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Transcription factor Stat5a/b as a therapeutic target protein for prostate cancer

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Abstract

Prostate cancer is the most common non-cutaneous cancer in Western males. The majority of prostate cancer fatalities are caused by development of castration-resistant growth and metastatic spread of the primary tumor. The average duration of the response of primary prostate cancer to hormonal ablation is less than 3 years, and 75% of prostate cancers in the United States progress to hormone-refractory disease. The existing pharmacological therapies for metastatic and/or hormone-refractory prostate cancer do not provide significant survival benefit. This review summarizes the importance of transcription factor Stat5 signaling in the pathogenesis of prostate cancer and discusses the molecular basis why inhibition of Stat5a/b could be used as a therapeutic strategy for prostate cancer.

Keywords

Stat5a/b; prostate cancer; therapy development

1. Introduction

Organ-confined primary prostate cancer is typically treated by surgery, radiation, hormone therapy, or different combinations of these three treatment modalities, depending on the age and operability of the patient (Pestell and Nevalainen, 2008). For a significant fraction of prostate cancers, the existing therapies only provide a temporary relief of the symptoms and the cancer growth, while the hormone-refractory and/or metastatic forms of prostate cancer develop. Currently, there are no effective pharmacological therapies for castration-resistant and/or metastatic prostate cancer (Pestell and Nevalainen, 2008). Identification of the specific mechanisms underlying growth and survival of prostate cancer cells provides a rational basis for development of new therapies for organ-confined and advanced prostate cancer. Transcription factor Stat5a/b is critical for prostate cancer cell survival and for prostate tumor growth (Ahonen et al., 2003; Dagvadorj et al., 2007; Dagvadorj et al., 2008; Kazansky et al., 2003). Recent reports indicate that the Stat5a/b signaling pathway may contribute to the

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progression of organ-confined prostate cancer to castration-resistant and/or metastatic disease. Based on these findings, we propose that the Jak2-Stat5a/b signaling pathway provides molecular targets for development of pharmacological intervention for prostate cancer. In addition, Stat5a/b may be a prognostic marker of primary, organ-confined prostate cancers that are most likely to become aggressive disease.

2. Stat5a/b Transcription Factors in Prostate Cancer Pathogenesis

2.1. Protein structure of Stat5

Stat5a and Stat5b belong to the Stat (Signal Transducer and Activator of Transcription) family of transcription factors which consists of seven members (Stat1, Stat2, Stat3, Stat4, Stat5a, Stat5b, and Stat6) (Hennighausen and Robinson, 2008; Tan and Nevalainen, 2008) (Fig. 1). Stat proteins have likely diverged from a single gene through several consecutive duplications into three separate genetic loci (Darnell et al., 1994). In humans, Stat1 and Stat4 map to chromosome 2 (bands q12 to q33) (Haddad et al., 1998; Yamamoto et al., 1997); Stat2 and Stat6 map to chromosome 12 (bands q13 to q14-1) (Goureau et al., 2001; Leek et al., 1997); and Stat3, Stat5a, and Stat5b map to chromosome 17 (bands q11-1 to q22) (Choi et al., 1996; Lin et al., 1996).

Stat5a was originally identified as a mammary-gland specific transcription factor (MGF) that mediates the effects of prolactin (Prl) in mice (Wakao et al., 1992) and sheep (Gouilleux et al., 1994; Wakao et al., 1994). The Stat5b isoform was later discovered in the mouse mammary gland, and is encoded by a separate gene (Lin et al., 1996; Liu et al., 1996; Liu et al., 1995). While both have the structurally and functionally conserved domains of the other Stat proteins (Schindler and Darnell, 1995), Stat5a has 20 amino acids that are unique to it's C-terminal sequence, while 8 amino acids in the C-terminus are specific to Stat5b. Both Stat5a and Stat5b are activated by Jak2 phosphorylation on a specific tyrosine residue (Liu et al., 1995). However, Stat5a transmits predominantly the signals initiated by the prolactin receptor (PrlR), while Stat5b mediates the biological effects of growth hormone (GH). In addition, Stat5a/b can also be activated by several other ligands including interleukin-2 (IL-2) (Hou et al., 1995), IL-3 (Mui et al., 1995a), IL-5 (Mui et al., 1995a), IL-7 (Foxwell et al., 1995), granulocyte-macrophage colony-stimulating factor (GM-CSF) (Mui et al., 1995b), insulin (Wartmann et al., 1996), erythropoietin (EPO) (Gouilleux et al., 1995; Wakao et al., 1994) and thrombopoietin (TPO) (Pallard et al., 1995).

