Activation of Janus Kinases During Tumorigenesis

Jeffrey W. Schmidt and Kay-Uwe Wagner

Abstract

Janus tyrosine kinases (JAKs) are important for the growth and homeostasis of a variety of normal tissues. Specifically, JAK1 and JAK2 are essential for mammalian development, and conventional knockout models in mice show that the absence of just one of these two kinases causes prenatal and postnatal lethality. Recent studies using JAK2 conditional knockout mice show that this tyrosine kinase plays key roles in mammary gland development, fertility, pancreatic β cell homeostasis, and the suppression of fatty liver disease in adult animals. Somatically acquired point mutations or structural abnormalities in the *JAK2* gene contribute to various hematopoietic malignancies. In contrast, a sustained activation of JAK1 and JAK2 in solid human cancers, such as those of the breast, prostate, lung, head and neck, skin, and gastrointestinal tract, is caused mainly by alternative mechanisms. These include the epigenetic silencing of negative regulators of JAKs as well as an aberrant autocrine stimulation of growth factors such as PRL, EPO, and IL-6. In addition to the canonical pathway through Signal Transducers and Activators of Transcription (STATs), JAKs are an integral part of a crosstalk with receptor tyrosine kinases and their substrates that promote the progression of solid cancers. The biological significance of JAKs within wider signaling networks, however, depends on the cell type and the stage of neoplastic

J.W. Schmidt
Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, 985950 Nebraska Medical Center, DRCII, Rm. 5012, Omaha, NE 68198-5950, USA

K.-U. Wagner (✉)
Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, 985950 Nebraska Medical Center, DRCII, Rm. 5012, Omaha, NE 68198-5950, USA

Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE, USA
e-mail: kuwagner@unmc.edu

Th. Decker and M. Müller (eds.), *Jak-Stat Signaling: From Basics to Disease*,
DOI 10.1007/978-3-7091-0891-8_15,

progression. For example, recent studies in breast cancer models that are conditionally deficient in JAK2 show that the importance of this kinase changes during disease initiation and progression, which may have significant implications for targeting this Janus kinase in a chemopreventive or therapeutic setting.

Preface

The four members of the Janus kinase family (JAK1, JAK2, TYK2, and JAK3) meditate signaling from multiple hormone and cytokine receptors that are crucial for normal development as well as the initiation and progression of hematopoietic malignancies and solid cancers. This chapter briefly summarizes the main biologically relevant functions of JAKs in normal tissue homeostasis and the mechanisms that mediate an aberrant activation of Janus kinases in human cancers. Also highlighted in this chapter will be the importance of JAKs as components of broader signaling networks, in particular their association to receptor tyrosine kinases and downstream effectors that are known to have pivotal roles in the genesis of solid cancers. In the main section of this chapter, there will be an overview about changes in the activation of Janus kinases in specific solid (i.e. non-hematopoietic) human cancers and their suggested effects on the proliferation, survival and invasive properties of cancer cells. Finally, there will be a discussion of important issues related to the targeted inhibition of JAKs for the prevention and treatment of human cancers. This book chapter will not review the protein structure of JAKs and the significance of specific posttranslational protein modifications that regulate the functionality of JAKs. The reader should refer to recent reviews to gain further insight into these specific molecular events (Ghoreschi et al. 2009; Schindler et al. 2007; Schindler and Plumlee 2008).

Janus Kinases and Normal Tissue Homeostasis

Janus tyrosine kinases (JAKs) are expressed in most tissues and mediate the downstream signaling of more than 50 cytokines and peptide hormones. Upon ligand binding to their corresponding receptors, the receptor-associated Janus kinases autophosphorylate themselves and become activated. Active JAKs phosphorylate specific tyrosine residues on the receptors thereby creating docking sites for signal transducers and activators of transcription (STATs). Following their recruitment to the receptors, STATs are subsequently activated by the JAKs through phosphorylation of critical tyrosine residues that serve as binding sites for the SH2 domains of other STAT proteins, mediating their homo- or heterodimerization. Active STATs undergo nuclear translocation and function as latent transcription factors by binding to consensus recognition sites. In mammals, the JAK family is composed of four members, JAK1, JAK2, JAK3, and TYK2,

Table 1 Phenotypes of Janus kinase knockouts

Gene	Knockout method	Phenotype	Reference(s)
Jak1	Conventional	Early postnatal lethality; neurological defects, SCID; cytokine insensitivity	Rodig et al. (1998)
Jak2	Conventional	Embryonic lethality; defective erythropoiesis	Krempler et al. (2004), Neubauer et al. (1998), Parganas et al. (1998)
	Conditional (mammary)	Impaired alveolar development and maintenance	Wagner et al. (2004)
	Conditional (neuroendocrine)	Impaired fertility and reproductive development	Wu et al. (2011)
	Conditional (pancreatic β-cells)	Impaired β cell homeostasis	Choi et al. (2011)
	Conditional (hepatocyte)	Fatty liver phenotype	Sos et al. (2011)
Jak3	Conventional	Viable and fertile; SCID; defective lymphoid development	Nosaka et al. (1995), Park et al. (1995), Thomis et al. (1995)
Tyk2	Conventional	Viable and fertile; impaired response to LPS and IL-12 signaling; defective cytokine signaling	Karaghiosoff et al. (2000, 2003), Shimoda et al. (2000)

SCID severe combined immunodeficiency, *IL* interleukin, *LPS* lipopolysaccharide

each capable of activating its own set of STATs. [For references regarding the coupling of specific JAKs to STATs and the ligands that activate them, see reviews by Schindler and Strehlow (2000), Kisseleva et al. (2002), and Rane and Reddy (2000).]

Since each Janus kinase transduces signals from multiple cytokines and receptors, gene knockout models have been generated in an attempt to better understand physiological functions of individual JAKs in vivo (Table 1). JAK1 knockout mice die perinatally due to neurological defects and also show insensitivity to cytokine signaling such as IL-2, IL-6, IFN and IL-10 (Rodig et al. 1998). JAK2 knockout mice are embryonic lethal and die at gestation day 12.5 due to defective erythropoiesis (Neubauer et al. 1998; Parganas et al. 1998; Krempler et al. 2004). The expression of JAK3 is largely limited to lymphoid tissues. Consequently, the knockout phenotype for the gene encoding this kinase is less severe than that of JAK1 or JAK2, and JAK3 deficient mice are both viable and fertile; however, they exhibit a severe combined immunodeficiency-like phenotype with defects in lymphoid development (Nosaka et al. 1995; Park et al. 1995; Thomis et al. 1995). The fourth member of the JAK family, the Tyrosine kinase 2 (TYK2), has been implicated in IFN-α, IL-6, IL-10 and IL-12 signaling. TYK2 knockout mice have an impaired response to LPS and IL-12 signaling (Karaghiosoff et al. 2000; Shimoda et al. 2000) and are unable to integrate signaling from multiple cytokine receptors (Karaghiosoff et al. 2003).

Since JAK2 deficiency results in early embryonic lethality, the role of this kinase in tissue homeostasis of postnatal animals is difficult to examine. The approach of transplanting cells and tissue fragments from JAK2 knockout embryos into adult mice was used by Shillingford and colleagues (2002) to study essential functions of JAK2 during mammary gland development. This study showed that JAK2 is required for epithelial cell proliferation, and the ablation of this kinase results in impaired formation of secretory alveoli. In order to better study the role of JAK2 in differentiated tissues of adult mice, Krempler et al. (2004) generated JAK2 conditional knockout mice by placing *loxP* sites around the first coding exon of the *Jak2* locus. In general, a Cre-mediated deletion of a gene such as *Jak2* provides a unique opportunity to assess important biological functions of a gene of interest beyond the initial block in development that often results from a conventional knockout. The final section of this chapter will describe how such a model can also be applied to investigate the role of Janus kinases in tumor initiation versus progression. To selectively ablate JAK2 in mammary epithelial cells in a spatially and temporally controlled manner at particular stages of mammary gland development, transgenic mice were used that express Cre recombinase in different epithelial subtypes in virgin, pregnant, and lactating females (Wagner et al. 1997, 2001). Specifically, the mouse mammary tumor virus (MMTV)-Cre-mediated deletion of *Jak2* from ductal progenitors led to a loss of activation of STAT5 in response to prolactin signaling, but deficiency in JAK2 had no effect on ductal elongation and branching morphogenesis (Wagner et al. 2004). Essential functions of JAK2 during postnatal mammary gland development in this model were restricted to alveolar cells in virgin females. In order to ablate JAK2 from differentiating alveolar cells, a whey acidic protein (WAP)-Cre-based JAK2 conditional knockout model was generated that exhibited a strong negative selection of JAK2-deficient secretory alveolar cells during late pregnancy and lactation. Collectively, both conditional knockout models demonstrated that JAK2 is required for the proliferation of alveolar progenitors and the maintenance of functionally differentiated alveolar cells during pregnancy and lactation. On a mechanistic level, these models provided clear evidence that JAK2 is an essential link between prolactin signaling and STAT5 activation in the normal mammary gland, which, as will be discussed later in this chapter, has important implications for the prevention of mammary cancer.

In addition to studying the role of JAK2 in mammary gland development, conditional knockout mice have been pivotal for the assessment of essential functions of this kinase in neuroendocrine cells, pancreatic β cells, and hepatocytes. The gonadotropin releasing hormone (GnRH) is a major regulator of the reproductive and sexual behavior of mammals. Loss of JAK2 in GnRH-producing neurons causes a number of abnormalities implicating this kinase to normal reproductive development and fertility in female mice (Wu et al. 2011). These conditional knockout mice exhibit a reduction in GnRH and luteinizing hormones, which causes a significant delay in puberty, first estrus, and irregular estrous cyclicity. Female mice showed impaired fertility as characterized by the prolonged time to produce their first litter, fewer pregnancies, and significantly smaller litter sizes. Another very recent study by Choi and colleagues (2011) using the JAK2

conditional knockout showed that erythropoietin (EPO) signaling through this particular kinase protects against the development of type 1 and type 2 diabetes. This study uncovered a key signaling pathway important for β-cell homeostasis with relevance for the treatment and prevention of diabetes. Finally, work by Sos and colleagues (2011) demonstrated that the Alb-Cre-mediated deletion of *Jak2 in* hepatocytes results in a profound fatty liver phenotype. This appears to be the result of a complex mechanism that starts with a reduction in serum insulin-like growth factor-1 (IGF-1), which, in turn, leads to an increase in serum growth hormone (GH) secretion due to a lack of feedback inhibition in the hypothalamus. While the liver-specific loss of JAK2 impairs hepatocellular GH signaling, this pathway is retained in adipocytes, and the increased level of serum GH causes enhanced lipolysis thereby releasing excess free fatty acids. These free fatty acids are then taken up by the GH-resistant hepatocytes at an enhanced rate due to increased expression of the free fatty acid transporter CD36. In support of this proposed mechanism, the fatty liver syndrome caused by the hepatocyte-specific deletion of *Jak2* could be completely reversed through abrogation of GH secretion.

Collectively, conventional and conditional knockout models for Janus kinases have been important to evaluate biologically relevant functions in signal transduction and tissue homeostasis. However there continues to be a need for these and similar kind of models to study cytokine and hormone signaling in normal versus neoplastic cell types. While JAK3 and TYK2 conventional null mutants are probably sufficient to examine the role of these kinases in hematopoietic cells, where these kinases are predominantly expressed, the availability of JAK2 conditional knockout mice now provides unique opportunities to study the role of this tyrosine kinase in a wide variety of tissues and in the context of neoplastic transformation. Similar to JAK2, JAK1 is activated in many normal tissues of adult mice, and, as discussed later, this kinase may be aberrantly activated by peptide hormones in cancer cells as part of a shift in signaling networks due to abnormal expression and activation of cytokine receptors and receptor tyrosine kinases. The necessity to genetically decipher these biological phenomena on a molecular level clearly underlines the need for the long overdue generation of a JAK1 conditional knockout mouse.

Mechanisms that Mediate an Aberrant Activation of JAKs in Cancer

Transgenic models expressing hyperactive JAK2 and STAT5 provide direct experimental evidence that Janus kinases can play a role as proto-oncogenes in the genesis of solid tumors. Particularly, a mammary-specific expression of the kinase domain of JAK2 linked to STAT5A and the transactivation domain of STAT6 prolongs cell survival and suppresses apoptosis that, in turn, induces the formation of sporadic adenocarcinomas (Iavnilovitch et al. 2002). The ability of hyperactive JAK2 to cause neoplastic transformation within the mammary epithelium depends largely on the activation of STAT5 as the main downstream effector of this Janus kinase. This notion is supported by the fact that mice overexpressing wildtype or a

hyperactive mutant of STAT5 develop mammary cancer following a similar latency period (Iavnilovitch et al. 2002; Vafaizadeh et al. 2010). Although these models are able to assess the mechanisms by which JAK2 and STAT5 contribute to the initiation of solid tumors, the precise genetic and epigenetic alterations that lead to the activation of these signaling mediators in cancer cells are not accurately recapitulated. In humans, missense mutations within Janus kinases and JAK fusion gene products are linked to the initiation and progression of myeloproliferative disorders and hematopoietic malignancies (Brisken et al. 2002; Brockman and Schuler 2005; Ruchatz et al. 2003; Slupianek et al. 2002). Although TEL-JAK2, BCR-JAK2, and PCM1-JAK2 fusion proteins are observed in various leukemia subtypes [for references see reviews by Valentino and Pierre (2006) and Ghoreschi et al. (2009)], chromosomal translocations that lead to the formation of hyperactivated Jak2 are not frequently detected in solid human tumors, and these cancers acquire active JAKs through alternative mechanisms.

