STAT3 as a target for inducing apoptosis in solid and hematological tumors

Khandaker Al Zaid Siddiquee¹, James Turkson¹

¹Biomolecular Science Center and Department of Molecular Biology and Microbiology, Burnett School of Biomedical Sciences, University of Central Florida College of Medicine, Orlando, FL 32826, USA

Studies in the past few years have provided compelling evidence for the critical role of aberrant Signal Transducer and Activator of Transcription 3 (STAT3) in malignant transformation and tumorigenesis. Thus, it is now generally accepted that STAT3 is one of the critical players in human cancer formation and represents a valid target for novel anticancer drug design. This review focuses on aberrant STAT3 and its role in promoting tumor cell survival and supporting the malignant phenotype. A brief evaluation of the current strategies targeting STAT3 for the development of novel anticancer agents against human tumors harboring constitutively active STAT3 will also be presented.

Keywords: STAT3, DNA-binding, apoptosis, small-molecule inhibitors, cell growth, human tumors

Cell Research (2008) 18:254–267. doi: 10.1038/cr.2008.18; published online 29 January 2008

Introduction

Signal Transducer and Activator of Transcription 3 (STAT3) belongs to the STAT family of proteins, which are both signal transducers and transcription factors. At least seven members in this family have been identified, namely, STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6, which are encoded by distinct genes. Alternative splicing also gives rise to naturally occurring truncated forms of some of the STAT proteins, such as STAT1 β and STAT3 β , which are splice variants of the wild-type, full-length STAT1 and STAT3 proteins, respectively, with deletions of the C-terminal domains. Structurally, STAT proteins have the following distinct domains: the N-terminal, coiled-coil, DNA binding, the Linker, Src-homology 2 (SH2) and C-terminal transactivation domains (Figure 1). Each of these domains has a distinct function. For example, the N-terminal domain is important in STAT dimer-dimer interactions; the DNA binding domain forms complexes between STAT proteins and DNA; the SH2 domain engages in dimerization between two activated STAT monomers through reciprocal phospho-tyrosine (pTyr)-SH2 domain interactions, while the C-terminal portion of the protein

functions as the transcriptional activation domain [1, 2].

The STAT proteins are differentially activated in a context-dependent manner in response to growth factors, cytokines, or other polypeptide ligands. They have important roles in fundamental processes, including proliferation, development, differentiation, inflammation, and apoptosis. STATs activation is initiated by the phosphorylation on a critical tyrosyl residue. Upon the binding of growth factors or cytokines to their cognate receptors on the cell surface, STATs are recruited to the cytoplasmic portions of the receptors, where they become phosphorylated on a critical tyrosyl residue (Y) in the C-terminus by Tyr kinases of growth factor receptors, or by cytoplasmic, non-receptor Tyr kinases, including Src, Janus kinases (Jaks) or Abelson (Abl) kinase (Figures 1 and 2). In the transactivation domain of some STAT proteins is a serine residue (S) (Figure 1), the phosphorylation of which maximizes the transcriptional activity of these proteins [3]. Tyrosine-phosphorylated STATs then dimerize through reciprocal pTyr-SH2 domain interactions, translocate into the nucleus and bind to specific STAT-response elements in the promoters of target genes, thereby inducing the transcription of those genes essential for their physiological functions. Under normal biological conditions, STATs activation is rapid and transient. However, aberrant activation of certain STAT proteins, particularly STAT3 and STAT5, is associated with many human cancers (for a comprehensive review, see [4–11]).

Correspondence: James Turkson

Tel: +1 407 882 2441; Fax: +1 407 384 2062

E-mail: jturkson@mail.ucf.edu

Aberrant STAT3 and malignant transformation

Constitutive activation of STAT3 was first observed associated with oncogenic transformation by the viral Src oncoprotein [12]. A similar observation was subsequently also reported for transformation by other oncogenic Tyr kinases, such as v-Ros, v-Fps, Etk/BMX, v-Abl, and Lck [13-18], or by viruses/viral proteins that directly or indirectly activate Tyr kinase pathways, including human T lymphotropic virus (HTLV)-1, polyomavirus middle T antigen, Epstein-Barr virus (EBV) and herpes virus saimiri [16, 19-24]. Subsequent genetic and molecular evidence indicated that aberrant STAT3 is a necessary requirement for malignant transformation [25, 26]. These studies were followed by another that provided genetic evidence sup-

porting STAT3 as an oncogene by showing that expression of an artificially engineered, constitutively dimerized STAT3C alone was sufficient to transform normal mouse fibroblasts, and that the STAT3C-transformed cells were able to form tumors in nude mice [27].

Compelling evidence has now established that aberrant STAT3 is a molecular abnormality that has a critical role in the development and progression of human tumors. In that regard, many human solid and hematological tumors harbor constitutively active STAT3 [7, 8, 10, 11]. A variety of molecular causes contribute to promoting constitutive STAT3 activation in malignant cells. In the absence of any known naturally occurring activating mutations in the *stat3* gene, aberrant STAT3 activation is predominantly due to persistent Tyr phosphorylation signals emanating

Figure 1 A schematic representation of STAT protein structure. Linear representation of the domain structures of the STAT proteins. The critical tyrosyl residue (Y) is shown, the phosphorylation of which initiates STAT activation and the dimerization between two STAT monomers through a reciprocal pTyr-SH2 domain interactions. A serine (S) residue is present in the C-terminal transactivation domain of some STAT proteins and is phosphorylated to enhance transcriptional activity. NH₂, amino (N) terminus; COOH, carboxy (C) terminus.

Figure 2 Activation of the STAT signaling pathway. STAT activation is induced by the binding of ligands, such as growth factors and cytokines to their cognate receptors (R) on the cell surface, which initiates the phosphorylation of the critical tyrosyl residue of STATs by growth factor receptor Tyr kinases (RTKs) or by non-receptor Tyr kinases (NRTKs), such as Jaks, Src or Abelson (Abl) kinase. NRTKs can also directly phosphorylate STATs in the absence of ligand-binding to receptors. Tyr-phosphorylated and activated STAT monomers engage in reciprocal pTyr-SH2 domain interactions for dimerization, and the resulting dimers translocate to the nucleus to regulate the transcription of specific genes by binding to specific STAT-responsive promoter sequences.

from dysregulated upstream Tyr kinases, such as hyperactive growth factor receptors or non-receptor Tyr kinases, including Src or Jaks, or the result of over-expression of stimulatory ligands, such as EGF or IL-6 [25, 28-36]. These molecular events are exemplified by the persistent stimulation of the IL-6/gp130 and the Jak/STAT pathway in multiple myeloma (MM), large granular lymphocyte (LGL) leukemia, and prostate cancer [28, 37, 38], and by the elevated EGFR-mediated signaling, as well as Src and Jak kinases activities in breast cancer, prostate cancer, non-small cell lung cancer (NSCLC), melanoma, pancreatic cancer, and head and neck squamous carcinoma (HNSCC) cells [16, 29-31, 34-36, 39-41], which result in constitutive STAT3 activation. While the molecular and biological mechanisms by which persistently activated STAT3 mediates cancer formation continue to be investigated, available evidence strongly supports the role of aberrant STAT3 in the promotion of uncontrolled cell proliferation and growth, cell survival, induction of angiogenesis, and the suppression

of host immune surveillance (Figure 3) [8, 10, 11, 42]. At the molecular level, evidence indicates that aberrant STAT3 causes expression changes of critical genes that dysregulate cell cycle and cell growth. Studies in transformed cells and using both solid and hematological tumor cells *in vitro* and *in vivo*, including HNSCC, NSCLC, glioma and breast cancers, show that constitutive activation of STAT3 is associated with the induction of Cyclin D1/Cyclin D2 and c-Myc expression, and down-regulation of expression of the cyclin-dependent kinase inhibitor, p21^{WAF} (see [4-11, 43] for extensive reviews).

Moreover, evidence indicates that abnormal STAT3 activity promotes tumorigenesis in part by up-regulating the expression of antiapoptotic proteins, such as Bcl-xL/Bcl-2, and Mcl-1 [28, 37, 44, 45]. Thus, the expression of the artificially designed, constitutively dimerized STAT3C alone induced the expression of Bcl-xL in transformed cells [27]. Furthermore, inhibition of constitutive STAT3 activation in malignant cells suppressed the induction of

Figure 3 Model of constitutive STAT3 activation and its role in oncogenesis. Aberrant signals from upstream growth factor RTKs, cytokine R, their overexpressed ligands, or activated NRTKs produce persistent Tyr kinase signals that induce constitutive STAT3 activation. Constitutive STAT3 activation results in dysregulation of cell cycle control and apoptosis genes, and genes that promote invasion, metastasis, and angiogenesis, and also leads to the suppression of host immune surveillance. These molecular changes together contribute to oncogenesis.

the *bcl-x* or *mcl-1* genes [28, 37, 46, 47]. Among the members of the Inhibitors of Apoptosis (IAP) family, Survivin is particularly highly expressed in various types of human cancers, including lung, colon, breast, pancreas, stomach, liver, prostate, ovarian, and hematopoietic malignancies, as well as melanoma [48]. It has recently been shown that constitutive activation of STAT3 induces the expression of Survivin in malignant cells [49, 50]. Mutation of Tyr705 in STAT3 or inhibition of the constitutively active STAT3 repressed Survivin expression in malignant cells, including human breast cancer cell lines and cells derived from patient samples, and induced apoptosis [49-51]. Aberrant STAT3 is also noted to repress the expression of the p53 tumor suppressor gene [52], thereby down-regulating pro-apoptotic genes and contributing to promoting the survival of tumor cells. As a pro-apoptotic transcription factor, the p53 protein up-regulates a number of genes, including those for the Bcl-2-associated X protein (BAX), an apoptotic protein that antagonizes Bcl-2/Bcl-xL function, the apoptotic peptidase activator factor (APAF1), caspase 6, and FAS [53, 54]. Studies have also shown that constitutive STAT3 activation is associated with the inhibition of death receptor- or FAS-mediated apoptosis, and cooperates with JUN in blocking FAS transcription in cancer cells [53]. STAT3 is also associated with the regulation of apoptosis by the noncanonical nuclear factor- κ B (NF- κ B) pathway [53]. Moreover, constitutive STAT3 activation promotes tumor angiogenesis via the induction of VEGF [55, 56] or hypoxia-inducible factor 1- α (HIF-1 α) [57], and also facilitates tumor migration, invasion and metastasis [58].