Stat5a/b proteins contain six functional domains in their protein structure (Fig. 1). The N-terminal domain of Stat5a/b (NTD; aa 1-126) stabilizes interactions between two Stat dimers to form tetramers. The Stat5-DNA interaction is strengthened by tetramerization, particularly at tandemly linked non-consensus and consensus Stat5 binding sites, which augments the transcriptional activation of weak promoters (John et al., 1999;Meyer et al., 1997;Soldaini et al., 2000). Post-translational glycosylation of threonine 92 of Stat5 allows interaction with the CREB-binding protein, which is required for Stat5 – mediated gene transcription (Gewinner et al., 2004). The coiled-coil domain (CCD; aa 138-330) interacts with chaperones (Xu et al., 2004), recruits co-activators (Zhu et al., 1999) and co-repressors (Maurer et al., 2002), and facilitates multiple protein-protein interactions crucial for transcriptional regulation. The central DNA-binding domain (DBD; aa 332-583) permits binding of Stat5a/b to consensus gamma-interferon activation sequence (GAS) (TTC(C/T)N(G/A)GAA) motifs contained in the regulatory elements of target genes (Decker et al., 1991;Horvath et al., 1995;Soldaini et al., 2000).

The linker domain (LD; aa 475-592) of Stats stabilizes the DNA-binding. Specifically, site-directed mutagenesis of the Stat1 linker domain resulted in a mutant dimer that binds and dissociates from DNA more rapidly than the wild type protein, suggesting a time-dependent

element in the linker domain that regulates gene transcription (Yang et al., 2002). The most highly conserved domain of Stat5a/b is the Src homology 2 domain (SH2; aa 593-670), which mediates receptor-specific recruitment and Stat dimerization (Stocklin et al., 1996) via the phosphorylated tyrosine residue of one Stat5 to the SH2 domain of another (Wakao et al., 1994). The most variable region is the transcriptional activation domain (TAD; Stat5a, aa 722-794;Stat5b, aa 727-787) in the C-terminus, which interacts with critical co-activators, such as the p300/CREB-binding protein (CBP)-associated co-activator NcoA-1 (Litterst et al., 2003), centrosomal P4.1-associated protein (CPAP) (Peng et al., 2002), P100 (Paukku et al., 2003) and Oct-1 (Magne et al., 2003).

2.2 The Canonical Prl-Jak2-Stat5a/b Signaling Pathway

The cytokine receptors that activate Stat5a/b generally lack tyrosine kinase activity. This kinase activity is provided by receptor-associated cytoplasmic Janus kinases (Jaks) (Hennighausen and Robinson, 2008). There are four members (Jak1, Jak2, Jak3 and Tyk2) in the Jak family. Jak3 is preferentially expressed in leukocytes. The other three Jak proteins (Jak1, Jak2, and Tyk2) are ubiquitously expressed in mammalian cells (Ghoreschi et al., 2009). The primary Jak protein that activates Stat5a/b is Jak2 (Gouilleux et al., 1994).

Prl binding induces a conformational change in the PrlR that brings the PrlR-associated Jak2 molecules into close proximity, allowing them to activate each other and phosphorylate specific tyrosine motifs of the PrlR (Fig. 2). Stat5a/b recognizes these phosphorylated tyrosine motifs through their SH2 domains, are recruited to the docking sites, and undergo rapid phosphorylation of a conserved tyrosine residue in the C-terminus by Jak2. The phosphorylation of tyrosine residues Y694 and Y699 activates Stat5a and Stat5b, respectively, leading them to homo- or heterodimerize through a phosphotyrosine–SH2 domain (Becker et al., 1998;Chen et al., 1998). A variety of protein kinases have been shown to phosphorylate Stats on serine residues, providing additional signaling pathways to potentiate the primary activating stimulus (Decker and Kovarik, 2000). However, the phosphorylation of the serine residues S726 of Stat5a (Yamashita et al., 2001), and S731 on Stat5b, may inhibit transcriptional activity (Yamashita et al., 1998).

Phosphorylated Stat5a/b dimers translocate from the cytoplasm into the nucleus, where they bind to an 8–10 base pair inverted repeat consensus sequence, TTC(C/T)N(G/A)GAA, referred to as the GAS element, and activate transcription of target genes (Horvath et al., 1995; Soldaini et al., 2000). Homodimers of Stat5a and Stat5b have equal binding specificities for palindromes that are spaced three base pairs apart (Ehret et al., 2001; Soldaini et al., 2000), while tetrameric binding occurs between tandem nonconsensus and consensus GAS motifs that are 6 base pairs apart (Soldaini et al., 2000). Therefore, the non-redundant functions of Stat5a and Stat5b during development are not a result of differences in DNA binding specificity, but could be attributed to their cell type-specific expression, or the interactions of their divergent C-terminus with different co-regulators (Ehret et al., 2001).

