Regulation of Tumor Immune Surveillance and Tumor Immune Subversion by TGF-β

Hae-Young Park1, Lalage M Wakefield2 and Mizuko Mamura1*

1Laboratory of Immunology, Lee Gil-Ya Cancer and Diabetes Institute, Gachon University of Medicine and Science, Incheon, Korea,
2Laboratory of Cancer Biology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA

Transforming growth factor-β (TGF-β) is a highly pleiotropic cytokine playing pivotal roles in immune regulation. TGF-β facilitates tumor cell survival and metastasis by targeting multiple cellular components. Focusing on its immunosuppressive functions, TGF-β antagonists have been employed for cancer treatment to enhance tumor immunity. TGF-β antagonists exert anti-tumor effects through #1 activating effector cells such as NK cells and cytotoxic CD8+ T cells (CTLs), #2 inhibiting regulatory/suppressor cell populations, #3 making tumor cells visible to immune cells, #4 inhibiting the production of tumor growth factors. This review focuses on the effect of TGF-β on T cells, which are differentiated into effector T cells or newly identified tumor-supporting T cells.

INTRODUCTION

Transforming growth factor-β (TGF-β) superfamily consists of a large family of structurally related cytokines, which are classified into two main groups: TGF-β/Activin and bone morphogenetic proteins (BMPs) and growth/differentiation factors (GDFs) (1).

Among them, TGF-β has been well characterized as a representative potent immunosuppressive cytokine, TGF-β has been reported to inhibit immune system by modulating both effectors and suppressors, TGF-β directly inhibits innate immune cells such as macrophages and NK cells and adoptive immune cells such as CD4+ T helper cells and CD8+ cytotoxic T cells, while expanding immunosuppressive regulatory T cells (Tregs) and inducing the recruitment of immunosuppressive myeloid-derived suppressor cells (2).

The immune surveillance system is one of the most important defense mechanisms against cancer progression. Attempts to enhance tumor immunity by various strategies have been tried as supportive anti-cancer therapies. The most potent immunosuppressive cytokine, TGF-β is massively produced and activated in tumor microenvironment, which allows invasive metastatic cancers to escape from immune surveillance (3). Thus, antagonizing TGF-β’s functions has been implicated as the most potent anti-tumor immune therapy and some TGF-β antagonists have been on preclinical and clinical trials (4-6).

PROGNOSIS AND THE SIZE/NUMBER OF METASTASIS

Tumor recurrence by remote metastasis affects the prognosis of cancer patients after radical surgery. Cancer staging is evaluated by TNM classification, which describes the size and the extent of the Tumor, degree of the spread to regional lymph Nodes and the presence of Metastasis. TNM staging is widely employed as a global standard to plan the treatments and to indicate the prognosis (7). Although there are no detailed parameters in metastasis defined in TNM staging, the size and the number of metastasis significantly affect the prognosis of the patients. In pulmonary metastasis from breast cancer, it has been reported that the size and the number of metastasis significantly correlate with prognosis and re-recurrence and the pulmonary metastasectomy is justified in case of small number of metastasis lesions (8). It has been reported that an increase in tumor size and an increase in number of re-
Regional lymph nodes with metastasis were associated with diminution in cellular immunity (9), which might be the consequence of the production of TGF-β by tumors themselves and tumor infiltrating immunosuppressive cells (10,11). Thus, one might expect that the number and the size of tumor and metastasis could be reduced by blocking TGF-β to achieve better prognosis.

**TGF-β AND TUMOR IMMUNE SURVEILLANCE-AFFECTING NUMBER OF METASTASIS**

Although some tumors express tumor specific antigens, which can be recognized by immune system, tumor specific immune responses often fail to eradicate tumors because tumors evade immune surveillance by variety of strategies. Tumors themselves, tumor stromal cells and the myeloid-derived suppressor cells produce large amount of TGF-β, which is activated in the tumor microenvironment (12-14). By its potent immunosuppressive effects, TGF-β strongly inhibits anti-tumor immunity.

Partly due to TGF-β, loss or down-regulation of MHC class I molecules is frequently observed in malignant cells, which makes them invisible to immune system (15). Poorly immunogenic tumors with low expression of MHC class I can escape from attack by cytotoxic CD8⁺ T cells but they become more sensitive to attack by NK cells (16), TGF-β also affects the tumor cell-NK cell interaction by modulating the expression of MHC class I homologues on the tumor cells and their receptors on NK cells. Expression of MHC class I homologues such as MHC class-I-chain-related protein A (MICA) and Rae-1γ human and murine NKG2D ligand, respectively, as well as NKG2D are reported to be down-regulated by TGF-β (17-19) Nam JS et al, demonstrated that anti-TGF-β antibody treatment up-regulated NKG2D expression on CD8⁺ T cells, not NK cells, which were already highly activated even without anti-TGF-β antibody treatment (19). Anti-TGF-β antibody treatment exerts anti-tumor efficacy by enhancing tumor-immune cell interaction (Fig. 1).

