Abstract

Maintaining the chromosome continuity and complete genetic information in human cells is crucial for cell survival and the whole organism. It prevents life-threatening pathologies and preserves genetic continuity. However, cellular DNA is exposed to both endogenous and exogenous stress damaging its content and integrity. This stress activates mechanisms involving detection and repair of these damaged sites (DDR). One of the most serious types of DNA damage double-stranded breaks (DSB) occuring when both strands are severed. DSBs trigger wave of PTMs that regulate protein interactions, nuclear localization and catalytic activity of hundreds of proteins. Such modifications include acetylation, methylation, SUMOylation, ubiquitinylation and especially phosphorylation. The most important kinases involved in DDR kinases are ATM, ATR and DNA-PK. These kinases are activated immediately after the detection of the damaged area.

DAXX (Death-associated protein 6) is an adapter and predominantly nuclear protein, which is involved in chromatin remodeling, gene expression modulation, antiviral response and depositing histone H3.3 variants into chromatin or telomeres. Daxx is essential for murine embryogenesis, since the homozygous deletion is lethal in E9.5-10. In 2006 a study mapping the substrates of kinases ATM, ATR and DNA-PK was published. About seven hundred proteins including DAXX were identified as targets of these kinases.

In our work, we studied the impact of DDR on the molecular and functional modulation of DAXX. We discovered, that DAXX is phosphorylated on serine 564 in response to DSB formation induced either chemically (etoposide, neocarzinostatin) or by IR. This phosphorylation is mediated by ATM kinase specifically, but not by ATR or DNA-PK. The key negative regulator of the S564 phosphorylation is WIP1 phosphatase. Consistent with the emerging oncogenic role of this phosphatase, dephosphorylation of S564 was most apparent in cancer cell lines harboring gain-of-function mutation of WIP1 which led to expression of more stable form of the protein. Furthermore, we showed that either the phosphorylation or S564 and DAXX itself have no effect on protein stability of DAXX, as well as the stability and transcriptional activity of p53. Either RNA interference or genetic deletion of DAXX did not influence the expression of transcriptional targets of p53 (GADD45, NOXA, MDM2, p21, PUMA, SESN2, TIGAR and WIP1). Thus, we disputed the long-standing model, according to which DAXX (including the S564 phosphorylation) plays a key role in stabilizing p53. In contrast, we identified a number of genes whose expression is DAXX-dependent and that the mechanism of this regulation is probably of epigenetic character.