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The role of signal transducers and activators of transcription in colon cancer

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1. ABSTRACT

A family of latent cytoplasmic transcription factors, Signal Transducers and Activators of Transcription (STATs), convey signals from numerous cytokines and growth factors to the nucleus. Their expression and their activity have been shown to be perturbed in a variety of malignancies, including colorectal cancer. Among the STAT family members, oncogenic STAT3 has been shown to be constitutively activated or overexpressed in colon cancers. In contrast, the expression levels of STAT1 have been found to be reduced in transformed intestinal epithelial cells, consistent with tumor suppressor properties of STAT1. We showed that transformation of intestinal epithelial cells with KRasV12 is sufficient to downregulate the expression of STAT1. Because both STAT1 and STAT3 are important regulators of genes that are involved in cell survival (BCL-x, survivin, caspases) and cell proliferation (c-Myc, p21, cyclin D1), their deregulation significantly impacts the homeostasis of intestinal tissues. The critical role of STATs in oncogenesis and in inflammation merits further investigation of targeted inhibitors of STATs activity that could be used alone or in combination with conventional chemotherapy.

2. INTRODUCTION

Signal transducers and activators of transcription (STATs) belong to a family of latent cytoplasmic transcription factors that mediate the responsiveness of cells to a variety of growth factors and cytokines. Today, seven members (STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b and STAT6) have been identified. The STAT family has evolved by duplications of a single primordial gene and all the members share several structural domains. The N-terminal domain of STATs is conserved among family members and harbors a coiled-coil domain, followed by the DNA binding domain (DBD) (1) and an SH2 domain (2, 3). In contrast, the carboxy-terminal domain , which functions as a transcriptional activation domain (TAD) (4, 5), differs in sequence among the STATs and thus contributes to STAT specificity (Figure 1).

Although STATs have not been found to be mutated in cancer, the activity of several STAT family members, such as STAT1, STAT3 and STAT5, has been shown to be altered in tumors. STAT1, STAT3 and STAT5 have been shown to be hyperactivated through

Figure 1. Structure of STAT proteins.NH2: N terminal domain, C-C: coiled coil domain, DBD: DNA binding domain, LIN: linker, Y: conserved tyrosine phosphorylation site, TAD: transcriptional activation domain.

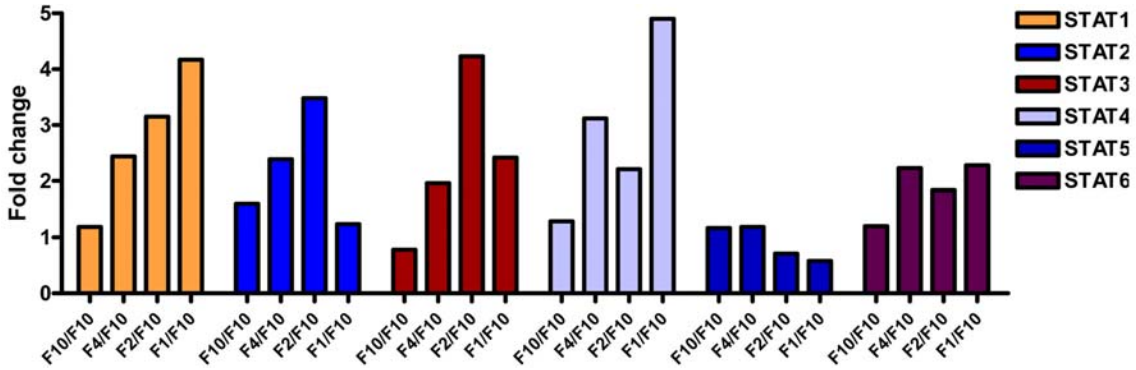

Figure 2. Expression of STATs along the crypt-villus axis in the mouse intestine. Fractions of intestinal epithelial cells were collected from the villus (F1), the crypt (F10) and from the space in between (F2 and F4) and gene expression arrays were performed as described in reference (9).

constitutive phosphorylation on tyrosine in a variety of tumors. In addition, the expression levels of STAT1 and STAT3 have been recently shown to be altered during transformation, which is significant, as both STAT1 and STAT3 can regulate the expression of a subpopulation of their targets genes in a phosphorylation-independent manner (6-8).

We recently adapted a method for sequential isolation of enterocytes from the villus tip to the crypts of mouse small intestine, and performed a genome-wide expression analysis on cells isolated from four different sections along the crypt villus axis (9). Cells from the crypt (fraction 10, F10) represent immature proliferating cells and they differentiate as they migrate and exit the proliferating zone in the villus (fraction 1, F1). We showed that in the mouse intestine the expression of STATs is regulated along the crypt-villus axis, suggesting that STATs may control the transition between proliferation, differentiation and apoptosis of intestinal epithelial cells. We found that the expression of STAT1, STAT2, STAT3, STAT4 and STAT6 increased as cells migrated from the crypt to the villus (Figure 2). In addition, two kinases, JAK1 and JAK2, that mediate the activation of STATs, were coordinately upregulated during differentiation (not shown, (9)). In contrast, the expression of STAT5A was the lowest in the differentiated compartment of the villus. Whether STATs contribute to maturation of epithelial cells that occurs along the crypt-villus axis remains to be determined.