Missense Mutations Within JAKs

Following the discovery and characterization of constitutively activating mutations of *JAK2* (e.g. $JAK2^{V617F}$) in myeloproliferative disorders (Baxter et al. 2005; James et al. 2005; Kralovics et al. 2005; Levine et al. 2005; Vainchenker and Constantinescu 2005), considerable effort has been placed in determining whether hyperactive JAK/STAT signaling observed in several human cancers is due, at least in part, to activating mutations in Janus kinases. Several recent studies, however, suggest that the occurrence of the $JAK2^{V617F}$ aberration and of homologous mutations in other JAKs remain a rare event in solid cancers. Thus far, these sequencing efforts identified mostly silent mutations and polymorphisms (Lee et al. 2006a; Motte et al. 2007). In prostate cancer, activation of STAT5 is associated with cancer cell survival and a high histological grade of primary tumors. Nonetheless, as a recent report by Gu and colleagues (2010c) showed a gain-of-function of JAK2 through the prominent V617F mutation is not the underlying cause for the increase in STAT5 phosphorylation in this type of cancer. Similarly, this mutation does not contribute to the activation of STAT3 in pancreatic cancers (Kocher et al. 2007). Sequencing efforts to identify mutations in *JAK1*, *JAK3*, and *TYK2* revealed a rare presence of somatic *JAK1* and *JAK3* missense mutations in breast, lung, and hepatocellular carcinomas (Table 2) (Jeong et al. 2008; Xie et al. 2009). The biological importance and functionality of these JAK mutations in carcinogenesis, however, remain unknown.

Epigenetic Silencing of Suppressors of JAK/STAT Signaling

A disruption of negative feedback loops occurs very frequently in human cancer, which is exemplified by the common loss of tumor suppressors that are antagonistic to proto-oncogenic pathways. Negative regulators of the JAK/STAT pathway

Table 2 Somatic JAK missense mutations observed in solid cancers

Gene	Solid cancer	Cancer subtype	Predicted amino acid change	Domain effected	References
JAK1	Lung	Non-small cell lung cancer	T782M	JH2	Jeong et al. (2008)
JAK1	Breast	Invasive ductal carcinoma	H647Y	JH2	Jeong et al. (2008)
JAK1	Liver	Hepatocellular carcinoma	Q646H	JH2	Xie et al. (2009)
JAK1	Liver	Hepatocellular carcinoma	H647F	JH2	Xie et al. (2009)
JAK3	Breast	Invasive ductal carcinoma	V715I	JH2	Jeong et al. (2008)

JH2 Janus kinase homology 2 (pseudokinase) domain

include the protein tyrosine phosphatases (PTPs), suppressors of cytokine signaling (SOCS), and protein inhibitors of activated STATs (PIAS) (Greenhalgh and Hilton 2001). JAK phosphorylation is a reversible process, and PTPs are a major regulator of JAK inactivation through catalyzing its dephosphorylation. SOCS proteins, on the other hand, suppress cytokine signaling through at least three distinct mechanisms. These include direct interaction with activated JAKs (i.e. SOCS1), association with phosphorylated residues on the receptor to block the binding of SH2 and PTB-domain containing proteins such as STATs (i.e. CIS), a combination of the two (i.e. SOCS3), or enhancing the proteosome-dependent degradation of Janus kinases (Greenhalgh and Hilton 2001; Kamizono et al. 2001; Ram and Waxman 1999). Unlike SOCS proteins that are upregulated in response to cytokine stimulation to silence the activation of JAKs, the PIAS family of proteins is ubiquitously expressed. They function by binding STATs directly and alter their localization, DNA binding, transcriptional activation, and additional STAT activities (O'Shea and Watford 2004). Among the three families of negative regulators of JAK/STAT signaling, PTPs and SOCS are epigenetically silenced through DNA methylation in a variety of human cancers. For example, the *SOCS1* gene is found to be aberrantly methylated in 60%–65% of hepatocellular carcinomas (Okochi et al. 2003; Yoshikawa et al. 2001), 50% of pancreatic tumors (Komazaki et al. 2004), and in a subset of colorectal cancers (Fujitake et al. 2004; Xu et al. 2009). Additional JAK/STAT inhibitors that are silenced through promoter methylation in hepatocellular carcinoma and colon cancer include the cytokine-inducible SH2-containing proteins (*CIS*), *SOCS2, SOCS3,* and *SHP-1* (Calvisi et al. 2006; Xu et al. 2009). Other reports have shown that *SOCS1* and *SOCS2* are hypermethylated in 14–24% of primary ovarian cancers, and silencing of the *SOCS1* gene also occurs in 9% of primary breast cancer cases (Sutherland et al. 2004). Recently, Sasi and colleagues (2010) examined the expression levels of SOCS1–7 during breast cancer progression. This study showed that a higher expression of *SOCS* genes was correlated with factors such as earlier tumor stage, disease-free survival, lack of disease recurrence, and an overall better clinical outcome. In light of these observations, the authors suggested that utilizing DNA methyltransferase inhibitors might provide an additive effect in a

targeted therapy against hyperactivated JAKs and STATs. Finally, the SOCS-related protein, caveolin-1, which is a potent suppressor of JAK2/STAT5 signaling, has been demonstrated to be epigenetically silenced through promoter methylation in a variety of human cancers (Chen et al. 2004; Cui et al. 2001; Park et al. 2002; Wiechen et al. 2001). Similar to transgenic models with a gain-of-function of JAK2 and STAT5, a knockout of caveolin-1 in mice accelerates the formation of multi-focal dysplastic lesions and mammary tumors (Park et al. 2002; Williams et al. 2003). The development of mammary neoplasms in this breast cancer model is closely associated with an increase in the activation of JAK2 and STAT5, which promotes mammary epithelial proliferation and premature differentiation in response to prolactin and other lactogenic hormones during pregnancy.

Autocrine Signaling

The hyper-activation of autocrine signaling networks is common in many human cancers. Rather than relying upon a constant supply of hormones and locally produced growth factors, cancer cells initiate the production of cytokines that bind to their corresponding receptors, which, in turn, activate growth and survival pathways. For example, autocrine signaling mediated by IL-6 is implicated in lung, colon, prostate and breast tumorigenesis (Giri et al. 2001; Grivennikov and Karin 2008; Sansone et al. 2007; Shirota et al. 1990). Similarly, the peptide hormone prolactin (PRL), which signals through JAK2 and STAT5, plays an important role in the etiology of breast cancer. High circulating levels of PRL are associated with an increased risk of developing breast cancer in humans (Tworoger and Hankinson 2006), and a sustained elevation of PRL has been shown to cause mammary cancers in transgenic mice (Tornell et al. 1991; Wennbo et al. 1997). In addition to the PRL that is released from the pituitary gland, breast cancer cells gain the ability to locally synthesize this hormone and enhance the expression of the PRL receptor (Clevenger et al. 1995; Ginsburg and Vonderhaar 1995). The importance of a PRL autocrine loop in the initiation of neoplastic transformation was further verified in a transgenic model (NRL-PRL) that expresses this hormone specifically in the mammary epithelium, and these transgenic mice develop both estrogen-receptor positive and negative lesions (Rose-Hellekant et al. 2003). Using JAK2 conditional knockout mice, its has been recently demonstrated that the mammary-specific ablation of this kinase completely prevented the onset of PRL-induced mammary tumors (Sakamoto et al. 2010). Therefore, the initiation of mammary tumors in the PRL overexpression model requires the activation of JAK2 and STAT5.

Collectively, the various studies that have assessed mutations within the coding regions of JAKs and STATs as well as the expression and activation of this pathway suggest that, unlike in hematopoietic malignancies, genomic alterations in these signal transducers are rare events in solid cancers. The activation of JAKs and their downstream mediators appears to be primarily regulated by alternative mechanisms that include the epigenetic silencing of negative regulators of JAK/STAT signaling in addition to an enhanced activation of Janus kinases through elevated autocrine

stimulation of growth factor receptors. Unfortunately, there are only a limited number of genetically engineered in vivo model systems available today that can be employed to systematically address the importance of negative regulators of JAK/STAT signaling in disease initiation and progression.

JAKs as Components of Broader Signaling Networks in Cancer

Within specific cell types, individual JAKs and STATs are in the lines of fire of diverse cytokine and hormone receptors such as receptor tyrosine kinases (RTKs) and their downstream mediators that are part of broader signaling networks. JAKs and STATs are an integral component of receptor crosstalk in normal cells, and it is known that their biological significance can change within signaling networks following malignant transformation. Therefore, the extent and type of receptor crosstalk that utilize JAKs and STATs not only depends on the cell type but also the stage of neoplastic progression. Examples for an extensive association of JAK/STAT signaling with RTKs and their downstream effectors that play pivotal roles in the genesis of solid cancers are members of the ERBB family and the PI3K/AKT pathway.

ERBB Family

Signaling through the ERBB family of receptor tyrosine kinases (EGFR; ERBB2-4) is frequently altered in human cancers through activating mutations, gene amplifications, or overexpression of individual receptors. Mutations within the human epidermal growth factor receptor (EGFR) are a common feature in adenocarcinomas of the lung. Previous studies have identified a close relationship between the extent of EGFR expression and a phosphorylation of JAK2 and STAT3 as well as a selective activation of JAK/STAT signaling by mutant EGFR in lung cancer cells (Lo et al. 2008; Sordella et al. 2004). Interestingly, the combined inhibition of the JAK/STAT pathway and the EGFR have been shown to inhibit tumor growth and cell survival more effectively than either agent alone (Dowlati et al. 2004; Lo et al. 2008). This suggests that inhibiting JAK/STAT signaling could serve as a synergistic approach to a targeted therapy against the EGFR. Such a therapeutic strategy would also affect the ability of the mutant EGFR to induce a JAK-dependent activation of STAT3 via upregulation of IL-6 production as recently suggested by Gao and coworkers (2007).

ERBB2 (HER2, neu) is amplified in a significant subset of breast cancer cases, and overexpression of this receptor tyrosine kinase is also frequently observed in lung cancer, ovarian cancer, and, at a lesser frequency, in colon cancer (Arteaga 2003; Brabender et al. 2001; Hellstrom et al. 2001; Hirsch and Langer 2004; Ochs et al. 2004). Following the binding of prolactin (PRL) or growth hormone (GH) to their corresponding receptors, JAK2 becomes activated and phosphorylates the cytoplasmic domains of the EGFR and ERBB2 (Yamauchi et al. 1997, 2000). The downstream activation of MAP kinases by GH and PRL has been shown to

depend on the phosphorylation of these two receptors. Both hormones, GH and PRL, also activate the cytoplasmic tyrosine kinase SRC, which phosphorylates various residues on the EGFR that leads to increased receptor signaling (Biscardi et al. 1999). On the other hand, the SRC family of tyrosine kinases is suggested to possess non-catalytic functions, and it was reported recently that SRC serves as a scaffold for the PRL-induced activation of JAK2 (Garcia-Martinez et al. 2010). JAK2 and STAT5 are important for the proliferation of epithelial subtypes in the mammary gland that are highly susceptible to ERBB2-induced neoplastic transformation (Henry et al. 2004). It has been demonstrated recently that the conditional deletion of the *Jak2* gene in these epithelial subtypes completely prevents the formation of mammary tumors in response to increased ERBB2 expression (Sakamoto et al. 2009). This study provides experimental evidence for the importance of receptor crosstalk between JAK/STAT signaling and RTK activation during the process of neoplastic transformation.

In adenocarcinomas of the breast and other organs, the ERBB2 receptor forms stable heterodimers with ERBB3, and both are suggested to function as an oncogenic unit (Holbro et al. 2003; Kim et al. 2005). Signaling through ERBB2/ERBB3 receptor complexes has also been shown to rapidly activate TYK2 and JAK3 and subsequently STAT3 and STAT5 in the lung epithelium (Liu and Kern 2002). Another example for important receptor crosstalk between ERBBs and JAK/STAT signaling is the proposed function of ERBB4 as a nuclear chaperone of active STAT5A in the mammary gland (Long et al. 2003; Williams et al. 2004). Clark and colleagues (2005) also demonstrated that ERBB4 modulates the activity of STAT5 by regulating the phosphorylation of additional serine residues besides the known JAK2-mediated tyrosine phosphorylation of this signal transducer. Collectively, these studies show that, in addition to their classical role as RTKs, ERBBs also possess important scaffold functions that can significantly modulate the activity of JAKs and STATs in normal and neoplastic cell types.

