

P077 The role of ubiquitination in DNA damage response: proteomic analysis to identify new DNA-damage induced ubiquitinated proteins

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The most well characterised function of ubiquitination is to target proteins for proteasomal degradation. However ubiquitination also regulates other cellular functions, such as DNA damage repair, through processes independent of proteasomal degradation. In the case of DNA-damage, evidence shows that modification of PCNA by ubiquitination enables it to promote post-replicative DNA repair. Similarly, the repair-associated Fanconi anaemia protein, FANCD2, has been identified as a target for mono-ubiquitination, strengthening the evidence that ubiquitination plays a critical role in the DNA-damage response in higher organisms. The aim is to identify new proteins ubiquitinated specifically in a DNA damage-dependant manner. Ubiquitinated proteins are purified from HEK293 cells (untreated and treated with DNA damaging agent) using agarose beads coupled to the Ubiquitin Associated Domain UQ1. The UBA domain binds efficiently to both mono- and polyubiquitin in a linkage independent manner. Purified samples will be characterized by 1D or 2D- gel electrophoresis, to detect ubiquitinated proteins specifically induced following DNA damage and these proteins will be identified by mass spectrometry.