STAT3 and STAT1 mediate IL-11–dependent and inflammation-associated gastric tumorigenesis in gp130 receptor mutant mice

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Deregulated activation of STAT3 is frequently associated with many human hematological and epithelial malignancies, including gastric cancer. While exaggerated STAT3 signaling facilitates an antiapoptotic, proangiogenic, and proliferative environment for neoplastic cells, the molecular mechanisms leading to STAT3 hyperactivation remain poorly understood. Using the gp130Y757F/Y757F mouse model of gastric cancer, which carries a mutated gp130 cytokine receptor signaling subunit that cannot bind the negative regulator of cytokine signaling SOCS3 and is characterized by hyperactivation of the signaling molecules STAT1 and STAT3, we have provided genetic evidence that IL-11 promotes chronic gastric inflammation and associated tumorigenesis. Expression of IL-11 was increased in gastric tumors in gp130Y757F/Y757F mice, when compared with unaffected gastric tissue in wild-type mice, while gp130Y757F/Y757F mice lacking the IL-11 ligand–binding receptor subunit (IL-11Rα) showed normal gastric STAT3 activation and IL-11 expression and failed to develop gastric tumors. Furthermore, reducing STAT3 activity in gp130Y757F/Y757F mice, either genetically or by therapeutic administration of STAT3 antisense oligonucleotides, normalized gastric IL-11 expression and alleviated gastric tumor burden. Surprisingly, the genetic reduction of STAT1 expression also reduced gastric tumorigenesis in gp130Y757F/Y757F mice and coincided with reduced gastric inflammation and IL-11 expression. Collectively, our data have identified IL-11 as a crucial cytokine promoting chronic gastric inflammation and associated tumorigenesis mediated by excessive activation of STAT3 and STAT1.

Introduction

Gastric cancer (GC) is the second most common cause of cancer-related deaths worldwide (1). Although the molecular mechanisms underlying the pathogenesis of GC remain to be fully defined, a causal correlation has been established between GC and chronic inflammation triggered by the Gram-negative bacterium Helicobacter pylori (2), which colonizes the epithelium of the gastric mucosa. Meanwhile, several genetic factors have also been linked to GC, including accumulation of (epi-)genetic alterations in p53 (3), tff1 (4), E-cadherin (5), and Cox2 (6), as well as genes encoding components of the TGF-β/Smad signaling cascade (7–10). More recently, persistent activation of the latent STAT3 has been proposed as a prognostic factor for poor survival of GC patients (11), while excessive STAT3 activation promotes the growth and survival of gastric cells (12, 13) and is associated with increased gastric angiogenesis (11). These observations are consistent with the capacity of STAT3 to induce expression of genes that promote angiogenesis (e.g., Vegf), cell-cycle progression (e.g., cyclin D1), and cell survival (e.g., Bcl-xL, survivin) (14, 15). Although no mutations have been identified in the human STAT3 gene, persistent STAT3 activity is associated with numerous hematologic malignancies and tumors of epithelial origin (11, 12, 14–16). This suggests that other (epi-)genetic events along the STAT3 signaling cascade(s) cause its activation, including oversupply of ligands for STAT3-activating cytokine or tyrosine (Y) kinase receptors. In this respect, elevated expression of IL-11, a member of the IL-6 cytokine family that activates STAT3, is a recurrent finding in human GC (17).

Besides IL-11, the IL-6 cytokine family comprises IL-27, IL-31, leukemia inhibitory factor (LIF), oncostatin M (OSM), and ciliary neurotrophic factor (CNTF), among others, and plays a crucial role in hematopoiesis, the immune response, inflammation, and cancer (18–20). IL-6 family cytokines execute their actions via the common signal-transducing receptor β-subunit gp130. In particular, binding of IL-6 or IL-11 to their specific receptor α-subunits, IL-6Rα and IL-11Rα, respectively, induces gp130 homodimerization, while other family members engage heterodimeric receptor complexes comprising gp130 and either the LIF receptor, OSM receptor, or WSX-1 β-subunits (21). Ligand-induced β-subunit dimerization subsequently activates receptor-associated JAK, leading to phosphorylation of cytoplasmic Y residues (22). Phosphor-
ylation of the 4 carboxyterminal Y residues in gp130 is required and sufficient for the activation of STAT3 and to a lesser extent of STAT1 (23). Meanwhile, the membrane-proximal phosphorylated Y residue in gp130 (pY757 in mouse, pY759 in human) provides a binding site for the tyrosine phosphatase Shp2 (24), which upon phosphorylation mediates activation of the Ras/ERK and PI3K/Akt pathways (25).

