Re-expression of DIRAS3 in KRAS Mutant Pancreatic and Ovarian Cancers increases Sensitivity to Autophagy Inhibitors

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Methods

1. Materials

• Mutant KRAS drives 30% of human cancers from several sites, including pancreatic ductal adenocarcinoma (PDAC) and low-grade serous ovarian cancer (LGSOC).
• More than 90% of PDACs and up to 40% of LGSOCs are driven by mutant KRAS.
• Despite the prevalence of RAS mutations in many different cancers, effective RAS-targeted treatment remains a challenge.
• KRAS is a molecular switch and when mutated becomes permanently stuck in the ON position, inducing oncogenic transformation.
• Genetic suppression of KRAS or pharmacological inhibition of its effectors, ERK/MAPK, enhances the reliance of PDAC on autophagy which can become a target for treatment.
• Our group has discovered that mutant KRAS protein can be targeted directly by DIRAS3 (ARHI), a novel endogenous physiological KRAS inhibitor.
• DIRAS3 is an imprinted tumour suppressor gene that encodes a protein that possesses a distinctive N-terminal extension that is required to reverse RAS function, suppressing oncogenesis.
• In this study, we tested whether inhibiting RAS signalling by re-expressing DIRAS3 would sensitize RAS-driven PDAC and LGSOC cell lines to autophagy inhibition.

Hypothesis

• DIRAS3 re-expression will enhance sensitivity to autophagy inhibition in cancer cells with KRAS mutations but not in cancer cells with wild type KRAS.

Materials/Methods

1. DIRAS3-inducible cell lines:
   - PDAC: AsPC1-DIRAS3 (KRASG12D), BxPC3-DIRAS3 (KRAS WT), Capan2-DIRAS3 (KRASG12V).
   - LGSOC: HOC7 (KRAS WT), HCC5075 (KRASG12V) and PM-LGSOC1-DIRAS3 (KRASG12V).
2. Autophagy inhibitors, chloroquine (CQ) and 3,5-dichloroquine (DC616): used to examine their effect on DIRAS3-inducible cells.

Methods:

- Western blot assays: performed to examine the levels of phospho-ERK1/2, LC3B-I, LC3B-II, p62 and DIRAS3 expression.
- Sulforhodamine B colorimetric (SRB) assays: used to examine cell viability in presence and absence of autophagy inhibitors, with or without DIRAS3 expression.

Results

Figure 1: Re-expression of DIRAS3 in KRAS mutant ovarian cancer cells inhibited KRAS signalling and increased autophagy. As evidenced by the western blot results which show decreased phosphorylation of ERK1/2 (pThr202/Tyr204) and increased conversion of LC3B to LC3B-I in only KRAS mutant cells.

Figure 2: Inhibition of DIRAS3-induced autophagy inhibits growth of mutant KRAS-drive pancreatic cancer cells. As evidenced by the SRB results showing decreased IC50 for both CQ and DC616 in the mutant KRAS-driven cancer cell line (HCC5075), but not in wild type KRAS cancer cell line (HOC-7).

Figure 3: Re-expression of DIRAS3 in KRAS mutant pancreatic cancer cells inhibited KRAS signalling and increased autophagy. As evidenced by the western blot results which show decreased phosphorylation of ERK1/2 (pThr202/Tyr204) and increased conversion of LC3B-I to LC3B-II only in KRAS mutant cells.

Figure 4: Inhibition of DIRAS3-induced autophagy inhibits growth of mutant KRAS-drive pancreatic cancer cells. As evidenced by the SRB results showing decreased IC50 for both CQ and DC616 in the mutant KRAS-driven cancer cell lines AsPC1.

Conclusions

1. Expression of DIRAS3:
   - Inhibits RAS-RAF-MEK-ERK signalling.
   - Induces autophagy.
   - Enhances sensitivity to autophagy inhibitors in KRAS mutant cancer cells but not in wild type (WT) KRAS cells.

2. The greater potency of DC616 should prove more effective for combinatorial therapies in patients with KRAS mutant PDAC and LGSOC.