

Introduction of Three Different Orientations of Nanoparticle-Conjugated Therapeutic Strategies in a Case of Triple-Negative Breast Cancer

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Abstract. Triple-negative breast cancer (TNBC) is a highly aggressive subtype of breast cancer with negative expression of estrogen receptors, progesterone receptors, and human epidermal growth factor receptor 2 (HER2). Albeit chemotherapy is the main treatment for TNBC patients, the efficacy is still limited, so there is pressing to search more effective treatments. Based on the expression of specific molecules and signaling pathways in TNBC, a variety of targeted treatment strategy has emerged. In the initial targeted therapy, the administration of monoclonal antibodies has (Mabs) problems such as poor efficacy, and the combination with chemotherapy drugs has problems such as short cycle half-life, and the emergence of nanotechnology in biomedicine provides new probabilities to address these constrains. Therefore, so far, a variety of nanoparticles (NPs)-conjugated therapy strategies have emerged. In this review, it is attempted to follow the development of nanotechnology in targeted therapy by addressing some limitations accordingly, with the introduction of three NPs-conjugated therapy strategies as examples. At the same time, it also aims to provide a reference for the subsequent development of nanotechnology combined targeted therapy.

Keywords: TNBC, Mabs, NPs-conjugates, targeted therapeutic, photothermal therapy.

1. Introduction

Breast cancer (BC) is the most common cancer type in women, and it has become the second leading cause of cancer mortality in women, moreover the incidence of a disease is increasing annually [1]. Triple-negative breast cancer (TNBC) has become a special subtype of BC because of its strong attack force, rapid division and proliferation, named for its lack of progesterone receptor (PgR), estrogen receptor (ER), and human epidermal growth factor receptor 2 (HER-2) [2]. Although TNBC is more sensitive to conventional chemotherapy than other subtypes, its therapeutic effect is not ideal and the prognosis is the worst due to the heterogeneity of the tumor and the rapid development of drug resistance [3]. Therefore, the urgent need for new treatment strategies for patients with TNBC.

Previous research has shown that targeted therapy is the treatment of 60-70% had a response to chemotherapy in patients with TNBC the most appropriate strategy [4]. In recent years, multiple regulatory agencies have approved the use of monoclonal antibodies (Mabs) against TNBC, particularly cetuximab (CET), an FDA-approved human/mouse chimeric Mab against epidermal growth factor receptor (EGFR) has been developed [5]. But because most Mabs have a survival rate of around 20%, they should be used in combination with chemotherapy drugs in clinical practice [5]. In addition, NPs have been used in clinical therapy as drug delivery systems, and its advantages such as improving targeting by combining drugs that is NPs-conjugated cancer drug, or targeting molecules as known as NPs-conjugated Mabs to improve cancer therapy, reduce cytotoxicity and enhance patient compliance have been successively demonstrated [5]. Moreover, non-targeted NPs, which is NPs that do not conjugate with any drugs or Mabs, circulating in blood vessels have been proven through enhanced permeability and retention (EPR) effect, greatly improve drug effectiveness in vivo and tumor site accumulation [5]. Because of the above advantages, the antibody engineering of nanomaterials and the nanomedicine of drug delivery have been greatly developed in cancer treatment, including the research on TNBC.

EGFR is affiliated as the ErbB family of receptor, can be ligands such as epidermal growth factor (EGF) activation. About 45% - 80% of the patients with TNBC EGFR expression. EGFR is therefore considered to be a potential key clinical therapeutic target [6]. To date, different NPs combined with Mabs, or combined with chemotherapy drugs, using different methods to treat TNBC research continues to emerge, and some of them have achieved strong therapeutic effect, with strong clinical transformation value and attracted wide attention. Therefore, based on the development of innovative therapeutic TNBC strategies, this review introduces some interesting new strategies in different directions of NPs-conjugated targeted therapy research in recent years.