3. SIGNALING BY STATS

STATs are latent cytoplasmic transcription factors that transduce signals from cytokines and growth factors to the nucleus and thereby regulate the expression of a variety of target genes. Activation of STATs in response to cytokine stimulation is mediated by the Janus family (JAK) tyrosine kinases. In mammals there are four members of the JAK family, JAK1, JAK2, JAK3 and TYK2. These kinases themselves have been shown to be constitutively activated in many hematopoietic malignancies and in certain carcinomas, and may therefore represent suitable targets for cancer therapy (10). Recently, activating mutations of JAK2 (JAK2V617F) have been found in almost all polycythaemia vera patients and in a subset of patients with essential thrombocythemia and myeloid metaplasia with myelofibrosis (11, 12), a mutation that occurs infrequently in other myeloid disorders (13). JAK2V617F has been shown to have constitutive tyrosine kinase activity, and is therefore able to transform hematopoietic cells and to activate JAK-STAT signaling.

The first step in cytokine signaling is binding of ligands to the cell surface receptors which results in receptor dimerization and in activation and trans-phosphorylation of Janus kinases (JAK) (Figure 3). This is followed by phosphorylation of STATs on a conserved tyrosine, their dimerization and translocation to the nucleus, where STATs bind DNA in a sequence specific manner, and modulate the expression of a variety of genes. STAT1 is transported to the nucleus after its association with importin alpha 5 (14). In the nucleus, STAT1 is dephosphorylated and actively exported back to the cytoplasm by the chromosome region maintenance 1 (CRM1) export receptor (15-19). TC45 has been identified as the nuclear tyrosine phosphatase for STAT1 and STAT3 (20, 21).

SOCS (suppressor of cytokine signaling) are cytokine inducible proteins that form a negative feed-back loop in cytokine signaling, and thereby ensure that signaling by cytokines remains transient. Eight members of the family have been described (CIS and SOCS1-SOCS7) and they interfere with cytokine signaling via distinct mechanisms. SOCS1 and SOCS3 inhibit the kinase

