



STATs in oncogenesis

Tammy Bowman^{1,2}, Roy Garcia^{1,2}, James Turkson^{1,2} and Richard Jove^{*1,2,3}

¹Molecular Oncology Program, H. Lee Moffitt Cancer Center and Research Institute, 12902 Magnolia Drive, Tampa, Florida, FL 33612, USA; ²Department of Biochemistry and Molecular Biology, University of South Florida College of Medicine, Tampa, Florida, FL 33612, USA; ³Department of Pathology, University of South Florida College of Medicine, Tampa, Florida, FL 33612, USA

Since their discovery as key mediators of cytokine signaling, considerable progress has been made in defining the structure-function relationships of Signal Transducers and Activators of Transcription (STATs). In addition to their central roles in normal cell signaling, recent studies have demonstrated that diverse oncoproteins can activate specific STATs (particularly Stat3 and Stat5) and that constitutively-activated STAT signaling directly contributes to oncogenesis. Furthermore, extensive surveys of primary tumors and cell lines derived from tumors indicate that inappropriate activation of specific STATs occurs with surprisingly high frequency in a wide variety of human cancers. Together, these findings provide compelling evidence that aberrant STAT activation associated with oncogenesis is not merely adventitious but instead contributes to the process of malignant transformation. These studies are beginning to reveal the molecular mechanisms leading to STAT activation in the context of oncogenesis, and candidate genes regulated by STATs that may contribute to oncogenesis are being identified. Recent studies suggest that activated STAT signaling participates in oncogenesis by stimulating cell proliferation and preventing apoptosis. This review presents the evidence for critical roles of STATs in oncogenesis and discusses the potential for development of novel cancer therapies based on mechanistic understanding of STAT signaling. *Oncogene* (2000) 19, 2474–2488.

Keywords: STAT; oncogenes; tyrosine kinases; serine kinases; cell transformation; human cancer; cancer therapy

Introduction

Delineation of cytokine signaling pathways that control cellular growth, differentiation, survival and development has defined a novel class of proteins known as STATs that regulate these processes by modulating the expression of specific target genes (for an overview of STATs, see Bromberg and Darnell, this issue). STAT proteins are activated by cytokine engagement of cognate cell surface receptors and induce the expression of ligand-dependent genetic programs that determine the biological response to the stimulus. Although originally discovered as effec-

tors of normal cytokine signaling, subsequent studies have demonstrated the participation of STATs in signaling by polypeptide growth factors and oncoproteins. Precise regulation of STAT activation is critical with regard to eliciting the appropriate responses to extracellular signals. In the event that control of STAT activation is deregulated, for example through constitutive ligand/receptor engagement or oncogenic tyrosine kinase (TK) activity, aberrant STAT signaling may contribute to malignant transformation by promoting cell cycle progression and/or cell survival. Because STATs directly regulate gene expression, implicit in the constitutive activation of STATs observed during oncogenesis is the acquisition of a permanent alteration in the genetic program. Moreover, better understanding of the mechanisms underlying aberrant STAT signaling during oncogenesis may lead to the development of novel cancer therapies based on interrupting key steps in this pathway. In this review, we summarize the mechanisms involved in STAT activation in the context of oncogenesis, and discuss the consequences of inappropriate STAT activation in malignant transformation.

Activation of STATs in normal signal transduction

Studies of interferon-dependent gene expression led to the recognition that STAT proteins are signaling molecules with dual functions (Darnell *et al.*, 1994; Stark *et al.*, 1998). STAT proteins not only transmit a signal from the cell surface to the nucleus but also directly participate in gene regulation (Darnell, 1997; Ihle *et al.*, 1995). Further studies revealed that STAT proteins are involved in signaling by many cytokines, such as IL-6 family cytokines (Hirano *et al.*, 1997; Taga and Kishimoto, 1997), and numerous growth factors, including EGF and PDGF (Leaman *et al.*, 1996; Schindler and Darnell, 1995). Seven mammalian STAT family members (Stat1–Stat6, with Stat5A and Stat5B encoded in distinct genes) have been molecularly cloned and likely represent the entire complement of STATs in mammalian cells (Darnell, 1997).

Figure 1 depicts the major structural features of STAT proteins. The C-terminal portion of STATs contains several key elements required for STAT activation and function (Shuai *et al.*, 1994; Wen and Darnell, 1997; Wen *et al.*, 1995). The Src-homology 2 (SH2) domain is a common structural motif among signaling molecules that mediates protein-protein interactions via direct binding to specific phosphotyrosines. Phosphorylation of a critical tyrosine residue activates STATs by stabilizing the association of two STAT monomers through reciprocal phosphotyrosine-

*Correspondence: R Jove, Molecular Oncology Program, Moffitt Cancer Center, 12902 Magnolia Drive, Tampa, FL 33612, USA

Signal Transducers and Activators of Transcription

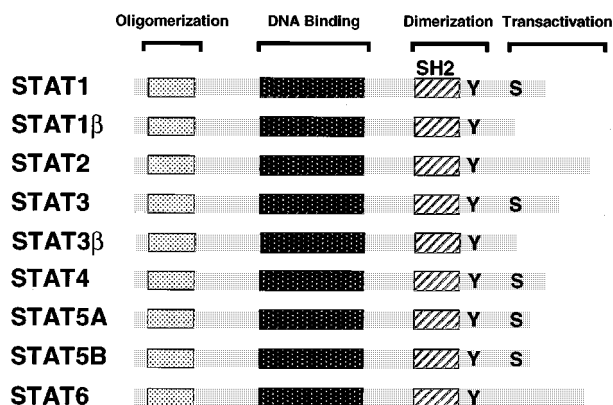


Figure 1 Structures of STAT proteins. Shown are the major structural features and their functions in the known mammalian STAT proteins. The N-terminal oligomerization region is involved in stabilizing interactions between two STAT dimers bound to adjacent sites in DNA. The DNA-binding domain directly contacts DNA and confers specificity of binding to palindromic sequences in promoters of genes. Located between the oligomerization region and the DNA-binding domain is a region of helical coils involved in interactions with other proteins important in transcriptional regulation. Phosphorylation of the invariant tyrosine (Y) residue in the dimerization region mediates interaction with the SH2 domain of another monomer that stabilizes STAT dimer formation. The C-terminal transactivation domain is involved in transcriptional activation of STAT-regulated genes. Located within the transactivation domain of some STAT proteins is a critical serine (S) residue that is required for maximal transcriptional activity. Shown are two naturally-occurring splice variants of Stat1 and Stat3, denoted as Stat1 β and Stat3 β , which have deletions within this transactivation domain. The closely related Stat5A and Stat5B proteins are encoded in distinct genes

SH2 interactions to form a dimer. The extreme C-terminal region of STATs contains the transactivation domain that is required for transcriptional activation. In addition to activation by tyrosine phosphorylation, phosphorylation of a serine residue in the transactivation domain of some STATs (particularly Stat1 and Stat3) contributes to maximal transcriptional activity (Wen *et al.*, 1995). Naturally-occurring splice variants of STATs such as Stat1 β and Stat3 β , which lack the C-terminal transactivation domain and this serine residue, can often block the function of the full-length proteins (or α forms) in a dominant-negative manner (Bromberg *et al.*, 1996; Caldenhoven *et al.*, 1996). In the N-terminal portion, STATs contain the DNA-binding domain and a region that mediates oligomerization between two STAT dimers bound to DNA (Horvath *et al.*, 1995; Xu *et al.*, 1996).

The following description and Figure 2 briefly illustrate the mechanism of STAT activation in normal signal transduction (Darnell, 1997; Darnell *et al.*, 1994). Signaling initiates when ligands such as cytokines bind to and activate their cell surface receptors, typically by inducing receptor aggregation. Cytokine receptors such as the interferon or IL-6 receptors, which lack intrinsic TK activity, recruit members of the Janus kinase (JAK) family of cytoplasmic TKs to act as intermediaries for activation of STATs (Ihle, 1996; Ihle *et al.*, 1995). The JAK family consists of Jak1, Jak2, Jak3 and Tyk2, each of

TK-STAT Signaling

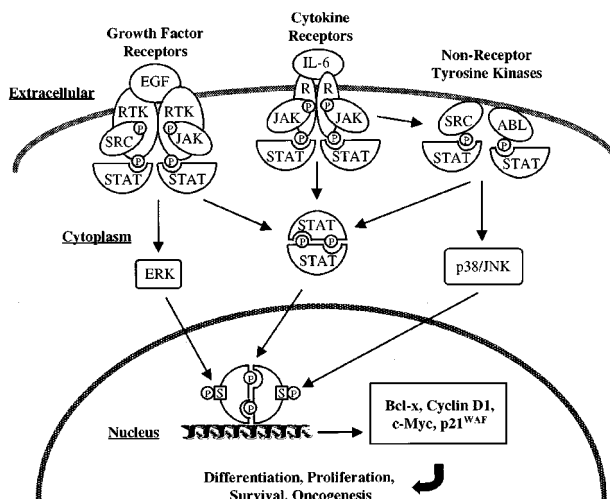


Figure 2 Mechanisms of STAT signaling. Shown are examples of different tyrosine kinase (TK) signaling pathways that can induce activation of STAT proteins. In the case of cytokines like IL-6 that bind to receptors lacking intrinsic TK activity, ligand-induced dimerization of the receptor (R) results in phosphorylation and activation of receptor-associated JAK family kinases. The activated JAKs in turn phosphorylate the receptor cytoplasmic tails on tyrosine, providing docking sites for recruitment of monomeric STATs. JAKs then phosphorylate the recruited STAT proteins on tyrosine, inducing dimerization, nuclear translocation, and DNA-binding activity. In some cases, other TKs such as SRC family kinases are also involved in cytokine-induced STAT activation. In the case of growth factors like EGF that bind to receptor tyrosine kinases (RTKs), the receptor can directly phosphorylate STATs and/or indirectly induce STAT phosphorylation through activation of intermediary kinases of the SRC and JAK families. In addition, non-receptor TKs such as activated SRC and ABL can directly phosphorylate STAT proteins in the absence of ligand-induced receptor signaling. Activation of TK signaling pathways often leads to simultaneous activation of serine/threonine kinases, including p38, JNK and ERK, which phosphorylate STATs on serine (S) residues in the C-terminal transactivation domain. Once in the nucleus, activated STAT proteins bind to specific DNA sequences in the promoters of genes and induce their expression through interactions with other transcriptional regulatory components. In the context of oncogenesis, constitutive activation of TK-STAT signaling pathways induces elevated expression of genes involved in controlling fundamental cellular processes such as cell proliferation and survival

which may be activated by a variety of receptors. Following ligand engagement, the receptor-associated JAKs become activated by phosphorylating themselves and subsequently phosphorylate tyrosine residues within the receptor cytoplasmic tails. The receptor phosphotyrosines serve as docking sites for recruitment of inactive cytoplasmic STAT monomers through interaction with the SH2 domain in STATs. JAK-mediated phosphorylation of an invariant tyrosine residue on the receptor-bound STAT monomer induces the phosphorylated STAT monomers to associate with each other through interaction of the phosphotyrosine of one molecule with the SH2 domain of another molecule, as described above. The activated dimers then translocate to the nucleus, where they bind to specific DNA-response elements (Seidel *et al.*, 1995) in the promoters of target genes and thereby induce unique gene expression programs.

In contrast to typical cytokine receptor signaling, activated growth factor receptors with intrinsic TK

activity, e.g. EGF receptor and PDGF receptor, may directly phosphorylate STAT proteins (Figure 2). In addition to growth factor receptors and JAKs, other TKs that directly phosphorylate STATs are cytoplasmic kinases such as Src and Abl. For example, c-Src is involved in STAT activation by the IL-3 receptor, EGF and PDGF receptors (Chaturvedi *et al.*, 1997, 1998; Olayioye *et al.*, 1999; Wang *et al.*, 2000). Thus, depending on the particular receptor and cellular context, different TKs are involved in activation of STAT signaling by both cytokine and growth factor receptors. For this reason, STAT signaling pathways might be more appropriately called 'TK-STAT' pathways. What determines which TK is involved in STAT activation by a given receptor, and why in some cases more than one TK appears to be involved, are questions that remain to be answered. One possibility is that the use of different TKs allows the simultaneous coupling of receptors to different combinations of signaling pathways that determine particular biological responses.