The exact molecular mechanisms of the nuclear import and export of Stat5a/b are still largely unclear. Nuclear and cytoplasmic pools of unphosphorylated Stat5a/b proteins may shuttle freely at high exchange rates in the absence of cytokine activation (Reich and Liu, 2006; Vinkemeier, 2004). In addition, recent reports demonstrate that the coiled coil domain is involved in constitutive nuclear import of latent and activated STAT5a (Iyer and Reich, 2008). While non-phosphorylated Stat5a/b proteins cycle between the cytoplasm and nucleus, the translocation of Stat5a/b dimers has been proposed to be an active, energy-dependent process (Reich and Liu, 2006; Vinkemeier, 2004) that utilizes components of Ran-dependent nuclear import machinery (Sekimoto et al., 1997; Sekimoto et al., 1996). MgcRacGAP, a chaperone protein that contains a nuclear localization signal, has been shown to be able to bind to the DNA-binding domain of the phosphorylated Stat5a/b dimer and, with GTP-bound Rac1,

form a shuttling complex between the nucleus and cytoplasm (Kawashima, et al. 2009). The karyopherin importin-b (p97) binds to this complex, and was identified as the carrier that transports Stat5a/b into the nuclear compartment. Intriguing new evidence also has suggested that unphosphorylated Stats may be able to bind to DNA in association with other transcription factors (Chatterjee-Kishore et al., 2000; Yang et al., 2005; Yang et al., 2007).

2.3. Active Stat5a/b in clinical progression of prostate cancer

2.3.1. Stat5a/b is highly critical for the viability of human prostate cancer cells in vitro and in vivo—Stat5a/b is the key mediator of the Prl signaling in both normal and malignant prostate tissue (Ahonen et al., 2002; Li et al., 2004; Nevalainen et al., 1996; Xu et al., 2001). Autocrine Prl acts as a powerful autocrine mitogen and survival factor for prostate epithelium (Ahonen et al., 1999; Dagvadorj et al., 2007; Nevalainen et al., 1997b). The functional importance of Stat5 in prostate tissue has been determined through studies of human prostate cancer cells (*in vitro* and *in vivo*), the TRAMP mouse prostate cancer model and the phenotype analysis of the *STAT5A* knockout mice. Specifically, the prostate epithelium of *STAT5A* null mice was comprised of deformed or broken prostate acini that contained desquamated, granular epithelial cells in a dense, coagulated secretory material (Nevalainen et al., 2000). Importantly, the tissue architecture of *STAT5A*−/− mice did not display the morphological hallmarks of prostate epithelial hyperplasia. Rather, the prostate characteristics of *STAT5A*−/− mice suggested that Stat5a is involved in the maintenance of integrated prostate epithelial structures.

In human prostate cancer cells, transcription factor Stat5a/b is critical for cell viability and prostate tumor growth *in vivo* (Dagvadorj et al., 2008). The novel concept that Stat5a/b controls growth and survival of prostate cancer cells has been demonstrated by several different studies. First, Ahonen et al. showed that inhibition of Stat5a/b by adenoviral expression of a dominant-negative mutant of Stat5a/b (DNStat5a/b) in Stat5-positive human prostate cancer cells induced massive apoptotic death as determined by cell viability assays and biochemical analysis (Ahonen et al., 2003). Second, examination of the TRAMP mouse prostate cancer model confirmed the central role of Stat5a/b in regulating the viability of prostate cancer cells (Kazansky et al., 2003). Third, Dagvadorj et al. recently demonstrated that Stat5 inhibition, regardless of the methodological approach (Stat5a/b siRNA, antisense oligonucleotides or adenoviral expression of DNStat5a/b), results in massive cell death in a variety of Stat5-positive human prostate cancer cell lines (Dagvadorj et al., 2008). In addition, inhibition of Stat5a/b decreased both incidence and growth of human prostate subcutaneous xenograft tumors in nude mice (Dagvadorj et al., 2008). *CYCLIN-D1* and *BCL-xL* are Stat5a/b target genes in human prostate cancer cells (Dagvadorj et al., 2008). The individual roles of Stat5a vs. Stat5b in the growth regulation of prostate cancer cells remain to be examined. Moreover, molecular mechanisms directing rapid apoptosis of prostate cancer cells as a result of Stat5a/b inhibition need to be understood since they may identify additional therapeutic target proteins for pharmaceutical intervention.

2.3.2. Active Stat5a/b as a prognostic factor for prostate cancer—Stat5a/b is persistently in the active state in human prostate cancer cells, but not in adjacent normal prostate acini (Ahonen et al., 2003). It has also been shown that activation of Stat5a/b is significantly more frequent in high grade human prostate cancer compared to intermediate or low grade prostate cancers (Li et al., 2004). The association of Stat5 activation with high histological grades of prostate cancer has been confirmed using tissue microarrays (Li et al., 2005). Furthermore, Stat5a/b is more likely to be active in the primary prostate cancer of a patient who had been treated with androgen ablation prior to radical prostatectomy vs. patients who have not received adjuvant androgen deprivation therapy (Tan et al., 2008). Moreover, active Stat5a/b in primary prostate cancer predicted early disease recurrence after initial treatment by

radical prostatectomy (Li et al., 2005). Significantly, if only prostate cancers of intermediate Gleason grades were analyzed, active Stat5a/b still remained an independent prognostic marker of early recurrence of prostate cancer (Li et al., 2005). The distribution of active Stat5a *vs.* Stat5b in prostate cancers of different histological grades remains to be determined, as well as the individual prognostic value of Stat5a *vs.* Stat5b in prostate cancer.

