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Signal Transducer and Activator of Transcription 5a/b: Biomarker and Therapeutic Target in Prostate and Breast Cancer

Mateusz Koptyra¹, Shilpa Gupta^{2,4}, Pooja Talati¹, and Marja T Nevalainen^{1,2,3}

¹Department of Cancer Biology, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA 19107.

²Department of Medical Oncology, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA 19107.

³Department of Urology, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA 19107.

⁴Genitourinary Oncology, H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL, 33612.

Abstract

The search for new therapeutic strategies for prostate and breast cancer is of significant interest. Signal Transducer and Activator of Transcription 5a/b (Stat5a/b) controls viability and growth of prostate cancer. Nuclear active Stat5a/b expression is clustered to high grade prostate cancers, predicts early disease recurrence and promotes metastatic dissemination of prostate cancer. In breast cancer, the role of Stat5a/b is more complex. In rodent model systems, Stat5a/b may promote malignant transformation and enhance growth of the breast tumors. In contrast, Stat5a/b activation in established human breast cancer positively correlates with tumor differentiation, prevents metastatic dissemination, and predicts favorable clinical outcome of node-negative breast cancer. Here we review the molecular structure and biological functions of Stat5a/b and discuss the potential applications of Stat5a/b for therapy development and as a prognostic marker for prostate and breast cancer.

Keywords

Stat5a/b; prostate and breast cancer; therapy and biomarker development

1. Introduction

Stat family of transcription factors has seven members: Stat1, Stat2, Stat3, Stat4, Stat5a, Stat5b and Stat6 – each are encoded by a separate gene. Stat5a was cloned in 1994 in Bernd Groner's laboratory from lactating ovine mammary tissue (Schmitt-Ney et al., 1991). It was called initially as a “mammary gland factor” (MGF) which was discovered to induce transcription of milk protein genes and mediate the effect of prolactin (Prl). Later, the Stat5b

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Address correspondence to: Marja T. Nevalainen, MD, PhD, Dept. of Cancer Biology, Urology, Medical Oncology, Kimmel Cancer Center, Thomas Jefferson University, 233 S. 10th Street, BLSB 309, Philadelphia, PA 19107. marja.nevalainen@jefferson.edu or Marja.Nealain@kimmelcancercenter.org Tel: 215-503-9250, Fax: 215-503-9245.

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isoform, encoded by a separate gene, was discovered in mouse mammary gland (Wakao et al., 1994). Stat5a and Stat5b are 96% conserved at the protein level and contain 793 and 786 amino acids, respectively (Darnell, 1997; Liao et al., 2010; Tan and Nevalainen, 2008). Both proteins share structurally and functionally similar domains with other Stat proteins. While Stat5a has 20 amino acids that are unique to its C-terminal sequence, 8 amino acids in the C-terminus are specific to Stat5b (Darnell, 1997; Liao et al., 2010; Tan and Nevalainen, 2008). Stat5a and Stat5b are activated by various cytokines and hormones such as interleukins (IL-2, IL-3, IL-5 and IL-7), erythropoietin, thrombopoietin (TPO), granulocyte-macrophage colony-stimulating factor (GM-CSF), growth hormone, insulin and prolactin (Darnell, 1997; Liao et al., 2010; Tan and Nevalainen, 2008). Furthermore, Stat5a/b is constitutively active and nuclear in the majority of leukemias and several solid tumors (Liao et al., 2010; Nikitakis et al., 2004; Tan and Nevalainen, 2008). Importantly, Stat5a/b is critical for prostate cancer cell viability and tumor growth (Ahonen et al., 2003; Dagvadorj et al., 2007, 2008). New evidence suggests the Stat5a/b signaling pathway may contribute to the transition of organ-confined prostate cancer to advanced disease (Gu et al., 2010; Tan et al., 2008). In breast cancer, the role of Stat5a/b is more complex and may include both promotion of tumor initiation as well as prevention of dissemination of established breast cancer (Liu et al., 1997; Miyoshi et al., 2001; Nevalainen et al., 2004; Sultan et al., 2005).