Control of normal biological processes by STATs

Activation of STATs results in expression of genes that control critical cellular functions including cell proliferation, survival, differentiation and development, as well as specialized cellular functions such as those associated with immune responses. Although the STAT family is highly structurally conserved, there are distinct differences both in primary sequence and in function. The contribution of specific STAT family members to control of normal cellular processes has been elucidated based on studies of homozygous deletion or more recently, by tissue-specific, conditional knockout of each STAT family member in mice (Akira, 1999). Stat1-deficient mice are impaired in their ability to respond to interferons and become susceptible to infections from bacterial and viral pathogens (Durbin *et al.*, 1996; Meraz *et al.*, 1996). Targeted disruption of the Stat4 (Kaplan *et al.*, 1996; Thierfelder *et al.*, 1996) or Stat6 (Shimoda *et al.*, 1996; Takeda *et al.*, 1996; Thierfelder *et al.*, 1996) gene in mice demonstrates that these STATs are required for IL-12- or IL-4-induced proliferation of activated T lymphocytes, respectively.

Although the Stat5A and Stat5B genes are highly related, both at the genetic and protein levels, targeted disruption of either gene exhibits a tissue-specific phenotype with respect to each gene. Stat5A knockout mice display defects in mammary gland development and lactation during pregnancy (Liu *et al.*, 1997), while Stat5B knockout mice display sexually dimorphic patterns of liver gene expression (Udy *et al.*, 1997). Female mice lacking functional Stat5A and Stat5B proteins are infertile and a role for Stat5 proteins in proper immune function has also been demonstrated in the double-knockout animals (Teglund *et al.*, 1998).

In the case of Stat2 (Kimura *et al.*, 1996) and Stat3 (Takeda *et al.*, 1997), homozygous deletion of the gene encoding either protein is embryonic lethal, indicating a critical role for each in normal development. Due to the limitations imposed by Stat3-null animals, i.e. mortality at day 6.5 to 7 during early development, investigators have recently created tissue-specific, Stat3-null conditions using the Cre-Lox system. Analysis of

mice lacking Stat3 expression in keratinocytes demonstrates a role for Stat3 signaling in control of cell motogenesis as it pertains to wound healing (Sano *et al.*, 1999). Furthermore, in contrast to wild-type littermates, IL-6 fails to prevent apoptosis in T lymphocytes from mice deficient for Stat3 signaling (Takeda *et al.*, 1998). Thus, from the above studies it is clear that STAT proteins are required for regulation of many diverse cellular functions. Future studies using tissue-specific STAT knockout animals will undoubtedly contribute to further elucidating the roles of STATs as they pertain to normal signaling.

Activation of STATs by oncogenic tyrosine kinases

Over the past two decades, progress on the cellular mechanisms that contribute to oncogenesis has evolved from the identification of viral and cellular oncoproteins that transform mammalian cells to definition of the signaling pathways that are disrupted during oncogenesis. Subversion of a cell's normal genetic program results in alterations in the expression patterns of genes involved in different facets of transformation, such as cell proliferation, anchorage-independent growth, survival, and morphological changes. These and other hallmarks of transformation are now being understood on a molecular level by functional analyses of key signaling components and their contribution to neoplastic transformation. In contrast to normal signaling, in which STAT activation is rapid yet transient, constitutive signaling by STATs has been increasingly associated with malignant progression. Because activation of STAT proteins requires tyrosine phosphorylation, and for some STAT family members serine phosphorylation, identification of the kinases that are responsible for catalyzing STAT phosphorylation has yielded valuable insight into the molecular mechanisms involved in activation of STATs in human tumors.

JAK family kinases

The first genetic evidence implicating constitutive STAT activation in oncogenesis was derived from studies of signal transduction in fruit flies. A lethal gain-of-function mutation induces hyperactive TK activity in the *Drosophila* JAK homolog, *hopscotch* (Harrison *et al.*, 1995; Luo *et al.*, 1995). As a result, fruit flies expressing the constitutively-activated JAK develop a hematopoietic neoplasia resembling leukemia. Dominant suppressors of this phenotype map to loss-of-function mutations in the *Drosophila* homolog of a mammalian STAT gene (Hou *et al.*, 1996; Yan *et al.*, 1996). Thus, these studies suggest that deregulated JAK kinase activity, inducing constitutive activation of a *Drosophila* STAT, directly leads to the formation of hematopoietic malignancies in fruit flies. Moreover, since *Drosophila* STAT is homologous with mammalian Stat5, the results obtained in fruit flies presaged the contribution of STATs to human cancer.

Involvement of aberrant JAK activation in human cancer is linked to a chromosomal abnormality in acute lymphocytic leukemia (ALL). Chromosomal translocation of the short arm of chromosome 9, containing the kinase domain of Jak2, to the short arm

of chromosome 12, containing the oligomerization domain of the Ets transcription factor, results in a fusion protein (Tel-Jak2) possessing constitutive kinase activity (Ho *et al.*, 1999; Lacronique *et al.*, 1997). Enforced dimerization due to the contribution from the Ets portion of the molecule mimics cytokine-induced activation of Jak2 at membrane-bound receptors. Subsequent studies demonstrated that introduction of the Tel-Jak2 fusion protein into hematopoietic cell lines results in constitutive activation of Stat1, Stat3, and Stat5 and induces factor-independent proliferation (Schwaller *et al.*, 1998). Furthermore, mice harboring cells that express Tel-Jak2 develop fatal myelo- and lympho-proliferative diseases.

Src family kinases

In mammalian cells, the original report demonstrating that STATs are constitutively activated in cells stably transformed by a specific oncoprotein linked activation of the oncogenic Src tyrosine kinase to activation of one STAT family member, Stat3 (Yu *et al.*, 1995). These studies demonstrated that an excellent correlation exists between activation of Stat3 and oncogenic transformation by Src. This observation, which was confirmed and extended by other investigators (Cao *et al.*, 1996; Chaturvedi *et al.*, 1997), raised the possibility that diverse oncoproteins of the receptor or non-receptor TK family may also activate STATs during oncogenic transformation. Src-family members and related kinases such as Lck and v-Fps have been reported to activate STATs (Garcia *et al.*, 1997; Lund *et al.*, 1997; Nelson *et al.*, 1998; Yu *et al.*, 1997). In T cells expressing a herpesvirus saimiri protein (Tip-484) required for transformation of T cells, activation of Lck by Tip-484 induces constitutive activation of Stat1 and Stat3 (Lund *et al.*, 1999a,b). In addition, v-Src activates Stat3 in cell lines derived from human gall bladder adenocarcinomas (Murakami *et al.*, 1998) and mammary epithelial cells (Smith and Crompton, 1998). In NIH3T3 cells, Jak1 and to a lesser extent Jak2 are constitutively activated by v-Src (Campbell *et al.*, 1997). Furthermore, recent studies indicate that Jak1 and v-Src cooperate in the activation of Stat3 in transformed NIH3T3 cells (Zhang *et al.*, 2000). These latter studies support a model in which Jak1 serves to recruit Stat3 to Src, which in turn directly phosphorylates and activates Stat3.

Significantly, two reports provided the first evidence that constitutive STAT activation by an oncoprotein directly participates in transformation (Bromberg *et al.*, 1998b; Turkson *et al.*, 1998). These studies demonstrated that constitutive Stat3 DNA-binding activity induced by the Src oncoprotein results in stimulation of Stat3-dependent gene expression. Moreover, abrogation of Stat3 signaling by co-expression of dominant-negative forms of the Stat3 protein blocks the transforming ability of Src (Bromberg *et al.*, 1998b; Turkson *et al.*, 1998). In contrast, co-expression of dominant-negative Stat3 together with the Ras oncoprotein, which does not activate Stat3 (Garcia *et al.*, 1997), does not block Ras-induced transformation. The combined results of these studies demonstrate that constitutive activation of Stat3 signaling is one pathway required for cellular

transformation by specific classes of oncoproteins like Src possessing TK activity. While Stat3 is almost certainly only one of probably many signaling pathways required for transformation by the Src oncoprotein, it is significant that disruption of this pathway is sufficient to block cell transformation. These studies suggest that activated Stat3 signaling contributes to oncogenesis by eliciting permanent changes in the genetic program required for the initiation and/or maintenance of transformation. Interestingly, dominant-negative Stat3 protein appears to block Src transformation by inducing cell cycle arrest and apoptosis, suggesting that activated Stat3 signaling contributes to transformation of these cells by promoting cell proliferation and preventing cell death (T Bowman and R Jove, unpublished). This conclusion is consistent with findings that candidate genes regulated by Stat3 in the context of oncogenesis are involved in controlling cell growth and survival, as described later.

Abl family kinases

Pre-B lymphocytes transformed by the oncogenic TK, v-Abl, possess constitutively activated Stat1 and Stat5 (Danial *et al.*, 1995). Constitutive activation of STATs by v-Abl appears to involve JAKs, since both Jak1 and Jak3 are activated in these cells. Indeed, recent studies indicate that activation of Jak1 is required for STAT activation by v-Abl via a direct, physical association between v-Abl and Jak1 (Danial *et al.*, 1998). Moreover, pre-B cell lines expressing a mutant v-Abl protein lacking the Jak1 interaction domain or defective for Jak1 signaling fail to support factor-independent growth or induce tumors in mice. In contrast, the oncogenic fusion protein, BCR-Abl, has been shown to activate Stat1 and Stat5 in hematopoietic cell lines in the absence of constitutive JAK activation (Carlesso *et al.*, 1996; Chai *et al.*, 1997; Frank and Varticovski, 1996; Ilaria and Van Etten, 1996; Shuai *et al.*, 1996), indicating a divergence in the mechanisms of STAT activation between v-Abl and BCR-Abl. Importantly, using dominant-negative and constitutively-activated Stat5 proteins, the activation of Stat5 has been shown to be essential for transformation by BCR-Abl (de Groot *et al.*, 1999; Nieborowska-Skorska *et al.*, 1999).

Other oncoproteins

The prediction that diverse oncoproteins may contribute to oncogenesis by activating specific STAT family members (Garcia and Jove, 1998; Yu *et al.*, 1995) has been borne out in numerous studies. Table 1 lists the viral and cellular oncogenes that are known to activate specific STAT family members. Oncoproteins such as v-Sis, v-Fps (Garcia *et al.*, 1997), v-Ros, insulin-like growth factor I (IGF-I) receptor (Zong *et al.*, 1998), and c-Eyk/v-Eyk (Besser *et al.*, 1999; Zong *et al.*, 1996) have been shown to activate predominantly Stat3 in fibroblast cell lines and to a lesser extent Stat1. In epithelial cells, the cellular tyrosine kinase, Etk/BMX, is able to activate Stat1, Stat3 and Stat5 (Wen *et al.*, 1999). The profile of STATs activated by a given oncoprotein may depend to some degree on the specificity of the oncoprotein as well as

Table 1 STAT activation by oncogenes

<i>Cell type</i>	<i>Oncogene</i>	<i>Activated STATs</i>	<i>References</i>
Fibroblasts	v-Src	Stat3	Cao <i>et al.</i> , 1996; Yu <i>et al.</i> , 1995
	c-Src	Stat3	Cao <i>et al.</i> , 1996; Yu <i>et al.</i> , 1995
	v-Fps (Fes)	Stat3	Garcia <i>et al.</i> , 1997; Nelson <i>et al.</i> , 1998
	v-Sis (PDGF)	Stat3	Garcia <i>et al.</i> , 1997
	Polyomavirus middle T antigen	Stat3	Garcia <i>et al.</i> , 1997
	SV40 large T antigen	—	Garcia <i>et al.</i> , 1997
	v-Ras	—	Garcia <i>et al.</i> , 1997
	v-Raf	—	Garcia <i>et al.</i> , 1997
	v-Ros	Stat3	Zong <i>et al.</i> , 1998
	IGF-I receptor	Stat3	Zong <i>et al.</i> , 1998
	c-Eyk, v-Eyk	Stat1, Stat3	Besser <i>et al.</i> , 1999; Zong <i>et al.</i> , 1996
Myeloid	v-Src	Stat1, Stat3, Stat5	Chaturvedi <i>et al.</i> , 1997
	v-Fgr	—	Chaturvedi <i>et al.</i> , 1997
T cell	Lck	Stat3, Stat5	Yu <i>et al.</i> , 1997; Lund <i>et al.</i> , 1997, 1999a,b
Mammary/lung epithelial	v-Src	Stat3	Smith and Crompton, 1998
	Etk/BMX	Stat1, Stat3, Stat5	Wen <i>et al.</i> , 1999
Gallbladder adenocarcinoma	v-Src	Stat3	Murakami <i>et al.</i> , 1998
Pre-B lymphocytes	v-Abl	Stat1, Stat5	Danial <i>et al.</i> , 1995, 1998
Erythroleukemia/blast cells/ basophils/mast cells	BCR-Abl	Stat1, Stat5	Carlesso <i>et al.</i> , 1996; Chai <i>et al.</i> , 1997; de Groot <i>et al.</i> , 1999; Frank and Varticovski, 1996; Ilaria and Van Etten, 1996; Nieborowska-Skorska <i>et al.</i> , 1999
Primary bone marrow	BCR-Abl	Stat5	Shuai <i>et al.</i> , 1996
	v-Mos	—	Shuai <i>et al.</i> , 1996

cell type. It is likely that the list of oncoproteins that activate STAT signaling will continue to grow, especially among oncoproteins that directly or indirectly induce tyrosine kinase signaling.

