# Involvement of STAT3 in immune evasion during lung tumorigenesis

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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 STAT3 activation 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* (*Stat3*$^{\Delta/\Delta}$), to show that STAT3 blunts antitumor immunity during carcinogen-induced lung tumorigenesis.[6]

In our study, tumors developed in both *Stat3*$^{\Delta/\Delta}$ and control mice, suggesting that STAT3 is dispensable for urethane-induced lung tumorigenesis. However, the total tumor volume was decreased in *Stat3*$^{\Delta/\Delta}$ 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 *Stat3*$^{\Delta/\Delta}$ mice. Concomitantly, antitumor inflammatory mediators, such as interferon γ (IFNγ) and tumor necrosis factor α (TNFα), were found to be increased in *Stat3*$^{\Delta/\Delta}$ mice. By contrast, we did not observe any differences in proliferation, apoptosis or angiogenesis between *Stat3*$^{\Delta/\Delta}$ 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 *Stat3*$^{\Delta/\Delta}$ 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.[5] 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
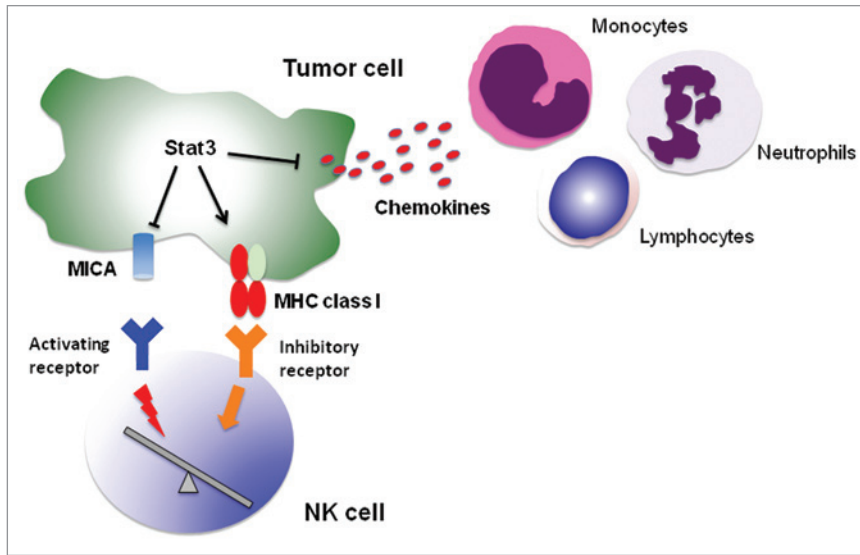
**Figure 1.** STAT3 activation allows tumor cells to evade antitumor immunity. STAT3 negatively regulates chemokines that play important roles in cancer-related inflammation by acting on various subpopulations of immune effector cells. STAT3 augments the expression of major histocompatibility complex (MHC) Class I molecules, a ligand for inhibitory natural killer (NK)-cell receptors and inhibits the expression of MHC Class I chain-related gene A (MICA), a ligand for activating NK-cell receptors. Consequently, the activation of NK-cell effector functions is inhibited by STAT3 activation in tumor cells.

reported to directly enhance natural killer (NK) cell cytotoxicity.[7] Furthermore, culture supernatants from STAT3-depleted NSCLC cells exhibited a higher chemotactic 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 *Stat3*$^{\Delta/\Delta}$ 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, $^{51}$Cr-release assays revealed that the transfection of NSCLC cells with STAT3-targeting siR-NAs increases their susceptibility to NK cell-mediated cytotoxicity (**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.[8] 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.[8] 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 immune effector cells.[5] 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.[9] Although side effects including autoimmune inflammatory bowel disease[10] 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.

### References

1. Bromberg JF, Wrzeszczynska MH, Devgan G, Zhao Y, Pestell RG, Albanese C, et al. Stat3 as an oncogene. Cell 1999; 98:295-303; PMID:10458605; http://dx.doi.org/10.1016/S0092-8674(00)81959-5.
2. Koskela HLM, Eldfors S, Ellonen P, van Adrichem AJ, Kuusanmäki H, Andersson EI, et al. Somatic STAT3 mutations in large granular lymphocytic leukemia. N Engl J Med 2012; 366:1905-13; PMID:22591296; http://dx.doi.org/10.1056/NEJMoa1114885.
3. Quintás-Cardama A, Kantarjian H, Cortes J, Verstovsek S. Janus kinase inhibitors for the treatment of myeloproliferative neoplasias and beyond. Nat Rev Drug Discov 2011; 10:127-40; PMID:21283107; http://dx.doi.org/10.1038/nrd3264.
4. Looyenga BD, Hutchings D, Cherni I, Kingsley C, Weiss GJ, Mackeigan JP. STAT3 is activated by JAK2 independent of key oncogenic driver mutations in non-small cell lung carcinoma. PLoS One 2012; 7:e30820; PMID:22319590; http://dx.doi.org/10.1371/journal.pone.0030820.
5. Yu H, Kortylewski M, Pardoll D. Crosstalk between cancer and immune cells: role of STAT3 in the tumour microenvironment. Nat Rev Immunol 2007; 7:41-51; PMID:17186030; http://dx.doi.org/10.1038/nri1995.
6. Ihara S, Kida H, Arase H, Tripathi LP, Chen YA, Kimura T, et al. Inhibitory roles of signal transducer and activator of transcription 3 in antitumor immunity during carcinogen-induced lung tumorigenesis. Cancer Res 2012; 72:2990-9; PMID:22659452; http://dx.doi.org/10.1158/0008-5472.CAN-11-4062.
7. Saudemont A, Jouy N, Hetuin D, Quesnel B. NK cells that are activated by CXCL10 can kill dormant tumor cells that resist CTL-mediated lysis and can express B7-H1 that stimulates T cells. Blood 2005; 105:2428-35; PMID:15536145; http://dx.doi.org/10.1182/blood-2004-09-3458.
8. Yu H, Pardoll D, Jove R. STATs in cancer inflammation and immunity: a leading role for STAT3. Nat Rev Cancer 2009; 9:798-809; PMID:19851315; http://dx.doi.org/10.1038/nrc2734.
9. Bluestone JA, Small EJ. The future of cancer treatment: will it include immunotherapy? Cancer Cell 2012; 22:7-8; PMID:22789534; http://dx.doi.org/10.1016/j.ccr.2012.06.009.
10. Takeda K, Clausen BE, Kaisho T, Tsujimura T, Terada N, Förster I, et al. Enhanced Th1 activity and development of chronic enterocolitis in mice devoid of Stat3 in macrophages and neutrophils. Immunity 1999; 10:39-49; PMID:10023769; http://dx.doi.org/10.1016/S1074-7613(00)80005-9.