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Stem Cell Factor LIFted as a Promising Clinical Target for Cancer Therapy

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Leukemia inhibitory factor (LIF) is a monomeric glycoprotein and belongs to the IL6 subfamily of the large "four-helix super-family" of cytokines. LIF acts on responding cells by binding to a heterodimeric membrane receptor complex composed of the ligand-specific LIF receptor (LIFR) and its coreceptor glycoprotein 130 (gp130 or IL6ST), which is shared by all the IL6 family receptor complexes. Despite common signal transduction mechanisms (JAK/STAT, ERK MAPK, and PI3K/AKT), LIF can exert opposite effects by stimulating or inhibiting cell differentiation, proliferation and renewal, and survival, depending upon cell type and maturity, and it has been regarded as the most pleiotropic member of the IL6 family of cytokines (1). LIF is now recognized to be at the heart of many physiologic processes throughout life, starting from embryo implantation to tissue-specific homeostasis, such as platelet formation, proliferation of some hematopoietic cells, bone formation, adipocyte lipid transport, adrenocorticotropic hormone production, neuronal survival and formation, muscle satellite cell proliferation, and acute-phase production by hepatocytes (2).

LIF is best known as a stem cell factor. Originally, it was independently purified and cloned as the embryonic stem cell differentiation inhibitory activity, which specifically suppresses the spontaneous differentiation of mouse embryonic stem cells *in vitro* (3-5). On the basis of this property, LIF is used extensively in experimental biology by virtue of its key ability to maintain the self-renewal and pluripotency of mouse embryonic stem cells and induced pluripotent stem cells. Moreover, studies on LIF knockout mice have revealed that LIF has physiologic, nonredundant actions in maternal receptivity to blastocyst implantation, placental formation, and in the development of the nervous system, and is essential for blastocyst implantation and the normal development of hippocampal and olfactory receptor neurons (6). In addition, in $LIF^{-/-}$ mice mammary gland involution is delayed and reduced apoptosis is seen, while mammary glands show precocious development during pregnancy,

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indicating that LIF also plays a crucial role in involution following weaning (7). Finally, LIF also has important immunosuppressive functions during embryo implantation by regulating the numbers and migration of macrophages, uterine natural killer cells, and eosinophils (8, 9), and similar effects were observed in a very recent study showing that LIF prevents $CD8^+$ T-cell infiltration into the tumor while at the same time activating protumoral macrophage function in the tumor microenvironment thus enhancing the immunosuppressive nature of the tumor (10).

LIF was uncovered in the late 1980s independently by several research groups, based on the different biological effects it has in distinct cellular models, and was hence given multiple names. It was first cloned as an inducer of differentiation and inhibitor of proliferation in the M1 myeloid leukemic cell line (11) and therefore called leukemia inhibitory factor (LIF), which has remained the most commonly used name, despite it being the least appropriate (2). At the same time, it was also purified as human IL DA (HILDA) based on its ability to stimulate proliferation of murine myeloid DA2 cells (12), and as melanoma-derived lipoprotein lipase inhibitor that can induce severe cachexia in tumor-bearing nude mice (13). Despite the potential link between LIF and cancer implied by these early discoveries, and some reports of LIF production by cancer cells and purported effects on certain cancer cell lines *in vitro*, its importance in cancer has only recently come to the fore, especially with the first direct *in vivo* physiologic evidence (14).

Over a decade ago, the expression of both *LIF* and *LIFR* was found to be upregulated and associated with the development of breast cancer, regulated by epigenetic modification in the gene promoter region with distinct DNA methylation patterns (15, 16). But it was only recently that *in vivo* evidence emerged from xenograft studies, showing that LIF is implicated in breast cancer tumorigenesis, EMT, and metastasis by activating multiple signaling pathways such as STAT3 and AKT-mTOR, with higher LIF levels being significantly associated with a poorer relapse-free survival in patients with breast cancer (17, 18). Furthermore, LIF acts in both autocrine and paracrine manners, and plays a role in cross-talk between tumor cells and fibroblasts to mediate the proinvasive activation of stromal fibroblasts (19). LIFR was also reported to be upregulated to confer drug resistance to HDAC inhibitors (20). Excitingly, a new study by Viswanadhapalli and colleagues published in this issue adds further *in vivo* evidence documenting the physiologic importance of LIF in breast cancer and also provides detailed mechanistic understanding and valuable insights into its translational application (21). One of the most intriguing findings in this study is that upregulated LIF expression occurs more prominently in triple-negative breast cancer (TNBC), the most aggressive breast cancer subtype with a higher propensity for metastasis and therapy resistance. Starting by profiling LIF and LIFR expression in various subtype-representative cell lines, the authors observed that by and large TNBC cells have higher expression of LIF and LIFR compared with ER^+ breast cancer cells and normal mammary epithelial cells. Consistently, the TNBC cell lines with higher LIF and LIFR expression had much better response to LIFR inhibition in cell viability assays. Moreover, the efficacy of LIFR inhibition as a therapeutic strategy was evaluated in TNBC patient-derived xenografts *ex vivo* and *in vivo*. Altogether, these findings highlighted the possibility that LIFR-mediated signaling can be an attractive therapeutic target for breast cancer, particularly TNBC. In this precision medicine era, it is crucial to find the proper patients for