2. Half-Chain Mabs-Conjugated NPs

Using therapeutic antibodies as anticancer the concept of the "panacea" is the earliest by Paul Ehrlich put forward in the last century [7]. After that, Mab development has continued to progress, with more than 70 chimeric, humanized, and whole-human monoclonal antibodies approved for use in 2017. In the past few decades, the application of monoclonal antibody provides great benefits for the treatment of certain cancers. However, several limitations, such as low tissue penetration, short cycle half-life, and the onset of drug resistance, have limited the routine treatment of unconjugated cetuximab (CTX) as same as other Mabs for the treatment of hypostatic tumors [3]. Therefore, based on CTX chemotherapy in early clinical trials failed to show significant clinical benefit for TNBC [8].

The cross combination of nanotechnology and bioscience brings great application prospects [9]. Mabs combined with NPs or incorporated into suitable nanocarriers have shown good antibody integrity, also certified tissue permeability, and heighten persistence in the blood circulation. Moreover, some reports have confirmed that the monoclonal coupling in the activity of colloid NPs effectiveness of targeted ability.

In some cases, some antibody fragments such as single chain variable fragment, nano-body and half-chain antibody demonstrated to be ponderable options for nanocoupled targeting applications in place of whole Mabs [3]. At the same time, the great potential of monoclonal antibody therapy has been reconsidered due to the advantages of antibody-conjugated NPs in treating highly invasive and refractory tumors [10]. Therefore, half-chain Mab-conjugated NPs as a substitute for therapeutic monoclonal antibodies in TNBC has been extensively studied.

TNBC chemotherapy patients typically retain adverse clinical outcomes, the most important cause is the development of chemotherapy resistance, this is inherent in TNBC genome instability of the direct consequences [3]. The activation of EGFR to adjust its downstream signaling pathways, including the mitogen-activated protein kinase cascade (originally called extracellular signal-regulated kinase, ERK) or MAPK/ERK and phosphatidylinositol-3 kinase (PI3K)/Akt pathways, to influence DNA repair, replication, and cancer gene transcription, induce migration of signals from the cell membrane to the nucleus, Increases proliferation and mediates chemical resistance [3]. Therefore, such receptor has been conceived for TNBC burgeoning therapeutic targets [3].

In a study of TNBC cell antibody therapy using half-chain cetuximab (HC-CTX) coupled with colloidal NPs, compared with full monoclonal conjugated colloid NPs, half-chain monoclonal conjugated colloid NPs can improve the chemical and physical properties of nanoconjugated compounds, especially in the case of stability of colloid in biological medium, but also improve the cycle half-life and targeting efficiency [3]. In the MDA-MB-468 and MDA-MB-231 cell lines, two TNBC-representative cell lines, HC-CTX-NP showed more durable anti-tumor efficacy than therapeutic CTX, possibly due to alterations in receptor cycling processes normally allowed by EGF affinity ligand and anti-EGFR antibodies [3]. At the same time, HC-CTX nanocoupling enhances the molecular mechanism of monoclonal antibodies promoting TNBC cell apoptosis by interfering with PI3K/Akt and RAS/MAPK expression signaling pathways [3]. In addition, it was found that HC-CTX-NPs significantly affected the cell cycle of HCC1937 cells and inhibited their proliferation by phosphorylating P38, blocking the rapid circulation of the receptor and preventing nuclear

translocation mechanism to inhibit TNBC resistance to CTX, which was different from uncoupled CTX [3].

Therefore, based on the above findings, there is strong evidence that HC-CTX-NPs can be used as a promising nanomedicine for a large number of TNBC-responding patients. It also suggests the potency of half-chain Mabs-conjugated NPs in TNBC therapy, which may provide a new prototype for therapeutic antibody delivery. In the future, this kind of nanomedicine may be a good research direction because of its minimal trace drug can improve the efficiency of treatment and reduce sensitive and resistant tumors [3].

