REVIEW

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Signal transducer and activator of transcription 5A/B in prostate and breast cancers

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Abstract

Protein kinase signaling pathways, such as Janus kinase 2-Signal transducer and activator of transcription 5A/B (JAK2-STAT5A/B), are of significant interest in the search for new therapeutic strategies in both breast and prostate cancers. In prostate cancer, the components of the JAK2-STAT5A/B signaling pathway provide molecular targets for small-molecule inhibition of survival and growth signals of the cells. At the same time, new evidence suggests that the STAT5A/B signaling pathway is involved in the transition of organ-confined prostate cancer to hormone-refractory disease. This implies that the active JAK2-STAT5A/B signaling pathway potentially provides the means for pharmacological intervention of clinical prostate cancer progression. In addition, active STAT5A/B may serve as a prognostic marker for identification of those primary prostate cancers that are likely to progress to aggressive disease. In breast cancer, the role of STAT5A/B is more complex. STAT5A/B may have a dual role in the regulation of malignant mammary epithelium. Data accumulated from mouse models of breast cancer suggest that in early stages of breast cancer STAT5A/B may promote malignant transformation and enhance growth of the tumor. This is in contrast to established breast cancer, where STAT5A/B may mediate the critical cues for maintaining the differentiation of mammary epithelium. In addition, present data suggest that activation of STAT5A/B in breast cancer predicts favorable clinical outcome. The dual nature of STAT5A/B action in breast cancer makes the therapeutic use of STAT5 A/B more complex.

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Introduction

Signal transducer and activator of transcription 5 (STAT5) was originally identified in the mouse mammary gland (Schmitt-Ney *et al*. 1991) and it was further characterized in the mouse system (Schmitt-Ney *et al*. 1992*a*,*b*, Wakao *et al*. 1992) before a 'mammary gland factor' (MGF) was cloned from sheep mammary gland. MGF was identified as the signaling protein that mediates the effects of prolactin (PRL) (Gouilleux *et al*. 1994, Wakao *et al*. 1994). It soon became clear that MGF had a significant sequence homology with the members of the STAT transcription factor family, and MGF was renamed STAT5 (Gouilleux *et al*. 1994, 1995*a*,*b*, Wakao *et al*. 1994). Later, another isoform of STAT5 was discovered in the mammary gland (Liu *et al*. 1995, 1996*a*, Lin *et al*. 1996), which was encoded by a separate gene. This STAT5 homolog was named STAT5B, whereas the original MGF carried the name STAT5A. Further studies revealed that STAT5B was a crucial signaling protein mediating the biological effects of growth hormone (GH), while the key function of STAT5A was to transduce the signals initiated by PRL receptors. In parallel with these significant findings, it was realized that STAT5A/B becomes activated by phosphorylation on a tyrosine residue (Gouilleux *et al*. 1994, Liu *et al*. 1995, 1996*a*). Concurrently, the PRL receptor-associated tyrosine kinase, Janus kinase 2 (JAK2), was discovered (Rui *et al*. 1994).

At the same time as the components of the PRL receptor-JAK2-STAT5A/B signaling pathway were identified, understanding of PRL as a classical peptide

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hormone underwent significant changes. Specifically, it was realized that PRL, which is known as a pituitary hormone secreted by the cells in the anterior lobe of the hypophysis, is actually produced as a local growth factor both in normal and malignant prostate (Nevalainen *et al*. 1997*a*,*b*, Li *et al*. 2004, Dagvadorj *et al*. 2007) and mammary glands (Ginsburg & Vonderhaar 1995). It was known that PRL has both mitogenic and secretory effects on breast epithelial cells. Unexpectedly, PRL was found to be a significant mitogen and survival factor for prostate epithelial cells as well (Nevalainen *et al*. 1991, 1997*b*, Wennbo *et al*. 1997, Kindblom *et al*. 2002, 2003). Autocrine PRL in prostate and breast tissue became important findings, since local production of PRL provided an explanation as to why the results of studies trying to link breast or prostate cancer incidence or progression with the circulating PRL levels yielded controversial results. Potential molecular mechanisms and factors, in addition to pituitary and autocrine GH and PRL underlying the constitutive activation of STAT5A/B signaling pathway in prostate and breast cancers will be discussed. Furthermore, involvement of STAT5A/B signaling pathway in the regulation of growth and progression of prostate and breast cancers will be reviewed.

Structure and function of STAT5A/B proteins

The STAT family of transcription factors has seven members (STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6) that are all encoded by separate genes (Darnell *et al*. 1994, Zhong *et al*. 1994*b*). The STAT proteins are likely to have diverged from a single gene through several consecutive duplications into three genetic loci. Specifically, in humans, the *STAT* genes map to three chromosomal regions (Copeland *et al*. 1995): *STAT3, STAT5A*, and *STAT5B* map to chromosome 17 (bands q11-1 to q22); *STAT1* and *STAT4* map to chromosome 2 (bands q12 to q33) (Yamamoto *et al*. 1997, Haddad *et al*. 1998); and *STAT2* and *STAT6* map to chromosome 12 (bands q13 to q14-1) (Leek *et al*. 1997, Goureau *et al*. 2001; Table 1). Differential splicing and proteolytic processing further increase the diversity of STAT function in cells (Haddad *et al*. 1998, Ihle 2001). In the mouse, *Stat1* and *Stat4* are located on chromosome 1 (band 1 C1.1) (Schindler *et al*. 1992, Yamamoto *et al*. 1994); *Stat2* and *Stat6* are on chromosome 10 (band 10 D3) (Fu *et al*. 1992, Quelle *et al*. 1995); and *Stat3*, *Stat5a*, and *Stat5b* map to chromosome 11 (band 11 D) (Zhong *et al*. 1994*a*, Copeland *et al*. 1995, Shi *et al*. 1996, Levy *et al*. 1998; Table 1).

STATs are proteins of 750–900 amino acids (90–115 kDa) with five structurally and functionally conserved domains that allow the transduction of ligand-specific signals (Schindler & Darnell 1995). The 94 kDa STAT5A (human, 794 amino acids; mouse, 793 amino acids) and 92 kDa STAT5B (human, 787 amino acids; mouse 786 aa) are distinct, but highly homologous isoforms (Mui *et al*. 1994, Liu *et al*. 1995, 1996*a*) (Table 1). Both carry five domains (Table 2) that are structurally and functionally conserved with other STAT proteins and allow the transduction of ligand-specific signals (Schindler & Darnell 1995) (Fig. 1). There is a unique stretch of 20 and 8 amino acids at the C-terminus of STAT5A and STAT5B respectively. The domain of STAT5A/B that is most highly conserved with other STAT proteins is the SH2 domain (aa 593–670, Table 1) that mediates both receptor-specific recruitment and Stat dimerization (Stocklin *et al*. 1996) through the phosphorylated tyrosine residue of one STAT5 to the SH2 domain of another (Wakao *et al*. 1994, Welte *et al*. 1994). The central DNA-binding domain (DBD, aa 332–583) of STAT5A/B is another conserved domain that allows binding of STAT5A/B to consensus GAS (γ-interferon activation sequence) sites (TTC(C/T)N(G/A)GAA)

Table 1 Chromosomal mapping and sequence identity between human and mouse signal transducer and activator of transcriptions (STATs)

	Human		Mouse			
STAT	Amino acid	Chromosome	Amino acid	Chromosome	% Identity	References
STAT1	750	2	749	1	93	Schindler *et al.* (1992) and Haddad *et al.* (1998)
STAT2	846	12	915	10	64	Fu *et al.* (1992) and Goureau *et al.* (2001)
STAT3	769	17	769	11	99	Zhong *et al.* (1994*a*) and Choi *et al.* (1996)
STAT4	748	2	748	1	94	Yamamoto *et al.* (1994, 1997)
STAT5A	794	17	793	11	96	Mui *et al.* (1995) and Lin *et al.* (1996)
STAT5B	787	17	786	11	96	Mui *et al.* (1995) and Lin *et al.* (1996)
STAT6	847	12	837	10	85	Quelle *et al.* (1995) and Leek *et al.* (1997)

Table 2 Location of conserved functional domains of human and mouse signal transducer and activator of transcription 5A/B (STAT5A/B)

	Human		Mouse	
	STAT5A	**STAT5B**	**STAT5a**	**STAT5b**
N-domain	1–126	1–126	1–126	1–126
Coiled-coil domain (STAT protein, all-α domain)	138–330	138–330	138–330	138–330
DNA-binding domain	332–583	332–583	332–583	332–583
Linker domain	475–592	475–592	475–592	475–592
SH2 domain	593–670	593–670	593–670	593–670
Phosphotyrosyl segment	686–701	686–706	686–701	686–706
Transactivation domain	722–794	727–787	721–793	726–786

within gene regulatory elements (Decker *et al*. 1991, Horvath *et al*. 1995, Soldaini *et al*. 2000). The linker domain (aa 475–592) of STATs, named based on crystallographic studies, in fact overlaps in function with the DBD (Yang *et al*. 2002). 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 (Yang *et al*. 2002). The N-terminal domain of STAT5A/B (aa 1–126) stabilizes interactions between two STAT dimers to form tetramers. STAT5–DNA interaction, particularly at adjacent non-consensus STAT5-binding sites, is reinforced by tetramerization that enhances transcriptional activation of weak promoters (Horvath *et al*. 1995, Meyer *et al*. 1997, John *et al*. 1999, Soldaini *et al*. 2000). Homodimers of STAT5A and STAT5B were found to share similar binding specificities for half palindromes that are spaced 3 bp apart (Soldaini *et al*. 2000, Ehret *et al*. 2001). The non-redundant functions of STAT5A and STAT5B during development are likely due to their cell type-specific expression or due to the interactions of their divergent C-terminus with different co-regulators rather than a result of differences in DNA-binding specificity (Ehret *et al*. 2001). Tetrameric binding is made possible by tandemly linked GAS (gamma - interferon activation sequence) motifs with non-consensus motifs that are optimally spaced 6 bp apart (Soldaini *et al*. 2000).

