STAT3 negatively regulates thyroid tumorigenesis

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\textbf{AUTHOR SUMMARY}

Members of the IL-6 cytokine family play a critical role in normal immune cell functions and diseases, such as rheumatoid arthritis and cancer. These cytokines act by binding to a specific cell surface receptor complexed with the signal-transducing molecule glycoprotein (gp) 130. This interaction induces phosphorylation of gp130-associated protein kinases called JAKs, which in turn, phosphorylate tyrosine residues on gp130, resulting in the recruitment and subsequent phosphorylation of the transcription factor STAT3. Activated STAT3 dimerizes, enters the nucleus, and binds to promoter sequences of target genes involved in controlling normal cellular functions and regulating many aspects of tumorigenesis (proliferation, survival, differentiation, metabolism, immune cell response, angiogenesis, migration, and invasion) (1). Normally, STAT3 is activated transiently by phosphorylation of a specific tyrosine residue. However, in many cancers, STAT3 is persistently activated by aberrant IL-6 signaling (2), and many lines of evidence indicate that STAT3 promotes tumorigenesis. In contrast, recent evidence indicates that STAT3 inhibits tumor growth and progression (3). Here, we determined that STAT3 suppressed the growth of thyroid cancer cell lines in vivo. This finding suggests that a careful analysis of the genetic, molecular, and cellular components of the tumor should be determined before targeting this transcription factor.

We examined the mechanism of STAT3 activation and its role in the most common endocrine cancer, papillary thyroid carcinoma (PTC). PTC results from oncogenic activation of the receptor protein tyrosine kinase rearranged in transformation (RET) by genomic rearrangements (RET/PTC) or mutations that activate the downstream effectors Rat Sarcoma (SRC) and BRAF, which in turn, activate the ERK/MAPK signaling pathway. Moreover, PTC frequently arises in association with chronic inflammation (thyroiditis). This study determines the mechanism of STAT3 activation and its functional role in PTC.

We analyzed STAT3 phosphorylation (pY-STAT3) in 146 primary human thyroid lesions and detected pY-STAT3 in a small fraction of tumor cells in most benign tumors (83%) and a subset of PTC (57%). In contrast, pY-STAT3 was present in only 25% of follicular thyroid carcinomas. In most lesions, cells in tumors positive for pY-STAT3 were associated with stromal cells (endothelial cells, fibroblasts, and immune cells) and were also positive for pSTAT3 found on the periphery of tumors. This result is consistent with published data, and it may reflect paracrine release of growth factors by the stroma.

We then examined the functional role of STAT3 in thyroid cancer by using in vitro and in vivo human (xenograft) and murine transgenic models, respectively. STAT3 knockdown in pY-STAT3-positive human cell lines using an interfering shRNA (shSTAT3) did not alter their growth in vitro, whereas short hairpin STAT3 generated significantly larger tumors in vivo (xenografts) compared with controls (Fig. P1). Similar results were obtained

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\caption{(A) STAT3 deficiency in thyrocytes of BRAFV600E mice increases thyroid tumor size and tumor cell proliferation (*P < 0.05). (B) The role of STAT3 during thyroid tumor growth. Small thyroid tumors are positive for pY-STAT3, which is regulated by stromal paracrine and oncogene-induced autocrine signaling. STAT3 may prevent excessive proliferation by promoting secretion of growth inhibitory factors, including insulin-like growth factor binding protein 7. As thyroid cancer cells grow farther from the periphery and blood supply, the oxygen levels drop, inducing stabilization of hypoxia-inducible transcription factor 1α and a decrease in the level of pY-STAT3. Such a decrease in activated STAT3 enhances aerobic glycolysis (increased glucose consumption, lactate production, and decreased oxygen consumption), conferring a growth advantage on these tumors.}
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using a transgenic murine model of BRAFV600E-induced PTC, where thyrocyte-targeted deletion of STAT3 led to enhanced tumor size and proliferation (Fig. P1). These data suggest that, in vivo, STAT3 restrained thyroid tumor growth rather than promoting it.

Given the hypoxic nature of tumors (5), we investigated whether STAT3 deficiency could alter the metabolic function of thyroid cell carcinomas. Inhibition of STAT3 expression in these cell lines led to increased glucose consumption and lactate production. Consistent with this phenotype, down-regulation of STAT3 increased the expression of hypoxia-inducible transcription factor 1α and genes encoding glycolytic enzymes. These results suggest that the absence of STAT3 confers a growth advantage under hypoxic stress caused by metabolic reprogramming.

Our data presented here show that STAT3 is a suppressor of thyroid tumor growth in preclinical models, suggesting that targeting this transcription factor should be used with caution. We conclude that a better understanding of the mechanisms and contexts that predict the dual-edged function of STAT3 in tumorigenesis must be obtained.