3. Drug-Mabs-Conjugated Nanocomposites

As is well-known, over the years, chemotherapy has been one of the most common cancer treatments. However, poor intracellular uptake, limited circulatory stability, normal cell damage, and low targeting reduce the ability of chemotherapeutic agents, so it needs to improve or find new treatments. NPs improve treatment by binding drugs or biomolecules, providing an opportunity to improve the efficacy of chemotherapy treatments. Previous studies have also confirmed that NPs used as cancer drug delivery carriers can effectively inhibit tumor growth in mice, and has the advantages of prolonging tissue retention time and reducing toxicity. Due to the wide application of NPs in the biomedical field, the potential toxicity of NPs is also being addressed. Therefore, some non-toxic and biocompatible nanomaterials necessary for clinical applications are also constantly appearing in the field of research. That is, the use of non-toxic and biocompatible nanomaterials. Among them, carbon nanomaterials such as nanodiamond (ND) particles, which have a wide range of biomedical applications, have attracted attention because they produce negligible cytotoxicity in a variety of cell types. Today, ND is being developed to deliver chemotherapy drugs. For the past few years, ND-polymer delivery systems have progressed as a promising platform for BC treatment [11].

With the development, targeted therapy is considered to be the most promising way in the treatment of TNBC, and the combination of chemotherapy drugs and Mabs is recommended, and now the treatment of TNBC with chemotherapy drugs and Mabs-conjugated NPs has become a new hotspot. In addition, due to the characteristics of TNBC and the EGFR targeting strategy of humanized Mabs [12], such as the development of cetuximab (CET), NPs combined with CET-conjugated to improve EGFR targeted therapy accounted for the largest proportion in relevant studies.

In a recent study [12], a nanocomposite design that pairs ND with paclitaxel (PTX) and CET-conjugated was used to target EGFR-positive TNBC cells. PTX is a microtubule inhibitor, which can lead to abnormal chromosome aggregation and mitotic mutations that cause tumor cell death, that has been extensively used in the treatment of patients with a variety of human cancers. The study found that people who ND - PTX inhibits several BC cell lines (MDA - MB - 231, the MCF - 7 and BT474) cell vitality and induce mutations in mitosis. On the contrary, ND alone is not the induced cell death. Further studies revealed that ND-PTX-CET specifically bound EGFR and strengthen anticancer effects, involved drug intake standards, mitotic mutations, and apoptosis, in MDA-MB-231 cells that expressed egfr, but not in MCF-7 cells that were EGFR-negative. The latter two are likely due to increases in p-H3S10 and active caspase-3 proteins (whose phosphorylation is a marker of mitosis associated with chromosome aggregation) and active caspase-3 (a well-known apoptotic effitor). In addition, ND-PTX-CET was more effective in reducing TNBC tumor volume than ND-PTX. So in summary, ND-PTX-CET nanocomposites enhance mitotic mutation and apoptosis by targeting EGFR in TNBC cells. And it as a treatment for TNBC provides a viable strategy, also hints at the NPs - drug - Mabs such nanocomposites can be used for targeting EGFR treatment TNBC. At the same time, the use of ND-PTX overcomes the drug resistance and side effects of PTX, which not only provides a new strategy for the use of PTX, but also inspires people to develop NPs-drug.

4. Photothermal Therapy (PTT)

Unlike conventional treatment principles and methods, the emergence of this strategy is entirely based on the development of nanotechnology. Because diseases occur in the human body, eradication needs to start from the mechanism, so the treatment mostly relies on chemical methods and biological methods. With the development of nanotechnology, the special properties of nanomaterials due to their extremely small size make people begin to consider their applications in the medical field. The PTT strategy is considered and named because of the photothermal effect of nanomaterials in some cases, and of course the discovery that near-infrared (NIR) light has excellent tissue penetration properties [13]. So PTT, an emerging and encouraging treatment strategy, that utilizes some NPs to generate heat and thermally ablate cancer cells under NIR laser irradiation [14]. Photothermal agents are an important factor in determining the therapeutic effect of PTT. They can effectively absorb light, directly convert light energy into heat energy, and cause tumor tissue hyperthermia, leading to tumor cell necrosis and apoptosis. Hitherto, various photothermal nanotherapies, involved precious metal nanomaterials, nanocarbons, transition metal sulfide/oxide nanomaterials, and organic nanomaterials, have been widely probed [14].

