Clinical PhD Project 2024

Exploring the biomarker potential and functional role of dysregulated microRNA expression in Wilms tumours

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Principal supervisor's CRUK CC theme: Paediatric Cancer Programme

Department for student registration: Department of Pathology
Department or institute where research will take place: Department of Pathology

Postgraduate scheme: **Clinical Research Training Fellow (3-year PhD)**

PhD project outline:

Wilms tumour (WT) is the most common renal malignancy of childhood. WT are clinicopathologically heterogeneous, thus clinical application of tissue biomarkers is complicated by variability across an individual tumour specimen. Although treatment is generally successful, ~10% of patients have poor outcomes, and novel therapeutic targets could greatly improve outcomes in these patients. There are no circulating biomarkers of WT in routine clinical practice for diagnosis and disease monitoring/follow-up.

One of the objectives of this project is to identify biomarkers which will address the urgent clinical need for improvements in diagnosis/treatment-stratification of WT. We have demonstrated that miRNAs, short, non-protein-coding RNAs that are dysregulated in cancer, are promising circulating cancer biomarkers, e.g., for patients with germ cell tumours (GCTs) e.g., [1-3], and other solid tumours of childhood, which included a small WT cohort [4], included in a recent meta-analysis [5]. We have already performed a discovery phase of WT circulating miRNA using TaqMan Low-Density-Array cards in a cohort of 47 patients (38 WT, nine age- and sex-matched controls), and identified candidate miRNAs segregating WT from controls. The first aim of this project is to confirm the 12 top-ranking miRNA candidate biomarkers in the initial discovery set of samples using targeted PCR (n=47), followed by the validation of the diagnostic potential of this panel in an independent cohort (n=170) of both serum and urine samples, to improve diagnostic accuracy for WT patients [6]. Importantly, all such biospecimens are already available in the laboratory.

The project next aims to elucidate the functional role of dysregulated miRNAs in WT, in order to better understand WT pathogenesis and discover novel treatment possibilities, as we have successfully undertaken in the host laboratory for other cancer types, such as GCTs e.g., [7-9].

PhD experimental plan:

The top-ranking 12 candidates that we have identified in the discovery phase represent a highly sensitive/specific candidate diagnostic circulating miRNA signature which segregates WT patients, regardless of subtype, and agnostic from other clinicopathological correlates, from non-cancer control samples. The top-ranking miRNA candidate biomarkers [each with area-under-the-curve (AUC)>0.90] will be confirmed in the discovery patient set (n=47) using another PCR based approach (targeted PCR instead of global discovery array cards), which is well established in the host laboratory. A smaller subset of candidates which were highly

overexpressed in some WT cases but had PCR Ct values similar to controls in others (AUC<0.90) will also be investigated to establish if they can be used for identification of WT subtypes or other clinic-pathological correlates.
The panel of miRNAs will then be validated in an independent cohort of both serum and urine samples (n=170). This total cohort (n=217) comprises 163 serum and 54 urine samples, from both WT patients and control patients, including patients who presented with an abdominal mass and thus represent a 'differential diagnosis' of WT – namely neuroblastoma, hepatoblastoma, rhabdomyosarcoma, and abdominal non-Hodgkin lymphoma.
Alongside the investigation of the potential use of these miRNAs as biomarkers, the role of dysregulated miRNAs in the biology of WT will be investigated. Using an integrated approach, sequencing/array analysis of a representative panel of WT cell lines recapitulating the clinical variability of these tumours, combined with analysis of existing publicly available WT tissue datasets, we will determine common dysregulated miRNAs suitable for study in vitro.
This miRNAs be then experimentally perturbed in WT cell lines as we have undertaken for other tumour types. Experiments will be performed to establish the downstream consequences of the replenishment of putative tumour-suppressor miRNAs and/or inhibition of putative oncogenic miRNAs in vitro, using a variety of approaches routinely available in the laboratory, informed by sequencing/array analysis of the global effects of such replenishment/inhibition.

Main techniques:

Patient biospecimen handling (e.g. serum/urine)
RNA and DNA extraction
qRT-PCR (singleplex and/or multiplex)
Cell culture
MicroRNA replenishment or targeting using established laboratory techniques (potentially including mimics/antogomirs, CRISPR/Cas9, lentiviral over-expression systems etc)
Arrays/sequencing
Functional assays, depending on the observed phenotypic and genotypic changes in Wilms tumour cell lines, but which may include cell cycle analyses (growth curves and flow cytometry), migration and invasion assays, angiogenesis assays etc
Western blot
Bioinformatic approaches to predict and confirm mRNA targets of dysregulated microRNAs
Data interpretation and analysis

Key references:

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