

ORIGINAL RESEARCH ARTICLE

Combining PD-1/PD-L1 inhibitor and PARP inhibitor: a new perspective on the treatment of triple negative breast cancer

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ABSTRACT

Compared with other types of breast cancer, triple negative breast cancer has a poor survival prognosis due to its high aggressiveness and lack of effective therapeutic targets. Immune checkpoint (PD-1/PD-L1 and CTL-4) inhibitors have emerged as a breakthrough therapy in the treatment in various metastatic cancers. PARP inhibitors promote DNA damage in tumor cells, not only promoting immune initiation through a series of molecular mechanisms, but also leading to adaptive upregulation of programmed death ligand 1 (PD-L1) expression. Therefore, the combination of the two inhibitors can improve the efficacy of tumor treatment. We reviewed the research progress of their combined use in triple negative breast cancer, and put forward relevant ideas for further development, hoping to find the best treatment mode of the combined use of the two.

Keywords: Immunotherapy; PARP Inhibitors; Combined Therapies; Triple Negative Breast Cancer

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1. Introduction

Triple negative breast cancer (TNBC) is that estrogen receptor (ER) progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER-2) are all breast cancers with negative expression. It accounts for about 15% of all breast cancer, and it is characterized by high aggression, easy metastatic recurrence, and poor prognosis. At present, according to the gene expression and biological characteristics of breast cancer, it is divided into four categories: ER +/Luminal group, normal breast group, HER2 + group, basal-like group. About 85% of basal-like group breast cancers belong to TNBC, and some of them express at least one of ER, PR and Her-2. However, there are some differences in gene expression profile and immunophenotype between TNBC and basal-like group breast cancers, so they cannot be completely identical. TNBC lacks endocrine therapy and corresponding treatment for HER2 target, so the treatment methods are limited. Although TNBC is relatively sensitive to chemotherapy, it is prone to recurrence and metastasis in the short term. Therefore, breast cancer researchers have been actively exploring new therapeutic approaches for TNBC, such as anti-tumor angiogenesis, EGFR inhibitors, multitarget drugs, PARP inhibitors and comprehensive utilization of various methods. According to the current preliminary research results, it is shown that the combination of immune detection point PD-1/PD-L1 inhibitor and PARPis can improve the therapeutic effect of TNBC. This paper reviews the role of PD-1/PD-L1 and PARP, the basic re-

search of their combination and the related research contents in TNBC.

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2. Programmed death protein-1/programmed death ligand-1(PD-1/PD-L1)

Tumor cells can use immune checkpoints to escape the attack of immune cells. At present, studies have confirmed that the immune checkpoint is cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), programmed cell death protein-1 (PD-1) and programmed death ligand-1 (PD-L1). By designing

and synthesizing these immune checkpoint inhibitors, the activity of immune checkpoint can be inhibited and reactivate the immune response effect of T cells on tumor, so as to achieve the anti-tumor effect. However, the roles of the two immune checkpoints are different: CTLA-4 mainly plays a role in the activation stage of T cells induced by antigen-presenting cells in lymph nodes, while PD-1 plays a role in the effector stage of T cells at tumor sites. Therefore, the antitumor activity of PD-1/PD-L1 antibody may be better than CTLA-4 antibody.

PD-1, a member of the immunoglobulin B7 family, was first identified in apoptotic T cell lymphomas, a transmembrane protein on the T cell surface and an important inhibitory receptor. It was named the programmed death receptor-1 (PD-1) for its promotion to programmed cell death. PD-1 is mainly expressed in activated T/B lymphocytes, NK cells, monocytes, mesenchymal stem cells (BMSCs) and dendritic cells (DCs)^[1], which plays an important role in immune cell differentiation and apoptosis and is an important immunosuppressive molecule for maintaining autoimmune tolerance^[2].

Currently, PD-1 has identified two ligands: PD-L1 and PD-L2. They all belong to the B7 family and are similar^[3]. The affinity between PD-L2 and PD-1 is about 3 times higher than PD-L1, but PD-L1 is the most dominant ligand of PD-1, which is highly expressed in multiple tumor tissues and is closely related to the pathological type, pathological stage, survival prognosis, etc^[4].

The immunosuppressive effect of PD-1/PD-L1 signaling pathway plays an important role in maintaining body health and internal environment stability. Its immunosuppressive activity has a great impact on the occurrence and development of malignant tumors. The combination of PD-1 and PD-L1 can inhibit the proliferation of CD4⁺ T lymphocytes and CD8⁺ T lymphocytes, so it weakens the tumor killing effect of T lymphocytes, leads to immune escape of tumor cells, and promotes the rapid growth of tumors^[5]. Therefore, blocking the combination of PD-1 and PD-L1 can restore the immune killing effect of T lymphocytes in tumor patients and enhance the efficacy of killing tumors, and finally block the development process of ma-

lignant tumors. Many studies have confirmed that tumor immunotherapy by blocking PD-1 and PD-L1 is very effective in the treatment of a variety of malignant tumors.

