Review
Signal transducers and activators of transcription as regulators of growth, apoptosis and breast development
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Abstract

STAT transcription factors were discovered 10 years ago as mediators of interferon-induced gene expression. They now form an important group, comprising seven members, that are activated by virtually every cytokine and growth factor. Their critical role in development and normal cell signaling has been largely determined through the analysis of transgenic mice lacking individual STAT genes. In addition, cell culture work has further delineated their importance in cellular transformation, apoptosis, differentiation and growth control. This review discusses the specific phenotypes of STAT-deficient animals with a focus on STAT5 and STAT3, as these two STAT molecules are required for normal breast development and involution, respectively, and may play an important role in breast carcinogenesis.

Keywords: apoptosis, breast development, cancer, involution, signal transducers and activators of transcription (STATs)

Introduction

Signal transducers and activators of transcription (STAT) proteins are latent transcription factors that become activated by phosphorylation on a single tyrosine (near the carboxy-terminal of the molecule), typically in response to extracellular ligands [1,2]. Virtually every cytokine and growth factor (polypeptide ligands) can cause STAT phosphorylation through either cytokine receptors plus associated Jak kinases or growth factors [eg epidermal growth factor (EGF), platelet-derived growth factor (PDGF), colony-stimulating factor-1] acting through intrinsic receptor tyrosine kinases. This clearly implicates STAT activation in many different biologic events. An active STAT dimer is formed via the reciprocal interactions between the Src homology domain-2 of one monomer and the phosphorylated tyrosine of the other [3]. The dimers accumulate in the nucleus, recognize specific DNA elements, and activate transcription. The STAT proteins are subsequently inactivated by tyrosine dephosphorylation and return to the cytoplasm [4,5].

The relevance of STAT activation to growth control is made apparent in experiments performed in cell lines or animals that lack specific STAT proteins, by the use of antisense molecules to specific STATs or by the use of dominant-negative STAT protein encoding constructs. Of the seven mammalian STATs: STAT2, STAT4, and STAT6 are activated by a small defined group of cytokines; and deficiencies in these STATs, as determined by murine knockout experiments, have been used to demonstrate their

Table 1

Cytokines, growth factors and oncogenes that activate signal transducers and activators of transcription (STAT) proteins

STAT1	STAT3	STAT5
IFNα	IL-6, IL-11, OSM, CNTF, LIF	IL-2, IL-7, IL-9, IL-15
IFNγ	IL-10	IL-3, IL-5, GM-CSF
EGF	Leptin	Growth hormone
PDGF	EGF	Prolactin
c/v-Eyk	PDGF	Erythropoetin
FGF	Insulin	Thrombopoetin
ACRII	c/v-Src, v-Eyk, v-Abl, v-Fps	EGF
	c-Fes, Lck, Tel-Jak, Ros, v-Sis	v-Abl
	Middle T antigen	

STAT2	STAT4	STAT6
IFNα	IL-12	IL-4
		IL-13

IFN, interferon; IL, interleukin, EGF, epidermal growth factor; PDGF, platelet-derived growth factor.

restricted roles in interferon-γ signaling and in T cell development (Schindler C, personal communication) [6–9]. In contrast, STAT1, STAT3, STAT5A, and STAT5B are activated by a large number of different ligands and frequently simultaneously by the same ligand, which raises the question regarding how these STATs participate in particular biologic responses (Table 1).

STAT1 promotes growth inhibition and apoptosis

Interferon-γ activates STAT1 almost exclusively, and mice that lack STAT1 have no innate response to either viral or bacterial pathogens because defense against these pathogens usually requires response to interferon [10,11]. Interestingly, over time STAT1-deficient mice develop spontaneous and chemically induced tumors more readily than wild-type animals, thus defining STAT1 as a 'tumor suppressor' (Schreiber B, personal communication) [12]. The mechanisms responsible for tumor suppression are not completely understood, but are in part due to the lack of tumor surveillance. Work done in tissue culture systems on STAT1-deficient cells [13,14] has demonstrated an important role for STAT1 in ligand-mediated growth arrest and apoptosis. Thus, there is growing evidence that STAT1 activation frequently leads to antiproliferative and proapoptotic events, and may partly explain why the lack of this molecule *in vivo* leads to increased tumor formation.