The glycosylation on threonine 92 of STAT5 is found to enhance interaction with the co-activator of transcription CREB-binding protein (CBP; Gewinner *et al*. 2004). Adjacent to the N-terminus of STAT5A/B is the coiled-coil domain (aa 138–330), consisting of a four-helix bundle (Soldaini *et al*. 2000) (Fig. 1; Table 2), which facilitates multiple protein–protein interactions crucial for transcriptional regulation by interacting with chaperones (Xu *et al*. 2004), with Nmi, which helps the recruitment of co-activators (Zhu *et al*. 1999), and with co-repressors (Nakajima *et al*. 2001, Maurer *et al*. 2002). The transcriptional activation domain in the C-terminus of STAT5A/B (STAT5A, aa 722–794; STAT5B, aa 727–787) is the most variable region and interacts with critical co-activators, including the p300/CBP-associated co-activator NcoA-1 (Litterst *et al*. 2003), centrosomal P4.1-associated protein (Peng *et al*. 2002), P100 (Paukku *et al*. 2003) and Oct-1 (Magne *et al*. 2003, Litterst *et al*. 2005).

In contrast to other STAT transcription factors that have a narrow activation profile, STAT5A and STAT5B transduce signals triggered by multiple ligands (Table 3). In addition to being activated by PRL (Kazansky *et al*. 1999), STAT5A/B are activated by interleukin-2 (IL-2; Hou *et al*. 1995), IL-3 (Mui *et al*. 1995), IL-5 (Mui *et al*. 1995)), and IL-7 (Foxwell *et al*. 1995), granulocyte–macrophage colony-stimulating

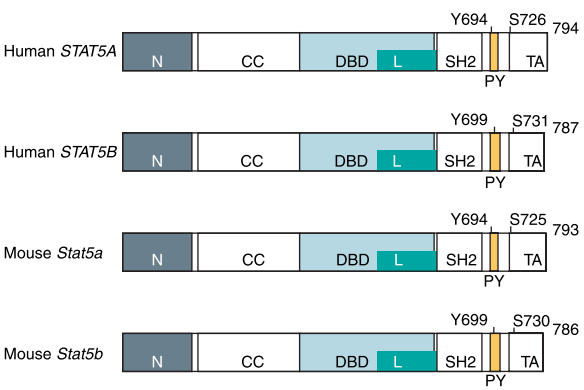

Figure 1 The major structural and functional domains and the phosphorylation sites of human and mouse STAT5A and STAT5B proteins. STAT5 proteins share an overall general structure that is organized into functional modular domains: N-terminal domain (N), coiled-coil domain (CC), DNA-binding domain (DBD), linker domain (L), SH2 domain (SH2), and transactivation domain (TA). All STAT molecules have a highly conserved tyrosine phosphorylation site (Y) at or around residue 700, labeled as the phosphotyrosyl segment (PY). Tyrosine phosphorylation follows ligand-induced activation and is required for dimerization and nuclear translocation.

Table 3 Ligands activating signal transducer and activator of transcription 5A/B (STAT5A/B)

Ligand	Reference
Prolactin	Gouilleaux *et al.* (1994) and Wakao *et al.* (1994)
IL-2	Hou *et al.* (1995)
IL-3	Mui *et al.* (1995)
IL-5	Mui *et al.* (1995)
IL7	Foxwell *et al.* (1995)
Granulocyte–macrophage colony-stimulating factor (GM-CSF)	Barahmand-pour *et al.* (1995), Gouilleux *et al.* (1995*a*), Mui *et al.* (1995), Pallard *et al.* (1995*a*) and Rosen *et al.* (1996)
Insulin	Wartmann *et al.* (1996) and Chen *et al.* (1997)
Erythropoietin (EPO)	Gouilleux *et al.* (1995*b*), Pallard *et al.* (1995*b*)
Thrombopoietin (TPO)	Pallard *et al.* (1995*a*)
GH	Gouilleux *et al.* (1995*b*) and Galsgaard *et al.* (1996)

factor (Barahmand-pour *et al.* 1995, Mui *et al.* 1995), insulin (Wartmann *et al.* 1996), erythropoietin (Wakao *et al.* 1994, Gouilleux *et al.* 1995*b*, Pallard *et al.* 1995*b*), thrombopoietin (Pallard *et al.* 1995*a*), and GH (Gouilleux *et al.* 1995*b*, Galsgaard *et al.* 1996) (Table 3).

Initially, insight into the distinct roles of STAT5A/B in mediating biological responses was gleaned mainly from gene targeting studies, which revealed that the phenotypes of *Stat5a/b* knockout (KO) mice are not fully functionally redundant. *Stat5a*-null female mice are defective in PRL-dependent mammary gland development (Liu *et al.* 1997), whereas *Stat5a*-null male mice exhibit defective prostate epithelium (Nevalainen *et al.* 2000). By contrast, *Stat5b*-null mice fail to respond effectively to GH (Udy *et al.* 1997, Teglund *et al.* 1998) and are severely anemic, indicating defective hematopoiesis due to impaired response to hematopoietins (Socolovsky *et al.* 1999). In *Stat5a/b* double KO mice, lymphoid development and differentiation were impaired (Yao *et al.* 2006). In addition, T-cell receptor γ-rearrangement and peripheral CD8+T-cell survival were abrogated in the absence of *Stat5a/b* (Yao *et al.* 2006).

The Jak-STAT5A/B signaling cascade

STAT5A/B-activating cytokine receptors do not typically posses tyrosine kinase activity that is provided by receptor-associated cytoplasmic proteins from the JAK family (Darnell 1997, Ihle 2001, Schindler 2002). In mammalian cells, there are four JAK proteins (120–130 kDa), JAK1, JAK2, JAK3, and TYK2 (tyrosine kinase 2) (Darnell 1997, Ihle 2001, Aaronson & Horvath 2002, Levy & Darnell 2002), which are, except for JAK3, ubiquitously expressed (Leonard & O'Shea 1998). JAK proteins have seven highly homologous domains. Located at the carboxyl terminus, the JAK homology domain 1 (JH1) has the kinase activity. Directly upstream of JH1 is the JH2 pseudokinase domain that resembles JH1 but has a negative regulatory function. The domains that mediate association with cytokine receptors are JH3–JH7 at the amino-terminus of JAKs, which constitute a four-point-one, ezrin, radixin, moesin domain (Schindler & Darnell 1995, Darnell 1997, Ihle 2001, Aaronson & Horvath 2002, Levy & Darnell 2002, Schindler 2002). The primary JAK protein that activates STAT5A/B is JAK2 (Gouilleux *et al.* 1994).

Ligand-induced receptor dimerization brings two JAK2 molecules into close proximity allowing them to phosphorylate specific tyrosine motifs of the receptor and activate each other (Fig. 2). STAT5A/B and other signaling molecules that recognize these tyrosine motifs, typically through their SH2 domains, are recruited to the docking sites. This is followed by rapid phosphorylation of a conserved tyrosine residue in the C-terminus of STAT5A/B by JAK2. Phosphorylation of the tyrosine residues Y694 and Y699 (identical in human and mouse) 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 phosphorylate STATs on serine residues, allowing additional signaling pathways to potentiate the primary STAT-activating stimulus (Decker & Kovarik 2000). By contrast, phosphorylation of the serine residues, S726 (human)/S725 (mouse) on STAT5A (Beuvink *et al.* 2000) and S731 (human)/S730 (mouse) on STAT5B, may inhibit the transcriptional activity of STAT5 (Kirken *et al.* 1997*a*,*b*, Yamashita *et al.* 2001).