Despite the finding that Stat1 is sometimes activated in conjunction with Stat3 or Stat5, it appears unlikely that Stat1 contributes to oncogenesis. The evidence accumulated to date favors the interpretation that Stat1 is predominantly growth inhibitory, consistent with the anti-proliferative response to interferon- γ , which activates exclusively Stat1 (Bromberg *et al.*, 1996, 1998a; Buard *et al.*, 1998; Chin *et al.*, 1996; Grimley *et al.*, 1998; Xu *et al.*, 1998). In addition, mice with homozygous deletion of Stat1 are predisposed to certain malignancies and develop tumors with more rapid frequency and kinetics than their wild-type littermates (Kaplan *et al.*, 1998), suggesting that Stat1 may actually have tumor suppressor functions. Moreover, p53-null mice that are defective for Stat1 signaling develop a broader tumor spectrum than their p53-null counterparts, suggesting that loss of Stat1 signaling further enhances oncogenesis (Kaplan *et al.*, 1998). Thus, activation of Stat1 in response to signaling by cytokines, such as IFN- γ , appears to be critical for mediating growth inhibition. This raises the question as to why Stat1 activation does not prevent oncogenesis in transformed cells where its activation is detected. One possibility is that the growth inhibitory effects of activated Stat1 can be overcome by the simultaneous activation of Stat3 or Stat5, which often accompanies Stat1 activation in oncogenesis. In this scenario, the pro-proliferative activities of Stat3 and Stat5 would predominate over the anti-proliferative activity of Stat1.

Serine phosphorylation of STATs

Concurrent induction of multiple signaling pathways occurs in response to stimulation by growth factors or oncoproteins, allowing coordination of signaling functions and the possibility of complex regulation by cross-talk among signaling pathways. Tyrosine phosphorylation of STATs constitutes an early event in the activation of these transcription factors that is required for their dimerization and DNA-binding activity. In addition, phosphorylation of a serine residue in the C-terminal transcriptional activation domain, corresponding to Ser-727 in both Stat1 and Stat3 (Figure 1), enhances the transcriptional activity of these STATs (Wen and Darnell, 1997; Wen *et al.*, 1995). This transcriptional enhancement involves mechanisms that are not fully understood but may include favorable interactions between STATs and co-activator proteins (Decker and Kovarik, 1999). The implications of this feature of STAT regulation are that both tyrosine and serine phosphorylations are essential for full activation of STAT signaling, and that STATs are points of convergence for tyrosine and serine kinases (Beadling *et al.*, 1996; Ng and Cantrell, 1997; Wen *et al.*, 1995). In contrast to the more well-characterized events of STAT activation by tyrosine phosphorylation, however, regulation of STATs by serine phosphorylation is understood less.

MAPK and PKC family kinases

Numerous studies suggest that members of the mitogen-activated protein kinases (MAPK) family

(Schaeffer and Weber, 1999), including extracellular signal-regulated kinases (ERKs) (Chung *et al.*, 1997; David *et al.*, 1995; Kuroki and O'Flaherty, 1999; Ng and Cantrell, 1997), c-Jun N-terminal kinase (JNK) (Lim and Cao, 1999; Turkson *et al.*, 1999) and p38^{mapk} (p38) (Gollob *et al.*, 1999; Turkson *et al.*, 1999), participate in the serine phosphorylation of Stat1 and Stat3 (Figure 2). Furthermore, there is evidence for a role of protein kinase C (PKC) in serine phosphorylation of STATs (Jain *et al.*, 1999). Also implicated in STAT serine phosphorylation are two undefined serine kinases, an H7-sensitive serine kinase (Boulton *et al.*, 1995; van Puijenbroek *et al.*, 1999), and MAPK kinase (MKK)-dependent, ERK-independent serine kinases (Ceresa *et al.*, 1997; Chung *et al.*, 1997). Direct phosphorylation of Stat3 or Stat1 *in vitro* by ERKs (Chung *et al.*, 1997), p38 and JNK (Turkson *et al.*, 1999) provides evidence that members of the MAPK family can induce STAT serine phosphorylation and regulate their activity *in vivo*. It is also relevant that the site of serine phosphorylation in both Stat3 and Stat1, -Pro-Met-Ser-Pro-, complies with the MAPK consensus sequence, -Pro-X-Ser/(Thr)-Pro- (Alvarez *et al.*, 1991; Schaeffer and Weber, 1999). Presumably, the relative contribution of each of these serine kinases to STAT signaling *in vivo* would depend on a variety of factors, including cell-type specific expression of the serine kinases and their interactions with individual STAT members.

Complexity of STAT serine phosphorylation

It is now becoming clear that regulation of STAT function by serine phosphorylation is more complex than originally expected. In addition to enhanced transcriptional activity induced by serine phosphorylation, repression of STAT signaling has also been associated with serine phosphorylation events under certain conditions. Several mechanisms have been proposed for this negative regulation: (1) an apparent direct influence of serine phosphorylation on preventing STAT tyrosine phosphorylation (Chung *et al.*, 1997); (2) indirect effects due to preferential association of STAT proteins with the serine kinases, precluding interaction with tyrosine kinases (Jain *et al.*, 1998, 1999; Lim and Cao, 1999); (3) a negative feedback effect of the serine kinase on an upstream protein tyrosine kinase (Sengupta *et al.*, 1998). One can envision a negative feedback mechanism in which serine phosphorylation of STATs promotes the induction of physiologic inhibitors of STAT signaling, such as those of the Suppressor of Cytokine Signaling (SOCS) family that inhibit at the level of JAKs (Endo *et al.*, 1997; Naka *et al.*, 1997; Starr *et al.*, 1997). A seemingly dual functional role is thus implied for STAT serine phosphorylation events, whereby the same serine kinases can apparently both enhance and repress STAT signaling. Noteworthy, however, is the 'super-active' states of the serine kinases that are present in some of the studies where negative regulation of STATs by serine phosphorylation is observed (Jain *et al.*, 1998). These findings are consistent with recent results indicating that moderate levels of ERKs enhance, while overexpression of ERKs inhibits, Stat3 transcriptional activity (Turkson *et al.*, 1999). Clearly, more work needs to be done to define mechanisms by

which serine phosphorylation regulates the activation and functions of STAT proteins.

STAT serine phosphorylation in oncogenesis

Given that Stat3 serine phosphorylation is required for maximal activation of Stat3 signaling, it is predicted that tyrosine phosphorylation is not sufficient for the obligatory role of Stat3 signaling in Src oncogenesis (Bromberg *et al.*, 1998b; Turkson *et al.*, 1998). Indeed, the contribution of serine phosphorylation of STATs to oncogenesis is becoming increasingly evident. Earlier reports of constitutive serine phosphorylation of Stat1 and Stat3 in human tumors, including leukemias (Frank *et al.*, 1997) and lymphomas (Nielsen *et al.*, 1997; Zhang *et al.*, 1996), suggested that serine phosphorylation participates in STAT signaling during oncogenesis. Direct support for this assertion comes from the finding that a dominant-negative Stat3 mutant with a Ser-727 to Ala-727 mutation, which blocks serine phosphorylation, suppresses Stat3 signaling and Src transformation (Bromberg *et al.*, 1998b). This observation was more recently confirmed in studies showing that blocking the serine kinase signaling pathways leading to induction of Stat3 serine phosphorylation also blocks Stat3 signaling and Src transformation (Turkson *et al.*, 1999).

The signaling pathways leading to Stat3 serine phosphorylation in Src oncogenesis were recently delineated and observed to be Ras-dependent (Turkson *et al.*, 1999), consistent with an essential role for Ras in Src transformation (Stacey *et al.*, 1991). Through use of both genetic and pharmacological approaches, it was determined that p38 and JNK are key serine kinases that mediate Stat3 serine phosphorylation in the context of Src transformation (Turkson *et al.*, 1999). Importantly, inhibition of p38 activity blocks constitutive Stat3 serine phosphorylation and transcriptional activity, as well as Src transformation (Turkson *et al.*, 1999). These observations demonstrate a convergence on Stat3 of tyrosine and serine kinase signaling pathways that have an essential role in oncogenesis by the Src oncoprotein. Similar to the wide-spread involvement of Stat3 tyrosine phosphorylation in a variety of human tumors, it appears likely that further investigation will reveal a wider role of constitutive Stat3 serine phosphorylation in malignant transformation.

Constitutively activated mutants of STATs

Importantly, studies utilizing mutations of STATs, rendering them constitutively active by various mechanisms, demonstrate that constitutive activation of STAT signaling is sufficient to induce at least some aspects of cell transformation. Mutations introduced into Stat5 generate a constitutively activated form that, when expressed in an IL-3-dependent mouse pre-B cell line, induces factor-independent proliferation (Onishi *et al.*, 1998). Acquisition of IL-3-independent proliferation in this cell line suggests that Stat5 may contribute to oncogenesis by promoting cell cycle progression and/or survival. A more recent report demonstrates that the constitutive Stat5 mutant induces expression of Bcl-x_L and Pim-1, suggesting that these and other

Stat5-dependent gene products contribute to factor-independent growth and survival (Nosaka *et al.*, 1999). In contrast, a constitutively-activated mutant form of Stat3, expressed in murine macrophages, mimics one of the effects of IL-10 by inhibiting proliferation but fails to suppress macrophage activation (O'Farrell *et al.*, 1998). Thus, constitutive activation of this particular Stat3 mutant replaces some but not all of the functions of IL-10 cytokine signaling, and suggests that cell-type specificity and/or tissue-specific context plays a key role in determining the biological outcome of aberrant STAT signaling.

Significantly, a recent study provides the first genetic evidence that Stat3 possesses intrinsic oncogenic potential in the absence of upstream TK activating events (Bromberg *et al.*, 1999). A constitutively-activated Stat3 molecule (Stat3-C) was genetically engineered that is capable of dimerization in the absence of tyrosine phosphorylation. As a result of this enforced dimerization, Stat3-C homodimers migrate to the nucleus, bind to Stat3 response elements in promoters and induce gene expression. Moreover, rodent fibroblast cells expressing Stat3-C form colonies in soft agar and induce tumors in nude mice (Bromberg *et al.*, 1999). Thus, constitutive activation of Stat3 DNA-binding leads to induction of a Stat3-dependent genetic program that is sufficient to recapitulate some hallmarks of cell transformation in the absence of other signaling pathways that might be activated by TKs. This finding provides further evidence for the causal role of constitutively-activated Stat3 signaling in oncogenesis, as opposed to being merely an adventitious event resulting from phosphorylation by promiscuous TKs. The identification of STAT-regulated genes, discussed below, will be important in further defining the underlying mechanisms of neoplastic transformation resulting from constitutive STAT signaling.