PI3K/AKT

The functional interactions between JAKs and ERBBs are prime examples for the engagement of different types of receptor tyrosine kinases involved in receptor crosstalk, but there are other RTKs such as the insulin receptor and the insulin-like growth factor-1(IGF-1) receptor that specifically interact with JAK1 and JAK2 and that play a role in a variety of solid cancers (Gual et al. 1998; Himpe and Kooijman 2009). In fact, many of these RTKs have overlapping biological activities in cancer cells. For example, ERBB2-overxpressing breast cancer cells are able to evade the antiproliferative action of a targeted therapy with trastuzumab through up-regulation of IGF-1 receptor expression or loss of PTEN function (Hynes and Lane 2005). These shifts in the signaling network are facilitated by downstream mediators such as phosphoinositide-3 kinase (PI3K) and AKT/PKB that are synchronously activated by various RTKs. The importance of PI3K signaling in cancer is highlighted by the fact that this is one of the most frequently deregulated pathways in human cancers

(Yuan and Cantley 2008). JAK/STAT signaling and the PI3K/AKT cascade share a number of similarities. Both promote survival, proliferation, and metabolism in a variety of cell types, and it is therefore reasonable to propose that these pathways converge within a signaling network. For example, it has been shown that active STAT3 and STAT5 can associate directly with the p85 regulatory subunit of the PI3 kinase in hematopoietic cells to initiate an activation of the PI3K/AKT pathway (Pfeffer et al. 1997; Santos et al. 2001). The functional association of p85 and STAT5 is suggested to play a role in myeloid leukemia (Harir et al. 2007; Nyga et al. 2005; Rosa Santos et al. 2000; Santos et al. 2001), but this interaction is not restricted to normal or neoplastic hematopoietic cells. Phosphorylated STAT5 was also shown to bind to p85 in mammary epithelial cells in vitro and in vivo following stimulation with PRL (Sakamoto et al. 2007). Prolactin signaling and activated JAK2 have previously been shown to promote PI3K activity (Tessier et al. 2001; Yamauchi et al. 1998), and it was proposed that active STAT5 may directly stimulate the activity of the PI3 kinase in the mammary epithelium. This notion is supported by the observation that the conditional deletion of the *Jak2* gene, which causes lack of STAT5 activation, leads to a synchronous reduction in the expression and activation of AKT1 (Sakamoto et al. 2007). This was not a consequence of a functional inhibition of SRC or MAP kinases since these signal transducers were still activated by PRL in the absence of JAK2. Deficiency in JAK2 leads to a dramatic reduction in the total levels of AKT1, and it was recently demonstrated that STAT5 controls the transcriptional expression of the *Akt1* gene in mammary epithelial cells. Nuclear STAT5 binds directly to consensus sites within the *Akt1* locus in a growth factor dependant manner and initiates transcription from a novel, mammary-specific promoter (Creamer et al. 2010). This proposed mechanism of a direct modulation of AKT1 expression and activation through STAT5 was verified in transgenic mice that overexpress a hyperactive mutant of this transcription factor in the mammary epithelium. The gain-of-function of STAT5 in vivo caused a sustained transcriptional upregulation of *Akt1*. The phenotypic consequence of this molecular association was a prolonged survival of functionally differentiated mammary epithelial cells despite activation of pro-apoptotic signaling pathways (Creamer et al. 2010). This phenotype is virtually identical to transgenic mice that overexpress wildtype or hyperactive AKT1 under the regulation of the MMTV LTR (Ackler et al. 2002; Hutchinson et al. 2001; Schwertfeger et al. 2001). Collectively, the results of these studies clearly show that JAK2/STAT5 signaling and the PI3K/AKT pathway can converge at various levels in particular cell types to execute similar biological functions.

Activation of JAKs in Specific Human Cancer Types

Breast Cancer

Elevated levels of prolactin (PRL) have been implicated in the occurrence of human breast cancer (Hankinson et al. 1999; Tworoger and Hankinson 2006), and this peptide hormone is suggested to play an important role in the establishment of an

aberrant autocrine loop that fuels the multiplication of breast cancer cells (Clevenger et al. 1995; Ginsburg and Vonderhaar 1995). Since PRL signals mainly through its receptor and the JAK2/STAT5 pathway in luminal breast epithelial cells, it is evident that these signal transducers are key for the genesis of human breast cancer subtypes that originate from this epithelial compartment (Wagner and Rui 2008). Unlike in normal mammary epithelial cells, PRL is also capable of activating STAT3 in human breast cancer cell lines (Cataldo et al. 2000), suggesting that signaling networks undergo a substantial rewiring process during neoplastic progression. Nelson and coworkers (2007) have recently shown that PRL activates JAK1 in a JAK2-dependent manner, and this may provide an underlying mechanism by which this hormone activates STAT3 and MAP kinases that play a pivotal role in breast cancer progression. The activation of JAK1 may correlate with particular breast cancer subtypes that result in a poor prognosis, and it has been reported that the inhibition of estrogen receptor expression in MCF-7 cells leads to an increase in the activation of JAK1 (Yeh et al. 2007).

Although PRL is a major growth factor for the multiplication of normal and neoplastic mammary epithelial cells, the activation of JAK/STAT signaling cascades in breast cancer is not restricted to this peptide hormone. Another growth factor that activates JAK2 and is known to play a significant role in human breast cancer is erythropoietin (EPO). It was reported recently that the receptor for erythropoietin (EpoR) is expressed in a significant subset of human breast tumor specimens and breast cancer cell lines (Larsson et al. 2009; Liang et al. 2010). Major side effects in patients treated with erythropoiesis-stimulating agents prompted the US Food and Drug Administration to issue a black-box warning for both epoetin alfa and darbepoetin alfa in 2008. It had been found that when these agents were given to patients with advanced breast cancer to achieve a target hemoglobin concentration, it shortened their overall survival and increased disease progression (Crouch and DeSantis 2009). Recently it has been shown that recombinant human EPO is also capable of counteracting the treatment of ERBB2-positive breast cancer cells with trastuzumab (Liang et al. 2010). The EPO-mediated activation of JAK2 and SRC as well as the inactivation of PTEN were identified as underlying mechanisms for this biological phenomenon. In addition to PRL and EPO, interleukin-6 (IL-6) is upregulated in primary human breast cancer specimens, and elevated expression of this cytokine is a poor prognostic indicator for breast cancer patients (Berishaj et al. 2007; Knupfer and Preiss 2007). IL-6 plays a key role in the activation of glycoprotein 130 (GP130) receptor-associated JAKs that are known mediators of STAT3 phosphorylation. Treatment of breast cancer cells with a pan-Jak inhibitor, blockade of the GP130 receptor, or sequestration of the IL-6 ligand each led to a decrease of active STAT3 in breast cancer cells (Berishaj et al. 2007). This may suggest that an inhibition of the IL-6/GP130-induced activation of JAK1 and JAK2 might be an effective strategy to target STAT3 in breast cancer. Besides the cytokines and their receptors that are known to directly activate JAK/STAT signaling such as PRL, EPO and IL-6, a recent report highlighted the role of a chemokine-like extracellular matrix protein, osteopontin (OPN), for the activation of STAT3 in a JAK2-dependent

manner (Behera et al. 2010). The study suggests that OPN promotes enhanced tumor growth and that increased expression of OPN and pSTAT3 correlates with breast cancer progression in clinical specimens.

It is evident that JAK1 and JAK2 are critical for the activation of STATs in breast cancer cells. Most clinical studies, however, focus solely on the examination of phosphorylated STATs (in particular STAT1, STAT3, and STAT5A/B) as biological readouts for the activation of JAK/STAT signaling cascades. The initial examination of the expression and activation of STAT5A in breast cancer showed that this signal transducer is nuclear localized and tyrosine phosphorylated in approximately 76% of human breast tumors, and its activation was positively correlated with tumor differentiation (Cotarla et al. 2004). Results from a larger study where more than 1,100 breast cancer specimens were analyzed revealed that active STAT5 is consistently present in healthy breast tissue. Its activity, however, is gradually lost during malignant progression, and less than 20% of metastases expressed active STAT5 (Nevalainen et al. 2004). Collectively, this study showed that STAT5 is as an independent prognostic factor for overall patient survival, but the molecular mechanism responsible for this phenomenon remained unknown. Recently, Johnson and colleagues (2010) provided evidence that an upregulation of the protein tyrosine phosphatase 1B (PTP1B) may account for the reduction in activate STAT5 in metastatic breast cancer cells. PTP1B functions as an inhibitor for active JAK2 by catalyzing the dephosphorylation of the Janus kinase, which consequently suppresses the activation of STAT5. The suggested biological role of active STAT5 as a proposed suppressor of metastasis is supported by the observation that STAT5 promotes differentiation as observed by the homotypic clustering of breast cancer cells, a reduction in invasive characteristics, and an increase in the cell surface levels of the adhesion molecule E-cadherin (Sultan et al. 2005). As the expression and activation of STAT5 declines, the level of phosphorylated STAT3 increases significantly during malignant progression. In fact, approximately 50–60% of primary breast cancers exhibit a constitutive activation of STAT3, and it has been shown in various independent studies that this member of the STAT family plays a key role in breast cancer cell growth, survival, and metastatic progression (Barbieri et al. 2010; Berishaj et al. 2007; Burke et al. 2001; Kunigal et al. 2009; Proietti et al. 2009; Ranger et al. 2009). As discussed earlier, STAT3 is synchronously phosphorylated in breast cancer cells by JAK1 and JAK2 in response to the aberrant activation of the receptors for PRL, EPO, and IL-6 as well as the crosstalk with cytoplasmic and receptor tyrosine kinases. It can therefore be expected that the targeted inhibition of STAT3, either directly or indirectly through inhibition of JAKs, will be of therapeutic value to treat advanced breast cancers.

Prostate Cancer

Recent evidence suggests a significant association between JAK/STAT activity and the development of androgen-refractory prostate cancer. Specifically, an increased expression of the IL-6 receptor and active cytoplasmic STAT3 have been linked to early relapse and reduced patient survival (Tam et al. 2007). This is in agreement with

previous work by Drachenberg and colleagues (1999) who observed a correlative increase in serum IL-6 in patients with hormone-refractory prostate cancer. In addition, cell culture studies have demonstrated that treatment with IL-6 or expression of constitutively active STAT3 are sufficient to promote androgen-independent growth (Lee et al. 2004). In support of this notion, the inhibition of STAT3 was reported to induce apoptosis in IL-6-dependent prostate cancer cells (Barton et al. 2004). In contrast to breast cancer, where an inverse relationship between STAT3 and STAT5 has been observed, both STATs are active in advanced prostate cancers where they promote disease progression. While STAT5 is required for cell viability and growth of the primary tumor, STAT3 is suggested to be an important driver for metastasis (Gu et al. 2010a, b). In contrast to STAT3, which can be activated by a variety of tyrosine kinases (e.g. JAK1, JAK2, JAK3, EGFR/HER family), the activation of STAT5 in the prostate epithelium is largely mediated by JAK2 in response to systemic and autocrine PRL signaling (Dagvadorj et al. 2007; Li et al. 2004; Nevalainen et al. 1997). The PRL/JAK2/STAT5 cascade has been implicated as a critical pathway for the growth, survival, and malignant progression of prostate cancer, and it is therefore a valid target for clinical therapy (Liao et al. 2010). This is supported by a number of studies demonstrating the efficacy of antagonizing PRL, JAK2, or STAT5 for the treatment and/or sensitization of prostate cancer cells by inhibiting their growth and viability (Dagvadorj et al. 2007, 2008; Li et al. 2004; Wu et al. 2007). STAT5, which is found to be active in 95% of clinical hormone-refractory prostate cancers, interacts with the ligand-bound androgen-receptor (AR) to synergistically promote the transcriptional activation of both AR and STAT5 (Tan et al. 2008). Since it has been shown that active STAT5 is necessary for the survival of androgen-sensitive as well as androgen-independent human prostate cancer cells, the therapeutic value for targeting JAK2 or STAT5 in hormone-refractory prostate cancer is of high clinical importance (Ahonen et al. 2003).

Lung Cancer

Atypical growth factor signaling is especially common in human lung cancers and frequently occurs through mutations in the epidermal growth factor receptor (EGFR). These mutant receptors transduce anti-apoptotic signaling selectively through the Phosphatidylinositol-3-Kinase (PI3K)/AKT and STAT signaling pathways. Similar to blocking EGFR signaling with gefinitinib, inhibiting the PI3K or JAKs was reported to cause extensive apoptosis in non-small-cell lung cancers (NSCLC) that are resistant to conventional chemotherapy (Sordella et al. 2004). The matrix metalloproteinase-10 (MMP-10) is a major contributor of lung tumor development and expansion through degradation of the extracellular matrix. Interestingly, protein levels of MMP-10 are significantly elevated in NSCLC compared to normal lung tissue, and this increase was reported to be JAK2-dependent through activation of the IL-6/JAK2/STAT3 signaling cascade (Zhang et al. 2009). Despite a clear relationship between JAKs and advanced lung cancer, additional studies are needed to better decipher their role in disease initiation and their potential for preventative and/or therapeutic intervention.