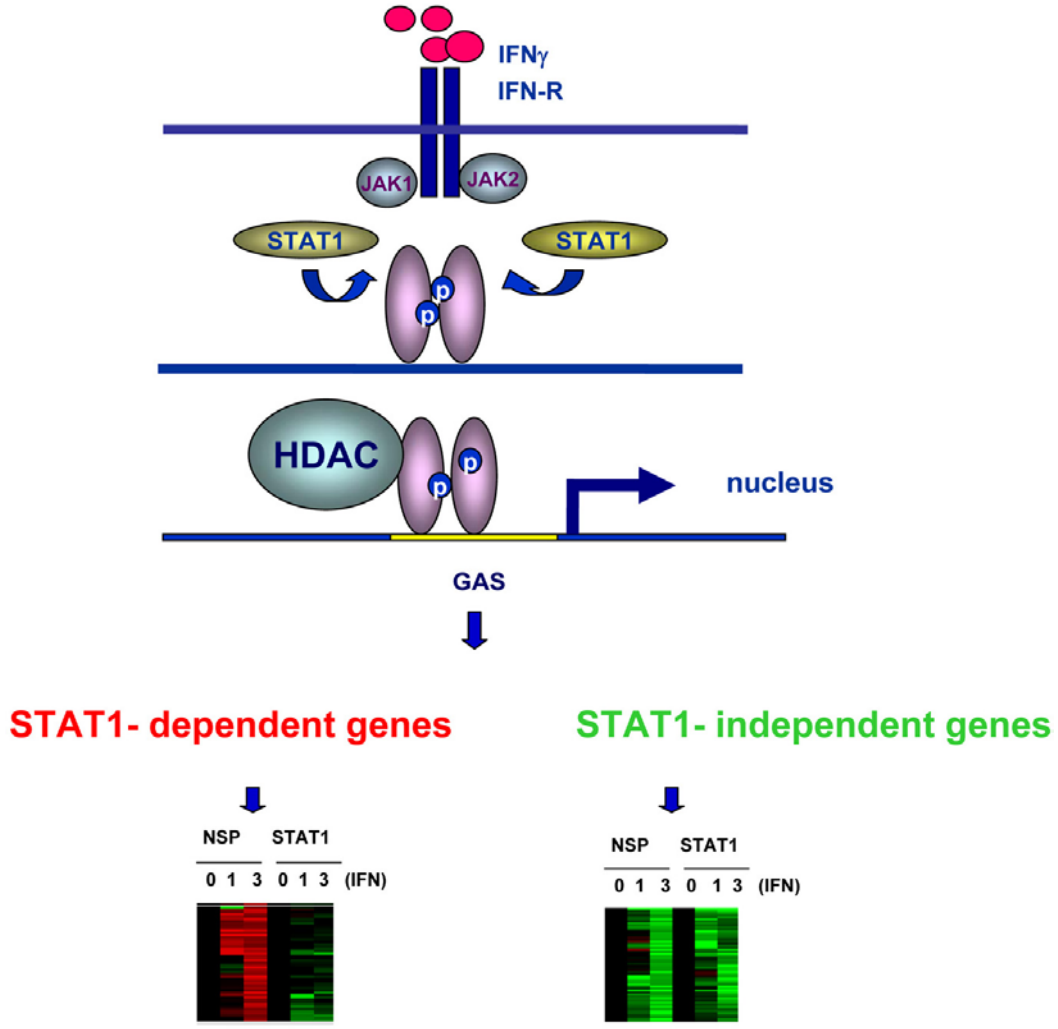

Figure 3. STAT1 dependent and STAT1 independent signaling by IFN gamma. Cells were transfected with nontargeting siRNA (NSP) or siRNA specific for STAT1, treated with IFN gamma for 1 or 3 hours and genome wide analysis was performed as described in ref (71). Note lack of induction, and intact inhibition of a subgroup of target genes upon silencing of STAT1.

activity of JAKs through direct binding to the activated JAKs (22), but SOCS3 can also bind to activated receptors, and CIS competes with STATs for binding to receptors (23). Gene knock-out studies have revealed prolonged activation of STAT1 and STAT3 and altered responses to IL-6 in the absence of SOCS proteins (24), confirming an important function of SOCSs in STAT signaling. Like STATs, the expression of SOCS proteins appears to be regulated along the crypt-villus axis in the mouse intestine. We showed that the expression of SOCS2 and SOCS4 is the highest in the crypt, while the expression of SOCS3 and SOCS5 increases as the enterocytes migrate from the crypt to the villus (data not shown, (9)).

Transcriptional inactivation of the SOCS-1 gene by hypermethylation has been shown to be involved in development and progression of gastric cancer (25) and aberrant methylation of the SOCS1 locus was observed in young colorectal cancer patients (26). SOCS3 is also frequently silenced by hypermethylation and has been shown to suppress cell growth in human lung cancer (27). Silencing of SOCSs expression through methylation is therefore an important mechanism of constitutive activation of the JAK/STAT pathway in cancer. SOCS2 has been shown to directly inhibit proliferation and to promote differentiation of intestinal epithelial cells (28). The weight and the length of the small intestine and colon were significantly increased in SOCS2 deficient mice, consistent with the anti-proliferative role of SOCS2 (28).

The signaling by STATs is regulated through the association of STATs with regulatory proteins, such as members of the PIAS family. There are four members of the family, PIAS1, PIAS3, PIASx and PIASy. Tyrosine phosphorylation of STAT1 is required for STAT1-PIAS1 interaction, demonstrating that PIAS1 is a specific inhibitor of STAT1 mediated gene activation.

The formation of Stat tetramers and higher order oligomers on DNA results from cooperative DNA binding

of Stat dimers that appears to be conserved throughout the Stat family, and plays an important role in signaling by STATs (29). For example, Stat1 defective in oligomerization of DNA-bound dimers was associated with prolonged interferon-induced nuclear accumulation (30).

Both STAT1 and STAT3 appear to shuttle from the cytoplasm to the nucleus in unstimulated cells and tyrosine phosphorylation appears to be dispensable for the nuclear localization of STATs in the absence of cytokine stimulation (31).

For example, although phosphorylation of STAT1 on tyrosine has been considered to be required for STAT1 function, a point mutant of STAT1 (Y701F), which cannot form dimers involving SH2-phosphotyrosine interactions, has been shown to bind DNA and to downregulate the constitutive expression of several genes, such as cyclin A, Hsp70, Bcl-x, and also to upregulate several genes, e.g. LMP2, Beta2M and caveolin 2 (32). Thus, STAT1 has the ability to regulate gene expression in a monomeric form. The mechanism whereby STAT1 modulates gene expression in the absence of tyrosine phosphorylation is, for now, unknown, but it likely involves cooperation of STAT1 with other proteins, or sequestration of transcription factors in the cytoplasm that physically associate with STAT1, such as Sp1 (33), HSF-1 (34) and others.

Like STAT1, STAT3 has been shown to regulate a subset of target genes in the absence of tyrosine phosphorylation (7). Recently, unphosphorylated STAT3 has been shown to drive the expression of genes such as RANTES, IL6, IL8, Met and MRas - genes that do not directly respond to phosphorylated STAT3 (8). The authors demonstrated that unphosphorylated STAT3 binds to NF-kappaB in competition with I-kappaB, and that this complex accumulates in the nucleus and drives the expression of target genes. Thus, it appears that STATs are not only classical nuclear transcription factors that regulate gene expression through binding to DNA, but also have a function as cytoplasmic signaling proteins.

