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Does PTEN Loss Impair DNA Double-Strand Break 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 PARP inhibitor sensitivity. *Clin Cancer Res; 18(4); 920–2. ©2011 AACR.*

In this issue of *Clinical Cancer Research*, Fraser and colleagues (1) report that prostate cancer cells lacking PTEN, also referred to as mutated in multiple advanced cancers (MMAC1) or TEP-1 (2, 3), show no loss of RAD51 expression or defect in DNA double-strand break (DSB) repair by homologous recombination and conclude, therefore, that PTEN status cannot be used as a biomarker for homologous recombination status or PARP inhibitor response in clinical trials 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 homologous recombination function in relation to PTEN status.

PTEN dephosphorylates phosphatidylinositol-3,4,5-triphosphate (PIP-3) to phosphatidylinositol-4,5-triphosphate (PIP-2), and in its absence, PIP-3 levels increase, resulting in sustained phosphoinositide 3 (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 process implicates PTEN in the DNA damage response because Chk1 has a critical role in maintaining genomic stability by delaying S- and G_2-phase progression of 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 and/or 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 ionizing radiation (IR) response in that abrogation of the cell-cycle checkpoint allows mitotic arrest bypass, resulting in resistance 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 radiotherapy, a result attributed to AKT activating DNA–protein kinase (PK) by phosphorylation (6), suppressing apoptosis and enhancing DNA repair. DNA-PK has a critical role in DNA DSB repair by nonhomologous end joining (NHEJ), which occurs mainly in the cell-cycle G_1 phase. The high frequency of chromosomal aberrations seen in PTEN-null cells, however, is more likely the consequence of impaired S-, G_2-, and mitotic-phase checkpoints, or defective homologous recombination (7, 8).

An early report came from Shen and colleagues (9) linked PTEN inactivation to reduced RAD51 expression; however, these results could not be confirmed even by using the same cell lines and reagents (8). However, Gupta and colleagues 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 a role for PTEN in checkpoints. Furthermore, PTEN-deficient cells were unable to maintain an active spindle checkpoint after paclitaxel treatment (8). Subsequent studies reporting a PTEN role in homologous recombination were indirect without describing a mechanism by which PTEN could regulate RAD51 expression or homologous recombination (10). Another recent report suggested PTEN involvement in homologous recombination, 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 PARP inhibitors, but resistant to cell killing by IR exposure (11). It is unclear how cells defective in homologous recombination could be radioresistant. The surrogate

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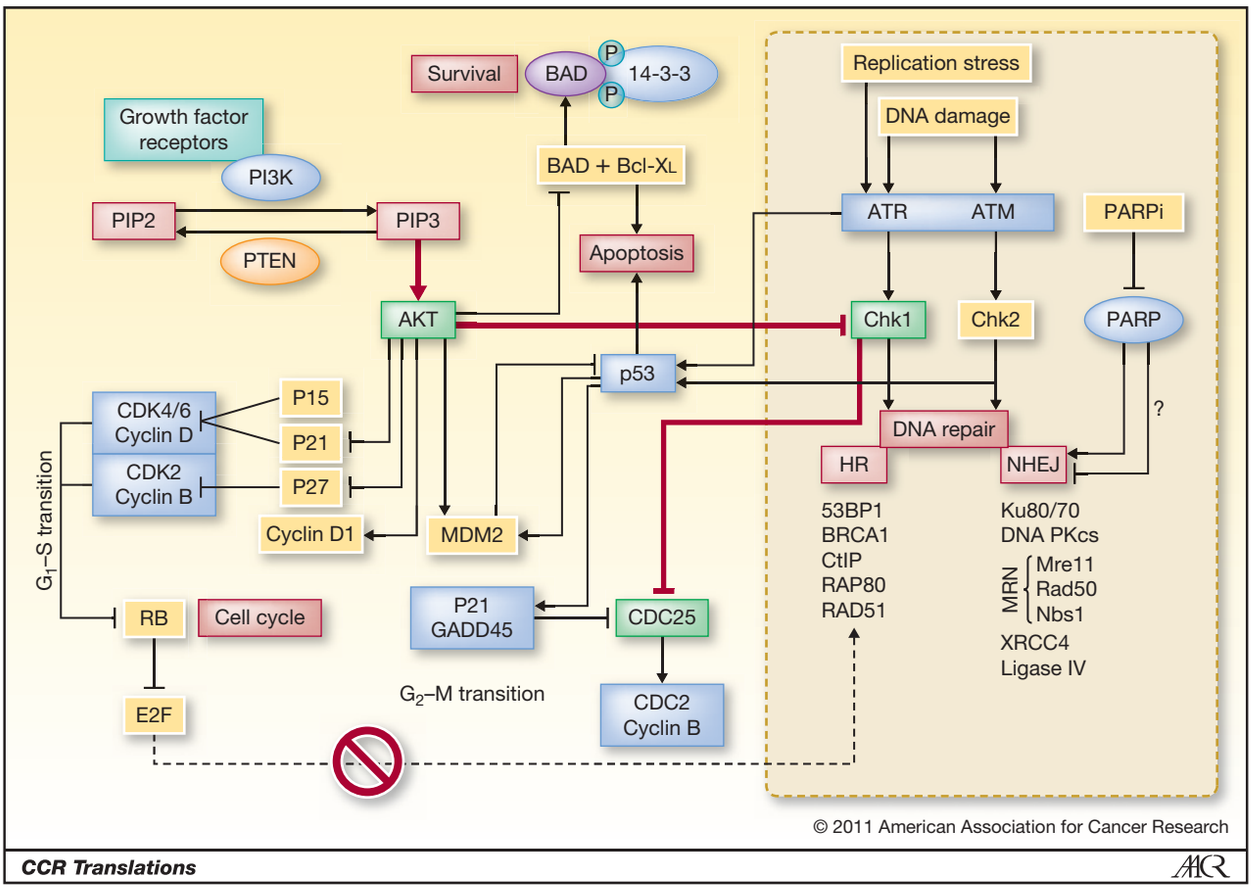