STAT5 promotes cell proliferation and is required for mammopoeisis

Two STAT5 genes exist, which are 96% homologous: *Stat5A* and *Stat5B*. Both STAT5 molecules are ubiquitously expressed and can be activated by many cytokines (see Table 1), including interleukins, growth hormone, prolactin, EGF, and erythropoietin. In addition, a number of

oncogenes such as those that encode Break Point Cluster Region/Abelson (BCR/ABL) tyrosine kinase and human T-cell leukemia virus (HTLV)-1, lead to persistent tyrosine phosphorylation of STAT5 [15,16]. In hematopoietic cells, STAT5 has been demonstrated to play a critical role in regulating apoptosis [17–20], and in promoting proliferation and cell cycle progression [21,22].

Mammary gland development is defined by the formation of ductal epithelial cells, which requires estrogen and EGF, and of lobular alveolar epithelium, which proliferates in response to prolactin and progesterone [23,24]. STAT5A, which can be activated by EGF and prolactin, is required for mammopoiesis and lactogenesis, as determined by knockout experiments [17,25].

The phosphorylated or activated form of STAT5 is quite low in mammary tissue of virgin animals and during early pregnancy, but rises dramatically after day 14 of pregnancy [26]. Upon weaning the breast involutes, which coincides with a drop in the levels of activated STAT5 [26]. Differentiation of ductal elements occurs in both wild-type and knockout mice. However, the extent or amount of epithelial duct development is decreased in STAT5 knockout animals [17]. Because both prolactin and EGF is required for ductal and alveolar growth, the absolute deficiency of STAT5 (despite the almost nonexistent levels of activated STAT5 in the virgin breast) may reflect the reduced growth or number of epithelial cells. The mammary tissue of STAT5A-deficient animals has a significant paucity of STAT5B protein [25]. STAT5B-deficient animals also have defects in mammopoiesis and lactogenesis (although not nearly as severe as in the STAT5A-deficient animals) [17,27].

In addition to STAT5, EGF, estrogen, and progesterone are factors that are critical to the development and proliferation of mammary tissue. The temporal and physical interaction between STAT5 and these other critical factors has not been well described. However, erbB-2, which can form a heterodimer with other erbB family members, may be essential for morphogenesis of the mammary ducts [28], and erbB-2 as a heterodimer with erB-4 can result in the activation of STAT5 [29]. The progesterone receptor can synergize with activated STAT5 in the induction of transcription from the β-casein gene promoter [30]. These examples suggest that there may be an interplay between these critical molecules and pathways in mammary development.

STAT3 as a regulator of cellular transformation and apoptosis in breast development

STAT3 deficiency leads to early embryonic lethality [31]. STAT3, like STAT5, is ubiquitously expressed in most tissues and is activated by a large number of different ligands, including EGF, PDGF, interleukin-6, ciliary neu-

rotrophic factor, oncostatin M, leukemia inhibitory factor, and granulocyte–macrophage colony-stimulating factor (see Table 1), as well as by a number of oncogenic tyrosine kinases. In normal cells and in animals, ligand-dependent activation of the STATs is a transient process, lasting for several minutes to several hours. In contrast, in many cancerous cell lines and tumors, where growth factor dysregulation is frequently at the heart of cellular transformation, STAT3 protein is persistently tyrosine phosphorylated or activated [32,33]. In studies performed in rodent fibroblasts, STAT3 activation has been demonstrated to be both required and sufficient to mediate cellular transformation. For example, v-*src* transformation requires activated STAT3, and a constitutively active form of STAT3 (STAT3-C) is sufficient for mediating transformation [34–36]. STAT3 activation appears to play an important role in preventing apoptosis in multiple myeloma-derived cell lines [37], squamous cell carcinomas [38], neurons [39,40], and mycosis fungoides tumor cells [41], and mediates cell cycle progression in a pro-B cell line [42].

The standard approach for circumventing embryonic lethality of gene knockouts is the creation of conditional knockouts, whereby only specific tissues lack the gene (protein) of interest [43]. Given that STAT3 deficiency results in death of the embryo, a number of STAT3-conditional knockouts have been generated, demonstrating the importance of STAT3 in preventing interleukin-6-mediated apoptosis of T cells [44], and keratinocytes that lack STAT3 do not migrate properly [45]. These findings, both *in vitro* and *in vivo*, suggest that STAT3 activation can prevent apoptosis and promotes proliferative processes, including cellular transformation.

The normal activation pattern of STAT3 in breast development and mammopoiesis reveals prominent levels of activated STAT3 in the virgin breast (presumably in response to EGF), which decrease somewhat during pregnancy and lactation, and subsequently increase during involution [26]. The marked increase in phosphorylated STAT3 during involution coincides with the initial burst of apoptosis.