We have previously demonstrated the physiological importance of tightly regulated gp130 signaling by STAT3-mediated transcriptional induction of the negative regulator SOCS3, which competes with Shp2 for binding to pY757/pY759 in gp130 (26). In particular, mice homozygous for a phenylalanine (F) knockin substitution of Y757 (gp130(Y757F/Y757F)) spontaneously develop gastric hyperplasia characterized by the presence of distinct tumors in the antral region of the glandular stomach, with histological features reminiscent of intestinal-type, metaplastic gastric tumors in humans (27). The molecular consequences of the Y757F substitution mutation, which simultaneously abolishes binding of SOCS3 and Shp2 to gp130, are exaggerated activation of STAT3 and STAT1 (14) and impaired activation of the Shp2/Ras/ERK and Shp2/PI3K pathways (27). We previously proposed a molecular mechanism whereby tumor formation is initiated through impaired expression of the Shp2/Ras/ERK target and gastric tumor suppressor gene tff1 (27). Meanwhile, the progressive growth of macroscopic lesions depends on STAT3 hyperactivation, because reduction of the pool of cellular STAT3 available for activation by monomeric gene ablation alleviates gastric tumorigenesis in the corresponding gp130(Y757F/Y757F)Stat3−/− compound mutant mice (14). The latter effect was partially attributed to the impairment of TGF-β-mediated cytostatic effects that arise from the transcriptional induction of Smad7 by STAT3 hyperactivation (14). Smad7, a key negative regulator of TGF-β-mediated signaling (28), is also induced by excessive STAT1 activation, which is associated with chronic infection with the gastric class I carcinogen H. pylori (29).

Our observation reported here of marked gastric overexpression of IL-11 in gp130(Y757F/Y757F) mice parallels recurrent observations of elevated IL-11 levels in human GC (17) and suggests that IL-11 may be the primary gp130-acting cytokine causing gastric tumorigenesis. Indeed, our present findings that gastric inflammation, hyperplasia, and tumor formation are suppressed in IL-11−/− unsponsive gp130(Y757F/Y757F)Il11ra1−/− compound mutant mice coincides with normalized activation of STAT3 and its target genes. Furthermore, genetic as well as pharmacological strategies to specifically reduce STAT3 activation in gp130(Y757F/Y757F) mice impaired gastric IL-11 expression and prevented the growth of gastric tumors. In addition, we also uncovered a hitherto unknown role for STAT1 in promoting gastric disease, since germline deletion of Stat1 in gp130(Y757F/Y757F) mice partially suppressed the growth of gastric tumors concomitant with a reduction in gastric inflammation and STAT3 activation. Collectively, these data implicate IL-11 on a genetic level as the primary cytokine driving gp130-mediated gastric tumorigenesis and provide important evidence for the potential oncogenic cooperation between STAT3 and STAT1 in the progression of inflammation-associated gastric tumors.

**Results**

**Increased expression of IL-6 family cytokines in gastric tumors of gp130(Y757F/Y757F) mice.** Elevated expression of IL-11 in human gastric adenocarcinomas (tumors) has recently led to the proposal that IL-11 may serve as a potential biomarker and oncogenic stimulus for human GC (17). We therefore quantified IL-11 expression in tumor-bearing antral tissues from adult gp130(Y757F/Y757F) mice (between 10 and 14 weeks of age) by quantitative RT-PCR and immunoblot analyses. Gastric IL-11 mRNA and protein levels were elevated approximately 30-fold and 15-fold, respectively, in tumors of gp130(Y757F/Y757F) mice compared with unaffected tissue from age-matched gp130(WT/WT) mice (Figure 1A and B). Meanwhile, gene expression for the gp130-acting cytokines IL-6 and LIF was elevated by only 5-fold in these lesions (Figure 1A). By contrast, expression of the ligand-specific receptors α-subunits IL-6Rα and IL-11Rα, as well as of the β-subunit gp130, in gastric tissue...
remained unaffected and was comparable in gp130<sup>Y757F/Y757F</sup> and gp130<sup>+/-</sup> mice (Figure 1C).

**IL-11 receptor signaling is essential for gastric tumorigenesis in gp130<sup>Y757F/Y757F</sup> mice.** Based on this augmented IL-11 expression in gp130<sup>Y757F/Y757F</sup> gastric tumors (Figure 1, A and B), we explored the notion of a possible causal link between the gastric phenotype and exaggerated signaling emanating from the IL-11 receptor complex. We inactivated IL-11 signaling by generating compound mutant gp130<sup>Y757F/Y757F</sup>Il11ra1<sup>–/-</sup> mice, which lack the widely expressed IL-11–specific ligand-binding receptor α-subunit (30), and found that the stomachs of these compound mutant mice were tumor free and indistinguishable in size and cellular morphology from the stomachs of age-matched wild-type mice even when beyond 14 weeks of age (Figure 2, A and B). Notably, gastric sections of gp130<sup>Y757F/Y757F</sup>Il11ra1<sup>–/-</sup> mice were characterized by the absence of chronic inflammatory (lymphoplasmacytoid) cell infiltrates in the submucosa and lamina propria (Figure 2C) and did not show any expansion of proliferating (proliferating cell nuclear antigen–positive [PCNA-positive]) gastric cell populations (Figure 2D). By contrast, genetic deletion of Il6 in gp130<sup>Y757F/Y757F</sup>Il6<sup>–/-</sup> mice failed to suppress tumorigenesis (31) and had no ameliorating effect on the inflammatory cell infiltrates and associated gastric hyperplasia characteristically found in gp130<sup>Y757F/Y757F</sup> mice (Figure 2). Collectively, these data identify an essential role for IL-11 receptor–mediated signaling in the initiation and progression of chronic gastritis and gastric adenomatous hyperplasia.

