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Modulating KRAS Expression for Pancreatic and Ovarian Cancers

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Introduction

- Women diagnosed with ovarian cancer have a higher mortality rate than women diagnosed with any other gynecological cancer (1)
- Pancreatic cancer is one of the deadliest cancers with a 5 year survival rate of <10% in the U.S. (2)
- SKOV3 is an ovarian cancer cell line (3)
- Mutations or increased expression in the KRAS genes causes the oncogene to become cancerous
- KRAS expression is regulated at the transcriptional level

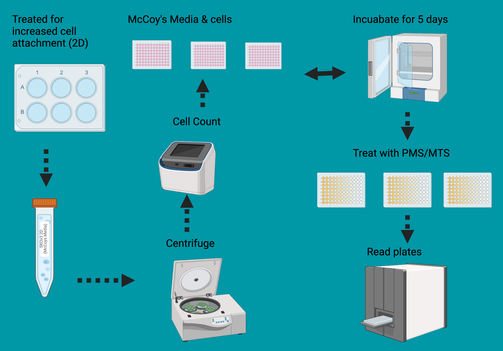
Unknown

- The efficacy of therapeutics stabilizing unique DNA structures (G-quadruplexes, G4s) in KRAS oncogene expression and ovarian/pancreatic cancer cells

Hypothesis

- Down regulation of KRAS through stabilizing of unique DNA structures in SKOV3 ovarian and CFPAC-1 pancreatic cell lines using oligonucleotides and/or novel indoloquinoline compounds to downregulate KRAS.
- JM49, JM65, JM55, and JM46 will stabilize the KRAS G4

Methods



- Plate SKOV-3 cells in 96-well plates in complete McCoys media
- CFPAC-1 cells in 96-well plates in IMDM or DMEM media
- The next day, treat SKOV-3 cells with a range of PPRH or compound doses. Incubate the plates at 37 C in 5% CO2 for 120 hr and image cells every 8 hr with a Cytena CellLinkX live cell imager
- After 120 hr, determine cell viability with the colorimetric MTS assay (supplemented with 5% PMS)
- After 4 hr of incubation, read absorbance of MTS at 490 nm and calculate the fold-change in cell viability with treatments. Use GraphPad Prism to determine cytotoxic effects

TERMS

CFPAC-1	CFPAC-1 is a ductal pancreatic adenocarcinoma derived by differential trypsinization of explant cultures from a metastatic lesion in the liver of a 26 year old Caucasian male with cystic fibrosis (CF) (4).
KRAS	A protein that communicates extracellular signals with intracellular proteins to signal for cell survival and proliferation (5). It is overexpressed or mutated in 30% of all cancers and is a validated therapeutic target.
PPRH	Polypurine (many C's and T's) strand of DNA that self-complements and forms a hairpin using intramolecular Hoogsteen hydrogen bonds (6).
G-Quadruplex	Non-B-DNA structure that occurs when guanines (Gs) base-pair with each other. They are held together by Hoogsteen hydrogen bonds (7).
SKOV3	Aggressive ovarian cancer cell line derived from a 64-year old Caucasian woman's adenocarcinoma (cancer) (2).

