Cells are constantly challenged by DNA damage and protect their genome integrity by activation of an evolutionary conserved DNA damage response pathway (DDR). A central core of DDR is composed of a spatiotemporally ordered net of posttranslational modifications among which protein phosphorylation plays a major role. Activation of checkpoint kinases ATM/ATR and Chk1/2 leads to stabilization of tumor suppressor p53, which results in a temporal arrest of cell cycle progression (checkpoint) and allows time for DNA repair. Following DNA repair, cells re-enter the cell cycle by checkpoint recovery. Wip1 phosphatase (also called PPM1D) dephosphorylates multiple proteins in DDR signaling and is essential for timely termination of the DDR.

Several key proteins of DDR machinery localize directly to the site of DNA breaks and form microscopically detectable nuclear foci. Their number reflects the level of DDR activation in a quantitative manner. The most commonly used markers of these foci are phosphorylated histone H2AX (γH2AX) and mediator protein 53BP1 which is recruited as a result of phosphorylation and ubiquitination-dependent events.

To identify novel chemical compounds that modulate DNA damage response, we set up the high-content microscopy screening of chemical compound library using Operetta system (PerkinElmer). The human osteosarcoma cell line U2OS was chosen as the well-characterized model that has been extensively used for studies of the DDR before. The cells were exposed to 3 Gy or mock-irradiated, seeded on the 396-well plates with chemical compounds, and fixed after 5 hours. Subsequently, the cells were immunostained for γH2AX and 53BP1 and analyzed by high-content fluorescent microscopy. The number, area and intensity of γH2AX and 53BP1 foci were used as readout of DDR activation. At the chosen timepoint (5h), the acute DDR is already in decline. In this setting, we can therefore score not only for compounds causing DNA damage in mock-irradiated cells, but also for the ones that interfere with timely termination of the DDR after irradiation, e.g. by inhibiting the Wip1. The identified hits will be further confirmed by dose response curve generation and studied for the mechanism of their action.

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