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PTEN has a role of radiosensitizer in H1299 cells.

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1. Introduction

PTEN (Phosphatase and Tensin homolog deleted on chromosome Ten) negatively regulates PI3K/Akt signaling, which is one of the most important pathways for cell survival and inhibition of apoptosis. PTEN tumor suppressor gene is dual phosphates with lipid and protein phosphates activities and antagonizes *phosphoinositide 3-kinase* (PI3K) by dephosphorylating phos-phatidylinositol-3, 4, 5-triphosphate (PIP3). The inactivation of PTEN function results in increased Akt activity and development of various cancers including breast, endometrial, prostate, giloblastoma and lung cancer [1, 5]

In this study, we have exploited novel mechanism of *PTEN* that inhibit the *PI3K/Akt* pathway as molecular targets of radiation sensitization for cancer treatment. Our data suggested that combined treatment of *PTEN* and radiation enhanced G_2/M phase accumulation of cell cycle through Akt inactivation and regulation of p21 and activity of CDK1 [2, 3, 4]

2. Methods and Results

1. PTEN enhanced irradiation-induced growth inhibition.

To determine the overexpression of *PTEN*, we performed western blot using PTEN antibody. H1299 cells expressing wild type PTEN significantly decreased the activity of Akt, but the H1299 cells containing pcDNA3 and mutant PTENs (C124S and G129E) did not. We confirmed exogenic PTEN in H1299 had normal function. To investigate whether PTEN had radiosensitization effect in H1299, the growth inhibitory effect of PTEN was analyzed by colony forming assay. As shown in Figure 1, ionizing radiation significantly inhibited the cell growth of wild-type PTEN containing H1299 cells in a dose-dependent manner, but did not inhibit the growth of pcDNA3 or mutant PTEN-containing H1299 cells (C124S and G129E). In particular, wild-type PTEN containing H1299 remarkably suppressed the fraction of surviving clones at 10 Gy. These findings indicated that PTEN enhanced growth inhibition by irradiation in H1299 cells.

2. PTEN induced irradiation-induced G_2/M phase arrest.

To investigate the mechanism responsible for growth regulation by PTEN, we performed propidium iodide (PI) staining by FACS analysis. As shown in Figure 2, radiation induced approximately 70% of G_2/M phase arrest by 10Gy for 24h in the wild type PTEN containing H1299 cells. However, it induced approximately 50% of G_2/M phase accumulation in the H1299 cells containing pcDNA3 and mutant PTEN (C124S and G129E). These results indicated that PTEN enhanced G_2/M phase arrest by radiation.

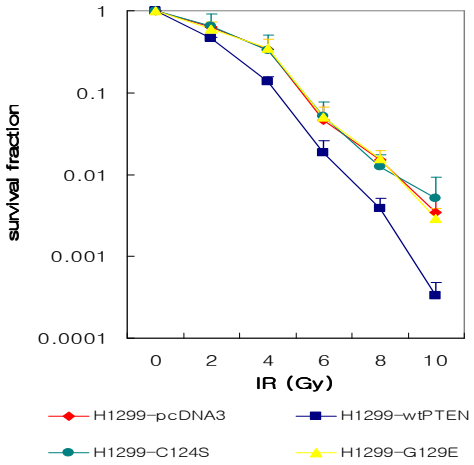

Figure 1. Clonogenic assay for combined effect of PTEN and radiation.
H1299 cells were stably transfected with pcDNA3, wild PTEN, mutant PTEN (C124S), or mutant PTENs (G129E). The H1299 cells expressing wild type and mutant PTEN (C124S and G129E) were seeded and treated with indicated doses of irradiation. The data represent average values of triplicate experiments with standard deviations (SD).

