Home Office

NON-TECHNICAL SUMMARY

Mechanisms of organ development and disassembly

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Organ, optogenetics, epithelium, brain, polarity

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Most organs in the body (including the brain) arise from tube-like structures, made from specialised cells called epithelial cells. The orientation of epithelial cells is critical for normal organ development

and function. If you were to cut a slice across a simple tube, it would look like a rosette, with all the cells aligned and pointing inwards towards the space (lumen) at the centre of the tube. To achieve this strict organisation, epithelial cells send particular proteins to their inner (apical) and outer (basal) ends. Therefore, epithelial cells are 'polarised'. Since epithelial tubes are so prevalent, the mechanism by which they initially become polarised is a fundamental process during the body's development. However, it is still not clear exactly how this polarity arises in the centre of a mass of cells. There is also some evidence to suggest that defects in cell polarity are linked to diseases such as cancer but so far it is not clear whether cell polarity defects are a cause or consequence of tissue disruption.

We will use zebrafish embryos for this research because they are transparent and develop rapidly. This means that we can put the in-tact embryo under a specialised microscope and can easily image the whole process of epithelial tube formation in their brain within approximately 12 hours. We will use a new technique, which uses light not only to see individual cells and their internal components, but also to manipulate them. We will use this to change the polarity of cells within the developing zebrafish brain and to test the consequences on cell behaviour. This will allow us to test how the polarity of individual cells drives the organisation of a whole organ. Part of this work will involve switching on and off cancer-linked signalling pathways, allowing us to understand how these pathways are linked to cell polarity and vice versa.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

The overall goal of this work is to further knowledge about the fundamental processes that are necessary for normal organ formation within vertebrate animals. By studying the effects of altering polarity and cancer-linked signalling pathways on cell behaviour and organ structure, this work should provide clues as to what happens to cells at the onset of diseases such as epithelial cancers, which may influence therapeutic strategy in the long term. Therefore, this work will increase knowledge in an important area of biomedical research.

Our work will also further develop an important bioscience tool - the ability to reversibly manipulate proteins and signalling within single cells within a whole organ using light (optogenetics). This can be used for a wide range of biomedical research.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

We expect to use up to 12,000 adult zebrafish for breeding purposes over 5 years. This number reflects the number of fish that it is necessary to establish and maintain to generate a robust breeding stock of fish in order to produce the embryos required for our experiments.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

The adult zebrafish will be housed in a dedicated aquarium within the department, run by trained staff. We will generate genetically altered zebrafish by introducing modified genetic material at the 1 cell embryo stage and growing these embryos to adulthood. We do not expect there to be adverse effects to adults from these alterations but sometimes younger larvae fail to thrive for unknown reasons following introduction of genetic material and very occasionally fish can show signs of being unwell only at older stages. If the larvae or adult fish appear unwell at any stage we will humanely kill them. We do not expect there to be any adverse effects from breeding the zebrafish. In order to know which fish contain genetic alterations we sometimes need to cut a small portion of the fish's tail fin under general anaesthetic and analyse the genetic code inside this tissue. The fish is then kept in a separate tank with fresh water and the fin then regrows relatively quickly. The severity level of this procedure is expected to be mild. It is unlikely but possible that fish might develop an infection following removal of a small part of the tail fin, in which case we will humanely kill the fish. We very occasionally need to anaesthetise fish for the collection of eggs and sperm and the expected severity level is also mild. For both of these procedures it is possible that fish may not recover from anaesthesia but this is very unusual (less than 1%). At the end of the protocols fish will be humanely killed or supplied to other project licences or recognised establishments with the authority to breed and maintain genetically altered zebrafish of this type.

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

This focus of this project is understanding how organs are built and how disease is initiated inside a living animal. It is important to use an animal rather than looking at cells in culture because the physical forces and interactions between cells are very different in a culture system. Therefore, it is only possible to fully understand the cellular behaviour during organ development by looking inside an in-tact animal. However, we are able to carry out some of our research using cells grown outside an animal (cell culture) – for example it is possible to ask specific questions like how cell-cell contact is involved in initiating cell polarisation. As we investigate the animal model alongside this culture system we will learn whether more of our work can be carried out in culture.

Reduction

Explain how you will assure the use of minimum numbers of animals.

All of our experimental work will be carried out in zebrafish embryos younger than 5 days old (which are not protected under The Animals (Scientific Procedures) Act 1986. Animals older than 5 days old will only be used for establishing genetically altered zebrafish for subsequent breeding. The number of

adult animals used is therefore solely related to the numbers required to maintain sufficient breeding stocks of animals. We are using several methods to reduce the numbers of adult animals used. First, we will share relevant fish stocks with other users within the facility. Second, we will try to limit repeated breeding to once per week to optimise breeding performance. Third, we will minimize the generation of transgenic lines and use wild type embryos wherever possible for our experiments. Fourth, we will freeze sperm from genetically altered lines of zebrafish for longer-term storage.

We will carefully design our experiments so that we use appropriate numbers of embryos for each experiment. Where necessary and possible, we will carry out pilot studies to determine the number of embryos required to achieve robust statistical analysis. If we require assistance in our experimental design, we will consult with a statistical expert. We will ensure that our publications conform to the ARRIVE guidelines: https://www.nc3rs.org.uk/arrive-guidelines.

To make our experiments robust, we will control for variability in the following ways:

We will reduce environmental variability by carefully housing breeding adult fish in the dedicated zebrafish facility and by keeping genetic background constant within each genetically modified line of fish.

We will assess normal levels of variability within experiments via pilot experiments, allowing us to select appropriate statistical methods and number of embryos

We will reduce bias by randomly selecting embryos collected from a pool of breeding adults and by assigning treatment and control groups in a way that is unknown to the person analysing the data (blinding).

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

The zebrafish brain is an ideal model system for studying cell polarity and cell division during epithelial tube development since there are some highly stereotyped cell divisions that occur at the same time as which polarity is being established. Zebrafish embryos are also small, transparent, develop rapidly and it is possible to alter their genetics in a reasonably straight-forward way. This means that they are an ideal system for using the optogenetic approaches that are integral to this project. We don’t envisage any suffering in licenced animals beyond the mild procedures described above. We will only use zebrafish embryos younger than 5 days old for our experiments, which are not yet capable of independent feeding or complex cognitive behaviours. We will aim to reduce any potential suffering of these embryos by promptly killing them using a humane, approved method at the end of the experiments and, where possible, by anaesthetising embryos that are sufficiently developed to be capable of initiating movement during imaging (those above 18 hours old).

Adult fish will be housed in a dedicated centralised zebrafish facility, where they will be looked after by full time staff, who will ensure their welfare. Numbers of fish per tank, water quality and food quality and quantity will be carefully controlled.