Cytokine Stimulation of Epithelial Cancer Cells: The Similar and Divergent Functions of IL-4 and IL-13

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Abstract
The Th2 cytokines interleukin (IL)-4 and -13 are acknowledged regulators of lymphocyte proliferation and activation. They have also been well studied in the regulation of various myeloid-derived populations in tumor biology. It has become clear, however, that both cytokines can have direct effects on epithelial tumor cells expressing appropriate receptors. Changes in tumor proliferation, survival, and metastatic capability have all been ascribed to IL-4 and/or IL-13 action. Here, we evaluate the evidence to support direct tumor-promoting roles of these cytokines. We also identify the questions that should be addressed before proceeding with therapeutic approaches based on neutralization of IL-4 or IL-13 pathways. Cancer Res; 72(24); 6338–43. ©2012 AACR.

Introduction
Cancer cell survival, proliferation, and metastasis are influenced by multiple factors including cytokines in the tumor microenvironment interacting with cells and regulating complex signaling pathways. Two such cytokines are the Th2 molecules, interleukin (IL)-4 and -13. IL-4 and IL-13 are structurally similar multifunctional peptides that can affect multiple cell types. IL-4 is a well-characterized regulator of proliferation and immunoglobulin class switching in B cells, and IL-13 stimulates changes in epithelial and smooth muscle cell functions leading to hypersensitivity reactions (1–3). These immunoregulating and effector cytokines are produced by macrophages, dendritic cells, mast cells, natural killer T-cells (NKT), natural killer (NK) cells, basophils, eosinophils, and T lymphocytes (4). As depicted in Fig. 1A, there are 4 different receptor subunits associated with these cytokines that can combine in different ways to generate various functional units.

Another receptor that is expressed and activated on tumor cells is IL-13Rα2. IL-13 binds IL-13Rα2, which is distinct from IL-13Rα1 and is present in 2 forms. A soluble form resulting from either alternative splicing or proteolytic cleavage has no signaling ability and has been called the decoy receptor (2), whereas a larger membrane-spanning form results in the activation of downstream effectors.

Cancer cell survival, proliferation, and metastasis are in part by mediating resistance to apoptosis. Epithelial cancers are often cross-resistant to both intrinsic and extrinsic apoptotic pathways as they have a common downstream effector protein, caspase-3. While TRAIL and other death ligands activate the extrinsic pathway

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of apoptosis, chemotherapeutic agents typically activate the intrinsic pathway. To examine the ability of IL-4 to protect against death ligand-induced apoptosis, Todaro and colleagues treated subcutaneous human colon and breast tumors in mice with an IL-4–neutralizing antibody and TRAIL. Treatment with the antibody alone modestly decreased the growth of breast and colon tumors, but strongly sensitized tumors to cotreatment with TRAIL. Reduction in tumor growth was attributed to the downregulation of anti-apoptotic proteins PED, cFLIP, Bcl-xL, and Bcl-2 in response to IL-4 blockade (9). This study and others have used the species specificity of IL-4/IL-4Rα binding to target the autocrine production of human IL-4 in mouse xenograft models. However, distinguishing the actions of autocrine versus paracrine IL-4 is less relevant in patients where both act similarly. Nonetheless, xenograft models have been instrumental in showing that IL-4 enhances the survival of epithelial cancer cells.

To establish IL-4 as a survival factor capable of inducing chemoresistance, Todaro and colleagues implanted human colorectal cancer spheroids containing colorectal cancer stem cells into the flanks of nude mice. Mice bearing colorectal tumors were treated with an anti-human IL-4–neutralizing antibody or IL-4 double “mutein” (IL-4DM), an antagonist of human IL-4Rα, to interfere with the IL-4/IL-4Rα interaction (16). A modest decrease in tumor size compared with controls was seen with either treatment alone. However, efficacy of chemotherapy with oxaliplatin and/or 5-fluorouracil (5-FU) was significantly enhanced when animals were cotreated with either the anti-IL-4 antibody or IL-4DM. Furthermore, these antitumor effects were sustained following cessation of treatment. Increased tumor cell death was again attributed to the downregulation of anti-apoptotic proteins PED, cFLIP, Bcl-xL, Bcl-2 (16), and survivin expression (9) following IL-4 blockade. With the prosurvival functions of autocrine IL-4 translating to...
chemoresistance in epithelial tumors in vivo, it follows that anti-IL-4/IL-4Rt treatments in combination with standard chemotherapeutics could have a synergistic effect in reducing tumor growth in patients. Because IL-4 levels are often elevated in the tumor microenvironment (8–10), anti-IL-4/IL-4Rt treatments may even be more potent in patients as total IL-4, both autocrine and paracrine, would be neutralized.