Phosphorylated STAT5 dimers translocate from the cytoplasm into the nucleus, where they bind to the 8–10 bp inverted repeat consensus, TTC(C/T)N(G/A)-GAA, referred to as the GAS element (γ-interferon activation sequence) (Decker *et al.* 1991, Horvath *et al.* 1995, Soldaini *et al.* 2000). Recent reports

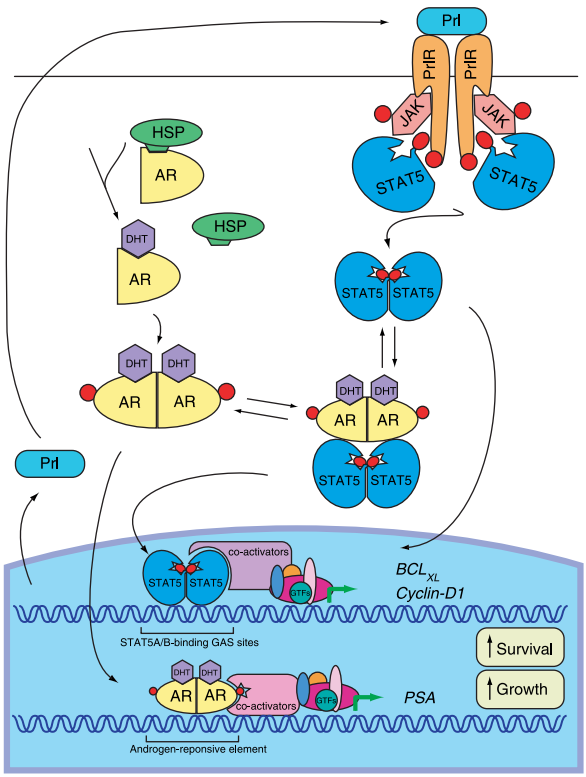

Figure 2 Functional interaction between JAK2-STAT5A/B and androgen receptor (AR) signaling in prostate cancer cells. Binding of prolactin (PRL) to prolactin receptor (PRLR) results in receptor dimerization and activates the receptor associated kinase, Janus kinase 2 (JAK2). JAK2 phosphorylates the PRLR on tyrosine residues, thereby creating docking sites for the SH2 domain of STAT5A/B monomers. STAT5A/B molecules are phosphorylated on unique tyrosine residues by JAK2, dissociate from the receptors, dimerize, and translocate to the nucleus where they bind to specific STAT5A/B-binding GAS (γ-interferon activation sequence) sites within the promoters of target genes. Binding of androgens (DHT) to the androgen receptor (AR) induces the dissociation from heat shock proteins (HSPs), receptor phosphorylation, and dimerization. Dimerized AR is translocated into the nucleus and bind to androgen response elements at the promoter of target genes. In prostate cancer cells, liganded AR interacts with activated STAT5A/B and enhances the nuclear translocation STAT5A/B. STAT5A/B, in turn, increases the nuclear translocation of AR. STAT5A/B and AR are both growth promoting signaling pathways in prostate cancer cells.

support the notion that nuclear and cytoplasmic pools of unphosphorylated STAT proteins, including STAT5A/B, shuttle freely at high exchange rates in the absence of cytokine activation (Meyer *et al*. 2002, Zeng *et al*. 2002, Marg *et al*. 2004, Vinkemeier 2004, Reich & Liu 2006). While non-phosphorylated STATs cycle between the cytoplasm and nucleus, the translocation of dimerized STAT proteins has been suggested to be an active, energy-dependent process (Vinkemeier 2004, Reich & Liu 2006) and utilize components of Ran-dependent nuclear import machinery (Sekimoto *et al*. 1996, 1997). The karyopherin importin-b (p97) has been identified as the carrier that transports importin complexed with STATs into the nuclear compartment. Intriguing recent evidence suggests 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, 2007*b*).

Negative regulators of STAT5 signaling

A number of different mechanisms regulate the duration and magnitude of STAT5 activation at the cytoplasmic and nuclear levels. First, both cytoplasmic and/or nuclear phosphatases inactivate STAT5 proteins. The protein tyrosine phosphatase (PTP), SHP-2, directly interacts with STATs in the cytoplasm and translocates as a complex into the nucleus (Chughtai *et al*. 2002, Chen *et al*. 2003). Other phophatases known to inactivate STATs are cytosolic PTP, PTP1B, and a nuclear phosphatase, TCPTP (T-cell protein tyrosine phosphatase) (Aoki & Matsuda 2000, 2002). The second mechanism includes protein inhibitors of activated STAT proteins (PIAS), which inhibit STAT protein activation by direct association to STATs. The mammalian PIAS family members include PIAS1, PIAS3, PIASx, PIASy, and alternative splicing variants of PIASx (Chung *et al*. 1997, Schmidt & Muller 2003). DNA binding of STAT1 and STAT3 is selectively inhibited by PIAS1 and PIAS3 respectively (Chung *et al*. 1997, Rogers *et al*. 2004). Moreover, PIAS proteins are E3 ligases for the small ubiquitin-like modifier (SUMO; Jackson 2001, Kahyo *et al*. 2001, Muller *et al*. 2001, Sachdev *et al*. 2001). SUMO is a family of four proteins of about 100 amino acids, SUMO-1, -2, -3, and -4, which is conjugated to the consensus site (Ψ(Psi)KXE) on protein substrates. Similar to ubiquitin conjugation, SUMO proteins are activated by E1 enzymes (Aos1 and Uba2) and conjugated by an E2 enzyme (Ubc9) (Muller *et al*. 2001, Seeler *et al*. 2007). Diverse SUMO ligases, including PIAS proteins (Sharrocks 2006, Shuai 2006), define the specificity of SUMOylation. SUMO modification of a protein may alter its function, localization, or extent of ubiquitination (Muller *et al*. 2001, Seeler *et al*. 2007).

A third mechanism for the down-regulation of signaling by STAT5 proteins involves cytokine-inducible suppressors of cytokine signaling (SOCS) proteins (Alexander & Hilton 2004). There are eight members of the SOCS family, including cytokine-inducible SH2 (CIS domain protein) and SOCS-1 to -7. These proteins are all structurally related and they possess a central SH2 domain and a

conserved C-terminal motif, termed the SOCS box (Alexander & Hilton 2004). The SOCS proteins also appear to target signal transducers for proteasomal destruction (Zhang *et al*. 1999, Kamizono *et al*. 2001, Ungureanu *et al*. 2002). SOCS are rapidly induced by activated STATs and act to block the cytokine signal by direct inhibition of JAKs (SOCS-1) and by competitive binding to tyrosine phosphorylated receptors so as to exclude further binding of signaling proteins such as STAT5, or by both mechanisms (Ram & Waxman 1999, Alexander & Hilton 2004).

The caveolin-1 (*CAV-1*) gene maps to chromosome 7q31.1 and encodes a 21–24 kDa integral membrane protein of caveolae, plasma membrane invaginations that are involved in vesicular transport, cholesterol homeostasis, signal transduction, and cell transformation (Williams & Lisanti 2005). The CAV-1 scaffolding domain shares homology with the SOCS pseudosubstrate domain, suggesting a negative regulatory function in the JAK/STAT5 signaling pathway. Indeed, CAV-1 was found to inhibit the kinase activity of JAK2 and suppress JAK2/STAT5A signaling (Park *et al*. 2001, Jasmin *et al*. 2006).

STAT5A/B in prostate cancer

Transcription factor STAT5A/B regulates the viability of prostate cancer cells

The understanding of the importance and function of transcription factor STAT5A/B in normal and malignant prostate tissues has been obtained from the analysis of *Stat5a* KO mice, studies in human prostate cancer cells *in vitro* and *in vivo*, and from the TRAMP (transgenic adrenocarcinoma of mouse prostate) mouse model of prostate cancer. Originally, STAT5A and STAT5B were identified as the key signaling proteins activated by PRL in both normal and malignant prostate tissues (Ahonen *et al.* 2002, Li *et al.* 2004). PRL, in turn, had been shown to be an autocrine mitogen and survival factor for androgen-deprived prostate cells (Ahonen *et al*. 1999). In determining the role of STAT5A/B in the growth of normal prostate epithelium, the first step was the analysis of the phenotype of *Stat5a* KO mice (Nevalainen *et al*. 2000). These studies revealed that the prostate epithelium of *Stat5a*-null mice was defective. Specifically, the prostate epithelium in *Stat5a*−/− mice was characterized by acinar cyst formation, local disorganization, and shedding of the epithelial cells to the glandular lumini (Nevalainen *et al*. 2000). The deformed prostate acini were filled with desquamated, granular epithelial cells embedded in dense, coagulated secretory material inside the broken acini (Nevalainen *et al*. 2000). The defective prostate tissue architecture in *Stat5a*−/− mice did not show increased prostate size or morphological hallmarks of epithelial hyperplasia. These results implied that STAT5A is not likely to mediate growth inhibition of prostate epithelium.

