UNIVERSITÀ DEGLI STUDI DI MILANO-BICOCCA

DOTTORATO DI RICERCA IN TECNOLOGIE CONVERGENTI PER I SISTEMI BIOMOLECOLARI (TeCSBi) – XXXIII CICLO

Project Supervisor: Prof. Maria Pia Longhese
Project Title: Regulation of DNA-end resection in the DNA damage response

Possible support for Phd students w/o a University fellowship: possible

Cancer is an evolutionarily disease fuelled by genome instability. To maintain genomic integrity, eukaryotic cells have evolved a DNA Damage Response (DDR), which is a network of hierarchically ordered proteins capable of detecting DNA lesions and signaling their presence to activate pathways that delay cell cycle progression (DNA damage checkpoint), repair DNA lesions, or induce cell death. The DDR acts as a physiological anti-cancer mechanism, as indicated by the cancer-prone phenotype of several DDR syndromes. Among the many types of DNA damage, the DNA double strand break (DSB) is one of the most severe ones because it has the potential to cause mutations and chromosomal rearrangements.

It is well known that the ends of a DSB are nucleolytically processed (resected) to generate 3'-ended ssDNA. DSB resection plays an important role in the regulation of the DDR. In fact, it promotes DSB repair by homologous recombination (HR) and regulates activation of the master checkpoint kinases Tel1/ATM and Mec1/ATR. While defects in DSB resection and HR increase the chance of developing cancer, resection-generated ssDNA is unstable and can lead to cancer-inducing mutation clusters. Furthermore, inactivation of resection increases the lifespan of prematurely aged mice, raising the possibility of delaying ageing by modulating resection. Thus, a better understanding of how DSB resection takes place will improve our knowledge of the mechanisms that drive genome instability in cancer and may identify novel therapeutic targets.

In both yeast and mammals, DNA end resection is thought to be a two-step process. The MRX (Mre11-Rad50-Xrs2)/MRN (Mre11-Rad50-Nbs1) complex catalyzes an endonucleolytic cleavage of the 5' strands at both DSB ends, which generates an entry site for the long-range resection nucleases Exo1 and Dna2, the latter working with the helicase Sgs1 (BLM in humans). Sae2 protein (CtIP in mammals) promotes the dsDNA endonucleolytic activity within MRX. This initial MRX-Sae2-mediated nick is particularly important to initiate resection at blocked or modified DNA ends that are not easily accessible to Exo1 and Dna2-Sgs1, such as camptothecin (CPT)-induced DNA lesions.

Cells lacking both Sgs1 and Exo1 are sensitive to camptothecin (CPT), a clinically anticancer drug that causes cell death by increasing the half-life of the Top1-DNA cleavage complex that can interfere with replication. Given the importance of end-resection in DSB repair pathway choice and in preventing oncogenic rearrangements, the understanding of how Sgs1 and Exo1 impact on DSB resection and other processes such as checkpoint activation and DNA

replication. To this aim, my laboratory has recently searched for mutations that reduced the sensitivity to CPT of *sgs1*Δ *exo1*Δ cells. This procedure allowed us to identify 30 single-gene suppressor mutants that fall into 10 distinct allelism groups. Illumina genome sequencing of 5 non-allelic suppressors and subsequent analysis showed that the mutations responsible for the suppression caused single amino acid substitutions in the following proteins: Ddc1, one of the subunits of the 9-1-1 checkpoint complex; Rad24, a protein required to recruit 9-1-1 to damaged DNA; Dpb11 (TopBP1 in humans), a scaffold protein involved in resection and checkpoint; Cdc55, a B-type regulatory subunit of protein phosphatase 2A (PP2A); Tpd3, an A-type regulatory subunit of PP2A. In both yeast and mammals, the 9-1-1 complex recruits Dpb11 to DSBs, which in turn enables Mec1/ATR activation by forming a complex with Rad9/53BP1 and Mec1/ATR. Both Dpb11 and 9-1-1 also interact with Fun30 (SMARCAD1 in humans) and this interaction is required for Fun30 to promote long-range resection by removing Rad9/53BP1 from DSBs. Thus, to understand the suppression mechanism(s), we will first determine whether suppression relies on Dbp11 and/or 9-1-1 functions in checkpoint activation or DSB resection. This will be done by testing whether the *dpb11*, *rad24* and *ddc1* suppressive mutations restore DSB resection in *exo1*Δ *sgs1*Δ mutant cells. Furthermore, separation-of-function Dbp11-ΔN and Dpb11-ΔC variants, which are specifically defective in Rad9 and Mec1 interaction, respectively, as well as Ddc1-T602A and Rad9-ST462,474AA variants, which are specifically defective in Dpb11 interaction, will be assayed for their ability to suppress MMEJ in *exo1*Δ *sgs1*Δ cells. In case resection and/or checkpoint functions of Dpb11 and/or 9-1-1 will turn out to be involved, we will undertake targeted approaches to identify the underlying suppression mechanism. Regarding PP2A, several hypotheses can be envisaged to explain the suppression effect of the identified Cdc55 and Tpd3 mutant variants. However, as Dpb11 acts as phospho-binding scaffold and the simultaneous presence of *cdc55* and *rad24* (or *dpb11*) alleles does not increase further CPT resistance of *sae2*Δ cells, the easier hypothesis is that Cdc55 and Tpd3 mutant variants might increase 9-1-1 and/or Rad9 phosphorylation, thus affecting subsets of Dpb11 interactions. If this will turn out to be the case, the role of PP2A in modulating Dpb11, 9-1-1, Mec1 and Rad9 functions and interactions will be investigated. Finally, we will identify and characterize the remaining 5 suppressive mutations.