Targeting a Novel KRAS-Integrin-Linked Kinase Regulatory Circuitry in Pancreatic Cancer

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Abstract

Acting KRAS mutations are the most frequent genetic abnormality in over 90% of pancreatic cancers. Evidence indicates that these mutations not only play a crucial role in initiating genomic carcinogenesis but are also required for tumor maintenance. From a clinical perspective, oncogenic KRAS represents a therapeutically relevant target in pancreatic cancer, of which the proof-of-concept was demonstrated by the effectiveness of ARMA-mediated silencing of KRAS to suppress pancreatic tumor growth in vivo. In this study, we have identified a novel KRAS-E2F1-ILK/hnRNP A1 regulatory circuitry that governs the expression of oncogenic KRAS. Integrin-linked kinase (ILK) is a serine/threonine kinase that mediates both intracellular signaling via cell–matrix interactions, angiogenesis and also plays a role in epithelial to mesenchymal transition (EMT) in cancer cells. Dysregulation of ILK expression has been observed in several tumors including breast, ovarian, melanoma, lung, prostate and pancreas and is related to correlated with tumor progression, metastasis and chemoresistance to gemcitabine in pancreatic adenocarcinoma cells, but the mechanisms by which ILK is required for the tumorigenesis in pancreatic cancer are yet to be understood. In our study, oncogenic KRAS induces ILK expression via an E2F1-dependent mechanism, while E2F1 regulates the ILK expression by promoting ILK transcriptional initiation of the KRAS gene. As a result, disruption of this circuit via siILK-mediated knockdown of any of these intermediary effectors (E2F1, ILK, or hnRNP A1), or pharmacological inhibition of ILK by T151, a novel ILK inhibitor developed in our laboratory, led to suppression of KRAS expression and reversal of the mesenchymal phenotype of pancreatic cancer cells. The therapeutic relevance of ILK in regulating this regulatory circuitry is evident in the susceptible effect of ILK knockdown in the EGFR-induced expression of EGFR-activated KRAS expression. Together, these findings provide a rationale for targeting ILK as a novel strategy to suppress oncogenic KRAS signaling.

Results

Figure 1. Mutant KRAS regulates ILK expression through transcriptional regulation in pancreatic cancer cells. A. Western blot analysis of differential protein expression levels of KRAS, E2F1 and ILK in pCMV-EGF1, pCMV-KRAS, pCMV-E2F1 and pCMV-E2F1-KRAS-transfected AsPC-1 cells. B. qRT-PCR analysis of the expression of endogenous KRAS, E2F1 and ILK (pCMV-E2F1 and pCMV-E2F1-KRAS) in the presence of pCMV-EGF1 (left) and pCMV-E2F1-KRAS (right), related to the pCMV control, on the protein expression of E2F1 and ILK, and on the ILK gene transcription as determined by qRT-PCR luciferase reporter assay (left) and independent experiments (right).

Figure 2. Evidence that regulation of KRAS expression through a KRAS-E2F1-ILK regulatory circuitry. A. Western blot analysis of the effects of ectopic expression of ILK on the expression levels of endogenous KRAS, E2F1 and hnRNP A1 using an shRNA lentiviral transduction approach and the analyses of the effect of siRNA-mediated knockdown of ILK on the protein expression levels of KRAS, E2F1 and hnRNP A1 in AsPC-1 cells. B. qRT-PCR analysis of the expression of endogenous KRAS and ILK (pCMV-E2F1 and pCMV-E2F1-KRAS) in the presence of pCMV-EGF1 (left) and pCMV-E2F1-KRAS (right), related to the pCMV control, on the protein expression of E2F1 and ILK, and on the ILK gene transcription as determined by qRT-PCR luciferase reporter assay (left) and independent experiments (right).

Figure 3. ILK regulates KRAS expression through hnRNP A1-mediated transcriptional suppression. A. Western blot analysis of the effect of sihnRNP A1-mediated knockdown of ILK on the protein expression levels of KRAS, E2F1 and hnRNP A1. B. qRT-PCR analysis of the effect of sihnRNP A1-mediated knockdown of ILK on the protein expression levels of KRAS, E2F1 and hnRNP A1 in AsPC-1 cells. C. Western blotting of the corresponding levels of hnRNP A1, ILK, and E2F1 (left) and hnRNP A1 and E2F1 (right) in AsPC-1 cells (left) and HeLa cells (right). D. qRT-PCR analysis of knockdown of hnRNP A1 silencing efficiency in AsPC-1 cells. E. Western blot analysis of knockdown of hnRNP A1 silencing efficiency in AsPC-1 cells (left) and HeLa cells (right). F. Western blot analysis of knockdown of hnRNP A1 silencing efficiency in AsPC-1 cells (left) and HeLa cells (right).

Figure 4. Evidence that ILK is involved in mediating the effect of oncogenic KRAS on EMT. A. Western blot analysis of the effects of ectopic inducible shhnRNP A1-mediated knockdown of ILK on the protein expression levels of EMT trans factors, including E-cadherin (E-cad), vimentin (Vim), and N-cadherin (N-cad), in AsPC-1 cells transfected with pCMV-shhnRNP A1 (left) and pCMV-EGF1 (right), related to the pCMV control, on the protein expression of E-cad, Vim, and N-cad therefore transcription as determined by qRT-PCR luciferase reporter assay (left) and independent experiments (right).

Figure 5. Summary of the EGFR-KRAS-ILK signaling pathway. Schematic diagram depicting the EGFR-KRAS-ILK/hnRNP A1 regulatory circuitry that governs the expression of oncogenic KRAS in pancreatic cancer cells.

• In this study, we have identified a novel KRAS-E2F1-ILK/hnRNP A1 regulatory circuitry that governs the expression of oncogenic KRAS in pancreatic cancer cells.

• It provides a rationale for targeting ILK as a novel strategy to suppress oncogenic KRAS signaling in pancreatic cancer.