OncoImmunology 2:5, e24036; May 2013; © 2013 Landes Bioscience

Prognostic value of the immune microenvironment in lung adenocarcinoma

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Keywords: FOXP3, interleukin-7 receptor, interleukin-12 receptor, lung adenocarcinoma

We analyzed the immune microenvironment in the neoplastic and stromal components of Stage I lung adenocarcinoma lesions, finding that a high ratio of tumor-infiltrating FOXP3$^+$ regulatory T cells to CD3$^+$ lymphocytes, an elevated expression of the interleukin-7 receptor, as well as a reduced expression of the interleukin-12 receptor β2 all constitute independent factors of poor prognosis.

Both the prognostic evaluation and the therapeutic management of lung adenocarcinoma patients currently rely on the tumor node metastasis staging system. Nevertheless, clinical outcomes after surgical resection are often unfavorable, even among patients presenting with early-stage disease. Accumulating evidence indicates that the tumor immune microenvironment constitutes a robust prognostic indicator. Thus, in some solid tumors, high levels of tumor-infiltrating CD4$^+$ helper, CD8$^+$ cytotoxic and CD45RO$^+$ memory T lymphocytes have been associated with favorable clinical outcomes.[1,2] Conversely, a high density of tumor-infiltrating FOXP3$^+$ regulatory T cells (Tregs) often represents an unfavorable prognostic factor.[3] FOXP3$^+$ Tregs are potent suppressors of adaptive antitumor immune responses and hence may sustain invasiveness and metastatic colonization.

Recently, in two large independent cohorts of patients affected by Stage I lung adenocarcinoma (total n = 956), we found that a high relative proportion of FOXP3$^+$ Tregs to CD3$^+$ lymphocytes infiltrating the tumor stroma is an independent factor of poor prognosis (**Fig. 1A**).[4] Among the tumors infiltrated by a high density of FOXP3$^+$ Tregs, high levels of CD3$^+$ T cells were associated with better clinical outcomes than relatively scarce CD3$^+$ T-cell infiltration. This finding suggests that the relative intensity of pro- and anti-tumor immune responses, as well as the type and density of tumor-infiltrating immune cells, constitute important prognostic markers.

In addition to tumor-infiltrating immune cells, we investigated the expression of genes involved in innate and adaptive immunity (including those coding for chemokines and their receptors), and some of these markers also turned out to convey a prognostic value.[5] In particular, we identified two independent prognostic factors in samples from patients affected by Stage I lung adenocarcinoma: the β2 subunit of the interleukin (IL)-12 receptor (IL-12Rβ2), which appears to mediate an antitumor effect, and the interleukin-7 receptor (IL-7R), which—conversely—seems to exert pro-tumor functions. IL-12Rβ2 is normally expressed by the lung epithelium, and its loss has been shown to correlate with the development of lung cancer in a mouse model.[6] Accordingly, the administration of IL-12 directly inhibits the growth of human IL-12R$^+$ lung adenocarcinoma cells in vitro and in vivo, an effect that is accompanied by the inhibition of angiogenesis following the downregulation of genes coding for angiogenic factors such as vascular endothelial growth factor (VEGF) C, VEGFD and IL-6.[7] In addition, IL-12 stimulates the secretion of interferon γ and the expression of cytotoxic proteins by T cells and limits the immunosuppressive functions of Tregs, resulting in increased cytotoxic T lymphocyte responses. Pegram et al. reported that IL-12$^+$ T cells retain cytotoxic capacity even in the presence of Tregs in vitro.[8] Interestingly, in our study, patients exhibiting high intratumoral expression levels of IL-12Rβ2 had favorable prognoses, as compared with patients whose lesions expressed low IL-12Rβ2 amounts (**Fig. 1B**). This effect persisted in a subset of patients whose tumors were infiltrated by a high relative proportion of Tregs to CD3$^+$ T cells.[4] This suggests that, even in the presence of an unfavorable immune microenvironment, elevated expression levels of IL-12Rβ2 may mediate antitumor effects. Thus, the administration of IL-12, especially to patients bearing IL-12R$^+$ tumors, may decrease tumor aggressiveness, control angiogenesis and limit the immunosuppressive effects of Tregs.

IL-7R is expressed by naïve and memory resting T cells and plays a central role in T-cell development and survival.[9] IL-7 is produced by stromal and epithelial cells in the bone marrow and the thymus, as well as by a variety of tumor cells. In lung cancer, IL-7 and IL-7R are co-expressed by tumor cells, and the IL-7/IL-7R signaling

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Submitted: 02/15/13; Accepted: 02/17/13
Citation: Kadota K, Nitadori J, Adusumilli PS. Prognostic value of the immune microenvironment in lung adenocarcinoma. OncoImmunology 2013; 2:e24036; http://dx.doi.org/10.4161/onci.24036