In addition to enhancing cancer cell survival for tumor growth, IL-4 has been shown to directly induce the rate of proliferation of colon, breast, head and neck, ovarian, and prostate cancer cells in vitro (4, 17, 18). The majority of these studies attributed IL-4–induced proliferation to the activation of downstream Stat6 signaling; however, the upregulation of survivin may also play a role. Survivin, a well-known inhibitor of apoptosis, can act as a mitotic regulator that directly influences cell proliferation by controlling cell-cycle entry (19). In a recent study, IL-4–induced proliferation of human prostate cancer cells correlated with increased expression of survivin in vitro. This finding translated to increased tumor cell proliferation in vivo, overall tumor progression, and increased mouse survival (18). Significantly, survivin mRNA levels did not differ between control and IL-4–stimulated prostate cancer cells, indicating that survivin upregulation was not controlled by a transcriptional mechanism, but rather by differences in mRNA translation (18). In contrast to proliferation-promoting roles, early in vitro models defined a possible growth inhibitory role for IL-4 that was also shown in vivo using tumor cell genetically modified to secrete IL-4 (20). However, it is important to note that, in such experiments, cancer cell IL-4 expression was driven by viral promoters resulting in consistent and copious overexpression that may not have been physiologically relevant. Importantly, clinical trials in which exogenous IL-4 was tested as an antitumor agent were not successful (20).

Because we now know that IL-4 levels are often already elevated in the tumor microenvironment of patients with cancer (6–8), the lack of effect of additional IL-4 is perhaps unsurprising.

Although the earlier studies examined the role of autocrine IL-4 in the growth of established epithelial tumors, the IL-4/IL-4Rt interaction may also contribute to tumor formation. Khaled and colleagues recently published that IL-4 and IL-13–induced Stat6 signaling is crucial for normal mammary gland development in vivo (21). It is plausible that epithelial tumor cells could exploit normal developmental pathways to promote tumor formation. In studies of established tumors, IL-4 has been shown to promote fibrosarcoma tumor formation in vivo by enhancing tumor cell survival (22). However, the relative contribution of tumor- versus host-derived IL-4 or the receptor was not examined in this study. This is significant because several cell types may contribute to IL-4/IL-4Rt–induced proliferation and survival during epithelial tumor development. For example, as reviewed elsewhere (15), tumor-promoting macrophages can be activated by IL-4.

In an attempt to distinguish the contribution of epithelial versus host IL-4Rt signaling in tumor development, Koller and colleagues used 2 mouse models: (i) implantation of wild-type murine colon cancer cells or short hairpin RNA (shRNA)-mediated IL-4Rt knockdown (IL-4Rt KD) clones into the ceca of C57BL/6 mice; and (ii) a carcinogen-induced model of colitis-associated colon cancer in IL-4Rt knockout (IL-4Rt−/−) mice (4). Results obtained from the implantation model indicated that tumor cell IL-4Rt was important for inducing proliferation but not survival in vivo. In the carcinoma model, IL-4Rt−/− mice had significantly smaller tumors compared with wild-type mice. The reduction in tumor size correlated with a trend in decreased proliferation and a significant increase in apoptosis, suggesting that cell death was mediated by host-cell IL-4Rt (4). However, because both host and tumor cells were deficient of IL-4Rt in the carcinogen-induced model, it is impossible to be certain of the relative contribution of each. These results established a role for IL-4Rt in promoting colon cancer development, yet they seem to contradict previous studies showing a strong prosurvival function of the IL-4/IL-4Rt interaction in epithelial tumor cells (9, 16, 18, 22). It is important to note that active survival-response pathways in developing compared with established tumors may differ. Other variables include colon cancer stem cell content as well as the presence and function of IL-13. It is also possible that in the colitis-associated colon cancer model, increased production of IL-13 by inflammatory cells in the tumor microenvironment may promote tumor development as IL-13 can also bind and activate IL-4Rt. The contribution of each cytokine remains to be distinguished, and this may be particularly important in models where immune infiltrates are prevalent.