Head and Neck Cancer

STAT5 and STAT3 are found to be highly expressed and activated in a number of squamous cell carcinomas of the head and neck (SCCHN), where these signal transducers contribute to cancer cell survival and proliferation (Lai and Johnson 2010). The constitutive activation of STAT3 is frequently observed in SCCHN and is suggested to be an early event in carcinogenesis (Grandis et al. 2000; Nagpal et al. 2002). STAT5, on the other hand, is thought to be activated during disease progression in response to EPO signaling. The abundance of EPO and its receptor in tumor biopsies correlates significantly with disease progression, and their highest expression is observed in the most malignant and invasive specimens. In support of these observations, Mohyeldin and coworkers (2005) showed that inhibition of JAK2 was sufficient to reduce both basal and EPO-induced invasiveness. In a study by Xi et al. (2003), active STAT5 was consistently elevated in head and neck tumors compared to normal epithelium, and there was a close correlation between phosphorylation of STAT5 and malignant progression. Particularly, the targeted inhibition of STAT5B resulted in a reduced proliferation of SCCHN cancer cells in vitro and tumor growth in vivo (Leong et al. 2002). Collectively, both studies suggest that targeting JAK2 and STAT5B could be clinically relevant for the treatment of advanced head and neck cancer.

Melanoma

As with cancers of the prostate and head and neck, STAT3 and STAT5 display significant levels of activity in melanocytes as they progress from a normal into a malignant stage, and both STATs have been shown to be needed for the survival and growth of melanoma cells (Hassel et al. 2008; Kortylewski et al. 2005; Mirmohammadsadegh et al. 2006; Niu et al. 2002). A previous study by Niu et al. (2002) suggested that STAT3 and STAT5 are predominantly activated by the SRC kinase, but a new report by Huang et al. (2008) showed that the increase in STAT3 activation was accompanied by an upregulation of JAK2 and a decrease in SOCS-1. The utilization of interferons (i.e. IFN-α) in the treatment of melanoma to provoke an anti-tumor response requires JAK/STAT signaling. Specifically, STAT1, which is activated by JAK1 and TYK2, is critical for the anti-proliferative effect of IFN-α and INF-γ (Kortylewski et al. 2004; Tassiulas et al. 2004). Samples from melanoma patients clinically resistant to IFN-α therapy frequently exhibited dysfunctional JAK/STAT signaling, including a reduction in STAT1 activity (Pansky et al. 2000). In addition to inducing an anti-tumor response via STAT1, it was also found that IFN-α causes phosphorylation of STAT5, which leads to resistance to cytokine-mediated antiproliferative therapy (Wellbrock et al. 2005). Therefore, overcoming interferon resistance in melanoma may lie in the ability to discriminate between the activation of particular STAT family members.

Gastrointestinal Cancers

The IL-6 and GH-mediated activation of STAT3 and STAT5 via JAK1 and JAK2 plays a key role in hepatocellular carcinoma (HCC) (Chow et al. 1996; Fuke et al. 2007; Lee et al. 2006b; Tan et al. 2010). In particular, expression and activation of STAT5B, which is the main target of JAK2 in response to GH signaling, is associated with a young age at tumor onset, metastatic progression, and overall poor patient survival (Fuke et al. 2007). Paradoxically, the loss of STAT5A/B in hepatocytes results in liver fibrosis and enhances chemically-induced tumor formation, presumably through increased activation of TGF-β and STAT3 (Hosui et al. 2009). Active STAT3 is found in approximately 50% of HCC specimens and 75% of metastatic lesions, whereas little or no activity of this signal transducer is observed in adjacent normal tissue. Inhibition of Janus kinases with AG490 has been shown to lower the activation of STAT3, which results in reduced cell proliferation and viability and enhances TNF-related apoptosis-inducing ligand (TRAIL)-induced apoptosis (Fuke et al. 2007). This suggests that inhibition of Janus kinases might be a suitable strategy for sensitizing HCC cells for TRAIL agonists currently under development.

The importance of JAK/STAT signaling in pancreatic cancer was recently highlighted in a study by Thoennissen and colleagues (2009) who tested the efficacy of the experimental anti-cancer drug Cucurbitacin B. This agent causes significant cell cycle arrest and apoptosis of pancreatic cancer cells associated with inhibition of JAK2, STAT3, and STAT5. Another study by Lee et al. (2007) suggested that enhanced JAK2 activity, which is observed in pancreatic cancers, might be a result of elevated levels of reactive oxygen species (ROS). According to this report, growth factor signaling promotes the formation of ROS, which, in turn, prolongs JAK2 phosphorylation and cell survival by inhibiting the low molecular weight-protein tyrosine phosphatase (LMW-PTP) responsible for JAK2 inactivation.

Sustained activation of Janus kinases 1 and 2 is commonly observed in human colon cancers, and the JAK1/2-mediated activation of STAT3 is suggested to control key events during early colonic tumorigenesis and its progression into malignant adenocarcinoma. According to a study by Xiong et al. (2008), the expression of active STAT3 increased from 26.7% in the normal epithelium to virtually 100% in adenocarcinomas as determined by immunostaining. This was accompanied by an increase in pJAK2 staining from 46.7% to 81.6%, respectively. The authors further demonstrated that JAK1, JAK2, and STAT3 are involved in controlling the growth, survival, and metastatic capabilities of colorectal cancer cells. Beside these changes, a functional loss of p53 is observed in the majority of colon cancers and has been shown to confer resistance to irinotecan, a topoisomerase 1 inhibitor. On the other hand, irinotecan is able to enhance TRAIL-induced apoptosis in a p53-independent manner, and this cellular phenomenon is reported to be a consequence of an inhibition of JAK2/STAT3/5 signaling which leads to reduced colon cancer metastases (Ravi et al. 2004). Finally, JAK2 is also implicated in mediating the growth-promoting anti-apoptotic effects of the potent growth factor glycine-extended gastrin (G-Gly). Overexpression of G-Gly in transgenic mice promotes colonic proliferation, and colon cancers are known to upregulate G-Gly as part of an autocrine signaling

mechanism (Koh et al. 1999; Stepan et al. 1999; Watson et al. 1999). According to a study by Beales and Ogunwobi (2006), JAK2 is activated in response to G-Gly and promotes the subsequent activation of AKT and STAT3. The authors suggested that targeting G-Gly directly or through key signaling nodes (i.e. JAKs) might be a useful approach for sensitizing colon cancers to chemotherapeutic agents. In a follow-up study, Ogunwobi and Beales (2007) also found that JAK2, in conjunction with AKT and STAT3, is required for the anti-apoptotic effects of leptin in colon cancers. Collectively, signaling through JAK1 and JAK2 and possibly even JAK3 (Lin et al. 2005; Mori et al. 2005) regulates important biological events during colon cancer initiation and progression, but it needs to be experimentally verified that JAKs are genuine targets for colon cancer prevention and therapy.

Additional Human Cancer Types

There is emerging evidence from a number of recent reports that JAK/STAT signaling plays a key role in the genesis of an even wider variety of malignant tumor subtypes including cancers of the brain, cervix and ovary. For example, JAK2 and STAT5 were suggested to be constitutively active in most brain tumors compared to normal brain tissue, which has a significantly lower activity of JAK2 and STAT5. This abnormal stimulation of JAK2 and STAT5 was reported to be a consequence of both ligand-dependent and ligand-independent mechanisms (Kondyli et al. 2010). Similar to breast cancer, enhanced signaling through the EPO and IL-6 receptors play a key role in cervical and ovarian cancers. Specifically, the EPO ligand and receptor are expressed in 88% and 92% of cervical tumor samples, respectively (Leo et al. 2006). In a new study, Sobti and colleagues (2010) observed a significant increase in STAT5B expression in cervical tumors compared to nearly undetectable levels of this signal transducer in normal tissues. The authors also reported that the expression of STAT5B was associated with the severity of the disease. Finally, a recent study by Colomiere et al. (2009) shows that ovarian cancers exhibit a significant increase in the activity of JAK2 and STAT3 compared to normal tissues, which was reported to be the consequence of receptor crosstalk between the EGFR and the IL6-R signaling through JAK2 and STAT3 during the process of epithelial to mesenchymal transition (Colomiere et al. 2009). Collectively, all these studies suggest that aberrant activation of JAK/STAT signaling cascades seem to play important roles during particular stages of disease initiation and malignant progression.

V. Targeting JAKs for the Prevention and Treatment of Cancers

The development of small molecular inhibitors against JAKs, in particular JAK2, to treat myeloproliferative disorders (MPDs) and hematopoietic cancers invigorates the concept to utilize these new agents for a pharmacological inhibition of Janus kinases in solid cancers. There is also sufficient experimental evidence to suggest that some of the newly developed drugs might be successful in future clinical settings. For example,

JANEX-1, a small molecule inhibitor of JAK3, is reported to be effective in preventing intestinal tumor development in the APC^{min} model for spontaneous intestinal adenoma formation (Uckun and Dibirdik 2010). This finding is encouraging since JAK3 has been recognized as a poor prognostic indicator in colon cancer (Lin et al. 2005; Mori et al. 2005). However, this finding brings along with it a note of caution as systematic inhibition of JAK3 can lead to severe combined immunodeficiency. Whether this or similar compounds will be useful as a chemopreventive drugs remains to be seen as these types of agents would have to be administered over long periods of time. Currently, there are more than a dozen investigational studies underway to test putative JAK2 inhibitors to treat MPDs (Geron et al. 2008; Pardanani 2008; Pardanani et al. 2010; Wernig et al. 2008). Among these agents, INCB018424 from Incyte might be of interest for the treatment of solid cancer since this drug was reported to inhibit both JAK1 and JAK2 that are often simultaneously hyperactive in solid cancers (Verstovsek et al. 2010). Despite these advances, it still remains to be determined whether all these new agents are genuine inhibitors that specifically target their corresponding Janus kinase(s). Also, unlike MPDs and other hematopoietic malignancies that originate through point mutations or structural abnormalities in the *JAK2* gene, the vast majority of solid cancers exhibit a constitutive activation of wildtype JAKs and STATs through alternative mechanisms. Therefore, it might be unreasonable to expect that drugs specifically designed to target the mutant JAK2 will have the same efficacy in the treatment of solid cancers that exhibit an upregulation of active, wildtype Janus kinases.

Much of the work demonstrating the importance of JAK/STAT signaling in human cancers has been performed using cancer cell lines by knocking down JAKs, STAT3, and STAT5 or by utilizing various JAK inhibitors, which have been shown to alter tumorigenic properties such as growth, survival, and invasion. While these studies provide detailed insights into particular pathways that promote malignant properties of cancer cells, they do so under non-physiological conditions in primary or metastatic cells. Specifically, the activation of Janus kinases in established cancers requires a ligand-inducible stimulation of the JAK-associated hormone and cytokine receptors, but there are known inter-species-related incompatibilities between growth factors that can significantly alter the outcome of a preclinical study (Wagner et al. 2004). For example, the hormone PRL is suggested to fuel the proliferation of breast cancer cells. While some breast cancer cell lines might synthesize PRL as part of an autocrine loop, the systemic hormone produced in the mouse failed to induce biologic responses mediated by the human PRL receptor such as cell clustering, proliferation, and signal transduction through STAT5, STAT3, ERK1/2, and AKT (Utama et al. 2006). Hence, in order to adequately reflect the endocrine environment in breast cancer patients, it would be necessary to "humanize" the recipient animal model through expression of hormones and cytokines that activate their receptors and downstream JAKs and STATs in a physiological manner.

Although the efficacy and specificity of putative JAK inhibitors remain to be more thoroughly assessed in patients and animal models that permit an activation of JAKs and STATs at physiologically relevant levels, there are appropriate genetic tools available to date to examine whether particular JAKs and STATs are required for disease initiation and progression. Specifically, conditional knockout mice for JAK2,

STAT5, STAT3, and STAT1 are available (Krempler et al. 2004; Cui et al. 2004; Takeda et al. 1998; Klover et al. 2010) that can be used to delete these signal transducers in a temporally controlled manner specifically within primary or metastatic cancer cells (i.e. following neoplastic transformation). In addition, transgenic mice that express wildtype or hyperactive STAT5 under regulation of the tetracycline-controlled transactivator have been generated (Creamer et al. 2010; Yamaji et al. 2009), and those can now be used to downregulate exogenous STAT5 in solid tumors or hematopoietic malignancies. Such an experimental design would address whether STATs that contribute to neoplastic transformation are equally required for the maintenance of a neoplasm. Based on these experimental concepts, Sakamoto and colleagues recently discriminated the importance of JAK2 in mammary tumor initiation versus progression in two established breast cancer models (Sakamoto et al. 2009, 2010). Collectively, the results of these two studies clearly show that the deletion of the *Jak2* gene from the mammary epithelium prior to tumor onset completely protected female mice from developing mammary tumors in response to an overexpression of ERBB2 as well as PRL. This suggests that signaling through JAK2 in the cancer-initiating epithelial subtype is required for neoplastic transformation, and therefore targeting JAK2 might be a suitable strategy for cancer prevention. In principle, this experimental design is similar to previous animal model studies on the basis of conventional knockout mice to assess the appearance of neoplasms in the absence of a gene-of-interest, for example *Stat5a* or *Cyclin D1* (Humphreys and Hennighausen 1999; Yu et al. 2001), and this also includes models that co-express Cre recombinase and an oncogene such as ERBB2 from a bicistronic construct in a conditional knockout background (Klover et al. 2010; Ursini-Siegel et al. 2008). The lack of tumorigenesis in particular knockout models, for example Cyclin D1 null mice (Yu et al. 2001), prompted the authors to suggest that targeting these genes is therapeutically relevant to treat established cancers. These conclusions are premature since these mice neither developed cancer nor expressed the "therapeutic target" a single day in their lives (Matulka and Wagner 2005). The use of the conditional JAK2 knockout mice has demonstrated that the timing of the functional ablation of a gene is critical for its impact on tumorigenesis. While the deletion of *Jak2* prior to tumor initiation was protective against mammary cancer, the ablation of this Janus kinase from fully neoplastic cells had no impact on tumor cell survival and proliferation in vitro or in vivo (Sakamoto et al. 2009, 2010). Collectively, both studies show that JAK2 is a moving target during neoplastic progression, and the gain-of-function of other tyrosine kinases, in particular RTKs, might substitute for the loss of JAK2 in particular tumor types. The role of JAK1 alone or in combination with JAK2 in tumorigenesis still needs to be determined once a conditional knockout mouse for JAK1 becomes available.