Aberrant STAT3 activation and human tumors

A large number of studies with tumor cell lines and patient samples have provided evidence for the incidence of constitutive STAT3 activity in human tumors. Solid tumors, including breast, brain, colon, prostate, lung, pancreatic, pituitary, gastrointestinal, ovarian, and cervical tumors, HNSCC, and melanoma, as well as hematological malignancies such as lymphomas and leukemias all harbor persistently activated STAT3 (see [4-11, 42] for reviews). Studies with experimental tumor models *in vitro* and *in vivo* indicate a requirement for aberrant STAT3 activity in tumor maintenance and progression, and illustrate the biological significance of the STAT3 protein in the context of malignant transformation and tumorigenesis, which are briefly discussed below.

Breast and prostate cancers, head and neck squamous cell carcinoma, melanoma

Incidence of aberrant STAT3 signaling has been observed in breast cancer. Studies in breast cancer cell lines,

tumor models, and human tumor samples have revealed the incidence of hyper-phosphorylation of STAT3 (on Tyr705) and constitutive STAT3 activity. While the mechanisms for constitutive STAT3 activation continue to be explored, available evidence has drawn a link to the activities of the non-receptor Tyr kinases Jaks and Src [29, 59]. The IL-6/gp130/Jak pathway and the Src kinase both mediate aberrant STAT3 activation [29, 60], such that the inhibition of either of these pathways results in attenuation of STAT3 activation. Although the EGFR family Tyr kinases and their ligands are over-expressed or hyperactivated in breast cancer [61], and further ligand-induced stimulation of the EGFR pathway induces additional STAT3 activation [29], there is little evidence at this time to suggest that the constitutive STAT3 activation in breast cancer cells is directly linked to aberrant activities of the EGFR pathway [16, 29]. However, given that signal transduction from the growth factor receptor Tyr kinases has been implicated in many cancers and cancer progression [62], and the role that Src and Jaks play in constitutive STAT3 activation, it is increasingly likely that EGFR, Src, Jaks, and STAT3 act in concert to promote breast carcinogenesis. Thus, inhibitors of Src or Jak kinases blocked STAT3 activation and induced apoptosis in breast cancer cells [29, 59]. Moreover, aberrant STAT3 activity is implicated in possible resistance to apoptosis of metastatic breast tumor cells [63]. Direct inhibition of STAT3 or disruption of STAT3 dimerization induced apoptosis of breast tumor cells in part by down-regulation of Bcl-xL, Bcl-2, Survivin and Mcl-1, and correlated well with the inhibition of human breast tumor growth in xenograft models [29, 46, 47, 49, 50, 59, 63].

The HNSCC exhibits constitutive activation of STAT3. Substantial evidence indicates the up-regulated EGFR signaling pathway is key in the induction of hyper-phosphorylation of STAT3 [64]. Evidence further suggests EGFR-mediated aberrant STAT3 activation contributes to HNSCC carcinogenesis [39]. Thus, abrogation of constitutive STAT3, either directly by decoy oligonucleotides or indirectly by blocking the EGFR Tyr kinase, inhibited cell growth, induced apoptosis, and led to decreased tumor volumes in animal models. Furthermore, down-regulation of aberrant STAT3 inhibited the induction of cell growth control and survival genes, including Cyclin D1, VEGF, and Bcl-xL [34, 55, 65, 66]. There is also some evidence indicating that Src kinase mediates aberrant STAT3 activity in HNSCC. In that regard, down-regulation of Src function with inhibitors or dominant-negative mutant suppressed STAT3 activation and inhibited the growth of tumor cells *in vitro* [65].

In melanoma, persistent STAT3 activation has been reported to be due to Src and Jak kinase activities [30, 57]. Aberrant STAT3 promotes the growth and survival of

melanoma cells [30, 67]. Recent evaluation of melanoma patient samples showed that constitutive STAT3 activation correlates with the expression of Bcl-xL and Mcl-1, consistent with the induction of antiapoptotic genes, which promote the survival and progression of melanoma [68]. Furthermore, studies reveal that persistent STAT3 activity promotes *in vivo* angiogenesis, in part by inducing the vascular endothelial growth factor (VEGF), a potent inducer of angiogenesis [56, 69], and stimulates invasion and metastasis by inducing matrix metalloproteinase-2 (MMP-2) *in vitro* and *in vivo* [69]. Thus, inhibition of aberrant STAT3 suppresses VEGF expression and angiogenesis [56, 69], potentially contributing to the antitumor effects associated with the inhibition [56]. These reports suggest potential therapeutic benefits through inhibition of constitutively active STAT3 in melanoma patients.

Prostate cancer is associated with persistent activity of STAT proteins. In that regard, the presence of activated STAT3 has been detected in prostate cancer tissue samples [70]. Investigative studies have shown that aberrant STAT3 activation in prostate cancer cells is associated with signal transduction from the IL-6 and IL-11 cytokines, and is mediated by kinase activities of the EGFR and Jak families [38, 71]. The mechanisms for STAT3-mediated prostate cancer development are currently under investigation. Available evidence suggests STAT3 promotes the dysregulation of cell cycle and cell growth, and enhances the survival of prostate cancer cells [70]. Studies further show that activation of the IL-6R/Jaks/STAT3 pathway is involved in the development of hormone-refractory prostate cancer [71]. Several reports further indicate a correlation between the constitutive activation of STAT3 and the expression of anti-apoptotic proteins Bcl-xL and Mcl-1 in prostate cancer cells. Inhibition of constitutively active STAT3 induces cell growth inhibition and apoptosis of prostate cancer cells [70, 72-75], and inhibits prostate tumor growth *in vivo* [76].

Brain, pancreatic, colon, ovarian, lung, and other solid tumors

There are several reports of constitutive activation of the Jak/STAT pathway in brain tumors [77-80]. Studies indicate that constitutive activation of STAT3 in brain tumors is mediated in part through signaling from the gp130 cytokines, including Oncostatin M and IL-6, and from the VEGFR-2 [77, 78, 81, 82]. Evidence further indicates that Jaks are the predominant Tyr kinases that induce the aberrant STAT3 activation. In support of the requirement of aberrant STAT3 for the malignant phenotype, blockade of persistent STAT3 activity by Jak kinase inhibitors, dominant-negative STAT3 mutant, or by RNAi in glioma cells leads to apoptosis [78, 79, 83].

Human pancreatic, ovarian, lung, renal, esophageal, cervical, colon, and gastrointestinal tumors also harbor aberrant STAT3 activity, which promotes the survival of the tumor cells and supports the malignant phenotype [84-90]. Evidence suggests that the persistent activation of STAT3 is induced in part by the IL-6/gp130 pathway in NSCLC [35], and by the growth factor receptor and non-receptor Tyr kinases in other tumors, including pancreatic, ovarian, and colon cancers. In that regard, there is an association between STAT3 activity and the overexpression and/or the hyperactive states of VEGF, EGFR family, Src, Jaks, and c-Met/HGFR pathways [41, 91-99]. The constitutive activation of STAT3 promotes the induction of Mcl-1, Bcl-xL, and Survivin, thereby promoting the survival of tumor cells [91, 92, 94]. Evidence further indicates that under hypoxic conditions activated STAT3 promotes VEGF induction [95], in part by inducing the HIF-1 α [99]. Increased expression of activated STAT3 has also been reported to be associated with tumor resistance to chemotherapy-induced apoptosis [84].