a targeted therapy so as to maximize therapeutic efficacy, and in this scenario LIFR inhibition specifically for TNBC subtype is attractive. Moreover, because TNBC is the more aggressive breast cancer subtype that is more likely to relapse and harder to treat, effective targeted therapies are urgently needed but lacking so far. However, further thorough validation in a large number of breast cancer cases is needed to confirm the specific correlation between LIF upregulation with the TNBC subtype, and then to verify the correlation between LIF levels and prognosis in patients with TNBC. In addition, characterization of the therapeutic effects in this study was focused solely on the tumor cells. Given the reported effects of LIF on both tumor cells and associated fibroblasts (19), future studies should also comprehensively examine the effects on the stromal components in the tumor microenvironment. In this connection it should be noted, however, that LIFR signaling has also been reported to function as a metastasis suppressor through the Hippo-YAP pathway (22) and confer a dormancy phenotype in breast cancer cells disseminating to bone (23), and therefore further evaluation in a more physiologically relevant context is needed to assess the roles of LIF on metastasis in breast cancer.

Genomic characterization by The Cancer Genome Atlas (TCGA) of over 20,000 primary tumors and matched normal samples spanning 33 cancer types revealed that the most highly dysregulated *LIF* expression occurs in pancreatic ductal adenocarcinoma (PDAC). Consistently, the physiologic significance and underlying cellular and molecular mechanisms of LIF action have been best illustrated in PDAC by several recent studies. In an effort to comprehensively investigate the paracrine interaction between the pancreatic cancer and stromal stellate cells (PSC), our systematic proteomic studies uncovered LIF as a key paracrine factor mainly generated by activated PSCs and inducing responses in cancer cells (14). We blocked LIF signaling by either genetic deletion of LIFR in cancer-deriving epithelial cells or by pharmacologic inhibition with a neutralizing anti-LIF antibody in the KPC genetically engineered mouse model of PDAC to evaluate the physiologic effects of LIF in PDAC. Both types of blockade markedly slowed tumor progression and augmented the efficacy of chemotherapy leading to prolonged survival of KPC PDAC tumor-bearing mice, mainly by enhancing cancer cell differentiation and reducing epithelial–mesenchymal transition status. Besides the functional significance of LIF in PDAC tumorigenesis, our studies also revealed that LIF can be an attractive circulating marker for monitoring tumor status and response to therapy. In a study focusing on the neural aspects of PDAC, the Tomasini group unraveled another function of LIF in PDAC to support PDAC-associated neural remodeling, suggesting a further therapeutic benefit of LIF blockade to alleviate pain and therefore improve life quality (24). In addition, in an effort to investigate the mechanisms underlying the diversity of cancer-associated fibroblast (CAF) heterogeneity, the Tuveson group discovered that LIF is a key driver for the development of inflammatory CAFs (25, 26). All these studies consistently revealed the activated PSCs are the major source of LIF production in pancreatic tumors; although, a fraction of tumor cells also express LIF as a secondary source (27). Elevated LIF in patients with pancreatic cancer could also be an underlying cause of cachexia, which is common in patients with PDAC and is the underlying cause of many disease-related complications and sometimes even death (28, 29).

