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NON-TECHNICAL SUMMARY

Mechanisms of metabolic regulation in health and disease

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

cancer, metabolism, obesity, diet, imaging

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

The primary goals of this project are to study how tumours change the metabolism of the host organism, and how, in turn, metabolic changes in both tumour and the host support tumour development.

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?

Despite significant progress in understanding the molecular basis of how tumours emerge and develop, cancer remains a significant cause of death worldwide. In particular, liver cancer, a major focus of this project, is the fifth most deadly tumour type, with no substantial improvement in patient mortality rates over the past three decades, in contrast to most other tumour types. The rise in liver cancer cases is, partly, due to an alarming increase in obesity-induced liver disease, which is typically associated with accumulation of fat and subsequent injury in the liver. Chronic liver injury, in turn, promotes cancer - almost all liver cancer patients have had some form of liver disease earlier in life and about 20% of patients with cirrhosis (an advanced form of liver disease, also associated with excessive alcohol consumption) will develop liver cancer. However, there are no clear indicators that predict *which* patients with liver disease will go on to develop liver cancer. Furthermore, there are limited therapeutic options and, even then, it is unclear which patients will respond to these therapies. Therefore, clinicians need to observe patients with liver disease over long periods of time in order to capture those that do develop cancer early, when surgical resection still offers hope for survival. Beyond the cost in human lives, such long-term patient monitoring adds a significant cost to health systems.

A common feature of cancer cells is that they change their metabolism, compared to normal cells, in order to survive and proliferate. Targeting cancer metabolism offers significant therapeutic potential as evidenced by the fact that the first chemotherapy, methotrexate, targets a metabolic pathway and is still successfully used today in the clinic. However, there is a high failure rate in producing effective drugs that target metabolism, in part because there are many gaps in our knowledge about basic metabolic processes. Specifically, it is unclear how changes in metabolism of the host organism, which are often found in patients with various types of cancer, help tumour initiation, maintenance and dissemination (metastasis). Alterations in the metabolism of the host, such as loss of fatty (adipose) tissue and muscle mass, also have profound effects on the quality-of-life of cancer patients and, in part, contribute to mortality.

The proposed work brings these two challenges under one umbrella by aiming to provide mechanistic insights into how tumours change host metabolism and how host metabolism, in turn, supports cancer formation, in order to identify new therapeutic strategies; and apply these insights into testing new therapeutic approaches for liver cancer. Some of the metabolic pathways we study play important roles in normal physiology and our studies will also provide insights into these normal functions. Finally, by using models other than liver cancer, such as mammary gland cancer, we also aim to explore whether our findings have broader relevance for oncology.

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

Broadly, we expect to produce new knowledge about the role of specific metabolic processes in normal body physiology and tumorigenesis, and to generate new genetic, chemical and computational tools

that will accelerate mechanistic understanding of the functions of metabolism in health and disease. More specifically:

(a) We will elucidate the mechanisms that tumours employ to alter the metabolism of the host organism and will assess whether and how these pathways can be targeted for cancer therapy.

(b) We will generate new genetically altered mouse lines that harbour knockouts of metabolic enzymes to assess the role of these enzymes in normal physiology, in metabolic disease and tumorigenesis.

(c) We will develop new small animal imaging methods to measure dynamically metabolic pathway activities in living animals and validate their usefulness as a tool to monitor disease progression and response to therapy.

(d) We will validate small molecules that alter the function of metabolic enzymes to assess their value as potential therapeutics against cancer.

(e) We will use our data from animal experiments to generate and train new computational models of animal physiology and metabolism that have improved predictive capacity.

The new knowledge acquired from our work will be distributed through a wide range of channels including presentations and posters at relevant conferences, and publications in relevant journals. In addition, we will also announce breakthroughs and updates through social media channels such as Twitter and the institutional website.