Target genes regulated by STATs

Signal transduction often involves the transmission of a stimulus from the cell surface to the nucleus such that specific genetic programs are induced that allow the cell to respond appropriately to the stimulus. In normal cells, these signal transduction mechanisms are tightly controlled to prevent unscheduled gene regulation and consequently inappropriate biological responses. In contrast, constitutive STAT-dependent gene expression may contribute to oncogenesis by inducing elevated expression of key gene products required to initiate and/or maintain transformation. While the identification of STAT-regulated genes is currently under intense investigation, there are a number of candidate target genes that have already been characterized. The genes discussed below have all been associated with oncogenesis through regulation of cell cycle progression and/or apoptosis, implicating a direct role for STATs in mediating transformation through induction of genes critical for these processes.

Bcl-x_L

From studies utilizing the constitutively-activated form of Stat3, Stat3-C, several key Stat3-regulated genes

have been identified. Expression of Stat3-C induces transcription of reporter constructs containing promoter sequences derived from the *bcl-x* gene (Bromberg *et al.*, 1999). A strong correlation exists between elevated levels of members of the Bcl-2 family of anti-apoptotic regulatory proteins, including Bcl-x_L protein, and human cancer (Reed, 1999). Significantly, in a human myeloma cell line dependent on IL-6 for survival, constitutive Stat3 activation directly contributes to induction of *bcl-x* gene expression (Catlett-Falcone *et al.*, 1999a,b). Furthermore, functional disruption of IL-6 mediated Stat3 signaling in these tumor cells inhibits *bcl-x* expression, correlating with induction of apoptosis. Consistent with these results, studies with TK inhibitors in NIH3T3 cells expressing v-Src indicate that the *bcl-x* gene is induced by v-Src in a Stat3-dependent manner (Karni *et al.*, 1999). The expression of another member of the Bcl-2 family, Mcl-1, has recently been shown to be dependent on Stat3 activation in multiple myeloma and large granular lymphocyte (LGL) leukemia (Epling-Burnette *et al.*, 2000; Puthier *et al.*, 1999). In addition, activation of Stat5 signaling in response to various cytokines such as IL-3 (Dumon *et al.*, 1999) or erythropoietin (EPO) (Silva *et al.*, 1999) is required for maximal expression of Bcl-x_L and correlates with cell survival. Moreover, Stat5 signaling has recently been shown to be required for the survival of EPO-dependent red blood cell precursors (Socolovsky *et al.*, 1999). Mice nullizygous for both Stat5A and Stat5B suffer fetal anemia due to increased apoptosis of erythroid precursors, correlating with decreased expression of Bcl-x_L protein. Consistent with these findings, recent studies demonstrate that Stat5 contributes to the induction of *bcl-x* gene expression by BCR-Abl (de Groot *et al.*, 2000). Together, these findings provide compelling evidence that constitutively-activated STAT signaling, particularly involving Stat3 and Stat5, contributes to malignant progression by preventing apoptosis.

Cyclin D1

Alteration in the expression levels of proteins required for regulating transit through the cell cycle has been detected in numerous human malignancies. Precise control of cell cycle progression depends on formation and activation of specific cyclin/cyclin-dependent kinase (CDK) complexes at defined points within the cell cycle (Sherr and Roberts, 1999). The expression of cyclin D1, which associates with cdk4 or cdk6 and controls progression from G1 to S phase, is elevated in Stat3-C expressing cells (Bromberg *et al.*, 1999). Since Stat3-C possesses oncogenic potential, it is possible that Stat3-mediated transcriptional regulation of key components of cell cycle control contributes to malignant progression by promoting inappropriate cell cycle traversal. Consistent with this possibility, cyclin D1 mRNA and protein levels are elevated in NIH3T3 fibroblast cells stably transformed by the v-Src oncoprotein compared to their normal counterparts (Sinibaldi *et al.*, 2000). Moreover, v-Src-induced Stat3 activation results in expression of a cyclin D1 promoter-based reporter, which is blocked by co-expression of a Stat3 dominant-negative protein. In addition, regulation of the cyclin D1 gene by Stat5 has also been observed (de Groot *et al.*, 2000; Matsumura

et al., 1999; Wen *et al.*, 1999). Thus, activation of the two STAT family members most strongly associated with oncogenesis, Stat3 and Stat5, results in increased expression of a critical regulator of cell cycle progression. These findings are consistent with the suggestion that inappropriate STAT activation contributes to oncogenesis by stimulating cell proliferation.

p21^{WAF1/CIP1}

It has been previously shown that Stat1 activation in response to interferon- γ results in induction of *p21^{WAF1/CIP1}* and growth arrest (Bromberg *et al.*, 1996; Chin *et al.*, 1996). The *p21^{WAF1/CIP1}* promoter contains three STAT binding sites, two of which bind Stat1 and one that binds Stat1 and Stat3 (Chin *et al.*, 1996). In NIH3T3 fibroblasts stably transformed by the v-Src oncoprotein and harboring activated Stat3, *p21^{WAF1/CIP1}* mRNA and protein levels are increased compared to non-transformed fibroblasts (Sinibaldi *et al.*, 2000). As observed for cyclin D1, Stat3 activation by v-Src induces expression of a *p21^{WAF1/CIP1}* promoter-based reporter, while co-expression of a Stat3 dominant-negative protein blocks this induction. Although increases in *p21^{WAF1/CIP1}* levels are normally associated with cell cycle arrest, a number of studies have also reported elevated levels of *p21^{WAF1/CIP1}* in human tumors (Barboule *et al.*, 1998; Kuwahara *et al.*, 1999; Pindzola *et al.*, 1998; Sparrow *et al.*, 1998). This apparent contradiction in *p21^{WAF1/CIP1}* function may be explained by the finding that, in addition to its growth inhibitory role, *p21^{WAF1/CIP1}* also performs an essential scaffolding function to promote the assembly of active cyclin D1/CDK complexes (LaBaer *et al.*, 1997). Thus, in Src-transformed cells that proliferate more rapidly than their normal counterparts, the observed coordinate increases in *p21^{WAF1/CIP1}* and cyclin D1 expression may be essential for stimulating cell cycle progression.

c-Myc

The *c-myc* gene is over-expressed or amplified in a number of human malignancies (Dang *et al.*, 1999). Itself a transcription factor, *c-Myc* protein is a critical regulator of both cell proliferation and survival. Endogenous *c-myc* mRNA levels are elevated in rodent fibroblast cell lines stably expressing Stat3-C, implicating a role for Stat3 in regulating *c-myc* expression either directly or indirectly (Bromberg *et al.*, 1999). Consistent with this finding, *c-myc* expression is induced in Src-transformed NIH3T3 cells, although the precise role of Stat3 in regulation of *c-myc* by Src remains to be determined (T Bowman, J Turkson, N Sinibaldi, R Croxton, D Cress and R Jove, unpublished data). Furthermore, in the context of mitogenic responses to growth factors like PDGF and EGF, transcriptional regulation of *c-myc* appears to be mediated by Src family kinases through a Ras-independent pathway (Barone and Courtneidge, 1995). Recent studies demonstrate that Stat3 mediates transcriptional regulation of the *c-myc* gene in response to IL-6 (Kiuchi *et al.*, 1999). Mutation of a consensus Stat3-binding site within the *c-myc* promoter abolishes IL-6-induced transcription of reporter constructs. In addition, using differential display

screening of gene expression in a murine pre-B cell line, a recent report demonstrates that the *pim-1* gene is upregulated in response to gp130-mediated signaling (Shirogane *et al.*, 1999). The *pim-1* gene is a proto-oncogene encoding a serine/threonine kinase associated with various murine lymphoid malignancies (Breuer *et al.*, 1989a,b; Cuypers *et al.*, 1984; van Lohuizen *et al.*, 1989, 1991). In murine pre-B cells defective for Stat3 signaling, co-expression of *pim-1* and *c-myc* is sufficient to rescue cells from a block in G1 to S phase cell cycle progression and prevent apoptosis (Shirogane *et al.*, 1999).

From the above studies, it is clear that Stat3 and Stat5 regulate multiple cellular genes that could contribute to oncogenesis by mechanisms that include promotion of cell cycle progression and prevention of apoptosis. However, it is likely that there are other genes regulated by STATs that may also contribute to oncogenesis, and identification of such genes is the focus of ongoing studies.

Activation of STATs in human cancer

Four major findings, discussed above, have identified STATs as critical mediators of oncogenic signaling and have led to the hypothesis that constitutive activation of STAT proteins may participate in development and progression of human tumors: (1) STATs are selectively activated by oncogenic tyrosine kinase signaling pathways; (2) dominant-negative STAT mutants block STAT-dependent transcription and transformation induced by activated TKs; (3) constitutively-activated mutants of STATs can induce some aspects of cell transformation; and (4) inappropriate activation of STATs in oncogenesis leads to induction of genes involved in controlling cell proliferation and survival. In further support of the critical role of STATs in oncogenesis, Table 2 lists the many different human tumor types for which elevated STAT DNA-binding activity has been detected in both tumor cell lines and primary tumor specimens. While Stat1 is also activated in some tumors, it is likely the major STATs involved in promoting oncogenesis are Stat3 and Stat5, for the reasons discussed earlier. These findings are consistent with critical roles for activated STAT signaling in malignant progression of human tumors, including various blood malignancies and solid tumors, some of which are discussed in more detail below.

Leukemias/lymphomas

Activation of specific JAKs and STATs has been demonstrated in various human blood malignancies, including lymphomas and leukemias (Migone *et al.*, 1995; Gouilleux-Gruart *et al.*, 1996; Weber-Nordt *et al.*, 1996; Zhang *et al.*, 1996). As discussed above, transient activation of STATs is critical to transmission of cytokine-induced proliferative, differentiation and survival signals in hematopoietic cell types. Under normal circumstances, ligand availability and negative feedback pathways tightly regulate cytokine-induced activation of STATs. A number of studies suggest that the constitutive activation of STATs in transformed

