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Efficacy of PARP inhibition combined with EZH2 inhibition depends on BRCA mutation status and microenvironment in breast cancer

Mei-Kuang Chen

Department of Molecular and Cellular Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030, USA

Abstract

The efficacy of the combination of a PARP inhibitor (PARPi) and an EZH2 inhibitor has been investigated in breast cancer cells with either *BRCA1* mutation or *BRCA2* mutation. However, earlier studies focused on the efficacy of this combination against BRCA-mutated but not BRCA-proficient breast cancer. Yang *et al.* observed that PARP1 depletion combined with EZH2 depletion via PRC2 depletion did not affect the growth of *BRCA1/2* wild-type breast cancer cells *in vitro*. Moreover, Yang *et al.* reported that this combination stimulated synthetic viability of *BRCA1/2*-proficient breast cancer cells *in vivo* by regulating the tumor microenvironment to induce angiogenesis and differentiation of M2-type macrophages. The findings of Yang *et al.* provided evidence that both *in vitro* and animal models should be employed in the studies of PARPi combination therapies in order to involve the alteration of the tumor microenvironment in these investigations. These studies of PARP inhibition combined with EZH2 inhibition in breast cancer showed that this combination may benefit breast cancer patients carrying *BRCA1*-mutated tumor, but the combination may also enhance recurrence of *BRCA2*-mutated tumor and may even promote BRCA-proficient cancer cell survival. Therefore, *BRCA1* mutation status should be used to select breast cancer patients for PARPi and EZH2 inhibitor combination treatment in clinical trials in the future.

Keywords

PRC2; macrophages; PARP inhibitor

Introduction

DNA repair deficiency and replication stress are both cellular stresses that contribute to genomic instability in breast cancer [1]. Poly(ADP-ribose) polymerase 1 (PARP1) plays a key role in regulating mechanisms to resolve DNA damage and replication fork stalling by consuming nicotinamide adenine dinucleotide (NAD+) to ADP(ribosyl)ate multiple proteins [2, 3]. Small-molecule PARP inhibitors (PARPis) are designed to compete with NAD+ for

Correspondence: Mei-Kuang Chen, Department of Molecular and Cellular Oncology, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd., Unit 1051, Houston, TX 77030, USA; phone: +1-832-750-6641; mchen6@mdanderson.org.

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PARP1 and thus inhibit PARP1 enzymatic activity and trap PARP1 on DNA, inducing DNA damage and replication fork stalling as a result [3]. In addition to PARP1-mediated pathways, BRCA1/2 proteins also facilitate DNA repair and restoration of replication forks [4, 5]. Therefore, targeting PARP1 using PARPis can induce synthetic lethality in cancer cells with BRCA deficiencies [2]. Indeed, PARPis have been shown to prolong progression-free survival of breast cancer patients carrying germline deleterious *BRCA1/2* mutations [6]. As the first strategy to successfully demonstrate synthetic lethality against BRCA-mutated breast cancer in the clinic [2], PARPis in cancer treatment have been extensively investigated to determine their effect on DNA damage repair and replication fork stalling [6]. However, the biological functions of PARP1 and BRCA1/2 proteins are not limited to DNA repair and gene transcription [7, 8]. For example, PARP1 also regulates NF-κB–mediated inflammation, mitosis, and cellular energetics, as it interacts with a variety of target proteins [8, 9]. In an investigation of the PARPi and EZH2 inhibitor combination, a recent study published in *The FEBS Journal* provides new insights showing that the interaction between tumor cells and the tumor microenvironment should be taken into consideration in the development of combinational therapies for targeting DNA damage repair pathways in cancer.