**IL-13 and its receptors**

Numerous studies have reported the expression and role of IL-4 in epithelial cancers; however, few have also focused on the implications of IL-13 and IL-13R expression. This gap in knowledge may be due to the complexity of the IL-13 system. IL-13 can signal through type II IL-4R (IL-4Rt and IL-13Rt1) or through IL-13Rt2, which was earlier believed to serve only as a decoy receptor. Elevated levels of IL-13 were detected in primary breast cancer tumor tissue (23) and in the peripheral blood of 131 other patients with cancer (pancreatic, esophageal, and gastric) when compared with healthy controls (24). Recent studies by Formentini and colleagues and Barderas and colleagues suggested possible roles for IL-13 and its receptors IL-13Rt1 and IL-13Rt2 in colorectal cancer (11, 25). In tumor tissue specimens, expression of IL-13Rt1 did not influence survival and IL-13 expression did not influence patient lymph node metastasis. Surprisingly, low IL-13 expression correlated with worse overall survival compared with high IL-13 expression (25). However, high expression of IL-13Rt2 in patients with colon cancer is a predictor of later stages of disease and poor outcome (11). Similarly, in highly invasive glioblastoma multiforme (GBM), IL-13Rt2 is overexpressed, whereas it is absent in normal brain tissue (13). Finally, IL-13Rt2 has been reported to be a biomarker of disease in ovarian cancer and targeting this receptor with a specific immunotoxin in mice resulted in decreased tumor burden and extended host survival (14). Although these expression findings suggest a possible contribution of IL-13Rt2 to tumor metastasis and survival, they do not establish a causal role for IL-13 in cancer cell growth or metastasis.
To establish functional relationships between IL-13 and tumor behavior, it is helpful to understand the various signaling pathways associated with receptor activation. The dominant signaling pathway activated by IL-13 binding to IL-13Rα1 in the context of the type II IL-4R, is the Stat6 pathway (Fig. 1B; ref. 2). This is the same pathway activated by IL-4 binding to the type II IL-4R, although signaling kinetics seem to be different (3). In many of the studies discussed previously, IL-4Rα was targeted to abolish IL-4 binding and resultant signaling. This does not affect IL-13 binding to IL-13Rα1, but in the absence of IL-4Rα, signaling is eliminated. Several studies identifying the role of IL-4R in tumorigenesis have assumed that the response is equal regardless of which cytokine, IL-4 or IL-13, is activating the signaling cascade. This, however, may be inaccurate (3) and further studies isolating the signaling pathways activated downstream of each cytokine are required. Additional mediators in tumorigenesis may include AP-1-dependent induction of TGF-β expression after IL-13 binding to IL-13Rα2, as shown in macrophages (Fig. 1B; ref. 26). Another mediator is 15-lipoxygenase-1 (15-LOX-1), which conversely occurs only in the absence of IL-13Rα2 (Fig. 1C; ref. 27).

A correlation between IL-13Rα2 expression and metastasis of tumor cells has been observed in multiple epithelial cancers. In an orthotopic model of human ovarian cancer, IL-13Rα2-expressing tumors metastasized to lymph nodes faster than IL-13Rα2-negative tumors and resulted in a higher rate of animal mortality (14). Exogenous IL-13 treatment further increased mortality in mice carrying IL-13Rα2-expressing tumors (14), linking IL-13/IL-13Rα2 to increased aggressiveness of tumor cells. Similarly, overexpression of IL-13Rα2 increased invasion and metastasis, and decreased survival in a mouse model of human pancreatic cancer (12). Recent studies in a mouse model of human colorectal cancer indicated that genetic knockdown of IL-13Rα2 in tumor cells reduced liver homing ability, resulting in increased survival of mice compared with those injected with IL-13Rα2-expressing cells (11). In addition, in mouse models of GBM, tumor cells that survive IL-13Rα2 targeted therapy, presumably due to low receptor expression, were less tumorigenic than untreated control cells (13). Possible molecular mediators of this prometastatic function of IL-13 include the transcription factor AP-1 and its target TGF-β. Treatment of IL-13Rα2-expressing tumor cells with exogenous IL-13 activated ERK1/2 followed by AP-1, in vitro, with expression confirmed in vivo (14). AP-1 is at least one of the transcription factors downstream of IL-13Rα2 signaling leading to expression of TGF-β in monocytic leukemia and macrophages as a result of IL-13 treatment (26). Therefore, we speculate this pathway may also be important in epithelial tumor cells.

Another important mechanism for promotion of metastasis is regulation of apoptosis. Interestingly, IL-13 can be both pro- and antiapoptotic depending on receptor presence and/or the signaling pathways activated. As described previously, signaling through type II IL-4R is strongly associated with increased expression of anti-apoptotic proteins. The IL-13–induced proapoptotic pathway is activated by 15-LOX-1 catalyzing the oxidation of arachidonic and linoleic acids, leading to activation of PPARG, whose ligands can then activate cell apoptosis. According to Hsi and colleagues, IL-13–dependent production of 15-LOX-1 occurs only in the absence of IL-13Rα2. In their model, decreased in vivo growth of GBM due to increased apoptosis was associated with IL-13 (27). Some researchers have postulated that this IL-13–induced apoptosis signaling pathway occurs through IL-4R; however, there have not been any studies to either support or dispute this contention.

