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NON-TECHNICAL SUMMARY

Dynamic homeostasis of stem cells from development to aging

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

stem cells, timing, embryo, aging, diapause

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

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?

A long-held question in biology is how biological timing operates at the cellular level. Throughout the animal kingdom, the duration of development and lifespan vary hugely despite many species sharing equivalent sets of genes. Further, some species can halt development for extended periods of time in response to adverse nutrient conditions (diapause). This project aims to compare the dynamics of stem cells in development and aging to discover the molecular mechanisms that encode timing in the genome.

The key objectives of this programme of work are (1) to understand dynamic properties of cells in embryos and in adult stem cells; and (2) To obtain insights into the impact of developmental timing on lifespan.

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?

How biological timing is encoded in the genome to track time across timescales remains unknown. Understanding the rules that determine how cells can precisely initiate and terminate processes at specified times and how do they modulate the rate at which they tick will help us explain when processes go awry. Examples of these are tissue overgrowth and deficits, or aging. Moreover, if we identify the molecular mechanisms that encode biological timing, we can harness them to speed up or stall in vitro stem cell differentiations to generate tissues in culture to study them in the lab or for transplantation therapy purposes.

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

The output from this programme of work will be in the form of new information (characterisation of developmental processes, differentiation and aging; whole genome profiling resources) that will be published and reagents, such as stem cell lines and transgenic mice to be used by our colleagues, other researchers and, potentially, the pharmaceutical industry.

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

The short-term benefits will be a better molecular understanding of how stem cells maintain their homeostasis from fertilization to death. Embryonic stem cell lines derived from mice, including those of genetically modified mice, will provide an ex vivo system with which to study the properties and potential of the tissue of origin. In the medium term, we expect to have generated a comprehensive understanding of the mechanisms that regulate the pace of development and adulthood, and the impact of lengthening development on lifespan. In the long term, we will increase knowledge about how can we slow down or speed up the pace of developmental and homeostasis processes. Ultimately, we expect to be able to translate our knowledge for application by us and others in regenerative systems and alternative systems that improve the health span of the organism.

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

The aim of this work is to advance our biological understanding of dynamics in development and homeostasis, and the outputs from this research will include new knowledge and publications in peer-reviewed journals as well as in scientific conferences.

An important feature of the project is the association of mechanisms in development to homeostasis and lifespan, as these tend to be studied in separate fields. Moreover, the protocols and techniques we will develop during the course of the work will benefit researchers studying similar problems. Our protocol on diapause is specialised, and we collaborate with one group in the UK and one in Germany to refine the protocols, avoid duplication, and maximize the output.

Species and numbers of animals expected to be used

- Mice: 14750

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.

The use of mice is necessary to achieve the proposed research goals, which require analysis of intact embryos and adult mice. Unlike other experimental systems, the mouse offers the most relevant in vivo model system, with the ability to genetically alter the genome, to address important biological questions that could impact on major advances in the stem cell field. This project will work with early developmental stages as well as with a small proportion of aged mice.

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

By far most of the work to be carried out under this licence involves breeding of genetically altered mice, for provision of early embryos from females culled using a Schedule 1 method. In some instances, females may undergo superovulation, diapause induction or embryo transfer. Some animals will be maintained for a long period of time to study aging.

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

It is expected that most animals will not experience greater than mild severity. Inducing agents will be used in some animals to control gene regulation/expression in cells and label molecules in vivo as required to meet our project objectives; it is not always possible to fully predict the phenotypic outcome in a novel environment.

In addition, some surgical procedures will be performed. Even though surgery is considered a moderate procedure, we expect the animals to promptly recover.

Ageing mice are more susceptible of distress. Regular monitoring will ensure that animals do not suffer greater than mild longer than 24h.

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)?

Five of the protocols (Protocols 1, 2, 3, 6 and 7) are classified as moderate because they involve routine surgical procedures such as embryo transfer or vasectomy. The proportion of mice exposed to these moderate protocols is expected to be less than 15% of the total number of mice utilised under this licence.

Minimal animal suffering is anticipated as most animals undergoing administration of substances or surgery are expected a mild severity as they may experience short-lived postoperative pain and discomfort.

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

- Used in other projects
- 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?

We replace the use of animals with cultured cell lines whenever possible. However, we sometimes need to breed mice carrying particular genetic modifications to provide early embryos for short-term experiments, for stem cell derivation or to analyse specific phenotypes such as diapause. This is particularly important when we do not fully know how the in vitro system performs in relation to in vivo development, as is the case for diapause. In addition, there is currently no culture alternative that exactly mimics normal development or aging.

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

We will make use of stem cell models of development to minimize the number of animals used. Our team has developed 2D differentiation models to generate different primary non-dividing cell types from established pluripotent embryonic stem cell (ESC) lines of mouse and human origin. Further, the lab will develop 3D stem cell models (namely mouse blastoids, gastruloids and motor neuron stem cell differentiations).

For aging, we may use terminally-differentiated neurons derived from stem cells cultured for long periods of time without any deterioration in survival. This has been successfully developed for human motor neurons, and a recent optimisation of the culture protocol that is applicable to mouse motor neurons demonstrated viability for at least 2 months.