2.3.3. Stat5a/b functionally interacts with androgen receptor in prostate cancer cells—Castration-resistant prostate cancer continues to express the androgen receptors (AR) and androgen-regulated genes, which implies that the AR signaling pathway remains active dispite low levels of circulatory androgens after androgen ablation therapy (Isaacs and Isaacs, 2004). Recently, Tan et al. demonstrated that active transcription factor Stat5a/b is expressed in 95% of castration-resistant clinical human prostate cancers (Tan et al., 2008). The study further showed that the activeated Stat5a/b signaling pathway increased the transcriptional activity of AR, and ligand-bound AR, in turn, increases transcriptional activity of Stat5a/b in prostate cancer cells (Tan et al., 2008) (Fig. 3). Furthermore, active Stat5a/b physically interacted with liganded AR, and both Stat5a/b and AR enhanced nuclear localization of each other when activated in prostate cancer cells (Tan et al., 2008). Recent findings indicate that recurrent prostate cancers develop the intracellular capacity to biosynthesize testicular androgens from adrenal androgens and cholesterol (Mohler et al., 2004; Penning and Byrns, 2009; Titus et al., 2005; Yuan and Balk, 2009). Given that Stat5a/b and AR are both anti-apoptotic and growth-promoting transcription factors in prostate cancer cells, promotion of AR transcriptional activity by Stat5a/b in the presence of low levels of androgens may therefore contribute to castration-resistant growth of prostate cancer. AR, in turn, by promoting transcriptional activity of Stat5a/b, may critically support viability of prostate cancer cells in growth conditions where prostate cancer cells would normally undergo apoptosis. Future work needs to identify the detailed molecular mechanisms underlying the co-action between Stat5a/b and AR, the effect of Stat5a/b-AR synergy on prostate cancer growth *in vivo*, and the Stat5 interaction with mutated AR liganded by adrenal androgens or anti-androgens.

2.3.4. Positive regulators of Stat5a/b in prostate cancer—The molecular mechanisms underlying high-level expression of active Stat5a/b in primary and castration resistant human prostate cancer are largely unclear. One mechanism may be autocrine Prl in prostate cancer cells responsible for the constitutive activation of Stat5a/b in human prostate cancer (Li et al., 2004; Nevalainen et al., 1997a; Nevalainen et al., 1997b). Prl is produced locally by normal prostate epithelium and prostate cancer, and is known to promote proliferation and survival of prostate cells (Ahonen et al., 1999; Dagvadorj et al., 2007; Kindblom et al., 2002; Kindblom et al., 2003; Nevalainen et al., 1997a; Nevalainen et al., 1996; Nevalainen et al., 1997b; Nevalainen et al., 1991; Wennbo et al., 1997). Prl is one of the predominant cytokines 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 protein and PrlR expression are associated with high histological grades of human prostate cancer (Li et al., 2004). Activating mutations of Jak2 have been recently described in hematopoietic malignancies resulting in constitutive activation of Stat5 (Baxter et al., 2005). Since Jak2 is the predominant kinase that activates Stat5a/b (Li et al., 2004), such somatic gain-of-function Jak2-mutations may also occur in advanced prostate cancer. A third potential mechanism for the high abundance of Stat5a/b in prostate cancer is amplification of Stat5a/b genes. Importantly, the *STAT5A/B* genes are located on chromosome 17 (Clark et al., 2003), which is frequently altered in both incidental and hereditary prostate cancer (Gillanders et al., 2004). Stat5a/b might also be activated by tyrosine kinases such as Src (Yu and Jove, 2004) or fusion proteins containing tyrosine kinase activity such as Bcr-Abl (de Groot et al., 1999) or Tel-Jak (Schwaller et al., 2000). Although GH is a principal activator of Stat5b in a number of tissues (Halmos et al., 2002; Letsch et al., 2003; Wang et al., 2005; Weiss-Messer et al., 2004), there is currently no evidence to support of GH-

Stat5-mediated stimulation of prostate cancer cell growth. Alternatively, reduced expression of Stat5a/b phosphatases or inhibitory proteins of Stat5a/b, such as PIAS, may result in active Stat5a/b in malignant prostate epithelium.

2.3.5. Negative Regulators of Stat5 Signaling in Prostate Cancer—Several different molecular mechanisms regulate the duration and magnitude of Stat5a/b activation in the cytoplasmic and nuclear compartments. These mechanisms involve: (1) protein inhibitors of activated Stat proteins (PIAS); (2) cytoplasmic and nuclear protein tyrosine phosphatases (PTP); and (3) suppressors of cytokine signaling (SOCS) proteins.

