Combinatorial targeting of anaplastic lymphoma kinase (ALK) signalling to treat cancer and obesity

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Postgraduate scheme: **MRes + PhD (1 + 3-year non-clinical applicants only)**

MRes project outline:

Non small-cell lung cancer (NSCLC) is often caused by translocations leading to the fusion of genes such as Echinoderm Microtubule Associated Protein Like 4 (ELM4) with anaplastic lymphoma kinase (ALK). ALK is a single-pass transmembrane receptor tyrosine kinase (RTK) that is predominantly expressed in the brain, but ELM4-ALK fusion proteins are broadly expressed and constitutively signal via PI3K/AKT and other pathways to promote oncogenesis. This aberrant kinase signalling can be blocked by small molecule drugs, but these have undesirable side effects and can upregulate the expression of other RTKs to facilitate clonal evolution and tumour “escape” from ALK inhibitor monotherapies. Pharmacological Alk inhibition also upregulates RTKs in the wild-type mouse brain, and can dramatically reduce food intake and body weight in diet-induced obese (DIO) mice, which is mirrored by lean phenotypes in mice lacking Alk or its peptide ligand Alkal2. To improve the therapeutic benefit of ALK inhibition in cancer and better understand the consequences of its prolonged inhibition that could be relevant to the treatment of metabolic disease, it is essential to understand not just ELM4-ALK signalling in NSCLC but also normal ALK signalling in the brain. To this end, we have shown that neurons derived from human pluripotent stem cells (hPSCs) express ALK and robustly respond to ALKAL2, making it an ideal system to characterise native ALK signalling in a well-defined and mechanistically relevant human cell type. We propose to address these questions in an MRes project:
Aim) Characterise ALK signal transduction signalling in human neurons
Objective 1) Confirm that human cortical neurons respond to ALKAL2
Objective 2) Test which candidate signalling pathways are induced by ALKAL2
Objective 3) Identify alterations in gene expression upon ALK inhibition

MRes experimental plan:

Objective 1) Confirm that human cortical neurons respond to ALKAL2
Human induced pluripotent stem cell (hiPSC)-derived hypothalamic neurons express ALK and robustly respond to a purified ALKAL2 peptide. We will now extend these studies to the KOLF2.1J ‘reference’ iPSC line that stably expresses doxycycline-inducible neurogenin 2 (NGN2) to rapidly generate relatively homogeneous cultures of glutamatergic cortical neurons (i3N). Specifically, we will confirm ALK expression by RT-qPCR, assess rapid functional

responses by calcium imaging in the presence of synaptic blockers, and quantify the fraction of responding cells at various doses of ALKAL2 by immunohistochemistry for the immediate early gene cFOS.
Objective 2) Test which candidate signalling pathways are induced by ALKAL2
ELM4-ALK fusion proteins have been reported to signal via the PI3K/AKT/mTOR, JAK/STAT3, RAS/MEK/ERK, PLCγ/PIP2/IP3 pathways. To test which of these pathways are active in human neurons expressing native ALK, we will expose i3N to several doses of ALKAL2 for several durations, and test for candidate protein phosphorylation by Western blotting.
Objective 3) Identify alterations in gene expression upon ALK inhibition
The small molecule ALK inhibitor Ceritinib paradoxically upregulates PI3K/AKT/mTOR signalling in the mouse brain, suggesting that the mechanisms facilitating clonal evasion of ALK signalling reliance in cancer are also active in non-transformed neurons. To define these changes and compare and contrast them to existing datasets from the mouse brain and human hypothalamic neurons, we will treat i3N with ALKAK2 or Ceritinib at various doses and durations and compare the resulting gene expression changes by bulk RNA sequencing.

Outcomes: Together, these studies will shed light on endogenous mechanisms of ALK signalling that will inform therapeutic strategies for cancer treatment.