**Absence of gastric tumors in gp130<sup>Y757F/Y757F</sup>Il11ra1<sup>–/-</sup> mice correlates with reduced IL-11 expression and STAT3 activation.** Since genetic reduction of the level of STAT3 activation in gp130<sup>Y757F/Y757F</sup>Stat3<sup>+/–</sup> mice impaired the growth of gastric lesions (14), we next investigated whether the extent of gastric STAT3 activation in gp130<sup>Y757F/Y757F</sup>Il11ra1<sup>–/-</sup> mice was affected. Indeed, basal STAT3 tyrosine phosphorylation and expression of the bone fide STAT3 target gene Socs3 (32) were similar in gp130<sup>Y757F/Y757F</sup> and gp130<sup>Y757F/Y757F</sup>Il11ra1<sup>–/-</sup> mice and markedly reduced when compared with those in tumor-bearing gp130<sup>Y757F/Y757F</sup>Il6<sup>–/-</sup> and gp130<sup>Y757F/Y757F</sup> mice (Figure 3, A and B). Strikingly, the proportion of phosphorylated STAT3 was less pronounced in the forestomach (fundus) than in the antrum (Figure 3A), and this was reflected by the modest increase in Socs3 expression observed in the fundus when compared with its antral expression (Figure 3B). Gastric expression of Il11 mRNA was reduced to wild-type levels in tumor-free gp130<sup>Y757F/Y757F</sup>Il11ra1<sup>–/-</sup> mice but remained elevated in gp130<sup>Y757F/Y757F</sup>Il6<sup>–/-</sup> mice (Figure 3C). These observations suggest that IL-11 was responsible for gastric STAT3 hyperactivation and that Il11 may constitute a potential STAT3 target gene (see below) whose augmented expression...
correlates with gastric disease. We also observed a tight correlation between the formation of gastric lesions and elevated antral expression of several STAT3 target genes implicated in the pathogenesis of human GC and promoting cellular processes crucial for tumorigenesis, namely cell-cycle progression (cyclin D1 and c-myc) (15), survival (Bcl-xL and survivin) (12, 14), and extracellular matrix degradation (Mmp13) (33) (Figure 3C). Collectively, the above data suggest a compelling correlation among the extent of gastric IL-11 expression, STAT3 phosphorylation, and the expression level of critical STAT3 target genes implicated in tumorigenesis.

Pharmacological interference of STAT3 hyperactivation in gp130Y757F/Y757F mice reduces gastric tumor burden. Since our analyses suggest that the IL-11/STAT3 signaling axis contributes to the development of gastric tumors in the gp130Y757F/Y757F mouse model, we next explored whether therapeutic targeting of Stat3 expression (and therefore of its activity) would alleviate established disease. We therefore treated adult gp130Y757F/Y757F mice systemically with 2′-methoxyethyl-modified (2′-MOE-modified) antisense oligonucleotides (ASOs) directed against mouse Stat3 (34). The 2′-MOE modification of the five most 5′ and most 3′ nucleotides provides the STAT3-ASO gapmers with favorable pharmacokinetic characteristics, including suppression of renal clearance due to plasma protein binding and an associated tissue half-life exceeding 14 days (35–37). The physiological responsiveness of individual mice to a 4-week STAT3-ASO treatment regime was confirmed by the rescue of thrombocytosis in gp130Y757F/Y757F mice (Figure 4A), since we have previously shown that normal platelet counts are retained throughout the lifetime of gp130Y757F/Y757F Stat3+/– mice (38). Indeed, we observed a dose-dependent reduction in blood platelet counts in STAT3-ASO–treated gp130Y757F/Y757F mice to numbers observed in gp130+/+ mice (15.5 × 10^8 platelets/ml prior to treatment compared with 6.7 × 10^8 platelets/ml 2 days after the last ASO treatment; Figure 4A). Importantly, STAT3-ASO treatment also resulted in a smaller overall burden of gastric tumors (Figure 4B) with a reduced abundance of larger (>3 mm in diameter) lesions. Histologically, STAT3-ASO treatment coincided with increased interglandular cell necrosis, fragmentation of glandular (epithelial) structures (Figure 4C), and reduced epithelial staining for the proliferation marker BrdU (Figure 4D).
Furthermore, treatment with the STAT3-specific ASO, but not the sequence-scrambled control ASO, resulted in a reduction of total STAT3 expression, with a concomitant reduction in the abundance of phospho-STAT3 within the gastric lesions (Figure 4E) and other tissues (data not shown). We also observed reduced expression of the STAT3 target gene Socs3 as well as of the Il11 gene in gastric lesions of STAT3-ASO–treated mice (Figure 4F).

