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Immunotherapies against HER2 positive breast cancer: Focusing on monoclonal antibodies and therapeutic vaccines

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Abstract. HER2 positive breast cancer is prevalent in females, accounting for 31% female cancer worldwide. The pathology is due to the fact that overexpressed HER2 protein dimerize will others to cause constitutively signalling cascades inside the cells. Eventually, tumour develops due to uncontrolled proliferation. Immunotherapies have been researched significantly in treating this type of cancer, and this article focuses on the monoclonal antibodies and the therapeutic vaccines. Monoclonal antibodies, especially trastuzumab, significantly benefit in clinical outcomes. However, resistance developed against trastuzumab, and this urged the development of other novels mAb and antibody drug conjugates. On the other hand, even though none of the therapeutic vaccines have been approved, they are actively researched in clinical trials. With immunogenic peptides and efficient platforms are chosen, the therapeutic vaccine is expected to activate immune cells, resulting in elimination of tumour cells. Both approaches have drawbacks including drug resistance and the suppressive tumour microenvironment. Therefore, combined immunotherapies may be considered as potent treatment in the future.

Keywords: HER2 positive breast cancer, monoclonal antibodies, therapeutic vaccines.

1. Introduction

In 2023, breast cancer is predicted to account for 31% of female cancer in the world [1]. HER2 (human epithermal growth factor receptor 2), also known as ERBB2, exerts tyrosine kinase activity as a transmembrane glycoprotein. HER2 positive breast cancer (HER2+BC) is an aggressive subtype. Due to the amplification and overexpression of HER2, HER2 dimerizes with other family members of HER proteins. This heterodimerization or homodimerization will cause autophosphorylation of tyrosine residues of the cytoplasmic domain of this dimer. Then a constitutively active cascade of the downstream signalling pathways will be initiated. As a result, uncontrolled cell proliferation will lead to tumour growth [2]. Therefore, targeting HER2 protein will promote significant outcomes.

Trastuzumab is the first monoclonal antibody (mAb) developed to target HER2, by binding to the extracellular domain of HER2. This binding leads to several effects, including preventing dimerization and internalisation of the receptors, inhibiting the downstream phosphoinositide 3-kinase (PI3K/AKT/mTOR) pathway, and bringing about the antibody-dependent cell-mediated cytotoxicity. Even though Trastuzumab achieved great success in treating HER2+BC combined with chemotherapy, patients became resistant to it and disease relapse is another concern [3]. Therefore, researchers looked into other therapeutic agents that targeted HER2 protein. A second humanised mAb, pertuzumab, targets the dimerization site of HER2. This binding will exclude the dimerization of HER2 to other HER

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proteins including HER1, HER2, HER3, and HER4. By this means, pertuzumab inhibits the HER2-mediated cell signalling via the PI3K/AKT/mTOR pathway [4]. These two mAb together can exert synergic effects on treating HER2+BC. The ADCC effect through Fc on trastuzumab is the main reason for high efficacy. To amount immune response, Fc receptors (FcR) on the effector immune cells will bind to Fc region on the mAb. A novel margetuximab enhances this binding, which could potentiate greater antitumour immunity [5].

With the safety profile and lifelong protection, therapeutic vaccines could benefit patients by readjusting their immune system to an antitumour state [6]. However, neither prophylactic nor therapeutic vaccines have been approved now, with many of them still in clinical trials. It is just a time issue [7]. Due to the overexpression of HER2 proteins, peptide-based vaccine becomes a potential substitute for conventional chemotherapy. The epitopes have been produced from the HER2 protein, including AE36, E75, and GP2 [6]. However, solely peptides are not immunogenic enough to elicit sufficient immune response. Therefore, immunostimulant agents (adjuvants) are included in the vaccine to amplify immune response against target peptides. GM-CSF adjuvant is widely used in HER2-targeted vaccines, to improve the antigen presenting activity of various cells [7]. Moreover, suitable platforms should be selected to deliver the antigens and facilitate the presentation by antigen presenting cells (APC). Nanoparticles, for instance virus-like nanoparticles, can achieve this purpose [8]. With all the necessary components, a peptide-based vaccine has an effect in cancer killing by mobilizing scytotoxic CD8+T cells and NK cells [7].

Treating by monoclonal antibodies is a type of passive immunotherapy, while therapeutic vaccine is the active one. By comparing these two treatments against breast cancer, the drawbacks of each can be identified. Through further analysing the reason, future direction of how to improve each treatment can be clearer. Besides, other immunotherapies can be used as combined therapy to better improve the outcome on the patients.