Concluding Remarks

Janus kinases have important functions in normal tissue homeostasis, and their constitutive activation can promote neoplastic transformation and cancer progression. Specifically, a sustained activation of Janus kinases 1 and 2 is commonly observed in a

variety of solid human cancers. However, unlike hematopoietic malignancies that originate through point mutations or structural abnormalities in the *JAK2* gene, solid cancers almost exclusively exhibit a hyper-activation of wildtype JAKs and STATs through alternative mechanisms. These include the epigenetic silencing of negative regulators of JAK/STAT signaling as well as an enhanced activation of Janus kinases through aberrant autocrine stimulation of growth factors such as PRL, EPO, and IL-6. Janus kinases and their associated STATs are an integral component of receptor crosstalk in normal cells, and recent studies in genetically engineered models show that their biological significance can change within signaling networks following malignant transformation. The extent and type of receptor crosstalk that utilize JAKs and STATs therefore not only depends on the cell type but also the stage of neoplastic progression. This is one reason why JAKs can become moving targets for chemoprevention and therapy. Due to the heterogeneity of solid cancers, the outcome for a successful treatment of primary and metastatic tumors with JAK inhibitors requires a stratification of cancer subtypes according to their molecular characteristics that should not be restricted to gene expression profiles but rather include the activation of particular JAK/STAT pathways. First reports on the successful development of JAK1/2 inhibitors and their use in the clinic to treat MPDs and other hematopoietic malignancies are encouraging. The efficacy and specificity of these agents remain to be thoroughly evaluated in patients with solid tumors or preclinical animal models for human cancers. In addition, genetically engineered mice in which individual JAKs and STATs can be deleted from normal and neoplastic cell types can be utilized to address whether they are required for disease initiation and progression. They may also determine whether targeting individual JAK/STAT signaling pathways are relevant for the prevention and/or treatment of specific cancer subtypes.

Acknowledgements This work was supported by the Public Health Service grants CA117930 from the National Cancer Institute to K.-U. Wagner. J.W. Schmidt received a graduate fellowship through the UNMC Cancer Research Training Program (CA009476), a Program of Excellence Graduate Assistantship from the UNMC Graduate Studies Office as well as a Breast Cancer Predoctoral Traineeship Award from the Department of Defense Congressionally Directed Medical Research Program (BC100147).

References

Ackler S, Ahmad S, Tobias C, Johnson MD, Glazer RI (2002) Delayed mammary gland involution in MMTV-AKT1 transgenic mice. Oncogene 21(2):198–206

Ahonen TJ, Xie J, LeBaron MJ, Zhu J, Nurmi M, Alanen K, Rui H, Nevalainen MT (2003) Inhibition of transcription factor Stat5 induces cell death of human prostate cancer cells. J Biol Chem 278:27287–27292

Arteaga CL (2003) Trastuzumab, an appropriate first-line single-agent therapy for HER2-overexpressing metastatic breast cancer. Breast Cancer Res 5(2):96–100

Barbieri I, Quaglino E, Maritano D, Pannellini T, Riera L, Cavallo F, Forni G, Musiani P, Chiarle R, Poli V (2010) Stat3 is required for anchorage-independent growth and metastasis but not for mammary tumor development downstream of the ErbB-2 oncogene. Mol Carcinog 49:114–120

Barton BE, Karras JG, Murphy TF, Barton A, Huang HF (2004) Signal transducer and activator of transcription 3 (STAT3) activation in prostate cancer: direct STAT3 inhibition induces apoptosis in prostate cancer lines. Mol Cancer Ther 3:11–20

Baxter EJ, Scott LM, Campbell PJ, East C, Fourouclas N, Swanton S, Vassiliou GS, Bench AJ, Boyd EM, Curtin N et al (2005) Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet 365:1054–1061

Beales IL, Ogunwobi O (2006) Glycine-extended gastrin inhibits apoptosis in colon cancer cells via separate activation of Akt and JNK pathways. Mol Cell Endocrinol 247:140–149

Behera R, Kumar V, Lohite K, Karnik S, Kundu GC (2010) Activation of JAK2/STAT3 signaling by osteopontin promotes tumor growth in human breast cancer cells. Carcinogenesis 31:192–200

Berishaj M, Gao SP, Ahmed S, Leslie K, Al-Ahmadie H, Gerald WL, Bornmann W, Bromberg JF (2007) Stat3 is tyrosine-phosphorylated through the interleukin-6/glycoprotein 130/Janus kinase pathway in breast cancer. Breast Cancer Res 9:R32

Biscardi JS, Maa MC, Tice DA, Cox ME, Leu TH, Parsons SJ (1999) c-Src-mediated phosphorylation of the epidermal growth factor receptor on Tyr845 and Tyr1101 is associated with modulation of receptor function. J Biol Chem 274(12):8335–8343

Brabender J, Danenberg KD, Metzger R, Schneider PM, Park J, Salonga D, Holscher AH, Danenberg PV (2001) Epidermal growth factor receptor and HER2-neu mRNA expression in non-small cell lung cancer is correlated with survival. Clin Cancer Res 7:1850–1855

Brisken C, Ayyannan A, Nguyen C, Heineman A, Reinhardt F, Tan J, Dey SK, Dotto GP, Weinberg RA (2002) IGF-2 is a mediator of prolactin-induced morphogenesis in the breast. Dev Cell 3:877–887

Brockman JL, Schuler LA (2005) Prolactin signals via Stat5 and Oct-1 to the proximal cyclin D1 promoter. Mol Cell Endocrinol 239:45–53

Burke WM, Jin X, Lin HJ, Huang M, Liu R, Reynolds RK, Lin J (2001) Inhibition of constitutively active Stat3 suppresses growth of human ovarian and breast cancer cells. Oncogene 20:7925–7934

Calvisi DF, Ladu S, Gorden A, Farina M, Conner EA, Lee JS, Factor VM, Thorgeirsson SS (2006) Ubiquitous activation of Ras and Jak/Stat pathways in human HCC. Gastroenterology 130:1117–1128

Cataldo L, Chen NY, Yuan Q, Li W, Ramamoorthy P, Wagner TE, Sticca RP, Chen WY (2000) Inhibition of oncogene STAT3 phosphorylation by a prolactin antagonist, hPRL-G129R, in T-47D human breast cancer cells. Int J Oncol 17(6):1179–1185

Chen ST, Lin SY, Yeh KT, Kuo SJ, Chan WL, Chu YP, Chang JG (2004) Mutational, epigenetic and expressional analyses of caveolin-1 gene in breast cancers. Int J Mol Med 14:577–582

Choi D, Schroer SA, Lu SY, Wang L, Wu X, Liu Y, Zhang Y, Gaisano HY, Wagner KU, Wu H, Retnakaran R, Woo M (2010) Erythropoietin protects against diabetes through direct effects on pancreatic beta cells. J Exp Med 207(13):2831–2842

Chow JC, Ling PR, Qu Z, Laviola L, Ciccarone A, Bistrian BR, Smith RJ (1996) Growth hormone stimulates tyrosine phosphorylation of JAK2 and STAT5, but not insulin receptor substrate-1 or SHC proteins in liver and skeletal muscle of normal rats in vivo. Endocrinology 137:2880–2886

Clark DE, Williams CC, Duplessis TT, Moring KL, Notwick AR, Long W, Lane WS, Beuvink I, Hynes NE, Jones FE (2005) ERBB4/HER4 potentiates STAT5A transcriptional activity by regulating novel STAT5A serine phosphorylation events. J Biol Chem 280(25):24175–24180

Clevenger CV, Chang WP, Ngo W, Pasha TL, Montone KT, Tomaszewski JE (1995) Expression of prolactin and prolactin receptor in human breast carcinoma. Evidence for an autocrine/paracrine loop. Am J Pathol 146:695–705

Colomiere M, Ward AC, Riley C, Trenerry MK, Cameron-Smith D, Findlay J, Ackland L, Ahmed N (2009) Cross talk of signals between EGFR and IL-6R through JAK2/STAT3 mediate epithelial-mesenchymal transition in ovarian carcinomas. Br J Cancer 100:134–144

Cotarla I, Ren S, Zhang Y, Gehan E, Singh B, Furth PA (2004) Stat5a is tyrosine phosphorylated and nuclear localized in a high proportion of human breast cancers. Int J Cancer 108:665–671

Creamer BA, Sakamoto K, Schmidt JW, Triplett AA, Moriggl R, Wagner KU (2010) Stat5 promotes survival of mammary epithelial cells through transcriptional activation of a distinct promoter in Akt1. Mol Cell Biol 30:2957–2970

Cui J, Rohr LR, Swanson G, Speights VO, Maxwell T, Brothman AR (2001) Hypermethylation of the caveolin-1 gene promoter in prostate cancer. Prostate 46:249–256

Cui Y, Riedlinger G, Miyoshi K, Tang W, Li C, Deng CX, Robinson GW, Hennighausen L (2004) Inactivation of Stat5 in mouse mammary epithelium during pregnancy reveals distinct functions in cell proliferation, survival, and differentiation. Mol Cell Biol 24(18):8037–8047

Dagvadorj A, Collins S, Jomain JB, Abdulghani J, Karras J, Zellweger T, Li H, Nurmi M, Alanen K, Mirtti T et al (2007) Autocrine prolactin promotes prostate cancer cell growth via Janus kinase-2-signal transducer and activator of transcription-5a/b signaling pathway. Endocrinology 148:3089–3101

Dagvadorj A, Kirken RA, Leiby B, Karras J, Nevalainen MT (2008) Transcription factor signal transducer and activator of transcription 5 promotes growth of human prostate cancer cells in vivo. Clin Cancer Res 14:1317–1324

Dowlati A, Nethery D, Kern JA (2004) Combined inhibition of epidermal growth factor receptor and JAK/STAT pathways results in greater growth inhibition in vitro than single agent therapy. Mol Cancer Ther 3:459–463

Drachenberg DE, Elgamal AA, Rowbotham R, Peterson M, Murphy GP (1999) Circulating levels of interleukin-6 in patients with hormone refractory prostate cancer. Prostate 41:127–133

Fujitake S, Hibi K, Okochi O, Kodera Y, Ito K, Akiyama S, Nakao A (2004) Aberrant methylation of SOCS-1 was observed in younger colorectal cancer patients. J Gastroenterol 39:120–124

Fuke H, Shiraki K, Sugimoto K, Tanaka J, Beppu T, Yoneda K, Yamamoto N, Ito K, Masuya M, Takei Y (2007) Jak inhibitor induces S phase cell-cycle arrest and augments TRAIL-induced apoptosis in human hepatocellular carcinoma cells. Biochem Biophys Res Commun 363:738–744

Gao SP, Mark KG, Leslie K, Pao W, Motoi N, Gerald WL, Travis WD, Bornmann W, Veach D, Clarkson B et al (2007) Mutations in the EGFR kinase domain mediate STAT3 activation via IL-6 production in human lung adenocarcinomas. J Clin Invest 117:3846–3856

Garcia-Martinez JM, Calcabrini A, Gonzalez L, Martin-Forero E, Agullo-Ortuno MT, Simon V, Watkin H, Anderson SM, Roche S, Martin-Perez J (2010) A non-catalytic function of the Src family tyrosine kinases controls prolactin-induced Jak2 signaling. Cell Signal 22(3):415–426

Geron I, Abrahamsson AE, Barroga CF, Kavalerchik E, Gotlib J, Hood JD, Durocher J, Mak CC, Noronha G, Soll RM, Tefferi A, Kaushansky K, Jamieson CH (2008) Selective inhibition of JAK2-driven erythroid differentiation of polycythemia vera progenitors. Cancer Cell 13(4): 321–330