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

- Basic science and the scientific community: Metabolism underlies all aspects of life and is linked to the biggest killers of human society, incl. diabetes, infectious diseases and cancer. Research into the metabolic basis of disease is rife and our work will provide important insights into the fundamental mechanisms mammals use to maintain a healthy metabolism, how these mechanisms are perturbed in disease and how we can intervene to restore a healthy outcome. Our work will also generate new genetic, chemical and computational tools that can be used to interfere with metabolic processes. In the short term, both the knowledge and tools generated from our work will be available to other researchers to be used and inform their work.

- 3Rs: In the longer term, our work holds great promise for helping to reduce the number of animals required for future metabolic research. Our proposed work will help us train computational models of mouse metabolism to improve their ability to predict which metabolic processes to target in order to stop tumour growth. In turn, better computational models will help us refine our hypotheses and design more targeted experiments that will enable us to achieve our scientific objectives using a smaller number of animals. Similarly, once validated, our imaging methodologies have the potential to either decrease the numbers of animals needed to obtain similar insights, or obtain more data-rich results from each animal without repeated sampling.

- Clinical translation/patients: Ultimately, we envision that our work will benefit human patients by improving our understanding of cancer’s impact on whole body metabolism. Liver cancer patient mortality has not improved for decades, in contrast to other tumour types. Patients with cancer of

diverse primary origin suffer both due to perturbation in the affected tissue's function but also due to disturbance of systemic metabolism, which leads to frailty and poor quality-of-life. Our work on the metabolic consequences of tumorigenesis, will illuminate new strategies towards ameliorating whole-body physiology and to halt tumour growth. Beyond our focus on liver cancer, our work will also test whether whole-body metabolic changes are also relevant in other cancers, such as breast cancer, and in metabolic disease.

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

A major goal of this project is to understand how tumour-induced changes in host metabolism occur and their role in tumorigenesis. Host metabolism alterations have been reported in patients with tumours of various tissue origins. So, although most of the planned work focuses on liver cancer, we are also proposing targeted experiments in other mouse cancer models to assess how our findings apply to other cancers, thereby expanding the scope of our work's translational applications.

Dissemination of newly acquired knowledge and experimental tools will also help maximise our work's outputs. We will report our findings to the broader scientific and clinical community through publications in scientific journals and presentations in national and international conferences. Newly generated tools, such as imaging protocols, genetic knockouts and computational models of mouse physiology will also be made available through appropriate channels, such as mouse and software depositories. These tools will catalyse further research by other researchers world-wide.

The proposed work will also facilitate existing, or instil new collaborations both with academic and industrial partners. For example, our mouse metabolism data will help to improve computational models by mathematical biologists that would, otherwise, not be able to readily test their models' capabilities. In turn, computational models will help us process large amounts of data and generate new, testable hypotheses. Furthermore, our newly discovered metabolite analogues with improved pharmacokinetics may be of interest to other academic or industrial partners who seek to use in vivo validated chemical tools to study the consequences of perturbing metabolic pathways in mice.

Species and numbers of animals expected to be used

- Mice: 48450

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.

Most human metabolic pathways and the processes that regulate them are highly conserved in rodents making the mouse the lowest animal in the evolutionary tree that is appropriate as a model species to study metabolic disease and cancer, the main focus of our work. In practice, mice have proven invaluable in elucidating mechanisms that maintain an organism in a healthy state. Consequently, mice

have also been a cornerstone of preclinical drug evaluation for most successful therapies. Furthermore, there are numerous available genetically modified mouse lines and refined procedures that can significantly accelerate testing new hypotheses into molecular mechanisms and alleviate the need to re-develop experimental approaches that would necessitate additional animals.

Given that cancer significantly affects ageing populations, most experiments will be performed in adult mice (typically up to 40 weeks of age). Metabolic deterioration during ageing (e.g. age-induced inflammation and diabetes) may contribute to higher tumour incidence in older individuals. Therefore, to assess whether metabolic pathways in the host that support tumorigenesis change as mice age, we will also perform metabolic analyses in older adults (typically approx. 1-1.5 years old). Conversely, some of the metabolic enzymes we study have been implicated in inborn errors of metabolism affecting neonates and children. Therefore, mice harbouring genetic deletion of these genes will be valuable models of these metabolic diseases. In such cases, in a small number or animals, some metabolic measurements will be performed in a small number of neonate or juvenile animals.

