The p97/VCP system is a Swiss multitool knife in the cellular response to ionising radiation

Ignacio Torrecilla[1], JR Hernández-Fernaud[2], Iolanda Vendrell[3], Benedict Kessler[3], Kristijan Ramadan[4]

[1] The MRC Weatherall Institute of Molecular Medicine, Department of Oncology, University of Oxford, UK.

[2] University Hospital of the Canary Islands, Tenerife, Spain.

[3] Target Discovery Institute, Nuffield Department of Medicine, University of Oxford, UK.

[4] Lee Kong Chian School of Medicine, Developmental and Regenerative Medicine Program, Nanyang Technological University, Singapore

p97 (VCP or cdc48) is a key segregase protein in the degradative ubiquitin system and plays an established role in damaged DNA repair. Irradiating cells with ionising radiation (IR) induces a cell-wide degradative response that is critical for the outcome of radiotherapy. To elucidate the roles of p97 in response to IR, we employed an unbiased mass spectrometry (MS) approach in HEK293 cells to identify the p97 interactome with temporal and subcellular resolution. Our study identified 966 proteins that exhibit an altered interaction with p97 following IR exposure.

Mining the MS datasets revealed four key responsive features. First, we observed post-translational modifications of p97 itself in the nucleus. Second, we detected the exchange of p97 adaptor proteins, with only a select few driving the response to IR. Third, we noted changes in the ubiquitin chain topologies that p97 binds to in the nucleus. Finally, the analysis of p97 substrates showed that chromatin exhibits the highest diversity of p97 interactions and associated pathways, with functions distinct from those in the nucleosol and cytoplasm. Processes such as DNA damage repair, DNA replication, DNA transcription, mRNA maturation and various signalling pathways appeared to be regulated by p97 after IR. Additionally, we identified common biochemical and biophysical properties of p97 substrates, enhancing our understanding of p97´s roles.

The generated map of p97 functions provides a comprehensive resource of human p97-interactions, uncovers previously unexploited protein regulation events affected by IR, and offers practical insights for developing novel therapeutic strategies that combine radiotherapy with other agents.