The PIAS family of proteins are localized within the nucleus and function as constitutive repressors of STAT activity by direct association (Schmidt and Muller, 2003; Shuai, 2006). The PIAS family members include PIAS1, PIAS3, PIASx, PIASy, and alternative splicing variants of PIASx (Palvimo, 2007). While PIASy acts as a co-repressor of AR (Gross et al., 2001; Junicho et al., 2000), other members PIAS1, PIAS3 (Wang and Banerjee, 2004) and PIAS-like proteins Zimp7 (Huang et al., 2005) and Zimp10 (Sharma et al., 2003) have been shown to function as co-activators of AR mediated transcription in human prostate epithelial cells. PIAS3 is the only member of the PIAS family that has been shown to directly interact with Stat5a/b and repress Stat5-mediated transcription in CHO and lymphoid NB2 cells (Rycyzyn and Clevenger, 2002). PIAS3 is expressed in prostate cancer tissues and cell lines (Wang and Banerjee, 2004), and PIAS1 expression has been shown to be 33% higher in primary prostate cancers compared to normal prostates, but this overexpression did not correlate with the Gleason score as determined by *in situ* hybridization of PIAS1 mRNA (Li et al., 2002). Furthermore, PIAS1 expression has been shown to be significantly lower in hormone-refractory prostate cancer than in untreated prostate tumors (Linja et al., 2004). However, interaction and the effects of PIAS1 on Stat5a/b activity in prostate cancer cells remain unclear. It will be important to determine the interaction of PIAS proteins with Stat5a/b and Stat5a/b-regulated gene transcription in prostate cancer cells to understand how PIAS protein expression contributes to prostate cancer progression.

PTPs are enzymes that regulate the dephosphorylation event of the Jak/Stat5 signaling components (Xu and Qu, 2008). One of the PTPs member, SHP-2 which contains two Src homology 2 (SH2) domains, directly interacts with Stats in the cytoplasm and translocates into the nucleus as a complex (Chen et al., 2003; Chughtai et al., 2002). Cytosolic PTP1B (Aoki and Matsuda, 2000) and the nuclear phosphatase TC-PTP (Aoki and Matsuda, 2002) are also known to dephosphorylate and inactivate Stat5a/b in mammary epithelial cells. However, to date there is no direct evidence of PTP regulation of Stat5a/b activity in prostate cancer cells.

A third mechanism for down-regulation of Stat5a/b signaling involves SOCS proteins family which comprises of eight members, including CIS (cytokine-inducible SH2 domain protein) and SOCS1–7 (Croker et al., 2008). SOCS proteins are rapidly induced by activated Stats and act to block the cytokine signaling by direct inhibition of the upstream activator Jaks and/or by competitive binding to tyrosine phosphorylated receptors (Alexander and Hilton, 2004). The expression levels of SOCS1, SOCS3, SOCS5, and CIS genes in PC-3 and DU145 human prostate cancer cells are significantly lower than in the normal RWPE-1 prostate cells (Evans et al., 2007). However, the expression of SOCS1, SOCS3, SOCS5 and CIS genes in LNCaP cells were at levels comparable to RWPE-1 cells indicating that SOCS proteins are not silenced in all human prostate cancer cell lines (Evans et al., 2007). Future studies need to evaluate whether SOCS proteins directly regulate the Jak2-Stat5a/b pathway in human prostate cancer cells in order to contribute to constitutive activation of Stat5a/b in clinical prostate cancer.

