A Novel Mechanism to Induce BRCAness in Cancer Cells

Changmeng Cai

Cancer cells with germline deleterious mutations of BRCA1 or BRCA2 are deficient in homologous recombination repair and therefore sensitive to PARP inhibitor treatment. However, wild-type BRCA1/2-expressing cells with defects in other DNA damage repair pathway components may also exhibit "BRCAness," which in combination with PARP inhibition can similarly induce synthetic lethality. In this issue of *Cancer Research*, Luo and colleagues report a novel mechanism by which BRCA1 protein degradation in response to DNA double-strand breaks is regulated by prolyl isomerase Pin1. Inactivation of Pin1 can establish BRCAness in cancer cells and thus sensitize cells to PARP inhibitor treatment.

See related articles by Luo et al., p. 3033

Breast cancer has been classified as three major molecular subtypes: luminal estrogen receptor (ER) and progesterone receptor (PR) positive, HER2-enriched, and triple-negative basal-like cancer (TNBC), the latter being characterized by the absence of expression for ER, PR, and HER2 (1). Unlike the first two breast cancer subtypes that can be treated with hormonal therapies or HER2 inhibitors, it is extremely challenging to treat TNBC with targeted therapies. Although only a small subset of breast cancers contain deleterious germline mutations in BRCA1/2, the majority of tumors with BRCA1 mutations are TNBC (2). BRCA1/2 play critical roles in DNA damage repair (DDR) by mediating stalled replication fork repair or homologous recombination repair (HRR) in response to replication fork stalling or DNA double-strand breaks (DSB; ref. 3). Therefore, BRCA mutation–associated breast cancers are more sensitive to DNA-damaging treatments such as PARP inhibitors. PARP is a key enzyme in the base excision repair pathway. It binds to sites with DNA single-strand breaks (SSB) and catalyzes the addition of poly (ADP-ribose) chains (PARylation) on histones and other DNA repair proteins (3). PARP inhibitor treatment induces SSBs that are converted into DSBs at the DNA replication fork and induces cytotoxicity through PARP trapping. Therefore, the synthetic lethality between the deficiency in the HRR pathway and PARP inhibition promotes death of cancer cells. However, non-BRCA–mutant tumors may also exhibit "BRCAness" through defects in other DDR-related proteins or loss of BRCA1/2 expression (2). Therefore, identifying a strategy to induce BRCAness is urgently needed to develop a combination treatment with PARP inhibitors.

In this issue, Luo and colleagues reveal a novel mechanism by which inactivation of prolyl isomerase Pin1 promotes the degradation of BRCA1 recruited to DNA damage foci inducing BRCAness in non-BRCA–mutant breast cancer cells (4). Proline-directed serine/threonine (Ser/Thr) phosphorylation is a common modification of a number of signaling pathways, such as MAPKs and cyclin-dependent kinase (CDK; ref. 5). Pin1 is a member of the peptidyl-prolyl *cis-trans* isomerases (PPIase) superfamily and is the only PPIase that mediates isomerization of the phosphorylated Ser/Thr-Pro motif, which results in a structural transformation of the targeted protein and thus may affect its expression and function including subcellular localization, protein stability, and protein–protein interactions (6). Transcriptionally, Pin1 expression is regulated by E2Fs as well as other pathways, and Pin1 also undergoes multiple posttranslational modifications (5). Pin1 deficiency causes neurodegeneration, which is linked to Parkinson disease, Alzheimer disease, and Huntington disease (6). In cancers, Pin1 is often aberrantly regulated or overexpressed and it upregulates many oncogenes and downregulates tumor suppressors. For example, Pin1 enhances p53-induced senescence and apoptosis, promotes CDK-induced phosphorylation, and inhibits PP2A-mediated dephosphorylation of Rb (5). Pin1 has also been implicated in DDR (2), but its role in DSB-induced HRR is not clear.

In this study, Luo and colleagues observed that Pin1 localized to DDR foci in response to ionizing radiation in breast cancer cell lines and that silencing Pin1 decreased the binding of DDR proteins such as BRCA1 and 53BP1. Furthermore, Pin1 decreased HRR activity in mouse embryonic stem (ES) cells with wild-type (wt) BRCA1 but not in ES cells with BRCA1 deficiency, suggesting that Pin1 may potentially affect HRR by regulating BRCA1 activity. Screening of the interaction domains established that the 1005–1313 amino acid fragment of BRCA1 was responsible for binding with Pin1. This site contains four Ser/Thr-Pro motifs, which are S1125, T1149, S1189, and S1191; the latter two were previously reported as the substrates of CDK1 during DDR (7). Mutagenesis analysis confirmed phosphorylated S1191/P1192 as the substrate of Pin1, although whether Pin1 can directly isomerize P1192 was not determined. Nonetheless, the authors sought to determine the role of Pin1 in regulating BRCA1 activity. They found that in response to ionizing radiation, Pin1 stabilized wtBRCA1 but not the S1191A mutant, which has a much shorter half-life due to a greater level of ubiquitination. Interestingly, the S1191A mutant cannot be efficiently recruited to DDR foci, suggesting another possible function of this Ser/Pro site in regulating BRCA1 activity in addition to protein degradation, although this was not further pursued. Moreover, the authors identified that ubiquitination at lysine 1037 was responsible for degrading BRCA1 and could be blocked by Pin1-mediated isomerization at P1192.

