Resolving DPC: analysis of repair pathway

P-02.5-13

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Common type of DNA lesions are DNA-protein crosslinks (DPC), a result of a covalent interaction of proteins involved in DNA maintenance and DNA molecule, due to endogenous and exogenous environmental conditions (previously published in: Stingele J, Jentsch, S (2015) Nat Rev Mol Cell Biol 16, 455–460). Spartan is one of the rare human proteins whose function is involved in resolving DPC (previously published in: Lopez-Mosqueda J et al. (2016) Elife 5:e21491). Mutations in SPRTN gene in humans cause hepatocellular carcinoma, as well as chromosomal breakage and Ruijs-Aalfs type of progeria (previously published in: Lessel D et al. (2014) Nat Genet 11,1239–1244). We hypothesized that Spartan protein regulates the activation of DPC repair pathway and that cells without Spartan function will have altered expression levels of genes related to DNA maintenance and repair. To test our hypothesis, we have compared cells with endogenous Spartan protein function with cells silenced for Sprtn gene and with or without reconstitution of Spartan with exogenous wild type or mutated Spartan. Cells were exposed to different types and intensities of genotoxic stress and the level of gene expression was measured on mRNA level using RT^2 profiler PCR array, as well as RNA sequencing. The results obtained were further confirmed with protein mass spectrometry, pulldown, western blot and flow cytometry analysis. The aim of our study was to determine how Spartan influences the activity of other DNA repair genes expression and to elucidate the mechanism of DPC resolving pathway.

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