Inhibition of Porcupine reduces Wnt-dependent colony formation and/or proliferation in both autocrine and paracrine cell models

BA Thompson1, KJ Messenger2, E Linnane1, L Sheehy1, SE Coupland3, PJ Calcral1 Contact: b.thompson@redxpharma.com

Redx Oncology, Liverpool, UK 1Department of Molecular and Clinical Cancer Medicine, Institute of Translational Medicine, University of Liverpool, UK.

Introduction

Ablation of the poro-suvalpro-related Wnt signalling pathway is a key determinant in numerous cancers (Nusse and Varmus, 2012) and therefore constitutes of this pathway are potentially promising therapeutic targets. The mammalian-bound acyltransferase porcupine (PORCN) is required for post-translational modification of all Wnts and is vital for Wnt secretion (Herr and Badler, 2012). Due to the complex and varied oncogenic roles of the known Wnt ligands, prevention of active ligand secretion provides an effective mechanism for inhibiting canonical and non-canonical Wnt signalling in both paracrine and autocrine systems.

We therefore sought to determine whether a PORCN inhibitor could effectively reduce Wnt-dependent proliferation in both paracrine and autocrine in vitro models.

PORCN inhibition restricts proliferation of CAPAN-2 cells

Figure 2 shows that REDX05562 potently inhibits Wnt signalling in CAPAN-2 cells. We therefore sought to determine the effect of PORCN inhibitors on the proliferation of in vitro models of pancreatic cancer cell lines (CAPAN-1 and HPAF-II).

- CAPAN-2 or HPAF-II cells were seeded in 96-well plates (2000 or 3000 cells/well respectively).
- After 24 hrs, media was removed and replaced with growth media containing compound or DMSO control (final DMSO concentration = 0.1%) and incubated for a further 24 hrs.
- The media was removed and transferred to cells stably expressing luciferase under control of Wnt pathway response elements (Enzo Life Sciences; seeded 24 hrs prior to media transfer).
- After 24 hrs, Wnt pathway activation was quantified with ONE-Glo Luciferase assay system (Promega) and luminescence was measured with an Envision plate reader (PerkinElmer).

REDX05562 potently inhibited Wnt-dependent proliferation of CAPAN-2 cells. Addition of recombinant Wnt3a restored Wnt signalling, confirming that the observed compound-mediated inhibition is occurring upstream of the Wnt receptor, Frizzled.

Having shown potent inhibition of Wnt signalling in our cell-based gene reporter assay we next determined whether REDX05562 inhibited Wnt pathway inhibition in a human pancreatic cancer cell model (CAPAN-2). Pathway engagement by REDX05562 was determined by the expression of AXIN2, an established marker of Wnt pathway activity (Jho et al., 2002).

- CAPAN-2 cells were grown to 70% confluence.
- Cells were treated with compound or DMSO control (final DMSO concentration = 0.1%) for 24 hrs before being harvested.
- Relative changes in AXIN2 mRNA with respect to 2 housekeeping genes (β-Actin and GAPDH) were tested by qPCR and processed using CTX manager software (BioRad).

At 10nM REDX05562 appeared to decrease AXIN2 mRNA expression more than 25-fold (Fig. 2). This inhibition of AXIN2 could be reversed by addition of recombinant human Wnt3a (250ng/mL) consistent with compound-mediated inhibition occurring upstream of Frizzled.

In contrast to HPAF-II cells, treatment of MCF-7 cells with REDX05562 did not inhibit SFE (Fig. 5A). SFE was significantly improved, however, by addition of 100nM REDX05562 to mammospheres of CAPAN-2 cells (Fig. 5B), suggesting that Wnt3a can bestow anoxia resistance in this model via a paracrine route.

Conclusions

- REDX05562 potently inhibit Wnt-mediated signalling in both a gene reporter assay and an in vitro model of pancreatic cancer proliferation.
- Conditioned media from patient derived CAFs increased anoxia resistance in MCF-7 cells. REDX05562 treatment of CAFs significantly inhibited colony formation in this paracrine model. This novel biology could be matched with matched patient samples to further investigate the role of paracrine Wnt pathway signalling in various human cancers.
- Consistent with the early position of PORCN within the Wnt pathway signalling cascade, inhibition with REDX05562 reduced the impact of Wnt ligand-mediated signalling in both paracrine and autocrine models.
- Taken together these data emphasise the broad therapeutic potential of PORCN inhibition in Wnt-dependent cancers.

References