STAT3-mediated gastric tumorigenesis is independent of antitumor immunity. While the above data are compatible with a cell-autonomous response of gastric epithelium to STAT3-ASO, tumor-mediated STAT3 hyperactivation in tumor-infiltrating immune cells may inhibit antitumor immunity (39). We therefore assessed cellular localization of activated STAT3 (pY-STAT3) by immunohistochemistry. In stomachs of wild-type mice, pY-STAT3 localized to cells in both the basal portion of the pyloric glands of the antrum and acid-secreting tubules of the corpus, as well as the foveolar epithelium of the gastric antrum and corpus. Although this pattern was retained throughout disease-free regions of stomachs of gp130Y757F/Y757F mice, widespread pY-STAT3 immunoreactivity was also observed throughout the epithelial elements of the adenosas and became prominently associated with dense, peritumoral inflammatory cell infiltrates (Figure 5A).

Due to the global presence of the mutation in gp130Y757F/Y757F mice and associated STAT3 hyperactivation in hematopoietic cell compartments (29, 36), we next assessed whether the tumor-suppressing effect of STAT3-ASO arose primarily from its activity on gp130-dependent STAT3 hyperactivation in mutant hematopoietic cells. Since 100% of naive gp130Y757F/Y757F mice show macroscopically visible gastric lesions by 6 weeks of age (40), we reconstituted lethally irradiated adult gp130Y757F/Y757F mice with bone marrow from either eGFP transgene–marked gp130+/+ mice or autologous gp130Y757F/Y757F mice. Two months later, we found nearly complete hematopoietic reconstitution with eGFP-positive gp130−/− mice or autologous gp130Y757F/Y757F mice. Following a further 4-week treatment with STAT3-ASO, we observed a similar reduction in gastric tumor burden in naive gp130Y757F/Y757F and reconstituted gp130Y757F/Y757F mice, irrespective of the genotype of the donor bone marrow (Figure 5B). These observations therefore suggest that the inhibitory effect of STAT3-ASO on the growth of gastric lesions arises independently...
of hematopoietic cells and that maintenance of these tumors is primarily dependent on the continuous availability of hyperactivated STAT3 in nonhematopoietic (i.e., tumor) tissue.

**STAT1 hyperactivation contributes to gastric tumorigenesis.** We next aimed to genetically dissect the relative contribution of gp130-mediated STAT3 relative to STAT1 hyperactivation to gastric tumorigenesis in gp130<sup>Y757F/Y757F</sup> mice (14). Since there is an absolute requirement for STAT1 to mediate type I and II IFN responses (41), we analyzed stomachs of IFN-responsive gp130<sup>Y757F/Y757F</sup> Stat1<sup>-/-</sup> mice (Supplemental Figure 1; supplemental material available online with this article; doi:10.1172/JCI4944DS1), as well as of IFN-unresponsive gp130<sup>Y757F/Y757F</sup> Stat1<sup>-/-</sup> mice. Surprisingly, either partial or complete global depletion of the STAT1 pool resulted in stomach sizes in the corresponding compound mutant mice that were noticeably smaller than those of age-matched gp130<sup>Y757F/Y757F</sup> mice (Figure 6A). Furthermore, macroscopically visible tumors were only observed in a proportion of these compound mutant mice when compared with the 100% penetrance of gp130<sup>Y757F/Y757F</sup> mice age 6 weeks or older (Figure 6B). In addition, the abundance of CD45-positive inflammatory cells was reduced in inter glandular spaces and submucosal aggregates in stomachs of gp130<sup>Y757F/Y757F</sup> Stat1<sup>-/-</sup> and gp130<sup>Y757F/Y757F</sup> Stat1<sup>-/-</sup> mice compared with gp130<sup>Y757F/Y757F</sup> mice (Figure 6, C and D) and reminiscent of our previous observation in gp130<sup>Y757F/Y757F</sup> Stat3<sup>−/−</sup> mice (42). While the complete absence of functional STAT1 protein in gp130<sup>Y757F/Y757F</sup> Stat1<sup>-/-</sup> mice failed to alleviate tumor burden as effectively as after monoallelic Stat3 inactivation in gp130<sup>Y757F/Y757F</sup> Stat3<sup>−/−</sup> mice (Figure 6, A and B), these data further support a correlation between the presence of inflammatory infiltrates and the occurrence of gastric lesions in gp130<sup>Y757F/Y757F</sup> mice.

