(Miething et al., 2007). This led to the identification of *Runx3* as a proviral target that facilitated tumor relapse upon targeted therapy with imatinib. These new mouse models and genomic techniques can provide us with unique reagents to uncover new resistance mechanisms, thereby allowing us to design strategies to overcome resistance that can subsequently be tested in cancer patients.

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Autocrine IL-6 Signaling: A Key Event in Tumorigenesis?

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Tumorigenesis is a multistep process that requires constitutive cell division, growth, and survival. One strategy used by cancer cells to upregulate growth and survival pathways is through autocrine production of growth and survival factors. Two recent papers by Gao et al. and Sansone et al. published in *The Journal of Clinical Investigation* outline the importance of autocrine interleukin 6 (IL-6) in lung and breast cancers and implicate IL-6 as an important activator of oncogenic STAT3 in lung adenocarcinomas and of Jagged-1/Notch signaling in breast tumor mammospheres.

IL-6 is a multifunctional cytokine that is important for immune responses, cell survival, apoptosis, and proliferation (Kishimoto, 2005). IL-6 signals via a heterodimeric IL-6R/gp130 complex, whose engagement triggers activation of Janus (JAK) kinases, and the downstream effectors STAT3, SHP-2/Ras, and PI3K/Akt (Kishimoto, 2005). Early studies implicated IL-6 and its major effector STAT3 as protumorigenic agents in many cancers, including breast, lung, colon, prostate, ovarian, and hematological cancers as well as melanoma; and IL-6 levels are significantly elevated in lung and breast cancer patients, associated with poor prognosis (Hodge et al., 2005).

Activating mutations in epidermal growth factor receptor (EGFR) were found to result in constitutive STAT3 activation in lung cancer (Gao et al., 2007). Although EGFR can also signal to STAT3 (Quesnelle et al., 2007), pharmacological inhibition of its tyrosine kinase activity did not prevent STAT3 phosphorylation in lung cancer cells, while it significantly inhibited Akt and ERK activation (Gao et al., 2007). By contrast, complete blockade of STAT3 phosphorylation was found upon treatment with pan-JAK inhibitor (Gao et al., 2007). The knockdown of STAT3 or inhibition of its phosphorylation delayed cell growth in culture and tumor growth in a xenograft model, underscoring the critical role of phospho-STAT3 in lung cancer driven by EGFR. Next the authors searched for the signal responsible for JAK and STAT3 activation in their system. Cancer cell lines carrying EGFR mutations were found to produce high amounts of IL-6, and introduction of mutated EGFR into breast epithelial cells in vitro rapidly induced IL-6 production along with cellular transforma-

tion. Blockade of IL-6, IL-6R, or gp130 completely abrogated this EGFR effect. There was also a good correlation between IL-6 expression and STAT3 activation in primary lung adenocarcinomas carrying EGFR mutations. Finally, the authors were able to show that ectopic expression of mutant EGFR activates the IL-6 promoter. In conclusion, oncogenic EGFR mutations activate transcription of IL-6 via an unidentified mechanism, and IL-6, acting in a paracrine and autocrine manner, contributes to cellular transformation and growth via STAT3 phosphorylation (Figure 1A).

Using a different approach, Sansone et al. also found a pivotal role of IL-6 in epithelial cancers. Working with mammospheres (MS)—multicellular spheroids, which when generated from breast cancer cells are enriched in tumor progenitors—the investigators found much higher levels of IL-6 mRNA when MS were produced from tumor samples rather than normal breast epithelium (Sansone et al., 2007). IL-6 neutralization blocked the self-renewal capability of tumor (T)-derived MS. The same applied for MS generated from MCF-7 cells stimulated with IL-6. The most intriguing observation made by Sansone et al. is that the Notch pathway is a critical downstream target of IL-6. Involvement of the Notch signaling pathway has been previously documented in breast cancers, and Notch is highly expressed in both normal (N)- and T-MS. However, this is the first time a relationship between IL-6 and Notch signaling has been described. IL-6 blockade induced a dramatic downregulation of Notch-3 gene expression, and recombinant IL-6 induced *notch-3* transcription. Notch-3 promoted MS survival via its interaction with Jagged-1. IL-6 also promoted secretion of Jagged-1, a cognate ligand for Notch-3 (Sansone et al., 2007) (Figure 1B), which has been confirmed by knockdown and inhibition approaches. Upregulation of Notch signaling by IL-6 culminated in a protumorigenic gene expression program. The authors also pointed out that the hypoxia resistance gene carbonic anhydrase (CA-IX) is activated in breast cancer cells by IL-6/Notch/Jagged action and provides survival advantages under hypoxic conditions. However, it remains to be determined whether IL-6-dependent Notch upregulation is also STAT3 dependent (Figure 1B).