Hematological malignancies

There is a high incidence of persistent STAT3 activation in many hematological malignancies (see [7, 9-11], for more extensive reviews). These include hyperactive STAT3 in MM, anaplastic large T cell lymphoma (ALCL), EBV-related Burkitt's lymphoma, and the cutaneous T-cell lymphoma, mycosis fungoides/Sezary syndrome. Aberrant STAT3 is also associated with the HTLV-1-dependent leukemia, erythroleukemia, acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia, acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), megakaryocytic leukemia and LGL leukemia [28, 37, 44, 100-104]. Mechanistically, there is evidence suggesting a strong association between constitutive STAT3 activation and autocrine/paracrine cytokine signaling. Studies indicate that signals from the IL-6/gp130 receptor pathway, through the Jak family kinases, promote constitutive activation of STAT3 in MM [28, 105, 106]. Also, constitutively active Jaks and STATs are associated in the EBV-infected B cell lines from patients with post-transplant lymphoproliferative disorder [20, 22]. Other cytokines and their related pathways that have been implicated in STAT3 activation include IL-12 in the cutaneous T-cell lymphoma, Sezary syndrome [102], and erythropoietin (EPO) in erythroleukemia [103], while IL-10-induced Jak/STAT activation is associated with B cell non-Hodgkin's lymphoma [44] and with the spontaneous lymphoblastoid cells from post-transplant lymphoproliferative disease [22]. Moreover, there is evidence to indicate an association between methylation-induced silencing and inactivation of the suppressor of cytokine signaling 1 (SOCS-1) in MM and AML [107, 108]. The

SOCS-1 protein is a physiological negative regulator of the Jak/STAT signaling pathway [109, 110]. Methylation-mediated inactivation of SOCS-1 expression would lead to STAT hyper-activation due to the lack of mechanisms to down-regulate the upstream receptor-mediated activation events. Recent evidence also indicates that the Bcr-Abl Tyr kinase promotes STAT3 activation via the Jak pathway [111]. Moreover, Src kinase is also reported to be important in STAT3 activation in hematological malignancies and in the development of leukemias [112]. Thus, inhibition or blockade of Jaks and Bcr-Abl has been reported to abolish constitutive STAT3 activation in hematological malignancies [28, 113] or down-regulate total STAT3 at protein and mRNA levels [111]. As with solid tumors, constitutive STAT3 activity up-regulates the expression of antiapoptotic genes, including Bcl-xL, Bcl-2, and Mcl-1, and promotes the survival of tumor cells, thus contributing to the resistance to Fas- or chemotherapy-induced apoptosis [28, 37]. Inhibition of STAT3 down-regulated Bcl-xL expression, induced apoptosis, and sensitized tumor cells to drug-induced cell death [28, 44, 45].

STAT3 as a regulator of cancer cell survival

Conditional knockout of the STAT3 gene or inhibition of STAT3 function blocked v-Src-induced transformation in cancer model systems [25, 26, 114], indicating a pivotal role for STAT3 in malignant transformation. As an important proof for the oncogenic potential of STAT3, an artificially engineered, constitutively dimerized STAT3C alone is sufficient to induce malignant transformation and tumor formation in mice [27]. Aberrant STAT3 dysregulates fundamental biological processes that culminate in malignant transformation [10, 11]. Apoptosis is a physiological cell suicide process to maintain tissue homeostasis and eliminate genetically altered and unstable cells [54, 115]. In cancer, this process is greatly compromised, leading to increased survival of cancer cells and the accumulation of cells with varying degrees of genetic instability. For many of the human tumors, evidence indicates a strong correlation between persistent STAT3 activity and the maintenance of the malignant phenotype (see [6-11] for extensive reviews). There is increasing evidence that persistent STAT3 activity dysregulates growth control and apoptosis. While the exact mechanisms by which constitutively active STAT3 mediates malignant transformation and human tumor formation continue to be investigated, there is sufficient evidence for the conclusion that persistent STAT3 activation induces gene expression changes that favor tumorigenesis. Gene expression changes induced by constitutively active STAT3 represent critical molecular events that lead to the dysregulation of cell cycle control and apoptosis, thereby

promoting cell growth and survival and contributing to malignant transformation and tumorigenesis (Figure 3). Among the genes affected by STAT3 activation are the cell cycle regulators, Cyclin D1 and Cyclin D2 [27, 116]. Constitutively active STAT3 further up-regulates the survival factors, Bcl-2, Bcl-xL, and Mcl-1 [28, 30, 37, 117, 118], and the inhibitor of apoptosis members, Survivin and c-IAP2 [49, 50, 118], promotes the induction of Akt [119], represses the expression of p53 [52], and facilitates the induction of the angiogenesis factor, VEGF [56, 57]. Consistent with these molecular changes, several studies have shown that pharmacological or genetic disruption of STAT3 signaling pathways leads to the inhibition of expression of Bcl-xL, Mcl-1, Bcl-2 [28, 37, 46, 117], and Survivin [49], activates the expression of the pro-apoptotic protein BAX [45, 120] in MM, LGL leukemia, breast cancer, prostate cancer, pancreatic cancer, melanoma, gastric cancer cell lines and xenograft models [55], and induces apoptosis of tumor cells [8-11]. Consistent with STAT3's role in preventing apoptosis, the combination of STAT3 inhibitors and chemotherapy sensitizes cancer cells to apoptosis [121, 122], thus supporting the potential use of small-molecule inhibitors of STAT3 as chemo-sensitizers. Altogether, numerous studies have provided compelling evidence in support of the critical role of constitutive STAT3 activation in the development and progression of human cancers.

STAT3 protein as a novel anticancer drug target

STAT3 is now well established as a critical molecular abnormality in the biological processes leading to cancer development. Constitutively active STAT3 mediates critical gene expression changes and molecular events that dysregulate cell growth and apoptosis, and promote angiogenesis, invasion, metastasis, and the development of resistance to apoptosis. STAT3's functions and its critical roles in tumorigenesis and tumor maintenance have qualified it as a valid target for the development of novel anticancer therapeutic modalities [8, 9, 11]. Initial work demonstrated that inhibition of persistently activated STAT3 specifically suppressed cancer cell survival and induced tumor regression. Therefore, effective and specific inhibition of aberrantly activated STAT3 or targets of STAT3 pathways may potentially alter the course of cancer pathogenesis. Significant evidence has been gathered from several investigations involving the use of dominant-negative STAT3 mutants, anti-sense oligonucleotides, and activated STAT3 mutants, as well as pharmacological modulators of STAT3 in cell-culture and animal models. These studies showed that the effect of antagonizing STAT3 includes inhibiting tumor cell survival and inducing apoptosis of cells harboring constitutively active STAT3, inhibiting angiogenesis,

and up-regulating host immunocompetence [67, 123–127]. As a now validated anticancer drug target, the stage is set for the development of inhibitors of the STAT3 pathway as a novel approach to inducing cancer cell apoptosis and hence treating cancer patients.

Strategies to target STAT3

There are several strategies for designing and identifying inhibitors of the aberrantly activated STAT3 signaling pathway (Figure 3). The upstream growth factor receptor Tyr kinases and non-receptor Tyr kinases (NRTKs) are logical target choices for blocking aberrant STAT3 activation and are extensively pursued for drug development. In that regard, several different kinds of Tyr kinase inhibitors have been developed that are active against their respective targets, and in doing so inhibit aberrant STAT3 activity and tumor growth in animal models. Some of these molecular-targeted agents are currently in clinical trials. An alternative approach is to directly target the STAT3 protein, which is currently being pursued by a number of groups both in the academia and in the pharmaceutical industry. One can also take the approach of mimicking the physiological negative modulators, thereby down-regulating the Tyr phosphorylation step. Some of these approaches are briefly discussed below.

Receptor antagonists

Aberrant activation of Tyr kinases has been implicated in many human tumors. In consideration of their key roles in inducing persistent activation of STAT3 and other signaling molecules and in mediating tumorigenesis, cell surface receptors represent attractive therapeutic targets for controlling the malignant phenotype. In that regard, growth factors and their receptors, such as the EGFR family, and receptors for cytokines, including the gp130/IL-6 receptor family, are among the targets presently considered for developing effective therapeutic modalities to control cancer (Figure 3, Step 1). These receptors either have intrinsic Tyr kinase activity, as with growth factor receptors, or are associated cytoplasmic Tyr kinases, as in the case of cytokine receptors. In experimental models, strategies targeting these receptors prevent or inhibit the activation of STAT3 in malignant cells. To modulate cell surface receptors, antibodies or other molecular entities could be designed that specifically compete against the physiological ligands for binding to the receptor [128, 129]. Such approaches are currently utilized to develop pharmacological agents against the EGFR family of receptors, which are frequently overexpressed in breast, pancreatic, ovarian, and lung cancers, as well as HNSCC and other tumors [128]. Monoclonal antibodies are one of the earliest and widely popular

approaches to inhibit the EGFR family. The early human anti-mouse EFGR antibody had low efficacy, and this led to the development of chimeric human mouse MAb 225 (monoclonal antibody, IM-C225, Cetuximab) that proved to be an effective standalone therapy (see [128] for a review on this subject). This MAb is also used in combination with chemotherapy or radiation therapy in the treatment of HNSCC, colorectal cancer and NSCLC [128]. Earlier work with IL-6 indicated that inhibition of the binding of this ligand to its receptor was sufficient to abolish STAT3 activation and modulate the malignant phenotype. In the U266 MM cell line, inhibition of the IL-6 receptor by the IL-6 “super antagonist” Sant7 suppressed aberrant STAT3 activation and the viability of these cells [28]. While this approach holds some potential, it has not been extensively exploited. More studies are needed to evaluate the effectiveness of ligand antagonists as therapeutic modalities for the many human cancers in which aberrant activation of STAT3 is implicated.