To begin to explore the hierarchical relationship between STAT3- and STAT1-dependent mechanisms underlying gastric disease in gp130<sup>Y757F/Y757F</sup> mice, we compared gastric expression of (tumor-associated) STAT3 and STAT1 target genes. Surprisingly, depletion of STAT1 in gp130<sup>Y757F/Y757F</sup> Stat1<sup>-/-</sup> mice reduced basal Stat3 expression and activation (i.e., phosphorylation; Figure 7A). This observation is likely to be of functional significance, because reduced STAT3 phosphorylation in gp130<sup>Y757F/Y757F</sup> Stat1<sup>-/-</sup> mice correlates with reduced expression of the STAT3 target genes Socs3, cyclin D1, survivin, and Mmp13 (Figure 7B). In addition, we found reduced expression of Il11 and the anticytostatic TGF-β signaling inhibitor Smad7 in gastric lesions of gp130<sup>Y757F/Y757F</sup> Stat1<sup>-/-</sup> mice when compared with gp130<sup>Y757F/Y757F</sup> mice. However, the complete absence of STAT1 was often less effective in reversing enhanced expression of STAT3 target genes in gp130<sup>Y757F/Y757F</sup> mice than observed following monoallelic ablation of Stat3. Although genetic restriction of the pool of cellular STAT3 in gp130<sup>Y757F/Y757F</sup> Stat3<sup>−/−</sup> mice also reduced basal STAT1 expression and activation (albeit to a lesser extent than observed in the reciprocal situation; Figure 7C), we observed impaired basal expression of the STAT1 target genes 2′-5′-Oas and Ip10 (Figure 7D). Collectively, these data are suggestive of a mechanism whereby the level of gp130-dependent activation of STAT3 and to a lesser extent STAT1 functionally mediates and determines the extent of IL-11-driven gastric disease.

**STAT1- and STAT3-mediated transcriptional activation of Il11.** Since the above data reveal a correlation between the basal activation levels of STAT1 and STAT3 and expression of Il11 in gastric lesions of gp130<sup>Y757F/Y757F</sup> mice, we next examined whether experimentally biased activation of either STAT1 (by IFN-α) or STAT3 (by IL-11) would directly promote Il11 expression in vivo. As expected, administration of IFN-α to gp130<sup>Y757F/Y757F</sup> mice predominantly activated the prototypical STAT1-response gene 2′-5′-Oas, while IL-11 primarily induced the STAT3-response gene Socs3 (Figure 8A). Gastric Il11 expression was strongly induced by IL-11, and to a lesser degree after IFN-α administration. The relative potency of response between the 2 cytokines correlated with their capacity to promote STAT3 tyrosine phosphorylation (Figure 8B), and the observed residual STAT3 activation by IFNs was consistent with previous reports (43, 44). Consistent with constitutively elevated activation of STAT3 (and to a lesser extent of STAT1) in gp130<sup>Y757F/Y757F</sup> mice, gastric lesions from these unchallenged mice also showed elevated expression of Socs3 and Il11, as well as a trend for increased 2′-5′-Oas expression.

In order to further characterize a potential autocrine signaling mechanism by IL-11, we tested the responsiveness of a 1.5-kb frag-
ment upstream of exon 1 of the Il11 gene promoter. We transiently expressed the corresponding pIL-11-luc reporter construct in wildtype mouse embryonic fibroblasts (MEFs) exposed to IFN-α (which activates STAT1 and STAT2), IFN-γ (which only activates STAT1), or the designer cytokine HYPERIL-6, comprising a fusion protein of IL-6Rα and IL-6 (which activates STAT3 and STAT1). Transfections were also performed in parallel using the STAT-specific reporter constructs pISRE-luc and pAPRE-luc to monitor for specificity of transactivation by STAT1 and STAT3, respectively. Since stimulation with all 3 cytokines induced IL-11–luc activity but only resulted in reciprocal activation of 1 of the 2 STAT-specific reporters (Figure 8C), we conclude that Il11 can serve as a direct target for STAT3 and to a lesser extent for STAT1. Indeed, nucleotide inspection of the murine Il11 promoter reveals three 5′-GACN₃GAA and one 5′-ATCN₃GAA sequence responsive to IL-6/STAT3 signaling and corresponding to sites II and III in the γ-fibrinogen gene (45), as well as one 5′-TTAN₃GAA sequence responsive to STAT1 (46) (Figure 8D). Collectively, these data suggest the existence of a positive feedback signaling loop whereby IL-11–mediated hyperactivation of STAT3 and STAT1 in gp130Y757F/Y757F mice augments the transcriptional activation of Il11.

Discussion
The gp130Y757F/Y757F mouse is a validated, reproducible, and genetically defined model for gastric tumorigenesis and shares many of the histological hallmarks of inflammation-associated intestinal-type GC in humans (14, 27, 40). Here, we extend the characterization of the gp130Y757F/Y757F mouse as an informative preclinical disease model by providing genetic proof for IL-11’s pivotal role in mediating aberrant STAT3 and STAT1 activation. In turn, aberrant activation of these latent transcription factors promotes atrophic gastritis that culminates in dysplastic and frequently metaplastic epithelial transformation and the outgrowth of distinct adenomatous polyps (27, 40). Furthermore, we demonstrate in an endogenous tumor model, for the first time to our knowledge, that therapeutic interference aimed at reducing the pool of systemic STAT3 available for activation ameliorates gastric tumor burden. This mechanistic insight is likely to be of clinical relevance, since recurring characteristics of human GC include elevated Il11 expression (47) and aberrant STAT3 activation, the latter being associated with poor patient survival outcome (11, 48).

