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Leukemia inhibitory factor may be a promising target against pancreatic cancer

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Pancreatic cancer is one of the deadliest forms of cancer, defying most treatments. Its ability to evade therapy may be attributable to the presence of cancer stem cells, a subset of cancer cells present in pancreatic tumors that drive tumor growth by generating bulk tumor cells. Cancer stem cells are notorious for their ability to resist traditional chemotherapies.

However, scientists at the University of California, San Francisco (UCSF), have discovered that two proteins — KRAS and leukemia inhibitory factor (LIF) — help create cancer stem cells and that the latter can be targeted to block them.

These results were presented at the American Association for Cancer Research's Pancreatic Cancer: Progress and Challenges conference, held here from June 18-21.

In many different types of tumors, a constitutively active, mutant form of the signaling protein KRAS helps drive the uncontrolled tumor cell proliferation that is a hallmark of cancer. In fact, more than 90 percent of pancreatic cancers exhibit KRAS mutations, but the link between KRAS and cancer stem cells has been tenuous — until now.

Using human pancreatic cancer cell lines and mouse fibroblasts and pancreatic cancer cells, Man-Tzu Wang, Ph.D., a postdoctoral researcher in the McCormick lab at the Helen Diller Family Comprehensive Cancer Center at UCSF, and colleagues showed that KRAS causes cells to

acquire and maintain stem cell-like properties.

"We know that KRAS is a very potent driver of pancreatic cancer, but we don't know how to drug it," said Wang. "Our results showed we can block KRAS-mediated cancer stem cells by blocking LIF activity."

KRAS is difficult to target therapeutically. Taking the next logical step, the researchers began looking for proteins that function downstream of KRAS in the generation of pancreatic cancer stem cells to determine if any of them could be potential drug targets. They found a number of candidates but focused on LIF, a protein known to regulate stem cell development. Moreover, they found that LIF is "druggable," making it a potential target for treatment.

Using neutralizing antibodies or shRNA, the team knocked down LIF activity or expression and found that each reduced the in vitro stem cell-like properties of mouse pancreatic cancer cells.

"We think our data indicate that blocking LIF can bring a significant improvement to cancer treatment," Wang said.

Knocking down cancer stem cells is critically important. Because these cells are slow-growing, they are often resistant to traditional chemotherapies, which target fast-growing cells. In addition, they also contain mechanisms that pump drugs out of the cell. Their survival allows them to continue to differentiate into mature cancer cells, leading to recurrent tumors.

Though there are no drugs currently available that target LIF, Wang is hopeful that these new data will spur the development of such products and generate new pancreatic cancer therapies.

More information:

Abstract

A role for leukemia inhibitory factor in oncogenic K-Ras-mediated stemness of pancreatic cancers. Man-Tzu Wang, Eric Collisson, Frank McCormick. UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco, CA.

Introduction: With a high degree of sequence homology as well as common sets of downstream effectors and upstream affecters, the three isoforms of Ras, N-, H- and K-Ras, have long been assumed to be functionally redundant. However, K-Ras, not N- or H-Ras, deficiency in mice leads to embryonic lethality, suggesting K-Ras may be required for the functions of stem cells. Activating mutations of K-Ras occur in over 90% of pancreatic cancers, but specific approaches to target oncogenic K-Ras have been difficult to develop. Thus, identification of essential factor(s) for K-Ras-mediated malignancy may provide an alternative way to block this —undruggable oncogene. Cancer stem cells (CSCs), sharing certain similar gene expressing signatures and biological functions with normal stem cells, have been identified in numbers of human malignancies, including pancreatic adenocarcinoma. Self-renewal and resistance toward conventional chemotherapies of CSCs can lead to tumor recurrence after treatments. Despite the putative role of K-Ras activation in pancreatic carcinogenesis, the roles of oncogenic K-Ras in CSCs have not convincingly demonstrated. Herein, we report that oncogenic K-Ras, differentially from H-Ras, causes CSC-like properties in transformed mouse fibroblast and pancreatic cancer cells. Furthermore, we identified the leukemia inhibitory factor (LIF), a stem cell regulatory chemokine, as a downstream effector essential for K-Ras-mediated stemness in pancreatic cancer cells.

Methods: NIH3T3 cells transformed by H-RasV12 or K-RasV12, human pancreatic cancer cell lines, PANC2.13 and PANC1, as well as mouse pancreatic duct carcinoma cells (PDAC) derived from transgenic mice

(FVB background; LSC-K-RasG12D; p53F/+,pDXCRE) were used as experimental models. Sphere forming assay and drug sensitization assays were used to evaluate in vitro stemness properties. The expression of stem cell markers was determined by quantitative PCR (qPCR) and western blotting. Microarray analysis (Mouse Gene ST1.0) was performed to evaluate the gene expression profiles. Knock-down of LIF or neutralization of LIF was conducted by shRNA or neutralizing antibodies against LIF, respectively.

Results: When compared to those transformed with H-RasV12, NIH3T3 cells with K-RasV12 demonstrated significantly enhanced sphere forming efficiency, increased resistance toward doxorubicin or cisplatin, and heightened sensitivity to the CSC inhibitor, salinomycin. Human and mouse pancreatic cancer cells in which K-Ras was knocked down possessed dramatically reduced in vitro stemness, suggesting that oncogenic K-Ras is required for the maintenance of pancreatic CSCs. Whole genome microarray analyses revealed oncogenic K-Ras specifically up-regulated the expressions of multiple stem cell-related factors at the RNA level. Among these genes, leukemia inhibitory factor (LIF) showed the greatest increase in K-RasV12-transformed cells (log fold change=2.29749) when compared to H-RasV12- transformed cells. Furthermore, oncogenic K-Ras induced mouse pancreatic cancer showed increased LIF expressions at RNA and protein levels in the comparison to B-Rafmt driven tumors. Human and mouse pancreatic cancer cells in which K-Ras had been knock down showed significantly reduced LIF expression. Repression of LIF expression or activity by shRNA or neutralizing antibodies suppressed in vitro stem-like properties in mouse PDACs driven by K-Rasmt, but not in B-Rafmt.

Conclusions: LIF plays essential roles in K-Ras-induced pancreatic cancer stemness properties, and may serve as a novel therapeutic target to eradicate pancreatic cancers with K-Ras activations.