4. REGULATION OF STAT ACTIVITY

The activity of STATs has been shown to be modified by phosphorylation, methylation, acetylation, and less understood modifications such as ubiqutinylation and sumoylation. All of these alterations have profound effects on the biological activity of STATs. The best studied of these modifications is tyrosine phosphorylation of STATs (2, 35, 36) which mediates dimerization of STATs, their nuclear translocation and DNA binding (37, 38). Serine phosphorylation has been shown to regulate the transcriptional activity of STAT1, STAT3 and STAT4 (39). Most constitutively active forms of STATs in tumors are phosphorylated on tyrosine, however constitutive serine phosphorylation of STAT1 and STAT3 has also been described in CLL (40).

Although phosphorylation of transcription factors remains a crucial posttranslational mechanism that regulates their transcriptional activity, several other modifications have been shown to regulate the activity of transcription factors. STATs have been recently shown to be acetylated on lysine 685 (the site which is conserved in STAT1, STAT2, STAT3, STAT4, STAT5 and STAT6) in response to cytokine stimulation, a modification that has been demonstrated to be crucial for STATs to form dimers and for their full transcriptional activity (41). Cytokine treatment has been shown to promote association of STAT3 with p300 (a histone acetylatransferase which adds the acetyl group to lysine), while HDAC appear to associate with STAT3 in the absence of cytokine stimulation.

STAT1 has been shown to be acetylated also on lysine 413 and 410 within STAT1 DNA binding domain (42). This modification depends on the balance between histone deacetylases (probably HDAC1 and HDAC3) and histone acetyltransferase, such as CBP. Acetylated STAT1 has been shown to interact with p65 subunit of the NF-kappa B and thereby inhibit NF-kappa B driven DNA binding, nuclear localization and its transcriptional activity (42). Thus, acetylation, like phosphorylation, modulates the interactions of STAT protein with other transcription factors and regulates their transcriptional activity.

In contrast, we and others have recently demonstrated that STAT1 signaling is blocked by inhibition of HDAC activity (43, 44). We showed that silencing of HDAC1, HDAC2 and HDAC3 through RNAi interference markedly decreased IFN gamma- driven gene activation and that, consistently, inhibitors of HDAC activity prevented STAT1 dependent gene activation (43). Finally, we demonstrated that HDAC inhibitors sensitized colon cancer cells to IFN gamma-induced apoptosis. Therefore, although the understanding of the role of STAT acetylation is far from complete, acetylation is emerging as a significant modification of STAT proteins that regulate their function. Is likely to play an important role also *in vivo,* as the levels of HDAC1, HDAC2 and HDAC3 are downregulated as epithelial cells migrate from the crypt to the villus axis in the mouse intestine (9, 45), a pattern of expression just the opposite to the expression of STATs (Figure 2). Finally, HDAC1, HDAC2 and HDAC3 have also been shown to be upregulated in a variety of human cancers, including colon cancer (9, 45).

The activity of STATs is regulated also through the interplay with other signaling pathways, such as Ras and Notch signaling, two pathways that play a significant role in maintaining the homeostasis in intestinal epithelium, and whose activities have been shown to be perturbed in colon cancer.

5. STAT1 AND COLON CANCER

STAT1 has been cloned as a transcription factor required for signaling by IFN (46). However, its role has expanded beyond being a transcription factor and several of it biological activities are mediated through transcription-independent processes. Endogenous IFN gamma and STAT1 form the basis of the surveillance system that controls the development of both chemically-induced and

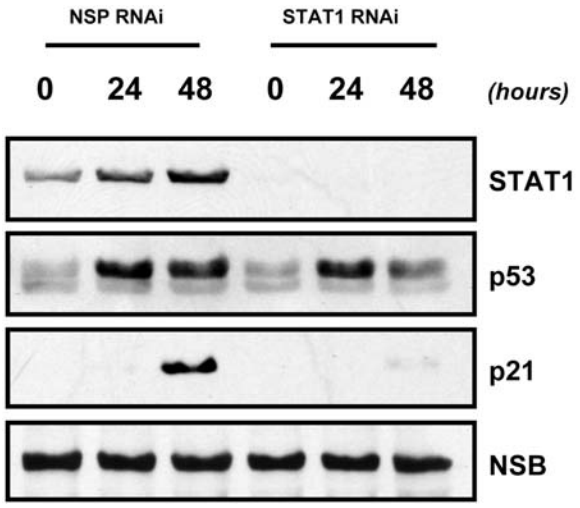

Figure 4. STAT1 dependent induction of p21 in response to camptothecin. The expression of STAT1, p53 and p21 was determined in Hke-3 cells transfected with nontargeting siRNA or siRNA specific for STAT1, that were left untreated or were treated with 10 nM camptothecin for 24 or 48 hours as indicated.

spontaneous tumors (47). Mice with a targeted deletion of the IFN gamma receptor or STAT1$^{-/-}$ mice display an increased incidence of tumor formation in response to methylcholanthrene (MCA), and STAT1 deficiency significantly accelerated formation of spontaneous tumors in mice with a p53 null background (47). Thus, although STAT1 deficiency is not sufficient to initiate tumor development, it has an important role in modulating tumor progression. In contrast, STAT1 deficient mice have been shown to be protected from leukemia, demonstrating that STAT1 can also act as a tumor promoter (48).