Therapeutic benefit of combination of PARPi and EZH2 inhibitor may be limited to *BRCA1*-mutated patients

Intrinsic and acquired PARPi resistance have been reported in germline BRCA-mutated cancer patients [10]; therefore, several combination therapies have been proposed to overcome PARPi resistance in breast cancer. Among the strategies to overcome PARPi resistance, the combination of a PARPi and an enhancer of zeste homolog 2 (EZH2) inhibitor showed synergism in BRCA-mutated breast cancer cell lines [11, 12]. EZH2 is a histone methyltransferase in polycomb repressive complex 2 (PRC2) [13]. PARP1-mediated poly-ADP(ribosyl)ation of EZH2 leads to dissociation of EZH2 from PRC2 in response to DNA damage in BRCA-mutated breast cancer cells [11, 12]. EZH2 and PARP1 also are promising targets because they were found to be co-overexpressed in about half of breast cancer patients [14], and high expression of PRC2 and PARP1 is associated with poor prognosis [15–17]. Yamaguchi *et al.* demonstrated that a DNA-damaging alkylating agent, methyl methanesulfonate, induced proteasome-mediated degradation of EZH2, whereas this EZH2 degradation could be reversed by combining methyl methanesulfonate with a PARPi [11]. Yamaguchi *et al.* further showed that the combination of a PARPi (olaparib) and an EZH2 inhibitor (GSK343) inhibited colony formation and tumor growth more than olaparib single-agent treatment did in *BRCA1*-mutated SUM149 breast cancer cells and a SUM149 orthotopic xenograft animal model [11]. However, in a *BRCA2*-depleted mouse breast cancer model, Rondinelli *et al.* demonstrated that the mice treated with the combination of a PARPi (olaparib) and an EZH2 inhibitor (GSK126) experienced early recurrence, and the tumor inhibition effect of the combination was similar to that of PARPi single-agent treatment [18]. Rondinelli *et al.* also showed that EZH2 played a critical role at the site of replication fork stalling in *BRCA2*-deficient tumor. These results suggest that the efficacy of the combination of an EZH2 inhibitor and a PARPi in breast cancer treatment is affected by the BRCA mutation status of the tumor.

While the studies published by Yamaguchi *et al.* and Rondinelli *et al.* focused on the effect of a PARPi combined with EZH2 inhibition on BRCA-mutated cancer cells [11, 18], Yang *et al.* reported that the tumor-promoting effect of PARP1 depletion combined with PRC2 depletion in BRCA-proficient breast cancer resulted from NF-κB signaling–induced angiogenesis and macrophage differentiation [19]. The PARP1 and PRC2 double-depleted cell was generated by knock-out of *PARP1* and knock-down of PRC2 protein expression by small hairpin RNA targeting of either EZH2 or SUZ12 [19]. Interestingly, the function of PARP1 in DNA damage repair was not the key determining factor in the BRCA-proficient tumor growth promoted by PARP1 and PRC2 double depletion. Yang *et al.* further demonstrated that the PARP1 and PRC2 double-depleted tumor cells not only proliferated faster than single- depleted counterparts in intra-tumoral regions, but also activated NF-κB signaling that secreted cytokines and chemokines to induce angiogenesis and increase differentiation into tumor-promoting M2 macrophages [19]. On the basis of these publications, the PARPi and EZH2 inhibitor combination may only benefit patients bearing *BRCA1*-mutated tumor (Figure 1). Furthermore, as demonstrated by Yang *et al.* together with the previous work, the involvement of the tumor microenvironment revealed an inconsistency in overall cancer cell growth inhibition between *in vitro* experiments and animal models. Further *in vivo* investigation should be pursued to determine the effect of *BRCA1/2* mutation status on the tumor response to PARPi and EZH2 inhibitor combination therapy.

Conclusion

The combination of a PARPi and an EZH2 inhibitor has been evaluated *in vitro* and *in vivo* in breast cancer and ovarian cancer cells with varying BRCA mutation status [11, 14, 19, 20] and has been proposed for clinical trial investigation (ClinicalTrials.gov identifier NCT04355858). However, the effect of this combination on the tumor microenvironment has not been thoroughly investigated, and it is not clear whether the synthetic viability of PARPi and EZH2 inhibitor is limited to BRCA-proficient breast cancer patients. Moreover, secondary BRCA mutation has been observed in patients with acquired PARPi resistance [21], and the response in patients bearing BRCA-reverted cancer may not be similar to that in patients carrying wild-type BRCA tumor. It is also noteworthy that EZH2 has a non-catalytic function in cancer cells [22], and whether EZH2 inhibitors can fully suppress its biological functions to the same degree as EZH2 depletion is not well studied yet. In conclusion, the effects of a PARPi and an EZH2 inhibitor on the tumor microenvironment and the recurrence of tumors with varying BRCA mutation status should be further investigated to identify which patient population will benefit from the combination.