A recent paper by Chapman *et al* [46] revealed a requirement for activated STAT3 in the involuting (apoptosing) breast. By crossing a β-lactoglobulin (BLG)–Cre mouse with a STAT3-null/STAT3-flox mouse, mice were generated that had a decrease in the levels of STAT3 in the lactating and involuting breast. The phenotype of the STAT3-deficient mammary gland revealed a delay in involution and apoptosis, suggesting a proapoptotic role for STAT3 in the involuting breast. At the molecular level a concomitant increase in the levels of STAT5 and STAT1 and an increase in the 'proapoptotic' proteins p21, p53 and Bax is observed in the BLG-Cre/Stat3$^{flox/-}$ breast tissue relative to the BLG-Cre/Stat3$^{flox/+}$ tissue. Given the wealth of evidence demonstrating a proliferative and antiapoptotic role

for STAT3 in some cell types, the findings of Chapman *et al* are somewhat surprising. Nevertheless, there are a few notable examples in which STAT3 activation is clearly responsible for a growth arrest in cultured cells. The first is in a myeloid tumor-derived cell line, M1, in which interleukin-6-mediated STAT3 activation is clearly responsible for inducing G1 arrest of these cells [47,48]. The second is in a macrophage-derived cell line, J774, in which interleukin-10, which activates STAT3, leads to antiproliferation, and expression of an inducibly active STAT3-gyraseB chimera mimics the effects of interleukin-10 [49].

The precise mechanism(s) by which STAT3 activation in the involuting breast leads to apoptosis are not known. Why activated STAT3 has this paradoxical effect as a function of cell type is presumably related to differences in the regulation of STAT3 target genes. For example, activated STAT3 can upregulate expression of the c-*myc* proto-oncogene [35,50]. It is well known that c-*myc* dysregulation can lead to both cellular transformation and apoptosis, depending on the cell type and the growth conditions [51]. Regardless of the mechanism, it is clear that STAT3 and STAT5 activation play critical roles in breast tissue development, probably through their ability to influence growth, differentiation, and apoptosis.

The relationship between the molecules responsible for organogenesis and cancer is not known. However, we believe that, in general, normal proteins and signaling pathways become abnormally regulated, which leads to the production of cancer. For example, in the case of breast cancer dysregulation of erbB-2, transforming growth factor-α, and estrogen is associated with the formation of breast cancer in human cells and murine models. STAT3 and STAT5 are clearly involved in breast organogenesis, and there is increasing evidence that the STAT proteins (in particular STAT3, STAT5 and STAT1) are persistently activated in primary breast cancers and breast cancer-derived cell lines (Bromberg J, *et al*, and Jove R, *et al*, unpublished observations) [32,52]. It is the persistent nature of STAT activation that is abnormal and is perhaps sufficient to mediate transformation. The potential mechanism(s) responsible for STAT tyrosine phosphorylation in primary breast cancer specimens are many and include the overproduction of the EGF receptor and EGF [38], PDGF receptor, Jak kinases, and c-*src* [32,53] (Reddy EP, personal communication), and perhaps mutations within the STAT protein itself. In addition, defects in the negative regulators of STAT phosphorylation may also be responsible for the persistence of tyrosine phosphorylated STAT3 in these specimens. These negative regulators include tyrosine phosphatases (ie SHP-2); the suppressor of cytokine signaling (SOC) proteins, which are negative regulators of the Jak kinases [54]; and the protein inhibitor of activated STATs (PIAS) [55], which inhibit activated STATs from binding DNA.

Interestingly, a number of STAT3 and STAT5 target genes (*cyclinD1*, c-*myc* [35,50,56] and *Bcl-x$_L$* [18,35]) are implicated either in breast cancer progression or in prognosis. Overexpression of cyclinD1 in transgenic models leads to hyperproliferation of breast tissue, as well as overt carcinoma [57]. CyclinD1 is overexpressed in preinvasive breast cancer, such as ductal carcinoma *in situ*, and in invasive breast carcinomas [58]. It has also been suggested that overexpression of cyclin D$_1$ protein is important in the detection of early stages of breast oncogenesis, and may play a crucial role in the development of breast cancer malignancy [58–61]. C-*myc*, another *Stat3* target gene, when overexpressed correlates with highly proliferative breast tumors [62–64]. Bcl-x$_L$, a member of the anti-apoptotic bcl-2 family of proteins, may be associated with a prognostically favorable phenotype in breast cancer [65–67]. Now, with the development of mice that lack the specific STAT proteins, we can begin to dissect the specific roles of the STATs in breast cancer pathogenesis.

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