Presently, no reports of prostate phenotypes of *Stat5b* or *Stat5a/b* double KO mice exist. Analysis of the prostate phenotype of *Stat5b* or *Stat5a/b* double KO mice would be essential for more complete understanding of the importance of STAT5A/B for the integrity and maintenance of normal prostate epithelium. This is because the prostate phenotype of *Stat5a*-null mice may have been undermined by redundant functions of other STAT family proteins, particularly STAT5B (Liu *et al*. 1998*b*, Nevalainen *et al*. 2002). Specifically, in the mammary glands of *Stat5a*−/− mice, STAT5B compensated for the lack of *Stat5a* after multiple pregnancies (Liu *et al*. 1998*b*, Nevalainen *et al*. 2002). Likewise, in the prostates of *Stat5a*-deficient mice, STAT5B may have compensated for the lack of STAT5A. Furthermore, the loss of *Stat5a* stemmed from a germ line mutation of the *Stat5a* gene and, therefore, allowed sufficient time for STAT5B to functionally offset the loss of STAT5A throughout the development of the mice. Based on these facts, more specific results on the significance of STAT5A/B in growth regulation of normal prostate epithelium would be obtained from the studies that utilize conditional prostate-specific targeting of *Stat5a* or *Stat5b* or *Stat5a/b*.

Transcription factor STAT5A/B is highly critical for the viability of human prostate cancer cells in culture. Moreover, STAT5A/B is crucial for the regulation of prostate tumor growth *in vivo*. The novel concept of STAT5A/B being a prostate cell growth-controlling protein is supported by the reports from several different groups. First, Ahonen *et al*. (2003) showed that inhibition of STAT5A/B in STAT5-positive human prostate cancer cells by adenoviral expression of a dominant-negative mutant of STAT5A/B induced massive apoptotic death of the cells as determined by cell morphology, cell viability assays, DNA fragmentation, and activation of caspase-3 and caspase-9. The demonstration of the vital contribution of STAT5A/B to the viability of prostate cancer cells was later confirmed by the studies in the TRAMP mouse prostate cancer model (Kazansky *et al*. 2003). In the TRAMP mouse prostate tumor cell lines, inhibition of STAT5A/B by inducible expression of a carboxy terminal-truncated STAT5B mutant decreased the growth of the cells in soft agar and tumor formation in nude mice. For further validation of STAT5A/B as a therapeutic target protein for prostate cancer, recent work from the Nevalainen Laboratory

(Dagvadorj *et al*. 2008) established the critical role of STAT5A/B for human prostate xenograft tumor growth in nude mice. The study also demonstrated that, regardless of the methodological approach, STAT5 inhibition in all STAT5-positive human prostate cancer cells resulted in massive cell death. In addition, *CYCLIN-D1* and *BCL-X_L* were identified as target genes of STAT5A/B in human prostate cancer cells (Dagvadorj *et al*. 2008). What remains unclear so far is the individual role of STAT5A versus STAT5B in the maintenance of growth regulation of prostate cancer cells. Moreover, identification of the molecular mechanisms underlying rapid apoptosis of prostate cancer cells upon STAT5A/B inhibition should be investigated, as it may reveal additional therapeutic target proteins for prostate cancer.

Active STAT5A/B in clinical progression of prostate cancer

STAT5A/B is constitutively activated in human prostate cancer cells, but not in the epithelium of adjacent normal prostate glands (Ahonen *et al*. 2003). Moreover, activation of STAT5A/B is associated with high-grade prostate cancer (Li *et al*. 2004). The distribution of active STAT5A/B in clinical prostate cancers of different histological grades was first demonstrated in a study that analyzed STAT5A/B activation in 114 paraffin-embedded prostate cancer samples (Li *et al*. 2004). Examination of tissue microarrays of an independent set of 357 prostate cancer patients further confirmed that activation of STAT5A/B associated with high Gleason grades of prostate cancer (Li *et al*. 2005). Active STAT5A/B in primary prostate tumors predicted an early recurrence of prostate cancer after the initial treatment of prostate cancer in the patient (Li *et al*. 2005). This finding further supported the concept of involvement of STAT5A/B in clinical progression of prostate cancer. Most importantly, active STAT5A/B remained an independent prognostic marker of early disease recurrence even if only prostate cancers of intermediate Gleason grades were analyzed (Li *et al*. 2005). It is therefore possible that the presence of active STAT5A/B in primary prostate cancers of intermediate histological grade is associated with progressive disease and would serve as a prognostic marker for the identification of prostate cancer patients who would benefit from a more aggressive therapeutic intervention. Future studies should determine the distribution of active STAT5A versus STAT5B in prostate cancers of different histological grades and assess the individual prognostic value of STAT5A versus STAT5B in prostate cancer. The ideal material for such studies would be primary prostate cancer specimens from patients who have undergone radical prostatectomy but have not received adjuvant therapies, since they may affect the activation of STAT5A/B.

Because active STAT5A/B promotes the growth of prostate cancer cells and active STAT5A/B in primary prostate cancer predicted early disease recurrence, which is often hormone-refractory cancer, the contribution of STAT5A/B to androgen-independent growth of prostate cancer is an important open question. Hormone-refractory prostate cancer is characterized by continued expression of the androgen receptor (AR) and androgen-regulated genes, suggesting that the AR signaling pathway remains active despite low levels of circulatory androgens (Isaacs & Isaacs 2004). Recently, the Nevalainen Laboratory (Tan *et al*. 2008) demonstrated that the active STAT5A/B signaling pathway increased transcriptional activity of AR in prostate cancer cells. Ligand-bound AR, in turn, increases transcriptional activity of STAT5A/B. AR expression is known to persist in hormone-refractory prostate cancer. Similar to AR, STAT5A/B was shown to be in the active state in 95% of hormone-refractory clinical human prostate cancers (Tan *et al*. 2008). The functional synergism between STAT5A/B and AR in prostate cancer cells was demonstrated to involve direct physical interaction between the two. Intriguingly, liganded AR was shown to enhance nuclear localization of STAT5A/B and active STAT5 promoted nuclear translocation of AR (Tan *et al*. 2008). These findings are important because STAT5A/B and AR are both transcription factors that inhibit apoptosis and promote the growth of prostate cancer cells (Fig. 2). Specifically, promotion of AR transcriptional activity by STAT5A/B in the presence of low levels of androgens may contribute to androgen-independent growth of prostate cancer. AR, in turn, by promoting transcriptional activity of STAT5A/B, may critically support the viability of prostate cancer cells in growth conditions where prostate cancer cells would normally undergo apoptosis. Among outstanding questions are the molecular mechanisms underlying the co-action between STAT5A/B and AR, the effect of STAT5A/B–AR synergy on prostate tumor growth *in vivo* and the STAT5 interaction with mutated liganded AR by non-testicular androgens.

Pathways leading to constitutive activation of STAT5A/B in prostate cancer cells

The molecular mechanisms underlying constitutive activation of STAT5A/B in primary and hormone-

refractory human prostate cancer are presently unclear. Such mechanisms may involve autocrine PRL (Nevalainen *et al*. 1997*a*,*b*, Li *et al*. 2004; Fig. 2). Specifically, PRL is one of the predominant peptide factors presently known to activate JAK2-STAT5A/B in normal and malignant prostate epithelia (Ahonen *et al*. 2002, Li *et al*. 2004, Dagvadorj *et al*. 2007). PRL promotes proliferation and survival of prostate cells, and PRL is produced locally by normal prostate epithelium and prostate cancer (Nevalainen *et al*. 1991, 1996, 1997*a*,*b*, Wennbo *et al*. 1997, Ahonen *et al*. 1999, Kindblom *et al*. 2002, 2003, Dagvadorj *et al*. 2007). Analysis of clinical human prostate cancer specimens showed that PRL protein expression is associated with a high histological grade of human prostate cancer (Li *et al*. 2004). Autocrine PRL in prostate cancer may be one of the factors responsible for the constitutive activation of STAT5A/B in human prostate cancer. It has been shown that JAK2 is the predominant kinase that activates STAT5A/B in prostate cancer cells (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). Such JAK2 mutations may also occur in advanced prostate cancer. A third potential mechanism for the high abundance of STAT5A/B in prostate cancer is the amplification of STAT5A/B genes. This is particularly important since the *STAT5A/B* genes are located on chromosome 17 (Clark *et al*. 2003) that is frequently altered in both incidental and hereditary prostate cancers (Gillanders *et al*. 2004). Chromosome 17q showed allelic imbalance in prostate cancer (Latil *et al*. 1994, Bova & Isaacs 1996, Alers *et al*. 2000, Kasahara *et al*. 2002, Wolter *et al*. 2002*a*,*b*, Verhage *et al*. 2003, von Knobloch *et al*. 2004), and gains in chromosome 17q were detected in five studies (Bova & Isaacs 1996, Alers *et al*. 2000, Kasahara *et al*. 2002, Wolter *et al*. 2002*a*,*b*). Moreover, three large studies linked a prostate cancer susceptibility gene to chromosome 17q (17q22) (Lange *et al*. 2003, Gillanders *et al*. 2004, Zuhlke *et al*. 2004), suggesting involvement of genes in this region in an inherited form of prostate cancer.