2. HER2 and development of cancer cells

HER1, HER2, HER3, and HER4 are cytoplasmic membrane-anchored receptor tyrosine kinases. They share similar structures and sequences, containing and extracellular ligand-binding domain, a transmembrane domain, and an intracellular tyrosine kinase domain. Structural analysis of HER2 revealed that the open conformation for its extracellular domain is similar to a ligand-activated state of other HER receptors. This finding indicates that these receptors can form homo/heterodimers without the bound ligands. In addition to the fact that there is no identification of HER2-specific ligands, the dimerization of HER receptors favours the binding of HER2. Therefore, the overexpression of HER2 in HER+BC promotes the homo/heterodimerization to initiate downstream signalling cascades that assist in cancer development [9].

As HER2 heterodimerizes with HER3, phosphorylation of HER3 will provide docking site for p85 subunit of phosphatidylinositol 3-kinase (PI3K). After PI3K is activated by binding of p110 subunit to p85 subunit, the catalytic domain of it will phosphorylate lipid phosphatidylinositol-4,5-bisphosphate [PI(4,5)P2] to phosphatidylinositol-3,4,5-bisphosphate [PI(3,4,5)P2]. PI(3,4,5)P2 is then able to recruit serine/ threonine protein kinase AKT through its pleckstrin homology interaction domains (PH domain). Once recruited to the membrane, AKT is further phosphorylated by both phosphoinositide-dependent protein kinase 1 (PDK1) and mammalian target of rapamycin complex 2 (mTOR2) at the threonine and serin residues [10]. Then fully activated AKT is able to phosphorylate the Tuberous Sclerosis protein 2 (TSC2). This phosphorylation inactivates TSC2. Inactive TSC2 fails to keep a Ras-related GTPase (Rheb) in its inactive form. Therefore, the Rheb can switch GDP for GTP to become active. Active Rheb ultimately causes the activation of mTORC1, leading to cell growth and protein synthesis [11]. Additionally, active AKT also phosphorylates glycogen synthase kinase-3 (GSK) and Foxhead O1 (FoxO1), exerting an inhibitory effect. Therefore, cell survival and cell proliferation are promoted [10]. Generally, the overexpression of HER2 proteins leads to uncontrolled and invasive tumour growth and survival through this PI3K/AKT/mTOR pathway.

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3. Monoclonal antibodies

The main mechanism of Trastuzumab to take its action is through inhibiting the PI3K/AKT/mTOR pathway. Trastuzumab binds to the extracellular domain of HER2 protein to inhibit its homodimerization, resulting in prevention of downstream activity. Besides, heterodimers of HER2 and HER3 have more oncogenic roles, and the activation of PI3K/AKT/mTOR pathway is highly correlated with HER3. Specifically, trastuzumab suppresses the phosphorylation of AKT unrelated to the PI3K activity and HER3 phosphorylation. Instead, trastuzumab reduces the phosphorylation of PTEN. This will increase the phosphatase activity of PTEN and recruit it to the plasma membrane. PTEN is a negative regulator of PI3K/AKT/mTOR pathway, it can dephosphorylate PI(3,4,5)P2 so that the PI3K signaling cannot continue [10]. Besides, trastuzumab can arrest the cell cycle during G1 phase. The progression of cell cycle is promoted by the formation of complexes including cyclin-dependent kinase (CDKs) and cyclin. Trastuzumab can activate the inhibitor of CDKs, the p27kip1, thereby arresting the cell cycle at G0/G1 phase [9]. Therefore, the proliferation of cancer cells can be inhibited by trastuzumab.

Another main mechanism through which trastuzumab carries out its anti-tumor activity is antibody-dependent cell-mediated cytotoxicity (ADCC). ADCC depends on the fragment crystallizable (Fc) region on the antibody to carry out immune response by innate immune cells. Trastuzumab can recruit effector cells, especially the natural killer (NK) cells, to the site with overexpressed HER2. NK cells present fragment crystallizable δ receptors (FcδRs) which bind to the IgG1 Fc region on the trastuzumab. This binding enables the formation of an immunological synapse. Different granzymes and perforins will be released from the NK cells into the synapse to induce apoptosis of tumor cells [12].

However, the resistance towards trastuzumab develops. The most prevalent mechanism in BC is the constitutive activation of PI3K/AKT/mTOR pathway. The loss-of-function mutation in PTEN and gain-of-function mutation in PI3KA can result in this effect. Besides, the overexpression of insulin-like growth factor-I receptor (IGF-IR) could decrease the growth arrest mediated by trastuzumab. Moreover, a truncated form of HER2 (p59HER2) can be generated and it lacks a binding site for trastuzumab. P59HER2 then is able to constitutively activate the kinase activity inside the cells [13]. Together with other mechanisms, the resistance towards trastuzumab develops. This then requires novel mAb or combined therapies to overcome.

