

Novel function of STAT1 in breast cancer

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In the past, the transcription factor STAT1 was considered as a tumor suppressor. We have recently discovered that STAT1 in malignant cells favors breast cancer progression as it stimulates immunosuppressive effects mediated by myeloid-derived suppressor cells (MDSCs). Inhibiting STAT1 activity offers a promising therapeutic approach against advanced breast cancer.

Signal transducer and activator of transcription 1 (STAT1) has been classically defined as an oncosuppressive transcription factor. Early studies showed that STAT1 activates anti-proliferative and pro-apoptotic genes in tumor cells. Moreover, a large body of evidence demonstrated that the interferon γ (IFN γ)-mediated activation of STAT1 is associated with antiviral and antitumor immunity.¹ In line with this notion, *Stat1*^{-/-} mice are more prone to mammary tumorigenesis than their wild-type counterparts.^{2,3} Nonetheless, evidence is mounting against the univocal role of STAT1 as a tumor suppressor. For example, STAT1 expression is upregulated in several late-stage human cancers, including metastatic lesions,^{4,5} and high STAT1 levels have been suggested to constitute a predictive marker for poor survival, as well as for chemo/radiotherapy resistance among breast carcinoma patients.⁶ Still, many of these studies only provided a correlative association between STAT1 and disease progression.

We have recently provided direct, causal evidence suggesting that STAT1 exerts tumorigenic effects in a syngeneic mouse model of breast cancer.⁷ In this model, TM40D-MB cells implanted into the mammary fat pads of BALB/c mice generated mammary tumors that were more aggressive and exhibited an increased

metastatic potential as compared with those developing from parental TM40D cells. A microarray-based comparison of the transcriptional signature of TM40D and TM40D-MB cells revealed alterations in genes related to the immune control of tumor progression. Interestingly, many IFN γ -activated genes were indeed upregulated in TM40D-MB cells, including *STAT1* (with a 4-fold change in expression levels). We next confirmed that *STAT1* is significantly overexpressed in human biopsies from invasive breast carcinoma patients, as compared with ductal carcinoma in situ (DCIS) specimens.

To determine whether STAT1 promotes breast cancer progression, mouse mammary carcinoma cell lines expressing STAT1 to various levels were generated. These cells were then implanted into the mammary fat pads of BALB/c mice and tumor progression was monitored. The constitutive overexpression of STAT1 in TM40D-STAT1 cells dramatically enhanced tumor growth and aggressiveness as compared with wild-type TM40D tumor cells. Conversely, a small hairpin RNA (shRNA) constitutively targeting STAT1 expression in TM40D-MB cells significantly delayed tumor growth.⁷

To gain further insights into the mechanisms whereby STAT1 stimulated breast cancer progression, we determined whether

the transcriptional activity of STAT1 regulates the expression of pro-inflammatory and immunosuppressive cytokines. Our data indicated that tumor necrosis factor α (TNF α), transforming growth factor β (TGF β), and interleukin-13 (IL-13) are all upregulated by STAT1. These factors are known to recruit and stimulate the function of cells that inhibit antitumor immune responses. We then proceeded to check what type of immunosuppressive cells is recruited to the STAT1-overexpressing tumor microenvironment. Using both human and mouse breast tumor samples, we showed that STAT1 overexpression results in significantly increased numbers of myeloid-derived suppressor cells (MDSCs). Indeed, the shRNA-mediated knockdown of STAT1 in murine tumors limited their infiltration by Gr1⁺ MDSCs. Functionally, these Gr1⁺ MDSCs exhibited high arginase activity and exert suppressive activity against effector T cells. Using both flow cytometry and immunohistochemistry, we also showed that the recruitment of MDSCs by STAT1-expressing tumors caused a significant decrease in the amount of tumor-infiltrating CD4⁺ and CD8⁺ T cells. This suggests that STAT1 expression by tumor cells suppresses the infiltration of CD4⁺ and CD8⁺ T cells, thereby disabling potent effectors of adaptive antitumor immunity.