STAT5A/B might also be activated by tyrosine kinases such as Src (Silva 2004, Yu & Jove 2004), Bcr-Abl (de Groot *et al*. 1999), or Tel-JAK (Schwaller *et al*. 2000). Although GH, a principal activator of STAT5B in a number of tissues, might be involved in activating STAT5A/B in malignant prostate epithelium (Chopin *et al*. 2002, Halmos *et al*. 2002, Letsch *et al*. 2003, Weiss-Messer *et al*. 2004, Stangelberger *et al*. 2005, Wang *et al*. 2005), there is presently no evidence of direct effects of GH on the stimulation of prostate cancer cell growth.

Negative regulators of STAT5A/B signaling in prostate cancer

Constitutive activation of STAT5A/B in malignant prostate epithelium may result from the loss of STAT5A/B phosphatases or inhibitory proteins of STAT5A/B (PIAS, CAV-1). STAT proteins are inactivated by both cytoplasmic and nuclear PTPs, such as SHP-1, SHP-2, CD45, cytosolic PTP, PTP1B, and a nuclear phosphatase, TCPTP (Aoki & Matsuda 2000, 2002, Shuai & Liu 2003). However, studies on direct regulation of STAT5A/B activation by tyrosine phosphatases in prostate cancer cells have not been reported. Nevertheless, expression of the tyrosine phosphatase SHP-1 has been detected in both PC-3 and LNCaP prostate cancer cells (Zapata *et al*. 2002). SHP-1 is also expressed in normal human prostate, benign prostate hyperplasia, and well-differentiated prostate cancer, but is undetectable in poorly differentiated advanced prostate cancer (Zapata *et al*. 2002).

The PIAS family of proteins are localized within the nucleus and function as constitutive repressors of STAT activity (Chung *et al*. 1997, Shuai 2000, Schmidt & Muller 2003). In addition, PIAS1, PIAS3 (Junicho *et al*. 2000, Gross *et al*. 2001, Wang & 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 to AR-mediated transcription in human prostate epithelial cells, while PIASy acts as a co-repressor of AR (Junicho *et al*. 2000, Gross *et al*. 2001). Importantly, the only member of the PIAS family that has been shown to interact with STAT5A/B is PIAS3. Specifically, the repressive action of PIAS3 on STAT5-mediated transcription was shown in CHO and lymphoid cells (Rycyzyn & Clevenger 2002), but not in prostate cells. PIAS3 is expressed in prostate cancer tissues and cell lines (Gross *et al*. 2001, Wang & Banerjee 2004). Moreover, PIAS3 acts as a co-regulator of AR-regulated transcription in LNCaP cells, and its expression is enhanced in response to DHT (dihydrotestosterone) treatment (Junicho *et al*. 2000, Gross *et al*. 2001). PIAS1 expression, in turn, has been shown to be 33% higher in primary prostate cancers compared with normal prostates, but this overexpression did not correlate with the Gleason score as determined by *in situ* hybridization of PIAS1mRNA (Li *et al*. 2002). Another study showed that PIAS1 expression is significantly lower in hormone-refractory prostate tumors 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. PIAS1 has been shown to act as an E3 ligase for AR (Nishida & Yasuda 2002), enhance the transcriptional activity of AR

in LNCaP cells (Gross *et al*. 2001), and inhibit STAT1-mediated transcription (Liu *et al*. 1998*a*). In contrast to PIAS1 and PIAS3, PIASy has been shown to interact with AR and act as an inhibitor of AR in prostate cancer cell without interfering with the DNA binding of AR (Gross *et al*. 2001). Additional studies on the interaction of PIAS proteins with STAT5A/B- and STAT5A/B-regulated gene transcription in prostate cancer cells would give more insight into how this interaction contributes to prostate cancer progression.

A comparison of SOCS expression in three prostate cancer cell lines (LNCaP, PC-3, and DU145) with a normal prostate cell line (RWPE-1) showed that the expression levels of SOCS-1, SOCS-3, SOCS-5, and CIS genes in PC-3 and DU145 cells are significantly lower than in the normal RWPE prostate cells. On the other hand, in LNCaP cells the expression of SOCS-1, SOCS-3, SOCS-5, and CIS genes were at levels comparable with RWPE cells (Evans *et al*. 2007). These results indicate that SOCS genes are not silenced in all prostate cancer cells. It remains to be determined whether the SOCS proteins directly regulate the JAK2-STAT5A/B pathway in human prostate cancer cells (Neuwirt *et al*. 2007) and, therefore, would contribute to constitutive activation of STAT5A/B in clinical prostate cancer.

In mammary epithelial cells, CAV-1 has been shown to repress JAK2-STAT5A/B signaling pathway (Park *et al*. 2002, Jasmin *et al*. 2006). In prostate cancer, overexpression of CAV-1 has been associated with higher Gleason score, positive surgical margins, metastasis to lymph nodes, aggressive PSA (prostate specific antigen) recurrence, and a higher likelihood of disease recurrence in patients treated with radical prostatectomy (Yang *et al*. 1999, Tahir *et al*. 2006, Karam *et al*. 2007). Moreover, it has been suggested recently that CAV-1 mediates angiogenesis during prostate cancer progression (Yang *et al*. 2007*a*). In the CAV-1 deficient human prostate cancer cell line, LNCaP, the overexpression of CAV-1 stimulated cell proliferation and promoted tumor growth in nude mice (Bartz *et al*. 2008). To date, there are no studies on the interaction of CAV-1 expression and JAK2/STAT5 signaling pathway in human prostate cancer cells. In summary, little is presently known about how changes in the expression patterns of negative regulatory proteins of STAT5A/B signaling contribute to the constitutive activation of STAT5A/B in advanced prostate cancer.

STAT5A/B and breast cancer

STAT5A/B regulation of normal mammary gland

The present understanding of the role of STAT5A/B in the regulation of normal mammary gland has been obtained for the most part from studies using murine mammary gland as a model system. Before and after the onset of puberty and during the reproductive cycle, the mouse mammary gland undergoes major morphological changes. Signals from steroid hormones, lactogenic hormones, and peptide growth factors all coordinate the expansion and differentiation of the alveolar compartment of the mouse mammary gland during the reproductive cycle (Hennighausen & Robinson 2005). Throughout all the stages of mammary gland development, the expression of STAT5A/B proteins is detected in the breast epithelium with a minor increase during the final stages of pregnancy and the onset of lactation (Kazansky *et al*. 1995, Liu *et al*. 1995). Of the two homologs of STAT5, STAT5A is more abundant in mammary epithelial cells than STAT5B (Liu *et al*. 1997). In contrast to the relatively steady expression pattern of STAT5A/B proteins, the kinetics of STAT5 tyrosine phosphorylation correlates with the developmental profile of the mammary gland and the activation of milk protein genes (Liu *et al*. 1995, 1996*b*, Nevalainen *et al*. 2002). In late pregnancy and early lactation, marked stimulation in STAT5 phosphorylation occurs, followed by a decrease during the involution phase of the post-lactational mammary gland (Liu *et al*. 1995, 1996*b*, Nevalainen *et al*. 2002). In addition to PRL, STAT5A/B is also activated in mouse mammary gland by GH or epidermal growth factor (EGF). However, while PRL activates STAT5A/B uniformly in the mammary epithelium, GH, and EGF activate STAT5A/B only in a subset of epithelial cells, and the main targets of GH and EGF in mouse mammary gland are mammary stromal cells (Gallego *et al*. 2001, Nevalainen *et al*. 2002).

