Home Office

NON-TECHNICAL SUMMARY

The role of the RASSF family in development and regeneration

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

tissue architecture, development, cell polarity, tissue growth, regeneration

Animal types	Life stages
Mice	adult, pregnant, embryo, neonate, juvenile

Retrospective assessment

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

Objectives and benefits

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

What's the aim of this project?

Our aim is to understand how the complex architecture of tissues and organs is built during animal development. In particular, we will study the RASSF (Ras association domain family) proteins, which have been implicated in this process, by generating knockout mouse models for these genes and studying the impact of these genes on development and tissue regeneration. We are particularly interested in how cell identity and polarity are precisely orchestrated in highly polarised organs such as the placenta, skin and kidneys.

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.

Why is it important to undertake this work?

In order to build functional organs, each cell within an animal has to adopt the right fate, shape and orientation to yield the appropriate tissue architecture. This organisation has to be maintained during adult life, and restored after injury through tissue repair. Normal tissue organisation is essential to prevent cells from becoming cancerous and disrupted tissue architecture is a hallmark of cancer. Studying how tissues are organised during development and maintained in adult life is therefore key to understanding not only how complex organs function, but also how to prevent tumour formation.

What outputs do you think you will see at the end of this project?

The main objective of our work is to generate new knowledge and promote a better understanding of how complex organs are built during development and maintained throughout adult life. The primary output will be publication of our results in peer-reviewed journals and deposition of raw data in the appropriate repositories to make our findings available to other researchers to build on.

Our work will also generate new lines of genetically altered mice that can be distributed and used by others in the field, for future research.

Who or what will benefit from these outputs, and how?

In the short term, new knowledge on the mechanisms of organ development will be beneficial to scientists working in developmental and stem cell biology.

In the longer term, we anticipate our findings will inform future work on several disease states. Unravelling how normal development proceeds is essential to understand how organ function fails in pathogenic conditions. For instance, our work on placenta development will promote a better understanding of how placenta failure or insufficiency occurs, which is a major cause of complications during gestation. Given the strong link between tissue architecture and cancer, as well as the genetic evidence pointing at a role for the RASSF family and the Hippo pathway in cancer formation, we also

anticipate that our work will benefit the cancer research community. For instance, although the RASSF family is frequently lost in a range of human tumours, there is little insight into why these genetic losses are favourable for cancer formation. Understanding the consequences of RASSF loss in tumours might lead to improved therapeutic options. Finally, our work on tissue regeneration may lead to improved strategies for tissue repair.

How will you look to maximise the outputs of this work?

In order to maximise outputs from this research, we will ensure the timely publication of our findings and we will promote our work on social media and by presenting it at international research conferences. To make our work available as early as possible, we will release pre-peer reviewed versions of manuscripts on appropriate preprint servers such as bioRxiv upon first submission to peer-reviewed journals.

Through internal and external collaborations, we will share both positive and negative results and best practice with our peers. We will share our mouse transgenic lines or tissue extracted from these models with others in the field in order to accelerate the progress of research and reduce overall animal usage.

Species and numbers of animals expected to be used

- Mice: 15600

Predicted harms

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

Explain why you are using these types of animals and your choice of life stages.

Our aim is to investigate how complex organs develop in mammalian systems, therefore mice represent the best available model, due to the many similarities with human development and the wealth of genetic tools available. The majority of our work will be performed during embryonic development and we will endeavour to focus on the earliest developmental times where a phenotype becomes manifest. For our work on tissue regeneration, we will also need to use adult animals, as the mechanisms for tissue repair during embryonic development and in adult animals are radically different.

Typically, what will be done to an animal used in your project?

Most of our experiments will involve the breeding of genetically altered animals, both for generating experimental animals and for maintenance of genetic lines for future experiments. For experimental animals, breeding will be followed by injection of a gene expression-modifying reagent (tamoxifen or doxycycline) and/or tracer dye if necessary. Upon reaching the appropriate stage, the animals (and the mother in the case of embryonic stages) will be killed and tissue will be harvested either for analysis (e.g. immunohistochemistry) or placed in ex vivo culture conditions.

For a small number of animals, adults of the appropriate genotype will undergo a standardised technique to create a small (4mm) shallow wound in loose skin on the back using a sterile biopsy punch. The aim of these experiments will be to evaluate the role of RASSF family members or functionally related proteins in tissue regeneration. Analgesia will be provided and the animals will be closely monitored to ensure their full recovery.

To generate new transgenic lines if necessary, a small number of animals will undergo surgical procedures to transfer embryos into the uterus of adult female mice. These animals will be closely monitored, and anaesthetics/analgesics will be used as appropriate.

What are the expected impacts and/or adverse effects for the animals during your project?

The majority of the phenotypes will be analysed at embryonic stages, less than two thirds into gestation, when the animals are not considered sentient. We will generate genetically modified animals, the vast majority of which are expected to display either no phenotypes or moderate phenotypes such as hair loss.