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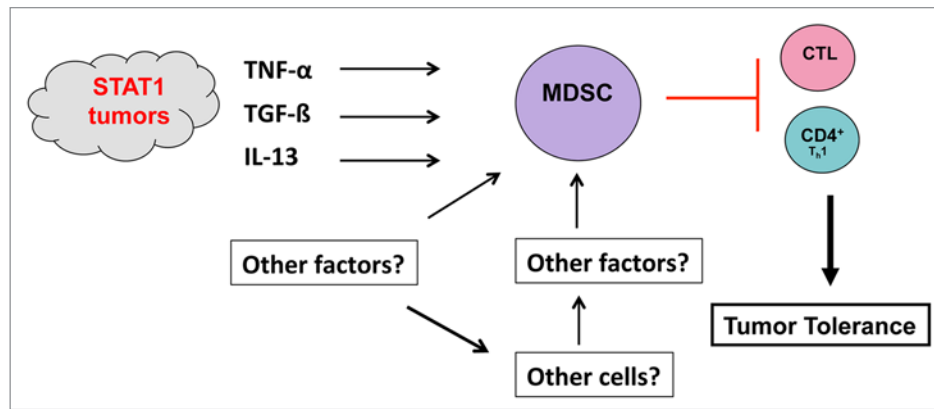


Figure 1. Pro-tumorigenic functions of STAT1 in breast cancer. Mammary tumors overexpressing signal transducer and activator of transcription 1 (STAT1) secrete tumor necrosis factor α (TNF α), transforming growth factor β (TGF β), and interleukin-13 (IL-13), which drive the recruitment and immunosuppressive activity of myeloid-derived suppressor cells (MDSCs). STAT1-overexpressing tumors may secrete additional factors that either attract MDSC directly or via other immune or stromal cells, in turn secreting MDSC-recruiting cytokines. MDSCs suppress the infiltration of tumors by cytotoxic T lymphocytes (CTLs) and CD4⁺ T cells, resulting in the evasion of malignant cells from immune control mechanisms.

The novel function of STAT1 that we uncovered is actually at odds against the prevailing view of STAT1 as an oncosuppressive transcription factor. Such a discrepancy between our data and those of others may reflect (at least in part) the expression levels and activation status of STAT1 in epithelial cells. In our study, tumor cells expressed constitutively active STAT1 and IFN-associated cytokines, which was shown to drive tumor progression. Moreover, the effects of STAT1 on tumor growth may depend on intrinsic properties of malignant cells and their microenvironment. It has been reported that stromal signals stimulate STAT1 and IFN-associated genes in estrogen receptor (ER)⁻ but not ER⁺ tumors or normal epithelia.⁸ This suggests that ER⁻, but not ER⁺, cells respond to inflammatory factors by activating a signal transduction cascade that impinges on STAT1.

Of note, recent data indicate that IFN γ , the main inducer of STAT1 signaling, also

plays contrasting roles in oncogenesis.⁹ Indeed, while IFN γ is usually associated with antiproliferative and pro-apoptotic effects for malignant cells, several lines of evidence point to IFN γ as to a pro-tumorigenic agent, at least in some circumstances. For instance, IFN γ has been shown to enhance the metastatic potential of TS/A tumor cells and their resistance to the cytotoxicity of natural killer (NK) cells.⁹ It appears that the pro- or anti-tumor effects of IFN γ exhibit a high degree of context-dependency, varying with tumor type, microenvironment factors, and signal transduction-related factor.⁹ The same may hold true for STAT1. Finally, while our study identified TNF α , a factor secreted from STAT1-overexpressing malignant cells, as a key player in the recruitment of MDSCs to neoplastic lesions, other tumor-derived factors/cytokines may be involved in this process. These factors can either recruit MDSCs directly or indirectly, through other stromal/immune cells that

in turn produce MDSC-recruiting cytokines (Fig. 1).