2. Stat5a/b structure

Stat5a and Stat5b map to chromosome 17 (bands q11-1 to q22) (Darnell, 1997; Liao et al., 2010; Tan and Nevalainen, 2008). Stat5a/b protein structure is composed of six functional domains: N-Terminal domain (NTD), coil-coiled domain (CCD), DNA binding domain (DBD), linker domain (LD), Src homology 2 domain (SH2) and transcriptional activation domain (TAD) (Fig. 1) (Darnell, 1997; Liao et al., 2010; Tan and Nevalainen, 2008). The well conserved N-Terminal domain (NTD) stabilizes interactions between two Stat dimers to form tetramers. Protein tetramerization reinforces Stat5a/b-DNA interaction and enhances the transcriptional activation of weak promoters (Darnell, 1997; Liao et al., 2010; Tan and Nevalainen, 2008). Post-translational glycosylation of NTD at threonine 92 of Stat5a/b is crucial for interaction with the CREB-binding protein. This interaction is essential for Stat5a/b-mediated gene transcription (Gewinner et al., 2004). Consisting of a four helix bundle, the coil-coiled domain (CCD) of Stat5a/b is responsible for various functions. These include: 1) Interaction with chaperones, 2) recruitment of co-activators and co-repressors, and 3) facilitation of multiple protein-protein interactions crucial for transcriptional regulation (Darnell, 1997; Liao et al., 2010; Tan and Nevalainen, 2008). The central DNA binding domain (DBD) determines Stat5a/b binding to DNA and recognizing consensus GAS (TTC(C/T)N(G/A)GAA) sites in the regulatory elements of target genes (Darnell, 1997; Liao et al., 2010; Tan and Nevalainen, 2008). The linker domain (LD) modifies stability of Stat5a/b DNA binding (Darnell, 1997; Liao et al., 2010; Tan and Nevalainen, 2008). The Src homology 2 domain (SH2) is the most highly conserved domain of Stat5a/b. Required for both receptors-specific recruitment and Stat5a/b dimerization, the SH2 domain is specifically crucial for binding of phosphorylated tyrosine residue of one Stat5a/b to the SH2 domain of the other subunit (Darnell, 1997; Liao et al., 2010; Tan and Nevalainen, 2008). The C-terminal transcriptional activation domain (TAD) facilitates the initiation of the transcription by directly interacting with critical co-activators including p300/CREB-binding protein (CBP)-associated co-activator NcoA-1, centrosomal P4.1-associated protein (CPAP), P100 and Oct-1 (Darnell, 1997; Liao et al., 2010; Tan and Nevalainen, 2008). CCD and/or N-terminal region is crucial for nuclear transport of Stat5b (Zeng et al., 2002).

3. Activation of Stat5a/b

Like for most Stats, Stat5a/b-activating cytokine receptors do not possess tyrosine kinase activity. This activity is provided by receptor-associated cytoplasmic kinases which belong to the Janus kinase (Jak) family. There are four known mammalian Jak proteins (Jak1, Jak2, Jak3, Tyk2), from which three are ubiquitously expressed (Jak1, Jak2, Tyk2) (Darnell, 1997; Liao et al., 2010; Tan and Nevalainen, 2008). Jak proteins range in size from 120–130 kDa and share seven regions of high homology (Darnell, 1997; Liao et al., 2010; Tan and Nevalainen, 2008). Stat5a/b is primarily activated by Jak2 (Darnell, 1997; Liao et al., 2010; Tan and Nevalainen, 2008).

