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NON-TECHNICAL SUMMARY

The role of DNA Damage response and replication factors in genome stability and cancer

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

DNA Damage response, Genomic stability, Tumorigenesis, Cancer therapy

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

During the course of the most recent licence, we generated several new DNA Damage Response (DDR) gene knockouts in mice, some of which presented with accelerated tumourigenesis, immunodeficiency, neurological problems and/or infertility. Hence, the overall aim of this project is to better define the role of DDR genes in tumorigenesis and the impact on other diseases/phenotypes including immunodeficiencies, neurological problems and/or infertility. We would like to determine which DDR genes are involved in tumorigenesis particularly and which strategies could be used to counteract the development of these tumours.

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?

The DDR is composed of multiple pathways and involves processes necessary to repair DNA lesions such as double-strand break, damaged bases, impaired replication and other genomic insults. The DDR is critical to healthy cells in order to preserve genomic stability and prevent tumor formation and other pathologies. In humans, hereditary mutations in DDR genes are associated with a wide range of cancer susceptibility, neurodegenerative, immunodeficiency and infertility disorders. Furthermore, the DDR is often somatically deregulated in many cancers, which renders the cells more susceptible to DNA damage and makes them more reliant on other pathways for survival. Targeting DDR is one of the most exciting therapeutic strategies for treating cancer, which can be exploited to selectively induce cancer cell death. Studying the DDR in more detail may reveal unappreciated vulnerabilities that could be targeted in cancer therapy. Interrogating the DDR in animals will also improve our understanding of the roles of these processes in immunity, fertility and/or neurophysiology.

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

The focus of my lab is to understand how DDR pathways, including homologous recombination (HR), contribute to organismal homeostasis and disease. Since DDR processes are commonly inactivated in hereditary human diseases and are frequently inactivated at a somatic level in many cancers, we aim to understand how these pathways impact on human pathologies including cancer, immunodeficiency, accelerated aging and fertility. By exploiting the respective experimental strengths of C. elegans, frog extracts and mammalian cell culture my lab has discovered new DNA repair genes relevant to human disease. The purpose of this project is to determine how these disease relevant genes contribute to genome stability, immunodeficiency, aging and cancer using mouse models. Our work also has the potential to inform on novel opportunities for directed cancer therapy and other human syndromes affecting fertility, immunity, development which we will also explore in mice.

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

Our studies aim to provide fundamental new insights into mechanisms by which cells and whole organisms repair DNA damage to maintain genomic integrity and how unrepaired DNA can affect cancer development and other pathologies (immunodeficiency, infertility, accelerated aging).

The questions addressed in our studies are clinically relevant as unrepaired/mis-repaired DNA damage has been shown to be an important first step in the process of carcinogenesis and also impact on the development of germ cells and the immune system. The data generated by this project will not only improve our understanding of DNA repair mechanisms and how this affects organismal homeostasis but will also inform on the development of targeted therapeutics. Only by inducing dysregulation of DNA damage repair pathways in vivo will we be able to uncover their roles in carcinogenesis and/or organismal physiology.

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

Findings will be made available to the broader scientific community through publication in peer-reviewed journals and presentations in scientific conferences. Data will also be deposited onto publicly accessible databases. Newly created transgenic animals will be distributed widely to the scientific community as these lines generated under the authority of this Project Licence will be very valuable to the research community.

Species and numbers of animals expected to be used

- Mice: 15200

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 offers an unparalleled level of genetic tractability in an organism that develops cancers, immune-deficiency or neurological problems (such as ataxia, impaired balance, coordination and/or behaviour) that are comparable to those occurring in humans. This is particularly relevant to our objective where we are attempting to provide evidence that DNA damage response factors are involved in specific diseases (any types of cancer, Riddle syndrome, Hoyeraal-Hreidarsson syndrome, FILS). We will first attempt to test our hypothesis in tissue culture with cells derived from these animals, but ultimately, it is experiments within the animal that will have the biggest impact on how human diseases such as cancer develop. The proposed experiments simply cannot be addressed in flies, worms or other model organisms, as they do not develop tumours, immune-deficiency or neurological problems. Where possible experiments on animals will be replaced with experiments on cultured cells as outlined above. However, in cases where this is not applicable, we will adhere to the three Rs (Replacement, Reduction and Refinement) to minimise the numbers of animal used and minimise the suffering of animals during experimental procedures.

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

Mice used will be generated to create conditional knockout, knockin, conditional over-expressing and/or BAC transgenic mice for genes we have previously implicated in the maintenance of genome stability (Protocol 1 and 2) or will be provided by other projects with authority to breed, maintain and/or supply transgenic mice.