Based on our findings, we argue that traditional therapies aimed at activating STAT1 may not be effective against specific types of breast cancer. Rather, a therapeutic approach against STAT1 should be used to treat advanced breast cancers, in particular ER⁻ and chemotherapy-resistant lesions that display high STAT1 expression levels. Since STAT1 is also required for the function of MDSCs,¹⁰ specific STAT1 inhibitors may block both the ability of tumor cells to recruit MDSCs and the immunosuppressive activity of the latter. For this reason, understanding the mechanisms that underlie the activity of IFN γ and STAT1 in oncogenesis and tumor progression may provide a paradigm shifting-strategy to combat advanced breast cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

- Durbin JE, Hackenmiller R, Simon MC, Levy DE. Targeted disruption of the mouse Stat1 gene results in compromised innate immunity to viral disease. *Cell* 1996; 84:443-50; PMID:8608598; [http://dx.doi.org/10.1016/S0092-8674\(00\)81289-1](http://dx.doi.org/10.1016/S0092-8674(00)81289-1)
- Lynch RA, Etchin J, Battle TE, Frank DA. A small-molecule enhancer of signal transducer and activator of transcription 1 transcriptional activity accentuates the antiproliferative effects of IFN-gamma in human cancer cells. *Cancer Res* 2007; 67:1254-61; PMID:17283162; <http://dx.doi.org/10.1158/0008-5472.CAN-06-2439>
- Chan SR, Vermi W, Luo J, Lucini L, Rickert C, Fowler AM, et al. STAT1-deficient mice spontaneously develop estrogen receptor α -positive luminal mammary carcinomas. *Breast Cancer Res* 2012; 14:R16; PMID:22264274; <http://dx.doi.org/10.1186/bcr3100>
- Manavi M, Hudelist G, Fink-Retter A, Gschwandtler-Kaulich D, Pischinger K, Czerwenka K. Gene profiling in Pap-cell smears of high-risk human papillomavirus-positive squamous cervical carcinoma. *Gynecol Oncol* 2007; 105:418-26; PMID:17306351; <http://dx.doi.org/10.1016/j.ygyno.2006.12.032>
- Schultz J, Koczan D, Schmitz U, Ibrahim SM, Pilch D, Landsberg J, et al. Tumor-promoting role of signal transducer and activator of transcription (Stat)1 in late-stage melanoma growth. *Clin Exp Metastasis* 2010; 27:133-40; PMID:20180146; <http://dx.doi.org/10.1007/s10585-010-9310-7>
- Weichselbaum RR, Ishwaran H, Yoon T, Nuyten DS, Baker SW, Khodarev N, et al. An interferon-related gene signature for DNA damage resistance is a predictive marker for chemotherapy and radiation for breast cancer. *Proc Natl Acad Sci U S A* 2008; 105:18490-5; PMID:19001271; <http://dx.doi.org/10.1073/pnas.0809242105>

7. Hix LM, Karavitis J, Khan MW, Shi YH, Khazaie K, Zhang M. Tumor STAT1 Transcription Factor Activity Enhances Breast Tumor Growth and Immune Suppression Mediated by Myeloid-derived Suppressor Cells. *J Biol Chem* 2013; 288:11676-88; PMID:23486482; <http://dx.doi.org/10.1074/jbc.M112.441402>
8. Buess M, Nuyten DS, Hastie T, Nielsen T, Pesich R, Brown PO. Characterization of heterotypic interaction effects in vitro to deconvolute global gene expression profiles in cancer. *Genome Biol* 2007; 8:R191; PMID:17868458; <http://dx.doi.org/10.1186/gb-2007-8-9-r191>
9. Zaidi MR, Merlino G. The two faces of interferon- γ in cancer. *Clin Cancer Res* 2011; 17:6118-24; PMID:21705455; <http://dx.doi.org/10.1158/1078-0432.CCR-11-0482>
10. Condamine T, Gabrilovich DI. Molecular mechanisms regulating myeloid-derived suppressor cell differentiation and function. *Trends Immunol* 2011; 32:19-25; PMID:21067974; <http://dx.doi.org/10.1016/j.it.2010.10.002>