The shared use of gp130 as the receptor signaling subunit for an entire family of cytokines together with the “silent” nature of the
Y757F substitution mutation in the absence of ligand has made it difficult to assign individual cytokines to specific pathologies in gp130Y757FY757F mice. Our findings that the genetic absence of functional IL-6 or IL-11 signaling in gp130Y757FY757F mice selectively suppresses the development of a systemic pan-inflammatory response or gastric inflammation–associated tumorigenesis, respectively, enables experimental compartmentalization of phenotypes in the corresponding compound mutant mice. Strikingly, gp130Y757FY757FIl1ra1–/– mice lacked inflammatory cell infiltrates in the stomach despite their persistent presence throughout most other organs, including liver, lung, and kidney (B.J. Jenkins and M. Ernst, unpublished observations). Conversely, despite the general absence of inflammatory cells from the latter organs in gp130Y757FY757Fo14-1 mice (B.J. Jenkins and M. Ernst, unpublished observations), submucosal infiltrates remained associated with the gastric tumors in these mice. These findings not only highlight the specific requirement of IL-11 to promote and maintain gastric inflammation and tumorigenesis in wild-type hosts (14). The mechanism underlying this observation relates to the finding that constitutive STAT3 activity in these STAT genes. Aberrant activation of the corresponding proteins, however, coincides with (and possibly results from) a variety of other genetic or biochemical alterations. For instance, overproduction and supply of ligand, mutations in receptor and (receptor-associated) cytoplasmic tyrosine kinases, or (epi-)genetic silencing of their negative regulators (15) account for events upstream of STATs that result in their persistent activation. Meanwhile, inappropriate expression of STAT-interacting proteins that alter the transcription-inducing activity of STATs (49), negative regulators of STAT dimerization (50), or STAT dephosphorylation (51) can modify the capacity of STATs to induce target gene expression. On the other hand, genetic data from mice suggest that normal physiological responses are unaffected by partial depletion of the cellular STAT pools in the corresponding heterozygous knockout animals. Collectively, these data suggest the likelihood a favorable therapeutic window to target disease-associated STAT hyperactivation in humans.

Here, we present evidence that therapeutic interference with STAT3 expression reduced the pool of endogenous STAT3 available for activation and ameliorated two of the gp130Y757FY757F mouse model’s independent disease phenotypes, namely thrombocytosis and inflammation-associated gastric tumorigenesis. It has been suggested that targeting STAT3 not only interferes with its cancer cell–autonomous properties, which is attributable to transcriptional induction of genes that promote cell-cycle progression, survival, and angiogenesis (15), but also elicits a potent “bystander” effect by inducing death of tumors cells that have not been reached by STAT3 inhibitors (39). The mechanism underlying this observation relates to the finding that constitutive STAT3 activity in tumor cells promotes immune evasion by tumor-specific production of cytokines (including IL-6 and IL-10) that activate STAT3 in adjacent stroma and cells of the immune system. In a tumor xenograph model, for instance, the immunosuppressive effect of aberrant STAT3 activation was particularly evident on tumor-infiltrating immune cells (e.g., dendritic cells, T cells, NK cells,
neutrophils) and the tumor-specific adaptive immune responses (52). Consequently, ablation or inhibition of STAT3 activity in host hematopoietic cells restored antitumor immunity (39). However, our observations that STAT3-ASO treatment resulted in reduced gastric tumor burden in gp130Y757F/Y757F mice irrespective of whether their immune cells carried the gp130(Y757F) mutation suggests a mechanism whereby the therapeutic benefit of STAT3 inhibition is unlikely to be mediated exclusively by an antitumor immune response. Indeed, the adaptive immune system appears to have a negligible effect on progression of tumorigenesis in gp130Y757F/Y757F mice, since the appearance of gastric lesions remained largely unaltered when these mice were crossed onto a Rag1-deficient background (31). Furthermore, STAT3 hyperactivation in the gp130Y757F/Y757F model promotes rather than suppresses a systemic pan-inflammatory response, which is reminiscent of emerging links between persistent STAT3 activation and inflammation-associated cancers of the liver (53) and colon (54, 55).