We will investigate the relationship and genetic interactions between the resulting mice and other mutant mice that exhibit genome instability phenotypes and/or meiotic defects (Protocol 3, 4, 8, 9).

We will also characterise the tumour predisposition, neurological, immunological and/or meiotic phenotype of these mice (Protocol 3, 4, 5, 6, 7, 8, 9).

The principle aim of objectives 1-3 will be the detailed examination of tumour incidence, meiotic, immune-deficiency or neurodegenerative phenotypes in mice mutated in novel genome maintenance genes. The significance of tumour predisposition, meiotic, immune-deficiency or neurological phenotypes conferred by a particular genetic background will be judged by comparison to established mice (Protocol 3), which should show clear statistically significant difference from controls (ANOVA). Tumour predisposition will also be assessed by treating mutant mice with gene inducers or modifiers, DNA damaging agents, carcinogens, tumour promotor (Protocol 3) or by injection of cells mutated for DNA damage response genes (as for example ALC1, Rad51, WRNIP) into nude mice (Protocol 4, 5, 6, 7). These objectives can only be addressed using whole animal models, as modelling tumour development to this level of detail is not possible in vitro. The minimum number of animals will be used and all suffering kept to the lowest possible level by ensuring that animals bearing tumours are sacrificed before the tumours reach a size that is likely to cause distress.

In general, determining tumour predisposition in mice bearing a mutation in DNA Damage response genes will be fulfilled by 2 complementary ways: first, we will monitor mice in aging cohorts by performing a daily health check (clinical examination, abdominal palpation and imaging if necessary) (Protocol 3). Second, we will use a small cohort of animals, which will be treated with chemicals and notably tumour inducers/promoters and/or carcinogens (Protocol 7, 8). These 2 complementary ways should allow us to demonstrate if a particular DNA damage response gene has a role in tumour formation. Chemical carcinogenesis is one of the best-established in vivo models for the study of tumour development and for evaluating tumour initiation, promotion and progression (Protocol 8). Well-established methods also require use of a procarcinogen such as diethylnitrosamine in combination with a high fat diet to allow tumour development (Protocol 7). The high fat diet will also induce obesity, which will be used as a tumour promotor. The quantities and frequencies of the chemical used should result in no more than transient discomfort and no lasting harm. Carcinogenesis protocols (chemical or UV) have been developed for the study of tumours in mice (i.e., tumour incidence, latency, multiplicity and progression). In complete carcinogenesis protocols, tumour development occurs either after the administration of a single high dose (or repeated applications of a lower dose) of a carcinogen or by repeated low dose exposure to ultraviolet (UV) light. UV/X-irradiation will also be used as a potential in vivo inducer of DNA damage that will in a time dependent manner induce tumour formation. Mutant animals as well as controls will be subjected to single or multiple doses of either UV or X-irradiation to allow us to determine the role of specific DNA damage response genes in DNA repair (Protocol 3, 4, 5, 6, 7, 8, 9).

Non-invasive imaging will be employed in Protocol 3, 4, 5, 6, 7, 8 to ensure that we can detect tumour(s) arising in mice as soon as possible and minimize animal suffering. We will use different types of imaging such as bioluminescence or fluorescence imaging to monitor tumour formation and growth (when for example using B16F10 cells expressing a fluorescent or bioluminescent transgene: B16-F10-Luc or B16-F10-RFP). In some cases, we may want to follow tumour progression by non invasive MRI or microCT. All imaging will be performed by experienced people working at the Crick imaging facility.

Clinical examination, abdominal palpations and imaging will be used to assess and confirm tumour presence in our animals. It will allow us to limit tumour burden to a minimum.

We will also assess neurological problems by employing SHIRPA behavioural tests in protocol 3.

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

An important aspect of our research is to monitor animals that have genetic modifications throughout the term of their natural lifespan (up to 24 months) in order to study and assess their phenotype (Protocol 3). Humane endpoints for mice used in such longevity studies have rarely been addressed, despite the expectation that health problems will become more common as the mice age. Natural and spontaneous death can occur without being linked to any genotype. In rare cases, aged animals can die without having shown any prior signs of illness or deterioration in health. We will monitor animals at risk with greater than usual frequency and any changes in their health status will be accurately recorded in a form specifically designed for animals at risk of death and archived. If animals are found dead without having shown prior clinical signs, post-mortem investigation will aid determination of cause of death and this data will then be analysed as soon as possible so that early euthanasia can be performed on other animals to promote their wellbeing as well as facilitating collection of valuable samples and measurements.

