

Molecular Pathways: IL11 as a Tumor-Promoting Cytokine—Translational Implications for Cancers

Matthias Ernst^{1,2} and Tracy L. Putoczki^{1,2}

Abstract

Emerging evidence suggests that cytokines produced by inflammatory cells act as rheostats to link the degree of wounding and local inflammation to epithelial cell survival, proliferation, and metabolism that collectively underpin the repair response. Among these cytokines, the GP130 family, which encompasses, among others, IL6 and IL11, plays a major role in orchestrating these complex processes through the activation of the latent signal transducer and activator of transcription 3 (STAT3) in the epithelium. However, many of the molecular mechanisms that govern and ensure effective epithelial wound healing and regeneration renewal also promote tumorigenesis and the progression of established cancers. Accordingly, GP130 cytokines endow the inflammatory tumor microenvironment with a capacity to promote "cancer hallmark capabilities" of the malignant epithelium, while simultaneously suppressing the antitumor response of innate and adaptive immune cells. Here, we review some recent insights derived from genetic and therapeutic inhibition of the IL6/IL11–GP130–STAT3 signaling cascade in the context of preclinical mouse models of cancer, which are likely to have implications to other solid malignancies. *Clin Cancer Res*; 20(22); 5579–88. ©2014 AACR.

Background

Tumor development and progression are multistep processes driven by the accumulation of genetic alterations in cancer-initiating (stem) cells. This is most powerfully illustrated for colorectal cancers, where more than 80% of sporadic human colorectal cancers are initiated by aberrant activation of the WNT pathway. During the progression from benign adenomatous polyps to frank metastatic cancer, neoplastic cells acquire additional mutations in the TGF β /SMAD4 signaling cascade, KRAS, and TP53 (1). A similar stepwise accumulation is also thought to underpin the development of intestinal-type gastric cancer (GC) with driver mutations including those in PI3K and ERBB2 (2). However, equally important to all stages of tumor initiation and progression are the contributions of the inflammatory tumor microenvironment (TME), where cancer cells conscript and corrupt normal, nonmutated stromal cells for their support (3, 4). Indeed, inflammation is a prevailing condition for initiation and progression of the majority of solid tumors. Although the adaptive immune response is important for immune surveillance and tumor suppression, chronic and overt inflammation more commonly stimulate

rather than prevent tumorigenesis. Cancers of the gastrointestinal tract illustrate this paradigm most powerfully, with compelling clinical correlations between inflammatory bowel disease (IBD) and colorectal cancers, or persistent *Helicobacter pylori* infection and GC (5), respectively. However, even sporadic cancers (e.g., those not associated with chronic inflammation) are embedded in a TME exhibiting the "smouldering inflammation" associated with wound-healing processes, including the recruitment and maturation of myeloid cells (4).

Among the panoply of cytokines involved with cancer-associated inflammation, traditionally emphasis has been given to IL1 β , IL6, IL23, and TNF α as the major cytokines. Their activities converge on NF- κ B and signal transducer and activator of transcription 3 (STAT3). These signaling hubs collectively mediate tissue repair, immune homeostasis, and balance the host's response to intestinal microbiota (6, 7). As a key coordinator of innate immunity and inflammation, uncontrolled activation of NF- κ B activity is involved in tumor initiation and progression in tissues in which cancer-related inflammation typically occurs, including the gastrointestinal tract and the liver (8). Like NF- κ B, which primarily promotes epithelial cell survival, persistent STAT3 activity in cancer and associated immune cells is a recurrent feature of many types of solid tumors and promotes both the survival and proliferation of neoplastic epithelium. Surprisingly, however, aberrant activation of these transcription factors in cancer cells more often reflects a cytokine-rich TME, rather than accumulation of oncogenic mutations in these pathways, or indeed the genes encoding these transcription factors (6). Collectively, these observations place the STAT3 signaling cascade at the center

¹The Walter and Eliza Hall Institute for Medical Research, Melbourne, Victoria, Australia. ²Department of Medical Biology, University of Melbourne, Melbourne, Victoria, Australia.

Corresponding Author: Matthias Ernst, The Walter and Eliza Hall Institute for Medical Research, 1G Royal Parade, Parkville, Melbourne, VIC 3052, Australia. Phone: 61-393452555; Fax: 61-393470852; E-mail: matthias.ernst@wehi.edu.au

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of an effective epithelial wound-healing reaction, and many of the upstream cytokines required for STAT3 activation are derived from myeloid cells. In this context, much attention has been given to the IL6/11 family of cytokines, which are defined by their shared use of the GP130 receptor.

