Deciphering DNA-protein crosslink repair in vivo using CRISPR/Cas genome editing in a zebrafish model

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DNA-protein crosslinks (DPCs) are very common DNA lesions that interfere with all DNA transactions including replication and transcription. The consequences of impaired DNA-Protein Crosslink Repair (DPCR) are severe. At the cellular level, impaired DPCR leads to the formation of double strand breaks, genomic instability and/or cell death, while at the organismal level, deficiency in DPCR is associated with cancer, aging and neurodegeneration. Induction of DPCs is used in medicine to treat many cancers and understanding the repair at the organismal levels could provide an impetus for the development of new drugs and combination therapies with currently used chemotherapeutics. We use zebrafish (Danio rerio), an established vertebrate model to study cancer, neurodegenerative and cardiovascular diseases, and CRISPR/Cas gene editing to knock-out or mutate genes of interest in order to study the interplay of DPCR factors and sub-pathways including proteolysis-, and tyrosyl-DNA phosphodiesterase-dependent repair at the biochemical and cellular level. I will present our recent discoveries from three new zebrafish strains generated with the CRISPR-Cas system: a catalytic mutant and a C-terminal mutant of the ACRC protease involved in DPCR, as well as a transgenic strain with the inactive DPCR factor, tyrosyl-DNA phosphodiesterase 1 (TDP1). We have found that ACRC is an essential protease in vertebrate development, as a catalytic mutation leads to early embryonic lethality. By injecting ACRC (WT) mRNA constructs into mutant embryos, we were able to grow the transgenic line and perform DPCR analysis. We found that ACRC is a DPCR protease with many cellular substrates and that the SprtT domain is essential for repair, while the intrinsically disordered region is dispensable. We also show that TDP1 is required for the resolution of topoisomerase 1- and histone-DPCs at the organismal level and we further characterise a novel TDP1-mediated repair pathway for histone-DPC repair.