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Title: Post-Translational Modulation of PTEN and its Role in genomic instability

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Multiple malignancies have been identified to frequently harbor mutations in the dual phosphatase tumor suppressor protein PTEN. The function of PTEN is not limited to its canonical function ie regulation of PI3K pathways. The genome of PTEN null cells is highly unstable and shows a greater number of aneuploidy. PTEN now emerges as a protein that seems to act as a caretaker of different cellular events like autophagy, senescence, DNA repair, etc. These multiple functions of PTEN are regulated by its post-translational modification or its specific association with other proteins. PTEN's subcellular localization has been reported to link with its PTM. Subcellular localization of PTEN is also associated with its catalytic activities like DNA repair, Activation of PI3K, maintenance of cell cycle, etc. However, how these post-translational alterations impact aneuploidy and cytological damage has not been thoroughly investigated. We concentrate on the relationship between phosphatase activity and PTEN's C terminal phosphorylation in light of cytological damage such as the creation of nuclear bridges, nuclear buds, and micronuclei. Our findings imply that in PTEN-deficient PC3 cells, wild-type PTEN greatly lessens cytological damage but not phospho-mutant PTEN. When PTEN is phosphatase dead, cytological damage markers rise throughout the first 24 hours following DNA damage. Utilization of phosphorylation and phosphatase-dead dual mutant PTEN showed the degree of cytological DNA damage parameters similar to phosphatase-dead PTEN. A failure in the mitotic phase of the cell cycle, persistent DNA damage, or an accumulation of aberrant replication intermediates can result in multinucleated cells and MNi development. Restoration of Wt-PTEN function reduces the generation of multinucleated cells caused by DNA damage, suggesting that PTEN is crucial for preserving genomic stability. We also discovered that both phosphatase activity and PTEN phosphorylation are necessary for preserving chromosome numbers. Significantly abnormal γ-tubulin pole development is seen in PTEN null cells during metaphase. It's interesting to note that PLK1, Aurora kinase A, and p-PTEN are all localized to spindle poles. The expression of phosphorylated Aurora kinase A (T288) and phosphorylated PLK1 (T210) increases in PTEN's phosphorylation and phosphatase mutant in comparison to cells expressing wild-type PTEN. Again, the Wild-type-PTEN can interact physically with Aurora Kinase A and PLK1 but not the phosphorylation dead mutant. As a result, our research reveals that PTEN physically interacts with and dephosphorylates Aurora kinase A and PLK1 in a manner that is dependent on C-terminal phosphorylation. PTEN prevents abnormal chromosomal segregation in metaphase by reducing the hyperphosphorylation state of these proteins.

Again, PTEN/PI3K/AKT pathway creates a vital signaling pathway regulating several biological processes like cell metabolism, cell growth, apoptosis, and cell proliferation. Phosphatidyl inositol-3 kinase, a class I PI3Ks family of lipid membrane constituents that are negatively charged formed by hydroxyl groups phosphorylation by specific kinases on 3, 4, 5 positions of the inositol ring of phosphatidyl inositol (PI). The phosphatase activity of PTEN dephosphorylates PIP3 to PIP2 at the position of D3 of the inositol ring by removing one

phosphate which is attributed to its canonical function. Thus, inhibiting the PI3K/AKT pathway. leads to tumor suppression. This function needs PTEN to localize in the membrane. Therefore, membrane localization of PTEN requires sumoylation, a post-translational modification that occurs at the K254 site of the PTEN protein. Cells containing PTEN mutation at K254R are reported not to respond to DNA damage. So, we investigated the significance of PTEN sumoylation with DNA damage-induced cytological damage, such as the generation of nuclear buds, nuclear bridges, and micronuclei. According to our data, sumo-dead PTEN suffers more cytological damage compared with Wt-type PTEN. The cytological damage indicators are elevated during the 24-hour recovery period following DNA damage in the case of sumo-dead PTEN. Next, we evaluated how well the sumo-dead mutant (K254R-PTEN) prevented aneuploidy and discovered that sumoylation is crucial for preserving chromosomal numbers. We analyzed the γ-tubulin pole generation in cells transfected with the K254R-PTEN clone qualitatively and quantitatively because chromosomal number fluctuation in daughter cells is caused by multiple spindle pole formation. In cells transfected with the K254R-PTEN clone, we discovered a considerably higher rate of aberrant pole formation as compared to the cells transfected with wild-type PTEN. Compared to cells expressing wild-type PTEN, the expression of the phosphorylated form of Aurora kinase A (T288) and phosphorylated form of PLK1 (T210) proteins increases when the sumoylation activity of PTEN is diminished in the presence or in the of absence an agent depolymerizing microtubule, nocodazole.

Thus, different post-translational modifications such as C-terminal phosphorylation along with phosphatase activity and SUMOylation of PTEN are involved in the catalytic activity as well as maintaining genomic stability of cells.

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