TGF-β directly suppresses functions, migration and expansion of cytotoxic CD8⁺ T cells, NK cells, NK T cells and γδ T cells, while inducing Foxp3⁺ Tregs (2). In poorly immunogenic syngeneic 4T1 mammary tumor metastasis model, TGF-β does not significantly affect the infiltration of NK cells and Foxp3⁺ Tregs, whereas depletion of CD8⁺ T cells abolished the effect of anti-TGF-β antibody treatment. Treatment with anti-TGF-β antibody increases the infiltration of acti-
Regulation of Tumor Immune Surveillance and Tumor Immune Subversion by TGF-β
Hae-Young Park, et al.

Figure 2. The role of TGF-β in subverting the CD8+ T cell response. TGF-β produced and activated in the tumor environment suppresses the immune surveillance, which mainly affects the number of metastasis by preventing the generation of cytotoxic T cells. On the other hand, TGF-β in combination with other factors, such as IL-6, subverts CD8+ T cells into IL-17 producing cells, which mainly affects the size of tumor and metastasis. IL-17 might promote survival of tumor cells in the condition with low nutrition or chemotherapy.

Figure 3. The role of TGF-β in subverting the CD4+ T cell response. TGF-β directly or indirectly suppresses cytotoxic T cells via the suppression of CD4+ Th1 cells. TGF-β, presumably in combination with other factors, subverts CD4+ T cells into Foxp3+ Tregs or Th17 cells.

over effector cell populations and suppress the effective anti-tumor immunity. Large amount of TGF-β produced and activated in tumor environment promotes the development and migration of these suppressor populations (21,22).

Recently, the novel subset of CD8+ T cells is identified, which promotes growth of primary and metastatic tumor (23). This CD8+ T cell subset is distinct from the reported CD8+ suppressor/regulatory T cells (24). IL-17 is produced by this subset, which favors tumor growth by preventing tumor apoptosis. The same combination of cytokines to induce Th17 CD4+ T cells in vitro, TGF-β and IL-6, subverts CD8+ T cells into this IL-17 producing CD8+ T cells (Fig. 2).

The importance of IL-17 in inflammatory autoimmune disorders was first addressed by Nakae S et al. (25) and the CD4+ T cell subset producing IL-17 was defined as Th17 by Stockinger B et al. (26). Although T cells infiltrated in tumors produce IL-17 (27), it has been controversial whether IL-17 promotes or inhibits tumor growth (28,29). Recently, it is reported that IL-17 produced by CD4+ T cells, not CD8+ T cells, promotes primary tumor growth through IL-6-Stat3 signaling pathway (30).

In poorly immunogenic 4T1 metastatic mammary tumor model, CD8+ T cell depletion decreases the primary tumor volume as well as metastasis size, indicating that some CD8+ T cells are not cytotoxic, but on the contrary, favor the tumor growth (23). IL-17 produced by tumor infiltrating CD8+ T cells was identified as the major soluble factor to prevent tumor cell apoptosis, and the knockdown of IL-17 response of tumor cells reduces tumor growth by enhancing apoptosis in vivo. IL-17 alone or in combination with TGF-β suppresses apoptosis of tumor cells under the condition with nutrient deprivation or the treatment with apoptosis inducer. It might not be universal phenomena for all the malignant tumors, because some tumor cell lines do not respond to IL-17 and in some tumors, IL-17 oppositely induces apoptosis. However, some patients exhibiting the up-regulation of IL-17 expression in tumor (23,27) might benefit from anti-TGF-β treatment through the same mechanism.

It has been reported that IL-23 is required for autoimmune inflammation mediated by Th17 cells in vivo. It is consistent with the results of lower numbers of IL-17+ T cells in IL-23 deficient mice. These findings suggest that IL-23-dependent signaling in Th-17 cells may depend on the transcription factor Stat3 (31).

Similarly, it is recently reported that Stat3 promotes IL-23-mediated procarcinogenic immune response while inhibiting IL-12-dependent antitumor immunity (32). However, it is not yet determined whether IL-23 is required for the development of IL-17-producing CD4+ T cells and IL-17-producing CD8+ T cells infiltrated in tumor in vivo. They also report that Stat3 is phosphorylated through IL-23 receptor to up-regulate the expression of Foxp3 and immunosuppressive cytokine, IL-10 in CD4+ T cells (Fig. 3). It is also to be de-
termined whether TGF-β is involved in this effect. The IL-17/IL-23 axis might be the new mechanism of tumor imm\nune subversion (33).

