Abstract

DNA damage response (DDR) represents a vital signaling network that protects genome integrity and prevents development of cancer. Therefore the study of DDR is of a crucial clinical importance and DDR proteins are promising therapeutic targets. Although the great advances have been made mapping out interactions between individual DDR proteins, better understanding of complex behavior of this network is still needed. One approach, which might help us in this task, is to describe the dynamics of key proteins under different conditions. The first objective of this study was to investigate whether the temporal dynamics of selected DDR proteins differ upon different genotoxic insults, particularly upon γ-irradiation and UV-C irradiation. We showed that under certain insult some DDR proteins exhibit a monotone continuous activation pulse, while the activation of others triggers a series of pulses. We observed a previously described pulsative dynamics of p53 after γ-irradiation in MCF7 cells. Interestingly, we detected a monotone increase of p53 in U2OS after γ-irradiation and similar dynamics upon UV-C irradiation. We suggest that p53 dynamics depends on the presence or absence of effective negative feedback loops between the upstream p53-activating kinases and Wip1 phosphatase. In the second part of this work, we focused on the dynamics and regulation of Wip1 upon genotoxic stress and during the cell cycle progression. We validated current and developed new tools, including new expression vectors and stable monoclonal cell lines expressing the Wip1 fusion proteins. We showed that Wip1 undergoes phosphorylation after UV-C irradiation. Additionally, we identified a new phosphorylation of Wip1 that occurs exclusively in mitosis, implicating that Wip1 is distinctly regulated during the cell cycle progression. Our findings contribute to the knowledge of DDR behavior and will be followed up in our future studies.