IL11, a member of GP130 cytokine family

IL11 forms part of a family of the phylogenetically related cytokines IL6, leukemia inhibitory factor (LIF), oncostatin M (OSM), ciliary neurotrophic factor (CNTF), neuropoietin (NPN), cardiotrophin-1 (CT-1), cardiotrophin-like cytokine (CLC), IL27, and IL31 (6, 9). Originally discovered as a soluble activity in the supernatant of cultured fibroblasts that could stimulate the growth of IL6-dependent plasmacytoma cells and associated IgG production (10),

IL11 has most extensively been characterized as a cytokine with thrombopoietic activity (11). However, other biologic activities of IL11 include its capacity to stimulate erythropoiesis, activation of megakaryocytes, and to improve the outcomes of adaptive bone marrow transfers in mice (10). IL11 also regulates polarization of T cells and macrophages, promotes maturation of bone-resorbing osteoclasts and associated net bone loss, retains tissue "stem cell" phenotypes (10), and, through an obligatory requirement of the IL11 receptor α -chain, enables the formation of a decidua during pregnancies (12). Like most of the other cytokines in this family, IL11 is produced by a variety of cells in response to inflammatory stimuli.

All ligands of the GP130 cytokine family form receptor complexes with the ubiquitously expressed signal-

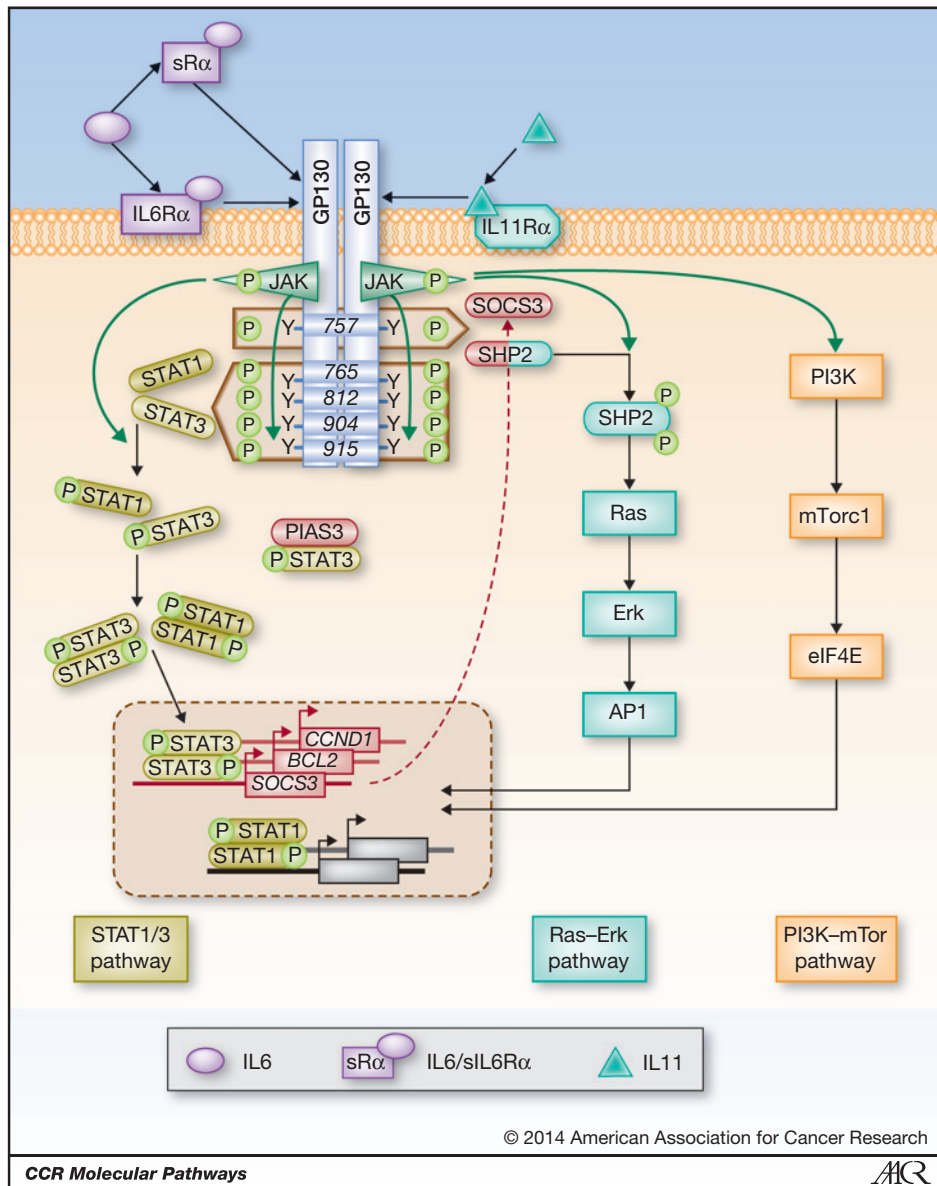


Figure 1. Simplified intracellular signaling events that occur in response to GP130 homodimerization as part of a hexameric complex comprising also two identical ligand-specific receptor α -subunits and two molecules of IL6 or IL11, respectively. A soluble version of the IL6R α (sR α) can confer trans-signaling in cells that do not express the transmembrane IL6R α receptor, and also activate the STAT1/3, Ras/Erk, and PI3K/mTorc1 signaling cascades. Tyrosine (Y) phosphorylation, mediated by GP130-associated JAK kinases, are indicated with green arrows; negative regulators of GP130 signaling are depicted in red, including the STAT3 target gene *Socs3*. In order for *Socs3* to exert its maximal negative regulatory activity on gp130 signaling, *Socs3* needs to simultaneously interact with the phosphorylated Y757 residue in gp130 and with the JAK kinases in a phospho-independent manner (78). Meanwhile, the tyrosine phosphatase SHP2 is required for activation of the Ras/Erk cascade, but SHP2 can also inhibit gp130 signaling through dephosphorylation of the receptor and the STAT proteins. STAT1 and STAT3 homodimers, formed in the cytoplasm in response to tyrosine phosphorylation (P) of STAT monomers, are actively transported into the nucleus (broken line) where they bind to DNA in a sequence-specific context to elicit transcription of target genes. The amino acid position of the Y residues required for binding and activation of STAT, SHP2, and *Socs3* are given for murine GP130 (please also refer to text).

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transducing GP130 (also called IL6st or CD130) subunit (Fig. 1). Most of these ligand-occupied receptor complexes consist of a α -receptors (IL6R α , IL11R α , CNTFR α , and CT-1R α) and signal-transducing β -receptors (GP130, LIFR, OSMR, IL27R/WSX-1, and IL31R/GPL), although LIF, OsM, and IL31 appear to form complexes comprising β -chains only (9). Binding of IL11 or IL6 to their respective ligand-specific α -receptors subunits results in sequential assembly with GP130 to form trimers with a geometry facilitating a cooperative transition into high-affinity, signaling-competent hexameric complexes comprising a 2:2:2 ratio of ligand, α -receptor and GP130 (13).