Ghoreschi K, Laurence A, O'Shea JJ (2009) Janus kinases in immune cell signaling. Immunol Rev 228:273–287

Ginsburg E, Vonderhaar BK (1995) Prolactin synthesis and secretion by human breast cancer cells. Cancer Res 55:2591–2595

Giri D, Ozen M, Ittmann M (2001) Interleukin-6 is an autocrine growth factor in human prostate cancer. Am J Pathol 159:2159–2165

Grandis JR, Drenning SD, Zeng Q, Watkins SC, Melhem MF, Endo S, Johnson DE, Huang L, He Y, Kim JD (2000) Constitutive activation of Stat3 signaling abrogates apoptosis in squamous cell carcinogenesis in vivo. Proc Natl Acad Sci USA 97:4227–4232

Greenhalgh CJ, Hilton DJ (2001) Negative regulation of cytokine signaling. J Leukoc Biol 70:348–356

Grivennikov S, Karin M (2008) Autocrine IL-6 signaling: a key event in tumorigenesis? Cancer Cell 13:7–9

Gu L, Dagvadorj A, Lutz J, Leiby B, Bonuccelli G, Lisanti MP, Addya S, Fortina P, Dasgupta A, Hyslop T et al (2010a) Transcription factor Stat3 stimulates metastatic behavior of human prostate cancer cells in vivo, whereas Stat5b has a preferential role in the promotion of prostate cancer cell viability and tumor growth. Am J Pathol 176:1959–1972

Gu L, Vogiatzi P, Puhr M, Dagvadorj A, Lutz J, Ryder A, Addya S, Fortina P, Cooper C, Leiby B et al (2010b) Stat5 promotes metastatic behavior of human prostate cancer cells in vitro and in vivo. Endocr Relat Cancer 17:481–493

Gu L, Zhu XH, Visakorpi T, Alanen K, Mirtti T, Edmonston TB, Nevalainen MT (2010c) Activating mutation (V617F) in the tyrosine kinase JAK2 is absent in locally-confined or castration-resistant prostate cancer. Anal Cell Pathol (Amsterdam) 33:55–59

Gual P, Baron V, Lequoy V, Van Obberghen E (1998) Interaction of Janus kinases JAK-1 and JAK-2 with the insulin receptor and the insulin-like growth factor-1 receptor. Endocrinology 139(3):884–893

Hankinson SE, Willett WC, Michaud DS, Manson JE, Colditz GA, Longcope C, Rosner B, Speizer FE (1999) Plasma prolactin levels and subsequent risk of breast cancer in postmenopausal women. J Natl Cancer Inst 91:629–634

Harir N, Pecquet C, Kerenyi M, Sonneck K, Kovacic B, Nyga R, Brevet M, Dhennin I, Gouilleux-Gruart V, Beug H et al (2007) Constitutive activation of Stat5 promotes its cytoplasmic localization and association with PI3-kinase in myeloid leukemias. Blood 109:1678–1686

Hassel JC, Winnemoller D, Schartl M, Wellbrock C (2008) STAT5 contributes to antiapoptosis in melanoma. Melanoma Res 18:378–385

Hellstrom I, Goodman G, Pullman J, Yang Y, Hellstrom KE (2001) Overexpression of HER-2 in ovarian carcinomas. Cancer Res 61:2420–2423

Henry MD, Triplett AA, Oh KB, Smith GH, Wagner KU (2004) Parity-induced mammary epithelial cells facilitate tumorigenesis in MMTV-neu transgenic mice. Oncogene 23 (41):6980–6985

Himpe E, Kooijman R (2009) Insulin-like growth factor-I receptor signal transduction and the Janus kinase/signal transducer and activator of transcription (JAK-STAT) pathway. Biofactors 35(1):76–81

Hirsch FR, Langer CJ (2004) The role of HER2/neu expression and trastuzumab in non-small cell lung cancer. Semin Oncol 31:75–82

Holbro T, Beerli RR, Maurer F, Koziczak M, Barbas CF 3rd, Hynes NE (2003) The ErbB2/ErbB3 heterodimer functions as an oncogenic unit: ErbB2 requires ErbB3 to drive breast tumor cell proliferation. Proc Natl Acad Sci USA 100(15):8933–8938

Hosui A, Kimura A, Yamaji D, Zhu BM, Na R, Hennighausen L (2009) Loss of STAT5 causes liver fibrosis and cancer development through increased TGF-{beta} and STAT3 activation. J Exp Med 206:819–831

Huang FJ, Steeg PS, Price JE, Chiu WT, Chou PC, Xie K, Sawaya R, Huang S (2008) Molecular basis for the critical role of suppressor of cytokine signaling-1 in melanoma brain metastasis. Cancer Res 68:9634–9642

Humphreys RC, Hennighausen L (1999) Signal transducer and activator of transcription 5a influences mammary epithelial cell survival and tumorigenesis. Cell Growth Differ 10(10): 685–694

Hutchinson J, Jin J, Cardiff RD, Woodgett JR, Muller WJ (2001) Activation of Akt (protein kinase B) in mammary epithelium provides a critical cell survival signal required for tumor progression. Mol Cell Biol 21(6):2203–2212

Hynes NE, Lane HA (2005) ERBB receptors and cancer: the complexity of targeted inhibitors. Nat Rev Cancer 5(5):341–354

Iavnilovitch E, Groner B, Barash I (2002) Overexpression and forced activation of stat5 in mammary gland of transgenic mice promotes cellular proliferation, enhances differentiation, and delays postlactational apoptosis. Mol Cancer Res 1:32–47

James C, Ugo V, Le Couedic JP, Staerk J, Delhommeau F, Lacout C, Garcon L, Raslova H, Berger R, Bennaceur-Griscelli A et al (2005) A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. Nature 434:1144–1148

Jeong EG, Kim MS, Nam HK, Min CK, Lee S, Chung YJ, Yoo NJ, Lee SH (2008) Somatic mutations of JAK1 and JAK3 in acute leukemias and solid cancers. Clin Cancer Res 14:3716–3721

Johnson KJ, Peck AR, Liu C, Tran TH, Utama FE, Sjolund AB, Schaber JD, Witkiewicz AK, Rui H (2010) PTP1B suppresses prolactin activation of Stat5 in breast cancer cells. Am J Pathol 177:2971–2983

Kamizono S, Hanada T, Yasukawa H, Minoguchi S, Kato R, Minoguchi M, Hattori K, Hatakeyama S, Yada M, Morita S et al (2001) The SOCS box of SOCS-1 accelerates ubiquitin-dependent proteolysis of TEL-JAK2. J Biol Chem 276:12530–12538

Karaghiosoff M, Neubauer H, Lassnig C, Kovarik P, Schindler H, Pircher H, McCoy B, Bogdan C, Decker T, Brem G et al (2000) Partial impairment of cytokine responses in Tyk2-deficient mice. Immunity 13:549–560

Karaghiosoff M, Steinborn R, Kovarik P, Kriegshauser G, Baccarini M, Donabauer B, Reichart U, Kolbe T, Bogdan C, Leanderson T et al (2003) Central role for type I interferons and Tyk2 in lipopolysaccharide-induced endotoxin shock. Nat Immunol 4:471–477

Kim A, Liu B, Ordonez-Ercan D, Alvarez KM, Jones LD, McKimmey C, Edgerton SM, Yang X, Thor AD (2005) Functional interaction between mouse erbB3 and wild-type rat c-neu in transgenic mouse mammary tumor cells. Breast Cancer Res 7(5):R708–R718

Kisseleva T, Bhattacharya S, Braunstein J, Schindler CW (2002) Signaling through the JAK/STAT pathway, recent advances and future challenges. Gene 285(1–2):1–24

Klover PJ, Muller WJ, Robinson GW, Pfeiffer RM, Yamaji D, Hennighausen L (2010) Loss of STAT1 from mouse mammary epithelium results in an increased neu-induced tumor burden. Neoplasia 12(11):899–905

Knupfer H, Preiss R (2007) Significance of interleukin-6 (IL-6) in breast cancer (review). Breast Cancer Res Treat 102:129–135

Kocher HM, Mears L, Lea NC, Raj K, Mufti GJ (2007) JAK V617F missense mutation is absent in pancreatic cancer. Gut 56:1174–1175

Koh TJ, Dockray GJ, Varro A, Cahill RJ, Dangler CA, Fox JG, Wang TC (1999) Overexpression of glycine-extended gastrin in transgenic mice results in increased colonic proliferation. J Clin Invest 103(8):1119–1126

Komazaki T, Nagai H, Emi M, Terada Y, Yabe A, Jin E, Kawanami O, Konishi N, Moriyama Y, Naka T et al (2004) Hypermethylation-associated inactivation of the SOCS-1 gene, a JAK/STAT inhibitor, in human pancreatic cancers. Jpn J Clin Oncol 34:191–194

Kondyli M, Gatzounis G, Kyritsis A, Varakis J, Assimakopoulou M (2010) Immunohistochemical detection of phosphorylated JAK-2 and STAT-5 proteins and correlation with erythropoietin receptor (EpoR) expression status in human brain tumors. J Neurooncol 100(2):157–164

Kortylewski M, Komyod W, Kauffmann ME, Bosserhoff A, Heinrich PC, Behrmann I (2004) Interferon-gamma-mediated growth regulation of melanoma cells: involvement of STAT1-dependent and STAT1-independent signals. J Invest Dermatol 122:414–422

Kortylewski M, Jove R, Yu H (2005) Targeting STAT3 affects melanoma on multiple fronts. Cancer Metastasis Rev 24:315–327

Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR, Tichelli A, Cazzola M, Skoda RC (2005) A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med 352:1779–1790

Krempler A, Qi Y, Triplett AA, Zhu J, Rui H, Wagner KU (2004) Generation of a conditional knockout allele for the Janus kinase 2 (Jak2) gene in mice. Genesis 40:52–57

Kunigal S, Lakka SS, Sodadasu PK, Estes N, Rao JS (2009) Stat3-siRNA induces Fas-mediated apoptosis in vitro and in vivo in breast cancer. Int J Oncol 34:1209–1220

Lai SY, Johnson FM (2010) Defining the role of the JAK-STAT pathway in head and neck and thoracic malignancies: implications for future therapeutic approaches. Drug Resist Updat 13:67–78

Larsson AM, Jirstrom K, Fredlund E, Nilsson S, Ryden L, Landberg G, Pahlman S (2009) Erythropoietin receptor expression and correlation to tamoxifen response and prognosis in breast cancer. Clin Cancer Res 15(17):5552–5559

Lee SO, Lou W, Johnson CS, Trump DL, Gao AC (2004) Interleukin-6 protects LNCaP cells from apoptosis induced by androgen deprivation through the Stat3 pathway. Prostate 60:178–186

Lee JW, Soung YH, Kim SY, Nam SW, Park WS, Lee JY, Yoo NJ, Lee SH (2006a) Absence of JAK2 V617F mutation in gastric cancers. Acta Oncol 45:222–223

Lee TK, Man K, Poon RT, Lo CM, Yuen AP, Ng IO, Ng KT, Leonard W, Fan ST (2006b) Signal transducers and activators of transcription 5b activation enhances hepatocellular carcinoma aggressiveness through induction of epithelial-mesenchymal transition. Cancer Res 66:9948–9956

Lee JK, Edderkaoui M, Truong P, Ohno I, Jang KT, Berti A, Pandol SJ, Gukovskaya AS (2007) NADPH oxidase promotes pancreatic cancer cell survival via inhibiting JAK2 dephosphorylation by tyrosine phosphatases. Gastroenterology 133:1637–1648

Leo C, Horn LC, Rauscher C, Hentschel B, Liebmann A, Hildebrandt G, Hockel M (2006) Expression of erythropoietin and erythropoietin receptor in cervical cancer and relationship to survival, hypoxia, and apoptosis. Clin Cancer Res 12:6894–6900

Leong PL, Xi S, Drenning SD, Dyer KF, Wentzel AL, Lerner EC, Smithgall TE, Grandis JR (2002) Differential function of STAT5 isoforms in head and neck cancer growth control. Oncogene 21:2846–2853

Levine RL, Wadleigh M, Cools J, Ebert BL, Wernig G, Huntly BJ, Boggon TJ, Wlodarska I, Clark JJ, Moore S et al (2005) Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. Cancer Cell 7:387–397

Li H, Ahonen TJ, Alanen K, Xie J, LeBaron MJ, Pretlow TG, Ealley EL, Zhang Y, Nurmi M, Singh B et al (2004) Activation of signal transducer and activator of transcription 5 in human prostate cancer is associated with high histological grade. Cancer Res 64:4774–4782

Liang K, Esteva FJ, Albarracin C, Stemke-Hale K, Lu Y, Bianchini G, Yang CY, Li Y, Li X, Chen CT, Mills GB, Hortobagyi GN, Mendelsohn J, Hung MC, Fan Z (2010) Recombinant human erythropoietin antagonizes trastuzumab treatment of breast cancer cells via Jak2-mediated Src activation and PTEN inactivation. Cancer Cell 18(5):423–435

Liao Z, Lutz J, Nevalainen MT (2010) Transcription factor Stat5a/b as a therapeutic target protein for prostate cancer. Int J Biochem Cell Biol 42:186–192