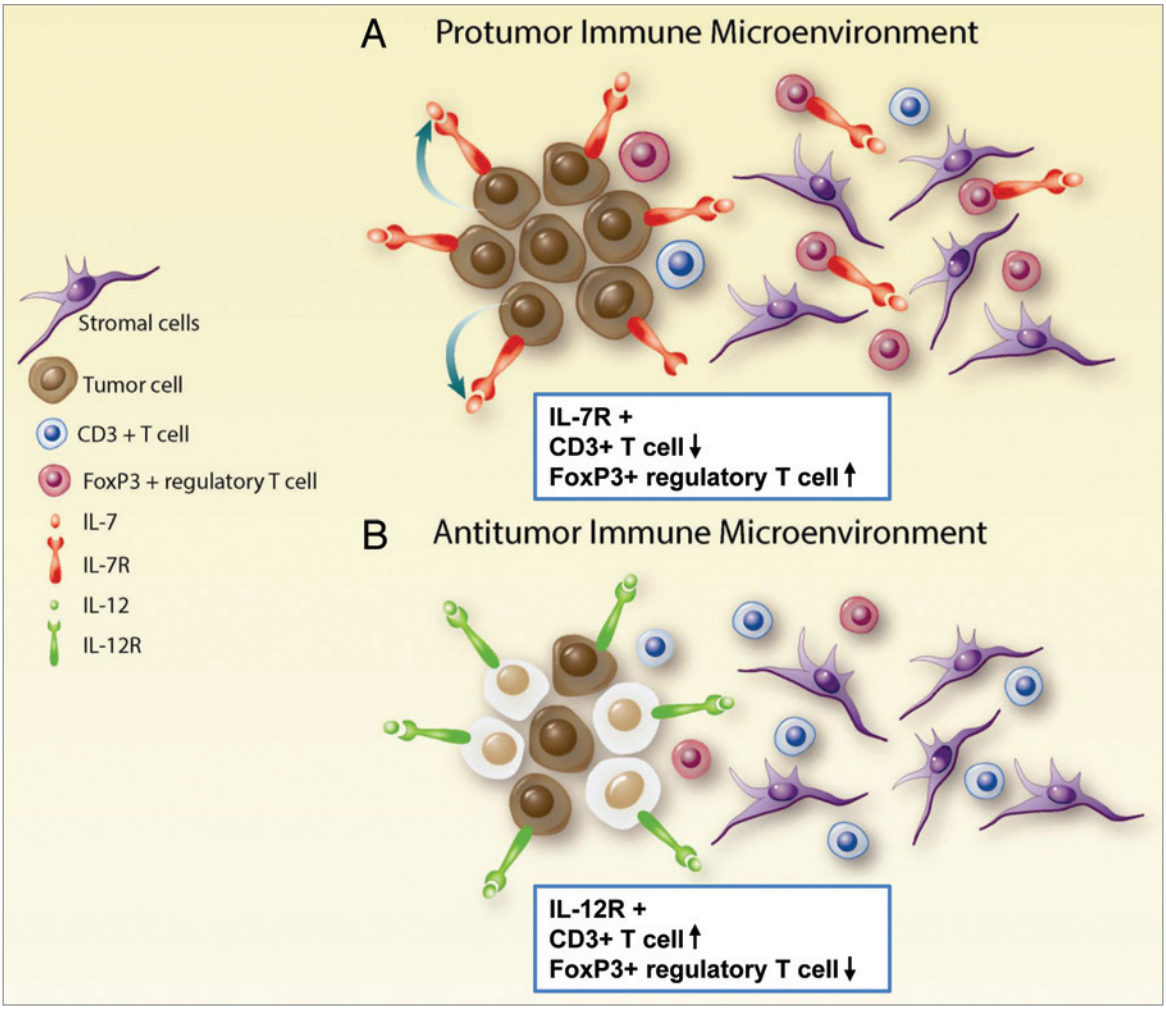

Figure 1. Impact of the immune microenvironment on lung adenocarcinoma. (**A**) A high relative proportion of tumor-infiltrating FOXP3$^+$ regulatory T cells (Tregs) to CD3$^+$ T cells as well as the expression of the interleukin (IL)-7 receptor (IL-7R) by tumor cells have been associated with poor clinical outcome. In this context, IL-7 produced by tumor cells not only operates in an autocrine fashion but also can act on Tregs, which express low levels of IL-7R. (**B**) A low relative proportion of tumor-infiltrating FOXP3$^+$ Tregs to CD3$^+$ T cells as well as the expression of the IL-12 receptor (IL-12R) by tumor cells have been shown to correlate with improved clinical outcomes, de facto favoring the establishment of an antitumor local microenvironment.

axis upregulates VEGFD and promotes lymphangiogenesis via the c-FOS/c-JUN pathway, resulting in unfavorable clinical outcomes.[10] These observations suggest that particular types of lung cancer may self-regulate aggressiveness by the co-expression of IL-7 and IL-7R. In our study, the expression of IL-7R by tumor cells was associated with unfavorable prognosis (**Fig. 1A**) with increased tumor size and pronounced lymphovascular invasion, at odds with the expression of IL-12Rβ2.[4] These findings suggest that the expression of IL-7R by lung adenocarcinoma cells may play an important role in tumor progression, presumably as it impacts both tumor growth and lymphangiogenesis. Interestingly, most human FOXP3$^+$ Tregs express low levels of IL-7R, and IL-7 contributes to the development and function of these cells.[9] Therefore, IL-7 may promote tumor progression as it activates IL-7R on both tumor cells and Tregs. Thus, therapeutic strategies that target the IL-7/IL-7R axis may decrease tumor growth and lymphangiogenesis while limiting the immunosuppressive effects of Tregs.

In conclusion, our findings suggest that, in early-stage lung adenocarcinoma, the immune microenvironment—including tumor-infiltrating lymphocytes (notably the relative proportion of Tregs to CD3$^+$ T cells) as well as the expression of cytokine receptors (i.e., IL-7R and IL-12Rβ2) by tumor cells—conveys robust prognostic information (**Fig. 1**). In addition, accumulating evidence suggests that both IL-7/IL-7R-targeting therapies and the administration of IL-12 may not only control the immunosuppressive effects of Tregs but also limit angiogenesis and lymphangiogenesis, resulting in better clinical outcomes. The role of the IL-7/IL-7R and IL-12/IL-12R signaling axes in tumor growth and antitumor immune responses, especially relative to Tregs, warrants further investigation. Further insights into these issues may indeed result in novel immunotherapeutic strategies for the clinical management of lung adenocarcinoma.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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