Abstract.

Background & Significance:

Pancreatic cancer is responsible for nearly 40,000 deaths in the U.S. annually, with a dismal 5-year survival below 7%. Poor therapeutic outcomes reflect (1) a paucity of new approaches targeting the genomic underpinnings of pancreatic ductal adenocarcinoma (PDAC, comprising 95% of pancreatic cancers) and (2) an inability to overcome the desmoplastic stromal barrier characteristic of PDAC. RNA interference holds promise in targeting key mutations, e.g. oncogenic Kras (mutated in >90% of PDACs). However, a small RNA delivery vehicle that homes to PDAC and breaches the stroma does not yet exist. iRGD, a new cyclic peptide from the Ruoslahti lab identified by phage display screening in tumors, mediates tumor targeting and *penetration* of small molecules by binding $\alpha_v\beta_{3/5}$ integrins while cyclized and signaling through neuropilin-1 after proteolytic cleavage reveals a cryptic CendR domain. Thus, we hypothesize that "tandem" peptides combining a cell-penetrating peptide (CPP) and iRGD can complex with siRNA or miRNA to form tumor-penetrating nanocomplexes (TPNs) effective in delivering siRNA to PDAC. A tumor-targeted and penetrating nanoscale carrier could address many of the delivery constraints and additionally provide a practical means for bridging the extensive compendia of *in vitro* RNAi hits and clinical translation by serving as a platform for validating genetic targets in realistic animal models.

Specific Aims:

I specifically propose to (1) design, characterize, and optimize modular iRGD-based TPNs for gene knockdown in pancreatic cancer *in vitro*; (2) formulate the preferred iRGD-based siRNA delivery vehicle for systemic delivery in clinically-relevant mouse models of pancreatic cancer through covalent and non-covalent chemical modifications; and (3) explore the translational potential of the technology through *in vivo* validation of candidate RNAi targets identified in human pancreatic cancer cell lines/samples, culminating in therapeutic trials in genetically-engineered mouse models of pancreatic cancer.

Methods:

In Aim 1, we will synthesize a library of peptide-based small RNA carriers composed of a tumor-penetrating domain and an siRNA-binding/cell-penetrating domain to study the key principles behind their function. We will use these studies to design new iRGD-based TPNs and characterize their suitability for mediating gene knockdown using *in vitro* assays. Finally, we will validate multi-gene RNAi and RNAi plus small molecule therapeutic combinations using TPNs *in vitro*.

In Aim 2, we will formulate TPNs to improve their pharmacokinetic profile in systemic administration. Specifically, we will employ non-covalent stabilizers (e.g. PEGylated peptides/lipids) as well as covalent modifications (fatty acid tails). We will compare the biodistribution, circulation time, and knockdown capacity of different particle formulations in mouse models – heterotopic and orthotopic transplant models and genetically engineered mouse (GEM) models such as the KPC PDAC model.

Finally, in Aim 3, we will establish a platform for profiling new siRNA targets *in vivo* to complement and follow-up on *in vitro* pooled shRNA screens performed by collaborators in the lab of Dr. William Hahn. Furthermore, we will conduct therapeutic pre-clinical mouse trials for promising target candidates from the literature and shRNA screens in orthotopic and KPC GEM models of pancreatic cancer (in collaboration with Dr. Tyler Jacks).