Abstract

Cells in our body respond to genotoxic stress by activation of a conserved DNA damage response pathway (DDR). Depending on the level DNA damage, DDR signaling promotes temporary cell cycle arrest (checkpoint), permanent growth arrest (senescence) or programmed cell death (apoptosis). Checkpoints prevent progression through the cell cycle and facilitate repair of damaged DNA. DDR represents an intrinsic barrier preventing genome instability to protect cells against cancer development. WIP1 (encoded by *PPM1D*) phosphatase is a major negative regulator of DDR pathway and is essential for checkpoint recovery. This thesis contributed to the understanding of molecular mechanisms of WIP1 function and revealed how WIP1 can be involved in tumorigenesis. Firstly, we described that WIP1 protein levels decline during mitosis by APC-Cdc20 dependent proteasomal degradation. WIP1 is phosphorylated at multiple residues which inhibit its enzymatic activity. We propose that inhibition of WIP1 in mitosis allows sensing of low levels of DNA damage that appear during unperturbed mitosis. Further, we identified novel gain-of-function mutations of *PPM1D* which result in expression of C-terminally truncated WIP1. These truncated WIP1 variants are enzymatically active and exhibit increased protein stability. As result, cells have more of catalytically active WIP1 that impairs the p53-dependent G1 checkpoint. These mutations were identified in cancer cell lines U2OS and HCT116 and also in the peripheral blood of breast and colorectal cancer patients. We suggest that these gain-of-mutations of *PPM1D* could predispose to cancer development. Finally, we validated commercially available inhibitors of WIP1 using cells with a CRISPR/Cas9-mediated knock-out of *PPM1D*. We confirmed the specificity of a small-molecule allosteric modulator GSK2830371 towards WIP1. Specific inhibition of WIP1 significantly reduced the cell proliferation in cancer cell lines which carry amplification of *PPM1D*. WIP1 inhibition did not affect the proliferation of non-transformed cells with low levels of WIP1. Importantly, we showed that inhibition of WIP1 by GSK2830371 sensitizes breast cancer cells with amplified *PPM1D* and wild-type p53 to DNA damage-induced chemotherapy (doxorubicin) and to MDM2 antagonist (Nutlin-3) treatment. In an effort to contribute the knowledge of WIP1 phosphatase we also aimed to determine its crystal structure. However, we have not optimized any crystallization condition for crystal growth. This part is included as unpublished results. In conclusion, the results obtained during the work on this thesis contribute to our knowledge of how the WIP1 negatively regulates DDR. Our results also support WIP1 phosphatase as a potential pharmacological target inhibition of which can sensitize cancer cells to chemotherapy.