Besides breast and pancreatic cancer, upregulated LIF secretion was also observed and shown to be associated with pathologic conditions in other solid tumors such as glioblastoma, nasopharyngeal carcinoma, prostate cancer, ovarian cancer, and osteosarcoma (30-35). However, due to the lack of inhibitory agents targeting the LIF/LIFR signal cascade, its value as a therapeutic target currently cannot be reliably evaluated. A small-molecule inhibitor of the LIF–LIFR axis would be highly desirable, due to the potential for oral bioavailability, greater tumor penetrance, and lower cost of production. However, the development of such an inhibitor is practically quite challenging, given the fact that LIF does not bind in a pocket but rather makes a number of contacts along a flat surface of the Ig-like domain of LIFR, making it hard to find a groove to dock a blocking molecule into.

Nevertheless, by screening an initial series of synthesized compounds rationally designed to dock into the interface of LIF–LIFR interaction based on the crystal structure of LIF/LIFR and subsequent optimization of a lead compound by medicinal chemistry modification, Viswanadhapalli and colleagues successfully developed a compound, EC359, that can bind at the LIF/LIFR binding interface to effectively block LIF-triggered LIFR signaling activation (21). Although the binding affinity of EC359 to LIFR, as measured by microscale thermophoresis technique (MST) assays, was approximately 10-fold lower than the approximately 1 nmol/L K_d for LIF binding to LIFR, surprisingly EC359 exhibited good *in vitro* and *in vivo* inhibitory activity with an IC_{50} of 10–50 nmol/L for *in vitro* cell viability assays. Moreover, the specificity of EC359 for inhibition of LIF–LIFR interaction was supported by at least two lines of evidence—a good correlation between the dose-dependent reduction of cell viability by EC359 treatment and LIF and LIFR expression levels in various breast cancer cell lines, and the effective inhibition of LIF-mediated STAT3 activation using STAT3-Luc reporter assays. Moreover, the low toxicity of EC359 *in vitro* (on low-LIFR–expressing ER^+ breast cancer lines or *LIFR*-knockout TNBC cells) and *in vivo* (on body weight) underscored its specificity. Last but not least, EC359 also possesses favorable pharmacologic features, supporting its therapeutic application *in vivo*. Notably, besides LIF, EC359 also blocked LIFR signaling elicited by other ligands, including Oncostatin M (OSM), Ciliary Neurotrophic Factor (CNTF), and Cardiotrophin 1 (CTF1). This might be an advantage or a problem depending on the specific case, broadening the spectrum of inhibition but in the meantime dampening the specificity and possibly leading to higher toxicity, and a careful evaluation will be necessary in each case. To inhibit the LIF–LIFR axis more specifically, a better strategy may be to block LIF activity with a neutralizing antibody, as we recently demonstrated (14), or by the use of soluble form of the receptor extracellular domain to act as a ligand-binding trap (36). Encouragingly, a humanized neutralizing anti-LIF antibody, MSC-1, has recently been developed as a LIF blocker and is being tested as a cancer therapeutic in a phase I clinical trial (ClinicalTrials.gov: NCT03490669).

An earlier effort to develop small-molecule inhibitors of cytokine signaling led to the discovery that bazedoxifene, originally designed as a selective estrogen receptor modulator, can act as an inhibitor of gp130 signaling (37), and was able to inhibit IL6 and IL11-induced activation of STAT3 in human pancreatic cancer cells and their proliferation in culture with an IC_{50} approximately 10 μmol/L, and also reduce xenograft tumor growth in mice (38). Although bazedoxifene was not tested for inhibition of LIF/LIFR signaling per se,

simulations showed that bazedoxifene could dock onto gp130 via the D1 domain, and experimentally prevented gp130 dimerization with the IL6 and IL11 receptors, bazedoxifene might also inhibit LIF/LIFR signaling. In this regard, the Forni group recently reported the results of testing bazedoxifene either as a monotherapy or in combination with chemotherapy in 13 patients with pancreatic and gastric cancer, with encouraging results, with 3 of the 6 patients who were biopsied showing reduced phospho-STAT3, including a patient with PDAC (39).

As commonly agreed, cancer frequently hijacks developmental programs to facilitate its progression and survival. Therefore, it should not be a surprise that LIF, one of the key molecules in development, has been coopted to play important roles in promoting tumorigenesis. However, LIF's physiologic significance in cancer has not been well recognized until recently. With the new development of the EC359 small-molecule LIFR inhibitor, the bazedoxifene gp130 inhibitor, and the anti-LIF antibody MSC-1, we are optimistic that the therapeutic potential of LIF blockade in many tumor types will be widely tested in the near future.

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