STAT3-mediated regulation of PD-L1 in breast cancer

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Introduction

- Signal transducer and activator of transcription 3 (STAT3) represents a crucial transcription factor for cell proliferation, survival and tumor development.
- STAT3 can also play a key role on the function of immune cells, by impairing effective antitumor immunity.
- Programmed death ligand-1 (PD-L1, CD274) represents an important immune checkpoint molecule which has proved to be a successful target in cancer immunotherapy

Aims of the study

- To elucidate the possible role of STAT3 in the regulation of PD-L1 expression
- To investigate the effects of STAT3 in the alteration of immune profile in breast cancer

Materials and Methods

In vitro system

- Breast cancer cell lines: MCF7, MDA-MB-231, BT549
- Protein expression levels were assessed by western blot analysis and immunohistochemistry (IHC) in cell lines
- Treatment of cell lines with the STAT3 inhibitor C-188-9 (XIII) and transfection with small interfering RNA (siRNA) of STAT3
- Chromatin immunoprecipitation (ChIP) analysis was performed confirming binding sites of phosphorylated STAT3 (pSTAT3) on CD274 gene promoter

In vivo model

- BALB/c female mice were injected with 4T1 mouse mammary carcinoma cells previously transduced with lentiviral vectors carrying short-hairpin RNA (shRNA) STAT3 plasmid
- Immune cell subpopulations were analyzed using flow cytometry.
- Breast cancer patient cohort
- Tissue microarrays (TMAs) were constructed from 541 human primary breast tumors
- PD-L1 tumor expression was assessed by IHC in FFPE TMA
- Gene expression profiling data were available in this cohort and correlations of a known pSTAT3-associated gene signature (pSTAT3-GS) with transcript and protein levels of PD-L1 were made

Conclusions

- STAT3 transcriptionally regulates PD-L1 expression
- STAT3 modulates antitumor immune response in breast cancer mainly through macrophage phenotype shift

Figure 1. PD-L1 and STAT3 expression in breast cancer cell lines

PD-L1 and pSTAT3 were strongly expressed in triple negative breast cancer (TNBC) cell lines as assessed by IHC (A) and western blotting (B)

Figure 2 (left). Regulation of PD-L1 by STAT3 in vitro. Both pharmacologic inhibition with the STAT3 inhibitor C-188-9 (A) and gene silencing in our breast cancer mouse model (B) led to decreased PD-L1 protein levels in TNBC cell lines. pSTAT3 was bound on CD274 gene promoter leading to PD-L1 upregulation, as revealed by ChIP analysis (C)

Figure 3 (left). STAT3 impact on tumor growth in vivo. STAT3 gene silencing in our breast cancer mouse model was associated with 36% lower tumor volume compared with controls (A) and with decreased tumor burden (B) upon injection of 4T1 cells (shCTRL-shSTAT3) into female BALB/c mice.

Figure 4. Effects of STAT3 gene silencing in immune subpopulations in vivo. Depletion of STAT3 (A) resulted in reduced PD-L1 transcript levels in 4T1 mouse mammary carcinoma cell line (B). A shift in macrophage polarization from M2 (decrease 16%; p=0.0229) to M1 (increase 37%; p=0.0109) phenotype was noted upon STAT3 depletion. Moreover, a significant increase in CD4+ T cells (p=0.00210) (E) and in natural killer (NK) cells (p=0.00750) (F) was also observed.

Figure 5. PD-L1 in breast cancer patient samples was expressed in 52/541 cases (9.6%), as assessed by IHC (A). pSTAT3-GS expression was positively correlated with CD274 (Spearman’s r=0.35) (B), while no difference in pSTAT3-GS expression between PD-L1 positive and negative cases (C) was noted.