The intracellular domain of the GP130 and LIFR β -receptor subunit constitutively associate through their membrane-proximal proline-rich domains with the JAK family kinases JAK1, JAK2, and TYK2. Upon β -chain dimerization and close apposition of JAK kinases, they undergo cross-phosphorylation and activation, and phosphorylate highly conserved tyrosine (Y) residues in these β -chains to provide docking sites for the SH2-domain containing STAT1/3, SHP2, and SOCS3 proteins. Specifically, the four membrane-distal phosphotyrosines (pY) at amino acid positions 765, 812, 904, and 914 in mouse (767,814,905,915 in human) GP130 are required and sufficient to activate the latent transcription factors STAT3, and to a lesser extent STAT1 (6, 8). Subsequently, pY-STAT proteins form homo- and heterodimers, and induce gene transcription upon sequence-specific DNA binding often in conjunction with other DNA-binding proteins. The GP130-elicited signaling events are transient in nature, because one of the STAT3 target genes is the negative regulator SOCS3, which binds to the membrane proximal pY757 residue in mouse (pY759 in human) GP130 to mediate receptor ubiquitination and degradation. Intriguingly, this pY-residue also provides a binding site for SHP2, thereby also enabling GP130 to engage the RAS/ERK signaling cascade as well as providing, through its phosphatase activity, some negative regulation of GP130 signaling. Meanwhile, the cytoplasmic E3 SUMO protein ligase PIAS3 specifically binds to activated STAT3 to prevent its translocation to the nucleus. Although there is clear evidence for GP130-dependent activation of the PI3K-mTOR pathway, there are conflicting findings about its dependence on the SHP2 and/or STAT3 pathway (9). However, Thiem and colleagues (14) have shown in isolated cells and mice that GP130-dependent activation of the PI3K-mTOR1 pathway occurs in the absence of STAT3 activation and independently of Y-phosphorylation of GP130.

The relative contribution of these three pathways is dependent on their individual capacity to activate specific transcription factors (e.g., STAT3, AP1, and eIF4E), the cellular context in which signaling occurs, as well as a likely signaling threshold at the level of GP130 (9). For instance, GP130-dependent ERK activation results in AP-1-mediated induction of the gastric tumor-suppressor gene *TFF1* (15), but promotes differentiation of embryonic stem cells (16). However, the majority of cellular responses to IL6 family cytokines are mediated by STAT3,

and therefore in the context of tumorigenesis, it is useful to focus on the capacity of the GP130-JAK-STAT3 signaling cascade to affect the behavior of both, the neoplastic tumor cells and the nontransformed cells of the TME.

STAT3 signaling during epithelial homeostasis and tumor formation

Excessive STAT3 activity in most epithelial cell types promotes cell-cycle progression through induction of MYC, cyclin D1/D2, and downregulation of p21, as well as cell survival chiefly mediated by induction of proteins of the BCL2 family and XIAP (7, 8). Likewise, STAT3 induces matrix remodeling enzymes (e.g., MMP2, MMP7, and MMP9) and, through the associated breakdown of the extracellular matrix, can facilitate epithelial-to-mesenchymal (EMT) transition. Importantly, STAT3 stimulates angiogenesis through induction of VEGFA and hypoxia-inducible factor-1 (HIF1), induces a metabolic switch toward (cancer cell-specific) aerobic glycolysis (6, 7), and helps to maintain the self-renewal capacity of various types of tissue and cancer stem cells (17).

