

IL-6 involvement in epithelial cancers

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Commentary

In this issue of the *JCI*, two reports provide intriguing new information on the role of the inflammatory cytokine IL-6 in breast and lung cancer. The study by Sansone et al. implicates IL-6 in the instigation of malignant properties in breast cancer stem cells (see the related article beginning on page 3988). The study by Gao et al. identifies mutant variants of EGFR as inducers of IL-6 in lung adenocarcinomas (see the related article beginning on page 3846). These studies add to our understanding of potential roles for IL-6 in cancer and further motivate investigations of IL-6–targeted therapeutics.

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taining chemical extracts from broccoli that boost production of protective enzymes in skin and protect from UV-induced erythema and inflammation in humans (20). With respect to cancer prevention, it is worth asking whether systemic CP-31398 might prevent cancer development in patients with Li-Fraumeni syndrome — a rare autosomal dominant hereditary disorder in which patients possess a mutation in the p53 tumor suppressor gene that greatly increases their susceptibility to cancer. The study by Tang et al. (17) reports exciting progress with clinical relevance and, like all outstanding papers, raises interesting questions for future work. Further studies and clinical translation of the findings of Tang et al. may lead to improved ways of preventing and treating UV light-induced skin cancers that afflict millions of people.

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In this issue of the *JCI*, two reports provide intriguing new information on the role of the inflammatory cytokine IL-6 in breast and lung cancer. The study by Sansone et al. implicates IL-6 in the instigation of malignant properties in breast cancer stem cells (see the related article beginning on page 3988). The study by Gao et al. identifies mutant variants of EGFR as inducers of IL-6 in lung adenocarcinomas (see the related article beginning on page 3846). These studies add to our understanding of potential roles for IL-6 in cancer and further motivate investigations of IL-6-targeted therapeutics.

IL-6 is a multifunctional cytokine that was originally characterized as a regulator of immune and inflammatory responses; however, elevated expression of IL-6 has been detected in multiple epithelial tumors (1). IL-6 binds to a heterodimeric receptor,

which contains the ligand-binding IL-6 α chain and the common cytokine receptor signal-transducing subunit gp130. IL-6 receptor engagement leads to activation of the JAK family of tyrosine kinases, which then stimulate multiple pathways involving MAPKs, PI3Ks, STATs, and other signaling proteins (2).

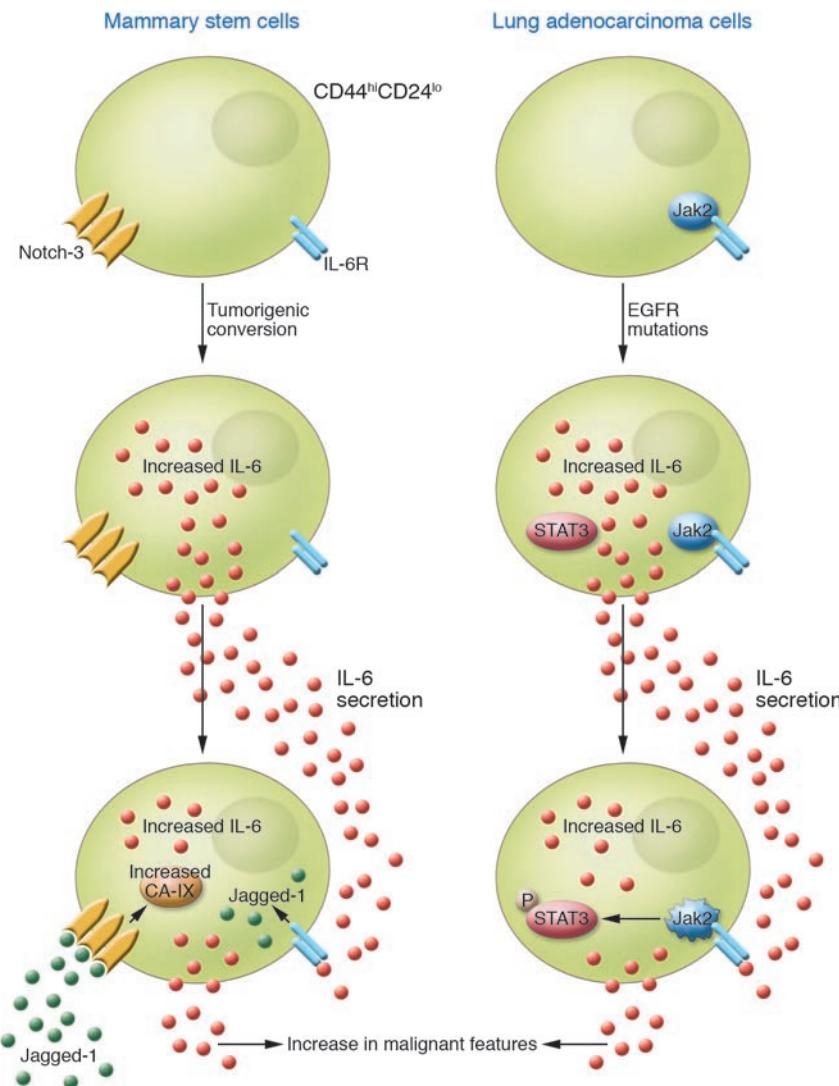
Given the reported involvement of IL-6 and its downstream targets in the regulation of cell proliferation, survival, and metabolism, it is not surprising that IL-6 signaling has also been implicated in

