Understanding how Primordial Germ Cells Repair DNA to Suppress Tumourigenesis

Investigators

Lead Investigator:  Dr Gerry Crossan  
MRC Laboratory of Molecular Biology  
gcrossan@mrc-lmb.cam.ac.uk  
http://www2.mrc-lmb.cam.ac.uk/group-leaders/

Co-Investigator:  Prof Azim Surani  
Wellcome Trust/Cancer Research UK Gurdon Institute and Department of Physiology Development & Neuroscience  
asurani@gurdon.cam.ac.uk  
http://www.gurdon.cam.ac.uk/research/surani

Project

The repair of damaged DNA is essential to ensure that the fidelity of the genome is maintained and to prevent mutations arising. Failure to counteract mutations in DNA can lead to neoplastic transformation. Therefore, gaining insight into the mechanisms that usually fix DNA damage is critical for us to understand how organisms prevent cancer.

This is of paramount importance in the germline where repair is essential to ensure the faithful transmission of the genome from one generation to the next; failure to do so has catastrophic consequences for the offspring. Furthermore, tumours arising from cells of the germline are the most common solid cancers in newborns. Whilst it is clear that repairing DNA damage in the germline is critical, very little is understood about how this is achieved.

In vertebrates, the germline is produced by extremely rare and specialised stem cells, primordial germ cells (PGCs), which arise early in embryogenesis. These cells are the most fundamental of all stem cells as they eventually give rise to every cell type in an organism, including all tissue-specific stem cells. As they migrate to the gonads, the ultimate site of gametogenesis, the PGCs undergo several maturation processes, each of which could potentially damage DNA.
Whilst preventing mutagenesis is an important mechanism to suppress tumourigenesis in general, it is crucial for the PGC population. These cells have the potential to give rise to an entirely new organism therefore if de novo mutations occur within key tumour suppressor genes, offspring can have an enormous predisposition to cancer. Indeed up to 20% of germline p53 mutations occur de novo within the germline of a parent. Additionally, tumours can arise from early PGCs themselves; these include both teratomas and yolk sac tumours. These solid tumours and are often associated with complex chromosomal rearrangements. Therefore, gaining insight into how DNA damage is repaired within PGCs will be key to understanding why complex germ cell tumours arise.

Aims
The following proposal sets out to address three key aims relating to how PGCs maintain genomic stability:
1. When is mutagenesis suppressed in the germline?
2. What is the nature of DNA damage in the PGCs?
3. How is neoplastic transformation suppressed in damaged germ cells?

1. When is mutagenesis suppressed in the germline?

Preliminary evidence suggests that mice unable to repair a crosslinked DNA, a particular form of DNA damage, are sterile. The pattern of sterility indicates that this is not due to a failure in meiosis. Gonads from these mice are almost entirely devoid of germ cells, with no accumulation of meiotic intermediates. This sterility is therefore likely to be due to failure during early germline development – a problem in the PGC pool. This suggests that during development PGCs accumulate sufficient DNA damage to render DNA repair essential. Understanding this process may also yield insight into the physiological source of damage that leads to neoplastic transformation in germ cell tumours.

In order to study this rare cell population, the student will establish a conditional mouse model of this specific form of DNA repair. The model will be used to delete repair either during PGC specification or during male or female meiosis by crossing the conditional mice with mice expressing Cre at a specific time during germ cell development (already established reagents). This will provide the first direct genetic evidence to explain the origin of sterility in these mice and identify a key mechanism to prevent mutagenesis in germ cells. This useful tool can then be used to address the questions below.

In order to assess when DNA damage occurs within the PGC pool, the student will employ mice in which the PGCs are fluorescently labeled (Stella-GFP, Azim Surani). These mice will allow the student to isolate wild type and DNA repair deficient PGCs at specific points during development. The student will ask if the crosslink repair pathway is activated at a specific point during development of wild type PGCs. They will then go on to ask if that activation coincides with the loss of the PGC pool in crosslink repair deficient mice.
2. What is the nature of DNA damage in the PGCs?
It will be critical to understand what kind of DNA damage accumulates in crosslink repair deficient PGCs. Do these cells show markers of specific lesions (e.g. γ-H2AX)? Do other repair pathways become activated in the absence of crosslink repair? This is important, as the nature of the DNA lesion will determine the pattern of mutagenesis in the germ cells.

Another key question is what causes the DNA damage necessitating repair? Several key processes occur during PGC development, such as global epigenetic reprogramming, that could potentially damage DNA. These very processes lead to the activation of DNA repair pathways. The student will ask if the loss of germ cells in crosslink repair deficient mice coincides with a defined process during PGC maturation (e.g. epigenetic reprogramming)?

This will provide a correlation between DNA damage and the activation of specific DNA repair pathways and physiological changes occurring in germ cells. If successful this would provide evidence showing when damage occurs, the nature of that damage (e.g. double strand break vs. single strand breaks) and the functional significance of that damage. It could potentially elucidate a physiological source of damage that threatens the integrity of the germline.

3. How is neoplastic transformation suppressed in damaged germ cells?
The accumulation of DNA damage is extremely deleterious, potentially leading to neoplastic transformation. However, cells usually repair this damaged DNA, exit the cell cycle or die. Tissue specific stem cells are often lost due to the activation of a checkpoint (typically p53) that then causes the cells to enter apoptosis. The ablation of p53 can lead to improved survival of tissue specific stem cells after DNA damage, albeit at the expense of compromised genetic integrity. Whilst p53 is critical in tissue specific stem cells, p63 is much more important in mature gametes.

The student will therefore generate PGCs which lack both DNA repair (generated in aim 1) and either p53 or p63 (both alleles already exist). They will ask if ablation of these checkpoint proteins rescues germ cell death but potentiates tumourigenesis. This would allow them to determine if DNA damage directly leads to PGC loss or if there is a checkpoint to ensure genomic stability. If this checkpoint is indeed different from tissue stem cells this may provide us with the ability to compare those checkpoints. This could allow the student to identify the checkpoint which suppresses neoplastic transformation in DNA damaged PGCs.

Further information

This project, by its very nature, will be both collaborative and also interdisciplinary. Prof Azim Surani has a great deal of experience in the study of PGCs and he is indeed a world leader in this
field. He will therefore share both unique reagents (e.g. Stella-GFP mice) and also his laboratory’s expertise to enable the graduate student to gain the necessary techniques to pursue this challenging but achievable project.

The student will have a significant support network throughout this PhD project. Azim Surani will act as a university supervisor having considerable experience in supervising PhD students and also the ability to provide very important insight into the PGC aspect of the project. Additionally, Julian Sale will act as second supervisor within the LMB. Julian is the director of studies for graduate students at LMB so is very well placed to offer support. Furthermore, he can offer important insight into the DNA repair aspects of the project.