

Pathophysiological role of microRNA-29 in pancreatic cancer stroma

Authors: Jason J Kwon, Sarah C Nabinger, Ravi K Alluri, Zachary Vega, Smiti S Sahu, Zahi Abdul Sater, Zhangsheng Yu, A Jesse Gore, Grzegorz Nalepa, Romil Saxena, Murray Korc, and Janaiah Kota

Abstract

Background: Dense fibrotic stroma associated with pancreatic ductal adenocarcinoma (PDAC) has been a major obstacle for drug delivery to the tumor bed and may impede attempts to slow down PDAC progression and metastasis. However, current anti-stromal drugs have not improved tumor response to chemotherapy or patient survival. Thus, a better understanding of the molecular mechanisms associated with tumor-stromal interactions is desperately needed to develop novel anti-stromal therapeutic approaches. MicroRNAs (miRNAs) are an abundant class of highly conserved, small non-coding RNAs that function as key regulators of eukaryotic gene expression and cellular homeostasis. miR-29 is known to play a paramount role in the fibrotic process of several organs by providing crucial functions downstream of pro-fibrotic signaling pathways such as TGF- β 1 and regulates the expression of extracellular matrix (ECM) proteins, a major component in the PDAC stroma. Upregulation of TGF- β 1 is associated with PDAC pathogenesis and is known to activate stromal cells. Furthermore, vascular endothelial growth factor (VEGF) that stimulates tumor angiogenesis is a predicted target of miR-29. We hypothesize that miR-29 may be misregulated in TGF- β 1 activated PDAC stromal cells and lead to excessive accumulation of ECM proteins and VEGF.

Restored expression of miR-29 could be therapeutically beneficial to modulate tumor-stromal interactions.

Methods: Northern blot or qPCR techniques were used to assess miR-29 levels *in vitro* stromal cells, murine PDAC model, and PDAC patient biopsies, and stromal deposition/fibrosis was determined by Sirius red staining. In murine and human PDAC samples, stromal specific miR-29 expression was determined via *in situ* hybridization by co-staining pancreatic tissues with glial fibrillary acidic protein a marker for stromal cells and miR-29. miR-29 functional studies were conducted by transfection of stroma cells with synthetic miR-29 mimics and locked nucleic acid, a miR-29 inhibitor, and ECM protein/VEGF expression was analyzed by western blot analysis. The effect of miR-29 overexpression in stromal cells on cancer colony growth was evaluated by direct co-culture of stromal cells ectopically expressing miR-29 with pancreatic cancer cells, and subsequently, cancer colony number and stromal accumulation was determined by crystal violet and sirius red stains respectively.

Results: In both *in vitro* and *in vivo* models as well as PDAC patient biopsies, we observed loss of miR-29 is a common phenomenon of activated stromal cells, and is associated with a significant increase in ECM and VEGF accumulation. Restored expression of miR-29 in stromal cells reduced the deposition of matrix proteins, VEGF expression, and cancer colony formation in direct co-culture.

Conclusion: These results provide insight into the mechanistic role of miR-29 in PDAC stroma and its potential use as an anti-stromal/angiogenic therapeutic agent.