tumorigenesis (3). However, the nature of IL-6's involvement in cancer has been quite controversial, as dichotomous roles for IL-6 in both tumor-promoting and -suppressive activities have been reported. For example, IL-6 signaling has been linked to both pro- and antiapoptotic activity in breast cancer cells (4, 5). Multiple studies have documented high IL-6 levels in the serum of patients with certain carcinomas (i.e., breast, lung, lymphoma) and have correlated high IL-6 levels with a poor clinical prognosis (2). These data imply an oncogenic role for IL-6; however, lacking is an understanding of the mechanisms governing IL-6 production in tumors and the biological role of this cytokine in tumorigenesis. Two reports in this issue of the *JCI* (6, 7) advance our understanding of both of these issues and provide a molecular rationale for the development of anti-IL-6 therapeutics (summarized in Figure 1).

Nonstandard abbreviations used: CA-IX, carbonic anhydrase IX.

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**Figure 1**

Models for role of IL-6 in breast and lung carcinomas. In this issue of the *JCI*, Sansone et al. (6) show that the tumorigenic conversion of mammary stem cells ($CD44^{hi}CD24^{lo}$) results in an increase in IL-6 expression and secretion. IL-6 secretion results in a positive feedback loop causing further IL-6 upregulation and secretion. Once secreted, IL-6 can bind the IL-6 receptor (IL-6R), causing the upregulation of the Notch-3 ligand Jagged-1, which triggers the upregulation of CA-IX. While not depicted here, it should be noted that Jagged-1 is a transmembrane ligand. The result of these changes is the promotion of malignant features in these mammary stem cells. Also in this issue, Gao et al. (7) show that EGFR mutations in lung adenocarcinoma cells cause an increase in IL-6 expression and secretion. This, in turn, promotes malignant features in these cells through the IL-6 receptor activation-mediated phosphorylation of STAT3.

IL-6 production by breast cancer mammospheres

The identification of putative stem cells in breast tumors has jump-started a rapidly growing field focused on the biology of cancer stem cells in solid tumors (8). The present study by Sansone et al. (6) implicates IL-6 as a potential regulator of normal and tumor stem cell self-renewal. Comparison of mammospheres (multicellular spheroids composed of anchorage-independent self-renewing cells and their derivatives) from normal and tumor tissue from the same patient revealed that *IL-6* mRNA is expressed at significantly higher levels in mammospheres derived from tumor tissue. In addition, spheroids cultured from the MCF-7 breast cancer cell line also contained high IL-6 levels, and treatment with IL-6-blocking antibodies suppressed spheroid formation. High expression of IL-6 was also

observed in basal-like breast carcinoma tissues, which are enriched in mammosphere and stem cell markers. The authors further show that IL-6 can stimulate secondary normal mammospheres and that inhibition of the interaction of IL-6 with its receptor blunts the size and capacity to form secondary tumor mammospheres, implicating IL-6 as a critical factor in tumor mammosphere self-renewal. Sansone et al. (6) further reveal that the ability of IL-6 to maintain mammosphere self-renewal is dependent on the MAPK-dependent upregulation of the transmembrane receptor Notch-3 (a member of the Notch signaling pathway essential for cellular differentiation in a variety of tissues), which has previously been demonstrated to be involved in mammosphere self-renewal (9, 10). Sansone et al. also describe how IL-6 can upregulate the Notch-3 ligand Jagged-1 to create a positive feedback loop

along the Notch-3/Jagged-1 axis (6). In addition, IL-6 is shown to further promote malignancy in breast cancer stem cells by upregulating the hypoxia response protein carbonic anhydrase IX (CA-IX), which the authors propose permits these cells to survive in hypoxic conditions.

While a role for an immune/inflammatory cytokine like IL-6 in epithelial tumor cells may be unexpected, Sansone et al. (6) propose that stimulation of epithelial stem cells may be part of a natural inflammatory repair program to activate stem cells to replace damaged cells. These studies implicate IL-6 as a critical mediator of mammary stem cell renewal in both normal and tumor contexts.