Tyrosine or serine kinase inhibitors

STAT3 is downstream of receptor and non-receptor Tyr kinases (RTKs and NRTKs). The occurrence of aberrant TKs has been noted in many human tumors, including breast, lung, prostate, colon, and pancreatic cancers, as well as glioblastoma and other cancers [130, 131]. As with the cell surface receptor antagonists, one of the strategies is to block the aberrant Tyr kinase activities of RTKs or NRTKs, thereby inhibiting constitutive activation of STAT3. The inhibition of the intracellular Tyr kinase activities of RTKs (Figure 3, Step 2) has been shown to sufficiently induce apoptosis and modulate tumor growth in part by suppressing constitutive activation of STAT3 [34, 35]. Recently, the NRTKs, Src and Jaks, which also mediate STAT3 activation [29, 35, 40], are receiving increasing attention for developing small-molecule inhibitors as novel therapeutic agents. Studies have shown that the small-molecule Src inhibitors, PD166285, SU6656, and PD180970, induced cell cycle arrest and apoptosis of tumor cells, including melanoma, lung and breast cancers by mechanisms involving the inhibition of aberrant STAT3 and down-regulation of STAT3 target genes, including those for Bcl-xL and Mcl-1 anti-apoptotic proteins [29, 35]. Also, the Src kinase inhibitor, Dasatinib (BMS-324825), induced antitumor effects in lung cancer cells that harbor mutations in the EGFR, in part by the inhibition of aberrant STAT3 activity [36]. In other studies, the irreversible EGFR Tyr kinase inhibitor, PD 0169414, was effective in controlling a number of epidermoid carcinomas in mouse xenografts, including lung and breast cancers [132]. Several other studies showed the activity of the Jak kinase inhibitor AG490 in malignant cells [28, 133]. AG490 blocked

STAT3 activation [28, 133], inhibited Bcl-xL expression [28], and induced apoptosis of malignant cells [28, 133], as well as inhibited the proliferation of ALL cells *in vitro* and *in vivo* [134]. In studies of the myeloproliferative disorder, myelofibrosis with myeloid metaplasia, in which aberrant Jak kinase activity is implicated in the pathogenesis, a Jak inhibitor blocked constitutive Jak/STAT3 activation and inhibited cell proliferation [135, 136].

An area that has not been explored much is the targeting of serine kinases, which are also known to have a key role in regulating STAT3 transcriptional activity [3]. It has been shown that phosphorylation of the serine727 residue is essential for maximal transcriptional activity of STAT3 [3]. Although more than one kinase has been reported to phosphorylate Ser727 within the transcriptional activation domain of STAT3, the identity of the key serine kinase(s) and the role of serine kinases in STAT3-mediated malignant transformation remain to be clearly defined. Initial work indicated that inhibition of the Ser phosphorylation blocked v-Src-mediated transformation [137], suggesting the potential to down-regulate the oncogenic activity of STAT3 by suppressing its transcriptional activity through inhibition of serine phosphorylation.

Direct inhibition of constitutively active STAT3

Activated STATs form a dimer through pTyr-SH2 domain interactions, and dimerization is essential for the DNA-binding activity of STATs [138]. Thus, disruption or prevention of dimerization (Figure 3, Step 3) can be an effective approach for controlling persistently active STAT3 and its functions [139]. This is supported by the finding that mutation of the critical Tyr705 residue of STAT3 to phenylalanine inactivates STAT3 signaling and blocks its biological functions [26]. Recently, a number of reports have highlighted approaches to develop direct inhibitors of STAT3. The development and evaluation of phosphopeptide and peptidomimetic analogs of the STAT3 SH2 domain-binding peptide have provided the proof-of-concept that small-molecule-mediated disruption of dimerization can inactivate the STAT3 protein and abolish its function [139, 140]. These peptidomimetics have paved the way for the design of non-peptide analogs with better physicochemical properties, which brought it a step closer to identifying STAT3 dimerization disruptors as potential novel anticancer therapeutics [47, 140]. Based on computational modeling along with structure-based virtual high throughput screening and design, a new molecule IS3-201 [46] has recently been identified that disrupts STAT3 dimerization. The evaluation of molecules including S3I-M2001 and S3I-201 in both cell-based studies and xenografts models of human breast tumors showed potent antitumor activities [46, 47]. Other approaches taken to directly inhibit aberrant

STAT3 include platinum (IV) complexes [75, 124], which may directly interact with the DNA-binding domain of the protein and inhibit its DNA-binding activity, G-rich oligodeoxynucleotides capable of forming four-stranded structures, called g-quartet molecules [141], peptide aptamers [142], cucurbitacin [125], and STA-21 (NSC 628869) and its derivatives [143, 144], as well as the small-molecule inhibitor of dimerization, Stattic [145]. Studies with these molecules show that direct inhibition of persistently active STAT3 induces apoptosis of a variety of malignant cells, including breast, prostate, and colon tumors, and induces tumor regression.

Physiological negative modulators of STAT3

Different physiological negative protein modulators of the STAT3 signaling pathway have been identified, including the cytokine-inducible SH2-containing (CIS) protein or suppressor of cytokine signaling protein (SOCS-1 and SOCS-3), Jak binding protein (JAB) and STAT-induced STAT inhibitor, and the protein inhibitors of activated STATs (PIAS) [109, 110, 146-148]. The SOCS proteins prevent the Jak kinases from activating STAT3. The activation of SOCS-3 inversely correlates with STAT3 activation. Of relevance to cancer, transcriptional silencing of SOCS-1 by hypermethylation has been reported in human hepatocellular carcinoma [149, 150], with evidence for a phenotype that is consistent with hyperactivated STAT3. These studies provide the initial indication that small-molecule mimics of SOCS-1 and SOCS-3 might be useful in suppressing events that lead to persistent STAT3 activation, thereby producing beneficial antitumor effects. Indeed, recent work showed that a cell-permeable SOCS-1-mimic peptide (Tkip) inhibited IL-6-induced STAT3 activation and blocked prostate cancer cell growth [151]. In addition, activation and inactivation of the STAT family proteins also depend on the modulation of phosphatase (PTPase) activities in cells. STAT3 (and other STATs) pathways are regulated by phosphatases SHP1 and SHP2 [152]. Serine phosphorylation and distribution of STAT3 in cutaneous T-cell lymphoma are regulated by the PP2A phosphatase [153]. Thus, selective modulation of phosphatase activity to control STAT3 activation in cancer cells might be a useful therapeutic approach.

Other approaches

The decoy antisense oligodeoxynucleotide (ODN) could be useful as a therapeutic approach. ODN will bind mRNA transcript of STAT3, preventing the expression of the protein and occluding the protein's functions. The use of STAT3 ODN in HNSCC has shown promise and demonstrated the potential to utilize this approach to inhibit tumor formation [39, 154, 155]. Additionally, the study

with dominant-negative STAT3 β in mouse melanoma that induced apoptosis of tumor cells through the down-regulation of anti-apoptotic Bcl-xL and Mcl-1 genes, and induced tumor regression suggests the potential that gene therapy approach could be applied to human tumors that harbor aberrant STAT3 [123].

The crystal structure of STAT3 dimer [156] provides important information on STAT3 DNA-binding mechanisms and on the critical amino acids that are involved in the interaction with DNA. Such information will be useful for designing disruptors of STAT3 DNA-binding activity. Moreover, STAT3 requires transcriptional co-activators for transcriptional activity. For example, the proteins CBP/p300 and Sp1 are known transcriptional co-activators of STAT3 in the induction of specific genes [157, 158]. Design of artificial co-factors could compete with these natural proteins and repress the ability of STAT3 to induce gene transcription.

Significance of aberrant STAT3 in clinical trials of molecular-targeted therapeutic agents

A number of novel molecular-targeted therapeutic modalities are either currently in different phases of clinical trials or have been approved for clinical application. Prior to the approval for patient treatment, these agents would have to go through extensive experimental evaluations regarding their activity and mechanism of action, efficacy, and toxicity, providing investigators a good assessment of the potential benefits to patients. Tyrosine kinase inhibitors (TKIs), such as modulators of EGFR, are perhaps the largest class of novel molecular-targeted therapeutics to be approved. Given that the molecular entities that are downstream from the growth factor receptor families and other Tyr kinases include STAT3, the monitoring of phospho-Tyr levels of STAT3 in tumor specimens from patients receiving TKIs could represent a useful surrogate marker for agents' activities, as well as provide a way to explore any correlation between efficacy, and the modulation of TK activity and its downstream targets in patient samples. In that regard, a similarly conducted study that monitored the skin biopsies of patients that participated in the phase I clinical trial of the EGFR TKI, ZD1839 (Gefitinib, Iressa) showed a significant effect of the TKI on EGFR-dependent downstream signaling molecules, including p27, phospho-Tyr STAT3 and phospho-MAPK [159-162].

Conclusion

The evidence is overwhelming regarding the critical role of abnormal STAT3 activity in diverse tumorigenic processes, including the dysregulation of cell cycle and

promotion of uncontrolled growth, induction of survival factors and inhibition of apoptosis, as well as the mediation of angiogenesis and the suppression of host's immune surveillance. Several important studies have provided convincing evidence establishing the proof-of-concept for the potential antitumor effects of inhibition of aberrant STAT3 activity, in both cell-culture and whole-animal models for a variety of human tumors. Several key studies to determine the impact on normal cells have also been performed which demonstrate a slow-down of growth of normal cells without significant apoptosis. The stage is therefore set for identifying and developing safe STAT3 inhibitors that will have potential clinical applications. Given the substantial biological and molecular evidence supporting STAT3 as a valid target and the increasing number of human tumors that harbor constitutively-active STAT3, novel anticancer therapeutic modalities based on STAT3 inhibition will have widespread therapeutic applications. They can either be used as standalone agents or in combination with chemotherapy or other molecular-targeted therapeutic agents. STAT3 inhibitors will also have applications as agents for sensitizing tumors in the case of drug resistance based on the initial evidence that aberrant activity of the STAT3 protein participates in the underlying molecular and biological mechanisms that promote resistance to apoptosis.