In the TME, GP130-dependent STAT3 signaling enables differentiation of Th17 cells at the expense of the production of inducible regulatory T cells (Fig. 2A; refs. 6, 7). Likewise, excessive STAT3 activity suppresses terminal differentiation of macrophages, dendritic cells, and polymorphonuclear leukocytes, thereby generally suppressing the host's immune response (18). On the other hand, activation of STAT3 in cancer-associated fibroblasts results in CCL2 expression that can stimulate a stem cell-specific phenotype (19) and further aids in the recruitment of monocytes.

As many of these activities are essential during wound healing, and because tumors share hallmarks of perpetual wound-healing reactions, one would predict that gastrointestinal tumors are highly sensitive to changes in STAT3 activity. Indeed, this is most powerfully illustrated with the colitis-associated colorectal cancer model in mice (20–23). Here, tumor formation is initiated largely by aberrant activation of the WNT signaling pathways (20), but tumor burden is enhanced by preconceived mutations in the GP130 signaling network that prolong activation of STAT3 expression (21–25). Meanwhile, genetic manipulations that reduce or ablate STAT3 expression or activation, or ablate IL6 production in myeloid cells reduces tumor incidence and growth (20, 23). Collectively, these findings suggest that epithelial GP130/STAT3 signaling may act as a "rheostat" for WNT signaling-mediated wound healing of the intestinal epithelium (26). Consistent with this, homozygous *gp130*^{ΔStat} mice, which harbor a mutation that renders GP130 incapable of activating STAT3, are prone to developing intestinal ulcers at sites of greatest mechanical stress (27). The phylogenetically highly conserved function of GP130/STAT3 signaling on the intestinal epithelium suggests that this pathway helps to confine rapid regeneration to sites of greatest damage and hence greatest inflammation (28). Incidentally, this mechanism is reinforced by