Mice on protocol 4, 5, 6, 7, and 8 will develop tumours as adverse effects. For mice used under protocol 4, 5, 6, tumours development will generally be monitored twice a week by either calliper measurement and/or imaging if possible. Animals developing tumours larger than 1.2cm or 1.5cm will be humanely sacrificed. These experiments should be short term experiment that may be no longer than 3 months. Mice under protocol 7 and 8 should also develop either external (protocol 7) or internal (protocol 8) tumours that will be monitored frequently. These protocols may last up to a year to follow induced tumours development in a genetically altered animal.

In this project, substance administration should not cause adverse effects that are longer than temporary and transitory as we will for most of them use doses that have already been described by the scientific community.

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)?

Only mice will be used in this project.

A high proportion of mice (75%) should develop phenotypes of moderate severity as a result of tumours development, immunodeficiency and/or neurological problems. A subset of these mice will also be used for tissue harvest and/or cells production.

25% will have a mild severity and the remaining would be subthreshold.

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 studies and those in the published literature on the DNA damage repair pathways and other DDR genes are suggestive of roles in maintenance of genome stability and tumour avoidance; Brca1, Rif1, Rtel1 and ALC1 have been observed to be either amplified or inactivated in tumours depending on context. What remains unclear is if these changes are a cause or a consequence of tumorigenesis. Animal experiments remain the only definitive test of whether inactivation/over-expression of these genes is relevant for tumour formation. These issues can only be addressed using whole animal models, as modelling tumour development to this level of detail is not possible in vitro. The use of mice offers an unparalleled level of genetic tractability in an organism that develops cancers, immune-deficiency or neurological logical problems that are comparable to those occurring in humans.

3D model such as cultured organoids are good model to answer a specific question but the relevance at the human level is very limited as it does not include the specific environment in which a tumour is growing. Only a genetically altered mouse model will be reproducing the same environment than the one observed in a human body. Moreover, genetic drift in organoids culture may happen quicker and won’t be as well controlled as it is in a mouse model.

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

All of our proposed studies aim to build on extensive groundwork in model organisms, mammalian cell culture and during the last 5 years in mouse models. The objectives outlined in this application represent the next and most pertinent step forward in our mouse studies, which is likely to provide novel insights into the impact of these factors on organismal biology and disease. We are currently using a multi system approach to answer questions addressed in our studies. For example, we use the C.elegans worm model as it is a very easy organism to genetically manipulate. It makes the study of synthetic lethality very accessible due to its quick reproduction and also is a very good model for studying meiosis as all steps of it are represented in the germline. We also use the frog model X.laevis to study replication-coupled DNA repair. One of our main tools is the use of mammalian cell culture, which was recently revolutionised by the discovery of CRISPR. Generation of genetically altered

mouse or human cell lines is proving to be easier and quicker with CRISPR techniques and these cell lines can be used to do targeted proteomic screens that will give us more insight on molecular mechanisms that we will be able to study more in depth at the level of a whole organism. Only by inducing dysregulation of DNA damage repair pathways in vivo will we be able to uncover their roles in carcinogenesis and/or organismal physiology.

Why were they not suitable?

The proposed experiments simply cannot be addressed in frogs, worms or other model organisms, as they do not develop tumours, immune-deficiency or neurological problems.

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?

For obtaining statistically significant and reproducible results, we will include a positive and a negative control group which will give validity to the ongoing experiment. Wherever possible, power calculation with a power of 80-90% and a type I error rate of 5% will be used to more accurately estimate the number of animals needed to ensure a statistically significant outcome and avoid unnecessary experiment duplication.

In all in vivo experiment, the number of mice required for a specific experiment is based on 3 criteria:

1. The number of animals for each set of experiments
2. The number of experiments (needs to be perform 2 independent times)
3. Pilot studies and previous experience performing a similar experiment

Where necessary we will also consult the bioinformatics and biostatistics group at the Crick during the experimental design stage to ensure that the appropriate numbers of mice are used per experiment.

All experiments/procedures notably tumour development measurement will be performed limiting the animals' number to the minimum required for a valid scientific outcome.

A specific subset/cohort of animals will be used under each protocol. We will also use animals to harvest tissue and/or produce cells (MEFs, ES cells). Some experiments could require up to five genetic modifications to be bred into one animal, which would require extensive breeding programmes.

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

Mouse breeding experiments will be planned in consultation with the Crick BRF and the basic principles of mouse breeding will be adhered to and gestation times, weaning age and litter sizes used to calculate the numbers of mice required to optimise the size of the colony. Mouse lines will be routinely maintained by keeping 2 breeding pairs. For crosses under analysis for specific phenotypes it is likely that 5-6 breeding pairs will be maintained for the duration of the experiment. However, every effort will be made to reduce the number of animals required for experiments, always keeping in mind that enough animals are used not to prejudice the generation of statistically relevant results. For exploratory (pilot) experiments the least number of animals will be used (usually 4-6 per genotype/experimental group) to provide a reasonable estimate of the numbers of animals needed to produce statistically relevant information in subsequent quantitative experiments. Where possible we will use data from published experiments to provide an estimate of the sample size.