Our observation that systemic ablation of Stat1 ameliorated tumor growth in gp130Y757F/Y757F mice is surprising in light of reports that STAT1 mediates IFN-dependent tumor suppressor activity (56) by promoting apoptosis, cell-cycle arrest (57), and tumor surveillance. However, STAT1 is also an essential component in mediating proinflammatory responses, highlighted by findings that IFN treatment rapidly induces gastritis in wild-type mice (58). Similarly, Socs1-deficient mice, which are characterized by excessive IFN-dependant STAT1 signaling, spontaneously develop chronic colitis and colorectal carcinomas (59), while antral tumor development in gastrin-deficient mice is accompanied by elevated IFN-γ production (60). These observations illustrate the gastrointestinal tract’s susceptibility to inflammation-associated tumorigenesis triggered by aberrant IFN signaling and underscore the paradoxical role of the immune system in cancer development (61). While it is unknown whether expression of IL-6 family cytokines is affected in the aforementioned situations, we note that inflammation-dependent colon tumorigenesis depends on gp130-mediated trans-signaling (18). However, our observation that monoallelic Stat1 ablation reduces tumor burden in the gp130Y757F/Y757F background without impairing the IFN-α response of the corresponding MEFs suggests that the tumorigenic activity of STAT1 in gp130Y757F/Y757F mice is unlikely...
to be related to IFN signaling. On the other hand, the complete absence of Stat1 in gp130Y757F/mice is less effective than monallelic Stat3 ablation in gp130Y757F/Stat3−/− mice, which implies a more prominent role for Stat3/Stat3 homodimers than for the corresponding Stat3/Stat1 heterodimers in mediating the transcriptional response(s) that promote(s) gastric tumorigenesis. At least in intestinal epithelium, however, gp130-dependent Stat1 activation also promotes survival (62). Thus, we propose here that overexpression of the gp130 ligand IL-11 not only induces a Stat3-dependent antiapoptotic, proangiogenic tumor-intrinsic gene expression program, but further fuels continuous gp130 signaling by disproportionate IL-11 expression in response to excessive Stat3 and Stat1 activation. Thus, in addition to targeting Stat3 activation, strategies that antagonize Stat1 may also be of potential therapeutic benefit for the treatment of inflammation-driven cancers characterized by deregulated gp130/Stat3 signaling.

In summary, our genetic dissection demonstrates that persistent Stat1-dependent activation of Stat3, and to a lesser extent of Stat1, specifically promotes inflammation-associated gastric tumorigenesis by a Stat-mediated feed-forward signaling mechanism on the II11 gene. Our findings validate, in what we believe to be the first preclinical cancer model of aberrant activation of endogenous Stat3, a concept originating from tumor xenograph models and touting Stat3 as a central signaling node (41) (generously provided by I. Campbell, University of Sydney, Sydney, New South Wales, Australia) were crossed with gp130Y757F/mice to generate the compound gp130Y757F/Stat1−/− and gp130Y757F/Stat1−/− mutant mice. All animals were housed under specific pathogen–free conditions. All research article

**Methods**

**Mice and treatments.** Mice homozygous for the gp130(Y757F) knockin mutation (gp130Y757F/Y757F), as well as their corresponding compound mutant derivatives lacking either the Il6 (gp130Y757F/Y757FIl6−/−) or Il11a1 (gp130Y757F/Y757FIl11a1−/−) genes were generated as previously described (30, 31). gp130Y757F/mice heterozygous for Stat3 (gp130Y757F/Y757FStat3−/+) have previously been reported (14, 36), and mice lacking a functional Stat1 gene (44) (generously provided by L. Campbell, University of Sydney, Sydney, New South Wales, Australia) were subjected to immunoblot analyses with the indicated primary antibodies and secondary antibodies as previously described (63). Immunohistochemical staining was performed with the Liquid DAB Substrate Chromogen System (Dako), and sections were counterstained with hematoxylin.

For cytokine administration, mice were subjected to a single i.p. injection of either human IL-11 (5 µg) or mouse IFN-α (10 IU). For Stat3-ASO treatments, adult gp130Y757F/mice were assessed for splenomegaly. Splenomegaly was assessed for splenomegaly. Splenomegaly was assessed for splenomegaly. Splenomegaly was assessed for splenomegaly.

Cytokines and antibodies. Recombinant human IL-11 was kindly provided by L. Robb (The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria, Australia), while mouse IFN-α1 and human IFN-γ were a gift from N. De Weerd (Monash Institute of Medical Research). The designer cytokine 

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**Cellular assays.** To assay for IFN-mediated antiviral cytopathic activity, MEFs generated from day 13 embryos were seeded at 104 cells/well in 96-well plates in DMEM containing 10% FBS. Cells were stimulated with virus stocks at an MOI of 1 (serotype 1) in the presence of IFN-α and simultaneously challenged with Semliki Forest virus (65) as indicated in Supplemental Figure 1. The extent of IFN-mediated cell survival was assessed by dimethyl thiazole diphenyl tetrazolium bromide (MTT) assay (63). For luciferase reporter assays, primary MEFs were maintained in DMEM containing 10% FBS and were plated into 24-well plates (5 × 104 cells/well) for 2 hours before transfections. Triplicate cultures were transfected (FuGENE 6 reagent; Roche Diagnostics) with the luciferase reporter plasmid pISRE-luc, the Stat3 reporter plasmid pAPRE-luc (14), or the IL-11 reporter plasmid pIL-11−luc together with the Stat3-independent Renilla luciferase plasmid (pTK-RL; Promega) to normalize for transfection efficiency. For construction of pIL-11−luc, a 1.5-kb fragment immediately upstream of exon 1 of murine Il11 (Essemlbl, release 46) was amplified with the sense (5′-CGGGTTACCGAGAAGGGTG-3′) and the antisense primer (5′-GGAAGATCTGGAGAGAATT-CAGGCACAACT-3′), which also included restriction enzyme recognition sites (underlined) in order to enable directional subcloning into the KpnI-BgIII sites of pGL3-Enhancer (Promega). Eight hours after transfection, cells were stimulated with the indicated amounts of either IFN-α or IFN-γ over a 48-h period.