Creation of *Stat5a*-null mice revealed a specific role for STAT5A in the mouse mammary gland during the reproductive cycle (Liu *et al*. 1997). The inactivation of *Stat5a* gene in mice by homologous recombination showed disrupted mammary gland development and a failure to lactate. Specifically, the *postpartum* lobulo-alveolar outgrowth was reduced and the alveolar lumina in the mammary gland failed to terminally differentiate (Liu *et al*. 1997). The mice developed otherwise normally and were indistinguishable from their wild-type litter mates in size, weight, and fertility (Liu *et al*. 1997). Interestingly, in the non-pregnant murine mammary gland, STAT5A/B is basally active within the ductal compartment (Nevalainen *et al*. 2002). However, the key functions of PRL-STAT5 signaling during pregnancy and lactation are located to the alveolar compartment of the gland (Horseman *et al*. 1997, Ormandy *et al*. 1997). These findings are in line with the results of the analysis of mice lacking two

alleles of the PRL receptor in mammary epithelial cells that exhibited normal morphogenesis of the ducts of the mammary glands but lacked milk-producing alveoli (Brisken *et al*. 1999, Miyoshi *et al*. 2001). Further studies using transgenic mice overexpressing the native ovine STAT5A (94% identical to mouse STAT5A) in the mammary glands showed that STAT5A promoted lobulo-alveolar development and delayed the onset of apoptosis (Iavnilovitch *et al*. 2002). This suggested that STAT5A is a survival factor for terminally differentiated non-malignant mammary epithelial cells. Collectively, these studies demonstrated that STAT5A is the principal mediator of lobulo-alveolar differentiation and expansion in mice during pregnancy, as well as the key mediator of lactogenic signaling. Moreover, transcriptionally active STAT5A critically promotes the viability and survival of terminally differentiated alveolar mammary epithelial cells.

Stat5b-null mice demonstrated a decreased lobulo-alveolar development, albeit a less severe phenotype than that of the *Stat5a*-null mice, with lactation unimpeded. The key function of STAT5B is to mediate the effects of GH, as *Stat5b*-null mice failed to respond effectively to GH (Udy *et al*. 1997, Teglund *et al*. 1998) and had severe anemia suggesting defective hematopoiesis due to impaired response to hematopoietins (Socolovsky *et al*. 1999). KO of both *Stat5a* and *Stat5b*, in turn, is perinatally lethal and the mice had severely impaired lymphoid development and differentiation. Absence of STAT5A/B also abrogated T-cell receptor γ-rearrangement and peripheral CD8+ T-cell survival (Yao *et al*. 2006). In addition, *Stat5a/b* KO mice were found to have a defect in the development of functional corpora lutea in the ovary, resultingin female infertility. Interestingly, while STAT5A primarily mediates biological effects distinct from those of STAT5B, it has been shown that STAT5B can compensate for the lack of STAT5A in the mammary gland function (Liu *et al*. 1998*b*, Nevalainen *et al*. 2002). After multiple pregnancies, lactation was partially established by activation of STAT5B in the mammary gland epithelium of the STAT5A-null mice. These results imply functional redundancy between STAT5A and STAT5B when sufficient time is provided, which is inherent to germ line KO studies.

STAT5A/B in tumorigenesis of rodent mammary gland

Present evidence suggests that STAT5A/B promotes tumorigenesis of rodent mammary gland. In a study using the rat mammary gland as a model, Shan *et al*. (2004) examined the immunohistochemical expression and activation of STAT5A in a panel of rat mammary gland carcinomas induced by the chemical carcinogens, 7,12-dimethylbenz[a]anthracene and 2-amino-1-methyl-6 phenylimidazo[4,5-b]pyridine, to assess the percentage of cells in carcinomas and preneoplastic lesions showing tyrosine phosphorylated and nuclear localized STAT5A. Nuclear immunostaining of STAT5A was detected in 65% of carcinomas, while STAT5A in control normal mammary gland tissue was cytosolic. STAT5A immunostaining patterns (cytoplasmic or nuclear) were compared with proliferating cell nuclear antigen immunostaining, tumor differentiation, nuclear grade, mitotic activity, and tumor size. The results indicated that intense nuclear immunostaining for STAT5A was associated with high-grade rat mammary gland carcinomas and STAT5A immunostaining was detected in intraductal proliferating cells and in ductal carcinomas *in situ* (Shan *et al*. 2004).

The effect of STAT5A on mouse mammary gland carcinogenesis has been tested in several different mouse breast cancer models. First, the effect of hemizygous loss of *Stat5a* was determined in a SV40-T antigen transgenic mouse model of mammary cancer. The breeding of WAP-TAg mice to mice carrying germ line hemizygous deletions of the *Stat5a* allele generated mice with reduced levels of STAT5A without altering mammary gland development or transgene expression levels. In comparison with mice carrying two wild-type *Stat5a* alleles, hemizygous loss of the *Stat5a* allele demonstrated a modest decrease in the number of tumor-bearing mice. Moreover, the tumors were smaller and the tumor formation was delayed (Ren *et al*. 2002). In another mouse breast cancer model system, *Stat5a*-null females exhibited delayed transforming growth factor-α-induced tumorigenesis (Humphreys & Hennighausen 2000). Third, a recent study by Oakes *et al*. (2007) suggested that PRL receptor deficiency may prevent early progression of mammary gland neoplasia to invasive carcinoma. However, it is unclear so far whether this inhibition of mammary gland cancer progression would be due to the lack of JAK2/STAT5 signaling or inhibition of other PRL receptor-activated signal transduction pathways. *Cav-1*, in turn, which inhibits JAK2-STAT5 signaling (Park *et al*. 2002), has been shown to affect mammary tumor formation in mice (Sotgia *et al*. 2006*b*). The mammary glands of *Cav-1* KO mice expressed a hyperactive JAK2-STAT5 pathway and developed hyperplastic, well-differentiated mammary tumors (Sotgia *et al*. 2006*b*). An interesting recent study reported that overexpression of wild-type or constitutively active *Stat5a* in a transgenic mouse model promoted the

occurrence of sporadic mammary cancers. Specifically, mammary-directed expression of wild-type *Stat5a* or constitutively active *Stat5a* developed well-differentiated micropapillary and papillary adenocarcinomas after latency periods of 8–12 months. Unexpectedly, mammary-directed overexpression of a carboxyl terminal-truncated STAT5 was able to promote mammary gland adenocarcinoma development in mice (Iavnilovitch *et al.* 2004). This result was surprising because C-terminally truncated STAT5A is transcriptionally inactive, and thus acts as a dominant-negative variant of STAT5A/B inhibiting endogenous STAT5A/B. Of note, the mammary gland adenocarcinomas in these mice were less well differentiated than carcinomas that occurred in wild-type or constitutively active STAT5 expressing transgenic mice (Iavnilovitch *et al.* 2004). Further observation showed that the expression of the carboxy terminally truncated *Stat5* in the mammary glands of transgenic mice inhibits cell proliferation during pregnancy, delays onset of milk secretion, and induces apoptosis upon involution (Iavnilovitch *et al.* 2006). In another study, Eilon *et al.* (2007) reported that in transgenic mice overexpressing *Stat5* or a constitutively activated *Stat5* variant, mammary tumorigenesis occurred more frequently in multiparous females compared with their age-matched virgin counterparts. The fact that mammary tumorigenesis was more frequent in multiparous females may be due to the use β-lactoglobulin gene promoter, which is a PRL-inducible promoter, to drive the expression of *Stat5*. The role of STAT5A/B in differentiation of malignant mammary epithelial cells is supported by the studies conducted in HC11 cells that are well-differentiated mouse mammary cancer cells. Specifically, inhibition of PRL-JAK2 signaling blocked cell differentiation and induced a hyperproliferative phenotype characterized by increased mitotic rate, reduced apoptosis, and reduced contact inhibition (Xie *et al.* 2002). In conclusion, several lines of evidence suggest that STAT5A/B may promote mammary gland tumorigenesis in rat and mice. The results of several independent studies suggest that STAT5A/B may also affect differentiation of mouse mammary gland carcinomas. However, it is unclear at this point to what extent the observed phenotypes in these studies with somewhat conflicting results were affected by crossing different mouse strains with one another.

STAT5A/B signaling pathway in human breast cancer

Activation of STAT5A/B in human breast cancer has been shown to positively correlate with the differentiation status of the tumor. In one study, STAT5A/B was found to be constitutively active in ~76% of human breast tumors ($n=83$) and the authors observed a positive correlation between tumor differentiation and active STAT5A (Cotarla *et al.* 2004). No association of active STAT5 was found with recognized prognostic indicators such as lymph node metastases, tumor size, ploidy, percentage of cells in S-phase, estrogen receptor (ER), ErbB2, or nuclear localized p21. In another study, STAT5A/B activation in primary breast cancer ($n=1100$) was found to predict favorable clinical outcome (Nevalainen *et al.* 2004). This study describes a gradual inactivation of STAT5A/B during metastatic progression of human breast cancer, with less than 20% of metastases exhibiting active STAT5. Importantly, loss of tyrosine phosphorylated STAT5A/B in the primary tumor of patients with lymph node-negative breast cancer was associated with an eightfold increased risk of death from breast cancer (Nevalainen *et al.* 2004). In multivariate analyses, STAT5A/B was an independent prognostic factor in node-negative breast cancer when other markers, including age, ER, progesterone receptor, HER2, (human epidermal growth factor receptor-2) tumor size, and grade were included. Subsequent studies focusing on histological differentiation of breast cancer and STAT5A/B activation confirmed that STAT5A/B levels positively correlated with breast cancer differentiation in the material of 517 cases (Yamashita *et al.* 2006). Moreover, high STAT5A/B levels predicted better response to endocrine therapy and longer survival after relapse than tumors that were STAT5A/B negative (Yamashita *et al.* 2006). Interestingly, examination of secretory carcinomas for the presence of STAT5A by immunohistochemistry showed that STAT5A was expressed at a high level in secretory carcinomas (11 invasive and 7 *in situ*, including 4 cases with both), but was absent in *in situ* or invasive ductal carcinomas and apocrine breast metaplasias (Strauss *et al.* 2006). Moreover, STAT5A was not expressed in other specialized histological types of breast carcinomas such as mucinous or clear cell carcinomas (Strauss *et al.* 2006). The authors suggested that STAT5A/B expression in the secretory breast carcinomas may be due to the secretory changes occurring in the cells. In addition, the levels of STAT5A were reported to be reduced in breast cancers in general compared with STAT5A levels in normal luminal breast epithelial cells (Bratthauer *et al.* 2006).

