ABSTRACT:

The overall goal of this project is to elucidate the contributions of tumor suppressor miRNAs to drug resistance and to use this information to develop new multi-targeted miRNA-based therapeutics for treating pancreatic ductal adenocarcinoma (PDAC). We recently discovered that miR-15a suppresses several elevated oncogenic targets in PDAC, including BMI1, CHK1, WEE1, and YAP1. BMI1 is a commonly overexpressed cancer stem cell marker, associated with poor prognosis and playing many roles in PDAC, including regulating proliferation, self-renewal, epithelial-to-mesenchymal transition, and metastasis. YAP1 is a transcriptional co-activator that activates oncogene expression downstream of the Hippo signaling pathway and promotes the bypass of oncogenic KRAS addiction, directly contributing to oncogenesis in PDAC. Both WEE1 and CHK1 are key G2 checkpoint kinases recently evaluated in clinical trials for treating PDAC. Although they are essential in its biology, the survival benefit of targeting WEE1 or CHK1 in patients with PDAC is minimal. We characterized the tumor suppressive function of miR-15a in PDAC by suppressing the expression of significant target genes (i.e., *Wee1*, *Chk1*, *Bmi1*, and *Yap1*). More importantly, our work features a novel concept of creating a miRNA-based biomimicry strategy based on tumor suppressor miR-15a using gemcitabine (Gem) that proved to be highly effective at eliminating PDAC cells while retaining target specificity. The novel miR-15a mimic was designed by replacing the cytidine (C) bases in the guiding miRNA strand with Gem. Using this approach, we combined the power of Gem and multi-targeted tumor suppressor miR-15a into a single synergistic entity. ***An important and unique feature of Gem-modified miRNAs is that they can be internalized by pancreatic cancer cells without a delivery vehicle (e.g., without lipid or magnetic nanoparticles or oligofectamine)***. *Collectively, these findings represent a major advance and a profound paradigm shift in developing miRNA-based therapeutics.* Our preliminary results revealed that gemcitabine (GEM) substitution improves the potency and stability of miR-15a and improves its capacity to inhibit PDAC metastasis *in vivo* without any observed toxicity, such as body weight loss.

The **Specific Aims** of the proposed project are as follows:

Specific Aim 1: We will define the direct targets and pathways in PDAC cells modulated by miR-15a and the Gem-miR-15a mimic and characterize the effects of Gem-miR-15a mimic on apoptosis, cell cycle control, and chemoresistance in vitro. We hypothesize that miR-15a and its mimics will exhibit critical tumor suppressor functions in PDAC cells via their capacity to suppress multiple oncogenic targets (WEE1, CHK1, YAP1, BMI1 and others) and pathways.

Specific Aim 2: We will develop and characterize the therapeutic potential of the Gem-miR-15a mimic in PDAC using *in vivo* model systems. We hypothesize that the Gem-miR-15a mimic will exhibit improved therapeutic efficacy compared to current regimens when administered either alone or in combination with gemcitabine to PDAC tumor models *in vivo*.