Lin Q, Lai R, Chirieac LR, Li C, Thomazy VA, Grammatikakis I, Rassidakis GZ, Zhang W, Fujio Y, Kunisada K et al (2005) Constitutive activation of JAK3/STAT3 in colon carcinoma tumors and cell lines: inhibition of JAK3/STAT3 signaling induces apoptosis and cell cycle arrest of colon carcinoma cells. Am J Pathol 167:969–980

Liu J, Kern JA (2002) Neuregulin-1 activates the JAK-STAT pathway and regulates lung epithelial cell proliferation. Am J Respir Cell Mol Biol 27:306–313

Lo HW, Cao X, Zhu H, Ali-Osman F (2008) Constitutively activated STAT3 frequently coexpresses with epidermal growth factor receptor in high-grade gliomas and targeting STAT3 sensitizes them to Iressa and alkylators. Clin Cancer Res 14:6042–6054

Long W, Wagner KU, Lloyd KC, Binart N, Shillingford JM, Hennighausen L, Jones FE (2003) Impaired differentiation and lactational failure of Erbb4-deficient mammary glands identify ERBB4 as an obligate mediator of STAT5. Development 130(21):5257–5268

Matulka LA, Wagner KU (2005) Models of breast cancer. Drug Discov Today 2(1):1–6

Mirmohammadsadegh A, Hassan M, Bardenheuer W, Marini A, Gustrau A, Nambiar S, Tannapfel A, Bojar H, Ruzicka T, Hengge UR (2006) STAT5 phosphorylation in malignant melanoma is important for survival and is mediated through SRC and JAK1 kinases. J Invest Dermatol 126:2272–2280

Mohyeldin A, Lu H, Dalgard C, Lai SY, Cohen N, Acs G, Verma A (2005) Erythropoietin signaling promotes invasiveness of human head and neck squamous cell carcinoma. Neoplasia 7:537–543

Mori D, Nakafusa Y, Miyazaki K, Tokunaga O (2005) Differential expression of Janus kinase 3 (JAK3), matrix metalloproteinase 13 (MMP13), heat shock protein 60 (HSP60), and mouse double minute 2 (MDM2) in human colorectal cancer progression using human cancer cDNA microarrays. Pathol Res Pract 201:777–789

Motte N, Saulnier P, Le Couedic JP, Soria JC, Delaloge S, Boige V, Pautier P, Vainchenker W, Bidart JM, Villeval JL (2007) Mutations in JAK2V617F homologous domain of JAK genes are uncommon in solid tumors. Int J Cancer 121:2113–2115

Nagpal JK, Mishra R, Das BR (2002) Activation of Stat-3 as one of the early events in tobacco chewing-mediated oral carcinogenesis. Cancer 94:2393–2400

Neilson LM, Zhu J, Xie J, Malabarba MG, Sakamoto K, Wagner KU, Kirken RA, Rui H (2007) Coactivation of janus tyrosine kinase (Jak)1 positively modulates prolactin-Jak2 signaling in breast cancer: recruitment of ERK and signal transducer and activator of transcription (Stat)3 and enhancement of Akt and Stat5a/b pathways. Mol Endocrinol 21:2218–2232

Neubauer H, Cumano A, Muller M, Wu H, Huffstadt U, Pfeffer K (1998) Jak2 deficiency defines an essential developmental checkpoint in definitive hematopoiesis. Cell 93:397–409

Nevalainen MT, Valve EM, Ingleton PM, Nurmi M, Martikainen PM, Harkonen PL (1997) Prolactin and prolactin receptors are expressed and functioning in human prostate. J Clin Invest 99:618–627

Nevalainen MT, Xie J, Torhorst J, Bubendorf L, Haas P, Kononen J, Sauter G, Rui H (2004) Signal transducer and activator of transcription-5 activation and breast cancer prognosis. J Clin Oncol 22:2053–2060

Niu G, Bowman T, Huang M, Shivers S, Reintgen D, Daud A, Chang A, Kraker A, Jove R, Yu H (2002) Roles of activated Src and Stat3 signaling in melanoma tumor cell growth. Oncogene 21:7001–7010

Nosaka T, van Deursen JM, Tripp RA, Thierfelder WE, Witthuhn BA, McMickle AP, Doherty PC, Grosveld GC, Ihle JN (1995) Defective lymphoid development in mice lacking Jak3. Science 270:800–802

Nyga R, Pecquet C, Harir N, Gu H, Dhennin-Duthille I, Regnier A, Gouilleux-Gruart V, Lassoued K, Gouilleux F (2005) Activated STAT5 proteins induce activation of the PI 3-kinase/Akt and Ras/MAPK pathways via the Gab2 scaffolding adapter. Biochem J 390:359–366

O'Shea JJ, Watford W (2004) A peek at PIAS. Nat Immunol 5:875–876

Ochs AM, Wong L, Kakani V, Neerukonda S, Gorske J, Rao A, Riggs M, Ward H, Keville L (2004) Expression of vascular endothelial growth factor and HER2/neu in stage II colon cancer and correlation with survival. Clin Colorectal Cancer 4:262–267

Ogunwobi OO, Beales IL (2007) The anti-apoptotic and growth stimulatory actions of leptin in human colon cancer cells involves activation of JNK mitogen activated protein kinase, JAK2 and PI3 kinase/Akt. Int J Colorectal Dis 22:401–409

Okochi O, Hibi K, Sakai M, Inoue S, Takeda S, Kaneko T, Nakao A (2003) Methylation-mediated silencing of SOCS-1 gene in hepatocellular carcinoma derived from cirrhosis. Clin Cancer Res 9:5295–5298

Pansky A, Hildebrand P, Fasler-Kan E, Baselgia L, Ketterer S, Beglinger C, Heim MH (2000) Defective Jak-STAT signal transduction pathway in melanoma cells resistant to growth inhibition by interferon-alpha. Int J Cancer 85:720–725

Pardanani A (2008) JAK2 inhibitor therapy in myeloproliferative disorders: rationale, preclinical studies and ongoing clinical trials. Leukemia 22(1):23–30

Pardanani A, Vannucchi AM, Passamonti F, Cervantes F, Barbui T, Tefferi A (2010) JAK inhibitor therapy for myelofibrosis: critical assessment of value and limitations. Leukemia 25 (2):218–225

Parganas E, Wang D, Stravopodis D, Topham DJ, Marine JC, Teglund S, Vanin EF, Bodner S, Colamonici OR, van Deursen JM et al (1998) Jak2 is essential for signaling through a variety of cytokine receptors. Cell 93:385–395

Park SY, Saijo K, Takahashi T, Osawa M, Arase H, Hirayama N, Miyake K, Nakauchi H, Shirasawa T, Saito T (1995) Developmental defects of lymphoid cells in Jak3 kinase-deficient mice. Immunity 3:771–782

Park DS, Lee H, Frank PG, Razani B, Nguyen AV, Parlow AF, Russell RG, Hulit J, Pestell RG, Lisanti MP (2002) Caveolin-1-deficient mice show accelerated mammary gland development

during pregnancy, premature lactation, and hyperactivation of the Jak-2/STAT5a signaling cascade. Mol Biol Cell 13:3416–3430
Pfeffer LM, Mullersman JE, Pfeffer SR, Murti A, Shi W, Yang CH (1997) STAT3 as an adapter to couple phosphatidylinositol 3-kinase to the IFNAR1 chain of the type I interferon receptor. Science 276(5317):1418–1420
Proietti CJ, Rosemblit C, Beguelin W, Rivas MA, Diaz Flaque MC, Charreau EH, Schillaci R, Elizalde PV (2009) Activation of Stat3 by heregulin/ErbB-2 through the co-option of progesterone receptor signaling drives breast cancer growth. Mol Cell Biol 29:1249–1265
Ram PA, Waxman DJ (1999) SOCS/CIS protein inhibition of growth hormone-stimulated STAT5 signaling by multiple mechanisms. J Biol Chem 274:35553–35561
Rane SG, Reddy EP (2000) Janus kinases: components of multiple signaling pathways. Oncogene 19(49):5662–5679
Ranger JJ, Levy DE, Shahalizadeh S, Hallett M, Muller WJ (2009) Identification of a Stat3-dependent transcription regulatory network involved in metastatic progression. Cancer Res 69 (17):6823–6830
Ravi R, Jain AJ, Schulick RD, Pham V, Prouser TS, Allen H, Mayer EG, Yu H, Pardoll DM, Ashkenazi A et al (2004) Elimination of hepatic metastases of colon cancer cells via p53-independent cross-talk between irinotecan and Apo2 ligand/TRAIL. Cancer Res 64:9105–9114
Rodig SJ, Meraz MA, White JM, Lampe PA, Riley JK, Arthur CD, King KL, Sheehan KC, Yin L, Pennica D et al (1998) Disruption of the Jak1 gene demonstrates obligatory and nonredundant roles of the Jaks in cytokine-induced biologic responses. Cell 93:373–383
Rosa Santos SC, Dumon S, Mayeux P, Gisselbrecht S, Gouilleux F (2000) Cooperation between STAT5 and phosphatidylinositol 3-kinase in the IL-3-dependent survival of a bone marrow derived cell line. Oncogene 19:1164–1172
Rose-Hellekant TA, Arendt LM, Schroeder MD, Gilchrist K, Sandgren EP, Schuler LA (2003) Prolactin induces ERalpha-positive and ERalpha-negative mammary cancer in transgenic mice. Oncogene 22:4664–4674
Ruchatz H, Coluccia AM, Stano P, Marchesi E, Gambacorti-Passerini C (2003) Constitutive activation of Jak2 contributes to proliferation and resistance to apoptosis in NPM/ALK-transformed cells. Exp Hematol 31:309–315
Sakamoto K, Creamer BA, Triplett AA, Wagner KU (2007) The Janus kinase 2 is required for expression and nuclear accumulation of cyclin D1 in proliferating mammary epithelial cells. Mol Endocrinol 21:1877–1892
Sakamoto K, Lin WC, Triplett AA, Wagner KU (2009) Targeting janus kinase 2 in Her2/neu-expressing mammary cancer: implications for cancer prevention and therapy. Cancer Res 69:6642–6650
Sakamoto K, Triplett AA, Schuler LA, Wagner KU (2010) Janus kinase 2 is required for the initiation but not maintenance of prolactin-induced mammary cancer. Oncogene 29:5359–5369
Sansone P, Storci G, Tavolari S, Guarnieri T, Giovannini C, Taffurelli M, Ceccarelli C, Santini D, Paterini P, Marcu KB et al (2007) IL-6 triggers malignant features in mammospheres from human ductal breast carcinoma and normal mammary gland. J Clin Invest 117:3988–4002
Santos SC, Lacronique V, Bouchaert I, Monni R, Bernard O, Gisselbrecht S, Gouilleux F (2001) Constitutively active STAT5 variants induce growth and survival of hematopoietic cells through a PI 3-kinase/Akt dependent pathway. Oncogene 20:2080–2090
Sasi W, Jiang WG, Sharma A, Mokbel K (2010) Higher expression levels of SOCS 1,3,4,7 are associated with earlier tumour stage and better clinical outcome in human breast cancer. BMC Cancer 10:178
Schindler C, Plumlee C (2008) Inteferons pen the JAK-STAT pathway. Semin Cell Dev Biol 19:311–318
Schindler C, Levy DE, Decker T (2007) JAK-STAT signaling: from interferons to cytokines. J Biol Chem 282:20059–20063