In the canonical Jak2-Stat5a/b signaling pathway, ligand induced receptor activation induces conformational change (Darnell, 1997; Liao et al., 2010; Tan and Nevalainen, 2008). This structure alteration brings PrIR associated Jak2 kinases in close proximity and allow them to phosphorylate specific tyrosine motifs of the receptor and to activate each other (Darnell, 1997; Liao et al., 2010; Tan and Nevalainen, 2008). Stat5a/b recognize these tyrosine motifs through SH2 domains and are recruited to the docking sites of the receptor, which causes rapid Stat5a/b C-terminal phosphorylation by Jak2 (Darnell, 1997; Liao et al., 2010; Tan and Nevalainen, 2008). Phosphorylation on Y694 and Y699 tyrosines activates Stat5a and Stat5b, respectively (Darnell, 1997; Liao et al., 2010; Tan and Nevalainen, 2008). Activated Stat5a/b undergoes homo- or hetero dimerization mediated by phosphotyrosine-SH2 domain (Darnell, 1997; Liao et al., 2010; Tan and Nevalainen, 2008). Stat5a/b can be also phosphorylated by other kinases, through various signaling pathways which enhance/amplify primary activation signal. While tyrosine phosphorylation is a part of activation signal, the serine (S726 (human)/S725 (mouse) on Stat5a and S731 (human)/S730 (mouse) on Stat5b) phosphorylation may abrogate the transcriptional activity of Stat5a/b (Yamashita et al., 2001). Active Stat5a/b dimers are transported from the cytoplasm to the nucleus and bind to the DNA, where they attach to the regulatory elements of their target genes to initiate transcription. Stat5a/b recognize 8–10 base pair inverted repeat consensus DNA-sequence TTC(C/T)N(G/A)GAA called γ -interferon activation sequence (GAS element) (Darnell, 1997; Liao et al., 2010; Tan and Nevalainen, 2008).

Non-phosphorylated Stat5a/b proteins shuttle freely between the cytoplasm and the nucleus in the absence of cytokine activation (Darnell, 1997; Liao et al., 2010; Tan and Nevalainen, 2008). However, the nuclear transport of dimerized Stat5a/b is an active, energy dependent process. This action is utilized by components of Ran-dependent nuclear import machinery. Specifically, Stat5a/b complex with importin is transported into the nuclear compartment by karyopherin importin- β (p97) (Darnell, 1997; Liao et al., 2010; Tan and Nevalainen, 2008). Interestingly, some reports reveal that unphosphorylated Stats may also be able to bind to DNA by interaction with other transcription factors (Chatterjee-Kishore et al., 2000; Yang et al., 2005, 2007).

4. Stat5a/b Promotes Prostate Cancer Growth and Disease Progression

Transcription factor Stat5a/b is critical for prostate cancer cell growth and viability. Stat5a/b is constitutively active in human prostate cancer but not in normal prostate epithelium (Ahonen et al., 2003; Li et al., 2004). The mechanisms responsible for Stat5a/b activation involve Prl. Specifically, Prl is known to activate Jak2-Stat5a/b in normal and malignant prostate epithelium (Ahonen et al., 2002; Dagvadorj et al., 2007; Li et al., 2004), and Prl is locally produced in clinical prostate cancers (Dagvadorj et al., 2007; Nevalainen et al., 1997). Stat5a/b is a critical survival factor in human prostate cancer cells in culture (Ahonen et al., 2003; Dagvadorj et al., 2007, 2008). Adenoviral expression of a dominant-negative (DN) mutant of Stat5a/b lacking the TA domain induces massive and rapid apoptotic death

of various human prostate cancer cell lines in culture (Ahonen et al., 2003; Dagvadorj et al., 2008; Gu et al., 2010). This finding was confirmed in the TRAMP mouse tumor model (Kazansky et al., 2003). Also, inhibition of Stat5a/b by other methods such as antisense oligo-nucleotides or RNA interference effectively killed Stat5-positive prostate cancer cells (Dagvadorj et al., 2008; Gu et al., 2010). Moreover, inhibition of Stat5a/b blocked human prostate cancer subcutaneous and orthotopic xenograft tumor growth in nude mice (Dagvadorj et al., 2008; Gu et al., 2010) and down-regulated levels of anti-apoptotic BclX_L and Cyclin-D1 protein expression in prostate cancer cells (Dagvadorj et al., 2008). Importantly, when compared side by side, Stat5a/b had a preferential role over Stat3 in promoting viability of prostate cancer cells and tumor growth *in vitro* and *in vivo* (Gu et al., 2010). However, it is important to emphasize that Stat3 effects on prostate cancer progression may be more context-dependent. Target genes of Stat5a/b in human prostate cancer include: *Bcl-X_L*, *cyclin D1* (Dagvadorj et al., 2008), *Bcl-2*, *KLF-4* and *PDC4D* (Gu et al., 2010).