Table 2 Activation of STATs in human primary tumors and tumor cell lines

<i>Tumor type</i>	<i>Activated STATs</i>	<i>References</i>
Breast cancer		
(a) Cell lines	Stat3	Garcia <i>et al.</i> , 1997; Sartor <i>et al.</i> , 1997
(b) Tumors	Stat1, Stat3	Watson and Miller, 1995; J Bromberg and J Darnell, unpublished results; P Chaturvedi and EP Reddy, unpublished results; R Garcia, C Muro-Cacho, S Minton, C Cox, N Ku, R Falcone, T Bowman and RJove, unpublished results
Multiple myeloma		
(a) Cell lines and tumors	Stat1, Stat3	Catlett-Falcone <i>et al.</i> , 1999b
Head and neck cancer		
(a) Cell lines and tumors	Stat1, Stat3	Grandis <i>et al.</i> , 1998
Leukemia (tumors and cell lines)		
(a) HTLV-I-dependent	Stat3, Stat5	Migone <i>et al.</i> , 1995; Takemoto <i>et al.</i> , 1997
(b) Erythroleukemia	Stat1, Stat5	Carlesso <i>et al.</i> , 1996
(c) Acute lymphocytic leukemia (ALL)	Stat1, Stat5	Gouilleux-Gruart <i>et al.</i> , 1996; Weber-Nordt <i>et al.</i> , 1996
(d) Chronic lymphocytic leukemia (CLL)	Stat1, Stat3	Frank <i>et al.</i> , 1997
(e) Acute myelogenous leukemia (AML)	Stat1, Stat3, Stat5	Chai <i>et al.</i> , 1997; Gouilleux-Gruart <i>et al.</i> , 1996; Weber-Nordt <i>et al.</i> , 1996
(f) Chronic myelogenous leukemia (CML)	Stat5	Chai <i>et al.</i> , 1997; Shuai <i>et al.</i> , 1996; Carlesso <i>et al.</i> , 1996
(g) Megakaryotic leukemia	Stat5	Liu <i>et al.</i> , 1999
(h) Large granular lymphocyte (LGL) leukemia	Stat3	Epling-Burnette <i>et al.</i> , 2000
Lymphoma (tumors and cell lines)		
(a) EBV-related/Burkitt's	Stat3	Weber-Nordt <i>et al.</i> , 1996
(b) Mycosis fungoides	Stat3	Nielsen <i>et al.</i> , 1997
(c) HSV saimiri-dependent (T cell)	Stat3	Lund <i>et al.</i> , 1997, 1999b
(d) Cutaneous T cell lymphoma	Stat3	Sun <i>et al.</i> , 1998; Zhang <i>et al.</i> , 1996
Lung cancer		
(a) Cell lines	Stat3	Fernandes <i>et al.</i> , 1999
Other cancers (tumors and cell lines)		
(a) Renal cell carcinoma	Stat3	L Mora, R Garcia, J Seigne, T Bowman, M Huang, N Patel, G Niu, J Pow-Sang, J Diaz, C Muro-Cacho, D Coppola, T Yeatman, J Cheng, S Nicosia, S Teng, D Reintgen, H Yu and R Jove, unpublished results
(b) Prostate carcinoma	Stat3	
(c) Melanoma	Stat3	
(d) Pancreatic adenocarcinoma	Stat3	
(e) Ovarian carcinoma	Stat3	

hematopoietic cells is associated with a permanent alteration in gene expression profiles that mimics cytokine-induced signaling and leads to ligand-independent expansion and/or survival of these cells. For example, in the myeloid cell line 32D c13, transformation by the v-Src oncoprotein results in abrogation of G-CSF-induced granulocyte differentiation and engraftment of v-Src-transformed cells induces tumors in nude mice (Kruger and Anderson, 1991). Subsequent studies suggest that abrogation of IL-3 growth dependence and G-CSF-induced differentiation is dependent on constitutive activation of STATs by v-Src (Chaturvedi *et al.*, 1997, 1998). Constitutive activation of STATs 1 and 5 has been detected in acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML) cells possessing the activated BCR-Abl tyrosine kinase (Chai *et al.*, 1997; Frank and Varticovski, 1996; Ilaria and Van Etten, 1996; Skorski *et al.*, 1998). Constitutive STAT activation in these cells mimics many aspects of IL-3-induced signaling such as mitogenesis and enhanced survival (Carlesso *et al.*, 1996) and plays important roles in malignant transformation (de Groot *et al.*, 1999; Nieborowska-Skorska *et al.*, 1999). Activation of STATs has also been detected in various lymphomas (Weber-Nordt *et al.*, 1996) and in multiple myeloma cells (discussed below) and appears to have similar roles in modulation of cytokine-independent growth and malignant transformation of these cell types.

Multiple myeloma

Previous studies have established the critical role of IL-6 in proliferation and survival of human multiple myeloma cells (Hallek *et al.*, 1998; Kishimoto *et al.*, 1994). Furthermore, IL-6 has been shown to induce activation of Stat3 and to up-regulate the anti-apoptotic protein Bcl-x_L (Kishimoto *et al.*, 1994; Schwarze and Hawley, 1995). The model human myeloma cell line, U266, possesses constitutively-activated Stat3, as do the majority of bone marrow tumor specimens from multiple myeloma patients (Catlett-Falcone *et al.*, 1999a,b). Using specific inhibitors, abrogation of IL-6 receptor-mediated signaling, inactivation of JAK family kinases, or inhibition of Stat3 transcriptional activity were shown to decrease Bcl-x_L mRNA and protein levels and induce apoptosis in U266 cells (Catlett-Falcone *et al.*, 1999b). Conversely, ectopic overexpression of Bcl-x_L rescues U266 cells from apoptosis induced by blocking this signaling pathway, providing evidence that Bcl-x_L contributes to the anti-apoptotic effect of Stat3 signaling (Oshiro *et al.*, 2000). Furthermore, inhibition of Stat3 signaling in primary cultures of human myeloma cells from patient bone marrows blocks expression of Bcl-x_L and induces apoptosis, suggesting that the mechanisms delineated in model U266 cells reflect those in actual tumors (L Moscinski, B Hill, M Huang, T Landowski, M Oshiro, W Dalton and R Jove, unpublished). Thus, in human myeloma cells, Stat3 activation is essential for

transduction of signals generated from the IL-6 receptor that result in increased cell survival, consistent with earlier results in murine pre-B cells (Fukada *et al.*, 1996). These findings provide evidence that, by preventing apoptosis, Stat3 activation may be a critical event involved in the accumulation of long-lived myeloma cells during progression of disease. Furthermore, these studies indicate that multiple myeloma provides a useful and relevant model for investigating the role of cytokine-mediated Stat3 signaling in human cancer.

Head and neck squamous cell carcinoma

Constitutive activation of STATs has been detected in extracts from human head and neck squamous carcinoma cells (Grandis *et al.*, 1998). In these cells, activation of STATs is dependent on TGF- α induced activation of the EGF receptor tyrosine kinase. Significantly, studies utilizing antisense oligonucleotides have demonstrated that Stat3, but not Stat1, mediates TGF- α induced oncogenic growth of these cells (Grandis *et al.*, 1998). These findings suggest that constitutive activation of Stat3 may also be critical to development and progression of human tumors possessing activated growth factor receptor signaling pathways. Similar to multiple myeloma, these studies further indicate that head and neck squamous cell carcinoma is a useful and relevant human tumor model for investigating the roles of Stat3 in TGF- α and EGF receptor mediated oncogenesis.

Breast cancer

Aberrantly elevated expression and/or activity of EGF receptor family kinases, particularly HER1/erbB-1 and HER2/neu, and aberrant expression of EGF-family ligands have been associated with the progression of human breast cancer (Khazaie *et al.*, 1993). In addition, activation of c-Src also occurs with elevated frequency during progression of human breast cancer (Ottenhoff-Kalff *et al.*, 1992) and has been associated with constitutive activation of HER1 and HER2 proteins (Luttrell *et al.*, 1994; Muthuswamy and Muller, 1995). EGF receptor and c-Src have been previously shown to cooperate in mitogenesis and transformation of model cell lines (Maa *et al.*, 1995). Significantly, constitutive activation of Stat3 has been detected in human breast carcinoma cell lines but not in mammary epithelial cell lines established from non-malignant tissues (Garcia *et al.*, 1997; Sartor *et al.*, 1997). Constitutive Stat3 activation in human breast cancer cells correlates with elevated EGF receptor and c-Src expression or activity (Garcia and Jove, 1998). Activation of Stat3, and to some extent Stat1, has been detected in primary breast tumor specimens compared to adjacent non-malignant tissue (J Bromberg and J Darnell, unpublished data; P Chaturvedi and EP Reddy, unpublished data). Activation of STATs appears to be associated with malignant transformation and is not found in benign lesions (R Garcia and R Jove, unpublished data). Furthermore, constitutive activation of STATs is also detected in tumor-infiltrated lymph nodes, suggesting it may be a property of more invasive tumor cells. Using specific TK-selective inhibitors, inhibition of signaling downstream of Src or JAKs was shown to abrogate

constitutive Stat3 DNA-binding, inhibit cell proliferation and induce apoptosis in model human breast carcinoma cell lines (Garcia *et al.*, 2000). Moreover, expression of dominant-negative Stat3 in these cells induces growth arrest and apoptosis (R Garcia, G Niu, H Yu and R Jove, unpublished data). These findings support a critical role for STAT proteins in oncogenic signaling by Src and JAKs during progression of breast carcinoma. In addition, because EGF can 'super-activate' STATs in human breast cancer cells, it is possible that activated STATs participate in cooperative oncogenic signaling by EGF receptor and c-Src as a result of the aberrant expression of EGF-related ligands within the mammary gland microenvironment during breast cancer progression. Like myeloma and head and neck squamous cell carcinoma, breast carcinoma appears to be a valuable model for understanding the roles of aberrant STAT signaling in development and progression of human cancer.

Other solid tumors

More recently, constitutive activation of STATs has also been detected in a wide diversity of primary tumors and cell lines derived from human tumors, including various prostate, renal cell, lung, ovarian, and pancreatic cancers as well as melanomas (refer to Table 2). Activation of STATs in lung cancer cells has been shown to be dependent on signaling by the HER2/neu receptor tyrosine kinase (Fernandes *et al.*, 1999). The signaling pathways responsible for STAT activation in the other tumor types have not been well characterized and are currently under investigation. It is likely that at least some of the mechanisms responsible for aberrant STAT activation in the various model transformed cell lines and human tumors described above will be functional in other solid tumor types as well. Thus, present evidence suggests that activated STAT signaling may participate in the development and progression of many different types of human blood malignancies and solid tumors.

STATs as markers of oncogenic transformation

Despite the diversity in oncogenic signals and the variety of tumor cell types involved, tumor cells harboring activated TKs acquire similar patterns of STAT activation, particularly involving Stat3 and Stat5. These findings suggest that the development of assays for detection of activated STATs may be useful in the diagnosis of human cancer. Specifically, activated STATs may provide a sensitive marker for detecting activation of oncogenic signaling pathways and aid in the selection of treatment modalities that may result in more efficient eradication of disease. Assays designed to detect STATs in their 'activated' states may include DNA-binding assays based on microplate assays that measure the relative levels of STAT-DNA complexes by colorimetric detection. Furthermore, antibodies designed to detect the activated phosphotyrosine forms of specific STAT family members can be employed to compare normal and tumor tissues by immunohistochemistry for nuclear localization and levels of activated STATs. Another useful application of STAT assays could be to provide a convenient molecular endpoint for monitoring the

effectiveness of tyrosine kinase inhibitors that are currently being tested or developed for clinical trials. In addition, the critical role of STATs in oncogenic signal transduction may provide the basis for development of novel cancer therapeutics, as discussed below.

Strategies for targeting STATs for cancer therapy

The implication of the studies discussed above is that aberrant STAT signaling contributes to a permanent alteration in the genetic program of cells that ultimately results in malignant progression. Because STAT proteins are involved in regulating fundamental biological processes, including apoptosis and cell proliferation, disruption of STAT signal transduction is a novel approach to blocking malignant progression in a wide variety of human tumors whose growth depends on activation of STATs. There are multiple strategies that can be taken to design inhibitors that disrupt STAT signaling. One approach involves identification of upstream STAT activators and design of pharmacologic inhibitors that specifically disrupt their function. Examples of such upstream targets are cytokines, growth factors, TKs and serine kinases to which antagonists and inhibitors can be synthesized (refer to Figure 2). Alternatively, a more direct approach is the rational design of small molecules that directly target STAT proteins and disrupt their function.

Indirect approaches to blocking STAT activation

Autocrine and paracrine activation of cytokine receptors participates in the progression of numerous hematologic and non-hematologic malignancies. One consequence of constitutive cytokine receptor activation is deregulation of STAT signaling. For example, malignant progression of multiple myeloma depends on the IL-6 signaling pathway for growth and survival (Keller and Ershler, 1995; Suematsu *et al.*, 1992). Molecular modeling of IL-6 interaction with the gp130 subunit of the IL-6 receptor has generated novel 'superantagonists' that have been shown to be effective in blocking IL-6 receptor activation (Savino *et al.*, 1994). For instance, using a human myeloma model, an IL-6 superantagonist results in significant reduction in Stat3 DNA-binding and inhibits proliferation (Dalton and Jove, 1999; Catlett-Falcone *et al.*, 1999a,b).

