Novel therapy targeting Mutant-KRAS^{G12D} and Galectin-1 in Pancreatic Cancer

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Introduction: Although, surgical resection and chemotherapy are the gold standard for treating Pancreatic Ductal Adenocarcinoma (PDAC), low patient survival rate remains the problem. The activating point mutation of the KRAS on codon-12 is present in 70–95% of PDAC cases and so far, no success has been achieved to inhibit KRAS. KRAS^{G12D} regulates cell proliferation, differentiation, apoptosis. Recent preliminary and published studies show high Galectin-1 (Gal-1) levels in both pancreatic cancer and stromal cells, which modulate tumor microenvironment and metastasis. Additionally, genetic deletion of gal1 inhibits metastasis and improves survival in KRAS mouse model of PDAC **(1)**. Therefore, our objective is to develop a novel combination therapy for PDAC by targeting mutated KRAS^{G12D} point mutation and Gal-1. This includes the delivery of KRAS^{G12D} inhibiting siRNA (siKRAS^{G12D}) using a superparamagnetic iron oxide nanoparticle (SPION) and a galectin inhibitor.

Methods: ASPC1/Panc-1 (human), KPC (mouse) cells were used. Our patented SPION nano-formulation **(2)** has been used to deliver siKRAS^{G12D} and investigated in conjunction with Gal-1 for its anticancer efficacy. Particles were investigated for size, physico-chemical characterization (Dynamic light scattering), hemocompatibility (hemolysis assay) and the complexation of siKRAS (gel retardation assay). Cellular internalization and uptake of the particles were investigated using FAM labelled siRNA and Prussian blue assay. KRAS^{G12D} silencing was confirmed at both mRNA and protein levels. Anti-cancer efficacy of the formulation was determined using *in vitro* functional assays for cell viability (MTT), migration (Boyden chambers), invasion (Matrigel), clonogenicity, tumor spheroid formation, and in nude mice.

Results: Our results demonstrate optimal particle size and zeta potential of SP-siKRAS formulation. SP-siKRAS efficiently internalized in PDAC cells and suppressed KRAS^{G12D} as well as its downstream targets, YAP and PDL-1. Combined targeting of siKRAS and Gal-1 inhibited cell proliferation. The formulation inhibited chemoresistance, cell proliferation, clonogenicity, migration, and invasion of pancreatic cancer cells. This resulted in activation of death related mechanisms, such as Bax, bcl-2, PARP cleavage in KRAS^{G12D} cells. Interestingly, the formulation was highly effective in inhibiting KRAS^{G12D} and growth of tumor spheroid in 3D cell models, which recapitulate the heterogeneity and pathophysiology of PDAC. This further provides a clinical validation demonstrating potential of SP-siKRAS particles to efficiently silence KRAS expression. SP-siKRAS also exhibited hemocompatibility, suggesting its potential of silencing KRAS without being toxic to the body. Additionally, the formulation was efficiently delivered in nude mice to exhibit KRas^{G12D} silencing and inhibit tumor growth.

Conclusion: This gene therapy targeting KRAS G12D mutation with a Gal-1 inhibition has a potential to modulate the oncogenic network and tumor microenvironment resulting in the repression of growth, metastasis, chemoresistance, and improvement in patient survival. This study will develop a novel sustainable therapeutic approach to target pancreatic cancer growth and improve patient survivability.

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1. Carlos A. Orozco, Neus Martinez-Bosch, Pedro E. Guerrero. Proceedings of the National Academy of Sciences Apr 2018, 115 (16) E3769-E3778; DOI: 10.1073/pnas.1722434115.
2. Khan S, setua S, kumari S, dan N, massey A, hafeez BB, yallapu MM, stiles ZE, alabkaa A, yue J, ganju A, behrman S, jaggi M, chauhan SC. Superparamagnetic iron oxide nanoparticles of curcumin enhance gemcitabine therapeutic response in pancreatic cancer. Biomaterials. 2019 jul;208:83-97. Doi: 10.1016/j.Biomaterials.2019.04.005. Epub 2019 apr 8. Pmid: 30999154.