Stat5a/b promotes prostate cancer progression to advanced disease. Immunohistochemical analysis demonstrated a strong positive correlation with nuclear Stat5a/b and histological grade prostate cancers (Li et al., 2004, 2005). Moreover, nuclear Stat5a/b in primary prostate cancer predicted early disease recurrence (Li et al., 2005). Stat5a/b is nuclear and in the active state in the majority of castrate-resistant recurrent human prostate cancers (Tan et al., 2008). Active Stat5a/b synergizes with androgen receptor (AR) and increases AR transcriptional activity in human prostate cancer cells (Fig. 2). There are several other examples of ligand-independent activation of the AR by cytokines and growth factor receptors such as EGF, HER-2/neu, IL-6 and Stat3 (Craft et al., 1999; Culig et al., 1994; De Miguel et al., 2003; Hobisch et al., 1998). Significantly, AR increases transcriptional activity of active Stat5a/b by 10-fold as well indicating a positive feedback loop between Stat5 and AR in prostate cancer cells (Tan et al., 2008). This interaction in prostate cancer cells may be potentially relevant for growth of prostate cancer during androgen deprivation in the presence of low levels of circulating androgens. In accordance, recent data implicated that knockdown of Stat5a/b enhanced AR degradation and delayed castrate-resistant prostate cancer progression *in vivo* (Thomas et al., 2011). While active Stat5a/b stimulated transcriptional activity of the AR, it is important to note that Stat5a/b inhibition induced rapid apoptotic cell death of also an AR-negative prostate cancer cell line DU145 (Gu et al., 2010). This is a significant observation since it indicates that Stat5a/b regulates prostate cancer cell viability not only through the AR but also through AR-independent mechanisms.

In addition to growth promotion of prostate cancer cells and tumors, Stat5a/b induces metastatic behavior of prostate cancer cells both *in vitro* and *in vivo* (Gu et al., 2010). Nuclear Stat5a/b expression is elevated in 61% of distant clinical prostate cancer metastases, and active Stat5a/b increases migration and invasion of human prostate cancer cells by induction of rearrangement of the microtubule network (Gu et al., 2010). Importantly, active Stat5 induced 10-fold increase in formation of lung metastases in an experimental *in vivo* metastases assay (Gu et al., 2010).

5. Stat5a/b Regulation of Breast Cancer Growth and Differentiation

Stat5a/b is well established as an important regulator of normal mammary gland development in mice (Liu et al., 1997; Miyoshi et al., 2001). Specifically, Stat5a is the primary mediator of lobulo-alveolar differentiation and expansion in mice during pregnancy, as well as the key mediator of lactogenic signaling (Liu et al., 1997; Miyoshi et al., 2001). Moreover, transcriptionally active Stat5a critically promotes survival and viability of terminally differentiated alveolar mammary epithelial cells (Briskin et al., 1999; Iavnilovitch et al., 2002; Miyoshi et al., 2001). In the context of breast cancer, active Stat5a/

b promotes tumorigenesis in rodents based on studies carried out in genetically engineered mice. Loss of one Stat5a allele in mice led to a reduced number of tumor incidence with a marked delay of tumor formation (Ren et al., 2002). However, in human breast cancer, Stat5a/b has been shown to positively correlate with the differentiation status of the tumors (Cotarla et al., 2004). Importantly, active Stat5a/b in human breast cancer predicted favorable clinical outcome (Nevalainen et al., 2004). In addition, active Stat5a/b, induced by Akt-1, positively correlated with mammary epithelial cell differentiation and possibly a better response to endocrine therapy (Creamer et al., ; Yamashita et al., 2006). Collectively, these studies suggest a dual role for Stat5a/b in the mammary gland as an initiator of tumor formation as well as a promoter of differentiations of established tumors.