PPRH's as regulators of KRAS

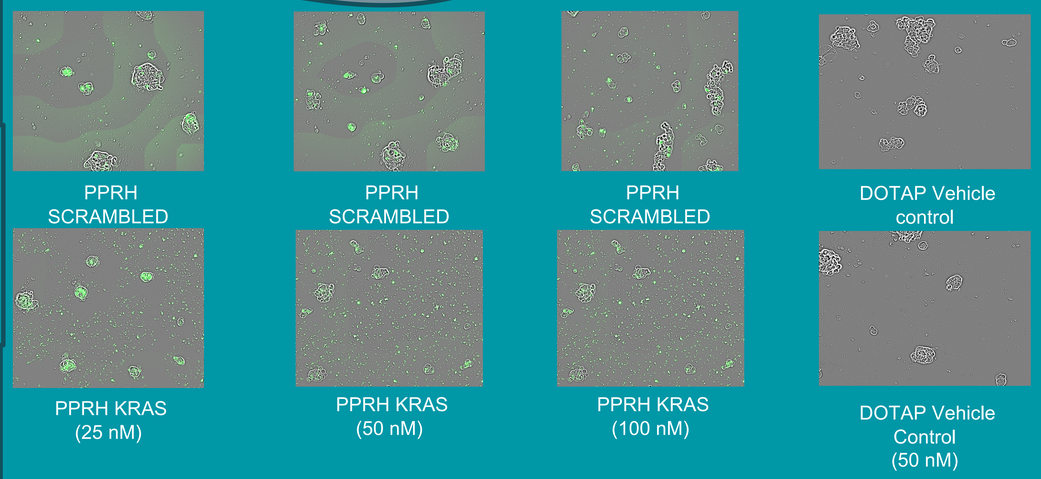


Figure 1: PPRH penetrance and effects on SKOV3 cells. SKOV3 ovarian cancer cells were transfected (with DOTAP) with a dose-range of either Scramble control or KRAS targeted KRAS's. PPRH's were FAM labeled and are visible as green fluorescence. Both the scramble and the targeted PPRHs penetrated the cells equally, and cellular uptake is evident. The targeted KRAS PPRH is more toxic to the SKOV3 cells than either the DOTAP transfection reagent vehicle control or the scramble PPRH control, indicated that downregulation of KRAS caused by G4 formation is toxic to these ovarian cancer cells.

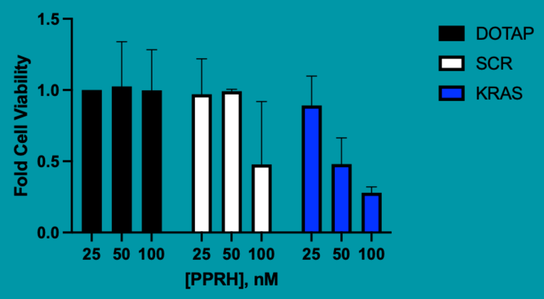


Figure 2: Dose-dependent cytotoxicity of PPRHs in SKOV3 cells. As above, SKOV3 cells were treated with a dose-range of PPRHs and allowed to incubate for 120 hr. Targeting KRAS (blue) has a more profound effect on SKOV3 cell growth than either the DOTAP vehicle control or the scrambled (SCR) PPRH control. Experiments were repeated in triplicate.

RESULTS

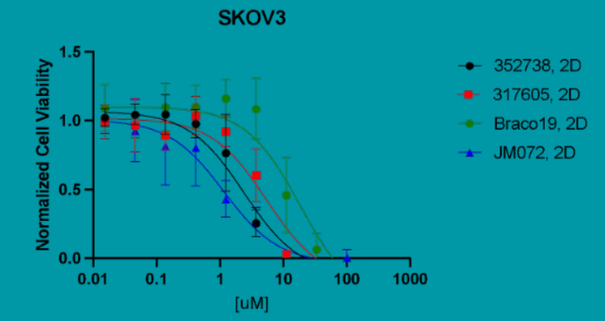


Figure 3: Compounds targeting the G4s, either broadly or selectively, demonstrate cytotoxicity in SKOV3 cells. SKOV3 cells were incubated in the presence of a dose-range of either the pan-G4-stabilizing BRACO-19 compound, or the KRAS G4-selective stabilizing compounds 352738, 317605 or JM072. The experiment was repeated in duplicate with technical triplicates.
Globally, the KRAS G4-selective stabilizing compounds were more effective than the pan-G4-stabilizing BRACO-19, and JM072 is the most cytotoxic of the tested agents with an IC50 of 1.2 μM.

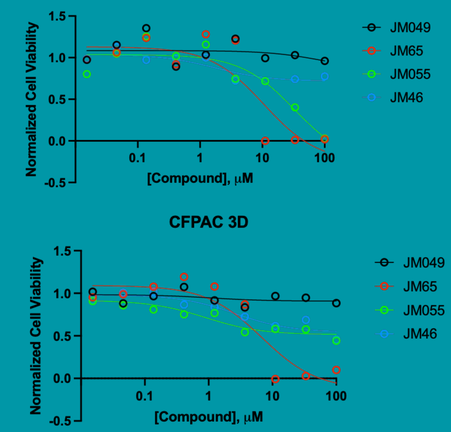


Figure 3: JM049, JM65, JM055, and JM46 were chemotherapeutics used to treat the CFPAC-1 2D and 3D cell lines. JM65 was the most promising compound that stabilizes the KRAS promoter structure, along with the oligonucleotides HpKRAS_G4 and HpKRAS_I1.

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