The relationship between replication stress survival and RECQL5 and MRE11

Background:
RECQL5 is one member of a family of five RECQ DNA helicases, which have roles in DNA replication, recombination, and repair (Hickson 2003). Homologs of these 5 proteins have been found in both man and mouse but only one RECQ helicase is found in yeast and Escherichia coli (Singh, Ahn et al. 2009).

RECQL5 has many roles within the cell, acting on stalled replication forks, stimulating flap endonuclease (FEN1) and maintaining genome stability. The different functions of RECQL5 have been discovered through analyzing the phenotype of mammalian cells after overexpression and knockout of the RECQL5 protein. Overexpression of RECQL5 has been shown to provide resistance to thymidine-induced replication stress (Blundred, Myers et al. 2010) and gene-knockouts of RECQL5 have been shown to increase susceptibility to replication stress induced by camptothecin treatment (Hu, Lu et al. 2009).

RECQL5 has been shown to interact with the DNA damage response protein MRE11. Zheng et al. (2009) performed an immunoprecipitation with RECQL5 and the MRE11 containing MRN complex, showing that the interaction is both DNA independent (cell lysates were treated with DNaseI or ethidium bromide) and constitutive (by testing the interaction at different stages of the cell cycle). Affinity pull-downs were performed with purified recombinant proteins in order to determine if the interaction is direct (with RECQL5 being expressed with a CBD tag). This was done with all 3 MRN component proteins and the results showed that the binding is to the MRE11 component of the MRN complex, independent of other factors (Zheng, Kanagaraj et al. 2009). It was also discovered that the exonuclease activity of MRN was significantly decreased when RECQL5 was added to a reaction mix of ATP and manganese. The interaction of RECQL5 and MRE11 does not require MRE11's exonuclease activity and is independent of the proteins BLM, WRN and ATM (Popuri, Ramamoorthy et al. 2012). Further experiments, using immunofluorescence, showed that not only do the MRN complex and RECQL5 co-localize at sites of DNA damage, but when MRE11 is depleted, the RECQL5 foci formed after HU treatment (creating stalled replication forks) are completely abolished (Zheng, Kanagaraj et al. 2009).

Recent work in our lab has shown that by knocking down RECQL5 expression, we increase the sensitivity to replication stresses camptothecin, thymidine and gemcitabine. While we have previously shown that overexpression of RECQL5 causes resistance to thymidine induced replication stress (Blundred, Myers et al. 2010).

Hypothesis:
Our hypothesis is that RECQL5 relies on MRE11 to bring it into the sites of stalled replication forks and that if MRE11 is depleted, RECQL5 will not be able to repair these stalled forks.

To test this hypothesis our aims are:
Knock down MRE11 protein expression in our RECQL5 over-expressing cell lines using siRNA,
Use this knock down system to test whether the thymidine-induced replication stress resistant phenotype we observe in RECQL5 overexpressing cells is reversed by MRE11 depletion.
If time allows we will also knock down both MRE11 and RECQL5 in non-overexpressing cell lines to investigate whether co-depletion further increases the sensitivity to replication stress compared to RECQL5 or MRE11 alone.

Translational relevance:
We have data demonstrating RECQL5 is overexpressed in bladder cancer.
If MRE11 is required for RECQL5-associated replication stress resistance, it could provide another target to inhibit RECQL5’s function and increase tumour’s sensitivity to existing chemotherapeutic agents.

Research Plan:
Week 1. The student will learn basic tissue culture and observe the Western blotting protocol. In this week they will also learn how to stain cells for a clonogenic survival assay and observe one such experiment being set up.
Week 2. Under close supervision the student will perform their first siRNA transfection, make protein extracts, and perform a Western blot to confirm the extent of MRE11 knockdown in MRE11 siRNA, control non-targeting siRNA and mock-transfected cells.
Weeks 3-6: Knockdown will be repeated in RECQL5 over-expressing cells, and in addition to Western blotting, a portion of the cells will be replated in the presence or absence of thymidine to induce replication stress and survival will be assessed using a clonogenic survival assay. This experiment takes a week and needs to be repeated 3 times. It is envisaged that the student will work with increasing independence as the 3 weeks go on.
Weeks 5-6: The student will also use siRNA transfection to knock down combinations of MRE11 and RECQL5 in non-overexpressing cells to observe the effects on proliferation in the presence or absence of replication stress. Proliferation will be assayed by counting cell numbers on 3 consecutive days.
We have already determined the conditions for knock down of both MRE11 and RECQL5 using siRNA.
In addition to the experiments above the student will assist/observe the supervisor in a number of other techniques routinely used to study DNA damage responses to replication stress, e.g. DNA fibre analysis, fluorescence microscopy and FACS cell cycle analysis.