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MOLECULAR CHARACTERIZATION OF THE CANCER SUSCEPTIBILITY PROTEIN WRAP53β IN CAJAL BODY FORMATION AND DNA REPAIR

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ABSTRACT

WRAP53β is a multifaceted protein involved in several biological processes including Cajal body maintenance, cancer cell survival and DNA damage repair. By directing factors to Cajal bodies and DNA double-strand breaks (DSBs), WRAP53β facilitates site-specific interactions necessary for proper biological responses.

The Cajal body is a subnuclear organelle implicated in cellular processes such as splicing machinery maturation and telomere maintenance. In Paper I, we reveal that WRAP53β is an essential structural component of Cajal bodies. Furthermore, WRAP53β is required for the intracellular targeting of factors to this site. WRAP53β associates with the survival of motor neuron (SMN) complex in the cytoplasm, mediates its nuclear import and subsequent Cajal body accumulation. In addition, we find that the interaction between WRAP53β and SMN is disrupted in the severe neurodegenerative disorder spinal muscular atrophy, suggesting clinical relevance of WRAP53β-mediated SMN transport.

In Paper II, we study the relationship between WRAP53β expression and cancer cell survival. We demonstrate that WRAP53β is overexpressed in a panel of different cancer cell lines in comparison to primary cells. WRAP53β depletion results in massive induction of cancer cell death, whereas normal human fibroblasts are largely insensitive to WRAP53β knockdown. The cell death associated with WRAP53β silencing occurs via the intrinsic mitochondrial pathway as demonstrated by Bax/Bak activation, loss of mitochondrial membrane potential and release of cytochrome c. Finally, we show that high WRAP53β expression levels correlate with poor prognosis and radioresistance of head and neck cancer patients.

In Paper III, we establish WRAP53β as a novel player in the DNA damage response. We show that WRAP53β rapidly and transiently localizes to DNA DSBs in an ATM- and PARP-dependent manner. WRAP53β binds the E3 ligase RNF8 and facilitates its interaction with MDC1, which is essential for the downstream recruitment of repair proteins 53BP1, BRCA1 and RAD51 to damaged sites. Knockdown of WRAP53β results in deficient DNA DSB repair, whereas WRAP53β overexpression enhances repair efficiency and provides resistance to DNA damaging agents. Furthermore, reduced expression of WRAP53β is related to decreased ovarian cancer patient survival.

In summary, our data identify WRAP53β as a novel structural and regulatory component of Cajal bodies as well as an important factor in carcinogenesis and DNA repair.