3. Therapeutic targeting of Stat5a/b in prostate cancer

Currently, there are no effective pharmacological therapies for castration-resistant and/or disseminated prostate cancer. We propose that Jak2-Stat5a/b signaling pathway provides several molecular targets for therapy development for prostate cancer. Stat5a/b activity and function in prostate cancer cells can be disrupted at multiple levels. First, activation of the PrlR can be blocked by specific PrlR antagonists, such as G129R-Prl (Llovera et al., 2000). The human Prl antagonist (Delta1-9G129R-hPrl) has been demonstrated to inhibit the Jak2/Stat5a/b signaling pathway by blocking the autocrine Prl activation of PrlR in prostate cancer (Dagvadorj et al., 2007). Second, small-molecule inhibitors for Jak2 are currently in active development (Gozgit et al., 2008) and may provide an effective therapeutic tool for those prostate cancers in which Jak2 is the primary kinase responsible for Stat5a/b activation. Third, direct blocking of the SH2 domain of Stat5a/b would theoretically result in the specific inhibition of Stat5a/b recruitment to an activated receptor (such as PrlR), in addition to inhibiting Stat5a/b phosphorylation, dimerization, nuclear translocation and DNA binding. Although SH2 domain binding phosphotyrosyl peptides or peptidomimetics have been developed to selectively inhibit Stat3 dimerization (Turkson et al., 2004; Turkson et al., 2001), no SH2-domain targeting compounds have been reported for Stat5a/b. In addition, peptide aptamers selected from combinatorial peptide libraries can potentially be used to block Stat5 dimerization or DNA binding (Nagel-Wolfrum et al., 2004). Another approach would be to design decoy oligonucleotides that mimic Stat5a/b binding sites, sequestering Stat5 from its target genes and inhibiting DNA binding (Guh et al., 2001). Transcriptional activation of Stat5a/b has been successfully disrupted by expression of Stat5 that lacks the C-terminal transactivation domain and therefore acts in a dominant-negative manner (Ahonen et al., 2003; Dagvadorj et al., 2008). Moreover, down-regulation of Stat5a/b gene expression by antisense oligonucleotides or RNA interference effectively induced apoptotic death of human prostate cancer cell lines (Dagvadorj et al., 2008). Finally, novel approaches for prostate cancer specific delivery of pharmacological agents are under development in a number of laboratories in order to overcome the adverse effects of systemic drugs (Isaacs, 2005). These delivery methods will eventually be available for pharmacological Stat5a/b inhibitors as a targeted treatment of prostate cancer.

Abbreviations

Stat	Signal Transducer and Activator of Transcription
Prl	prolactin
PrlR	prolactin receptor
AR	androgen receptor

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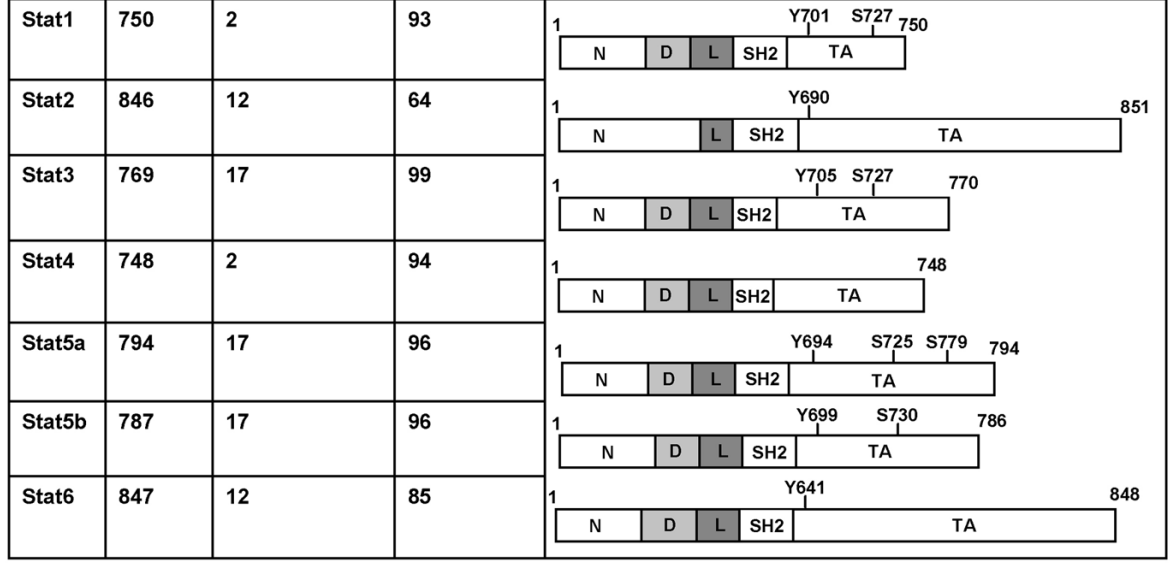
(A)

Stats	Amino Acids	Chromosome	Homology (%)	Structure
Stat1	750	2	93	
Stat2	846	12	64	
Stat3	769	17	99	
Stat4	748	2	94	
Stat5a	794	17	96	
Stat5b	787	17	96	
Stat6	847	12	85	



(B)

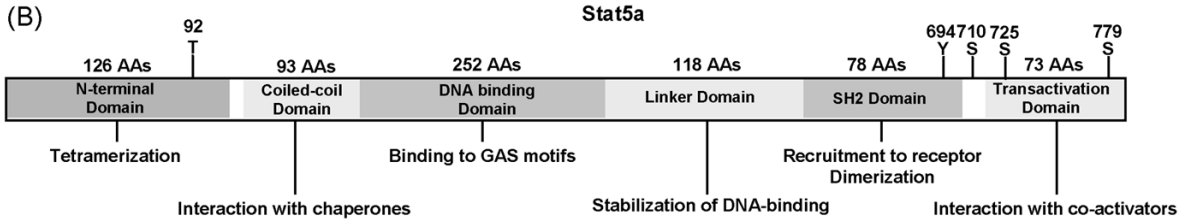


Figure 1. The members of Stat family of transcription factors

A. Chromosomal location in human, sequence identity between human and mouse Stats and the major structural features and phosphorylation sites. **B.** Schematic Stat5a domains and specific amino acids mediating important functions. Glycosylation of threonine 92 (T92) is crucial for interaction with p300/CREB-binding protein. Phosphorylation of tyrosine 694 (Y694) is essential for Stat5a activation. Mutation of serine 710 to phenylalanine (S710F) confers constitutive activation to Stat5a (gain of fuction). Phosphorylation of serine 725 has an impact on signal duration.