Acknowledgments

We thank all colleagues and members of our laboratory for stimulating discussions. This work was supported by the National Cancer Institute Grant CA106439, USA (JT).

References

- Schindler C, Darnell JE Jr. Transcriptional responses to polypeptide ligands: the JAK-STAT pathway. *Annu Rev Biochem* 1995; **64**:621-651.
- Darnell JE Jr. STATs and gene regulation. *Science* 1997; **277**:1630-1635.
- Wen Z, Zhong Z, Darnell JE Jr. Maximal activation of transcription by Stat1 and Stat3 requires both tyrosine and serine phosphorylation. *Cell* 1995; **82**:241-250.
- Bromberg JF. Activation of STAT proteins and growth control. *Bioessays* 2001; **23**:161-169.
- Darnell JE Jr. Transcription factors as targets for cancer therapy. *Nat Rev Cancer* 2002; **2**:740-749.
- Darnell JE. Validating Stat3 in cancer therapy. *Nat Med* 2005; **11**:595-596.
- Bowman T, Garcia R, Turkson J, Jove R. STATs in oncogenesis. *Oncogene* 2000; **19**:2474-2488.
- Buettner R, Mora LB, Jove R. Activated STAT signaling in human tumors provides novel molecular targets for therapeutic intervention. *Clin Cancer Res* 2002; **8**:945-954.
- Turkson J, Jove R. STAT proteins: novel molecular targets for cancer drug discovery. *Oncogene* 2000; **19**:6613-6626.

- 10 Turkson J. STAT proteins as novel targets for cancer drug discovery. *Expert Opin Ther Targets* 2004; **8**:409-422.
- 11 Yu H, Jove R. The STATS of Cancer-New molecular targets come of age. *Nat Rev Cancer* 2004; **4**:97-105.
- 12 Yu CL, Meyer DJ, Campbell GS, *et al.* Enhanced DNA-binding activity of a Stat3-related protein in cells transformed by the Src oncoprotein. *Science* 1995; **269**:81-83.
- 13 Danial NN, Pernis A, Rothman PB. Jak-STAT signaling induced by the v-abl oncogene. *Science* 1995; **269**:1875-1877.
- 14 Besser D, Bromberg JF, Darnell JE Jr, Hanafusa H. A single amino acid substitution in the v-Eyk intracellular domain results in activation of Stat3 and enhances cellular transformation. *Mol Cell Biol* 1999; **19**:1401-1409.
- 15 Zong CS, Zeng L, Jiang Y, Sadowski HB, Wang LH. Stat3 plays an important role in oncogenic Ros- and insulin-like growth factor I receptor-induced anchorage-independent growth. *J Biol Chem* 1998; **273**:28065-28072.
- 16 Garcia R, Yu CL, Hudnall A, *et al.* Constitutive activation of Stat3 in fibroblasts transformed by diverse oncoproteins and in breast carcinoma cells. *Cell Growth Diff* 1997; **8**:1267-1276.
- 17 Wen X, Lin HH, Shih HM, Kung HJ, Ann DK. Kinase activation of the non-receptor tyrosine kinase Etk/BMX alone is sufficient to transactivate STAT-mediated gene expression in salivary and lung epithelial cells. *J Biol Chem* 1999; **274**:38204-38210.
- 18 Lund TC, Prator PC, Medveczky MM, Medveczky PG. The Lck binding domain of herpesvirus saimiri tip-484 constitutively activates Lck and STAT3 in T cells. *J Virol* 1999; **73**:1689-1694.
- 19 Migone TS, Lin JX, Cereseto A, *et al.* Constitutively activated Jak-STAT pathway in T cells transformed with HTLV-I. *Science* 1995; **269**:79-81.
- 20 Weber-Nordt RM, Egen C, Wehinger J, *et al.* Constitutive activation of STAT proteins in primary lymphoid and myeloid leukemia cells and in Epstein-Barr virus (EBV)-related lymphoma cell lines. *Blood* 1996; **88**:809-816.
- 21 Lund TC, Garcia R, Medveczky MM, Jove R, Medveczky PG. Activation of STAT transcription factors by herpesvirus Saimiri Tip-484 requires p56lck. *J Virol* 1997; **71**:6677-6682.
- 22 Nepomuceno RR, Snow AL, Robert Beatty P, Krams SM, Martinez OM. Constitutive activation of Jak/STAT proteins in Epstein-Barr virus-infected B-cell lines from patients with post-transplant lymphoproliferative disorder. *Transplantation* 2002; **74**:396-402.
- 23 Samaan A, Mahana W. Constitutive and induced activation of JAK/Stat pathway in leukemogenic and asymptomatic human T-cell lymphotropic virus type 1 (HTLV-1) transformed rabbit cell lines. *Immunol Lett* 2007; **109**:113-119.
- 24 Tomita M, Kawakami H, Uchihara JN, *et al.* Inhibition of constitutively active Jak-Stat pathway suppresses cell growth of human T-cell leukemia virus type 1-infected T-cell lines and primary adult T-cell leukemia cells. *Retrovirology* 2006; **3**:22.
- 25 Turkson J, Bowman T, Garcia R, Caldenhoven E, De Groot RP, Jove R. Stat3 activation by Src induces specific gene regulation and is required for cell transformation. *Mol Cell Biol* 1998; **18**:2545-2552.
- 26 Bromberg JF, Horvath CM, Besser D, Lathem WW, Darnell JE Jr. Stat3 activation is required for cellular transformation by v-src. *Mol Cell Biol* 1998; **18**:2553-2558.
- 27 Bromberg JF, Wrzeszczynska MH, Devgan G, *et al.* Stat3 as an oncogene. *Cell* 1999; **98**:295-303.
- 28 Catlett-Falcone R, Landowski TH, Oshiro MM, *et al.* Constitutive activation of Stat3 signaling confers resistance to apoptosis in human U266 myeloma cells. *Immunity* 1999; **10**:105-115.
- 29 Garcia R, Bowman TL, Niu G, *et al.* Constitutive activation of Stat3 by the Src and JAK tyrosine kinases participates in growth regulation of human breast carcinoma cells. *Oncogene* 2001; **20**:2499-2513.
- 30 Niu G, Bowman T, Huang M, *et al.* Roles of activated Src and Stat3 signaling in melanoma tumor cell growth. *Oncogene* 2002; **21**:7001-7010.
- 31 Chan KS, Carbajal S, Kiguchi K, Clifford J, Sano S, DiGiovanni J. Epidermal growth factor receptor-mediated activation of Stat3 during multistage skin carcinogenesis. *Cancer Res* 2004; **64**:2382-2329.
- 32 Selander KS, Li L, Watson L, *et al.* Inhibition of gp130 signaling in breast cancer blocks constitutive activation of Stat3 and inhibits *in vivo* malignancy. *Cancer Res* 2004; **64**:6924-6933.
- 33 Greulich H, Chen TH, Feng W, *et al.* Oncogenic transformation by inhibitor-sensitive and -resistant EGFR mutants. *PLoS Med* 2005; **2**:e313.
- 34 Song JI, Grandis JR. STAT signaling in head and neck cancer. *Oncogene* 2000; **19**:2489-2495.
- 35 Song L, Turkson J, Karras JG, Jove R, Haura EB. Activation of Stat3 by receptor tyrosine kinases and cytokines regulates survival in human non-small cell carcinoma cells. *Oncogene* 2003; **22**:4150-4165.
- 36 Song L, Morris M, Bagui T, Lee FY, Jove R, Haura EB. Dasatinib (BMS-354825) selectively induces apoptosis in lung cancer cells dependent on epidermal growth factor receptor signaling for survival. *Cancer Res* 2006; **66**:5542-5548.
- 37 Epling-Burnette PK, Lui JH, Catlette-Falcone R, *et al.* Inhibition of STAT3 signaling leads to apoptosis of leukemic large granular lymphocytes and decreased Mcl-1 expression. *J Clin Invest* 2001; **107**:351-362.
- 38 Lou W, Ni Z, Dyer K, Twardy DJ, Gao AC. Interleukin-6 induces prostate cancer cell growth accompanied by activation of stat3 signaling pathway. *Prostate* 2000; **42**:239-242.
- 39 Grandis JR, Zeng Q, Drenning SD. Epidermal growth factor receptor—mediated stat3 signaling blocks apoptosis in head and neck cancer. *Laryngoscope* 2000; **110**:868-874.
- 40 Nam S, Kim D, Cheng JQ, *et al.* Action of the Src family kinase inhibitor, dasatinib (BMS-354825), on human prostate cancer cells. *Cancer Res* 2005; **65**:9185-9189.
- 41 Trevino JG, Gray MJ, Nawrocki ST, *et al.* Src activation of Stat3 is an independent requirement from NF-kappaB activation for constitutive IL-8 expression in human pancreatic adenocarcinoma cells. *Angiogenesis* 2006; **9**:101-110.
- 42 Haura EB, Turkson J, Jove R. Mechanisms of disease: Insights into the emerging role of signal transducers and activators of transcription in cancer. *Nat Clin Pract Oncol* 2005; **2**:315-324.
- 43 Kim DJ, Chan KS, Sano S, DiGiovanni J. Signal transducer and activator of transcription 3 (Stat3) in epithelial carcinogenesis. *Mol Carcinog* 2007; **46**:725-731.
- 44 Alas S, Bonavida B. Rituximab inactivates signal transducer and activation of transcription 3 (STAT3) activity in B-non-Hodgkin's lymphoma through inhibition of the interleukin 10 autocrine/paracrine loop and results in down-regulation of Bcl-2 and sensitization to cytotoxic drugs. *Cancer Res* 2001; **61**:5137-5144.
- 45 Alas S, Bonavida B. Inhibition of constitutive STAT3 activity sensitizes resistant non-Hodgkin's lymphoma and multiple

