Does PTEN Loss Impair DNA DSB Repair By Homologous Recombination?

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The tumor suppressor PTEN is frequently lost in cancer cells resulting in altered radiation and drug sensitivity. However, the role of PTEN in DNA repair is controversial. Detailed studies in prostate cancer cells now indicate PTEN does not regulate RAD51 expression or homologous recombination and is not a biomarker for PARPi sensitivity.
In this issue of *Clinical Cancer Research*, Fraser and colleagues (1) report that prostate cancer cells lacking PTEN (phosphatase and tensin homolog deleted from chromosome 10) also named as MMAC1 (mutated in multiple advanced cancers) or TEP-1 (2, 3) show no loss of RAD51 expression or defect in DNA DSB repair by homologous recombination (HR), and conclude, therefore, that PTEN status cannot be used as biomarker for HR status or PARP inhibitor (PARPi) response in clinical trails of patients with prostate cancer. The authors arrive at this conclusion after evaluating multiple cell lines (prostate, lung, colon and osteosarcoma) and primary prostate tumors for Rad51 expression and the integrity of HR function in relation to PTEN status.

PTEN dephosphorylates PIP-3 (phosphatidylinositol-3,4,5-triphosphate) to PIP-2 (phosphatidylinositol-4,5-triphosphate) and in its absence PIP-3 levels increase, resulting in sustained PI-3/AKT cascade signaling AKT kinase activity and phosphorylation of AKT substrates, one of which is the cell cycle checkpoint kinase Chk1 (Fig. 1). This implicates PTEN in the DNA damage response (DDR) since Chk1 has a critical role in maintaining genomic stability by delaying S and G2 phase progression in cells containing DNA damage to allow time for repair before exit to mitosis. Sustained activation of AKT in PTEN deficient cells results in Chk1 serine 280 phosphorylation which triggers ubiquitination and cytoplasmic sequestration, thus reducing Chk1 levels in the nucleus for phosphorylation/activation by nuclear ATM/ATR (4). Activated Chk1 phosphorylates cdc25A resulting in its degradation and cell cycle arrest (Fig. 1). The DSBs that arise when Chk1 is inhibited are apparently related to a specific S phase role whereby
Chk1 suppresses aberrant initiation of DNA replication that would generate DNA lesions (5). Thus, the mechanism by which PTEN loss results in genomic instability involves abrogation of critical cell cycle checkpoints. This assumption is consistent with the PTEN-null cellular phenotypes for IR response in that abrogation of the cell cycle checkpoint allows mitotic arrest bypass resulting in resistant to IR-induced cell killing.

Loss of PTEN results in PI3/AKT cascade activation, and tumor cells with constitutive AKT activation are reportedly resistant to chemo- and radiation-therapy, a result attributed to AKT activating DNA-PK by phosphorylation (6), suppressing apoptosis and enhancing DNA repair. DNA-PK has a critical role in DNA DSB repair by non-homologous end joining (NHEJ) which occurs mainly in the cell cycle G1-phase. The high frequency of chromosomal aberrations seen in PTEN null cells, however, is more likely the consequences of impaired S, G2 and mitotic-phase check points or defective HR (7, 8).