Schindler C, Strehlow I (2000) Cytokines and STAT signaling. Adv Pharmacol 47:113–174
Schwertfeger KL, Richert MM, Anderson SM (2001) Mammary gland involution is delayed by activated Akt in transgenic mice. Mol Endocrinol 15(6):867–881
Shillingford JM, Miyoshi K, Robinson GW, Grimm SL, Rosen JM, Neubauer H, Pfeffer K, Hennighausen L (2002) Jak2 is an essential tyrosine kinase involved in pregnancy-mediated development of mammary secretory epithelium. Mol Endocrinol 16:563–570
Shimoda K, Kato K, Aoki K, Matsuda T, Miyamoto A, Shibamori M, Yamashita M, Numata A, Takase K, Kobayashi S et al (2000) Tyk2 plays a restricted role in IFN alpha signaling, although it is required for IL-12-mediated T cell function. Immunity 13:561–571
Shirota K, LeDuy L, Yuan SY, Jothy S (1990) Interleukin-6 and its receptor are expressed in human intestinal epithelial cells. Virchows Arch B Cell Pathol Incl Mol Pathol 58:303–308
Slupianek A, Hoser G, Majsterek I, Bronisz A, Malecki M, Blasiak J, Fishel R, Skorski T (2002) Fusion tyrosine kinases induce drug resistance by stimulation of homology-dependent recombination repair, prolongation of G(2)/M phase, and protection from apoptosis. Mol Cell Biol 22:4189–4201
Sobti RC, Singh N, Hussain S, Suri V, Bharadwaj M, Das BC (2010) Deregulation of STAT-5 isoforms in the development of HPV-mediated cervical carcinogenesis. J Recept Signal Transduct Res 30:178–188
Sordella R, Bell DW, Haber DA, Settleman J (2004) Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways. Science 305:1163–1167
Sos BC, Harris C, Nordstrom SM, Tran JL, Balázs M, Caplazi P, Febbraio M, Applegate MAB, Wagner KU, Weiss EJ (2011) Profound fatty liver in mice with hepatocyte-specific deletion of JAK2 is completely rescued by abrogation of growth hormone secretion. J Clin Invest (in press)
Stepan VM, Krametter DF, Matsushima M, Todisco A, Delvalle J, Dickinson CJ (1999) Glycine-extended gastrin regulates HEK cell growth. Am J Physiol 277:R572–R581
Sultan AS, Xie J, LeBaron MJ, Ealley EL, Nevalainen MT, Rui H (2005) Stat5 promotes homotypic adhesion and inhibits invasive characteristics of human breast cancer cells. Oncogene 24:746–760
Sutherland KD, Lindeman GJ, Choong DY, Wittlin S, Brentzell L, Phillips W, Campbell IG, Visvader JE (2004) Differential hypermethylation of SOCS genes in ovarian and breast carcinomas. Oncogene 23:7726–7733
Takeda K, Kaisho T, Yoshida N, Takeda J, Kishimoto T, Akira S (1998) Stat3 activation is responsible for IL-6-dependent T cell proliferation through preventing apoptosis: generation and characterization of T cell-specific Stat3-deficient mice. J Immunol 161(9):4652–4660
Tam L, McGlynn LM, Traynor P, Mukherjee R, Bartlett JM, Edwards J (2007) Expression levels of the JAK/STAT pathway in the transition from hormone-sensitive to hormone-refractory prostate cancer. Br J Cancer 97:378–383
Tan SH, Dagvadorj A, Shen F, Gu L, Liao Z, Abdulghani J, Zhang Y, Gelmann EP, Zellweger T, Culig Z et al (2008) Transcription factor Stat5 synergizes with androgen receptor in prostate cancer cells. Cancer Res 68:236–248
Tan SM, Li F, Rajendran P, Prem Kumar A, Hui KM, Sethi G (2010) Identification of {beta}-escin as a novel inhibitor of STAT3/JAK2 signaling pathway that suppresses proliferation and induces apoptosis in human hepatocellular carcinoma cells. J Pharmacol Exp Ther 334 (1):285–293
Tassiulas I, Hu X, Ho H, Kashyap Y, Paik P, Hu Y, Lowell CA, Ivashkiv LB (2004) Amplification of IFN-alpha-induced STAT1 activation and inflammatory function by Syk and ITAM-containing adaptors. Nat Immunol 5:1181–1189
Tessier C, Prigent-Tessier A, Ferguson-Gottschall S, Gu Y, Gibori G (2001) PRL antiapoptotic effect in the rat decidua involves the PI3K/protein kinase B-mediated inhibition of caspase-3 activity. Endocrinology 142:4086–4094
Thoennissen NH, Iwanski GB, Doan NB, Okamoto R, Lin P, Abbassi S, Song JH, Yin D, Toh M, Xie WD et al (2009) Cucurbitacin B induces apoptosis by inhibition of the JAK/STAT pathway and potentiates antiproliferative effects of gemcitabine on pancreatic cancer cells. Cancer Res 69:5876–5884

Thomis DC, Gurniak CB, Tivol E, Sharpe AH, Berg LJ (1995) Defects in B lymphocyte maturation and T lymphocyte activation in mice lacking Jak3. Science 270:794–797

Tornell J, Rymo L, Isaksson OG (1991) Induction of mammary adenocarcinomas in metallothionein promoter-human growth hormone transgenic mice. Int J Cancer 49:114–117

Tworoger SS, Hankinson SE (2006) Prolactin and breast cancer risk. Cancer Lett 243:160–169

Uckun FM, Dibirdik I (2010) Chemoprevention of colorectal cancer by targeting Janus kinase 3 with a rationally designed small molecule inhibitor. Nutr Cancer 62:968–972

Ursini-Siegel J, Hardy WR, Zuo D, Lam SH, Sanguin-Gendreau V, Cardiff RD, Pawson T, Muller WJ (2008) ShcA signalling is essential for tumour progression in mouse models of human breast cancer. EMBO J 27(6):910–920

Utama FE, LeBaron MJ, Neilson LM, Sultan AS, Parlow AF, Wagner KU, Rui H (2006) Human prolactin receptors are insensitive to mouse prolactin: implications for xenotransplant modeling of human breast cancer in mice. J Endocrinol 188:589–601

Vafaizadeh V, Klemmt P, Brendel C, Weber K, Doebele C, Britt K, Grez M, Fehse B, Desrivieres S, Groner B (2010) Mammary epithelial reconstitution with gene-modified stem cells assigns roles to Stat5 in luminal alveolar cell fate decisions, differentiation, involution, and mammary tumor formation. Stem Cells 28:928–938

Vainchenker W, Constantinescu SN (2005) A unique activating mutation in JAK2 (V617F) is at the origin of polycythemia vera and allows a new classification of myeloproliferative diseases. Hematology Am Soc Hematol Educ Program 2005:195–200

Valentino L, Pierre J (2006) JAK/STAT signal transduction: regulators and implication in hematological malignancies. Biochem Pharmacol 71:713–721

Verstovsek S, Kantarjian H, Mesa RA, Pardanani AD, Cortes-Franco J, Thomas DA, Estrov Z, Fridman JS, Bradley EC, Erickson-Viitanen S, Vaddi K, Levy R, Tefferi A (2010) Safety and efficacy of INCB018424, a JAK1 and JAK2 inhibitor, in myelofibrosis. N Engl J Med 363 (12):1117–1127

Wagner KU, Rui H (2008) Jak2/Stat5 signaling in mammogenesis, breast cancer initiation and progression. J Mammary Gland Biol Neoplasia 13:93–103

Wagner KU, Wall RJ, St-Onge L, Gruss P, Wynshaw-Boris A, Garrett L, Li M, Furth PA, Hennighausen L (1997) Cre-mediated gene deletion in the mammary gland. Nucleic Acids Res 25:4323–4330

Wagner KU, McAllister K, Ward T, Davis B, Wiseman R, Hennighausen L (2001) Spatial and temporal expression of the Cre gene under the control of the MMTV-LTR in different lines of transgenic mice. Transgenic Res 10:545–553

Wagner KU, Krempler A, Triplett AA, Qi Y, George NM, Zhu J, Rui H (2004) Impaired alveologenesis and maintenance of secretory mammary epithelial cells in Jak2 conditional knockout mice. Mol Cell Biol 24:5510–5520

Watson SA, Morris TM, Varro A, Michaeli D, Smith AM (1999) A comparison of the therapeutic effectiveness of gastrin neutralisation in two human gastric cancer models: relation to endocrine and autocrine/paracrine gastrin mediated growth. Gut 45(6):812–817

Wellbrock C, Weisser C, Hassel JC, Fischer P, Becker J, Vetter CS, Behrmann I, Kortylewski M, Heinrich PC, Schartl M (2005) STAT5 contributes to interferon resistance of melanoma cells. Curr Biol 15:1629–1639

Wernig G, Kharas MG, Okabe R, Moore SA, Leeman DS, Cullen DE, Gozo M, McDowell EP, Levine RL, Doukas J, Mak CC, Noronha G, Martin M, Ko YD, Lee BH, Soll RM, Tefferi A, Hood JD, Gilliland DG (2008) Efficacy of TG101348, a selective JAK2 inhibitor, in treatment of a murine model of JAK2V617F-induced polycythemia vera. Cancer Cell 13(4):311–320

Wennbo H, Gebre-Medhin M, Gritli-Linde A, Ohlsson C, Isaksson OG, Tornell J (1997) Activation of the prolactin receptor but not the growth hormone receptor is important for induction of mammary tumors in transgenic mice. J Clin Invest 100:2744–2751

Wiechen K, Diatchenko L, Agoulnik A, Scharff KM, Schober H, Arlt K, Zhumabayeva B, Siebert PD, Dietel M, Schafer R et al (2001) Caveolin-1 is down-regulated in human ovarian carcinoma and acts as a candidate tumor suppressor gene. Am J Pathol 159:1635–1643

Williams CC, Allison JG, Vidal GA, Burow ME, Beckman BS, Marrero L, Jones FE (2004) The ERBB4/HER4 receptor tyrosine kinase regulates gene expression by functioning as a STAT5A nuclear chaperone. J Cell Biol 167(3):469–478

Williams TM, Cheung MW, Park DS, Razani B, Cohen AW, Muller WJ, Di Vizio D, Chopra NG, Pestell RG, Lisanti MP (2003) Loss of caveolin-1 gene expression accelerates the development of dysplastic mammary lesions in tumor-prone transgenic mice. Mol Biol Cell 14:1027–1042

Wu S, Divall S, Hoffman GE, Le WW, Wagner KU, Wolfe A (2011) Jak2 is necessary for neuroendocrine control of female reproduction. J Neurosci 31(1):184–192

Wu W, Zanello L, Walker AM (2007) S179D prolactin sensitizes human prostate cancer cells such that physiological concentrations of 1, 25 dihydroxy vitamin D3 result in growth inhibition and cell death. Prostate 67:1498–1506

Xi S, Zhang Q, Gooding WE, Smithgall TE, Grandis JR (2003) Constitutive activation of Stat5b contributes to carcinogenesis in vivo. Cancer Res 63:6763–6771

Xie HJ, Bae HJ, Noh JH, Eun JW, Kim JK, Jung KH, Ryu JC, Ahn YM, Kim SY, Lee SH et al (2009) Mutational analysis of JAK1 gene in human hepatocellular carcinoma. Neoplasma 56:136–140

Xiong H, Zhang ZG, Tian XQ, Sun DF, Liang QC, Zhang YJ, Lu R, Chen YX, Fang JY (2008) Inhibition of JAK1, 2/STAT3 signaling induces apoptosis, cell cycle arrest, and reduces tumor cell invasion in colorectal cancer cells. Neoplasia 10:287–297

Xu SB, Liu XH, Li BH, Zhang Y, Yuan J, Yuan Q, Li PD, Yang XZ, Li F, Zhang WJ (2009) DNA methylation regulates constitutive expression of Stat6 regulatory genes SOCS-1 and SHP-1 in colon cancer cells. J Cancer Res Clin Oncol 135:1791–1798

Yamaji D, Na R, Feuermann Y, Pechhold S, Chen W, Robinson GW, Hennighausen L (2009) Development of mammary luminal progenitor cells is controlled by the transcription factor STAT5A. Genes Dev 23(20):2382–2387

Yamauchi T, Kaburagi Y, Ueki K, Tsuji Y, Stark GR, Kerr IM, Tsushima T, Akanuma Y, Komuro I, Tobe K et al (1998) Growth hormone and prolactin stimulate tyrosine phosphorylation of insulin receptor substrate-1, -2, and -3, their association with p85 phosphatidylinositol 3-kinase (PI3-kinase), and concomitantly PI3-kinase activation via JAK2 kinase. J Biol Chem 273:15719–15726

Yamauchi T, Ueki K, Tobe K, Tamemoto H, Sekine N, Wada M, Honjo M, Takahashi M, Takahashi T, Hirai H, Tushima T, Akanuma Y, Fujita T, Komuro I, Yazaki Y, Kadowaki T (1997) Tyrosine phosphorylation of the EGF receptor by the kinase Jak2 is induced by growth hormone. Nature 390(6655):91–96

Yamauchi T, Yamauchi N, Ueki K, Sugiyama T, Waki H, Miki H, Tobe K, Matsuda S, Tsushima T, Yamamoto T, Fujita T, Taketani Y, Fukayama M, Kimura S, Yazaki Y, Nagai R, Kadowaki T (2000) Constitutive tyrosine phosphorylation of ErbB-2 via Jak2 by autocrine secretion of prolactin in human breast cancer. J Biol Chem 275(43):33937–33944

Yeh YT, Ou-Yang F, Chen IF, Yang SF, Su JH, Hou MF, Yuan SS (2007) Altered p-JAK1 expression is associated with estrogen receptor status in breast infiltrating ductal carcinoma. Oncol Rep 17:35–39

Yoshikawa H, Matsubara K, Qian GS, Jackson P, Groopman JD, Manning JE, Harris CC, Herman JG (2001) SOCS-1, a negative regulator of the JAK/STAT pathway, is silenced by methylation in human hepatocellular carcinoma and shows growth-suppression activity. Nat Genet 28:29–35

Yu Q, Geng Y, Sicinski P (2001) Specific protection against breast cancers by cyclin D1 ablation. Nature 411(6841):1017–1021

Yuan TL, Cantley LC (2008) PI3K pathway alterations in cancer: variations on a theme. Oncogene 27(41):5497–5510

Zhang X, Yin P, Di D, Luo G, Zheng L, Wei J, Zhang J, Shi Y, Xu N (2009) IL-6 regulates MMP-10 expression via JAK2/STAT3 signaling pathway in a human lung adenocarcinoma cell line. Anticancer Res 29:4497–4501