With these novel molecular insights, Luo and colleagues hypothesized that Pin1 inhibition may sensitize non-BRCA–mutant breast cancer cells to PARP inhibition. In addition to a number of successful *in vitro* studies, they utilized a breast cancer cell line–derived xenograft model to assess Pin1 depletion in combination with olaparib, an FDA-approved PARP inhibitor used as monotherapy to treat metastatic breast cancer with BRCA1/2 mutations (2). The results showed that Pin1 depletion greatly enhanced the tumor-suppressive effect of

Center for Personalized Cancer Therapy, University of Massachusetts Boston, Boston, Massachusetts.

Corresponding Author: Changmeng Cai, University of Massachusetts Boston, 100 Morrissey Blvd, ISC/4/4720, Boston, MA 02215. Phone: 617-287-3537; Fax: 617-287-6650; E-mail: changmeng.cai@umb.edu

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olaparib. Moreover, the authors also assessed a recently reported Pin1 inhibitor, all trans retinoic acid (ATRA; ref. 8), in combination with olaparib in BRCA1-proficient TNBC patient-derived xenograft (PDX) models (DDR pathway mutations were not found in these PDXs). The results were very consistent with the effect of Pin1 depletion in the cell line–derived xenograft model and clearly showed that ATRA greatly increased the efficacy of olaparib in these PDXs. Because ATRA is an FDA-approved treatment to treat acute promyelocytic leukemia, this preclinical study may be quickly translated into clinical trials combining PARP and ATRA inhibitors in patients with non-BRCA–mutant breast cancer. However, ATRA being a potent agonist of the retinoic acid receptor may have broader molecular and cellular effects other than inhibiting Pin1. Another covalent Pin1-specific inhibitor, KPT-6566, was shown to inhibit tumor growth and metastasis in preclinical breast cancer models, however, it has not been tested clinically. Nonetheless, more potent and specific Pin1 inhibitors with better therapeutic potential are clearly needed for the future development of combination therapies with PARP inhibitors.

Interestingly, another recent study also reported that Pin1 may regulate BRCA1 through enhancing BRCA1–BARD1 activity for stalled fork repair (9). BRCA1 functioned to protect the replication fork independent of BRCA1–PALB2 interaction and this function required the region of BARD1 containing the RAD51-binding site but did not require the E3 ligase activity of the BRCA1–BARD1 complex. Moreover, BRCA1 Ser114 was phosphorylated by CDK1 or CDK2 to promote fork protection and the phosphorylated-S114/P115 became a substrate of Pin1. Pin1-mediated isomerization at this site facilitated the binding of RAD51 to the stalled replication fork, promoting genomic stability. Importantly, cells expressing the cancer-associated BRCA1 mutations close to the S114 site were generally insensitive to cisplatin or olaparib, further supporting that this specific regulation of BRCA1 is not related to its function in the HRR pathway. Overall, this work revealed a distinct mechanism by which Pin1 promotes stalled fork repair through isomerization of the BRCA1 S114/P115 site independent of its activity on the repair of DSBs, which is mediated by the S1191/P1192 site.

In conclusion, the study by Luo and colleagues provides some novel molecular insights on how BRCA1 expression and activity during DNA DSB repair is regulated by Pin1-mediated isomerization at the phosphorylated S1191/P1192 site. This unique mechanism provides a rationale to induce BRCAness in non-BRCA mutation–associated breast cancer by inactivating Pin1, which sensitizes the cells to PARP inhibitor treatment. In addition to breast and ovarian cancers, HRR gene mutations or alterations are also frequently detected in prostate cancer and this frequency is further increased in metastatic castration-resistant prostate cancer (mCRPC; over 20% are primarily BRCA1/2 and ATM mutations; ref. 10). PARP inhibitor treatments are currently being tested in clinical trials of mCRPC with HRR pathway defects (olaparib, phase III, NCT02987543; rucaparib, phase III, NCT02975934; and niraparib, phase II, NCT02854436). Because Pin1 is overexpressed in prostate cancer, it is plausible that this combination strategy of PARP inhibitors with Pin1 inactivation can also be applied to prostate cancer treatment.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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