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Biol 506

Term Paper

**Naturally occurring germline and tumor-associated mutations within the ATP-binding motifs of PTEN lead to oxidative damage of DNA associated with decreased nuclear p53**

Objectives:

PTEN is a phosphatase and tensin homolog which functions in tumor suppression by regulating cell growth, apoptosis, and anchorage-dependent growth. Mutant forms of PTEN have been shown to be involved in both sporadic and hereditary breast cancers. Breast cancer is the second leading cause of death of women in the western world, with an increased risk and earlier age of onset for Cowden Syndrome (CS) patients. Because Cowden Syndrome is caused by mutations in PTEN, the increased risk of breast cancer is likely related to specific mutant forms of the protein. Researchers studied the effects of mutations found in the ATP-binding motif of PTEN, which block nuclear export and result in its localization in the nucleus of the cell. Previous studies have shown that mislocalization of PTEN affects its function. Therefore, in this study researchers analyzed the change in the function of ATP-binding mutants PTENK62R, PTENY65C, and PTENK125E due to mislocalization in the nucleus and whether the novel function contributes to breast carcinogenesis.

Experimental Approach and Results:

In order to determine the effects of increased levels of wild type PTEN and ATP-binding mutants in breast cancer cells, cells from a human breast carcinoma line, MCF-7, were transfected with plasmids containing PTEN wild type and ATP-binding mutants which produced proteins with a FLAG tag on the C-terminal end. Empty vectors were transfected into the control cells. The cells were allowed to culture and overexpression of PTEN was induced. The cells were then probed by immunoblotting. Antibodies identifying FLAG-PTEN were used to determine levels of each type of PTEN protein. It was shown that all PTEN proteins were present at the same level. Levels of molecules regulated by PTEN were then examined. Phospho-AKT (P-AKT) was analyzed due to its function in cell cycle arrest and role in lipid phosphatase activity. Levels of cyclin D1 were also analyzed to determine effects on the cell cycle checkpoint which regulates transition of cells from G1 to S phase. Researchers found that overexpression of wild type PTEN resulted in lowered levels of P-AKT and cyclin D1 compared with control cells, while overexpression of ATP-binding mutants showed no change compared with the control. This suggests that mutations result in impaired phosphatase activity and inappropriate regulation of the cell cycle.

To confirm previous studies which demonstrated a decreased G1/S ratio and apoptotic rate in cells possessing PTEN ATP-binding mutations, researchers used subcellular fractionation of MCF-7 cells overexpressing PTEN in order to determine levels of nuclear phospho-RB and p53, which function in tumor suppression through induction of apoptosis. Cells overexpressing PTEN ATP-binding mutations had decreased levels of p53 and increased levels of phospho-RB compared to those overexpressing the wild type. In order to validate these results, levels of nuclear p53 in MCF-7 cells overexpressing PTEN as well as a control which was transfected with an empty vector were examined using immunofluorescence confocal analyses and immunostaining. This resulted in an 85% increase in p53 immunostaining intensity in the cells possessing wild type PTEN compared with the control. Cells overexpressing ATP-binding mutants had a decrease in p53 immunostaining intensity. When treated with a proteasome inhibitor, however, all cells had a similar level of p53 protein, suggesting a post-translational degradation of the p53 protein in cells containing PTEN ATP-binding mutations.

After concluding the existence of a post-translational degradation of the p53 protein in cells containing PTEN ATP-binding mutations, researchers decided to investigate the mechanism of degradation. Because a main pathway of p53 degradation is ubiquitination induced by MDM2, researchers observed the level of MDM2 and phospho-MDM2 (P-MDM2) in MCF-7 cell lines transfected with each type of PTEN using an immunoblotting technique. They found increased levels of MDM2 and P-MDM2 in cells overexpressing the wild type PTEN, but decreased levels in cells overexpressing the PTEN ATP-binding mutations. Researchers also tested cellular levels of SOD1, a superoxide dismutase, using Western blot. They found increased expression in mutant cells, despite the corresponding elevated levels of ROS.

Due to the predominant nuclear localization of PTEN ATP-binding mutants, researchers tested the induction of double-stranded breaks (DSB’s) in the presence of these mutants. Senile cells (>40 passage cells) from the MCF-7 breast cancer line were treated with histone H2AX. After DBSs, H2AX is phosphorylated on serine 139, resulting in γ-H2AX. γ-H2AX was then stained and detected by immunofluorescence microscopy. Intensity of γ-H2AX in the cells transfected with PTEN WT and the control vector did not change. However, the intensity of γ-H2AXin the ATP-binding mutants was significantly greater than in the control. This suggests that the presence of PTEN ATP-binding mutants induces DSBs in senile cells.