The favorable prognosis associated with active STAT5A/B may be a result of reduced metastatic dispersal of breast cancer cells from the primary tumor caused by STAT5A/B. Specifically, STAT5A/B had an

invasion-suppressive role in human breast cancer cells (Sultan *et al*. 2005) as determined by cell clustering assays, E-cadherin expression, matrix metalloproteinase secretion, cell migration, and invasion assays (Sultan *et al*. 2005). On the other hand, hypoxia, a common consequence of solid tumor growth in breast cancer or other cancers, was reported to activate STAT5A/B and increase its transcriptional activity and binding to the GAS element in breast cancer cells (Joung *et al*. 2005, Lee *et al*. 2006). In addition, inhibition of STAT5A/B by adenoviral expression of a dominant-negative STAT5 mutant in T47D cells was described to cause apoptosis of the cells (Yamashita *et al*. 2003, 2004). These studies, however, did not include critical controls for the cytopathic effects of adenovirus, and therefore it is difficult to interpret the results. In conclusion, further studies are needed to determine whether STAT5A/B is not only a promoter of differentiation of breast cancer, but also a survival factor for established human breast tumors.

Negative regulation of STAT5 signaling in breast cancer

The molecular mechanisms underlying constitutive activation of STAT5A/B in clinical human breast cancer are presently unclear. First, the down-regulation of STAT5A/B phosphorylation during breast cancer progression to metastatic disease could be due to decreased STAT5A/B protein expression caused by genetic or epigenetic changes occurring in breast cancer cells. Second, STAT5A/B phosphorylation may be reduced because of increased expression or activation of phosphatases that dephosphorylate STAT5A/B. Both TCPTP and PTP1B were shown to form a complex with and dephosphorylate STAT5A/B, resulting in inactivation of STAT5A/B-mediated gene expression in mammary epithelial cells (Aoki & Matsuda 2000, 2002). Analysis of SHP-1 expression in 72 primary breast cancers showed a 2- to 12-fold increase in 58% of the samples compared with normal breast epithelial cells (Yip *et al*. 2000). No reports on the distribution of TCPTP or PTP1B protein expression in human breast cancers of different histological grades exist to date. Third, changes in the expression levels of PIAS proteins may result in reduced transcriptional activity of STAT5A/B in high-grade breast cancer. PIAS3 has been shown to inhibit PRL-induced STAT5A/B-mediated transactivation of the β-casein promoter (Rycyzyn *et al*. 2000). This inhibitory interaction between PIAS3 and STAT5A/B activity was disrupted by increasing amounts of a complex formed by intranuclear PRL and the peptidyl prolyl isomerase cyclophilin B, suggesting an indirect regulatory role of nuclear PRL on STAT5A/B transcriptional activity (Rycyzyn & Clevenger 2002).

Elevated expression levels of SOCS-1, SOCS-2, SOCS-3, and CIS were found in breast carcinomas and breast cancer cell lines compared with normal breast tissue and cell lines (Raccurt *et al*. 2003). In another study, the mRNA expression levels of SOCS-1, SOCS-2, SOCS-3, CIS, and the STAT5-induced growth factor, insulin-like growth factor-I, were examined by RT-PCR followed by immunohistochemical analysis of SOCS-2 protein expression (Haffner *et al*. 2007). SOCS-2 expression level was inversely correlated with histopathological grade of breast cancer. Moreover, high SOCS-2 expression were found in ER-positive tumors and correlated with higher survival rates.

The mammary glands of *Cav-1* null mice showed premature lactation, with accelerated development of the lobulo-alveolar compartment and hyperactivation of the JAK2/STAT5A signaling compared with the mammary gland of normal mice (Park *et al*. 2002). *Cav-1*−/− mammary epithelia were hyperproliferative *in vivo*, with dramatic increases in terminal end bud areas and mammary ductal thickness as well as increases in bromodeoxyuridine incorporation, extracellular signal-regulated kinase 1/2 hyperactivation, and up-regulation of *Stat5a* and *Cyclin-D1*. Consistent with these findings, loss of *Cav-1* dramatically exacerbated mammary lobulo-alveolar hyperplasia in *Cyclin-D1* transgenic mice, whereas overexpression of *Cav-1* caused reversal of this phenotype (Williams *et al*. 2006). However, the role of CAV-1 human cancers has been controversial (Lee *et al*. 1998, Hurlstone *et al*. 1999, Chen *et al*. 2004, Sagara *et al*. 2004, Williams & Lisanti 2005). CAV-1 is believed to be a tumor suppressor gene (Williams & Lisanti 2005, Sotgia *et al*. 2006*a*) based on the high frequency of deletions of 7q31 in human cancers (Lee *et al*. 2002), the presence of CAV-1 gene promoter methylation (Engelman *et al*. 1999), and inactivating gene mutation (Hayashi *et al*. 2001). By contrast, other reports suggest that CAV-1 may behave as an oncogene in breast (Hurlstone *et al*. 1999, Van den Eynden *et al*. 2006, Pinilla *et al*. 2006) and prostate (Thompson *et al*. 1999, Timme *et al*. 2000) cancers.

Reduced CAV-1 expression in breast carcinomas has been reported in several independent studies (Chen *et al*. 2004, Park *et al*. 2005). In another study, mRNA levels of CAV-1 were analyzed in breast cancer tissues by RT-PCR and showed that the down-regulation of CAV-1 mRNA levels in breast cancer tissues compared with normal tissues and correlated positively

Table 4 Findings on the role of signal transducer and activator of transcription 5A/B (STAT5A/B) in mammary gland and prostate in mouse models versus human

	Mouse	**Human** (cell lines)	**Human**
Breast	1. *Stat5a*−/− female mice were defective in PRL-dependent mammary gland development and failed to lactate (Liu *et al*. 1997) 2. In *Stat5a*−/− mice, STAT5B compensated for the lack of STAT5A after multiple pregnancies (Liu *et al*. 1998*b*, Nevalainen *et al*. 2002) 3. *Stat5b*−/− mice showed decreased lobulo-alveolar development (less severe than *Stat5a*−/−) and failure to respond effectively to GH (Udy *et al*. 1997, Teglund *et al*. 1998) 4. PRLR deficiency may prevent early progression of mammary gland neoplasia to invasive carcinoma (Oakes *et al*. 2007) 5. The overexpression of native ovine STAT5A in the mammary glands of transgenic mice promoted lobulo-alveolar development and delayed the onset of apoptosis (Iavnilovitch *et al*. 2002) 6. Immunohistochemical staining of rat mammary gland carcinomas induced by DMBA and PhIP for active STAT5A/B correlated with high histological grade of the carcinomas (Shan *et al*. 2004) 7. Hemizygous *Stat5a* mice crossed to SV40-T antigen transgenic mouse model of mammary gland cancer showed a delay in tumor formation, decrease in tumor size, and decrease in the number of tumor-bearing mice (Ren *et al*. 2002) 8. *Stat5a*−/− females showed delayed TGF-α-induced tumorigenesis (Humphreys & Hennighausen 2000)	1. The expression of STAT5A/B in human breast cancer cells T47-D and BT-20 suppressed metastatic dispersal (Sultan *et al*. 2005) 2. Phosphatases TCPTP and PTP1B formed a complex with and dephosphorylated STAT5A/B, resulting in inactivation of STAT5A/B-mediated gene expression in mammary epithelial cells (Aoki & Matsuda 2000, 2002) 3. About 58% of primary breast cancers (n=72) analyzed showed a 2- to 12-fold increase in phosphatase SHP-1 expression compared with normal breast epithelial cells in (Yip *et al*. 2000) 4. PIAS3 inhibited PRL-induced STAT5A/B-mediated transactivation of the β-casein promoter (Rycyzyn *et al*. 2000, Rycyzyn & Clevenger 2002) 5. Breast carcinomas and breast cancer cell lines expressed elevated levels of SOCS-1, SOCS-2, SOCS-3, and CIS compared with normal breast tissue and cell lines (Raccurt *et al*. 2003)	1. STAT5A/B was found to be constitutively active in ~76% of human breast tumors (n=83) and with a positive correlation between tumor differentiation and active STAT5A (Cotarla *et al*. 2004) 2. STAT5A/B activation in node-negative primary breast cancer (n=1100) predicted favorable clinical outcome (eight-fold increased risk of death from breast cancer associated with the loss of active STAT5A/B). STAT5A/B was found to be gradually inactivated during metastatic progression of human breast cancer (Nevalainen *et al*. 2004) 3. STAT5A/B levels positively correlated with breast cancer differentiation (n=517) and high STAT5A/B levels predicted better response to endocrine therapy and longer survival after relapse (Yamashita *et al*. 2006) 4. STAT5A expression, as shown by immunohistochemistry was high in secretory carcinomas, but was absent in *in situ* or invasive ductal carcinomas, apocrine breast metaplasias, or mucinous or clear cell carcinomas (Strauss *et al*. 2006) 5. Levels of STAT5A were reduced in breast cancers compared with STAT5A levels in normal luminal breast epithelial cells (Bratthauer *et al*. 2006) 6. SOCS-2 expression level was inversely correlated with histopathological grade of breast cancer and high SOCS-2 expression was found in ER-positive tumors and correlated with higher survival rates (Haffner *et al*. 2007)