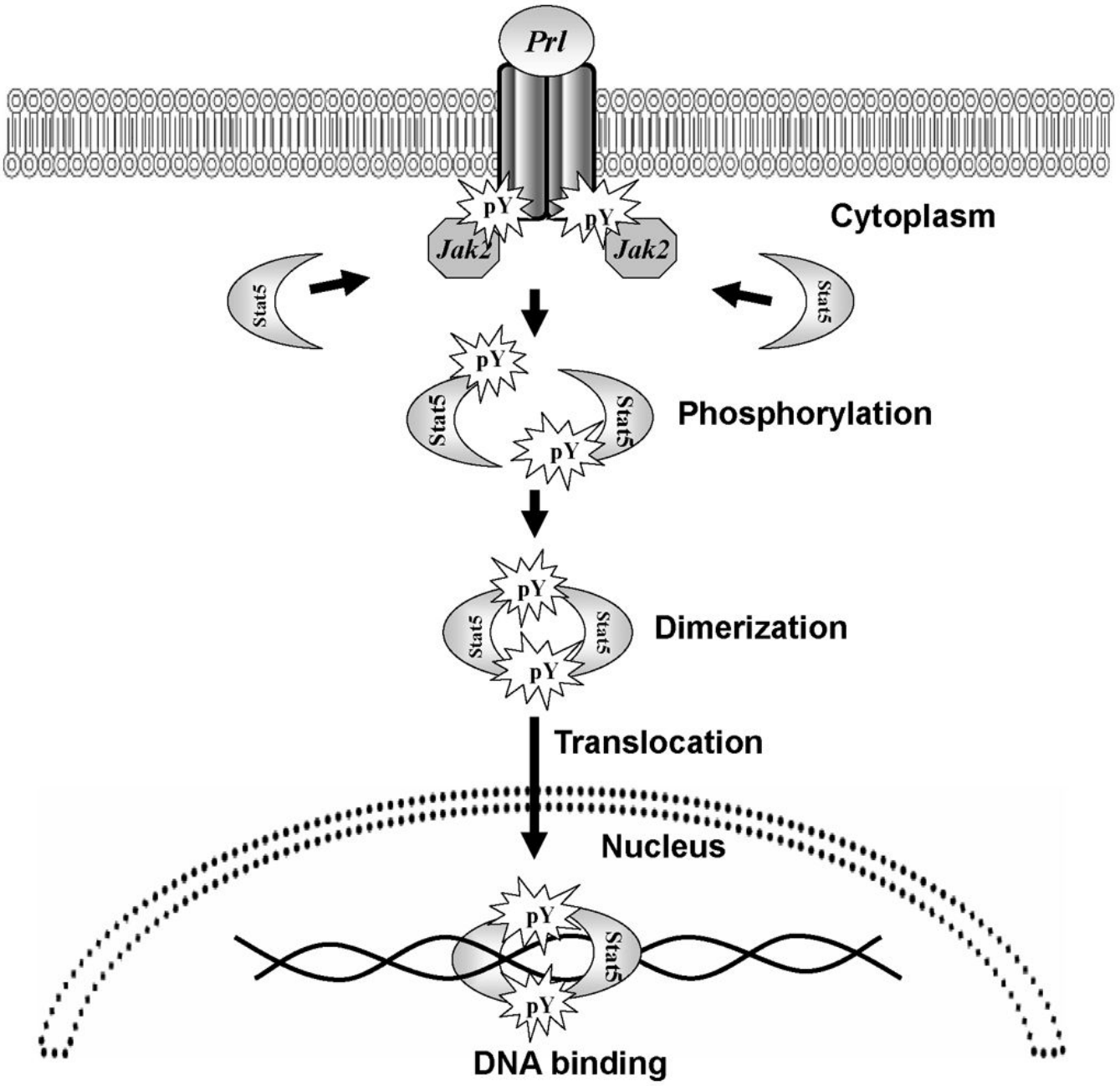


Figure 2. The canonical Prolactin (Prl)-Jak2-Stat5a/b signaling pathway

Prl binding brings the PrlR-associated Jak2 molecules into close proximity and subsequent activation. Cytoplasmic Stat5a/b proteins are recruited to the activated Prl-receptor-Jak2 complex, and Jak2 phosphorylates tyrosine residues Y694 and Y699 of Stat5a and Stat5b, respectively, leading them to homo- or heterodimerize through a phosphotyrosine SH2 domain interaction. Phosphorylated Stat5a/b dimers translocate from the cytoplasm into the nucleus, where they bind to DNA to regulate transcription.