Pertuzumab binds to a different epitope on HER2. This binding inhibits both homo and heterodimerization of HER proteins. Through inhibiting dimerization, pertuzumab can restrain the PI3K/AKT/mTOR pathway inside the cancer cells. Since the binding sites of trastuzumab and pertuzumab do not overlap, the actions of these two mAb can complement each other to inhibit signal transduction more efficiently. Besides, margetuximab is a novel IgG1 mAb targeting HER2. It has an engineered FC region so that the FC region binds to the activating Fcδ receptors with a higher affinity, while binding to the inhibiting Fcδ receptors with a decreased affinity. It has been shown that margetuximab is able to support an enhanced ADCC activity compared to both trastuzumab and pertuzumab in vitro [3]. Moreover, a microtubule inhibitor emtansine (DM1), a cytotoxic drug, is conjugated to trastuzumab to produce T-DM1. T-DM1 has been shown to improve the overall survival rate of trastuzumab-resistant HER2+BC patients [14]. As combined therapies, trastuzumab or pertuzumab is usually given to patients together with chemotherapy, leading to promising clinical outcomes. Besides, as the trastuzumab leads to ADCC effect based on innate immunity, the adaptive immunity can be activated at the same time to bring about greater efficacy. Programmed death 1 (PD-1) is an inhibitory receptor largely expressed on tumor-infiltrating lymphocytes, and BC cells express its ligand PD-L1. Studies showed that treating mice with trastuzumab and either an inhibitor of PD-1 or PD-L1 can lead to greater tumor regression than treating with trastuzumab alone. The inhibition of negative regulators can result in greater activity of effector T cells [15]. Therefore, with the combined therapy, the overall survival for HER2+ BC patients can be more promising.

4. Therapeutic vaccine

Due to the abnormal overexpression of HER2 in cancer cells, peptide-based vaccine becomes a potent therapy. The immune system will be modulated by the vaccine to attack the cancer cells that highly

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express HER2. The immunological memory provided by the vaccine can benefit in solving the relapse. Peptide-based vaccines are the most common strategy against breast cancer in clinical trials. E75 is derived from extracellular region of HER2 being HLA-A2/A3 restricted. E75 is usually incorporated in the NeuVaxTM vaccine together with granulocyte-macrophage colony-stimulating factor (GM-CSF), a secreted cytokine, to enhance antigen immunogenicity. Even though NeuVaxTM vaccine achieved success in early stages, it failed in phase 3 study showing no difference in disease-free survival. Despite this unsatisfying outcome, latest studies on GP2 showed promising results. GP2 is derived from transmembrane domain of the HER2 being HLA-A2/A3 restricted. At the end of phase 2, this type of vaccine showed recurrence rate of 0% in HER2 3+ patients after treatment of trastuzumab [16].

Apart from the GM-CSF, virus-like particle (VLP)can be used as adjuvant as well. Viral particles are immunogenic to elicit innate immune response, due to the presence of pathogen associated molecular patterns. Therefore, they are generally used as a delivery vehicle, benefiting from the fact that they won't infect the host due to the lack of mammalian-replicable genetic material. For example, cowpea mosaic virus (CPMV) is widely studied and effectively conjugated to HER2 epitopes. This conjugation led to increase in HER-2 specific antibodies in marine models [17].

The immunological foundation for developing anti-cancer therapeutic vaccine starts from that the peptide in the vaccine will be processed and presented by antigen presenting cells (APCs), especially dendritic cells (DCs). With the use of CPMV, the VLP will bind and be internalized by APC. In both circumstances, activation of DC will be promoted. Mature DC now can prime immune cells to become effector cells which then recognize and kill the cancerous cell displaying the corresponding peptide [18].

One of the big hurdles in developing therapeutic vaccines is that the low immunogenicity due to the antigen selected. To address this problem, studies have shown that the use of synthetic long peptides could be beneficial, as the short peptide will be loaded to non-professional APCs which will not present stimulatory signals to cytotoxic T cells (CTLs). Without sufficient CTLs being primed, they won't be able to exert killing effects. Besides, tumor microenvironment (TME) remains to be a major obstacle [7]. Tumor cells come up with several strategies to escape tumor surveillance after they survive from the selection pressure posed by immune system to become less immunogenetic. Especially, the PD-1 will be regulated on the tumor-infiltrating lymphocytes, and at the same time, upregulation of its ligand PD-L1 is seen on tumor cells and APC. The binding of PD-1 to PD-L1 will result in T-cell dysfunction and apoptosis. By this means, tumor cells can escape the killing. Consequently, using immune checkpoint inhibition of either PD-1 or PD-L1 will result in synergic effects [19].

