Investigating the role of cell migration in the pathogenesis of extragonadal germ cell tumours

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Principal supervisor's CRUK CC theme: Cell and Molecular Biology Programme

Department for student registration: Physiology, Development and Neuroscience
Department or institute where research will take place: Physiology, Development and Neuroscience; Anatomy Building

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

MRes project outline:

Primordial germ cells (PGCs) are the embryonic precursors of gametes. In many animals, including mice and humans, specification of PGCs occurs at a remote site in the embryo, following which PGCs must migrate to the gonad. Germ cell tumours (GCTs) account for 3-4% of childhood cancers and are derived from PGCs. In childhood, half of GCTs occur outside the gonad, typically in the midline from coccyx to cranium, suggesting a role for mismigration of PGCs in the pathogenesis of such extragonadal GCTs. In keeping with this, variants in BAK1, SPRY4, GAB2, and KITLG are associated with paediatric GCTs; each of the associated pathways are also implicated in mouse PGC migration. Despite this, little is known about the mechanisms of mammalian PGC motility or why and how mismigration occurs.

The Paluch lab has extensive experience developing confined microenvironments in order to study cell migration in vitro, and have recently been characterising the motility of mouse PGCs and PGC-like cells (PGCLCs – stem cell-derived PGC analogues) in PDMS microchannels mimicking physiological confinement. In this project, the student will perturb known pathways involved in germ cell tumorigenesis and quantitively assess the effects on mouse PGC migration.

MRes experimental plan:

The student will be introduced to culture of embryonic stem cells and PGCLCs, alongside microfabrication and microscopy techniques. Using PGCLCs as a model for PGCs, the student will perturb some of the target pathways (with relevance to cell migration & germ cell tumorigenesis), and quantify the effects on PGCLC migration. We will particularly focus on assessing the mode of migration used, and quantifying migration characteristics such as velocity and persistence. Key findings will then be verified in primary mouse PGCs to address physiological and clinical relevance.

Main Techniques:
1. Stem Cell Culture
2. Flow Cytometry
3. Microfabrication

4. Confocal Microscopy
5. Quantitative image analysis

PhD project outline:

The PhD project will build on the MRes project, and focus on investigating human PGC migration. Based on the key regulators of mouse PGC migration identified during the MRes project (alongside other ongoing work from the lab), the student will use existing labelled human pluripotent stem cell lines, from which human PGCLCs (hPGCLCs) can be derived for mechanistic studies of human PGC migration. The student will assess the mode of migration of hPGCLCs, and analyse key migration characteristics in microfabricated environments. It is anticipated that markers of PGC migration will be identified which may be used in clinical practice to assess GCTs histologically and which may differ by tumour site. With existing collaborators, this will be assessed by performing immunohistochemistry on paediatric GCT tissue. Taken together, the project will shed light on the mechanisms of germ cell tumours formation and offer insights into mechanisms of chemotherapy sensitivity and resistance of extra gonadal tumours.
PhD experimental plan:
In addition to those techniques developed within the MRes project, the student will be introduced to culture of human embryonic/pluripotent stem cells and hPGCLC derivation. The student will use image analysis software to quantitatively characterise PGC(LC) migration, including a pipeline developed within the lab for quantitively assessing cell morphology and protrusion types.

Main techniques:
1. Cell Culture
2. Derivation of human PGC-like cells
3. Flow Cytometry
4. Microfabrication
5. Confocal Microscopy
6. Quantitative image analysis
7. Cryosectioning
8. Immunohistochemistry

Key references:

1. Murray, M. J. & Nicholson, J. C. Germ cell tumours in children and adolescents. Paediatrics and Child Health 20, 109–116 (2010).
2. Kanamori, M., Oikawa, K., Tanemura, K. & Hara, K. Mammalian germ cell migration during development, growth, and homeostasis. Reproductive Medicine and Biology 18, 247–255 (2019).
3. Hayashi, K., Ohta, H., Kurimoto, K., Aramaki, S. & Saitou, M. Reconstitution of the Mouse Germ Cell Specification Pathway in Culture by Pluripotent Stem Cells. Cell 146, 519–532 (2011).
4. Cooke, C. B. & Moris, N. Tissue and cell interactions in mammalian PGC development. Development 148, dev200093 (2021).
5. Marcotte, E. L. et al. Variants in BAK1, SPRY4, and GAB2 are associated with pediatric germ cell tumors: A report from the children's oncology group. Genes Chromosomes Cancer 56, 548–558 (2017).
6. Paluch EK and Raz E. The role and regulation of blebs in cell migration. Curr Opin Cell Biol. 25:582-590 (2013).
7. Bodor DL, Pönisch W, Endres RG, Paluch EK. Of Cell Shapes and Motion: the Physical Basis of Animal Cell Migration. Dev Cell, 52: 550-562 (2020).