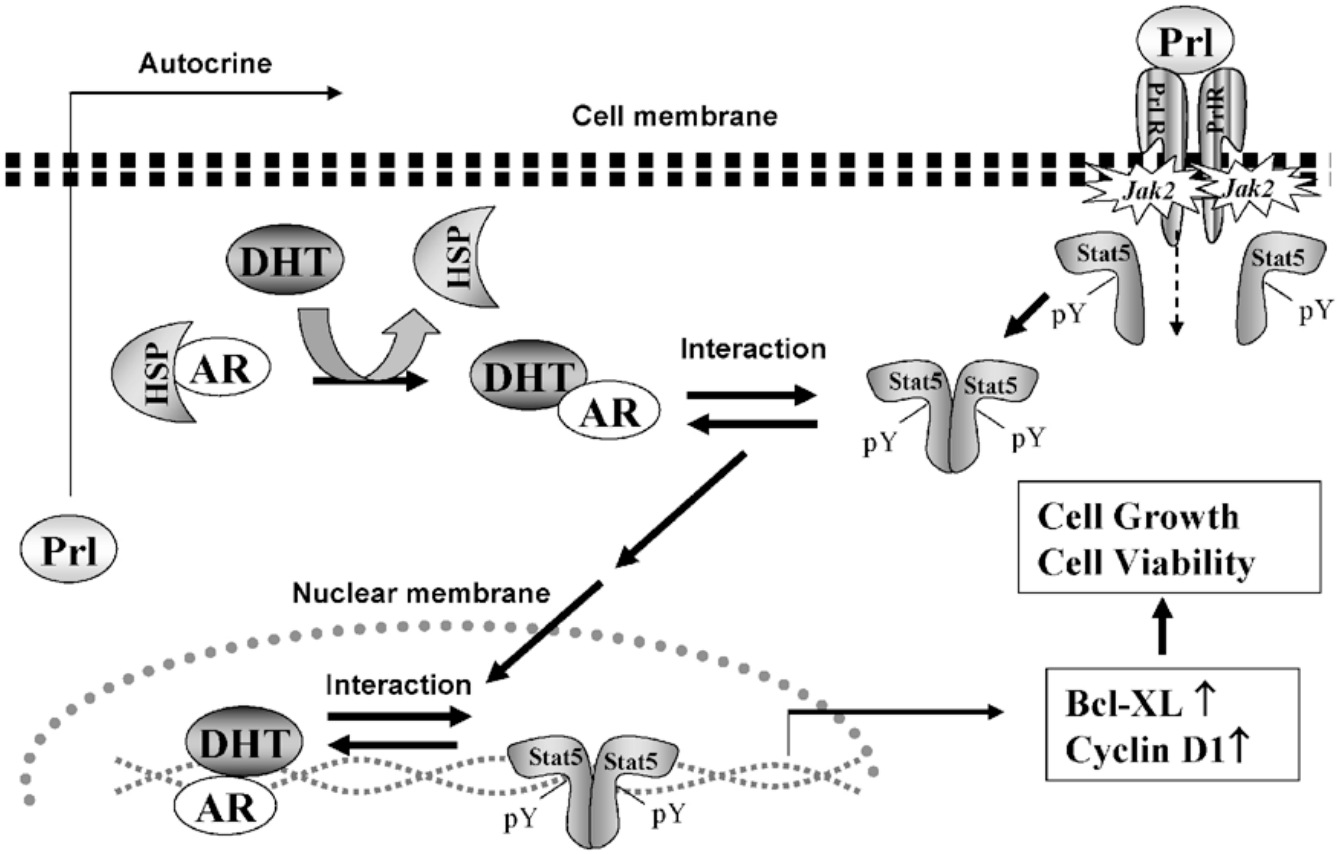


Figure 3. Interaction of Stat5a/b and androgen receptor (AR) signaling pathways in prostate cancer cells

Binding of Prl leads to Prl-receptor (PrlR) dimerization and activation of Jak2 proteins pre-associated with the cytoplasmic domains of PrlR. Cytoplasmic Stat5a/b are recruited to the activated PrlR-Jak2 complex, Stat5a/b is phosphorylated on a conserved tyrosine residue in the C-terminus of Stat5a/b by Jak2 resulting in Stat5 dimerization. Ligand binding to the AR leads to its dissociation from the heat shock proteins (HSPs) and dimerization. Liganded AR physically interacts with Stat5a/b in prostate cancer cells. Liganded AR and active Stat5a/b promote nuclear translocation and transcriptional activity of each other. Both AR and Stat5 signaling pathways are critical regulators of growth, viability and apoptosis of prostate cancer cells.