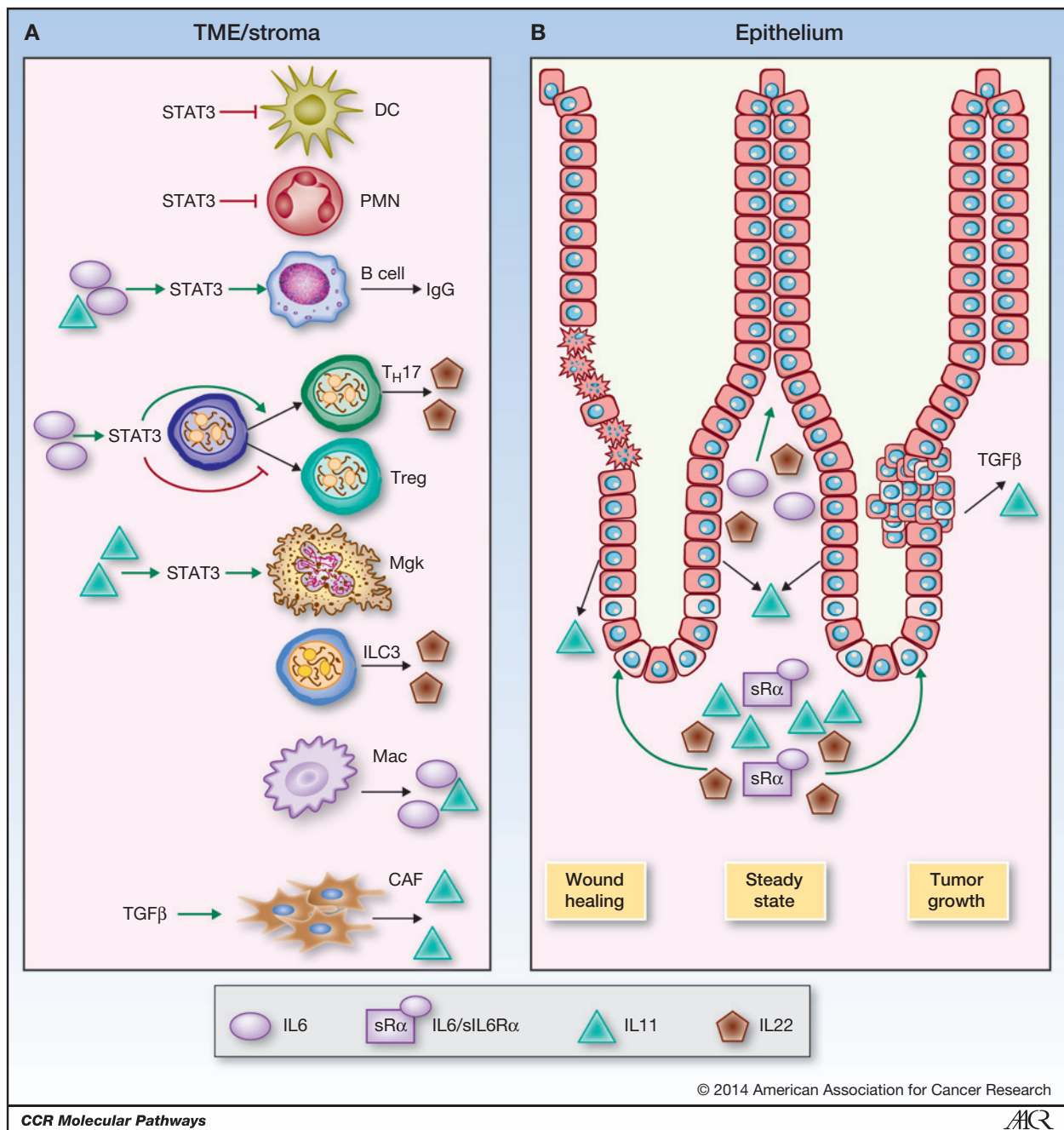


Figure 2. A, schematic representation of some of the major cell types in the stroma and the TME that respond to cytokines via STAT3 (green, stimulation; red, inhibition) and/or produce (black arrow) IL6, IL11, IL22, and other cell type-specific products. B, IL6-, IL11-, and IL22-dependent STAT3 signaling ensures effective wound healing (left side of diagram) and enables neoplastic growth of the gastrointestinal epithelium (right side of diagram). Meanwhile, these cytokines are individually dispensable for homeostatic renewal of the mucosa (middle part of diagram). A subset of the proliferating cells (light pink) toward the bottom of the crypt and within the tumors, preferentially respond (green arrows) to IL11 and a preformed complex comprising IL6 and the soluble IL6Rα ("trans-signaling"), rather than to IL6 through the membrane-bound IL6Rα ("conventional signaling"). Terminally differentiated cells (dark pink) express membrane-associated receptor for IL6 and IL11. Normal intestinal epithelium and its transformed counterpart, which can also produce TGFβ, are a source of IL11. The epithelial target cells for IL22, which is produced primarily by ILC3 cells (see A), have not been characterized. IL6, IL11, and IL22 also act on cells of the stroma and TME (see A). CAF, cancer-associated fibroblasts; DC, dendritic cells; ILC3, innate lymphoid cells; Mac, macrophages; Mgc, megakaryocytes; PMN, polymorphonuclear cells; Treg, regulatory T cells.

the capacity of the inflamed stroma to convert differentiated intestinal epithelium through a NF-κB-dependent mechanism to intestinal stem cells (29).

At least for epithelium at mucosal interfaces (e.g., in the intestine and lung), the IL22 family of cytokines has recently spurred considerable interest as an alternate

inducer of epithelial STAT3, because genetic reduction of IL22 availability in mice reduced their capacity to undergo intestinal wound healing (30). The directed activity of IL22, as a cytokine produced chiefly by Th17 lymphocytes and innate lymphocyte type (ILC) 3 cells (Fig. 2A), and acting exclusively on epithelial cells (31), provides a mechanism by which immune cells influence epithelial stem cells (17) and the wound-healing response (Fig. 2B).

IL11 promotes tumorigenesis

Although an ever increasing number of reports correlate excessive GP130/STAT3 signaling with the progression of various solid cancers, some of the most compelling functional evidence is derived from mouse models for gastrointestinal cancers. Originally spurred by observations of elevated IL6 levels in the circulating blood and intestines of patients with IBD (32), the availability of mouse models to reliably and reproducibly induce cancer in genetically modified backgrounds enabled the functional dissection of the contributions of this cytokine family to disease. In colitis-associated colorectal cancer models, excessive GP130-mediated STAT3 signaling in epithelial cells increases tumor burden, whereas epithelial ablation of STAT3 confers the opposite effect (21, 23). Surprisingly, genetic ablation of IL11R α had a much more profound effect than ablation of the gene encoding IL6, and completely protected mice from colitis-associated colorectal cancer development (22). Adaptive bone marrow transfer experiments revealed that this occurred independent of the capacity of bone marrow to respond to IL11. Likewise, in an inflammation-associated model of GC, which is based on excessive GP130 ligand-dependent STAT3 activation in *gp130^{Y757F}*-mutant mice, IL11 signaling rather than IL6 signaling enabled tumorigenesis. This raises the question as to whether the two cytokines, despite engaging GP130 homodimers, confer subtle differences to intracellular signaling events, or whether the response of distinct epithelial (stem) cell populations is limited to IL11. Different lines of circumstantial evidence favor the latter. Expression profiling, for instance, revealed that among the nonconcomitantly activated genes, IL11 stimulation yielded more of an epithelial-specific response, whereas IL6 stimulation favored a more hemopoietic cell-specific response (22). Indeed, IL6-dependent survival of inflammatory T cells (33), which perpetuate chronic inflammation, accounts for a mechanism by which IL6 promotes colitis-associated colorectal cancer in mice. Interestingly, tumor burden in this model could also be increased by administration of an IL6 hybrid protein that enabled trans-signaling, but not by conventional IL6 (23). The former refers to a process in which shedding of the extracellular domain of IL6R α enables formation of an IL6-soluble IL6R α complex, which can activate GP130 on cells that lack expression of the membrane-bound IL6R α . Collectively, these observations suggest a neoplastic epithelial subpopulation that lacks the membrane-bound IL6R α and where GP130/STAT3 signaling promotes

cellular expansion independently of conventional IL6 signaling (Fig. 2B).