- myeloma to chemotherapeutic drug-mediated apoptosis. *Clin Cancer Res* 2003; **9**:316-326.
- 46 Siddiquee K, Zhang S, Guida WC, *et al.* Selective chemical probe inhibitor of Stat3, identified through structure-based virtual screening, induces antitumor activity. *Proc Natl Acad Sci U S A* 2007; **104**: 7391-7396.
- 47 Siddiquee K, Glenn M, Gunning P, *et al.* An oxazole inhibits Stat3 activity and blocks malignant cell phenotype and growth of human breast tumor xenografts. *ACS Chem Biol* 2007; **2**:787-798.
- 48 Duffy MJ, O'Donovan N, Brennan DJ, Gallagher WM, Ryan BM. Survivin: a promising tumor biomarker. *Cancer Lett* 2007; **249**:49-60.
- 49 Gritsko T, Williams A, Turkson J, *et al.* Persistent activation of stat3 signaling induces survivin gene expression and confers resistance to apoptosis in human breast cancer cells. *Clin Cancer Res* 2006; **12**:11-19.
- 50 Diaz N, Minton S, Cox C, *et al.* Activation of stat3 in primary tumors from high-risk breast cancer patients is associated with elevated levels of activated SRC and survivin expression. *Clin Cancer Res* 2006; **12**:20-28.
- 51 Kim KW, Mutter RW, Cao C, *et al.* Inhibition of signal transducer and activator of transcription 3 activity results in down-regulation of Survivin following irradiation. *Mol Cancer Ther* 2006; **5**:2659-2665.
- 52 Niu G, Wright KL, Ma Y, *et al.* Role of Stat3 in regulating p53 expression and function. *Mol Biol Cell* 2005; **25**:7432-7440.
- 53 Barré B, Vigneron A, Perkins N, Roninson IB, Gamelin E, Coqueret O. The STAT3 oncogene as a predictive marker of drug resistance. *Trends Mol Med* 2007; **13**:4-11.
- 54 Chauhan D, Anderson KC. Apoptosis in multiple myeloma: therapeutic implications. *Apoptosis* 2001; **6**:47-55.
- 55 Xi S, Gooding WE, Grandis JR. *In vivo* antitumor efficacy of STAT3 blockade using a transcription factor decoy approach: implications for cancer therapy. *Oncogene* 2005; **24**:970-979.
- 56 Niu G, Wright KL, Huang M, *et al.* Constitutive Stat3 activity up-regulates VEGF expression and tumor angiogenesis. *Oncogene* 2002; **21**:2000-2008.
- 57 Xu Q, Briggs J, Park S, *et al.* Targeting Stat3 blocks both HIF-1 and VEGF expression induced by multiple oncogenic growth signaling pathways. *Oncogene* 2005; **24**:5552-5560.
- 58 Kortylewski M, Jove R, Yu H. Targeting STAT3 affects melanoma on multiple fronts. *Cancer Metastasis Rev* 2005; **24**:315-327.
- 59 Nam S, Buettner R, Turkson J, *et al.* Iridin derivatives inhibit Stat3 signaling and induce apoptosis in human cancer cells. *Proc Natl Acad Sci USA* 2005; **102**:5998-6003.
- 60 Berishaj M, Gao SP, Ahmed S, *et al.* Stat3 is tyrosine-phosphorylated through the interleukin-6/glycoprotein 130/Janus kinase pathway in breast cancer. *Breast Cancer Res* 2007; **9**:R32.
- 61 Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol* 2001; **2**:127-137.
- 62 Azambuja E, Durbecq V, Rosa D, *et al.* HER-2 overexpression/amplification and its interaction with taxane-based therapy in breast cancer. *Ann Oncol* 2007; doi:10.1093/annonc/mdm352.
- 63 Real PJ, Sierra A, De Juan A, Segovia JC, Lopez-Vega JM, Fernandez-Luna JL. Resistance to chemotherapy via Stat3-dependent overexpression of Bcl-2 in metastatic breast cancer cells. *Oncogene* 2002; **21**:7611-7618.
- 64 Grandis JR, Chakraborty A, Zeng Q, Melhem MF, Tweardy DJ. Downmodulation of TGF-alpha protein expression with antisense oligonucleotides inhibits proliferation of head and neck squamous carcinoma but not normal mucosal epithelial cells. *J Cell Biochem* 1998; **69**:55-62.
- 65 Xi S, Zhang Q, Dyer KE, *et al.* Src kinases mediate STAT growth pathways in squamous cell carcinoma of the head and neck. *J Biol Chem* 2003; **278**:31574-31583.
- 66 Kijima T, Niwa H, Steinman RA, *et al.* STAT3 activation abrogates growth factor dependence and contributes to head and neck squamous cell carcinoma tumor growth *in vivo*. *Cell Growth Differ* 2002; **13**:355-362.
- 67 Niu G, Shain K, Huang M, *et al.* Overexpression of a dominant-negative signal transducer and activator of transcription 3 variant in tumor cells leads to production of soluble factors that induce apoptosis and cell cycle arrest. *Cancer Res* 2001; **61**:3276-3280.
- 68 Zhuang L, Lee CS, Scolyer RA, *et al.* Mcl-1, Bcl-XL and Stat3 expression are associated with progression of melanoma whereas Bcl-2, AP-2 and MITF levels decrease during progression of melanoma. *Mod Pathol* 2007; **20**:416-426.
- 69 Xie TX, Huang FJ, Aldape KD, *et al.* Activation of Stat3 in Human Melanoma Promotes Brain Metastasis. *Cancer Res* 2006; **66**:3188-3196.
- 70 Mora LB, Buettner R, Seigne J, *et al.* Constitutive activation of Stat3 in human prostate tumors and cell lines: direct inhibition of Stat3 signaling induces apoptosis of prostate cancer cells. *Cancer Res* 2002; **62**:6659-6666.
- 71 Tam L, McGlynn LM, Traynor P, Mukherjee R, Bartlett JM, Edwards J. Expression levels of the JAK/STAT pathway in the transition from hormone-sensitive to hormone-refractory prostate cancer. *Br J Cancer* 2007; **97**:378-383.
- 72 Lee SO, Lou W, Qureshi KM, Mehraei-Ghomi F, Trump DL, Gao AC. RNA interference targeting Stat3 inhibits growth and induces apoptosis of human prostate cancer cells. *Prostate* 2004; **60**:303-309.
- 73 Pu YS, Hour TC, Chuang SE, Cheng AL, Lai MK, Kuo ML. Interleukin-6 is responsible for drug resistance and anti-apoptotic effects in prostatic cancer cells. *Prostate* 2004; **60**:120-129.
- 74 Kotha A, Sekharam M, Cilenti L, *et al.* Resveratrol inhibits Src and Stat3 signaling and induces the apoptosis of malignant cells containing activated Stat3 protein. *Mol Cancer Ther* 2006; **5**:621-629.
- 75 Turkson J, Zhang S, Mora LB, Burns A, Sebt S, Jove R. A novel platinum compound inhibits constitutive Stat3 signaling and induces cell cycle arrest and apoptosis of malignant cells. *J Biol Chem* 2005; **280**:32979-32988.
- 76 Gao L, Zhang L, Hu J, *et al.* Down-regulation of signal transducer and activator of transcription 3 expression using vector-based small interfering RNAs suppresses growth of human prostate tumor *in vivo*. *Clin Cancer Res* 2005; **11**:6333-6341.
- 77 Schaefer LK, Ren Z, Fuller GN, Schaefer TS. Constitutive activation of Stat3alpha in brain tumors: localization to tumor endothelial cells and activation by the endothelial tyrosine kinase receptor (VEGFR-2). *Oncogene* 2002; **21**:2058-2065.
- 78 Rahaman SO, Harbor PC, Chernova O, Barnett GH, Vogelbaum MA, Haque SJ. Inhibition of constitutively active Stat3 suppresses proliferation and induces apoptosis in glioblastoma multiforme cells. *Oncogene* 2002; **21**:8404-8413.
- 79 Konnikova L, Kotecki M, Kruger MM, Cochran BH. Knockdown of STAT3 expression by RNAi induces apoptosis in astrocytoma cells. *BMC Cancer* 2003; **3**:23.

