Abstract

Genome integrity is essential to safeguard the faithful propagation of genetic information that is required for maintenance of all life forms. The DNA damage response (DDR) is an elaborate system that functions to preserve genome integrity. The canonical DDR pathways are involved in inducing cell cycle arrest, and DNA repair or programmed cell death (apoptosis) to counter DNA damage from endogenous and exogenous sources. The tumor suppressor p53 is a key regulator of the DDR, and functions to drive apoptosis in response to irreparable DNA damage by transcriptionally activating pro-apoptotic genes. p53 is also the most commonly gene in majority of human cancers, and most cancer-associated p53 mutations are missense mutations that compromise the tumor suppressive transcriptional activity. As a result, damaged cells fail to undergo apoptosis, leading to uncontrolled proliferation and cancer formation.

In this study, we employ *Caenorhabditis elegans*, to gain a deeper understanding of the mechanisms through which the DNA damage checkpoint signaling pathways impact the apoptotic DNA damage response. The core apoptotic pathway is distinctly conserved in *C. elegans*, where *C. elegans* p53-like, CEP-1, transcriptionally induces the BH3-only domain pro-apoptosis factors to trigger the apoptosome upon DNA damage. This model provides distinct advantages for the genetic investigation of apoptosis –the germline of the worm shows a specific and easy to visualize apoptotic response to DNA damage, enabling quantifiable evaluation of genetic and chemical interventions.

We recapitulated the most prevalent p53 hotspot mutation in the worm, and generated a genetic tool for high throughput screening of novel regulators of *cep- 1*/p53 dependent apoptosis in whole live organism. Ionizing radiation-induced apoptosis is abrogated in worms with the hotspot mutation, and in the forward genetic screen performed, we were able to isolate mutant worms which showed restored apoptosis despite a mutated p53. A mutation in a DNA damage repair gene was found to be involved in suppression of the apoptotic defect in the p53/*cep-1* mutant worms. Our data further indicate that the restored apoptotic response occurs in a delayed manner and involves a dysregulated ERK/MAPK signaling pathway. We also established an in vitro system using human cancer cell lines carrying the p53 hotspot mutation to further investigate the mechanisms underlying these observations in cancer cells. Overall, the findings in this work point towards a secondary wave of DNA damage response that is set in motion if the primary response regulated by *cep-1*/p53 fails when it is mutated.