3. Poly ADP-ribose polymerase (PARP)

PARP is a kind of poly ADP ribose polymerase, which plays an important role in DNA damage repair and cell apoptosis. It is activated by identifying DNA fragments with structural damage, participates in base excision repair (BER), and is used to repair single-strand breaks (SSBs) closely related ribozymes^[6]. So far, more than 18 members of PARP family have been found, of which PARP-1 and PARP-2 are the earliest and most mature members and PARP-1 is involved in the PARP activities of most enzymes^[6,7]. PARP-1 includes three domains: (1) N-terminal DNA binding domain (DBD), which is composed of two zinc finger structures and a nuclear localization signal region. Zn I and Zn II recognize damaged DNA, and Zn III participates in the connection between domains and activates proteins; (2) intermediate autoregulation domain (AD), which is rich in glutamate residues and a BRCA1 carboxyl terminal, as well as Caspase-3 digestion function; (3) The C-terminal catalytic domain is the main binding region of adenine dinucleotide. PARP catalyzes the decomposition of nicotinamide adenine dinucleotide (NAD⁺) into nicotinamide and ADP ribose through its own glycosylation, and then takes ADP ribose as substrate to “PAR” the receptor protein and PARP1 itself to form PARP-ADP ribose branched chain^[8].

By activating NF- κ B, PARP-1 upregulates of pro-inflammatory cytokines such as tumor necrosis factor α (TNF α) and IL-6, and their inhibitors down regulate these cytokines and reduce the occurrence of local inflammation^[9]. The balance of pro-inflammatory and anti-inflammatory responses was maintained by PARP-1 and its inhibition^[10]. In monocytes, PARP-1 controls the development of dendritic cell (DC). PARP inhibitors suppress CD86, CD8, IL-12, and IL-10 expression^[11]. CD86 expression can be restored by exogenous IL-12. As a result, depending on the expression of cytokines, PARPis may have a role in the maturation and

function of DC^[10,11]. Activated T nuclear factor (NFAT), which is required for T cell activity, is regulated by PARP-1. Therefore, increased trans-activation is caused by PARPis that NFAT depends on and the delay of NFAT nuclear export^[12]. PARPis can shield CD8⁺ T lymphocytes from oxygen radicals produced by phagocytes, and it can also protect CD8⁺ lymphocytes from apoptosis caused by oxygen radicals^[13]. Therefore, PARPis may be used in conjunction with immunotherapy ionizing radiation treatment of tumors with significantly invasive CTL.

BRCA1/2 is a tumor suppressor gene. The loss of BRCA1 and BRCA2 expression causes homologous recombination repair damage, followed by genomic instability, and increases frequency of DNA mutations. Its genetic mutant phenotype induces the risk of breast and ovarian cancer, and improves sensitivity to DNA damage drugs. BRCA1/2 mutant cells are defective in DNA damage repair. Cells cannot repair duplex damaged DNA by homologous recombination when cellular PARP activity is inhibited, and repair the damaged DNA by another error-prone repair pathway, resulting in chromosomal group instability, cell cycle inhibition and apoptosis.

The close relationship between PARP and the development of multiple malignant tumors, which, as a new target of tumor treatment, has become a hot target in recent years.

4. A combination of PD-1/PD-L1 inhibitors and PARP inhibitors to treat TNBC

PARPis and immune checkpoint inhibitors cooperate with and enhance the tumor antigen-specific activation of the CD8⁺ T lymphocyte-mediated antitumor immune response. PARPis promotes tumor-infiltrating lymphocytes (TILs) by upregulating chemokines and induces a CTL-mediated immune response. PARPis induces the upregulation of PDL-1, suppresses CTL, and promotes the escape of immune tumors. Anti-CTLA-4 immunotherapy stimulates T cells and works in tandem with PARP inhibitors to trigger an anti-tumor immune response. Anti-PDL-1/PD-1 immunother-

apy restores the PARPis-induced increase of PDL-1-mediated CTL inhibition. Therefore, anti-PDL-1/Pd-1 antibodies can work together with PARPis to stimulate an anti-tumor immune response^[14].

Lymphocytes inoculated in a mouse model identified in the BR5FVB1-Akt cell line “BRCA-1 deficient ovarian cancer”, PARPis elicited a local immune response^[15]. Intraperitoneal cytotoxic CD8⁺ T cells and NK cells increased, producing greater IFN γ and α . Furthermore, the proportion of MDSCs (bone marrow-derived suppressor cells) dropped. MDSCs aid tumor growth, particularly by inhibiting T cells^[16]. Therefore, PARPis boosts antitumor immunity by boosting TIL (like NK cells and CTL) and lowering MDSC levels. The overexpression of CCL2 and CCL5 in this model can explain the rise in TILs^[16], because CCL2 can deliver TIL in ovarian disease patients^[17]. PARPis’ capacity to influence the makeup and function of TILs is being used in conjunction with immunotherapy to maximize response. Several mouse models reported the advantages of PARPis and immunotherapy^[15,18,19]. The emergence of immunotherapy and PARPis in the treatment of some cancers has prompted research on their combination. The majority of malignancies discovered by these combinations are tumors that lack DNA repair activity, such as BRCA defective tumor cells. PARPis^[20] and immune checkpoint inhibitors like anti-PD-1 work effectively on these cancers^[21]. Immune checkpoint inhibiting agents might be used to increase TAA expression in tumors that have been mutated, promoting a particular immune response^[22]. High mutation load is related to improving survival rate where anti-PD-1 therapy was used to treat melanoma patients, even as tumors from responding sufferers are rich because of mutations in DNA restore function, which include BRCA-2^[23]. These findings reinforce the rationale for combining immunodetection point inhibitors with PARP inhibitors in malignancies with DNA repair defects.