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Abbreviations:

PARP1	poly(ADP-ribose) polymerase 1

PARPi	PARP inhibitor
EZH2	enhancer of zeste homolog 2
PRC2	polycomb repressive complex 2

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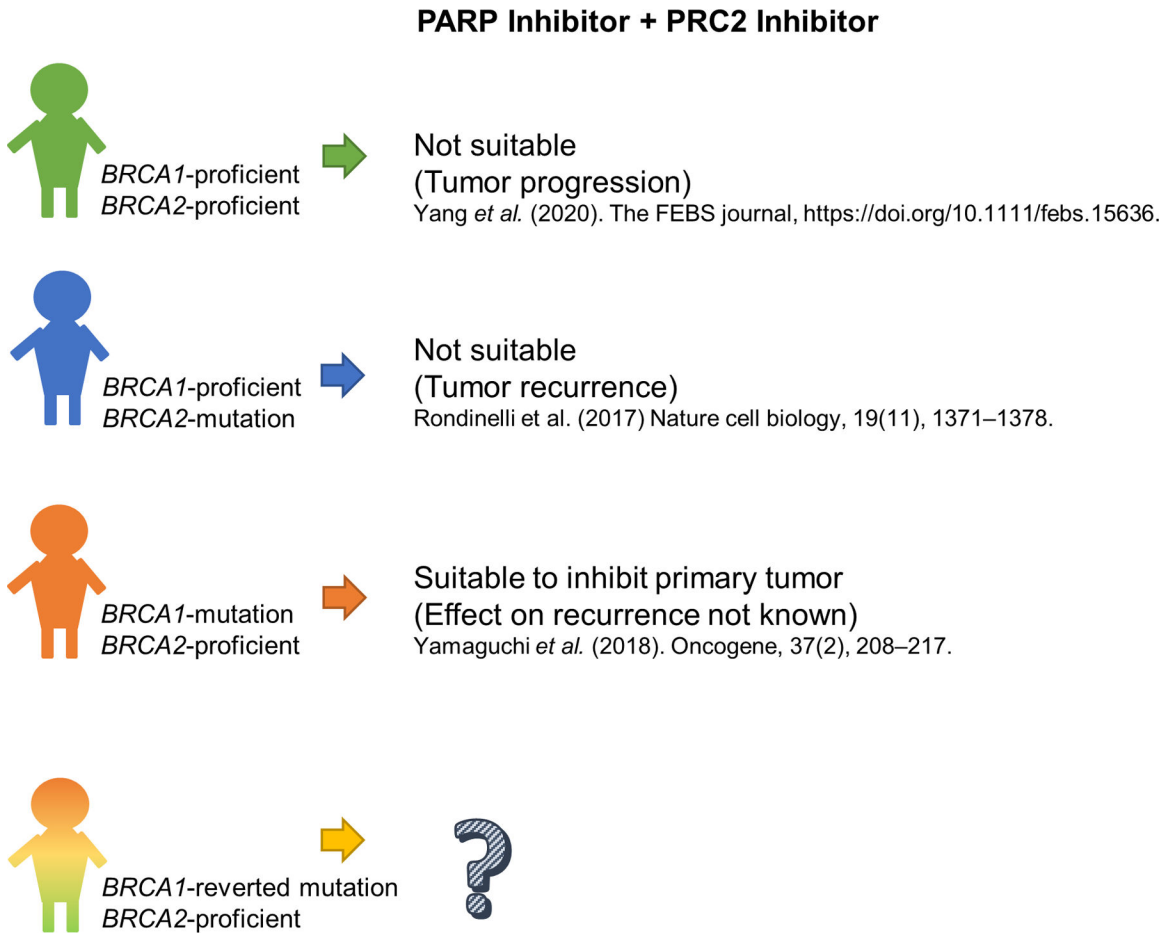

Figure 1. Efficacy of PARP inhibitor combined with EZH2 inhibitor may depend on *BRCA1/2* mutation status in breast cancer patients.

Using breast cancer cell lines in xenograft mouse models, studies showed that the tumor response to the PARP inhibitor and EZH2 inhibitor combination varies between models with differing *BRCA1* and *BRCA2* mutation status.