An early report linking PTEN inactivation to reduced RAD51 expression came from Shen and coworkers (9) but these results could not be confirmed by using the same cell lines and reagents (8) to support the role of PTEN in HR. However, Gupta and coworkers found that caffeine treatment specifically increased IR-induced chromosome aberrations and mitotic index only in cells with PTEN, and not in PTEN deficient cells, suggesting the role of PTEN in checkpoints. Furthermore, PTEN-deficient cells were unable to maintain active spindle checkpoint after taxol treatment (8). Subsequent studies reporting a PTEN role in HR were indirect without describing a mechanism by which PTEN
could regulate RAD51 expression or HR (10). Another recent report suggested, PTEN involvement in HR, but this report did not confirm that RAD51 protein levels in PTEN-null astrocytes were different from PTEN levels in astrocytes with wild-type PTEN (11). These investigators also reported that PTEN deficient cells were hypersensitive to MNNG, camptothecin and PARPi, but resistant to cell killing by IR exposure (11). It is unclear how cells defective in HR could be radioresistant? The surrogate marker for HR is RAD51 foci formation post irradiation as a result of multiple stochastic nucleation events along a DNA molecule. Loss of RAD51 function should manifest as an elevated mutation rate and increased cancer risk as well as increased sensitivity to IR-induced cell killing. Pappas and coworkers reported that the repair of IR-induced DSBs was retarded in H1299 cells expressing PTEN as compared to H1299 lacking PTEN consistent with cells deficient in PTEN having better survival and further suggesting that PTEN has a minimum role in HR DNA DSB repair (12). Thus the studies described above raise concerns about the conceptual basis used to design specific therapies for tumors with dysfunctional PTEN.

Fraser and coworkers (1) have now made direct comparisons between PTEN and RAD51 expression in primary prostate tumors and failed to demonstrate that PTEN deficient cells have reduced levels of Rad51. Furthermore, they found that PTEN deficient cells are not sensitive to camptothecin or PARPi nor defective in HR mediated DSB repair. These results are important, as it is clear that PTEN status cannot therefore be used as a biomarker for defective HR repair or PARPi sensitivity.
Why are there so many discrepancies in the reported role of PTEN? One possible explanation is that cancer cells that have developed within the context of a PTEN-null genotype may function differently than cells in which PTEN has been removed experimentally, possibly due to accumulated secondary genetic aberrations. Recent studies have suggested that PTEN deletion is a fairly late event during prostate cancer development, suggesting that additional genomic alterations occur in these tumors in a PTEN-independent manner before PTEN loss. However, when PTEN is disrupted under normal non-cancerous situations, such as in transgenic mice, an alternative role of PTEN in HR could be highlighted and the defect would be measurable. Indeed, it has not been shown whether the silencing PTEN actually reduces the expression of RAD51 in normal cells. Frazer and coworkers have additionally demonstrated that even in early stage of cancer cells, E2F1 expression, which is a critical transcription factor in RAD51 activation, could be high enough before PTEN loss since Rb-pathway is likely compromised already at the early stage of tumorigenesis. If this is the case, Rad51 expression is high regardless of PTEN status in advanced stage of cancer, refuting that PTEN interacts with E2F1 for transcriptional activation of RAD51.

The majority of cancer patients will receive IR as part of their therapy. Ionizing radiation dependent killing of tumor cells primarily results from the production of DNA DSBs, however, both tumor and normal cells have highly efficient DNA repair systems that must be overcome before cell death occurs. Strategies are being developed to make the tumor cells sensitive to IR-induced cell death. Tailoring radiotherapy requires an understanding of the pathways
implicated in IR-induced DNA damage repair. A major experimental focus has been to identify targets/mutations unique to tumor cells and not found in normal tissue, that render the cells more sensitive to combinatorial therapeutic approaches like synthetic lethality. While the consistent loss of PTEN in many tumors may represent a potentially important target, evolving studies indicate that PTEN, RAD51, HR, and PARPi are not connected within a simple mechanistic line and their relationship could be quite cell type specific. Our search for a universal biomarker for PARPi sensitivity, therefore, continues since RAD51 is now demonstrated not to be a biomarker for prostate cancer response during clinical trials.

**Figure Legend**

**Fig. 1:** Summary of current relationships between PTEN, AKT, and DNA repair pathways. PTEN loss leads to cellular PIP3 accumulation resulting in constitutive activation of AKT kinase. AKT phosphorylates Ser280 on Chk1 and inhibits CDC25-dependent activation of CDC2/cyclin B, in addition to the direct regulation on G1/S checkpoint (see text). Note that PTEB, RAD51, HR and PARPi are not connected within a simple mechanistic line and their relationship could be quite cell type specific.
Disclosure of Potential Conflict of Interest

No conflict of interest.

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