Dissertation Abstract

The E3 ubiquitin ligase UFD-2 interactome linked to DNA damage repair and apoptosis

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Homologues recombination (HR) and non-homologues end joining (NHEJ) are the two main repair pathways for DNA double-strand breaks (DSBs) that are conserved through evolution and need to be strictly regulated. Regulation can be achieved by several post-translational modifications such as protein ubiquitylation. Ubiquitylation is known for supporting recognition, signaling, and repair of DNA DSBs. However, it remained poorly understood how the repair process of DSBs is coordinated with DNA damage-induced apoptosis and how ubiquitylation regulates this coordination. The E3/E4 ubiquitin ligase UFD-2 has been identified as a conserved regulator of DNA HR repair and DNA damage-induced apoptosis in the nematode *Caenorhabditis elegans* and human cells. In the absence of UFD-2, DNA damage-induced apoptosis is reduced, and the removal of the main HR factor RAD-51 is delayed as indication for ongoing repair. Moreover, upon initiation of HR, ubiquitylation hubs at the chromatin are formed for substrate processing by the ubiquitin proteasome system (UPS) factors: UFD-2, the ubiquitin-selective segregase CDC-48 (p97), the deubiquitylation enzyme Ataxin-3 (ATX-3), and the 26S proteasome.

In this study, we identified the UFD-2 interactome in the *C. elegans* germline upon DNA DSBs induction, and revealed novel insights into the functional interactome of UFD-2 with DNA repair factors like PCN-1 and ABL-1. Furthermore, this work discovered F49C12.9 as a new physical and functional UFD-2 binding partner. We found that F49C12.9 harbors ubiquitin like domain and ubiquitin associated domain (UBL-UBA), and it is the homolog of the yeast DSK2 and paralog of the *C. elegans* ubiquilin UBQL-1. The F49C12.9 requires UFD-2 ubiquitylation for its stabilization and interacts with UFD-2 at the chromatin upon inducing DSBs. *f49c12.9(tm7934)* deletion mutant could suppress the high sensitivity of *ufd-2(tm1380)* deletion mutant to ionizing radiation (IR) together with suppressing the delay in RAD-51 foci removal. In addition, we show here that F49C12.9 is expressed strongly in spermatocyte nuclei and accumulate in residual bodies in spermatogenesis. Therefore, we suggest here a functional interaction between UFD-2, F49C12.9 and other reported UPS factors in spermatogenesis.

Altogether, this study suggests that UFD-2 interacts with F49C12.9 and other UPS factors to form ubiquitylation hubs at the chromatin to regulate DSBs repair factors and consequently the DNA damage-induced apoptosis. By discovering the new ubiquilin F49C12.9 and revealing a novel UFD-2/F49C12.9 interaction and its role in DSBs repair, this work contributes to the ongoing global attempts to understand the physiological regulation of DNA repair pathways and how UPS factors might be utilized for potential therapeutic treatments in DNA damage-related diseases.