STAT1 is known to regulate cell proliferation. The regulatory region of the p21 gene harbors multiple STAT1 binding sites, suggesting that STAT1 supports transcriptional activation of p21 (49). Indeed, we and others have shown that the lack of STAT1 expression impedes the induction of p21, an inhibitor of cell cycle progression, in response to several agents, such as 5-Aza-CdR (50), inhibitors of HDAC activity (51), and camptothecin (Figure 4), establishing an important role of STAT1 in the expression of p21. It has been demonstrated that p21 deficiency increases the frequency and the size of intestinal tumors in Apc1638$^{+/-}$ mice which inherit a mutant allele of the Apc gene (52), confirming tumor suppressor properties of p21 and STAT1.

In addition to its role in proliferation, STAT1 also plays an important role in programmed cell death. However, its role in apoptosis appears to be more complex than its role in cell cycle progression. In a number of cell types STAT1 has been shown to promote apoptosis (53). Its ability to support the expression of several caspases (6) may at least in part underlie its pro-apoptotic ability. However, STAT1 has been shown to be overexpressed in radioresistant tumors and its overexpression has been shown to confer resistance from radiation-induced cell death (54). Consistent with these data, genome-wide analysis of genes associated with resistance of ovarian cancer cells to platinum compounds has identified STAT1 as a gene associated with resistance to cisplatin. Accordingly, overexpression of STAT1 in sensitive cell line conferred resistance to cisplatin (55).

We showed that colon cancer cells with silenced STAT1 expression via siRNA displayed increased sensitivity to apoptosis induced by inhibitors of HDAC activity (51). We demonstrated that STAT1 deficient colon cancer cells failed to activate p21 in response to butyrate treatment and that p21 protects cells from apoptosis induced by HDAC inhibitors. Thus, the ability of STAT1 to regulate the expression of p21 is important for its role in both proliferation and apoptosis.

Many tumor cells acquire resistance to IFN gamma, a major activator of STAT1 and important anti-proliferative cytokine. Some cancer cells have been shown to acquire resistance to IFN through deletion of the IFN gamma locus (56) or downregulation of the IFN gamma receptors (57). However, several cancers resistant to the anti-proliferative action of IFNs have been shown to have defects in the expression of transcription factors required for IFN signaling, including STAT1 (58-61). The reconstitution of STAT1 suppressed the tumorigenicity of RAD-105 cells *in vivo* , which correlated with decreased expression of proangiogenic molecules such as bFGF, MMP-2 and MMP-9, demonstrating that STAT1 acts as an important inhibitor of tumor angiogenesis and therefore negatively regulates tumor growth and metastasis (62).

A study by Karpf *et al* (63) demonstrated that STAT1, STAT2 and STAT3 are silenced by DNA methylation in the HT29 colon carcinoma cell line, resulting in inhibition of IFN-responsive genes and, importantly, in reduced sensitivity of cells to IFN alpha. Treatment of HT29 cells with 5'Aza cdR restored the expression of STAT1, STAT2 and STAT3, which coincided with transcriptional induction of IFN-responsive genes, and increased sensitivity to IFN alpha (63). Consistently, epigenetic silencing of STAT1 and of multiple IFN target genes by methylation has been shown in spontaneously immortalized Li-Fraumeni fibroblasts (64), suggesting that silencing of this growth suppressing pathway represents an early step in cellular immortalization and transformation. The methylase inhibitor 5-Aza-2-deoxycytidine (5-Aza-CdR) restored the expression of STAT1 and its target genes, and subsequently inhibited growth of human pancreatic cancer cells (50). In addition, microarray analysis has identified STAT1 and IFN-inducible genes and as a major transcriptional target of the human tumorigenic Papillomavirus type 31 (65). Likewise, cells transformed with the adenoviral E1A oncogene also exhibit reduced IFN signaling and display impaired IFN-gamma driven gene expression due to reduced cellular levels of STAT1 and p48 (66-68).

