
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.