Abstract

Cells employ an extensive signalling network to protect their genome integrity, termed DNA damage response (DDR). The DDR can trigger cell cycle checkpoints which prevent cell cycle progression and allow repair of DNA damage. The failures in these safeguarding mechanism are represented by serious human malignancies, most predominantly by cancer development. This work aims to contribute to the understanding of how do the cells negatively regulate DDR and cell cycle checkpoint signalling. We focused mainly on Wip1 (*PPM1D*) phosphatase, which is a major negative regulator of DDR and is indispensable for checkpoint recovery. Firstly, we have shown that Wip1 is degraded during mitosis in APC-Cdc20 dependent manner. Moreover, Wip1 is phosphorylated at multiple residues during mitosis, resulting in inhibition of its enzymatic activity. We suggest that the abrogation of Wip1 activity enables cells to react adequately even to low levels of DNA damage encountered during unperturbed mitosis. In the following publication, we have investigated why the mitotic cells trigger only early events of DDR and do not proceed to the recruitment of DNA repair factors such as 53BP1. We showed that 53BP1 is phosphorylated within its ubiquitination-dependent recruitment domain by CDK1 and Plk1. These phosphorylations prevents 53BP1 to bind ubiquitinated histones, to localize to sites of DNA damage and ultimately hampers DNA repair. In included unpublished results we showed that Wip1 is phosphorylated upon genotoxic stress by MK2 and p38 kinases. The functional relevance of these modifications still remains to be elucidated. In next part, we identified novel gain-of-function mutations of *PPM1D* which result in expression of C-terminally truncated Wip1. The truncated Wip1 retains its catalytic activity, while exhibit increased protein stability. As result, cells have more of catalytically active Wip1, that efficiently shut down the p53-dependent G1 checkpoint. These mutations were identified in cancer cell lines U2OS and HCT116 and importantly also in peripheral blood of breast and colorectal cancer patients. We propose that these mutations could predispose to cancer development. Finally, we showed *in vitro* that inhibition of Wip1 by small molecule drug GSK2830371 specifically sensitizes breast cancer cells with amplified *PPM1D* and wild-type p53 to chemotherapy treatment with DNA damaging drugs and to Mdm2 antagonist such as Nutlin3. In conclusion, the results obtained during the work on this thesis contribute to our knowledge of how the cells negatively regulate DDR. We believe that better understanding to molecular regulation of DDR will eventually lead to better diagnostics and to development of more targeted cancer treatments.