6. Stat5a/b as a Therapeutic Target Protein

The role of Stat5a/b in pathogenesis and progression of prostate cancer provides a compelling rationale for development of pharmacological inhibitors of Jak2-Stat5a/b signaling pathway. The Prl/Jak2/Stat5a/b signaling pathway provides various molecular targets for development of pharmacological Stat5a/b inhibitors: First, PrlR activation can be blocked by PrlR antagonists, such as S179D-hPrl (Xu et al., 2001) and the specific human Prl antagonist Del1-9G129R-hPrl (Dagvadorj et al., 2007; Llovera et al., 2000). Second, blocking the SH2 domain of Stat5a/b could directly block Stat5a/b dimerization and recruitment to an activated receptor (such as PrlR) (Liao et al., 2010). Third, Jak2 kinase, the major upstream activating kinase of Stat5a/b, can be directly inhibited by various small-molecule inhibitors currently in early clinical trials for myeloproliferative disorders (Pardanani, 2008). AZD1480 is one such small-molecule Jak2 inhibitor with significant pre-clinical activity (Hedvat et al., 2009). Furthermore, specific targeting of Stat5a/b function would inhibit Stat5a/b gene expression and transcriptional activation through various methodological approaches (adenoviral expression of a dominant-negative Stat5a/b, siRNA or anti-sense inhibition of Stat5a/b protein expression) (Ahonen et al., 2003; Dagvadorj et al., 2008). The role of Stat5a/b in breast cancer growth and differentiation is yet-to-be determined. However, since Stat5a/b is an indicator of favorable prognosis in node-negative human breast cancer (Nevalainen et al., 2004), Stat5a/b may be a successful biomarker of favorable clinical outcome. In contrast, nuclear Stat5a/b expression in prostate cancer may provide a biomarker of early disease recurrence. Development of pharmacological inhibitors of Stat5a/b activity may have the most potential for therapeutic use in prostate cancer.

Acknowledgments

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Fig. 1. Schematic structure of Stat5a and Stat5b with domains and specific amino acids mediating important functions

N-terminal domain (oligomerization domain) is responsible tetramerization. Coiled Coil domain is essential for interaction with chaperones and recruitment of co-activators and co-repressors. DNA-binding domain recognizes and binds to GAS element on DNA. Linker domain mediates stabilization of the DNA binding. SH2 domain is essential for the recruitment of Stat5a/b to the receptor and for Stat5a/b dimerization. Transactivation domain is responsible for interaction with co-activators and transcriptional activity. Glycosylation of threonine 92 (T92) is crucial for interaction with p300/CREB-binding protein. Phosphorylation of tyrosine 694/699 (Y694/699) is essential for Stat5a/b activation. Phosphorylation of serines 710/715, 725/730, 779 may inhibit transcriptional activity and signal duration.

Figure 2. The canonical Jak2-Stat5a/b pathway and its functional interaction with androgen receptor signaling in prostate cancer cells

The Jak2-Stat5 signaling cascade involves stimulation of the prolactin receptor (PrIR) followed by transphosphorylation and activation of receptor – associated kinase Jak2. Stat5a/b is recruited to the active receptor through its SH2 domain. Following Jak2-mediated Stat5a/b phosphorylation, active Stat5a/b forms a dimer and translocates to the nucleus. The Stat5a/b dimer recognizes and binds GAS elements (TTC(C/T)N(G/A)GAA) on the DNA, initiating transcription of target genes. In human prostate cancer cells, active Stat5a/b transcriptionally synergizes, physically interacts, and enhances nuclear localization of active androgen receptor (AR).