Table 4 *continued*

	Mouse	**Human** (cell lines)	**Human**
	9. *Cav-1*−/− mice have a hyperactive JAK2-STAT5 pathway, premature lactation, and developed hyperplastic, well-differentiated mammary tumors (Park *et al*. 2002) 10. Transgenic mice overexpressing constitutively active and a C-terminally truncated dominant-negative STAT5A had enhanced mammary gland carcinoma development (Iavnilovitch *et al*. 2004). Mammary tumors in these mice were more frequent in multiparous females (Eilon *et al*. 2007)		
Prostate	1. *Stat5a*−/− male mice had a defective prostate epithelium (Nevalainen *et al*. 2000) 2. In the TRAMP mouse prostate tumor model, inducible expression of a carboxy-terminally truncated STAT5B inhibited STAT5A/B and decreased the growth of the cells in soft agar (Kazansky *et al*. 2003) 3. Prostate-specific expression of PRL in transgenic mouse induced prostate hyperplasia and enlargement of prostate (Wennbo *et al*. 1997, Kindblom *et al*. 2003)	1. Inhibition of STAT5A/B in human prostate cancer cells by adenoviral expression of a dominant-negative STAT5A/B induced apoptotic cell death (Ahonen *et al*. 2003) 2. Inhibition of STAT5A/B in all STAT5-positive human prostate cancer cells by siRNA or antisense oligonucleotides resulted in massive cell death. STAT5A/B was shown to be critical for human prostate xenograft tumor growth in nude mice. The target genes of STAT5A/B in human prostate cancer cells include *CYCLIN D1* and *BCL-*X_L (Dagvadorj *et al*. 2008) 3. Active STAT5A/B increased transcriptional activity of AR; ligand-bound AR increases transcriptional activity of STAT5A/B. Liganded AR enhanced nuclear localization of STAT5A/B and active STAT5 promoted nuclear translocation of AR. This synergism involved direct physical interaction between AR and STAT5A/B (Tan *et al*. 2008)	1. STAT5A/B is constitutively activated in human prostate cancer cells but not in normal prostate epithelium (Ahonen *et al*. 2003) 2. Activation of STAT5A/B was associated with high Gleason grades of clinical prostate cancer (Li *et al*. 2004) 3. Activation of STAT5A/B in primary human prostate cancer predicted early prostate cancer recurrence (Li *et al*. 2005) 4. STAT5A/B was shown to be in the active state in 95% of hormone-refractory clinical human prostate cancers (Tan *et al*. 2008) 5. PRL is expressed in normal prostate epithelium and prostate cancer cells and activates STAT5A/Bin prostate cancer through JAK2 (Nevalainen *et al*. 1997*b*, Li *et al*. 2004) 6. Pharmacological inhibition of autocrine PRL in prostate cancer cells by a PRL antagonist inhibited STAT5A/B activation in induced prostate cancer cell death (Dagvadorj *et al*. 2007)

with tumor size and negative ER status (Sagara *et al*. 2004) A comparison of CAV-1 expression in normal breast tissues, benign lesions, breast cancer precursors, metastatic, and invasive breast cancers using a combination of immunofluorescence, ultrastructural analysis, and immunohistochemistry showed preferential CAV-1 expression in myoepithelial cells (MECs), fibroblasts, and endothelial cells in normal breast tissue (Savage *et al*. 2007). CAV-1 was found to be expressed in 90% metaplastic breast carcinomas and in 9.4% invasive breast carcinomas, and, importantly, the expression of CAV-1 in the latter group was associated with basal-like immunophenotype, shorter disease free, and lower survival based on univariate analysis.

Targeting STAT5A/B activation

There are multiple levels at which STAT5A/B function could be targeted in cells. These include STAT5A/B gene expression, STAT5A/B recruitment to a receptor, STAT5A/B tyrosine phosphorylation, STAT5A/B dimerization, STAT5A/B nuclear translocation, STAT5A/B DNA binding, and its interaction with other transcriptional co-regulators. Down-regulation of STAT5A/B expression by RNA interference in both mammary epithelial cells and prostate cancer cells (Dagvadorj *et al*. 2008) has been performed successfully. SH2 domain-binding phosphotyrosyl peptides or peptidomimetics that have been used to selectively inhibit STAT3 dimerization (Turkson *et al*. 2001, 2004) could be applied to inhibit STAT5A/B receptor recruitment and dimerization. In addition, decoy oligonucleotides that mimic STAT5A/B-binding sites could theoretically sequester STAT5 from its target genes and inhibit DNA binding as shown by the inhibition of CYCLIN-D1 protein expression in NRK-49F kidney cells (Guh *et al*. 2001). Furthermore, peptide aptamers selected from combinatorial peptide libraries could potentially be used to block STAT5 dimerization or DNA binding (Nagel-Wolfrum *et al*. 2004). Another approach to inhibit STAT5A/B would be a disruption of transcriptional activation of STAT5 by the expression of a truncated dominant negative variant that lacks the C-terminal transactivation domain (Ahonen *et al*. 2003, Dagvadorj *et al*. 2008). The involvement of human autocrine PRL in activating the JAK2/STAT5A/B signaling pathway in breast and prostate epithelial cells inspired the development of hPRL antagonists such as G129R-PRL (Llovera *et al*. 2000) that may have potential for therapeutic use (Dagvadorj *et al*. 2007). Finally, identification of pharmacological small-molecule lead compounds that would disrupt STAT5A/B dimerization or DNA binding would be critical for the development of conventional pharmaceuticals for the inhibition of STAT5A/B.

Conclusions

Transcription factor STAT5A/B is constitutively active in human prostate and breast cancers. In prostate cancer, activation of STAT5A/B is associated with high histological grade of clinical prostate cancer, and activation of STAT5A/B in primary prostate cancer may predict early disease recurrence. Moreover, STAT5A/B functionally interacts with AR in prostate cancer cells. Importantly, STAT5A/B is highly critical for the viability of human prostate cancer cells and therefore presents a potential therapeutic target protein for high-grade prostate cancer (Table 4).

In breast cancer, the role of STAT5A/B is more complex (Table 4). In mouse models of breast cancer, STAT5A/B promotes tumor formation. By contrast, activation of STAT5A/B in human breast cancer is a robust predictor of favorable clinical outcome, and STAT5A/B inhibits metastatic behavior of human breast cancer cells. Moreover, expression of active STAT5A/B is lost during histological dedifferentiation of human breast cancer. It is therefore possible that STAT5A/B has a dual role in breast cancer. STAT5A/B may promote the growth of the early malignant lesions, whereas it may maintain differentiation of established breast cancer. It is also possible that the role of STAT5A/B in mouse mammary gland regulation is different from that in humans. The specific and possibly different roles of STAT5A versus STAT5B in the regulation of breast cancer cells are not yet clear and some of the conflicting data may have resulted from the analysis of STAT5A and STAT5B collectively in the majority of the studies carried out to date. Finally, little is known about STAT5A/B activation and regulation in human breast cancer stem cells and how STAT5A/B activation in different cell types of the human breast translates into the effect of STAT5A/B on growth promotion versus differentiation of breast cancer.

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