When we performed genome-wide analysis of gene expression in isogenic colon cancer cell lines that

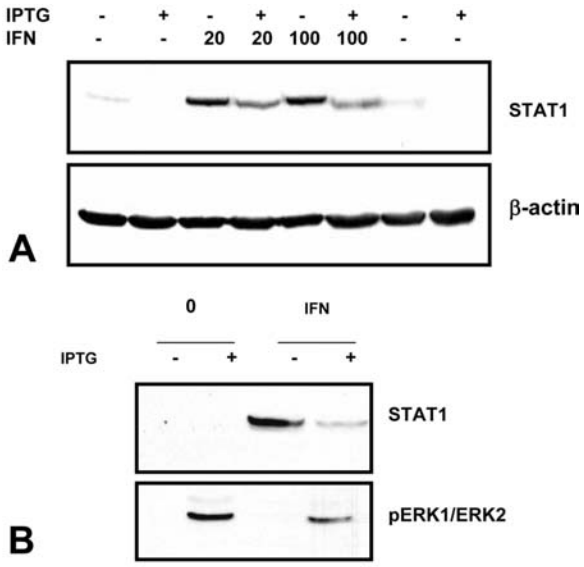

Figure 5. Activation of RasV12 interferes with the basal and IFN-induced expression of STAT1. IEC cells with IPTG-inducible kRasV12 (IECiKRas cells) (111) were treated with IFN-gamma in the absence or the presence of IPTG as indicated and the levels of total STAT1, pERK1/2 and beta-actin were determined by immunoblotting.

differ only by the presence of mutant Ras (69) we found that both STAT1 and a cluster of STAT1 responsive genes were reduced in cells that carry mutant Ras and subsequently demonstrated that activated Ras interferes with STAT1 dependent transcription (70). Our results therefore revealed a novel mechanism of negative regulation of STAT1 and its target genes. Indeed, we demonstrated that oncogenic transformation of intestinal epithelial cells with kRas is sufficient to significantly reduce the expression of STAT1 (70, 71) (Figure 5). Consistently, genome-wide analysis of a panel of 30 colon cancer cell lines revealed that the expression of STAT1 and of its target genes is specifically reduced in cell lines that harbor oncogenic kRas (70), while the levels of STAT3, STAT5 and STAT6 were not affected by the presence of the oncogenic kRas (data not shown).

IFN/STAT signaling has been shown to be inhibited during prostate tumor progression (72), which is frequently driven by oncogenic Ras activation (73). Because Ras mutations are found in a variety of human cancers, it is likely that they represent a frequent mechanism for the deregulation of STAT1 and STAT2 expression and subsequent reduced sensitivity of tumor cells to IFNs. In line with our data, activation of PI3K and MAPK, two downstream effectors of Ras signaling, have been shown to negatively regulate JAK/STAT signaling (74). Accordingly, tyrosine phosphorylation of STAT3, STAT5 and JAK2 was increased in melanoma cells treated with pharmacologic inhibitors of the MEK-ERK pathway or the PI3K pathway, confirming that constitutively active MAPK or PI3K interfere with signaling by JAK/STATs (74). Finally, a study by Huang *et al* demonstrated that colon cancer cell lines with wt k-ras display higher sensitivity to IFN gamma than cell lines harboring a mutant k-ras (75). We recently obtained data demonstrating that inducible expression of mutant k-ras in normal rat intestinal cells is sufficient to abrogate the anti-proliferative effect of IFN gamma (Klampfer, unpublished). Consistently, activation of the Ras/Raf/MEK pathway has been shown to inhibit IFN-mediated antiviral response (76).

What are the consequences of STAT1 deficiency? Although cytokines such as IFN gamma can transmit signals in the absence of functional STAT1 (43, 77-79), the absence of STAT1 profoundly alters the biological activity of several cytokines that play a role in inflammation and tumorigenesis. For example, IFN gamma inhibits c-Myc expression and inhibits proliferation in wt cells, but activates c-Myc and accelerates proliferation in STAT1 deficient cells (78). Similarly, IFN gamma induced apoptosis in a colon cancer cell line with intact JAK/STAT1 signaling, but actually promoted proliferation upon inactivation of STAT1 (80). The authors demonstrated that induction of the pro-apoptotic mediator TRAIL is STAT1 dependent and did not occur in STAT1 deficient cells. Endogenous IFN gamma promotes the host response to primary tumors and IFN gamma- insensitive tumors display increased tumorigenicity, increased cell proliferation, and can evade tumor surveillance mechanism (81). It is therefore likely that tumors with reduced STAT1 expression can escape tumor surveillance mechanisms more readily. Finally, STAT1 deficiency has been shown to result in enhanced activation of STAT3 in response to IFN gamma, an oncogenic STAT that is usually not induced by this cytokine (82). However, STAT1 deficiency in colon cancer cell lines that we examined did not affect the expression or the activity of STAT3 (not shown).

