NON-TECHNICAL SUMMARY

Epigenetic control of mammalian development and genome function

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

Epigenetic, imprinting, genome, mechanisms, development

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Required at inspector’s discretion

Objectives and benefits
What’s the aim of this project?

Our genes are the DNA code that is the blueprint for life. Epigenetics is a layer of chemical marks that sit on top of our DNA that make our different cells (which all contain the same DNA and genes) behave in a specific way. For example, it makes liver cells behave like liver cells and brain cells behave like brain cells etc. Genes are present in two copies. One copy is inherited from the mother and one copy is inherited from the father. Some genes are epigenetically marked whereby only one copy is switched on depending on whether it is inherited from the mother or the father. These important processes contribute to mammalian development and their failure can influence human health.

The aim of the research in our laboratory is to understand the function of epigenetic marks and the DNA and genes that they regulate. We want to understand how they control growth and development of the baby, in the uterus and after birth when breastfed, focusing on normal mammary gland development, as this field is highly biased toward breast cancer while knowledge of healthy mammmary development and of cells that do not transform into tumours is lacking. the placenta and the brain and We will study how these genes regulate metabolic processes and normal growth, and how they associated with diseases such as obesity and diabetes in adulthood, as well as the effect of breastfeeding on the long-term health status of the offspring and the mother and how their altered regulation causes disease and aging.

Many studies have shown that breastfed children tend to be less susceptible to develop obesity and diabetes, however the underlying mechanism is as yet unknown, and we hypothesise that epigenetic regulation could play an important role in this association.

In addition, we are investigating the causes and the consequences of normal aging process and epigenetic marks.

We also aim to understand epigenetic marks controlling the amount of gene product present in the body which when altered can cause cancer and other diseases. From our studies, we have discovered that the amounts of some genes is also involved in tissue regeneration. From these results we can investigate the regeneration process in our genetically altered animal models.

This project will generate genetically altered mice in which these processes are perturbed and will shed new light on our understanding of this crucial level of regulation which is vastly affected by environment. These insights will potentially help us understand the long-term consequences of lifestyle choices, embryonic environment, breastfeeding and how faulty epigenetic regulation could lead to detrimental results and compare them with normal mice. We wish to understand important epigenetic marks and how their control contributes to all stages of development. To help us to understand these mechanisms we will study the effects of these alterations on the well-being of the mice and their offspring. We will also study environmental factors which affect the epigenetic marks such as diet and aging. To complement our studies we will also use zebrafish, which have many of the same genes and pathways as mice but are simpler to study.

**A retrospective assessment of these aims will be due by 19 July 2022**

The PPL holder will be required to disclose:
• Is there a plan for this work to continue under another licence?
• Did the project achieve its aims and if not, why not?

**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 findings from our research will provide more information on how epigenetic marks contribute to normal processes in all stages of development from pre-implantation to perinatal to the adult. This includes the regeneration process which can occur in our cells following tissue damage. This information will also help us to understand how these processes are perturbed causing disease states such as obesity, diabetes and cancer. This in turn will help in understanding the benefits of breastfeeding, and how it influences long-term health status of the offspring, in addition to the development of therapies in tissue regeneration and to target epigenetic changes that cause disease.

**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 will use two animal models to conduct our experiments; mice and zebrafish. Over a period of 5 years our projected use of mice is 18700 and 20500 zebra fish (larvae, juveniles and adults). These include a number of protocols and as we continue to work on our reduction and refinement we believe that we will work below these numbers.

**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?**

We have protocols that are classed as mild and moderate severity. However, many of our animals actually only experience a mild or less than mild severity level. New genetically altered mice strains that we generate are closely monitored for adverse effects. An adverse effect that they may encounter is restricted growth during development in the mother, which is sometimes recoverable after birth as the mouse gets older. We also study the regeneration of cells in animals. In mice we administer a substance into the muscle to cause cell damage. This is done using the lowest dose possible to achieve an effect and is performed under anaesthetic. The mice may only suffer from mild inflammation and they are expected to make a full recovery. It is this recovery process that we will investigate.

**A retrospective assessment of these predicted harms will be due by 19 July 2022**
The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

## Replacement

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

We cannot conduct all our experiments on cultured cells as we are assessing the health of the whole organism. Epigenetic states in vitro are also very different to their natural states in vivo. We are studying mechanisms and pathways in the developing organism and we therefore need to look at different time points during development. As cultured cells are exact copies of each other this means that changes occurring in developing cells cannot be seen. However we use in house and public databases and reanalyse existing data instead of rerunning experiments wherever possible. We perform initial experiments in cultured cells and in zebrafish to test some of our hypothesis and choose important genes from these results before moving into mouse experiments.

A retrospective assessment of replacement will be due by 19 July 2022

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

## Reduction

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

We have been working with mice for over 25 years and we have expertise in statistical analysis and optimal experimental design to determine the minimum number of animals needed to achieve robust, meaningful data. We collaborate with other groups, share mice tissues and data. We statistically analyse mouse numbers for use in experiments, plan experiments responsibly and communicate between lab members to make the best use of our resources. We use databases to find candidates genes and conduct studies first in cell lines whenever possible. We are collaborating with local colleagues who are experts in theoretical and mathematical modelling who will provide added value and novel insights to the animal work. Where appropriate we use control litter mates or use control tissue from the same animal. Where appropriate we randomly assign animals to control and test groups and analyse samples blind to avoid bias. Breeding colonies are generally kept small following good colony management strategies.

A retrospective assessment of reduction will be due by by 19 July 2022

The PPL holder will be required to disclose:
How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

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 mouse is the best model for these studies because a catalogue of all the genes in the mouse exists and there are well-established procedures that are not harmful for the animals which can be used to mutate the genes or change their regulation. Many of the genes found in mice are found in humans too. In addition we can breed the mice selectively and follow the effects in their offspring for multiple generations.

We keep up-to-date with new technologies and developments that allow us to refine our experiments. We have improved the method to generate genetically altered mice so we can use significantly fewer animals to study. We continuously monitor our animals and work closely with the vet and the staff in the animal unit to ensure the animals reach a humane end point and receive the best welfare possible.

A retrospective assessment of refinement will be due by by 19 July 2022

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?