5. Discussion

Generally, the mAb used to treat HER2+BC acts by directly inhibiting the intracellular signal to inhibit proliferation and carry out the ADCC effects through the binding of its Fc region to FcR on immune cells. In contrast, the mechanism of therapeutic vaccines to take action is through educating the active immune cells, for example, CTLs, to eliminate cancer cells. The passive and active immunotherapies take different ways of action resulting in different challenges.

Even though there are several strategies to resolve the resistance to trastuzumab, resistance remains to be the main challenge for all mAb in general. In addition to the action through trastuzumab in T-DM1, and DM1 can cause cytotoxicity after the antibody-drug conjugate (ADC) is taken up and possessed within lysosome to produce lysine-MCC-DM1. Lysine-MCC-DM1, after being transported to the cytoplasm, can bind to tubulin, thereby preventing microtubule polymerization. This deteriorates the ability to form micro spindle and eventually causes apoptosis. However, resistance develops towards T-DM1 as well, and some of its mechanisms can be challenging to all HER2-targeted mAb. First, the loss of HER2 expression. This will affect Trastuzumab, T-DM1, and all the HER2-targeted mAb binding. Furthermore, the reduction of HER2 will impair the internalization of T-DM1 and its cytotoxity of DM-1. Besides, the binding affinity of T-DM1 will be decreased as HER2 is truncated to p95HER2 [20]. In general, all the mechanisms of resistance to trastuzumab will eventually be applied to T-DM1, because T-DM1 is trastuzumab-based. The resistance to HER2-targeted mAb urges the resolving methods.

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When it comes to therapeutic vaccines, it is always a challenging approach. When the vaccine is peptide-based, the immunogenicity of the peptide always needs thorough consideration. One approach to increase the immunogenicity is to include multiple peptides within the vaccine in addition to the HER2-related peptide. For instance, one study included MUC-1, CEA, and three co-stimulatory molecules in one single vaccine. Moreover, drawbacks in developing therapeutic vaccines always include the TME. Except for the PD-1 therapy mentioned above, anti-CTLA4 can be investigated to solve this problem. The activation of naïve T cells needs a costimulatory stimulation through the binding of CD28 to B7 on APCs. However, CTLA-4 on regulatory T cells has higher affinity for CD28 and this binding will exerts inhibitory effects, resulting in T cell anergy [21]. Thus, insight into anti-CTLA as a combined therapy needs more attention.

However, as the mAb and therapeutic vaccines act in different ways, the different mechanisms can be taken advantage of and combine these two therapies together. Surprisingly, according to previous study, combining trastuzumab and E75 vaccine could cause synergic effects [22].

6. Conclusion

Unmet medical needs still exist for HER2+BC. First, HER2+BC is still a prevalent disease among females. Even though chemotherapies remain to be the conventional treatment for this cancer, immunotherapies used as a combined therapy may bring about more promising effects. mAb can act by binding to extracellular regions of the HER2 protein, thereby inhibiting signaling cascades inside to restrain the proliferative ability of cancerous cells. Moreover, the Fc region can direct ADCC effects with the help of NK cells to impose extra killing on the cancerous cells. Although, trastuzumab was quite effective, patients developed resistance towards it through different mechanisms. Therefore, novel mAb and ADCs were developed to resolve the problem to certain extent. However, resistance still develops against these approaches. In the future, the researchers still need to pay more attention in resolving resistance when developing mAb in treating HER2+BC. For therapeutic vaccines, even though there are a lot of them in the clinical trials, none of them have been approved yet. To get expected results, the immunogenetic antigens should be chosen. In addition, certain platforms, like VLP, are added to increase the immunogenicity and to deliver the peptide. CTLs will then be activated to initiate killing effects on tumor cells. However, there are several hurdles exist in the development of therapeutic vaccines, and the main one could be the suppressive tumor microenvironment. In both scenarios of applying mAb and therapeutic vaccines, immune checkpoint inhibition of PD-1/PD-L1 will bring synergic effects. As conclusion, individual immunotherapies are always not good enough in terms of bringing clinical benefits. However, combining two or more, for example even combining mAb and therapeutic vaccines, can result in promising outcomes, because they can act in different ways to achieve the ultimate goal—treating cancer. Furthermore, when considering combined therapies, more immunotherapies can be considered, including adoptive cell transfer, CAR-T cell therapy and so on.

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