Because stress-induced senescence can result from DSBs caused by reactive oxygen species (ROS), researchers examined basal levels of ROS production and tumor protein 53-induced nuclear protein 1(TP53INP1). ROS levels were determined using cells from the previous test. Cells overexpressing PTENWT had lower levels of ROS than the control; however, significantly increased levels were found in ATP-binding mutants. Researchers then compared mRNA levels of TP53INP1, which mediates p53 antioxidant function, in the same cell lines by using qRT-PCR. They found that mRNA levels of TP53INP1 were lower in PTEN ATP-binding mutants, especially PTENK62R and PTEN Y65C. These results indicate that PTEN mutations increase the oxidative stress on the cell and decrease expression of antioxidant functions.

Conclusion:

The results demonstrate the impairment of tumor-suppression in PTEN ATP-binding mutants. One way that wild type PTEN suppresses the emergence of tumors is through regulation of the cell cycle. PTENWT has a role in cell cycle arrest through its ability to phosphorylate molecules such as AKT. PTEN ATP-binding mutants have decreased phosphatase activity, most likely due to the position of mutation within the phosphatase domain. In addition, ATP-binding mutations caused an inability to regulate cyclin D1, and a corresponding inability to regulate the G1/S cell cycle checkpoint. This inability to manage cell cycle properly results in a diminished function in tumor-suppression, which may promote breast carcinogenesis.

Reduced nuclear levels of the p53 protein, which functions in tumor suppression through induction of apoptosis, were found in cells containing PTEN ATP-binding mutations. However, levels of P-MDM2 and MDM2, which normally initiates p53 degradation, were found in lower concentrations in ATP-binding mutants than in the wild types. This suggests that degradation of p53 in cells containing PTEN ATP-binding mutations is independent of MDM2 and phosphatase activity. Although this was not tested, researchers speculate that the degradation may be due to an inability of ATP-binding mutants to bind to p300. In wild type cells, this complex helps maintain high acetylation of p53, which stabilizes the protein. The inability to stabilize p53 leads to decreased transcriptional activity of genes which induce apoptosis. In addition, PTEN mutants decrease expression of proteins, such as TP53INP1, which mediate antioxidant function. The decreased transcription due to an increase in nuclear PTEN levels from mislocalization represents a lack of the necessary balance of PTEN and p53 for normal cell function. Although expression of SOD1, a superoxide dismutase, is increased in mutant cells, this increase is accompanied by increased levels of ROS, suggesting aberrant expression of SOD1 in mutants. The imbalance of PTEN and p53 in the nucleus as well as the resulting lack of functional apoptosis and antioxidant proteins can promote breast tumorigenesis.

This study also shows that PTEN ATP-binding mutants show increased levels of ROS, leading to oxidative stress, especially in senile cells. This oxidative stress can lead to DNA damage, which has been demonstrated by the large increase in spontaneous DSBs. DSBs may result in irreparable damage to the genetic information, including disruption or loss of genes. As demonstrated by this and other studies, oxidative stress plays an important role in breast tumorigenesis by promoting proliferation of tumor cells and causing DNA damage.

Further Research/Critique:

There are many areas for further research based on the results obtained in this study. When the researcher discovered an increase in the amount of DSBs in PTEN ATP-binding mutants, they stated that it was due to a mechanism beyond disrupting binding to centrosomes. Experiments used to determine the cause of DSBs may eventually lead to a treatment preventing these breaks in patients with high risks of cancer. Also, although the researchers postulated a possible mechanism for degradation of p53 in cells containing PTEN ATP-binding mutations, further research could determine this mechanism. In addition, research in the mechanism by which oxidative stress is increased in cells containing PTEN ATP-binding mutations could lead to a development of a therapy for patients with these mutations, which would also help prevent DNA damage.

This experiment was very extensive. Although the researchers had the goal of analyzing the functional consequences of mislocalization of PTEN ATP-binding mutants, when they found an interesting discovery such as increased degradation of p53 without an increase in MDM2, they continued with that research before finishing those experiments that seemed more pertinent to their purpose. These results, while interesting, were secondary to those of their original purpose yet were discussed more thoroughly than other parts of the paper. In addition, the results section for the oxidative stress test was not described thoroughly, especially the section on SOD1. The purpose and results of this test would have been more easily understood had the authors discussed it in more detail.