Animals undergoing the punch wound or embryo transfer protocols will experience mild to moderate post-operative pain which will be controlled with analgesics. Full recovery is expected within one or two weeks.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Moderate severity is expected for up to 14% of animals. The rest of the animals will reach a maximum of mild severity.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Our project aim is to understand the precise cellular interactions that allow the development and maintenances of highly complex organs. In order to tackle this question, we have used and will continue to use simple models such as fruit flies, mammalian cells in culture, computational models and different types of ex vivo cultures systems such as gastruloids. However, in order to fully recapitulate the complexity of organ development, it is necessary to observe how this occurs in an animal model, as

there are currently no cell culture models that fully reconstitute this process. Since ultimately our aim is to gain insights into human development, mice are the in vivo system of choice due to the close similarities between mice and humans during development and the number of genetic tools available in the mouse system.

Which non-animal alternatives did you consider for use in this project?

As outlined above, we have used several less complex systems (fruit flies, cell culture, computational simulations) to minimise the number of vertebrate animals used in our experiments. We will continue to monitor the rapid progress in ex vivo differentiation and organoid models with a view to incorporating as many of these emerging technologies into our research as possible.

Why were they not suitable?

Cell culture-based models such as blastoids and gastruloids are showing a great deal of promise to study development, particularly its early phases. However, these models remain imperfect and fail to capture the complexities of organ formation, which involve complex interactions between many different cell types, and in many cases communication between different organs. One example relevant to our work is the interplay between the mother and embryo during the development of the placenta. Although we will take full advantage of cell culture, it remains necessary to use animals to capture the real complexity of development.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Our animal use projections are based on several factors:

1. Number of genetically modified lines we are currently maintaining.

2. Complexity of the crossing schemes to generate experimental animals.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We carefully reviewed the cross schemes required to generate the desired genotypes in order to ensure we use the minimal number breeding steps to generate the maximum number of experimental animals. We regularly review our mouse colony and freeze sperm and embryos in order to archive lines that are not immediately required. To achieve efficient colony use, we work closely with the mouse technicians in our animal unit and our institution's colony management team. Our institution also supports sharing of

useful genetically modified animals such as Cre lines with other licensees in order to minimise animal usage at the institutional level. This is enabled by our sophisticated electronic colony management system.

The RASSF gene family contains 10 members, which likely perform redundant function, making the analysis of all possible mutant combinations using standard genetic approaches hugely costly in terms of animal breeding. To circumvent this problem we will screen for phenotypes in mutant combinations using mouse embryonic stem cells and in vitro differentiation protocols such as gastruloids. This will allow us to screen for combinations that elicit phenotypes prior to generating mouse lines and will therefore allow us to focus only on informative combinations. This approach will considerably reduce mouse usage for this project. Whenever possible, we will use established protocols to derive stem cells from mutant and control animals, and differentiate them in vitro into the cell types we want to study. For instance, to support our work on placenta development, we will derive trophoblast stem cells and differentiate them in vitro to compare gene expression profiles between mutant and control animals.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

In addition to the measures outlined above, when we generate genetic combinations for analysis, we will maintain as many of the genetic elements as possible in the same line without causing harmful phenotypes in order to minimise the numbers required to generate experimental animals. Where we generate a new mutant line to investigate its phenotype, we will perform pilot studies with a small number of animals in order avoid generating a large cohort that will not present any phenotype we want to study.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We have chosen to use mice for these studies for a number of reasons. They are ideal model organisms to investigate mammalian development and regeneration - their biology is close enough to that of humans for our findings to be relevant to human disease. Mouse embryonic development and biology are well described, so we will be able to identify and characterise abnormalities easily. As our primary interest is development, we will seek to study phenotypes at early developmental stages where the animal is less sentient in order to minimise suffering. Our analyses will primarily be performed on dissected tissue following the killing of the subjects via humane methods.

To study regeneration, we have chosen a punch wound protocol that induces relatively little discomfort compared with many other injury models such as liver regeneration upon partial hepatectomy or toxic

substance ingestion. This protocol was chosen because the Hippo signalling pathway, which we are investigating, has been shown to affect epidermal regeneration using this experimental system.

Why can’t you use animals that are less sentient?

Most of our work will be carried out on embryos prior to two thirds of gestation, which are not considered sentient. Wherever possible, we will derive stem cells from our animals in order to reduce the number of animals we need to breed for our experiments (e.g. trophoblast stem cells for out placenta work). For our regeneration work, we need to use adult animals as the wound healing process is very different in adults compared with embryonic stages. Our work on mice will continue to be guided by our work in fruit flies and cell culture, which allows us to reduce mouse usage.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Our close collaborative relationship with the staff in our animal facility ensures that animals having undergone procedures such as our epidermis regeneration protocol are closely monitored. New genetic combinations which may develop unanticipated phenotypes will also be closely monitored. In these cases, the animals will be monitored daily or twice-daily depending on the situation. Animals exhibiting unexpected harmful effects will be killed using an approved method, except in rare cases where these animals are essential for an experiment, in which case we will seeks advice from the Home Office Inspector.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We follow guidance and best practice provided by the NC3Rs, as well as our animal unit and scientific colleagues.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Our animal unit communicates best practice and new advances in animal handling through regular newsletters and annual refresher sessions for project and personal license holders. In addition, we will keep abreast of new advances in ex vivo culture experiments through conferences and publications. Finally, we will regularly monitor the NC3R web site for updates (https://www.nc3rs.org.uk/3rs-resources).