Histological and immunohistochemical analyses. Following dissection, stomach specimens were fixed in 10% neutral buffered formalin (pH 7.4) solution and embedded in paraffin. For general histology, sections were stained with H&E. Immunohistochemical stainings were performed with antibodies against CD45, pY-STAT3, and PCNA (Dako) on sections from untreated mice. Cellular proliferation was also assessed by staining with an anti-BrdU antibody (BD Biosciences — Pharmingen) of tissues collected 4 hours after injection of 50 µg/kg BrdU (Amersham Biosciences; GE Healthcare). In each case, immunoperoxidase staining was detected with the Liquid Diaminobenzidine (DAB) Substrate Chromogen System (Dako), and sections were counterstained with hematoxylin.

**Protein extraction and immunoblot analysis.** Lysates were prepared from snap-frozen gastric tissue in ice-cold lysis buffer, after which they were subjected to immunoblot analyses with the indicated primary antibodies as previously described (63). Proteins were visualized using either

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**Cellular assays.** To assay for IFN-mediated antiviral cytopathic activity, MEFs generated from day 13 embryos were seeded at 10^4 cells/well in 96-well plates in DMEM containing 10% FBS. Cells were stimulated with varying concentrations of IFN-α and simultaneously challenged with Semliki Forest virus (65) as indicated in Supplemental Figure 1. The extent of IFN-mediated cell survival was assessed by dimethyl thiazole diphenyl tetrazolium bromide (MTT) assay (63).

For luciferase reporter assays, primary MEFs were maintained in DMEM containing 10% FBS and were plated into 24-well plates (5 × 10^4 cells/well) for 2 hours before transfections. Triplicate cultures were transfected (FuGENE 6 reagent; Roche Diagnostics) with the luciferase reporter plasmid pISRE-luc, the Stat3 reporter plasmid pAPRE-luc (14), or the IL-11 reporter plasmid pIL-11−luc together with the Stat3-independent Renilla luciferase plasmid (pTK-RL; Promega) to normalize for transfection efficiency. For construction of pIL-11−luc, a 1.5-kb fragment immediately upstream of exon 1 of murine Il11 (Essemlbl, release 46) was amplified with the sense (5′-CGGGTTACCGAGAAGGGTG-3′) and the antisense primer (5′-GGAAGATCTGGAGAGAATT-CAGGCACAACT-3′), which also included restriction enzyme recognition sites (underlined) in order to enable directional subcloning into the KpnI-BgIII sites of pGL3-Enhancer (Promega). Eight hours after transfection, cells were stimulated with the indicated amounts of either IFN-α or IFN-γ over a 48-h period. Dual luciferase activity was measured using Promega’s Dual-Luciferase assay, and firefly activity was normalized to Renilla luciferase activity (14).

Histological and immunohistochemical analyses. Following dissection, stomach specimens were fixed in 10% neutral buffered formalin (pH 7.4) solution and embedded in paraffin. For general histology, sections were stained with H&E. Immunohistochemical stainings were performed with antibodies against CD45, pY-STAT3, and PCNA (Dako) on sections from untreated mice. Cellular proliferation was also assessed by staining with an anti-BrdU antibody (BD Biosciences — Pharmingen) of tissues collected 4 hours after injection of 50 µg/kg BrdU (Amersham Biosciences; GE Healthcare). In each case, immunoperoxidase staining was detected with the Liquid Diaminobenzidine (DAB) Substrate Chromogen System (Dako), and sections were counterstained with hematoxylin.

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the enhanced chemiluminescence (ECL) detection system (Amersham Biosciences; GE Healthcare) or Odyssey Infrared Imaging System (LI-COR Biosciences) with the appropriate secondary antibodies as per the manufacturer’s instructions.

RNA isolation and quantitative expression analysis. Total RNA was extracted from snap-frozen tissues using TRIzol reagent (Invitrogen) according to the manufacturer’s protocol. To eliminate any contaminating genomic DNA, on-column DNase digestion was performed using the RNeasy Mini Kit (Qiagen). CDNA was prepared from 1 µg of total RNA using the SuperScript III First-Strand Synthesis System (Invitrogen) as per the manufacturer’s instructions.

Quantitative RT-PCR gene expression analyses were performed on triplicate samples with SYBR Green (Invitrogen) using the 7900HT Fast RT-PCR System (Applied Biosystems) over 40 cycles (95°C/15 s, 60°C/1 min), following an initial denaturation step at 95°C/10 min. Primers to specifically amplify 18S were used to normalize CDNA concentrations of target genes. Data acquisition and analyses were performed with Sequence Detection System version 2.3 software (Applied Biosystems). Sequences for the mouse primer sets used are provided in Supplemental Table 1.

Statistics. Comparisons between mean values were performed using ANOVA and 2-tailed Student’s t-tests as appropriate. A P-value of less than 0.05 was considered statistically significant.

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