Main techniques
• Human pluripotent stem cell culture and neuronal differentiation
• RT-qPCR, RNA sequencing, and statistical analysis
• Calcium imaging
• Immunohistochemistry
• Western blotting

PhD project outline:

Non small-cell lung cancer (NSCLC) and other cancer types can result from fusion proteins involving the receptor tyrosine kinase (RTK) anaplastic lymphoma kinase (ALK). ALK-dependent tumours often evolve to escape pharmacological ALK inhibition by upregulating other receptors. This finding suggests that combination therapies targeting ALK alongside other candidate genes can lead to more effective cancer treatment by providing fewer paths for clonal evolution. Combination therapies may also permit lower drug concentrations to be used to reduce side effects such as liver toxicity and nausea that are commonly seen with ALK inhibitors. However, ALK signalling has been predominantly studied in culture-adapted cancer cell lines that only model certain patient populations, suggesting that considerable value could be derived by studying ALK signalling in its native context. In healthy individuals, ALK is predominantly expressed in the brain where it is activated by endogenous peptide ligands including ALKAL2, and may play a role in appetite regulation, making it a therapeutic target for metabolic disease. We will characterise ALK signalling in human neurons and established NSCLC cellular lines expressing ELM4-ALK fusion proteins in which ALK is constitutively active to reveal fundamental biological insights and design new candidate therapies in three Aims:

Aim 1) Characterise the effects of ALK activation and inhibition in human neurons
Objective 1a) Generate cortical neurons from human pluripotent stem cells (hPSCs)
Objective 1b) Identify signalling pathways engaged by ALK activation and inhibition
Objective 1c) Compare and contrast genes upregulated in response to ALK inhibition in neurons to those upregulated in cancer cell lines

Aim 2) Test combinatorial treatments in cellular models of NSCLC in vitro
Objective 2a) Select candidate drugs targeting signalling pathways and genes identified in Aim 1

Objective 2b) Test the ability of candidate drugs to slow cell growth in at least two cellular models of ALK-driven cancer, alone or in combination with ALK inhibitor drugs

Aim 3) Test combinatorial treatments animal models of cancer
Objective 3a) In a humanised mouse model of NSCLC, test for the ability of candidate drug combinations to slow tumour growth
Objective 3b) Since ALK inhibition can dramatically reduce body weight in mice fed a high fat diet, we will also test for changes in body weight and body composition in lean and obese mice treated with combination therapies.

PhD experimental plan:
Overactive ALK signalling participates in cancer formation but its role in other tissues is still poorly understood, including the brain where it is predominantly expressed. We will therefore test the hypothesis that characterising endogenous ALK signalling will reveal new strategies for treating ALK-driven cancer. To so do, we will first generate neurons from human pluripotent stem cells (hPSCs), and then characterise the signalling pathways and transcriptional changes induced by ALK inhibitors commonly used to treat cancer using targeted methods such as RTqPCR and Western blotting, as well as unbiased methods such as RNA sequencing and phosphoproteomics. Next, since upregulation of genes that promote growth and survival is one mechanism by which clones of ALK-driven cancer cells escape ALK inhibition, we will then test the ability of drug combinations targeting both ALK and candidate genes to slow the cancer cell line growth, using a combination of time-lapse imaging and sequencing to characterise "escaping" cell clones. Finally, we will test the ability of individual or combinatorially-added candidate drugs to suppress tumour growth in a humanised mouse model of ALK-driven cancer. Since ALK inhibition can have significant metabolic effects in mouse models of diet-induced obesity (DIO), we will also characterise the effects of drug treatment on body weight, body composition, and other metabolic parameters such as glucose homeostasis.

Expected outcomes and significance: Together, this project has the potential to reveal more potent strategies for the treatment of ALK-driven cancers, reduce the side effects associated with treatment, and shed light on the role of ALK in metabolic regulation, potentially also suggesting candidate treatments for metabolic disease. These results would be highly significant, since NSCLC is one of the most common forms of cancer affecting millions of people each year, and obesity and diabetes affect billions.

Main techniques:

- Human pluripotent stem cell culture and neuronal differentiation
- Drug screening in cellular models of cancer
- RT-qPCR, RNA sequencing, and statistical analysis
- Histology and immunohistochemistry
- Western blotting and phosphoproteomics
- Animal models of cancer and metabolic disease, and metabolic phenotyping

Key references:

Soda, M., Choi, Y. L., Enomoto, M., Takada, S., Yamashita, Y., Ishikawa, S., ... & Mano, H. (2007). Identification of the transforming EML4–ALK fusion gene in non-small-cell lung cancer. Nature, 448(7153), 561-566.

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neuroblastoma independent of MYCN status. Nature communications, 10(1), 5428.

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