The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL

PROJECT DETAILS	
Project Title:	Structures and mechanisms of EXD2 nuclease in DNA repair

SUPERVISORY TEAM	
Primary Supervisor(s):	**Dr Wojciech Niedzwiedz / Dr Alessandro Vannini**
Other supervisory team members:	**Professor Jessica Downs**
Lead contact person for the project:	**Wojciech Niedzwiedz**

DIVISIONAL AFFILIATION	
Primary Division:	Cancer Biology
Primary Team:	Genome instability and Cancer
Other Division (if applicable):	Structural Biology
Other Team (if applicable)):	Vannini team

PROJECT PROPOSAL

BACKGROUND TO THE PROJECT

DNA double-strand breaks (DSBs) are amongst the most toxic lesions that cells can suffer. Their presence can trigger genome rearrangements and the loss of genetic information at the break site. The faithful repair of DSBs is therefore essential not only for cell survival, but also for organismal development, as mutations in genes involved in this process underline a plethora of inherited human syndromes characterised by a predisposition to cancer, immunodeficiency, infertility, neurodegeneration and premature ageing. DSBs are also thought to be the predominant lethal lesion driving cell death, a property that has been exploited for cancer treatment. Thus, exploiting our knowledge of DSB repair mechanisms is also highly relevant to the development of novel cancer therapeutics.The two major pathways involved in the repair of DSBs in eukaryotic cells are an error prone non–homologous end-joining (NHEJ) process that involves the simple ligation of broken DNA ends (but often with losses of bases), and an error free homology directed repair (HR) that utilises an intact DNA template.

We recently identified a novel DNA repair protein called EXD2 and demonstrated that it is required for homology directed repair and resistance to large number of anti-cancer treatments. However, the molecular mechanism of its action is unknown.

The primary aim of this PhD project will be to determine the structure of unbound EXD2 as well as in complex with DNA substrate and/or other interaction partners. This work will provide an integrated picture of its DNA binding and enzymatic function laying foundations for future targeting of its nuclease activity with small molecules as a potential treatment for diseases such as cancer.

PROJECT AIMS

1) **Purification of recombinant EXD2 fro structural studies**
2) **Identification of nucleic acid substrate**
3) **Characterization of cellular partners in the replication fork protection pathway**
4) **Structural characterization of EXD2-containing complexes**

RESEARCH PROPOSAL

The ability of cells to divide allows organisms to grow and reproduce. This process requires copying and maintaining a vast amount of genetic information. Therefore, accurate replication of DNA is essential not only for the preservation of genomic integrity but also the continuation of life. To accomplish this, cells have evolved complex mechanisms to both replicate cellular DNA with high fidelity and to preserve its integrity[1,2]. Nevertheless, genomic integrity is challenged during every S-phase by lesions present in the DNA template that can collapse replication forks, contributing to tumour progression by driving chromosomal instability[1,3-5]. To counteract this threat, replicative stress, the most important of which is the replication fork protection and checkpoint pathway, which relies on the activation of the ATR kinase. This surveillance pathway ensures inhibition of cell cycle progression, suppression of late origin firing and helps to stabilize and restart stalled replication forks. Together these events allow for efficient completion of DNA synthesis. Work from my laboratory and others show that if this pathway functions sub-optimally accumulate DNA damage including DNA double-strand breaks (DSBs)[6,7]. Accordingly, many human diseases, including cancer, are associated with defective repair of damaged DNA[1-2]. Recently, our and others' work have identified the EXD2 nuclease as a novel factor required for DSB repair by homologous recombination (HR)[8] and a non-classical NHEJ pathway[9].

In collaboration with the group of Dr. Alessandro Vannini in the Division of Structural biology, we are planning to understand the molecular basis of EXD2 nuclease using an integrated structural biology approach. Biochemical assays will be carried out in order to identify the preferred nucleic acid substrate as well as interaction partners that might play a role in the replication fork protection pathway. The identified complexes will be structurally characterized using x-ray crystallography and/or cryo-EM.

Obtaining a mechanistic understanding of EXD2 nuclease in the context of a complex with its natural substrate and interaction patterns will be paramount to validate it as novel anticancer target. Furthermore, the structural work will provide a solid framework to design EXD2 small molecule inhibitors.

LITERATURE REFERENCES

1. Kass, E. M., Moynahan, M. E. & Jasin, M. When Genome Maintenance Goes Badly Awry. *Mol Cell* **62**, 777- 787, (2016).

2. Jackson, S. P. & Bartek, J. The DNA-damage response in human biology and disease. *Nature* **461**, 1071- 1078, (2009).

3. Burrell, R. A., McClelland, S. E., Endesfelder, D., Groth, P., Weller, M. C., Shaikh, N., Domingo, E., Kanu, N.,

Dewhurst, S. M., Gronroos, E. *et al.* Replication stress links structural and numerical cancer chromosomal instability. *Nature* **494**, 492-496, (2013).

4. Zeman, M. K. & Cimprich, K. A. Causes and consequences of replication stress. *Nat Cell Biol* **16**, 2-9, (2014).

5. Jeggo, P. A., Pearl, L. H. & Carr, A. M. DNA repair, genome stability and cancer: a historical perspective. *Nat Rev Cancer* **16**, 35-42, (2016).

6. Schwab, R. A., Blackford, A. N. & Niedzwiedz, W. ATR activation and replication fork restart are defective in FANCM-deficient cells. *EMBO J* **29**, 806-818, (2010).

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8. Broderick, R., Nieminuszczy, J., Baddock, H. T., Deshpande, R. A., Gileadi, O., Paull, T. T., McHugh, P. J. & Niedzwiedz, W. EXD2 promotes homologous recombination by facilitating DNA end resection. *Nat Cell Biol* **18**, 271-280, (2016).

9. Biehs, R., Steinlage, M., Barton, O., Juhasz, S., Kunzel, J., Spies, J., Shibata, A., Jeggo, P. A. & Lobrich, M. DNA Double-Strand Break Resection Occurs during Non-homologous End Joining in G1 but Is Distinct from Resection during Homologous Recombination. *Mol Cell*, (2017).

CANDIDATE PROFILE

Note: the ICR's standard minimum entry requirement is a relevant undergraduate Honours degree (First or 2:1)

Pre-requisite qualifications of applicants: e.g. BSc or equivalent in specific subject area(s)	**Biology, Biochemistry or other allied science.**

Intended learning outcomes:

1) biochemistry of DNA helicase
2) protein-nucleic acids, protein-protein interaction studies
3) Integrated structural biology (x-ray crystallography and/or cryo-EM)