- 80 Paillaud E, Costa S, Fages C, *et al.* Retinoic acid increases proliferation rate of GL-15 glioma cells, involving activation of STAT-3 transcription factor. *J Neurosci Res* 2002; **67**:670-679.
- 81 Schaefer LK, Menter DG, Schaefer TS. Activation of Stat3 and Stat1 DNA binding and transcriptional activity in human brain tumour cell lines by gp130 cytokines. *Cell Signal* 2000; **12**:143-151.
- 82 Krona A, Järnum S, Salford LG, Widegren B, Aman P. Oncostatin M signaling in human glioma cell lines. *Oncol Rep* 2005; **13**:807-811.
- 83 Ren W, Duan Y, Yang Y, Ji Y, Chen F. Down-regulation of Stat3 induces apoptosis of human glioma cell: a potential method to treat brain cancer. *Neurol Res* 2007 Aug 22 [Epub ahead of print].
- 84 Duan Z, Foster R, Bell DA, *et al.* Signal transducers and activators of transcription 3 pathway activation in drug-resistant ovarian cancer. *Clin Cancer Res* 2006; **12**:5055-5063.
- 85 Ikuta K, Takemura K, Kihara M, *et al.* Overexpression of constitutive signal transducer and activator of transcription 3 mRNA in cisplatin-resistant human non-small cell lung cancer cells. *Oncol Rep* 2005; **13**:217-222.
- 86 Kanda N, Seno H, Konda Y, *et al.* STAT3 is constitutively activated and supports cell survival in association with survivin expression in gastric cancer cells. *Oncogene* 2004; **23**:4921-4929.
- 87 Scholz A, Heinze S, Detjen KM, *et al.* Activated signal transducer and activator of transcription 3 (STAT3) supports the malignant phenotype of human pancreatic cancer. *Gastroenterology* 2003; **125**:891-905.
- 88 Kusaba T, Nakayama T, Yamazumi K, *et al.* Expression of p-STAT3 in human colorectal adenocarcinoma and adenoma; correlation with clinicopathological factors. *J Clin Pathol* 2005; **58**:833-838.
- 89 Kusaba T, Nakayama T, Yamazumi K, *et al.* Activation of STAT3 is a marker of poor prognosis in human colorectal cancer. *Oncol Rep* 2006; **15**:1445-1451.
- 90 Yakata Y, Nakayama T, Yoshizaki A, Kusaba T, Inoue K, Sekine I. Expression of p-STAT3 in human gastric carcinoma: significant correlation in tumour invasion and prognosis. *Int J Oncol* 2007; **30**:437-442.
- 91 Burke WM, Jin X, Lin HJ, *et al.* Inhibition of constitutively active Stat3 suppresses growth of human ovarian and breast cancer cells. *Oncogene* 2001; **20**:7925-7934.
- 92 Toyonaga T, Nakano K, Nagano M, *et al.* Blockade of constitutively activated Janus kinase/signal transducer and activator of transcription-3 pathway inhibits growth of human pancreatic cancer. *Cancer Lett* 2003; **201**:107-116.
- 93 Lin Q, Lai R, Chirieac LR, *et al.* 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* 2005; **167**:969-980.
- 94 Chen CL, Hsieh FC, Lieblein JC, *et al.* Stat3 activation in human endometrial and cervical cancers. *Br J Cancer* 2007; **96**:591-599.
- 95 Wei D, Le X, Zheng L, *et al.* Stat3 activation regulates the expression of vascular endothelial growth factor and human pancreatic cancer angiogenesis and metastasis. *Oncogene* 2003; **22**:319-329.
- 96 Wei LH, Kuo ML, Chen CA, *et al.* Interleukin-6 promotes cervical tumor growth by VEGF-dependent angiogenesis via a STAT3 pathway. *Oncogene* 2003; **22**:1517-1527.
- 97 Leu CM, Wong FH, Chang C, Huang SF, Hu CP. Interleukin-6 acts as an antiapoptotic factor in human esophageal carcinoma cells through the activation of both STAT3 and mitogen-activated protein kinase pathways. *Oncogene* 2003; **22**:7809-7818.
- 98 Horiguchi A, Oya M, Marumo K, Murai M. STAT3, but not ERKs, mediates the IL-6-induced proliferation of renal cancer cells, ACHN and 769P. *Kidney Int* 2002; **61**:926-938.
- 99 Jung JE, Lee HG, Cho IH, *et al.* STAT3 is a potential modulator of HIF-1-mediated VEGF expression in human renal carcinoma cells. *FASEB J* 2005; **19**:1296-1298.
- 100 Zamo A, Chiarle R, Piva R, *et al.* Anaplastic lymphoma kinase (ALK) activates Stat3 and protects hematopoietic cells from cell death. *Oncogene* 2002; **21**:1038-1047.
- 101 Nasr MR, Laver JH, Chang M, Hutchison RE. Expression of anaplastic lymphoma kinase, tyrosine-phosphorylated STAT3, and associated factors in pediatric anaplastic large cell lymphoma: A report from the children's oncology group. *Am J Clin Pathol* 2007; **127**:770-778.
- 102 Eriksen KW, Kaltoft K, Mikkelsen G, *et al.* Constitutive STAT3-activation in Sezary syndrome: tyrphostin AG490 inhibits STAT3-activation, interleukin-2 receptor expression and growth of leukemic Sezary cells. *Leukemia* 2001; **15**:787-793.
- 103 Kirito K, Nagashima T, Ozawa K, and Komatsu N. Constitutive activation of Stat1 and Stat3 in primary erythroleukemia cells. *Int J Hematol* 2002; **75**:51-54.
- 104 Spiekermann K, Biethahn S, Wilde S, Hiddemann W, Alves F. Constitutive activation of STAT transcription factors in acute myelogenous leukemia. *Eur J Haematol* 2001; **67**:63-71.
- 105 Hodge DR, Xiao W, Wang LH, Li D, Farrar WL. Activating mutations in STAT3 and STAT5 differentially affect cellular proliferation and apoptotic resistance in multiple myeloma cells. *Cancer Biol Ther* 2004; **3**:2.
- 106 Burger R, Bakker F, Guenther A, *et al.* Functional significance of novel neurotrophin-1/B cell-stimulating factor-3 (cardiotrophin-like cytokine) for human myeloma cell growth and survival. *Br J Haematol* 2003; **123**:869-878.
- 107 Chen CY, Tsay W, Tang JL, *et al.* SOCS1 methylation in patients with newly diagnosed acute myeloid leukemia. *Genes Chromosomes Cancer* 2003; **37**:300-305.
- 108 Galm O, Yoshikawa H, Esteller M, Osieka R, Herman JG. SOCS-1, a negative regulator of cytokine signaling, is frequently silenced by methylation in multiple myeloma. *Blood* 2003; **101**:2784-2788.
- 109 Heinrich PC, Behrmann I, Müller-Newen G, Schaper F, Graeve L. Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. *Biochem J* 1998; **334** (Pt 2):297-314.
- 110 Krebs DL, Hilton DJ. SOCS: physiological suppressors of cytokine signaling. *J Cell Sci* 2000; **113**(Pt 16):2813-2819.
- 111 Coppo P, Flamant S, De Mas V, *et al.* BCR-ABL activates STAT3 via JAK and MEK pathways in human cells. *Br J Haematol* 2006; **134**:171-179.
- 112 Warmuth M, Damoiseaux R, Liu Y, Fabbro D, Gray N. SRC family kinases: potential targets for the treatment of human cancer and leukemia. *Curr Pharm Des* 2003; **9**:2043-2059.
- 113 Frost MJ, Ferrao PT, Hughes TP, Ashman LK. Juxtamembrane mutant V560GKit is more sensitive to Imatinib (STI571) compared with wild-type c-kit whereas the kinase domain mutant D816VKit is resistant. *Mol Cancer Ther* 2002; **1**:1115-1124.
- 114 Schlessinger K, Levy DE. Malignant transformation but not

