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TITLE: "*CONNECTION BETWEEN POST-TRANSLATIONAL MODULATION OF PTEN AND NON-HOMOLOGOUS END JOINING PATHWAY*"

ABSTRACT: The fundamental cause of tumor development is the constitutive activation of oncogenic proteins and mutational inactivation of DNA repair proteins. Thus, maintaining genomic stability is the fundamental cause of preventing cancer predisposition as neoplastic transformation shows genomic aberration. PTEN is a multifunctional tumor suppressor protein that was identified in 1997. It is a phosphatase that negatively regulates phosphatidylinositol 3-kinase signaling pathway. PTEN is made up of several domains, such as the N-terminal phosphatase domain, the core C2 domain, and the C-terminal tail which has several phosphorylation sites. However, PTEN has become known as a DNA repair protein and is crucial for preserving genomic stability throughout the last ten years. The replication progression of PTEN-null cells has been demonstrated to be hampered, but no specific studies have been done on the relationship between the catalytic function of PTEN that is controlled by post-translational regulation and the cellular response to replication stress. We found that p-PTEN accumulates in the nucleus after S phase arrest induced by double thymidine block followed by DNA damage induced by etoposide. Accumulation of p-PTEN indicates the PTEN association with chromatin and is necessary for restoration of arrested replication fork which was established in our previous study. Phosphatase-dead PTEN cannot sense replication stress though it can be associated with chromatin. So, it provokes us to investigate the role of post-translation modulation of PTEN in the recovery of stalled replication. Subsequently, we utilized aphidicolin to generate replication stress and explore the necessary post-translational modification of PTEN during replication stress. If the cell does not sense replication stress, aberrant replication may occur which is one of the factors that contributes to genomic instability. Together, our data suggest that the interruption of DNA replication caused by aphidicolin drives the development of heterochromatin by stabilizing and up-regulating H3K9me3 foci, increases CHK1 activation, and enables PTEN chromatin binding through C-terminal phosphorylation. Thus, PTEN functions as a stress-sensing protein, maintaining genomic stability during replication arrest. Further, p-PTEN promotes heterochromatin formation to arrest replication and allow the repair process initiation.

PTEN can translocate into the nucleus and be associated with chromatin in response to double-strand break. DNA DSB is detrimental as it can cause cancer-driving mutation and chromosomal arrangements unless it is successfully repaired. DSB repair is mediated by either HR or NHEJ which involve several proteins. It has been reported that PTEN is involved in both HR and NHEJ pathways. Mechanistically, it is physically linked to the Rad51 promoter and functions in combination with the transcriptional factor E2F1 to regulate Rad51 synergistically. Though several papers emphasized the role of PTEN in HR but

role of PTEN in NHEJ is yet to be studied. There is one report suggesting that PTEN increases NHEJ frequency by up-regulating XLF protein. Further post-translational modulation of PTEN also regulates its activity and chromatin association. Additionally, we found that PTEN phosphorylation is necessary for both DNA repair and chromatin association. Our in-vivo plasmid-based reporter assay also suggests that PTEN-WT increases NHEJ repair frequency. Further, it has been found that loss for PTEN causes chromatin disorganization and compromised expression of HP1α and H3K9me3 protein. H3K9me3 is an epigenetic marker that is critical to the 53BP1 protein-mediated NHEJ repair process. To develop novel therapeutic approaches for targeting the NHEJ pathway in cancer cells, it is necessary to investigate how PTEN post-translational regulation and its catalytic activity influence chromatin modulation and the NHEJ repair pathway in response to DNA damage. In this study, we investigated the epigenetic regulation of PTEN, which is related to NHEJ. Our study reveals that phosphorylation as well as the phosphatase activity of PTEN is needed for the NHEJ-mediated DSB repair. Here, reduced expression of Ku70/80, DNA-PKcs, XRCC4, and XLF was observed in PTEN-null PC3 cells upon DNA damage. A recent study also indicates that nuclear PTEN may interact with RNA POLII-mediated transcription machinery and thus regulate the expression of many genes including NHEJ factors. PTEN-WT expression can rescue compromised NHEJ unlike phospho and phosphatase-dead PTEN transfected cells. Furthermore, we demonstrated that a DNA-PKcs inhibitor prevents etoposide-induced PTEN C-terminal phosphorylation following DNA damage, suggesting that PTEN may be a target of DNA-PKcs. In addition, we found that PTEN dephosphorylates DNA-PKcs by binding to its C-terminal region. Therefore, after DNA damage, crosstalk between PTEN and DNA-PKcs modulates the NHEJ pathway. PTEN phosphorylation causes its attachment to chromatin, therefore upon DNA damage, PTEN is phosphorylated by DNA-PKcs and attaches to chromatin, resulting in the dephosphorylation of DNA-PKcs and the recruitment of other NHEJ factors on chromatin occurs for efficient execution of the NHEJ pathway. Thus, our research provides a molecular understanding of the epigenetic regulation of PTEN and its significant role in the NHEJ pathway.

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