Figure 1. Summary of current relationships among PTEN, AKT, and DNA repair pathways. PTEN loss leads to cellular PIP-3 accumulation, resulting in constitutive activation of AKT kinase. AKT phosphorylates serine-280 on Chk1 and inhibits CDC25-dependent activation of CDC2/cyclin B, in addition to the direct regulation on G_1–S checkpoint (see text). Note that PTEN, RAD51, homologous recombination (HR), and PARP inhibitors are not connected within a simple mechanistic line and their relationship could be quite cell-type specific. NHEJ, nonhomologous end-joining.

marker for homologous recombination is RAD51 foci formation postirradiation 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 colleagues reported that the repair of IR-induced DSBs was retarded in H1299 cells expressing PTEN as compared with H1299 lacking PTEN, consistent with cells deficient in PTEN having better survival and further suggesting that PTEN has a minimum role in homologous recombination 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 colleagues (1) have now made direct comparisons between PTEN and RAD51 expression in primary prostate tumors and failed to show that PTEN-deficient cells have reduced levels of RAD51. Furthermore, they found that PTEN-deficient cells are not sensitive to camptothecin or PARP inhibitors, nor defective in homologous recombination-mediated DSB repair. These results are important, as it is clear that PTEN status cannot, therefore, be used as a biomarker for defective homologous recombination repair or PARP inhibitor 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 noncancerous situations, such as in transgenic mice, an alternative role of PTEN in homologous recombination could be highlighted and the defect would be measurable. Indeed, it has not been shown whether the silencing of PTEN actually reduces the expression of RAD51 in normal cells. Fraser and colleagues have additionally shown that even in early-stage cancer cells, E2F1 expression, which is a critical transcription factor in RAD51 activation, could be high before PTEN loss because Rb-pathway is likely

compromised already at an early stage of tumorigenesis. If this is the case, RAD51 expression is high regardless of PTEN status in advanced-stage cancers, refuting that PTEN interacts with E2F1 for transcriptional activation of RAD51 promoter.

The majority of cancer patients will receive IR as part of their therapy. IR-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 and/or mutations unique to tumor cells and not found in normal tissue, which render the cells more sensitive to combinatorial therapeutic approaches like synthetic lethality. Although the consistent loss of PTEN in many tumors may represent a potentially important target, evolving studies indicate that PTEN, RAD51, homologous recombination, and PARP inhibitors are not connected within a simple mechanistic line and their relationship could be quite cell-type specific. Our search for a universal biomarker for PARP inhibitor sensitivity, therefore, continues because RAD51 is now shown not to be a biomarker for prostate cancer response during clinical trials.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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