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

1) Generation of mouse models of cancer and metabolic disease.

In general, we will generate mice that develop tumours by various genetic or chemical methods. In most cases, mice will also be fed diets high in sugar and fat, which are known to cause obesity and are linked to increased human cancer incidence. In some cases, mice will undergo surgical procedures, e.g. to remove an endocrine organ that is implicated in producing tumour-promoting signals. All surgical procedures are relatively short (up to 30 minutes).

2) Monitoring metabolic body parameters and tumour growth.

Most animals will be scanned by non-invasive imaging methods (primarily MRI) to monitor tumour development, metabolic parameters or response to therapy. Imaging will be done at regular intervals throughout tumour development, and up to the time that tumours approach the humane end point. In cases where response to therapy is assessed, tumour monitoring may be extended for up to a time required to confirm no re-emergence of therapy-resistant tumours.

3) Measurement of metabolic activities *in vivo*.

To measure metabolic activities in tissues of living mice, most experimental animals will be fasted between 6-18 h and then administered with labelled metabolites. The metabolic state of some experimental animals will be assessed by administering a single dose (bolus) of a metabolite (e.g. glucose) or hormones (e.g. insulin) followed by sampling of a small volume of blood.

4) Testing effects of pharmacological or genetic modulation of target pathway upon tumour development.

In some cases, to test whether a metabolic process is needed for tumour emergence or development, an agent that alters the activity of such a process will be administered over a period of time that is long enough to observe an effect upon tumour growth. Suitable compound doses, administration routes and frequencies will first be determined in pilot experiments. Substance administration will not exceed predetermined volume and frequency limits that are approved in this licence.

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

Experimental procedures proposed for this project have either been established or will be refined to minimise the possibility of adverse effects. Animals that develop tumours may experience weight loss, appetite loss, hunching, or temporary shivering. Animals will be monitored daily and weighted weekly, and any animals experiencing more than 15% weight loss between these measurements will then be weighed daily and killed if approaching the humane endpoint. Notably, in some cases weight loss during therapeutic treatment regimens may be associated with improving, rather than deteriorating, overall health, e.g. when animals are switched from an obesity-promoting to a normal chow diet. Objective assessment of whether weight loss is a cause of concern will be aided by assessing the overall body condition and behaviour of animals (e.g. for signs of distress or piloerection).

Surgical or chemical damage to the liver is reversible and full liver mass and function are expected to recover within 6-7 days after treatment. Surgical procedures and cell injections can cause internal bleeding. Animals are closely monitored during procedures and directly after recovery and any animal showing evidence of bleeding will be humanely killed.

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 (60-70%)

Mild (30-40%)

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?

To identify mechanisms of growth and metabolism that are relevant in the context of the whole intact animal and are therefore meaningful for human clinical studies, it is necessary to utilise animal experiments. This is because, organ- and cancer-specific metabolic pathways are highly influenced by the complex physiological processes inside the body, the local tissue microenvironment, and metabolic crosstalk between different organs and tumours; none of these features are yet possible to faithfully recapitulate *in vitro.*

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

Our current and future research makes extensive use of alternatives to animals. *In vitro* cultures of mammalian cells/tissues, under conditions made more physiologically relevant as informed by the experiments described in this project, can be used for some of our studies that focus on cell-autonomous mechanisms and are undoubtedly an important source of replacement.

We are also making extensive use of computational models of mouse metabolism, whereby, metabolic processes that are predicted to be important for cellular functions related to cancer (such as biomass production to support proliferation). To provide meaningful predictions, these models require input from prior experimental measurements but can be used to test or refine hypotheses before experimental validation in animals.