**PERSPECTIVE**

Researchers used to focus on tumor immune surveillance by effector cell populations to enhance tumor immunity. Recent accumulation of data shows the importance of regulating tu\nmor immune subversion by suppressor cell populations to re\nverse immune suppression against tumor. Antagonizing TGF-\nβ successfully enhances tumor immune surveillance and re\nverses immune subversion, which results in the decrease in\nthe number of metastasis and the size of tumor and meta\nstasy. So far, TGF-β antagonists are applied to the treatment\nof terminal stage patients of certain highly malignant cancers\nonly in clinical trials. By further confirmation of their effic\nacy and the number of metastasis, this result in the decrease in\nthe number of metastasis and the size of primary tumor and to\nthe patients before the metastasectomy to reduce the size and\nthe number of metastasis.

**ACKNOWLEDGEMENTS**

We thank Dr. Jeong-Seok Nam, Jin-Ah Park, Ji-Hyun Park and\nthe members of the Laboratory of Immunology for helpful\ndiscussions.

**CONFLICTS OF INTEREST**

The authors have no financial conflict of interest.

**REFERENCES**

1. Miyazono K, Kusanagi K, Inoue H: Divergence and con\ncvergence of TGF-beta/BMP signaling, J Cell Physiol 187:265-276, 2001

2. Li MO, Wan YY, Sunjabi S, Robertson AK, Flavell RA: Tran\nsforming growth factor-beta regulation of immune responses, Ann Rev Immunol 24:99-146, 2006

3. Roberts AB, Wakefield LM: The two faces of transforming growth factor beta in carcinogenesis, Proc Natl Acad Sci U S A 100:8621-8625, 2003

4. Yang YA, Dukhanina O, Tang B, Mamura M, Letterio JJ, MacGregor J, Patel SC, Khorin S, Liu ZY, Green J, Anver MR, Merlino G, Wakefield LM: Lifetime exposure to a solu\nable TGF-beta antagonist protects mice against metastasis without adverse side effects, J Clin Invest 109:1607-1615, 2002

5. Ge R, Rajeev V, Ray P, Lattime E, Rittling S, Medicherla S, Prother A, Murphy A, Chakravarty J, Dugar S, Schreiner G, Barnard N, Reiss M: Inhibition of growth and metastasis of mouse mammary carcinoma by selective inhibitor of transforming growth factor-beta type I receptor kinase in\nvivo, Clin Cancer Res 12:4315-4320, 2006

6. Wrzesinski SH, Wan YY, Flavell RA: Transforming growth factor-beta and the immune response: implications for anti\ncancer therapy, Clin Cancer Res 13:5262-5270, 2007

7. Denoix PF: Enquête permanent dans les centres anticance\nreaux, Bull Inst Nat Hyg 1:70-75, 1946

8. Chen F, Fujinaga T, Sato K, Sonobe M, Shoji T, Sakai H, Miyahara R, Bando T, Okubo K, Hirata T, Toi M, Dute H: Clinical features of surgical resection for pulmonary meta\nstasy from breast cancer, Eur J Surg Oncol 35:293-297, 2009

9. Humphrey LJ, Singla O, Voleneck F: Immunologic re\nsponsiveness of the breast cancer patient, Cancer 65:893-\n898, 1980

10. Ghiringhelli F, Puig PE, Roux S, Parcellier A, Schmitt E, Solary E, Kroemer G, Martin F, Chauffert B, Zitvogel L: Tumor cells convert immature myeloid dendritic cells into TGF-beta-secreting cells inducing CD4+CD25+ regulatory T cell proliferation, J Exp Med 202:919-929, 2005

11. Allegra D, Burger C, Elgert KD: Tumor-induced regulation of suppressor macrophage nitric oxide and TNF-alpha production, Role of tumor-derived IL-10, TGF-beta, and prostaglandin E2, J Immunol 153:1674-1686, 1994

12. Bierie B, Moses HL: Tumour microenvironment: TGFBeta: the molecular Jekyll and Hyde of cancer, Nat Rev Cancer 6:506-520, 2006

13. Mantovani A, Allavena P, Sica A, Balkwill F: Cancer-related inflammation. Nature 454:436-444, 2008

14. Yang L, Moses HL: Transforming growth factor beta: tumor suppressor or promoter? Are host immune cells the answer? Cancer Res 68:9107-9111, 2008