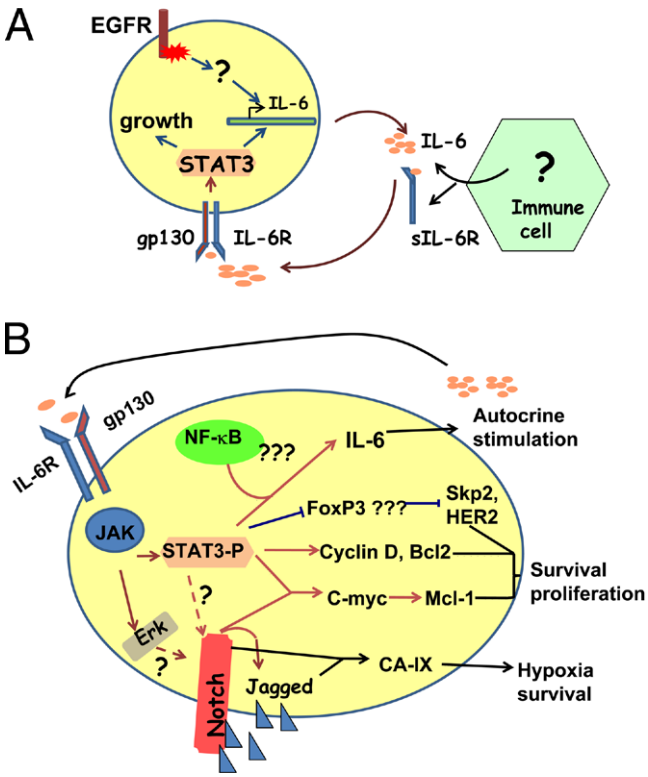

Figure 1. The Role of IL-6 Signaling in Cancer Cells

(A) In lung adenocarcinoma cells, somatic mutations in EGFR activate IL-6 transcription by an as yet unidentified mechanism. IL-6 is secreted by cancer cells and activates STAT3, which sustains tumor growth and proliferation. Contribution of infiltrating immune/inflammatory cells in vivo is also possible, and they can provide both IL-6 and soluble IL-6R (sIL-6R).

(B) IL-6-dependent STAT3 activation results in numerous downstream events required for tumor growth. Sansone et al. found an important role of the IL-6/Notch/Jagged pathway, which activates expression of the hypoxia resistance gene CA-IX. It remains to be determined whether Notch induction by IL-6 requires STAT3 or the SHP2/Erk pathway. Other IL-6/STAT3-dependent pathways include production of IL-6, activation of genes required for cell survival and proliferation (c-Myc, Cyclin D, Bcl2), and inactivation of tumor suppressor (FoxP3).

These two studies significantly expand our understanding of IL-6 involvement in epithelial cancers and stress the importance of autocrine/paracrine IL-6 signaling along with the work of others (Sasser et al., 2007; Yeh et al., 2006). These studies also pose several mechanistic and conceptual questions.

(1) While many normal epithelial cells express gp130, they do not express significant amounts of IL-6R (Mitsuyama et al., 2006). It would be of interest to investigate whether, during malignant transformation, tumor cells acquire high IL-6R expression along with IL-6 secretion. Interestingly, tumor-infiltrating inflammatory/immune cells may secrete both IL-6 and soluble IL-6R, which then signal to gp130+ IL-6R− cells, a phenomenon called IL-6 trans-signaling (Mitsuyama et al., 2006). Macrophages and dendritic cells are potent IL-6 producers and can be activated by molecular "danger" signals produced by dying cancer cells (or in the case of lung cancer by tobacco smoke or air pollutants) (Karin et al., 2006).