Why were they not suitable?

The main aim of this project is to understand how organs interact to maintain whole-body metabolic homeostasis, the perturbation of which is a hallmark of many severe diseases such as cancer, infectious disease and metabolic syndrome. It is, therefore, fundamentally impossible to study this problem in any other model than the intact animal. In addition, the effects of manipulations of the diet can only be meaningfully studied *in vivo*.

Furthermore, cultured cell systems alone are inadequate to address most of this project's aims because they cannot recapitulate the metabolic input from the gut and tissue microbiomes; they cannot mimic circadian effects; they lack the multitude of cell types found within different tissues and overall tissue architecture (known as "zonation" in the liver). All these parameters are known to have profound influence on cell metabolism.

Finally, although our improved computational models are increasingly making better predictions that help refine experiments and reduce animal numbers, they need to be validated experimentally before making any meaningful decisions about the translational potential of our discoveries.

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?

Most experimental models proposed in this project have already been established in the lab. We therefore have a clear picture of assay limitations in the context of the expected variability from animal physiology and we can empirically estimate how many animals each experimental group requires for our studies. For new methods, we have consulted the literature and colleagues with relevant experience.

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

Our animal studies design is aided by the NC3Rs Experimental Design Assistant and uses the PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) guidelines. We consult with a statistician with regards to the experimental design to minimise the number of animals used whilst ensuring meaningful data can be collected.

We always strive to invent, improve or implement novel technologies to obtain more information from each animal. Then we incorporate those techniques that are suitable to obtain the desired output while minimising animal numbers at strategic points in our experimental plan. For example, when we need to study a specific pathway (rather than the entire metabolic network, which requires specialised measurements in tissues from terminal experiments) we use magnetic resonance spectroscopy (MRS) to obtain dynamic metabolic activity measurements in a tissue-specific manner. This approach provides, from a single mouse, data that would otherwise require dozens of animals. We use MRS to measure lipid content in a spatially resolved manner without killing the animal, which can then be used for other measurements. For therapeutic treatment studies, longitudinal MR imaging (MRI) ensures cohorts have the desired tumour burden, negating the need for increased animal numbers that would otherwise be used to offset therapeutic response variability due to diverse starting tumour size, or to avoid losing animals that unexpectedly reach the humane end point before the completion of the study.

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

Before embarking on any animal experiments, we will collect as much evidence as possible to determine whether a candidate genetic or environmental manipulation has a reasonable chance of regulating mammalian growth and metabolism in vivo. Evidence will be collected from our own initial studies using cancer cells cultured under physiologically relevant conditions, as well as by surveying the relevant literature. In addition, we will use non-regulated procedures to collect expression data from fixed non-GM mammalian tissues and functional/expression data from genetically and/or environmentally manipulated in vitro mammalian cell lines.

Where possible, mouse lines will be maintained in a homozygous state, thereby obviating the generation of a large excess offspring with inappropriate genotypes. In other cases, homozygotes will be generated from heterozygote inter-crosses, with littermates genotyped as heterozygous or wild type used as age and gender matched controls. In the case of our liver studies, when scientifically justified, we will make use of viral vectors with tropism to the liver (e.g. adeno-associated virus serotype 8 – AAV8), to decrease the need for breeding multiple alleles to obtain tissue-specific genotypes.

For most of the quantitative experiments, design will be based on PREPARE guidelines. Otherwise, we will use the minimum number of animals to provide an adequate description of the projected outcome, determined on the basis of previous experience (our own, or from the literature).

This programme of work will make optimal use of several tissues, fluids and cell types per individual mouse. We will aim to collect organ samples from multiple body sites and to provide other affected tissues to appropriate scientists, so that they do not have to breed mice specifically for their experiments. This highly integrative approach will maximise the information obtained from the minimum

resources. Cryopreservation of gametes, embryos, tissues and cells is routine at our establishment and will ensure that the minimum number of mice is bred.