We showed that the inducible expression of iNOS and the production of NO in intestinal epithelial cells require STAT1 (83). In contrast, we demonstrated that IFN gamma inhibits the expression of COX-2 in intestinal epithelial cells through a pathway that requires the activity of JAKs, but bypasses STAT1. Furthermore, we performed global analysis of IFN responsive genes in intestinal epithelial cells in which STAT1 was specifically silenced by siRNA and confirmed that gene activation in response to IFN gamma generally requires STAT1, but that gene repression often involves pathways that circumvent STAT1 entirely, or include both STAT1 dependent and STAT1 independent signaling (71) (Figure 3). Our results therefore suggest that IFN gamma activates and represses gene expression via distinct pathways that can be separated, at least in part, by their requirement for STAT1.

Importantly, we showed that Ras transformation of epithelial cells alters the balance between STAT1 dependent and STAT1 independent signaling due to impaired expression of STAT1 in Ras transformed cells. This is likely to have important consequences for the local inflammatory response, and, therefore, the progression of intestinal tumors. Therefore, although Ras mutations and consequent reduction of STAT1 expression do not eliminate the responsiveness of cells to IFN gamma, they

are likely to alter the biological response to IFN significantly.

6. STAT3 AND COLON CANCER

A growing number of tumor cell lines and primary tumor tissues have been shown to express constitutively active STAT3, the oncogenic STAT (84-86). Over-activation of STAT3 (attained through constitutive tyrosine phosphorylation) has been ascribed to deregulation of the EGF pathway in head and neck cancer (87), overproduction of IL-6 in multiple myelomas (88), or deletion of PIAS or silencing of SOCS1, two negative regulators of STAT activity (89, 90). Constitutive activation of STAT3 has been also found in leukemias, lymphomas and several types of solid tumors, such as breast and prostate cancer.

Much less is known about the status of STAT3 and its biological role in colon cancer. Activation of Src, Fer and autocrine or paracrine secretion of IL-6 have been suggested to underlie the constitutive phosphorylation of STAT3 in colon cancer. Recently, STAT3 has been found to be constitutively activated in 72% of colorectal adenocarcinomas, but only in 18% of adenomas (91). The authors showed that phosphorylation of STAT3 significantly correlated with the depth of tumor invasion, venous invasion, lymph node metastasis and increasing stage according to the Duke's classification (91). Constitutive STAT3 activity was found in both de-differentiated cancer cells and in infiltrating lymphocytes, but was absent in normal epithelium (92). Intriguingly, cell lines derived from malignant cancers lost persistent STAT3 activity, suggesting that microenvironment plays a crucial role in STAT3 activation in colon cancer. Consistently, only a fraction of established colon cancer cell lines express P-STAT3 (Klampfer, unpublished). Nevertheless, inhibition of STAT3 activation in colon cancer xenografts slowed their growth, confirming that activated STAT3 contributes to tumorigenesis (92).

Inhibition of constitutive active STAT3 in two colon cancer cell lines, SW480 and HT29, induced caspase-3 dependent apoptosis and cell cycle arrest through downregulation of BCL-2, BCl-x, MCl-1 and cyclin D2 and upregulation of p21 (93). The authors attributed constitutive STAT3 activity in these cells to the activation of JAK3, which is somewhat surprising, as the expression of JAK3 has been thought to be restricted to lymphoid and myeloid cells. Analysis of primary tumors revealed that pSTAT3 was present in 12.5% adenomas and in 72.7% of carcinomas (93), suggesting that STAT3 activation plays a role in the progression of colon cancer.

Nuclear accumulation of Beta catenin is a key event in the development of colon cancer (94). Inhibition of STAT3 has been shown to induce translocation of Beta catenin from the nucleus to the cytoplasm and to significantly impair Beta catenin/TCF driven transcription, followed by the inhibition of cell proliferation (95). Although the authors did not detect physical interaction between Beta catenin and STAT3, they demonstrated that 90% of primary cancer tissues that were positive for pSTAT3 were also positive for nuclear Beta catenin and that patients with nuclear pSTAT3 had significantly lower disease-specific survival rate (95).

Activation of STAT3 has been shown to result in enhanced expression of matrix metalloproteinases MMP1, MMP3, MMP7 and MMP9, which is likely to underlie the increased invasiveness of colon cancer cells with activated STAT3 (96). TFF3 and VEGF, cytokines that are involved in proliferation, survival and angiogenesis of intestinal epithelial cells, have been shown to signal through STAT3 (97). Inhibition of STAT3 has been shown to interfere with TFF3 and VEGF-induced cellular invasion and to reduce the growth of colon cancer cell lines (97). Consistently, cucurbitacin, STAT3 specific inhibitor, restrained proliferation and induced apoptosis in colon cancer cell lines, and inhibited growth of colon cancer xenografts.

IL6 has been recently shown to be upregulated in a mouse model of colon cancer, the ApcMin mice. Significantly, Myd88 deficiency, which significantly reduced the number of tumors in these mice, also resulted in reduced expression of IL6 in intestinal mucosa (98). The status of STAT3 in these models of intestinal cancer remains to be determined. In addition to having STAT3 activated, some colon cancers have been shown to have STAT3 overexpressed (7), a significant finding as STAT3 can signal and regulate the expression of a variety of genes in the absence of phosphorylation (8).