(2) The IL-6/STAT3 pathway induces expression of SOCS3, the molecule that inactivates IL-6 signaling through a negative feedback loop (Kishimoto, 2005). While SOCS3 expression was not analyzed in these studies, tumors that rely on continuous autocrine IL-6 signaling may evolve a strategy to prevent SOCS3-dependent inactivation of IL-6 signaling.

(3) Only about 10% of lung cancers display EGFR mutations, but up to 50% of such tumors contain constitutively activated STAT3. The mechanism by which active EGFR leads to IL-6 induction is not known, but the involvement of NF-κB, AP-1, C/EBP, and their activating kinases can be proposed. Obviously, EGFR is not a unique trigger of IL-6 expression in cancer cells, and other signaling pathways should also lead to IL-6 production, given the importance of IL-6 in cancer. While it is not known whether the *erbB2/HER2* oncogene can activate the IL-6 gene, activated Ras, which is observed in 30% of lung cancers, may also lead to IL-6 induc-

tion, as secreted IL-6 has recently been shown to be required for Ras-dependent carcinogenesis (Ancrile et al., 2007).

(4) Does EGFR-induced IL-6 production contribute to tumorigenesis in other tissues? An interaction between gp130 and EGF signaling was noted also in breast cancer, and EGFR mutations are found in various tumors. Therefore, it appears likely that mutated EGFR can also induce IL-6-dependent tumorigenesis in other cancers, but this requires further investigation.

Another interesting question is why tumors choose IL-6 to constitutively activate STAT3? STAT3 can be activated by many IL-6-like cytokines and by means of crosstalk with other signaling pathways. While Onconstatin M and LIF were ruled out (Gao et al., 2007), other cytokines like IL-11 or IL-22 could also contribute to STAT3 phosphorylation and tumor development. One plausible hypothesis is that immune/inflammatory cells in a close interaction with cancer cells are capable of producing prodigious amounts of "start-up" IL-6 (but not other family members) required for early tumor promotion.

The last but not the least question that arises from these studies concerns the importance of the IL-6/STAT3 axis in cancer, or in other words, what is the role of STAT3 in carcinogenesis? Early studies on colon cancer implicated STAT3 as a major regulator of cell proliferation and survival, particularly due to its ability to regulate expression of c-Myc, Mcl-1, Cyclin D, and Bcl-2 (Figure 1) (Becker et al., 2005). In chemically induced hepatocellular carcinoma (HCC), IL-6 appears to play a dual role: on one hand it facilitates cell injury and subsequent compensatory proliferation of hepatocytes, and on the other hand it provides growth signals to transformed hepatocytes (Naugler et al., 2007). Being part of a positive IL-6 autocrine loop, STAT3 could also cooperate with NF-κB in IL-6 induction (Figure 1B). Importantly, Sansone et al. provide a link between IL-6 and Notch in cancer and show how IL-6 signaling can eventually drive expression of Notch-dependent genes. The IL-6, STAT3, and Notch pathways synergize in induction of c-Myc, but it remains to be determined whether the same cooperation extends toward expression of other typical IL-6 targets, such as Bcl-2 and Cyclin D.

Another idea that awaits experimental confirmation comes from the studies of T helper cell differentiation. In T cells, IL-6-activated STAT3 antagonizes FoxP3 and thus drives differentiation of proinflammatory instead of regulatory T cells (Dominitzki et al., 2007). A recent report reveals FoxP3 within epithelial cells as an important tumor suppressor in breast cancer acting via repression of the *erbB2*/*HER* and *skp2* oncogenes (Zuo et al., 2007). Expression of Foxp3 and its targets HER2 and SKP2 was not analyzed by Gao et al. or Sansone et al., so it would interesting to test whether IL-6/STAT3 could work through a FoxP3-dependent mechanism in addition to previously described Notch and c-Myc mechanisms (Figure 1B).

Only a few targeted therapeutics that specifically inhibit tumor growth with minimal general cytotoxicity are currently available. However, several drugs, including antibodies and soluble receptors that block IL-6 signaling, have been developed and are currently in the clinic or under clinical trials. The studies by Gao et al. and Sansone et al., along with other recent papers (Becker et al., 2005; Naugler et al., 2007), create the rationale for anti-IL-6-specific therapy in a variety of carcinomas.

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