The dominance of IL11 over IL6 as the cytokine enabling tumor outgrowth from the gastrointestinal epithelium also extends into clinically more prevalent situations of cancers that occur independently of overt inflammation and/or colitis. In *Apc^{Min}* mice, a model for human familial adenomatous polyposis, IL11R α deficiency confers significantly more protection than IL6 deficiency. On the other hand, IL17A, which is a cytokine that is produced for instance by IL6-induced Th17 cells during infection with enterotoxigenic *Bacteroides fragilis*, promotes tumorigenesis in *Apc^{Min}* mice (34). Indeed, a contribution of these adaptive immune cells may also be sustained by microbially activated, tumor-infiltrating myeloid cells that produce IL23 to reinforce Th17 cell polarization (35). Finally, we have also found that IL11R α deficiency confers significant protection against chemically induced colonic tumorigenesis (22) in models that mimic the sporadic forms of colorectal cancer that most profoundly affects the human population. Recently, Calon and colleagues (36) have also identified a role for IL11 in sustaining survival and growth of colorectal cancer cells at metastatic site in the liver, where cancer cell-derived TGF β promotes the production of IL11 by cancer-associated fibroblasts (Fig. 2). The latter link has also been purported as one of the mechanism whereby the release of matrix-bound TGF β facilitates the osteolytic capacity of breast cancer cells through their release of IL11 and other cytokines that stimulated the formation of bone-resorbing osteoclasts (37). Accordingly, low IL11 levels correlate with reduced resistance toward chemotherapy of human breast tumors and prolonged relapse-free survival (38). Meanwhile, IL11 expression by malignant breast epithelium at the primary site causes "encapsulation" by fibroblasts and promotes a desmoplastic reaction (39).

Besides the long suspected involvement of IL11 in bone metastasis, IL11 signaling may facilitate the growth of osteosarcoma through upregulation of their IL11R α (40), and IL11 is suspected to facilitate cell motility in human chondrosarcoma cells (41). IL11R α is also expressed by the neoplastic glandular epithelium of the endometrium (42), and IL11 is elevated in endometrial adenocarcinoma and more abundant in uterine lavage, and is thought to facilitate disease progression through STAT3-mediated cell migration (43). Although IL6 promotes growth of cervical carcinoma cell lines, and STAT3 predicts poor prognosis for this cancer, IL11 expression is elevated in endocervical polyps (44). However, it is still unclear whether IL11 plays a major role in gynecologic cancers, as IL11 expression appears low in ovarian carcinoma and the expression of IL11R α remains comparable between malignant and nonmalignant ovarian epithelium (45). Meanwhile, elevated serum IL11 has been suggested as a potential tumor marker for prostate cancer progression (46), and human prostate carcinoma shows increased IL11R α expression (47), resulting in a report that this receptor may serve as a candidate target for metastatic prostate cancer (48). Although elevated serum IL6 level is associated with reduced survival of patients with advanced

pancreatic cancer, IL11 is also highly expressed in pancreatic cancer cell lines and primary tumors (49). Given the prominent role of KRAS mutations in pancreatic cancer, and the observation that this may result in excessive release of IL6 and IL11 of these cells (50), it remains to be established whether these cytokines activate STAT3 expression, as part of an autocrine/paracrine feed-forward loop (51). There is also emerging evidence for a role of IL11 in liver cancer, and akin to its role in gastrointestinal cancers, possibly evolving from a wound-healing mechanism. Upon acute liver injury and exposure to reactive oxygen species, we have shown in mice that IL11 is produced and released from dying hepatocytes to induce STAT3 activation in adjacent healthy hepatocytes to mediate their compensatory proliferation (52). Accordingly, this wound-healing reaction is impaired in IL11R α -deficient mice (52), and expression of IL11, but not IL6, is increased in inflammatory hepatocellular adenomas (53). Incidentally, inflammatory hepatocellular adenomas are also frequently associated with constitutively active somatic mutations of GP130 or of STAT3 (53, 54). Moreover, a recent report confirmed in the liver the tight correlation between TGF β and IL11 signaling referred to before. Specifically, excessive expression of a TGF β -dependent long-noncoding RNA resulted, via suppression of miR200, in the induction of the EMT regulators Zeb1/2 and, via stabilization of IL11 mRNA, in the induction of STAT3 activity (55). As a result, EMT facilitated invasion of tumor cells, while persistent IL11/STAT3 activity enabled cancer cell colonization at distant sites.