Table 1 lists the variety of oncoproteins that have been discovered thus far to constitutively activate STAT family members in transformed cell lines. Overexpression and/or elevated kinase activity of Src, BCR-Abl, JAKs, IGF-I receptor, EGF receptor and other cellular TKs are associated with numerous human malignancies shown in Table 2. Thus, identification of the TKs and serine kinases that activate specific STAT family members provides the basis for development of another class of inhibitors of STAT signaling. Tyrphostins, compounds that selectively inhibit TK activities, have been shown to be therapeutically successful in several recent studies (Levitzki, 1999). For example, in a model of human acute lymphocytic leukemia (ALL), the tyrphostin AG490 inhibited proliferation of ALL cells in mice without adverse effects on normal hematopoiesis

(Meydan *et al.*, 1996). Moreover, AG490 blocks activation of Stat3 DNA-binding and inhibits proliferation in model human and mouse myeloma cells by inducing apoptosis (Burdelya *et al.*, 2000; Catlett-Falcone *et al.*, 1999b). Other examples include TK inhibitors that selectively target the EGF receptor and Src family kinases and thus may block tumor cell growth through similar mechanisms involving inhibition of Stat3 signaling (Fry *et al.*, 1994; Kraker *et al.*, 2000). Another TK inhibitor, which is selective for the BCR-Abl kinase and is predicted to inhibit Stat5 signaling, shows promise for treatment of CML patients (Druker *et al.*, 1996).

In addition, elevated serine kinase activity is associated with oncogenesis (Frank, 1999), and increased levels of serine phosphorylation of Stat1 and Stat3 have been detected in chronic lymphocytic leukemia (CLL) (Frank *et al.*, 1997). Moreover, blocking serine phosphorylation of Stat3 in fibroblast cells transformed by the Src oncoprotein inhibits their growth in soft agar (Turkson *et al.*, 1999), suggesting that serine kinases may possess potential as promising chemotherapeutic targets. Additional therapeutic strategies might be based on modulation of biological suppressors of STAT signaling, including the SH2 domain-containing tyrosine phosphatase family (SHP-1 and SHP-2) (Kharitonov *et al.*, 1997; Stofega *et al.*, 1998; Yu *et al.*, 2000), the SOCS/JAB/SSI family of JAK regulators (Endo *et al.*, 1997; Naka *et al.*, 1997; Starr *et al.*, 1997), the PIAS family of STAT regulators (Liu *et al.*, 1998), and degradation of STATs by proteasomes (Yu and Burakoff, 1997) (reviewed in Naka *et al.*, 1999; Starr and Hilton, 1999).

Methods to directly block STAT function

Detailed structure-function relationships of STAT proteins have been elucidated based on genetic, biochemical and crystallographic analyses, facilitating the design of compounds that directly disrupt STAT function. For example, one potential structural element against which to design selective inhibitors of STAT function is the phosphotyrosine-SH2 interaction domain (refer to Figure 1). Peptidomimetics that disrupt the association of STAT SH2 domains with phosphotyrosines may inhibit STAT recruitment to receptors and STAT dimerization, thereby blocking nuclear translocation and STAT-dependent gene regulation. Molecular modeling studies based on the crystal structures of activated STAT proteins bound to DNA could provide promising leads for design of novel STAT inhibitors (Becker *et al.*, 1998; Chen *et al.*, 1998).

Another potential functional domain by which to modulate the effects of STAT activation is the DNA-binding domain. Small molecule inhibitors that directly interfere with the STAT DNA-binding domain or oligodeoxynucleotide (ODN) strategies utilizing decoy STAT consensus binding sites may prevent activated STATs from inducing transcription of target genes. One therapeutic approach involves the use of STAT anti-sense ODNs that specifically block expression of STAT mRNA transcripts. In a recent study, introduction of anti-sense Stat3 ODNs into human head and neck squamous carcinoma cell lines inhibits proliferation *in vitro* and correlates with loss of Stat3 DNA-

binding (Grandis *et al.*, 1998), suggesting that *in vivo* delivery of anti-sense STAT ODNs is a potentially promising anti-cancer therapy.

Compounds that interfere with STAT functions such as dimerization, DNA-binding or transcriptional activation can be screened utilizing high-throughput assays based on inhibition of these STAT biochemical properties. In addition, the efficacy and cytotoxicity of these compounds can be evaluated utilizing cultured human tumor cells and *in vivo* animal models. Initial studies utilizing normal mouse fibroblasts demonstrate that disrupting Stat3 signaling is not deleterious to normal cell growth (Niu *et al.*, 1999; Turkson *et al.*, 1998). Thus, blocking Stat3 signaling may not grossly impair normal cellular functions. One possible explanation for the increased sensitivity of transformed cells compared to normal cells is that tumor cells may have become irreversibly dependent on STAT signaling to sustain their growth and survival, while normal cells may be able to use alternative pathways to compensate for loss of STAT signaling.

Although gene therapy approaches to cancer treatment are still in relatively early stages of development, gene therapy offers a powerful experimental tool to establish 'proof-of-principal' that a particular molecular pathway is a valid target for cancer treatment. Using a mouse melanoma cell line harboring constitutively-activated Stat3, a recent study employed a gene therapy technique with a dominant-negative form of Stat3 to block endogenous Stat3 signaling (Niu *et al.*, 1999). Syngeneic mice with tumors induced by the melanoma cell line were electroinjected intratumorally with a vector expressing the dominant-negative Stat3 or empty vector. Tumor regression due to massive apoptosis *in vivo* occurred in mice that received dominant-negative Stat3, in contrast to those that received empty vector (Niu *et al.*, 1999). Interestingly, the number of apoptotic cells appeared to greatly exceed the number of transfected cells, suggesting that a 'bystander' effect may enhance the therapeutic potential of this approach. These findings are consistent with earlier observations that blocking Stat3 signaling induces apoptosis in human myeloma cells *in vitro* (Catlett-Falcone *et al.*, 1999b). Taken together, these studies provide further evidence that Stat3 is a valid molecular target for cancer therapy by various methods including genetic, oligonucleotide and small molecule disruption of STAT signaling.

Conclusions and future directions

Numerous studies provide evidence that a causal relationship exists between aberrant STAT activation and oncogenesis, suggesting that therapeutic modalities designed to disrupt STAT signaling hold considerable

promise for prevention and treatment of human cancers. Importantly, many types of tumors, particularly aggressive cancers, are initially refractory to chemotherapy or eventually become resistant to the therapies. Thus, there is an urgent need for identification of new molecular targets that may provide more effective therapeutic responses. One of the mechanisms of tumor cell killing by anticancer agents involves apoptosis. As discussed above, constitutive activation of Stat3 and Stat5 signaling in many cases confers resistance to apoptosis in tumor cells. Thus, tumor cells possessing activated Stat3 or Stat5 are predicted to be resistant to chemotherapeutic modalities that may utilize similar apoptosis pathways. Consequently, in addition to the direct killing of tumor cells, another potential advantage of disrupting STAT signaling in tumors is that inactivation of STATs may sensitize drug-resistant tumor cells to chemotherapeutic agents. Such an approach may potentially reduce side effects associated with conventional, aggressive chemotherapeutic regimens and improve the overall response. Development of selective inhibitors of STAT activation for use in combination therapy with more conventional chemotherapy may be a promising area in the field of novel anticancer therapeutics. Additional clinically important benefits from the discovery of the contribution of STAT activation to oncogenesis include development of new diagnostic and prognostic assays based on the molecular STAT profile of tumors.

In summary, the preponderance of evidence is consistent with the conclusion that inappropriate activation of STAT signaling contributes to oncogenesis by subverting some of the very same biological processes controlled by STATs in normal cells, namely cell proliferation and survival. This conclusion is further supported by the findings that mutationally-activated forms of Stat3 and Stat5 are sufficient to induce some of the characteristics of transformed cells, and that constitutive activation of Stat3 or Stat5 in the context of oncogenesis regulates the expression of genes known to be involved in cell cycle control and apoptosis. Ongoing studies will likely lead to identification of additional STAT-regulated genes that will provide more insights into the roles of STATs in oncogenesis as well as novel molecular approaches to interrupt STAT signaling pathways for potential cancer therapies.

Acknowledgments

We thank members of the laboratory and our collaborators for their important contributions to some of the work described here, and Moffitt Cancer Center, the Angela Musette Russo Foundation, and the National Cancer Institute for their generous support.

References

- Akira S. (1999). *Stem. Cells*, **17**, 138–146.
- Alvarez E, Northwood IC, Gonzalez FA, Latour DA, Seth A, Abate C, Curran T and Davis RJ. (1991). *J. Biol. Chem.*, **266**, 15277–15285.
- Barboule N, Baldin V, Jozan S, Vidal S and Valette A. (1998). *Int. J. Cancer*, **76**, 891–896.
- Barone MV and Courtneidge SA. (1995). *Nature*, **378**, 509–512.
- Beadling C, Ng J, Babbage JW and Cantrell DA. (1996). *EMBO J.*, **15**, 1902–1913.
- Becker S, Groner B and Muller CW. (1998). *Nature*, **394**, 145–151.