Approximately 20% of human cancers are estimated to develop as a result of chronic intestinal inflammation. Inflammatory bowel diseases (IBD), such as ulcerative colitis and Crohn's disease, significantly increase the risk of development of colorectal cancer. IL-22 has been shown to synergize with IFN gamma in inducing iNOS in a STAT3 dependent manner (99), suggesting that STAT3 has an important role in regulating NO production, an important mediator of both inflammation and intestinal tumorigenesis. STAT3 has been shown to be strongly activated in human UC and Crohn's disease, as well as in DSS-induced colitis in mice (100) and STAT3 activity has been shown to correlate with the severity of Crohn's disease. In addition, IL6, a major activator of STAT3 in inflammation, has been shown to be overproduced in many types of colitis. STAT3 has been found to be constitutively activated in T cells isolated from colon mucosa of patients with Crohn's disease, but not from healthy controls (101), suggesting that STAT3 represents an important link between intestinal inflammation and colorectal cancer. Whether the presence of activated STATs can predict the risk of developing cancer in patients with IBD remains to be determined. SOCS1 deficient mice, which develop spontaneous colorectal carcinomas, have constitutively activated STAT1 and STAT3 present in the colons (102). Accordingly, STAT3 target genes such as BCl-x and c-Myc were upregulated in SOCS1 deficient mice. Intestinal specific inactivation of SOCS3 resulted in increased number of tumors following AOM/DSS treatment, accompanied by increased STAT1, STAT3 and NF-kappa B activation (103). Tumor infiltrating T lymphocytes have been shown to be associated with a better prognosis of

colorectal cancer patients (104). TGF beta production in TIL have been shown to suppress colon cancer growth through inhibition of IL6 production, a cytokine that is elevated in serum of colon cancer patients, and signals though STAT3 (105). The authors also demonstrated that blockade of IL6 signaling and STAT3 activation inhibits the formation of colon cancer in the model of DSS colitis.

How does unrestrained signaling by STAT3 promote tumor development? Constitutive signaling by STAT3 has been shown to result in upregulation of several anti-apoptotic genes, such as BCL-x and MCL1 and thereby inhibit apoptosis. In, addition recent studies also found that inhibition of STAT3 signaling leads to restoration of p53 function, further supporting the role of STAT3 in apoptosis (106). The ability of STAT3 to induce the expression of genes such as c-Myc and cyclin D1 and cylcin D2 is critical for its control of cell proliferation. Therefore, cells with constitutive activation of STAT3 have perturbed balance between cell proliferation and apoptosis, they proliferate uncontrollably and they resist apoptosis. In addition, STAT3 has been shown to be required for VEGF signaling in many types of cells, including endothelial cells, and inhibition of STAT3 signaling prevented VEGF-induced migration of endothelial cells and vessel formation (107), demonstrating a potent anti-angiogenic activity of STAT3 inhibitors.

Finally, persistent STAT3 signaling in tumor cells has been shown to restrain immune surveillance and to promote evasion of cancer cells from immune surveillance. Inhibition of STAT3 signaling in tumors increased the production of proinflammatory cytokines and chemokines, such as IFN gamma, TNF, IL-6 and IP-10 ("danger signals"), which activate innate immunity and ensure efficient tumor-specific T-cell response (108). Consistently, hyperactive STAT3 has been shown to interfere with functional maturation of dendritic cells (108, 109). Inhibitors of STAT3 activity are therefore likely to interfere with tumor progression at multiple levels.

7. CONCLUSIONS

Tumor cells acquire a number of capabilities, such as self-sufficiency in proliferation, insensitivity to negative growth factors, evading apoptosis, limitless replicative potential, sustained angiogenesis, invasion and metastasis (110). In addition, tumors develop mechanisms to evade recognition by the immune system. STATs are transcription factors whose activity and/or the expression have been shown to be deregulated in a variety of tumors, including colon cancer. Oncogenic mutations have been described in multiple tyrosine kinase pathways that all converge onto STAT proteins. Because STATs regulate most of the processes involved in tumor formation, they are emerging as promising targets for therapy. Indeed, blocking STAT3 in tumors has been shown to inhibit proliferation, induce apoptosis, suppress angiogenesis and stimulate immune response against tumors. As tumor cells become addicted to STAT signaling, the hope is that STAT inhibitors would be selective for transformed cells, and would have minimal effect on normal cells.

8. ACKNOWLEDGMENTS

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Abbreviations: IBD, inflammatory bowel disease; UC, ulcerative colitis; IFN, interferon; JAK, Janus kinase; STAT, signal transducer and activator of transcription

Key Words: IBD, inflammatory bowel disease; UC, ulcerative colitis; IFN, interferon; JAK, Janus kinase; STAT, signal transducer and activator of transcription, Review

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