We also expect that, as we gain better understanding of the metabolic processes involved in biological systems under study, we will be able to define more tailored methods to investigate metabolism while maximizing the amount of information derived from each animal, e.g. by defining new biomarkers or in vivo imaging probes that would allow longitudinal observations of metabolic functions and thereby reduce animal numbers. This is likely to have application not only for this project but also for the broader research community.

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 use mice to study inter-organ communication in health and disease because, although relatively low in the evolutionary tree, their overall anatomical distribution of metabolic functions and metabolic network organisation are highly similar to those in humans. Furthermore, there are many genetic tools and highly refined techniques available for mice, which aid scientific discovery.

The genetic and chemical carcinogenesis models of liver and mammary gland cancer are well established, are known to have relatively minimal adverse effects and have been extensively characterised in many labs around the world. Furthermore, the selected liver cancer and tissue damage models, alongside diet manipulation, recapitulate specific aspects of human disease, that, collectively, contribute to morbidity.

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

One of our primary scientific goals is to understand how organs communicate to achieve whole-body homeostasis, and less sentient species, such as the fly, do not recapitulate the overall anatomical and functional distribution of metabolic activities across organs (e.g. the fly tissue that is thought to be the liver equivalent only performs some of the mammalian liver functions, in addition to other functions that are performed by the mammalian adipose tissue). Most of our measurements with metabolic tracers are done under terminal anaesthesia. However, for studies on cancer growth and metabolic disease development, both of which are intimately linked to ageing, and develop over several months, we need to use adult mice as this is the most physiologically relevant life stage.

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

Wherever appropriate, we will minimise the adverse effects associated with genetic alterations by using inducible or conditional alleles to delete gene activity from specific tissues rather than from the entire mouse. To minimise stress during breeding and maintenance, we will follow best practice guidelines and follow local refinements of husbandry such as cage enrichment and sufficient amounts of nesting material. On receipt or generation of a new line, we will minimize suffering by ensuring increased observation and monitoring until a detailed phenotypic analysis for each line is accomplished. If any welfare implications are identified, they will be acted upon and refinements considered in consultation with the NVS and NACWO.

For all manipulations we will adhere to local or national guidelines that aim to minimize suffering. Many of the genetic, dietary, temperature and oxygen manipulations as well as the administrations of gene inducers/repressors or other agents are standard and previous refinements from the literature will be used. If, however, there is insufficient information available, new manipulations will be pre-screened in small-scale pilot studies to obtain indications of the minimum dose and exposure time that is likely to be effective, thereby minimising any potential suffering.

In all surgery, analgesia will be provided according to contemporary best practice and advice from the NVS/NACWO. In work done under a previous licence, we have optimised surgical techniques, such as partial hepatectomy, to accelerate the procedure, minimise incision size and reduce the probability of collateral tissue damage. Good aseptic surgical techniques, heat & fluid therapy will be provided. In the case of cancer models for example, we will follow the guidelines in Workman et al, British Journal of Cancer (2010) 102, 1555 – 1577. For each protocol where there are a number of optional steps, the maximum number of steps is clearly defined within the adverse effects section.

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

We will follow the guidance given in the NC3Rs ‘Resource Hub’ (https://nc3rs.org.uk/resource-hubs) for example on blood sampling (https://www.nc3rs.org.uk/blood-sampling-mouse) and effective use of genetically altered mice (https://www.nc3rs.org.uk/GAmice). We will also refer to the National Cancer Research Institute guidelines on using animals in cancer research published by Workman et al. 2010 (British Journal of Cancer 102, 1555 – 1577).

For surgery, we will follow the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (https://www.lasa.co.uk/wp-content/uploads/2018/05/Aseptic-Surgery.pdf).

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

We will keep up to date with the latest developments on refining animal research methods via the NC3Rs website (https://www.nc3rs.org.uk), also complemented by information we obtain from regular newsletters prepared by our animal facility. Animal house staff will ensure that any advances are fully implemented throughout the facility.