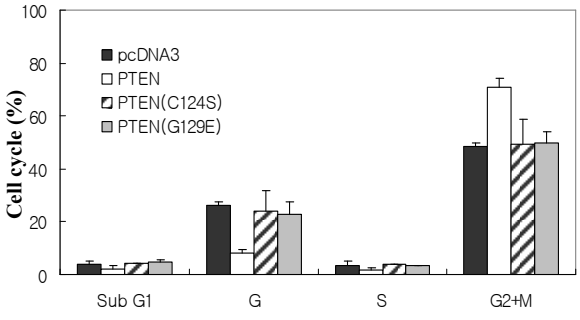

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Figure 2. Modulation of cell cycle distribution.
The H1299 cells containing wild type and mutant PTEN (C124S and G129E) were seeded and treated with 2 mM of thymidine for 17 hours. Cells treated with 10 Gy of radiation for the indicated time and analyzed by PI staining. The change of cell cycle phase at 24 hours was shown in Figure. The data represent average values of triplicate experiments with standard deviations (SD).

3. PTEN increased the level of p21 protein and decreased the activity of CDK1 by radiation

To investigate the mechanism responsible for G_2/M phase arrest by PTEN, we performed Western blot. As shown in Figure 3, we observed the activated Akt regulated G_2/M phase arrest in wild type-PTEN containing H1299 cells. After radiation irradiation, the activation of Akt increased in the H1299 cells expressing pcDNA3 and mutant PTENs (C124S, G129E), but not in the H1299 cells expressing wild PTEN. This indicated radiation-induced phospho-Akt (pAkt) was suppressed by PTEN. Next, we observed activated Akt regulated cell cycle regulatory proteins, specially focused upon the proteins involved in those G_2/M phase transition. We showed the level of cyclin B, CDK1 and p27 protein were not changed by radiation in all H1299 clones. However, p21 protein significantly increased by radiation in the H1299 cells expressing wild type PTEN. Finally, we observed the activity of CDK1, key regulator of G_2/M transition was reduced by radiation in the H1299 cells containing wild type PTEN. As shown in Figure 3B, radiation induced the activity of CDK1 in the H1299 cells expressing pcDNA3 and mutant PTENs (C124S and G129E). However, the H1299 cells expressing wild tpye PTEN had reduced activity of CDK1 by radiation. These fundings suggested the inactivation of Akt by PTEN induced the expression of p21 protein and sequentially the induction of p21 suppressed the activity of CDK1.

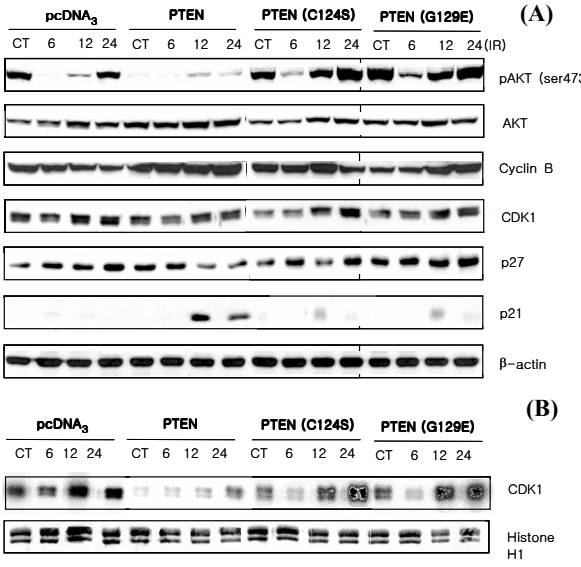

Figure 3 Effect of phosphorylated Akt (pAKT), Akt, and cell cycle regulatory proteins after radiation in PTEN clones.
(A) The H1299 cells containing wild type and mutant PTENs (C124S and G129E) treated with 10Gy radiation for the indicated time and analyzed by western blot using pAkt, Akt, cyclin B, CDK1, p27, p21 and β-actin. (B)Kinase activity assay. H1 kinase from extracts of treated cells was immuno-precipitated with anti-CDK1 antibody/ protein G-agarose and assayed for kinase activity. Activity was visualized by autoradiography of the phosphorylated histone H1 substrate after separation on polyacrylamide gel. Similar results were obtained from at least three independent experiments.

3. Conclusion

Our data indicated that *PTEN* enhanced cell growth inhibition and apoptosis by radiation through the inactivation of AKT signaling and induction of cell cycle arrest in non small lung cancer cell line, H1299. These findings suggested that tumor suppressor *PTEN* might play an important role as a radiosensitizer and might be used for overcoming radioresistance. These results also showed a possibility of combined therapy of gene therapy and radiotherapy for lung cancer treatment having a genetic disorder.

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