Where necessary we will also consult the bioinformatics and biostatistics group at the Crick during the experimental design stage to ensure that the appropriate numbers of mice are used per experiment. We will also use the Experimental Design Assistant from NC3R to help us design our experiments (ensure the use of minimum number of animals consistent with our scientific objectives).

For multiple genetic modification, we will investigate alternative methodologies to enable reduced numbers of animals to be used. This will be to use viral delivery of the expression modifying genes CRE and tetracycline activator via different routes (intratracheal, intraperitoneal, topical application). This would require two less genetic modifications to be present in the mice, so would greatly reduce the size of the breeding programme.

We will also reduce numbers of mice by cryopreserving sperm or embryos with the help of the Genetic Modification Services of the Crick.

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

We are always looking for ways to reduce the number of animals that we produce. Breeding efficiency is key and for strains that are known to have low breeding efficiency, we feed females with baby milk mash. This allow us to use less mice for breeding and also increases the chance to have a higher number of embryos/pups in some of our C57BL6 mice. Moreover, most of our xenograft studies start with pilot experiment using small animal cohorts that lead to a refinement in term of cell number injected, and/or doses used for specific compounds and give us insight into experiment duration; and where possible we will use a single group of animals as a control for several treatment groups.

Other ways in which we will aim to reduce mouse numbers will include freezing down transgenic lines; deriving cell lines from mice for specific cell culture experiments; providing tissues from our mice to other labs.

The breeding of genetically altered mice will be reduced through collaborative access to strains.

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.

Mice will be our model of choice during this project. The use of mice offers an unparalleled level of genetic tractability in an organism that develops cancers, immune-deficiency or neurological problems that are comparable to those occurring in humans. This is particularly relevant to our objective where we are attempting to provide evidence that DNA damage response factors are involved in specific diseases. We will first attempt to test our hypothesis in tissue culture with cells derived from these animals, but ultimately, it is experiments within the animal that will have the biggest impact on human disease. We will minimise the numbers of animal used and minimise the suffering of animals during experimental procedures.

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

Mice and Human are biologically and genetically very similar. By using mice, we ensure that we can study the effect of a single specific genetic alteration (for example found in human breast cancer or any other disease that have a deregulated DNA Damage repair pathway) in a very controlled organism. Moreover, spontaneous tumourigenesis resembling human tumorigenesis cannot be reproduced in other organisms (flies, worm, frog, cell culture) than a mouse model, as they do not develop tumours, immune-deficiency or neurological problems. Where possible experiments on animals will be replaced with experiments on cultured cells, C.elegans and X.laevis as outlined above. We will first attempt to test our hypothesis in tissue culture with cells derived from these animals, but ultimately, it is experiments within the animal that will have the biggest impact on human disease and new therapy findings. We will minimise the numbers of animal used and minimise the suffering of animals during experimental procedures.

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

We will monitor animals at risk with greater than usual frequency and any changes in their health status will be accurately recorded in a form specifically designed for animals at risk of death and archived. If animals are found dead without having shown prior clinical signs, post-mortem investigation will aid determination of cause of death and this data will then be analysed as soon as possible so that early euthanasia can be performed on other animals to promote their wellbeing as well as facilitating collection of valuable samples and measurements. We will use palpation and imaging in order to limit adverse effects due to tumour development.

The weight of the animals going through a procedure will be monitored closely to ensure weight is stably increasing and is not affected by any procedure that a mouse went through.

Most of the protocols that we are using in this project are well established protocols that have been widely used by the scientific community. We will follow them closely by administering the recommended concentration of substances (such as chemical carcinogen, tumours initiator and promotor, IR/UV doses, antigen concentration).

To improve our breeding efficiency with difficult to breed mice, animals will be fed with mashed food supplemented with milk powder.

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

FELASA “Working Group on Pain and distress” and NCRI guidelines will be employed to aid in the assessment of pain and distress and used to determine the earliest endpoint possible to allow a valid scientific outcome.

When assessing tumour burden, the NCRI Guidelines for the Welfare and Use of Animals in Cancer Research will be followed.

In addition, we will follow the PREPARE guidelines when designing experiments and ARRIVE guidelines when publishing to allow better reproducibility and avoid work duplications.

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

We are currently subscribing to the NC3R monthly newsletter which gives us information on any techniques’ improvement and 3R advances. We are also in relation with the regional programme manager for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) who can give us advice and support on the 3Rs for our project.