Collectively, there is an emerging picture for a role of IL11 to mediate a tumor-promoting signaling outcome in neoplastic cells, which is likely to be very similar to that assigned to IL6. However, we predict that in compartments where tissue regeneration and wound healing is critical for tissue homeostasis, IL11 has become a preferred regulator. We hypothesize that the more restricted distribution of IL11-responsive cells over a broader distribution of IL6-responsive cells may have been the driving force to select for IL11 as the regulator for STAT3-dependent wound healing and tissue regeneration. We would argue that this allows these processes to be restricted and confined to sites of injury rather than being widely induced by cells expressing the membrane-bound IL6R α and becoming activated by systemic increase of IL6 during generic acute-phase responses. Incidentally, it has been observed that neutrophils are a major source of sIL6R α , which they release upon apoptosis at the site of wounding (56), thereby maximizing STAT3 activation via IL6 trans-signaling in epithelial cells that lack the transmembrane IL6R α .

Clinical-Translational Advances

The compelling functional and correlative evidence for an involvement of the GP130-JAK-STAT3 signaling cascade alongside IL6 and IL11 in many epithelial cancers in mice and human patients, respectively, makes these mole-

cules potential therapeutic targets. On the basis of the available evidence, targeting of each individual component is likely to confer specific benefits that need to be evaluated with side effects conferred by on- and off-target activities.

Ligands

To date, the biggest efforts have been devoted to the development of antibody-based reagents to interfere with binding of IL6 to its cognate receptor, including antibodies directed against IL6 (i.e., siltuximab) or IL6R α (i.e., tocilizumab) approved for rheumatoid arthritis, juvenile idiopathic arthritis, and Castleman disease (57). Interestingly, long-term administration of IL6 mAbs resulted in accumulation of the antibody-bound ligand in the serum, although this was circumvented with the development of IL6R α mAb. Meanwhile, the most frequent side effects associated with IL6R α therapy were infections and impaired liver function (58). IL6 mAb treatment showed efficacy in advanced-phase clinical trials for various solid cancers as single-agent therapy and in an adjuvant setting (57). Notwithstanding the limited efficacy of genetic IL6 ablation discussed above, IL6R α administration also resulted in a reduction of colitis-associated intestinal cancer in mice (23). However, at this stage, clinical efforts with antibodies that target IL6 signaling in the gastrointestinal tract are limited to curbing the excessive inflammation in Crohn's disease. In addition to antibodies, smaller IL6-targeting entities, including single-chain antibodies (nanobodies) and avimers, are currently undergoing clinical testing (59) and may help to overcome some of the accessibility limitations of conventional antibodies in the context of the constricted tumor vasculature. In an attempt to specifically target IL6 trans-signaling, a human Fc fragment linked to the extracellular domain of GP130 is currently being tested for IBD (60). The underlying premise of this sGP130Fc reagent is to selectively target (inflammatory) effects mediated by IL6 trans-signaling (61), including those on cancer-promoting (epithelial) cells that are revealed only upon administration of the Hyper-IL6 fusion protein comprising IL6 linked to the extracellular portion of sIL6R α (23, 51).

At this stage, neutralizing antibodies for IL11 or IL11R α have not been reported. However, systemic administration of IL11-Mutein, a peptide-based signaling antagonist that binds the IL11R α with 20 \times higher affinity than IL11, reduced colitis-associated and mutagen-induced sporadic colorectal cancer in mice (22). Likewise, IL11-Mutein also reduced inflammation-associated GC in *gp130*^{Y757F} mice, and, when administered systemically to the host, also the growth of gastrointestinal cancer cell line xenografts (22). Meanwhile, excessive IL11 production, associated with *H. pylori* infections, is associated with hyperplasia of the gastric epithelium (61), and elevated IL11 expression has been described in various other genetic mouse models for GC (62). Unlike IL6, which is primarily produced by myeloid cells, we also identified the gastrointestinal epithelium as a

source for IL11 (22). Indeed in human colon and GCs, expression of IL11 correlates better with profound STAT3 than with IL6 expression (22). In these situations, excessive STAT3 activity is detected in the core and invasive tumor edge, and in turn correlated with poorer survival of these patients (63).

As mentioned above, IL11 is best recognized for its capacity to stimulate megakaryocyte maturation and platelet production, for which it has been given an FDA approval (10). However, at least in mice, hemopoietic steady-state parameters are neither affected in IL11R α -deficient animals (10), nor following long-term administration of IL11-Mutein (22), consistent with findings that platelet homeostasis is chiefly maintained by thrombopoietin signaling (64). Because these observations do not preclude that anti-IL11 signaling therapy may affect local concentration of platelets, it is intriguing to consider that the association of circulating tumor cells with platelets is believed to be important for cancer cell extravasation (65).

