teng MW. Targeting regulatory T cells in tumor immunotherapy. *Immunol Cell Biol.* 2014; 92:473-474.
  87. Kohrgruber N, Gröger M, Meraner P, Kriehuber E, Petzelbauer P, Brandt S, Stingl G, Rot A, Maurer D. Plasmacytoid dendritic cell recruitment by immobilized CXCR3 ligands. *J Immunol.* 2004; 173:6592-6602.
  88. Basset L, Chevalier S, Danger Y, Arshad MI, Piquet-Pellorce C, Gascan H, Samson M. Interleukin-27 and IFN $\gamma$  regulate the expression of CXCL9, CXCL10, and CXCL11 in hepatitis. *J Mol Med (Berl).* 2015; 93:1355-1367.

(Received September 1, 2017; Revised October 17, 2017; Accepted October 24, 2017)