- normal cell growth depends on signal transducer and activator of transcription 3. *Cancer Res* 2005; **65**:5828-5834.
- 115 Yu J, Zhang L. Apoptosis in human cancer cells. *Curr Opin Oncol* 2004; **16**:19-24.
- 116 Sinibaldi N, Wharton W, Turkson J, Bowman T, Pledger WJ, Jove R. Induction of p21WAF1/CIP1 and cyclin D1 expression by the Src oncoprotein in mouse fibroblasts: role of activated STAT3 signaling. *Oncogene* 2000; **19**:5419-5427.
- 117 Iwamaru A, Szymanski S, Iwado E, *et al.* A novel inhibitor of the STAT3 pathway induces apoptosis in malignant glioma cells both *in vitro* and *in vivo*. *Oncogene* 2007; **26**:2435-2444.
- 118 Bhattacharya S, Ray RM, Johnson LR. STAT3-mediated transcription of Bcl-2, Mcl-1 and c-IAP2 prevents apoptosis in polyamine-depleted cells. *Biochem J* 2005; **392**:335-344.
- 119 Park S, Kim D, Kaneko S, *et al.* Molecular cloning and characterization of the human AKT1 promoter uncovers its up-regulation by the Src/Stat3 pathway. *J Biol Chem* 2005; **280**:38932-38941.
- 120 Nielsen M, Kaestel CG, Eriksen KW, *et al.* Inhibition of constitutively activated Stat3 correlates with altered Bcl-2/Bax expression and induction of apoptosis in mycosis fungoides tumor cells. *Leukemia* 1999; **13**:735-738.
- 121 Lee YK, Isham CR, Kaufman SH, Bible KC. Flavopiridol disrupts STAT3/DNA interactions, attenuates STAT3-directed transcription, and combines with the Jak kinase inhibitor AG490 to achieve cytotoxic synergy. *Mol Cancer Ther* 2006; **5**:138-148.
- 122 Yau CY, Wheeler JJ, Sutton KL, Hedley DW. Inhibition of integrin-linked kinase by a selective small molecule inhibitor, QLT0254, inhibits the PI3K/PKB/mTOR, Stat3, and FKHR pathways and tumor growth, and enhances gemcitabine-induced apoptosis in human orthotopic primary pancreatic cancer xenografts. *Cancer Res* 2005; **65**:1497-1504.
- 123 Niu G, Heller R, Catlett-Falcone R, *et al.* Gene therapy with dominant-negative Stat3 suppresses growth of the murine melanoma B16 tumor *in vivo*. *Cancer Res* 1999; **59**:5059-5063.
- 124 Turkson J, Zhang S, Palmer J, *et al.* Inhibition of constitutive signal transducer and activator of transcription 3 activation by novel platinum complexes with potent anti-tumor activity. *Mol Cancer Ther* 2004; **3**:1533-1542.
- 125 Blaskovich MA, Sun J, Cantor A, Turkson J, Jove R, Sefti SM. Discovery of JSI-124 (cucurbitacin I), a selective Janus kinase/signal transducer and activator of transcription 3 signaling pathway inhibitor with potent antitumor activity against human and murine cancer cells in mice. *Cancer Res* 2003; **63**:1270-1279.
- 126 Wang T, Niu G, Kortylewski M, *et al.* Regulation of the innate and adaptive immune responses by Stat-3 signaling in tumor cells. *Nat Med* 2004; **10**:48-54.
- 127 Kortylewski M, Kujawski M, Wang T, *et al.* Inhibiting Stat3 signaling in the hematopoietic system elicits multicomponent antitumor immunity. *Nat Med* 2005; **11**:1314-1321.
- 128 Kamath S, Buolamwini JK. Targeting EGFR and HER-2 receptor tyrosine kinases for cancer drug discovery and development. *Med Res Rev* 2006; **26**:569-594.
- 129 Xu H, Yu Y, Marciniak D, *et al.* Epidermal growth factor receptor (EGFR)-related protein inhibits multiple members of the EGFR family in colon and breast cancer cells. *Mol Cancer Ther* 2005; **4**:435-442.
- 130 Thomas CY, Chouinard M, Cox M, *et al.* Spontaneous activation and signaling by overexpressed epidermal growth factor receptors in glioblastoma cells. *Int J Cancer* 2003; **104**:19-27.
- 131 Dhesy-Thind B, Pritchard KI, Messersmith H, O'malley F, Elavathil L, Trudeau M. HER2/neu in systemic therapy for women with breast cancer: a systematic review. *Breast Cancer Res Treat* 2007; doi: 10.1007/s10549-007-9656-y.
- 132 Vincent PW, Bridges AJ, Dykes DJ, *et al.* Anticancer efficacy of the irreversible EGFR tyrosine kinase inhibitor PD 0169414 against human tumor xenografts. *Cancer Chemother Pharmacol* 2000; **45**:231-238.
- 133 Sriuranpong V, Park JJ, Amornphimoltham P, Patel V, Nelkin BD, Gutkind JS. Epidermal growth factor receptor-independent constitutive activation of STAT3 in head and neck squamous cell carcinoma is mediated by the autocrine/paracrine stimulation of the interleukin 6/gp130 cytokine system. *Cancer Res* 2003; **63**:2948-2956.
- 134 Meydan N, Grunberger T, Dadi H, *et al.* Inhibition of acute lymphoblastic leukemia by a Jak-2 inhibitor. *Nature* 1996; **379**:645-648.
- 135 Pikman Y, Lee BH, Mercher T, *et al.* MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. *PLoS Med* 2006; **3**:e270.
- 136 Pardanani A, Hood J, Lasho T, *et al.* TG101209, a small molecule JAK2-selective kinase inhibitor potently inhibits myeloproliferative disorder-associated JAK2V617F and MPLW515L/K mutations. *Leukemia* 2007; **21**:1658-1668.
- 137 Turkson J, Bowman T, Adnane J, *et al.* Requirement for Ras/Rac1-mediated p38 and c-Jun N-terminal kinase signaling in Stat3 transcriptional activity induced by the Src oncoprotein. *Mol Cell Biol* 1999; **19**:7519-7528.
- 138 Shuai K, Horvath CM, Huang LH, Qureshi SA, Cowburn D, Darnell JE Jr. Interferon activation of the transcription factor Stat91 involves dimerization through SH2-phosphotyrosyl peptide interactions. *Cell* 1994; **76**:821-828.
- 139 Turkson J, Ryan D, Kim JS, *et al.* Phosphotyrosyl peptides block Stat3-mediated DNA-binding activity, gene regulation and cell transformation. *J Biol Chem* 2001; **276**:45443-45455.
- 140 Turkson J, Kim JS, Zhang S, *et al.* Novel peptidomimetic inhibitors of signal transducer and activator of transcription 3 dimerization and biological activity. *Mol Cancer Ther* 2004; **3**:261-269.
- 141 Jing N, Li Y, Xu X, *et al.* Targeting Stat3 with G-quartet oligodeoxynucleotides in human cancer cells. *DNA Cell Biol* 2003; **22**:685-696.
- 142 Nagel-Wolfgram K, Buerger C, Wittig I, Butz K, Hoppe-Seyler F, Groner B. The interaction of specific peptide aptamers with the DNA binding domain and the dimerization domain of the transcription factor Stat3 inhibits transactivation and induces apoptosis in tumor cells. *Mol Cancer Res* 2004; **2**:170-182.
- 143 Song H, Wang R, Wang S, Lin J. A low-molecular-weight compound discovered through virtual database screening inhibits Stat3 function in breast cancer cells. *Proc Natl Acad Sci USA* 2005; **102**:4700-4705.
- 144 Bhasin D, Cisek K, Pandharkar T, *et al.* Design, synthesis, and studies of small molecule STAT3 inhibitors. *Bioorg Med Chem Lett* 2008; **18**:391-395.
- 145 Schust J, Sperl B, Hollis A, Mayer TU, Berg T. Stattic: a small-molecule inhibitor of STAT3 activation and dimerization. *Chem Biol* 2006; **13**:1235-1242.
- 146 Shuai K. Modulation of STAT signaling by STAT-interacting proteins. *Oncogene* 2000; **21**:2638-2644.
- 147 Shuai K, Liu B. Regulation of gene-activation pathways by PIAS

- proteins in the immune system. *Nat Rev Immunol* 2005; **5**:593-605.
- 148 Yasukawa H, Sasaki A, Yoshimura A. Negative regulation of cytokine signaling pathways. *Annu Rev Immunol* 2000; **18**:143-164.
- 149 Nagai H, Kim YS, Konishi N, *et al.* Combined hypermethylation and chromosome loss associated with inactivation of SSI-1/SOCS-1/JAB gene in human hepatocellular carcinomas. *Cancer Lett* 2002; **186**:59-65.
- 150 Yoshikawa H, Matsubara K, Qian GS, *et al.* 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* 2001; **28**:29-35.
- 151 Flowers LO, Subramaniam PS, Johnson HM. A SOCS-1 peptide mimetic inhibits both constitutive and IL-6 induced activation of STAT3 in prostate cancer cells. *Oncogene* 2005; **24**:2114-2120.
- 152 Yamada S, Shiono S, Joo A, Yoshimura A. Control mechanism of JAK/STAT signal transduction pathway. *FEBS Lett* 2003; **534**:190-196.
- 153 Woetmann A, Nielsen M, Christensen ST, *et al.* Inhibition of protein phosphatase 2A induces serine/threonine phosphorylation, subcellular redistribution, and functional inhibition of STAT3. *Proc Natl Acad Sci USA* 1999; **96**:10620-10625.
- 154 Grandis JR, Drenning SD, Chakraborty A, *et al.* Requirement of Stat3 but not Stat1 activation for epidermal growth factor receptor-mediated cell growth *in vitro*. *J Clin Invest* 1998; **102**:1385-1392.
- 155 Grandis JR, Drenning SD, Zeng Q, *et al.* Constitutive activation of Stat3 signaling abrogates apoptosis in squamous cell carcinogenesis *in vivo*. *Proc Natl Acad Sci USA* 2000; **97**:4227-4232.
- 156 Becker S, Groner B, Muller CW. Three-dimensional structure of the Stat3beta homodimer bound to DNA. *Nature* 1998; **394**:145-151.
- 157 Gray MJ, Zhang J, Ellis LM, *et al.* HIF-1alpha, STAT3, CBP/p300 and Ref-1/APE are components of a transcriptional complex that regulates Src-dependent hypoxia-induced expression of VEGF in pancreatic and prostate carcinomas. *Oncogene* 2005; **24**:3110-3120.
- 158 Loeffler S, Fayard B, Weis J, Weissenberger J. Interleukin-6 induces transcriptional activation of vascular endothelial growth factor (VEGF) in astrocytes *in vivo* and regulates VEGF promoter activity in glioblastoma cells via direct interaction between STAT3 and Sp1. *Int J Cancer* 2005; **115**:202-213.
- 159 Albanell J, Rojo F, Baselga J. Pharmacodynamic studies with the epidermal growth factor receptor tyrosine kinase inhibitor ZD1839. *Semin Oncol* 2001; **28**:56-66.
- 160 Han SW, Hwang PG, Chung DH, *et al.* Epidermal growth factor receptor (EGFR) downstream molecules as response predictive markers for gefitinib (Iressa, ZD1839) in chemotherapy-resistant non-small cell lung cancer. *Int J Cancer* 2005; **113**:109-115.
- 161 Burtneß B, Goldwasser MA, Flood W, Mattar B, Forastiere AA, Group ECO. Phase III randomized trial of cisplatin plus placebo compared with cisplatin plus cetuximab in metastatic/recurrent head and neck cancer: an Eastern Cooperative Oncology Group study. *J Clin Oncol* 2005; **23**:8646-8654.
- 162 Burtneß B. The role of cetuximab in the treatment of squamous cell cancer of the head and neck. *Expert Opin Biol Ther* 2005; **5**:1085-1093.