JAK inhibitors

Spurred by the need to develop inhibitors for the constitutive active V617F JAK2 mutation associated with myeloproliferative diseases, many JAK inhibitors have been designed that are based on competitive binding with ATP (66). Accordingly, all of these inhibitors show some activity against the closely related JAK1, JAK2, and TYK2 proteins with usually significantly less activity toward JAK3, the major kinase associated with the common γ -chain and underlying severe combined immunodeficiency (67). Thus, where adverse (on target) effects were reported for JAK inhibitors, they included anemia, neutropenia, and thrombocytopenia (68). Predictably, JAK1/2 inhibitors, such as AZD1480, impair GP130 signaling, and reduce colitis-associated colon and inflammation-associated GC development in mice (69). We have shown that this occurs through inhibition of STAT3 signaling in the epithelium (69), while others found that AZD1480 also reduced the abundance of tumor-associated myeloid cells and tumor angiogenesis (70). Consistent with our observation that GP130 signaling becomes rate-limiting for the expansion of *Apc*-mutant intestinal tumors, therapeutic JAK inhibition also reduced tumor burden in *Apc*^{Min} mice, without affecting homeostatic intestinal renewal of the mucosa (71).

STAT3 inhibition

Because STAT3 provides a signaling node both in cancer cells and in the many (immune) cell components of the TME (6, 7), many molecular approaches have been explored to inhibit its activity, including inhibition of STAT3 phosphorylation, dimerization, DNA binding, and expression. We have shown that STAT3 antisense oligonucleotides reduce gastric tumor burden in *gp130*^{Y757F} mice (51) and a similar molecule (AZD9150) is currently undergoing a phase I/IIb trial for advanced hepatocellular carcinoma. Meanwhile, an oral inhibitor that suppresses STAT3/5 phosphorylation is undergoing phase II testing

for the same indication in Japan and shows efficacy in various blood-borne cancers that are driven by STAT3 or STAT5 (72). Although the complete absence of STAT3 is associated with impaired immune response, the existence of naturally occurring germline-null mutations in STAT3 in humans, suggest that the associated Hyper IgE syndrome only occurs if STAT3 activity is below 25% (73). On the other hand, systemic ablation of one STAT3 allele is sufficient to impair tumor growth in the stomach of *gp130*^{Y757F} mice (74) and also to reduce colonic tumor burden that arise in response to mutations in *Apc* (71). Collectively, this suggests therapeutically favorable differences in sensitivity to STAT3 signaling between normal and tumor cells.

Nonmutated pathway

In analogy to the concept of oncogene addiction in tumor biology, Luo and colleagues (75) have coined the term "non-oncogene addiction" to refer nonmutated pathways that become rate-limiting for the proliferation of neoplastic cells. We have identified GP130-dependent activation of the PI3K-mTor pathway as a requirement for neoplastic gastrointestinal epithelium to proliferate in response to excessive STAT3 activation. Thus, at least in the context of IL11-GP130-STAT3-dependent inflammation-associated gastric or colitis-associated colorectal cancer, tumor growth can be suppressed by intracellular targeting of either the STAT3 or the PI3K-mTor pathway (27). Interestingly, mTor signaling has been proposed to be rate limiting for the tumor growth in *Apc*^{Min} mice (76), although mTor inhibitors have proved to be disappointing as single agents for the treatment of sporadic colorectal cancers (77). This is in notable contrast to activation of the unmutated IL11-GP130-STAT3 pathway playing a rate-limiting function for the growth of *Apc*-mutant intestinal tumors (71), suggesting nonoverlapping mechanism by which mTor and STAT3 signaling limit the growth cancers driven by mutations in the WNT pathway.

Conclusions

STAT3 provides a central signaling node in the gastrointestinal epithelium and many of the surrounding (immune) cells to ensure an effective intestinal wound-healing response. Many of these hardwired mechanisms also underpin the vital homeostatic renewal of the extensive intestinal surface that provides a barrier to the outside. It appears that cancer cells have evolved to exploit these mechanisms to help ensure their survival and growth. GP130-dependent STAT3 activation is now firmly recognized as providing a functional link by which the inflammatory response of the TME supports many of the cancer-enabling hallmarks. Complementing evidence from *in vitro* and *in vivo* models, and supported by correlative studies in patients, strongly suggests that gastrointestinal tumors become highly addicted to persistent STAT3 activity. The latter occurs predominantly from the oversupply of cytokines rather than the less frequent acquisition of somatic mutations in the associated signaling cascades. Therefore, the activity of IL6, IL11, and

the GP130-associated JAK kinases acquire rate-limiting gate-keeper roles that are amenable for therapeutic targeting, with compounds already in advanced clinical trials for other indications.

Disclosure of Potential Conflicts of Interest

M. Ernst and T.L. Putoczki have ownership interest (including patents) in CSL Limited.

Authors' Contributions

Conception and design: M. Ernst, T.L. Putoczki

Writing, review, and/or revision of the manuscript: M. Ernst, T.L. Putoczki

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M. Ernst, T.L. Putoczki

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