Involvement of STAT3 in immune evasion during lung tumorigenesis

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Abbreviations: MHC, major histocompatibility complex; NK, natural killer; NSCLC, non-small cell lung carcinoma; STAT3, signal transducer and activator of transcription 3

In a recent study, we have shown that STAT3 expressed by tumor cells blunts antitumor immunity during carcinogen-induced lung tumorigenesis. STAT3 inhibits the production of pro-inflammatory chemokines and MHC Class I chain-related gene A. In contrast, STAT3 promotes the expression of MHC Class I molecules. Consequently, STAT3 promotes tumor cell resistance to NK cell-mediated cytotoxicity.

In normal cells and under physiological conditions, the binding of various cytokines to their receptors rapidly and transiently activates signal transducer and activator of transcription 3 (STAT3). STAT3 is negatively regulated by various proteins, such as suppressor of cytokine signaling (SOCS) and protein inhibitor of activated STAT (PIAS). When constitutively activated, STAT3 promotes malignant transformation.1 Accordingly, somatic mutations in the Src homology 2 (SH2) domain of STAT3 are frequently found in large granular lymphocytic leukemia.2 Furthermore, mutations in Janus kinase 2 (JAK2) that result in constitutive activation of STAT3 have also been identified in myeloproliferative disorders.3 However, similar mutations in the JAK/STAT3 pathway have not been reported in lung cancer patients.

To date, several driver oncogenes, including Kirsten rat sarcoma viral oncogene homolog (KRAS), epidermal growth factor receptor (EGFR), and anaplastic lymphoma kinase (ALK), have been identified in non-small cell lung carcinoma (NSCLC), a common type (accounting for 80% of all cases) of lung cancer. Formerly, STAT3 was thought to act in pathways downstream of such oncogenes. However, it is now known that STAT3 is activated by various inflammatory mediators in the cancer microenvironment, including interleukin-6 (IL-6), independently of driver oncogenes.4 Thus, it appears that the role of STAT3 in lung cancer is to mediate the crosstalk between malignant cells and their microenvironment.5 In an effort to define the role of STAT3 in lung cancer in vivo, we used mice bearing an epithelium-specific knockout of Stat3 (Stat3Δ/Δ), to show that STAT3 blunts antitumor immunity during carcinogen-induced lung tumorigenesis.6

In our study, tumors developed in both Stat3Δ/Δ and control mice, suggesting that STAT3 is dispensable for urethane-induced lung tumorigenesis. However, the total tumor volume was decreased in Stat3Δ/Δ mice compared with control mice. The number of inflammatory cells found in the bronchoalveolar lavage fluid revealed that tumor-related inflammation increases so to inhibit tumor growth in Stat3Δ/Δ mice. Concomitantly, antitumor inflammatory mediators, such as interferon γ (IFNγ) and tumor necrosis factor α (TNFα), were found to be increased in Stat3Δ/Δ mice. By contrast, we did not observe any differences in proliferation, apoptosis or angiogenesis between Stat3Δ/Δ and control mice. Based on these results, we concluded that STAT3 deficiency in tumor cells promotes antitumor inflammatory responses in urethane-induced tumorigenesis. To further explore the mechanisms underlying the inflammatory responses evoked by the absence of STAT3 in lung tumor cells, we performed a comparative microarray analysis using RNA extracted from tumors developing in Stat3Δ/Δ and control mice, and hypothesized two mechanistic pathways.

The first pathway involved the STAT3-dependent negative regulation of inflammatory chemokines, which was previously identified in other cancer cell models, in vitro.7 To confirm that this pathway is also activated in lung cancer, we suppressed STAT3 expression in various NSCLC cells using specific small-interfering RNAs (siRNAs) and showed that STAT3 negatively regulates various chemokines, including CCL5 (RANTES) and CXCL10 (IP-10). These mediators play an important role in cancer-related inflammation by acting on various subpopulations of immune effector cells. Of particular interest, CXCL10 has been

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reported to directly enhance natural killer (NK) cell cytotoxicity. Therefore, culture supernatants from STAT3-depleted NSCLC cells exhibited a higher chemo- tactic activity than those from control cells. The mechanism by which STAT3 silencing affects the production of inflammatory mediators remains unknown and should be explored in the future.

The second pathway involved the STAT3-dependent evasion of NK cell-mediated cytotoxicity by cancer cells. In general, NK-cell activation depends on the balance between signals from inhibitory and activating surface receptors. One of the major inhibitory receptor ligands is constituted by major histocompatibility complex (MHC) Class I molecules. Consistent with this notion, we found that MHC Class I expression is reduced in both tumor-bearing lung tissue from Stat3Δ/Δ mice and STAT3-depleted NSCLC cells. On the other hand, one of the major ligands for activating NK-cell receptors is MHC Class I chain-related gene A/B (MICA/B). In line with this notion, we found that MICA expression increases in response to the siRNA-mediated down-regulation of STAT3 in all NSCLC cells examined so far (S.I., unpublished data).

Consequently, STAT3 blockade in cancer cells results in NK-cell activation. Consistent with these results, Cr-release assays revealed that the transfection of NSCLC cells with STAT3-targeting siRNAs increases their susceptibility to NK cell-mediated cytoxicity (Fig. 1).

Depending on multiple variables, the immune system can respond to malignant cells in two opposite ways. Cancer-promoting inflammation is associated with various types of tumors, including gastric and intestinal neoplasms, and this inflammation is predominantly regulated by STAT3. On the other hand, anticancer immune responses, such as that observed in our study, involve the IFNγ/STAT1-mediated activation of innate effectors (such as NK cells), the Type 1 helper T (T H1) response and cytotoxic T cells. In both these scenarios, blocking STAT3 influenced the immune system in a manner that inhibited tumor growth, that is, blocking STAT3 ameliorates cancer-promoting inflammation and promotes anticancer immunity. Previous studies have shown that STAT3 suppresses the antitumor activities of innate effector cells. These studies and our findings suggest that blocking STAT3 constitutes one strategy to overcome the resistance of lung cancer to antitumor immunity. Recently, it has been reported that antibodies blocking the immunosuppressive pathway mediated by programmed death-1 (PD-1) are a promising treatment for lung cancer. Although side effects including autoimmune inflammatory bowel disease constitute a potential concern, STAT3 inhibition alone or combined with conventional chemotherapeutics may become the next generation strategy for the treatment of lung cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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