These multiple studies established contradictory roles for IL-13 in promoting and combating cancer progression that is seemingly dependent on receptor expression. For example, when all receptors are expressed, IL-13 can: (i) bind to IL-13Rα1, recruit IL-4Rα, and signal through this heterodimer to phosphorylate Stat6 and promote proliferation and/or apoptotic resistance; (ii) bind to soluble IL-13Rα2 decoy receptor and result in no signaling; or (iii) bind transmembrane monomeric IL-13Rα2 and induce TGF-β, resulting in increased metastasis (Fig. 1B, C). In addition, in mice genetically deficient for IL-4Rα, where any IL-13 signaling must be through IL-13Rα2, Ko and colleagues showed increased development of precancerous lesions in an intestinal carcinogen model (28). These mice also had increased levels of TGF-β. It is important to note that induction of AP-1 by IL-13 signaling through IL-13Rα2 can also activate other factors including matrix metalloproteinases, which are active in tumor growth, invasion, and metastasis (14). In contrast, in the absence of IL-13Rα2 expression, IL-13 signaling through type II IL-4R is possibly increased and the IL-13–induced apoptotic pathway is activated, although it is not yet clear if this IL-13–induced apoptotic pathway is downstream of type II IL-4R. Overall, then, it is clear that IL-13Rα2–targeted therapy may be just as important as IL-4Rα–targeted therapy.

Implications and Future Directions

As indicated by the foregoing discussion, there is now significant evidence supporting IL-4/IL-13 and their receptors as valid therapeutic targets in many different cancers. Even without considering the biologic pathways involved, the high level expression of IL-4Rα (also known as CD124) or IL-13Rα2 on several types of tumor cells has allowed tumor cell-selective delivery of various toxins or lytic peptides, resulting in reduced tumor load (29). Therefore, it is plausible to target the IL-4R or IL-13Rα2 signaling pathways in patients as anticancer therapy? There are several key concerns to address before the use of anti-IL4 or -13 receptor treatments: (i) IL-4Rα–inhibiting treatments would inhibit signaling through both type I and II IL-4Rs. To our knowledge, there are no comprehensive studies evaluating the expression of type I IL-4R in epithelial cancers. Type I IL-4R should be ruled out as a potential player in tumor cell behavior, but is clearly a critical regulator of immune cell behavior. When given systemically, agents that target IL-4Rα will affect not only cancer cells but also tumor-associated macrophages, other myeloid cell types, and lymphocytes. It is this last group of cells that may be of concern. It is known that there are polymorphisms in the gene for IL-4RA that result in enhanced receptor activity. Small genetic studies have suggested that patients with colorectal cancer with these polymorphisms actually have a better prognosis (30). This is likely due to more effective B- and T-cell function, although actual
experimental evidence is lacking. Therefore, therapies that specifically target cancer cells via the type II IL-4R, but avoid the lymphocyte-expressed type I receptor, may be most beneficial; (ii) targeting of I receptor type may impact the activity or ligand-binding of other related receptors. One of the most exciting recent studies in the IL-4/IL-13 field defines the crystal structures of the various receptors and shows IL-4 and IL-13 having different potencies through the same receptor in tumor cells (3). IL-4 and IL-13 have differing affinities for the various receptor subunits, which can make I or the other receptor the more likely target when all are available. However, this will be changed if 1 subunit is no longer able to bind ligand. For example, in the absence of IL-13Rα2, will there be increased binding of the type II IL-4R by IL-13 that would have a different outcome to when that receptor is bound by IL-4? We are currently undertaking studies using single and dual receptor knockdown in tumor cells expressing these receptors at various levels to answer these complex, yet pertinent; and (iii) efficacy may be different in primary and metastatic tumors. Many studies have found particularly strong evidence for pro-survival functions of IL-4 and IL-13. This might suggest that metastatic colonization and resistance to chemotherapy, both dependent on survival programming, are key roles for IL-4 and IL-13 signaling. In mouse models, we have preliminary evidence that growth of primary tumors is unaffected, whereas metastatic burden is strongly reduced when IL-4Rα is ablated on mammary tumor cells (Venmar and Fingleton, unpublished data). Further analysis of the different mechanisms involved will be necessary before making recommendations about which patient populations would most benefit from IL-4/IL-13 pathway-directed therapies.

In conclusion, IL-4 and IL-13 as well as their receptors seem to have significant functions on tumor cell biology that is distinct from their roles in regulation of immune cells. Although complex and still in need of further elucidation, the signaling pathways activated by these Th2 cytokines may offer novel means to target cancer-specific behaviors.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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