EGFR-driven IL-6 production in lung tumors

In a study of non-small-cell lung adenocarcinomas, Gao et al. (7) provide additional



evidence for the involvement of IL-6 in cancer and identify an EGFR/IL-6/STAT3 signaling cascade that is important for tumorigenesis. Mutations of the EGFR have been observed in about 10% of lung adenocarcinomas, and patients whose tumors contain these mutations show increased sensitivity to EGFR tyrosine kinase inhibitors gefitinib (Iressa) or erlotinib (Tarceva) (11, 12). In studies using both mice and human non-small-cell lung adenocarcinoma cell lines, Gao et al. discovered a correlation between activated STAT3 (a downstream target of IL-6) and EGFR mutations in lung tumors (7). Using small hairpin RNA, blocking antibodies, and reconstruction experiments, they demonstrate that activated EGFR induces expression of IL-6, which leads to activation of STAT3. Furthermore, they show that IL-6 expression is substantially elevated in, and IL-6 is secreted by, multiple lung cancer cell lines that harbor EGFR mutations. Gao et al. (7) have characterized a novel mechanism for IL-6 secretion that suggests that anti-IL-6-based therapies may have impact in patients with lung adenocarcinomas. Given that approximately 50% of tumors have activated STAT3 and only 10% contain activating EGFR mutations, it is possible that additional alterations may lead to IL-6 secretion and subsequent STAT3 activation. In support of this, a recent report has demonstrated that induction of tumorigenesis in mice by activated Ras, which is mutated in approximately 30% of human lung adenocarcinomas (13), is dependent on the secretion of IL-6 (14).

The results of Gao et al. (7) nicely complement the work of Sansone et al. (6) by implicating an IL-6 autocrine loop in lung adenocarcinoma. Comparison of the findings in these reports raises obvious questions about the extent to which distinct aspects of each report relate to the other's results. For example, is EGFR involved in IL-6 production in basal breast tumors, as it is in lung tumors? EGFR is enriched in the same breast tumor subclass (basal tumors) that is enriched for IL-6 and Notch-3, so it is plausible that EGFR could regulate IL-6 production in these tumors (15, 16). ErbB2, a receptor closely related to EGFR, is amplified in approximately 25% of breast tumors and activates many of the same pathways as EGFR (12). Therefore, ErbB2 could also stimulate the IL-6 pathway. While Sansone and coworkers did not examine upstream inducers of IL-6 production in breast tumors or mammospheres, they

did find that IL-6 itself upregulates *IL-6* mRNA in breast tumor mammospheres, thus creating a positive feedback loop to enhance IL-6 production (6).

Perhaps a more interesting question is: Do lung tumors contain self-renewing cells driven by an IL-6/Notch pathway, as in breast tumors? Gao et al. (7) did not address which IL-6/STAT3 downstream targets are critically involved in lung tumorigenesis; however, the report by Sansone et al. (6) clearly implicates the Notch pathway in stem cell self renewal. Bronchoalveolar stem cells have been identified in normal tissues and lung tumors (17), so it will be of interest to examine involvement of IL-6 and Notch-3 in both contexts.

While Sansone et al. (6) focused on the Notch-3 pathway and CA-IX as critical mediators of IL-6 in breast tumorigenesis, the results of the Gao et al. study implicate the JAK/STAT pathway in lung carcinogenesis (7). It is possible that Notch and JAK/STAT pathways both contribute to IL-6-mediated effects in breast and lung tumors. Indeed, IL-6 has been shown to activate STAT3 in breast tumor cells lines (18), and ErbB2-induced STAT3 has been shown to regulate tumorigenesis in mouse mammary tumors (19). Crosstalk between these pathways has been reported to occur via facilitation of the interaction of JAK2 and STAT3 by the Notch effectors Hes1 and Hes5 (20). Thus it would be interesting to determine whether a relationship exists among IL-6, Notch, and JAK/STAT in these tumors.

A future for therapeutic targeting of IL-6 signaling?

The reports highlighted here (6, 7) provide important new insights into potential roles for IL-6 in epithelial carcinomas and raise the question of whether IL-6-targeted therapies may be effective in treating patients with basal cell breast carcinomas or lung adenocarcinomas carrying EGFR mutations. IL-6 or IL-6 receptor antagonists (i.e., CNTO 328, a human-mouse chimeric antibody to human IL-6, and Tocilizumab, humanized anti-IL-6 receptor antibody) are currently in either phase I or phase II clinical trials in a small subset of cancers and other diseases (21, 22). It is difficult to predict the outcome of IL-6 antagonism in human tumors, because the studies in these reports did not address whether inhibition of IL-6 would lead to tumor regression or merely prevent expansion of existing tumors that are IL-6 dependent. It is also

unclear whether IL-6 antagonism would provoke tumor cell death. In the absence of tumor cell killing, the opportunity for selection of drug-resistant cells is highly probable. Future studies in mouse tumor models may provide more meaningful predictions of the therapeutic efficacy of IL-6 antagonists. Regardless, these studies strongly implicate IL-6 in 2 types of epithelial carcinomas and represent significant conceptual advances in our understanding of the role of this cytokine in cancer.

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