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Title of Project: Characterisation and targeting of Lin28 for the control of paediatric medulloblastoma

Summary: (approximately 1,000 words)

Hypothesis vs Findings

Medulloblastoma (MB) is the most common malignant childhood brain tumour. MBs are genetically subdivided into 4 groups of Wingless (WNT), Sonic hedgehog (SHH), Group 3 and Group 4. Children with Group 3 and 4 MBs (>60% of all MBs) have a high incidence of metastasis and a poor survival rate (~60%). Current treatment options include surgery, followed by radiotherapy and chemotherapy. Surgery is highly unlikely to remove all tumour without damaging surrounding healthy brain tissue. Radio and chemotherapy are not completely effective and also harm healthy tissues. As the current treatments for these aggressive subgroups are harsh, surviving children frequently show severely impaired physical, cognitive, social and emotional function for the rest of their lives. Clearly, new treatments are urgently needed to improve and save the lives of children with MB.

We hypothetised that Lin28B is an important protein in medulloblastoma (MB) progression. Our findings supported this hypothesis as presented below.

In this study, we first characterised the mRNA and protein expression of Lin28B in five different MB cell lines belonging to different molecular subgroups. We then identified that inhibition of Lin28B with its pharmacological inhibitor in MB cells with high levels of Lin28B, reduces the growth of cells as measured by two independent assays (ATP and WST-1).

Enforced expression of Lin28B (using plasmid overexpression) in MB cells with low levels of Lin28B, enhanced the production of cellular ATP, suggesting that Lin28B may promote cancer cell growth by enhancing cellular energy levels.

Our studies also determined simultaneous expression of a short and a long form of Lin28B in some MB cells. The roles of these newly identified isoforms are yet to be determined.

Intriguingly, we also identified that Lin28B expression is highly correlated with the expression of another gene, CaV3.2. Importantly, there is a selective inhibitor of

CaV3.2, mibefradil, that is used clinically for treatment of hypertension providing a unique opportunity for rapid drug repurposing for the treatment of MB.

We showed that CaV3.2 inhibition with mibefradil suppresses the growth and invasive abilities of MB cells.

Combination therapy is a critical approach to enhance the effect of current treatment options (eg. chemo/radio therapies) and/or use lower the doses of these agents in order to reduce side effects. We therefore also explored the effect of mibefradil together with current MB chemotherapeutic agent, vincristine. We identified that low concentrations of mibefradil (where by itself doesn't have any significant effect on MB growth), when combined with vincristine, improves the IC50 of vincristine in suppression of MB cell growth. Indeed, this results in very low concentrations of vincristine (where alone does not show any significant effect), to be effective in suppression of MB cell proliferation.

Understanding the mode of action of a drug is an important step towards enhancing the likelihood of bringing it into clinic. We hence performed proteomics analysis to further understand the mechanism of action of mibefradil. These studies strongly suggest that mibefradil results in a metabolic switch in cells by reducing mitochondrial function and enhancing lipid metabolism. Our further studies showed that this metabolic witch results in an induction of cell death in cancer cells.

Based on these studies, we are currently preparing two research manuscripts for publication. Findings from this study will be also used as preliminary data for this round of NHMRC Ideas Grant.

Unanswered Questions

-What are the roles of small and large isoforms of Lin28B?
-Is CaV3.2 expression controlled by Lin28B?
-Is mibefradil (or its similar structure compounds) an appropriate drug for MB treatment?

What these research outcomes mean

This study identified the critical roles of Lin28B and CaV3.2 proteins in MB growth. It also introduced mibefradil as a potential drug for treatment of MB ether alone or in combination with current chemotherapeutic agents. Future studies will further confirm the suitability of targeting Lin28B and/or Cav3.2 as a potential therapeutic strategy for MB treatment.