Recently, Jiao *et al.* have observed the combination of PARPis (olaparib) and anti-PD-1/PD-L1 in the treatment of TNBC by *in vivo* and *in vitro* experiments. The expression of PARP protein is opposite to that of PD-L1 in human breast cancer. In the syngeneic mouse model inoculated with

TNBC cell system, PARPis up-regulated PD-L1 expression on the surface of EMT6 tumor cells. This was accomplished via generating a reduction in TILs and facilitating the deactivation of the GSK3 β mechanism, so PARPis plays an immunosuppressive role by reducing the level of TILs. Anti-PD-L1 reversed the inhibitory effect on TILs, and combined with PARPis, enhanced expression of TILs with antitumor responses over PARPi and anti-PD-L1 alone. These data further support the study of PARPis combined with anti-PD-L1/PD-1 immunotherapy^[19]. *In vivo*, inhibition of PARP has been demonstrated to trigger PD-L1 upregulation, which may be the result of the described interferon expression. However, *in vitro* and xenotransplantation are also viable options^[24]. The latter result implies that PD-L1 regulation downstream of PARP is parallel to the internal mechanism of cells, but independent of external signals. The adaptable and inherent overexpression of PD-L1 may stamp down PARP inhibitor-mediated downstream immune response and may be overcome by binding of PARP inhibitors to PD-1/PD-L1 inhibitors.

Because the response rate of TNBC against PDL-1 or anti-PD-1 treatment is still unsatisfactory, only 10%–20% of TNBC patients respond partially. Glycosylation PD-L1 is the operative part of PD-L1 which is of necessity to the interaction between PD-L1 and PD-1. TNBC cells had much greater levels of glycosylated PD-L1 than non-TNBC cells. Bin *et al.* discovered that 2-deoxyglucose (2-DG) may be utilized as an analog of glucose to diminish PD-L1 glycosylation during the selecting of glucose to prevent PD-L1 glycosylation. Because PARP inhibition increases PD-L1 expression, 2-DG lowers glycosylated PD-L1 expression mediated by PARP inhibition. PARP inhibition in combination with 2-DG provides a powerful anticancer effect^[25]. Therefore, the results provide a strong theoretical basis for the combination of PD-1/PDL-1 inhibitors and PARPis in the treatment of TNBC.

Till now, only three PARP inhibitor/anti-PD-1/L1 combos have been studied: olaparib/durvalumab^[26,27], niraparib/pd-1 inhibitor^[28,29], and BGB-A 317/BGB-290^[30]. The premier combination of the two was well-tolerated, and its toxicity was consistent with that of the related drugs observed in

single drug environment. The latter, on the other hand, had a higher risk of hepatotoxicity, showing the tolerance of the combining of PARPis and PD-1/PD-L1 inhibition agents may vary depending on the drug used and/or the exact environment. Niraparib/pbrobroli-zumab combined treatment for advanced three negative breast cancer (100 cases) also showed initial activity. The ORR was 28% and 60% in patients with BRCA 1/2 mutations, again consistent with PARP inhibitor monotherapy^[31]. These preliminary breast and ovarian findings back up the theory for further exploration of joint application. Because compared with PARPis with single treatment, combined application may bring benefits to a wider population without DDR defects, or have longterm benefits for all patients; the latter is likely because the benefits of ICB are mainly considered to improve survival^[32].

5. Conclusion

At present, the preliminary research shows that the composition between PD-1, PD-L1 inhibitor and PARP inhibitor has better clinical efficacy in the treatment of TNBC, and provides the basic research basis for the combination, which allows us to see a little breakthrough in the treatment of TNBC, brings a glimmer of confidence to clinical workers and a glimmer of dawn to patients, but there are still several key questions to be further answered: (1) Compared with monotherapy, what are the advantages of comprehensive treatment? Are potential disadvantages beneficial to long-term survival? (2) What is the best dose or time arrangement for combination therapy? (3) Is it possible to improve the curative effect by combining other treatment methods, such as chemotherapy, radiotherapy, anti-vascular growth, ATR inhibitors, etc? Can the patient tolerate it? How to optimize the treatment plan? These need our vast number of breast cancer researchers to actively explore and research, to find effective treatment for TNBC patients, so that patients can achieve long-term survival purposes.

Conflict of interest

No conflict of interest was reported by the au-

thor.

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