- Besser D, Bromberg JF, Darnell Jr JE and Hanafusa H. (1999). *Mol. Cell. Biol.*, **19**, 1401–1409.
- Boulton TG, Zhong Z, Wen Z, Darnell Jr JE, Stahl N and Yancopoulos GD. (1995). *Proc. Natl. Acad. Sci. USA*, **92**, 6915–6919.
- Breuer ML, Cuypers HT and Berns A. (1989b). *EMBO J.*, **8**, 743–748.
- Breuer ML, Slebos R, Verbeek S, van Lohuizen M, Wientjens E and Berns A. (1989a). *Nature*, **340**, 61–63.
- Bromberg JF, Fan Z, Brown C, Mendelsohn J and Darnell Jr JE. (1998a). *Cell Growth Differ.*, **9**, 505–512.
- Bromberg JF, Horvath CM, Besser D, Lathem WW and Darnell Jr JE. (1998b). *Mol. Cell. Biol.*, **18**, 2553–2558.
- Bromberg JF, Horvath CM, Wen Z, Schreiber RD and Darnell Jr JE. (1996). *Proc. Natl. Acad. Sci. USA*, **93**, 7673–7678.
- Bromberg JF, Wrzeszczynska MH, Devgan G, Zhao Y, Pestell RG, Albanese C and Darnell Jr JE. (1999). *Cell*, **98**, 295–303.
- Buard A, Vivo C, Monnet I, Boutin C, Pilatte Y and Jaurand MC. (1998). *Cancer Res.*, **58**, 840–847.
- Burdelya L, Catlett-Falcone R, Levitzki A, Coppola D, Sun JZ, Sebti S, Dalton WS, Jove R and Yu H. (2000). Manuscript submitted.
- Caldenhoven E, van Dijk TB, Solari R, Armstrong J, Raaijmakers JAM, Lammers JWJ, Koenderman L and de Groot RP. (1996). *J. Biol. Chem.*, **271**, 13221–13227.
- Campbell GS, Yu CL, Jove R and Carter-Su C. (1997). *J. Biol. Chem.*, **272**, 2591–2594.
- Cao X, Tay A, Guy GR and Tan YH. (1996). *Mol. Cell. Biol.*, **16**, 1595–1603.
- Carlesso N, Frank DA and Griffin JD. (1996). *J. Exp. Med.*, **183**, 811–820.
- Catlett-Falcone R, Dalton WS and Jove R. (1999a). *Current Opinion Oncology*, **11**, 490–496.
- Catlett-Falcone R, Landowski TH, Oshiro MM, Turkson J, Levitzki A, Savino R, Ciliberto G, Moscinski L, Fernandez-Luna JL, Nunez G, Dalton WS and Jove R. (1999b). *Immunity*, **10**, 105–115.
- Ceresa BP, Horvath CM and Pessin JE. (1997). *Endocrinology*, **138**, 4131–4137.
- Chai SK, Nichols GL and Rothman P. (1997). *J. Immunol.*, **159**, 4720–4728.
- Chaturvedi P, Reddy MV and Reddy EP. (1998). *Oncogene*, **16**, 1749–1758.
- Chaturvedi P, Sharma S and Reddy EP. (1997). *Mol. Cell. Biol.*, **17**, 3295–3304.
- Chen X, Vinkemeier U, Zhao Y, Jeruzalmi D, Darnell Jr JE and Kuriyan J. (1998). *Cell*, **93**, 827–839.
- Chin YE, Kitagawa M, Su WC, You ZH, Iwamoto Y and Fu XY. (1996). *Science*, **272**, 719–722.
- Chung J, Uchida E, Grammer TC and Blenis J. (1997). *Mol. Cell. Biol.*, **17**, 6508–6516.
- Cuypers HT, Selten G, Quint W, Zijlstra M, Maandag ER, Boelens W, van Wezenbeek P, Melief C and Berns A. (1984). *Cell*, **37**, 141–150.
- Dalton WS and Jove R. (1999). *Seminars in Oncology*, **26**, 23–27.
- Dang CV, Resar LM, Emison E, Kim S, Li Q, Prescott JE, Wonsey D and Zeller K. (1999). *Exp. Cell Res.*, **253**, 63–77.
- Danial NN, Losman JA, Lu T, Yip N, Krishnan K, Krolewski J, Goff SP, Wang JY and Rothman PB. (1998). *Mol. Cell. Biol.*, **18**, 6795–6804.
- Danial NN, Pernis A and Rothman PB. (1995). *Science*, **269**, 1875–1877.
- Darnell Jr JE. (1997). *Science*, **277**, 1630–1635.
- Darnell Jr JE, Kerr IM and Stark GR. (1994). *Science*, **264**, 1415–1421.
- David M, Petricoin E III, Benjamin C, Pine R, Weber MJ and Larner AC. (1995). *Science*, **269**, 1721–1723.
- de Groot RP, Raaijmakers JA, Lammers JW, Jove R and Koenderman L. (1999). *Blood*, **94**, 1108–1112.
- de Groot RP, Raaijmakers JA, Lammers JW and Koenderman L. (2000). Manuscript submitted.
- Decker T and Kovarik P. (1999). *Cell. Mol. Life Sci.*, **55**, 1535–1546.
- Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Zimmermann J and Lydon NB. (1996). *Nature Medicine*, **2**, 561–566.
- Dumon S, Santos SC, Debierre-Grockiego F, Gouilleux-Gruart V, Cocault L, Boucheron C, Mollat P, Gisselbrecht S and Gouilleux F. (1999). *Oncogene*, **18**, 4191–4199.
- Durbin JE, Hackenmiller R, Simon MC and Levy DE. (1996). *Cell*, **84**, 443–450.
- Endo TA, Masuhara M, Yokouchi M, Suzuki R, Sakamoto H, Mitsui K, Matsumoto A, Tanimura S, Ohtsubo M, Misawa H, Miyazaki T, Leonor N, Taniguchi T, Fujita T, Kanakura Y, Komiya S and Yoshimura A. (1997). *Nature*, **387**, 921–924.
- Epling-Burnette PK, Lui JH, Catlett-Falcone R, Turkson J, Oshiro M, Kothapalli R, Li Y, Wang JM, Yang-Yen HF, Jove R and Loughran TP. (2000). Manuscript submitted.
- Fernandes A, Hamburger AW and Gerwin BI. (1999). *Int. J. Cancer*, **83**, 564–570.
- Frank DA, Mahajan S and Ritz J. (1997). *J. Clin. Invest.*, **100**, 3140–3148.
- Frank DA. (1999). *Mol. Med.*, **5**, 432–456.
- Frank DA and Varticovski L. (1996). *Leukemia*, **10**, 1724–1730.
- Fry DW, Kraker AJ, McMichael A, Ambroso LA, Nelson JM, Leopold WR and Bridges AJ. (1994). *Science*, **265**, 1093–1095.
- Fukada T, Hibi M, Yamanaka Y, Takahashi-Tezuka M, Fujitani Y, Yamaguchi T, Nakajima K and Hirano T. (1996). *Immunity*, **5**, 449–460.
- Garcia R and Jove R. (1998). *J. Biomed. Sci.*, **5**, 79–85.
- Garcia R, Sun Y, Niu G, Bowman TL, Zhang Y, Minton S, Muro-Cacho C, Ku NN, Falcone R, Cox C, Kraker A, Levitzki A, Parsons S, Yu H, Sebti S and Jove R. (2000). Manuscript submitted.
- Garcia R, Yu CL, Hudnall A, Catlett R, Nelson KL, Smithgall T, Fujita DJ, Ethier SP and Jove R. (1997). *Cell Growth Differ.*, **8**, 1267–1276.
- Gollob JA, Schnipper CP, Murphy EA, Ritz J and Frank DA. (1999). *J. Immunol.*, **162**, 4472–4481.
- Gouilleux-Gruart V, Gouilleux F, Desaint C, Claisse JF, Capiod JC, Delobel J, Weber-Nordt R, Dusanter-Fourt I, Dreyfus F, Groner B and Prin L. (1996). *Blood*, **87**, 1692–1697.
- Grandis JR, Drenning SD, Chakraborty A, Zhou MY, Zeng Q, Pitt AS and Tweardy DJ. (1998). *J. Clin. Invest.*, **102**, 1385–1392.
- Grimley PM, Fang H, Rui H, Petricoin EF III, Ray S, Dong F, Fields KH, Hu R, Zoon KC, Audet S and Beeler J. (1998). *Blood*, **91**, 3017–3027.
- Hallek M, Leif Bergsagel P and Anderson KC. (1998). *Blood*, **91**, 3–21.
- Harrison DA, Binari R, Nahreini TS, Gilman M and Perrimon N. (1995). *EMBO J.*, **14**, 2857–2865.
- Hirano T, Nakajima K and Hibi M. (1997). *Cytokine Growth Factor Rev.*, **8**, 241–252.
- Ho JM, Beattie BK, Squire JA, Frank DA and Barber DL. (1999). *Blood*, **93**, 4354–4364.
- Horvath CM, Wen Z and Darnell Jr JE. (1995). *Genes Dev.*, **9**, 984–994.
- Hou XS, Melnick MB and Perrimon N. (1996). *Cell*, **84**, 411–419.
- Ihle JN. (1996). *Cell*, **84**, 331–334.
- Ihle JN, Witthuhn BA, Quelle FW, Yamamoto K and Silvennoinen O. (1995). *Annu. Rev. Immunol.*, **13**, 369–398.

- Ilaria Jr RL and Van Etten RA. (1996). *J. Biol. Chem.*, **271**, 31704–31710.
- Jain N, Zhang T, Fong SL, Lim CP and Cao X. (1998). *Oncogene*, **17**, 3157–3167.
- Jain N, Zhang T, Kee WH, Li W and Cao X. (1999). *J. Biol. Chem.*, **274**, 24392–24400.
- Kaplan DH, Shankaran V, Dighe AS, Stockert E, Aguet M, Old LJ and Schreiber RD. (1998). *Proc. Natl. Acad. Sci. USA*, **95**, 7556–7561.
- Kaplan MH, Sun YL, Hoey T and Grusby MJ. (1996). *Nature*, **382**, 174–177.
- Karini R, Jove R and Levitzki A. (1999). *Oncogene*, **18**, 4657–4662.
- Keller ET and Ershler WB. (1995). *J. Immunol.*, **154**, 4091–4098.
- Kharitonov A, Chen Z, Sures I, Wang H, Schilling J and Ullrich A. (1997). *Nature*, **386**, 181–186.
- Khazaie K, Schirmacher V and Lichtner RB. (1993). *Cancer Metastasis Rev.*, **12**, 255–274.
- Kimura T, Kadokawa Y, Harada H, Matsumoto M, Sato M, Kashiwazaki Y, Tarutani M, Tan RS, Takasugi T, Matsuyama T, Mak TW, Noguchi S and Taniguchi T. (1996). *Genes Cells*, **1**, 115–124.
- Kishimoto T, Taga T and Akira S. (1994). *Cell*, **76**, 253–262.
- Kiuchi N, Nakajima K, Ichiba M, Fukada T, Narimatsu M, Mizuno K, Hibi M and Hirano T. (1999). *J. Exp. Med.*, **189**, 63–73.
- Kraker AJ, Hartl BG, Amar AM, Barvian, MR, Hollis HD, Showalter HDH and Moore CW. (2000). Manuscript submitted.
- Kruger A and Anderson SM. (1991). *Oncogene*, **6**, 245–256.
- Kuroki M and O'Flaherty JT. (1999). *Biochem. J.*, **341**, 691–696.
- Kuwahara M, Hirai T, Yoshida K, Yamashita Y, Hihara J, Inoue H and Toge T. (1999). *Dis. Esophagus*, **12**, 116–119.
- LaBaer J, Garrett MD, Stevenson LF, Slingerland JM, Sandhu C, Chou HS, Fattaey A and Harlow E. (1997). *Genes Dev.*, **11**, 847–862.
- Lacronique V, Boureux A, Valle VD, Poirel H, Quang CT, Mauchauffe M, Berthou C, Lessard M, Berger R, Ghysdael J and Bernard OA. (1997). *Science*, **278**, 1309–1312.
- Leaman DW, Leung S, Li X and Stark GR. (1996). *FASEB J.*, **10**, 1578–1588.
- Levitzki A. (1999). *Pharmacol. Ther.*, **82**, 231–239.
- Lim CP and Cao X. (1999). *J. Biol. Chem.*, **274**, 31055–31061.
- Liu B, Liao J, Rao X, Kushner SA, Chung CD, Chang DD and Shuai K. (1998). *Proc. Natl. Acad. Sci. USA*, **95**, 10626–10631.
- Liu RY, Fan C, Garcia R, Jove R and Zuckerman KS. (1999). *Blood*, **93**, 2369–2379.
- Liu X, Robinson GW, Wagner KU, Garrett L, Wynshaw-Boris A and Hennighausen L. (1997). *Genes Dev.*, **11**, 179–186.
- Lund TC, Coleman C, Horvath E, Sefton BM, Jove R, Medveczky MM and Medveczky PG. (1999a). *Cell Signal.*, **11**, 789–796.
- Lund TC, Garcia R, Medveczky MM, Jove R and Medveczky PG. (1997). *J. Virol.*, **71**, 6677–6682.
- Lund TC, Prator PC, Medveczky MM and Medveczky PG. (1999b). *J. Virol.*, **73**, 1689–1694.
- Luo H, Hanratty WP and Dearolf CR. (1995). *EMBO J.*, **14**, 1412–1420.
- Luttrell DK, Lee A, Lansing TJ, Crosby RM, Jung KD, Willard D, Luther M, Rodriguez M, Berman J and Gilmer TM. (1994). *Proc. Natl. Acad. Sci. USA*, **91**, 83–87.
- Maa MC, Leu TH, McCarley DJ, Schatzman RC and Parsons SJ. (1995). *Proc. Natl. Acad. Sci. USA*, **92**, 6981–6985.
- Matsumura I, Kitamura T, Wakao H, Tanaka H, Hashimoto K, Albanese C, Downward J, Pestell RG and Kanakura Y. (1999). *EMBO J.*, **18**, 1367–1377.
- Meraz MA, White JM, Sheehan KC, Bach EA, Rodig SJ, Dighe AS, Kaplan DH, Riley JK, Greenlund AC, Campbell D, Carver-Moore K, DuBois RN, Clark R, Aguet M and Schreiber RD. (1996). *Cell*, **84**, 431–442.
- Meydan N, Grunberger T, Dadi H, Shahar M, Arpaia E, Lapidot Z, Leeder JS, Freedman M, Cohen A, Gazit A, Levitzki A and Roifman CM. (1996). *Nature*, **379**, 645–648.
- Migone TS, Lin JX, Cereseto A, Mulloy JC, JJ OS, Franchini G and Leonard WJ. (1995). *Science*, **269**, 79–81.
- Murakami Y, Nakano S, Niho Y, Hamasaki N and Izuhara K. (1998). *J. Cell. Physiol.*, **175**, 220–228.
- Muthuswamy SK and Muller WJ. (1995). *Oncogene*, **11**, 271–279.
- Naka T, Fujimoto M and Kishimoto T. (1999). *Trends Biochem. Sci.*, **24**, 394–398.
- Naka T, Narazaki M, Hirata M, Matsumoto T, Minamoto S, Aono A, Nishimoto N, Kajita T, Taga T, Yoshizaki K, Akira S and Kishimoto T. (1997). *Nature*, **387**, 924–929.
- Nelson KL, Rogers JA, Bowman TL, Jove R and Smithgall TE. (1998). *J. Biol. Chem.*, **273**, 7072–7077.
- Ng J and Cantrell D. (1997). *J. Biol. Chem.*, **272**, 24542–24549.
- Nieborowska-Skorska M, Wasik MA, Slupianek A, Salomoni P, Kitamura T, Calabretta B and Skorski T. (1999). *J. Exp. Med.*, **189**, 1229–1242.
- Nielsen M, Kalltoft K, Nordahl M, Ropke C, Geisler C, Mustelin T, Dobson P, Svejgaard A and Odum N. (1997). *Proc. Natl. Acad. Sci. USA*, **94**, 6764–6769.
- Niu G, Heller R, Catlett-Falcone R, Coppola D, Jaroszeski M, Dalton W, Jove R and Yu H. (1999). *Cancer Res.*, **59**, 5059–5063.
- Nosaka T, Kawashima T, Misawa K, Ikuta K, Mui AL and Kitamura T. (1999). *EMBO J.*, **18**, 4754–4765.
- O'Farrell AM, Liu Y, Moore KW and Mui AL. (1998). *EMBO J.*, **17**, 1006–1018.
- Okuda K, Golub TR, Gilliland DG and Griffin JD. (1996). *Oncogene*, **13**, 1147–1152.
- Olayioye MA, Beuvink I, Horsch K, Daly JM and Hynes NE. (1999). *J. Biol. Chem.*, **274**, 17209–17218.
- Onishi M, Nosaka T, Misawa K, Mui AL, Gorman D, McMahon M, Miyajima A and Kitamura T. (1998). *Mol. Cell. Biol.*, **18**, 3871–3879.
- Oshiro MM, Landowski TH, Catlett-Falcone R, Jove R and Dalton WS. (2000). Manuscript submitted.
- Ottenhoff-Kalff AE, Rijksen G, van Beurden EA, Hennipman A, Michels AA and Staal GE. (1992). *Cancer Res.*, **52**, 4773–4778.
- Pindzola JA, Palazzo JP, Kovatich AJ, Tuma B and Nobel M. (1998). *Pathol. Res. Pract.*, **194**, 685–691.
- Puthier D, Bataille R and Amiot M. (1999). *Eur. J. Immunol.*, **29**, 3945–3950.
- Reed JC. (1999). *Curr. Opin. Oncol.*, **11**, 68–75.
- Sano S, Itami S, Takeda K, Tarutani M, Yamaguchi Y, Miura H, Yoshikawa K, Akira S and Takeda J. (1999). *EMBO J.*, **18**, 4657–4668.
- Sartor CI, Dziubinski ML, Yu CL, Jove R and Ethier SP. (1997). *Cancer Res.*, **57**, 978–987.
- Savino R, Ciapponi L, Lahm A, Demartis A, Cabibbo A, Toniatti C, Delmastro P, Altamura S and Ciliberto G. (1994). *EMBO J.*, **13**, 5863–5870.
- Schaeffer HJ and Weber MJ. (1999). *Mol. Cell. Biol.*, **19**, 2435–2444.
- Schindler C and Darnell Jr JE. (1995). *Annu. Rev. Biochem.*, **64**, 621–651.

