APPLICATION FOR A GOSHCC SURGICAL SCIENTIST PHD STUDENTSHIP

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1. Title.
Trachea-oesophageal birth defects: determining the key gene expression changes during fetal development

2. Portfolio summary.

Aims: Faulty separation of the trachea and oesophagus leads to severe human birth defects. This project aims to use animal models and human fetal material to determine the gene expression changes that occur during trachea-oesophageal separation, and to investigate changes that fail in fetuses developing foregut malformations.

Hypothesis: Specific gene expression is required for separation of the trachea and oesophagus during normal development, and is absent from fetuses developing trachea-oesophageal fistula.

Background: Oesophageal atresia (OA) and tracheo-oesophageal fistula (TOF) are common birth defects, affecting around 1 in 3500 births and frequently requiring emergency surgery in the neonatal period [1]. While advances have been made in understanding the genetic aetiology of syndromic forms of OA/TOF, such as VACTERL, the majority of cases are non-syndromic and their cause is unknown [2]. Although some molecular pathways are emerging as candidates to mediate the early development of the trachea-oesophagus [3], how these function to specify the separation of the foregut into tracheal and oesophageal components remains poorly understood. This lack of knowledge is hampering progress towards development of molecular therapies, e.g. that might be delivered in utero, to reduce the severity or prevent the development of OA/TOF. Most information to date on tracheo-oesophageal separation has come from studies of animal models. OA/TOF results when mid-gestation rodent embryos are exposed to Adriamycin (doxorubicin), an anthracycline antibiotic that interferes with DNA replication and transcription. We observed partial or complete failure of tracheo-oesophageal separation in 47% of Adriamycin-treated mouse embryos and fetuses [4]. We also studied the embryonic pathogenesis of OA/TOF in mice lacking function of sonic hedgehog (Shh) and found many morphological similarities to the Adriamycin model [4]. Hence, both teratogen-based and genetic mouse models of OA/TOF are available in our laboratory. Tracheo-oesophageal separation has been rarely studied in humans, mainly owing to the early fetal stage at which the developmental events occur. The Human Developmental Biology Resource (HDBR; www.hdbr.org) based at ICH and Newcastle collects and provides researchers with human embryonic and fetal tissues from ‘social’ terminations of pregnancy, from 4 to 18 weeks post-fertilization. This material is available for the PhD project and will enable an extension of the animal model studies to normal human fetuses.

Proposed methodology to be adopted:
Mouse studies. RNA sequencing analysis will be applied to foregut samples dissected from embryos and fetuses at different stages of tracheo-oesophageal separation. In the Adriamycin model, treated fetuses will be compared with untreated controls matched for stage of OA/TOF development. In the sonic hedgehog model, Shh-/- fetuses will be compared with wild-type littermates at key developmental time points.
developmental stages. Bioinformatics analysis of the data will indicate which genes differ in expression at different stages of tracheoesophageal development, and which are mis-regulated in OA/TOF. Spatial expression patterns of key genes from this analysis will then be studied in whole or sectioned embryos by in situ hybridisation to evaluate possible mechanistic involvement in the process of trachea-oesophageal separation.

**Human studies.** The developmental events of trachea-oesophageal separation will be studied in staged human fetuses, using dissected tissues and histological sections. RNA sequencing may be performed using the human samples, or candidate genes from the mouse studies may be directly analysed for expression patterns in human fetal samples.

**Skills to be achieved by the PhD trainee:**
The student will gain skills in the genetics and developmental biology of mouse models of OA/TOF, including dissection and handling of fetuses. He/she will learn methods for performing RNA seq, bioinformatics analysis of the resulting data, and testing of candidate genes for expression in normal and abnormal embryos.

**Relevance to the area of paediatric surgery:**
OA/TOF is a frequent indication for surgical intervention in young babies at GOSH. While surgical correction techniques is effective, the long-term outlook for such children can be poor. This project aims to provide insights into the developmental mechanisms of TOF, and hence may suggest molecular targets for drug therapy in utero. As such, it is ideal for a surgical trainee who aims to develop a career in paediatric surgery.

**References:**