Over the past century, traditional cancer treatments have not been as effective as desired. With the continuous development of research, emerging therapeutic modalities have flourished in the past few decades, and among various innovative cancer treatment strategies, PTT has attracted much attention in clinical research due to its advantages of high specificity, minimally invasive, and accurate temporal and spatial selectivity [15]. And compared with traditional treatment methods, the use of NIR greatly improves the spatio-temporal accuracy of the treatment process and quite reduces the accidental injury to ambient healthy tissue [15]. More valuably, only need to heat the tumor cells to nearly 50 °C, the high efficiency of PTT can cause tumor tissue ablation [16].

PTT can be used alone, under the guidance of multimodal imaging, or in combination with existing treatments to achieve their respective therapeutic effects. For the past few years, the synergistic effect of PTT combined with chemotherapy on tumor metastasis has been continuously studied [14]. For example, combination chemotherapy in the treatment of TNBC, as it is a typical representative of highly aggressive cancers, a potential immune-stimulating nanomodulator, coupling the transforming growth factor- α inhibitor rovalpitavir and the nucleic acid recognition aptamer on the surface of black phosphorus NPs, was designed, and by blocking the TGF- β pathway, synergistic PTT, vascular normalization, and initiation of the tumor-suppressing immune microenvironment are used to treat TNBC [17]. In particular, Emami F et al. developed a photoimmunotherapy (PIT) strategy based on PTT that uses the targeted photosensitizer cetuximab-conjugated gold nanorods (CTX-AuNR) in combination with antibodies targeting EGFR to treat TNBC by altering the tumor-associated macrophage phenotype and reducing tumor resistance [6]. And it provides a potential, attractive PIT treatment strategy for TNBC. In addition, for TNBC for which there is currently no accurate targeted molecular therapy, NPs-based PTT also has the potential to reduce or indeed eliminate tumors in locally advanced BC and reduce the incidence of preoperative treatment [18]. As mentioned above, PTT or a combination of them could offer an important and promising treatment modality that offers new expectation for the future fight against cancer metastasis.

5. Conclusion

TNBC is characterized by high invasiveness, heterogeneity and malignancy, which leads to drug resistance and poor prognosis. At present, although the targeted therapy is the most promising solutions, but in view of the patients with TNBC is still short of effective targeted treatment strategy, so chemotherapy is still the major therapies. As for TNBC in-depth analysis, and the development of nanotechnology in the medical field, clues have been provided for the precise treatment of TNBC patients.

The occurrence and development of TNBC and its vicious cycle involve the abnormal activation of various signaling pathways. At present, the exploration of a variety of relevant signaling pathways,

such as MAPK/ERK, PI3K/Akt, etc., will help to better understand the pathogenesis of TNBC and provide theoretical foundation for molecular targeted tumor therapy. It also gives NPs-conjugated therapy more development.

With the development of molecular target research, nano combination therapy technology has been improved step by step in a certain direction, such as the conjugation of Mabs and NPs, the development of NPs-drug-Mabs nanocomposites, PTT for physical killing and PIT for physico-chemical attack, as mentioned in this review. However, these methods still have some problems to be solved. Just as drug resistance issues also arise in nanocouplings and composites, the potential long-term toxicity of nanomaterials used in PTT and the specificity and selectivity of PTT for normal tumor cells and metastatic cells need to be carefully considered and improved.

So although nanotechnology brings a lot of convenience to treatment, existing TNBC treatment methods are still limited. Improving treatment outcomes for TNBC patients is an urgent issue and a daunting challenge. In the future, it is hoped that the discovery of more feasible clinical targets for TNBC patients will lead to better treatment strategies to overcome drug resistance.

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