- Schwaller J, Frantsve J, Aster J, Williams IR, Tomasson MH, Ross TS, Peeters P, Van Rompaey L, Van Etten RA, Ilaria Jr R, Marynen P and Gilliland DG. (1998). *EMBO J.*, **17**, 5321–5333.
- Schwarze MM and Hawley RG. (1995). *Cancer Res.*, **55**, 2262–2265.
- Seidel HM, Milocco LH, Lamb P, Darnell Jr JE, Stein RB and Rosen J. (1995). *Proc. Natl. Acad. Sci. USA*, **92**, 3041–3045.
- Sengupta TK, Talbot ES, Scherle PA and Ivashkiv LB. (1998). *Proc. Natl. Acad. Sci. USA*, **95**, 11107–11112.
- Sherr CJ and Roberts JM. (1999). *Genes Dev.*, **13**, 1501–1512.
- Shimoda K, van Deursen J, Sangster MY, Sarawar SR, Carson RT, Tripp RA, Chu C, Quelle FW, Nosaka T, Vignali DA, Doherty PC, Grosveld G, Paul WE and Ihle JN. (1996). *Nature*, **380**, 630–633.
- Shirogane T, Fukada T, Muller JM, Shima DT, Hibi M and Hirano T. (1999). *Immunity*, **11**, 709–719.
- Shuai K, Halpern J, ten Hoeve J, Rao X and Sawyers CL. (1996). *Oncogene*, **13**, 247–254.
- Shuai K, Horvath CM, Huang LH, Qureshi SA, Cowburn D and Darnell Jr JE. (1994). *Cell*, **76**, 821–828.
- Silva M, Benito A, Sanz C, Prosper F, Ekhterae D, Nunez G and Fernandez-Luna JL. (1999). *J. Biol. Chem.*, **274**, 22165–22169.
- Sinibaldi D, Wharton W, Turkson J, Bowman T, Peters G, Pledger WJ and Jove R. (2000). Manuscript submitted.
- Skorski T, Nieborowska-Skorska M, Wlodarski P, Wasik M, Trotta R, Kanakaraj P, Salomoni P, Antonyak M, Martinez R, Majewski M, Wong A, Perussia B and Calabretta B. (1998). *Blood*, **91**, 406–418.
- Smith PD and Crompton MR. (1998). *Biochem. J.*, **331**, 381–385.
- Socolovsky M, Fallon AE, Wang S, Brugnara C and Lodish HF. (1999). *Cell*, **98**, 181–191.
- Sparrow LE, Eldon MJ, English DR and Heenan PJ. (1998). *Am. J. Dermatopathol.*, **20**, 255–261.
- Stacey DW, Roudebush M, Day R, Mosser SD, Gibbs JB and Feig LA. (1991). *Oncogene*, **6**, 2297–2304.
- Stark GR, Kerr IM, Williams BR, Silverman RH and Schreiber RD. (1998). *Annu. Rev. Biochem.*, **67**, 227–264.
- Starr R and Hilton DJ. (1999). *Bioessays*, **21**, 47–52.
- Starr R, Willson TA, Viney EM, Murray LJ, Rayner JR, Jenkins BJ, Gonda TJ, Alexander WS, Metcalf D, Nicola NA and Hilton DJ. (1997). *Nature*, **387**, 917–921.
- Stofega MR, Wang H, Ullrich A and Carter-Su C. (1998). *J. Biol. Chem.*, **273**, 7112–7117.
- Suematsu S, Matsusaka T, Matsuda T, Ohno S, Miyazaki J, Yamamura K, Hirano T and Kishimoto T. (1992). *Proc. Natl. Acad. Sci. USA*, **89**, 232–235.
- Sun WH, Pabon C, Alsayed Y, Huang PP, Jandeska S, Uddin S, Platanius LC and Rosen ST. (1998). *Blood*, **91**, 570–576.
- Taga T and Kishimoto T. (1997). *Annu. Rev. Immunol.*, **15**, 797–819.
- Takeda K, Kaisho T, Yoshida N, Takeda J, Kishimoto T and Akira S. (1998). *J. Immunol.*, **161**, 4652–4660.
- Takeda K, Noguchi K, Shi W, Tanaka T, Matsumoto M, Yoshida N, Kishimoto T and Akira S. (1997). *Proc. Natl. Acad. Sci. USA*, **94**, 3801–3804.
- Takeda K, Tanaka T, Shi W, Matsumoto M, Minami M, Kashiwamura S, Nakanishi K, Yoshida N, Kishimoto T and Akira S. (1996). *Nature*, **380**, 627–630.
- Takemoto S, Mulloy JC, Cereseto A, Migone TS, Patel BK, Matsuoka M, Yamaguchi K, Takatsuki K, Kamihira S, White JD, Leonard WJ, Waldmann T and Franchini G. (1997). *Proc. Natl. Acad. Sci. USA*, **94**, 13897–13902.
- Teglund S, McKay C, Schuetz E, van Deursen JM, Stravopodis D, Wang D, Brown M, Bodner S, Grosveld G and Ihle JN. (1998). *Cell*, **93**, 841–850.
- Thierfelder WE, van Deursen JM, Yamamoto K, Tripp RA, Sarawar SR, Carson RT, Sangster MY, Vignali DA, Doherty PC, Grosveld GC and Ihle JN. (1996). *Nature*, **382**, 171–174.
- Turkson J, Bowman T, Adnane J, Zhang Y, Djeu JY, Sekharam M, Frank DA, Holzman LB, Wu J, Sebt S and Jove R. (1999). *Mol. Cell. Biol.*, **19**, 7519–7528.
- Turkson J, Bowman T, Garcia R, Caldenhoven E, De Groot RP and Jove R. (1998). *Mol. Cell. Biol.*, **18**, 2545–2552.
- Udy GB, Towers RP, Snell RG, Wilkins RJ, Park SH, Ram PA, Waxman DJ and Davey HW. (1997). *Proc. Natl. Acad. Sci. USA*, **94**, 7239–7244.
- van Lohuizen M, Verbeek S, Krimpenfort P, Domen J, Saris C, Radaszkiewicz T and Berns A. (1989). *Cell*, **56**, 673–682.
- van Lohuizen M, Verbeek S, Scheijen B, Wientjens E, van der Gulden H and Berns A. (1991). *Cell*, **65**, 737–752.
- van Puijenbroek AA, van der Saag PT and Coffey PJ. (1999). *Exp. Cell Res.*, **251**, 465–476.
- Wang YH, Wharton W, Garcia R, Kraker A, Jove R and Pledger WJ. (2000). *Oncogene*, in press.
- Watson CJ and Miller WR. (1995). *Br. J. Cancer*, **71**, 840–844.
- Weber-Nordt RM, Egen C, Wehinger J, Ludwig W, Gouilleux-Gruart V, Mertelsmann R and Finke J. (1996). *Blood*, **88**, 809–816.
- Wen X, Lin HH, Shih HM, Kung HJ and Ann DK. (1999). *J. Biol. Chem.*, **274**, 38204–38210.
- Wen Z and Darnell Jr JE. (1997). *Nucleic Acids Res.*, **25**, 2062–2067.
- Wen Z, Zhong Z and Darnell Jr JE. (1995). *Cell*, **82**, 241–250.
- Xu X, Fu XY, Plate J and Chong AS. (1998). *Cancer Res.*, **58**, 2832–2837.
- Xu X, Sun YL and Hoey T. (1996). *Science*, **273**, 794–797.
- Yan R, Small S, Desplan C, Dearolf CR and Darnell Jr JE. (1996). *Cell*, **84**, 421–430.
- Yu CL and Burakoff SJ. (1997). *J. Biol. Chem.*, **272**, 14017–14020.
- Yu CL, Jin YJ and Burakoff SJ. (2000). *J. Biol. Chem.*, **275**, 599–604.
- Yu CL, Jove R and Burakoff SJ. (1997). *J. Immunol.*, **159**, 5206–5210.
- Yu CL, Meyer DJ, Campbell GS, Larner AC, Carter-Su C, Schwartz J and Jove R. (1995). *Science*, **269**, 81–83.
- Zhang Q, Nowak I, Vonderheid EC, Rook AH, Kadin ME, Nowell PC, Shaw LM and Wasik MA. (1996). *Proc. Natl. Acad. Sci. USA*, **93**, 9148–9153.
- Zhang Y, Turkson J, Carter-Su C, Smithgall T, Levitzki A, Kraker A, Krolewski JJ, Medveczky P and Jove R. (2000). Manuscript submitted.
- Zong C, Yan R, August A, Darnell Jr JE and Hanafusa H. (1996). *EMBO J.*, **15**, 4515–4525.
- Zong CS, Zeng L, Jiang Y, Sadowski HB and Wang LH. (1998). *J. Biol. Chem.*, **273**, 28065–28072.