15. Geiser AG, Letterio JJ, Kullarni AB, Karlsson S, Roberts AB, Sporn MB: Transforming growth factor beta 1 (TGF-beta 1) controls expression of major histocompatibility genes in the postnatal mouse: aberrant histocompatibility antigen expression in the pathogenesis of the TGF-beta 1 null mouse phenotype, Proc Natl Acad Sci U S A 90:9944-9948, 1993

16. Ljunggren HG, Kärre K: In search of the "missing self": MHC molecules and NK cell recognition, Immunol Today 11: 237-244, 1990

17. Zwirner NW, Fuertes MB, Giart MV, Domaica CI, Rossi LE: Cytokine-driven regulation of NK cell functions in tumor immunity: role of the MICA-NKG2D system, Cytokine Growth Factor Rev 18:159-170, 2007

18. Lee JC, Lee KM, Kim DW, Heo DS: Elevated TGF-beta se\ncretion and down-modulation of NKG2D underlies im\npaired NK cytotoxicity in cancer patients, J Immunol 172:7335-7340, 2004

19. Nam JS, Tenabe M, Mamura M, Kang MJ, Chae H, Stuelten G, Kohn E, Tang B, Sahnovar H, Anver MR, Lawrence S, Danielpour D, Lorenz S, Berzofsky JA, Wakefield LM: An anti-transforming growth factor beta antibody suppresses metastasis via cooperative effects on multiple cell compart-
Regulation of Tumor Immune Surveillance and Tumor Immune Subversion by TGF-β
Hae-Young Park, et al.

20. Thomas DA, Massagué J: TGF-beta directly targets cytotoxic T cell functions during tumor evasion of immune surveillance, Cancer Cell 8;369-380, 2005
21. Yamaguchi T, Saleaguchi S: Regulatory T cells in immune surveillance and treatment of cancer, Semin Cancer Biol 16;115-123, 2006
22. Kitamura T, Kometani K, Hashida H, Matsunaga A, Miyoshi H, Hoosugi H, Aoki M, Oshima M, Hattori M, Takabayashi A, Minato N, Taketo MM: SMAD4-deficient intestinal tumors recruit CCR1+ myeloid cells that promote invasion, Nat Genet 39;467-475, 2007
23. Nam JS, Terabe M, Kang MJ, Chae H, Voong N, Yang YA, Laurence A, Michalowska A, Mamura M, Lonning S, Berzofsky JA, Wakefield LM: Transforming growth factor beta subverts the immune system into directly promoting tumor growth through interleukin-17, Cancer Res 68;3915-3923, 2008
24. Kapp JA, Bucy RP: CD8+ suppressor T cells resurrected, Hum Immunol 69;715-720, 2008
25. Nakae S, Komiyama Y, Nambu A, Sudo K, Iwase M, Homma I, Sekikawa K, Asano M, Iwakura Y: Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses, Immunity 17;375-387, 2002
26. Veldhoen M, Hocking RJ, Atkins CJ, Lodgesley RM, Stockinger B: TGF beta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells, Immunity 24;179-189, 2006
27. Miyahara Y, Odanski K, Chen W, Peng G, Matsuzaki J, Wang RF: Generation and regulation of human CD4+ IL-17-producing T cells in ovarian cancer, Proc Natl Acad Sci U S A 105;15505-15510, 2008
28. Numasaki M, Fukushima J, Ono M, Narula SK, Zavodny PJ, Kudo T, Robbins PD, Tahara H, Lotze MT: Interleukin-17 promotes angiogenesis and tumor growth, Blood 101;2620-2627, 2003
29. Kryczek I, Wei S, Szela H, Vatan L, Zou W: Endogenous IL-17 contributes to reduced tumor growth and metastasis, Blood 114;357-359, 2009
30. Wang L, Yi T, Kortylewski M, Pardoll DM, Zeng D, Yu H: IL-17 can promote tumor growth through an IL-6-Stat3 signaling pathway, J Exp Med 206;1457-1464, 2009
31. McGeachy MJ, Chen Y, Tait CM, Laurence A, Joyce-Shaikh B, Blumenschein WM, McClanahan TK, O'Shea JI, Gua DJ: The interleukin 23 receptor is essential for the terminal differentiation of interleukin 17-producing effector T helper cells in vivo, Nat Immunol 10;314-324, 2009
32. Kortylewski M, Xin H, Kujawski M, Lee H, Liu Y, Harris T, Drake C, Pardoll D, Yu H: Regulation of the IL-23 and IL-12 balance by Stat3 signaling in the tumor microenvironment, Cancer Cell 15;114-123, 2009
33. Martin-Orozco N, Dong C: The IL-17/IL-23 axis of inflammation in cancer: friend or foe? Curr Opin Investig Drugs 10;543-549, 2009