Why were they not suitable?

Stem cell models are appropriate for the study of cell-intrinsic timing mechanisms but tissue development and homeostasis is multifaceted and an emergent feature for which we still do not have integrated models that recapitulate all of its entirety. We are developing more complex models that allow us to recapitulate in vivo processes. These are likely to further increase the rate at which we replace animal use with in vitro models. Nevertheless, we are still in the development and validation phase and it is essential to carefully compare and benchmark in vivo methods to the in vivo situation.

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?

The numbers have been estimated considering the number of samples and embryos needed for the experiments proposed within the objectives set. These are informed by similar experiments we performed in the past, where we can estimate with accuracy the number of animals needed. For those experiments that we have never performed, such as lifespan studies, we have checked in the literature, talked to peers and reached out to bio-statisticians to seek advice.

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

We started the process by reading relevant literature and studied how other researchers had performed similar experiments. We planned essential experiments only, and sought advice from experts. At the stage where we started designing the experiments, we made use of the NC3R's Experimental Design Assistant. We planned how would we group the animals, which variables would we need to analyse and planned an unbiased statistical approach that would fit our experimental plan for our analyses. We also are in continuous contact with a bio-statistician that is helping us with the experimental design, and the whole licence was reviewed by an internal committee.

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

We minimise the numbers of mice we need to use primarily by making sure our breeding programmes are the most efficient for providing the genetically modified embryos we require. Our extensive experience in managing breeding programmes for the generation of embryos for research contributes considerably to the efficiency with which we can reduce the numbers of mice we maintain.

The methodologies for generating genetic modifications have now evolved sufficiently to enable us to delete specific genes in an inducible manner. Conventional deletion of the copies of such essential genes from both parents (known as 'homozygous null') in an embryo results in developmental failure. This means that homozygous null embryos can only be produced by mating male and female mice carrying one deleted copy of the gene each, and therefore, by Mendelian genetics, only 25% of embryos would be expected to be homozygous null. However, being able to breed mice carrying special modifications on both copies of the gene of interest that allow its deletion only when the embryo is exposed to a deleting agent means that all the derivative embryos from a mating have the potential to be made homozygous null. As a result, up to 75% fewer genetically modified mice need to be maintained to generate as many homozygous null embryos as would be required from animals carrying only a single deleted gene.

For early pre-implantation stages, we use superovulation to generate large amounts of oocytes per mouse which reduced the overall amount of mice that need to be used for oocyte supply.

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.

Mouse models are advantageous for biological discovery. They are small and easy to breed, reaching sexual maturity within two months from birth, and have the capacity to produce large numbers of embryos. Furthermore, mice are the best animals for our research because they can provide all the genetic modifications we require. Our gene alterations aim to induce the deletion of genes or label specific cell populations and molecules, and we do not anticipate they will cause any adverse effect.

Most animals on this project are not expected to be subject to any pain, suffering, distress or lasting harm. A small proportion of our animals undergoing regulated procedures (~8%) will be subjected to surgery, which involves preparation for procedures required for making female mice receptive to transplantation of embryos, or for extending the period of development just before implantation (known as 'diapause'). In both instances, the surgical procedures may be substituted for alternative non-surgical methods that are currently being piloted. All animals undergoing surgery will be provided with pain relief, and are not expected to suffer more than mild discomfort. If signs of ill health are apparent or animals experience more than mild discomfort, the animals will be humanely killed.

Some of the animals (<3%) in the project will be maintained for a long period of time to investigate ageing. There are no specific impacts or adverse effects expected on mice ageing healthily during this project, and regular monitoring of aged animals will prevent for any unnecessary animal distress.

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

The study of biological timing in development and homeostasis aims to identify molecular mechanisms that are relevant for human biology. Overall, the identification of physiological mechanisms that modulate timing and its translation to stem cell models may have important implications in the field of human assisted reproduction, regenerative medicine, and aging. As a proxy to human biology, we use mice for the in vivo part of our research because they represent the mammalian model system with the best characterised development, most comprehensive availability of validated transgenics, small physical size, large litters and short inter-generational interval. Their husbandry is well established; highly trained and dedicated teams of technicians are already in place for their care and maintenance.

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

We will follow the latest advice for specific procedures, and we will bring in external experts when needed to refine our methods.

For diapause induction, we will liase with researchers that have recently refined the method for training and advice.

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

Refinements from the NC3Rs such as the ARRIVE guidelines on design and reporting; the LASA aseptic guidelines; LASA Diehl guidelines on volumes and frequency limits and the most up-to-date veterinary knowledge.

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Finally, protocols in this licence can be found in Manipulating the Mouse Embryo: A Laboratory Manual, Fourth Edition from CSHL by Richard Behringer, University of Texas, M.D. Anderson Cancer Center; Marina Gertsenstein, Toronto Centre for Phenogenomics, Transgenic Core; Kristina Vintersten Nagy, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto; Andras Nagy, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto.

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

I routinely monitor news and progress in the NC3Rs web page, and the Biological Support unit in the Institute also updates the researchers when it is needed. Further, I interact regularly with a member of Felasa.

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