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Antibody engineering promotes nanomedicine for cancer treatment

"Targeted nanomedicines decorated with antibodies can significantly improve the therapeutic effectiveness of conventional chemotherapeutics or gene therapy in cancer."

KEYWORDS: antibody ■ cancer ■ drug delivery ■ targeted nanomedicines

At present, cancer is the second leading cause of death worldwide. In 2005, out of 58 million deaths worldwide, 7.6 million people died of cancer. Classical cancer treatments, including surgery, radiation therapy and chemotherapy, all cause serious side effects by the loss of normal cells or organ function. Tumor-targeted therapy, aiming at eliminating cancer cells and protecting normal tissue, is being developed rapidly and achieves many innovative improvements [1]. One of the most significant advances in tumor-targeted therapy is the application of monoclonal antibodies (mAbs) in oncology [1,2]. MAbs have been widely used alone or in combination with chemotherapy agents in cancer therapy [1–3]. The use of mAbs in cancer therapy is growing rapidly, owing to their ability to bind with high specificity and affinity to overexpressed antigens in cancer cells and then exert antitumor effects [4]. So far, there are ten mAbs approved for cancer therapy [5].

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Monoclonal antibodies or their derivatives (e.g., Fab' fragments and single-chain variable fragments, among others) are often adopted as the targeted ligands in cancer nanomedicines, owing to their highly specific associations with antigens [6–8]. Targeted nanomedicines decorated with antibodies (short for targeted nanomedicines) can significantly improve the therapeutic effectiveness of conventional chemotherapy or gene therapy in cancer [9–12]. When conjugated with antibodies as targeting ligands, targeted nanomedicines can be used to target tumor cells and the tumor microenvironment (e.g.,

tumor stroma and tumor vasculatures) with high specificity and affinity, achieving significantly improved antitumor activity over the untargeted nanomedicines [6,9–12]. The development of targeted nanomedicines, which perfectly combine antibody engineering and nanomedicine, is becoming a possible state-of-the-art in nanomedicine research. In this article, the recent advances in the field of targeted nanomedicines for cancer therapy are summarized as follows: the achievements of mAbs and antibody engineering; how antibody engineering can promote cancer nanomedicine development; and the challenges and future perspective of targeted nanomedicines.

MAbs & antibody engineering

A century ago, Paul Ehrlich envisioned antibodies as 'magic bullets' that could be developed to selectively target disease [13]. This imagination became true when Köhler and Milstein developed the hybridoma technology, which provided mAbs capable of highly specific associations with their targeted antigens [14]. However, the first generation of murine mAbs was severely limited in the clinic by their human antimouse antibodies [15]. These hurdles were greatly overcome by the generation of chimeric and humanized mAbs that contain the human Fc domain and retain targeting specificity by incorporating portions of the murine variable regions. Chimeric mAbs comprise murine variable regions attached to human constant regions, while in humanized mAbs, only the antigen-binding complementarity-determining region loops derive from murine mAbs [16–18]. New technologies have also been developed that allow the production of fully human mAbs using either phage display technology or transgenic mice carrying human immunoglobulin genes [19]. Fully human mAbs are increasingly important for cancer therapy due to their low immunogenicity [20].

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Whole mAbs can be reduced to antibody fragments lacking the Fc portion (Fab, Fv or single chain Fv [scFv]), dissected into minimal binding fragments, such as the V_H domain, and rebuilt into multivalent high-avidity reagents (i.e., diabodies, triabodies or tetramers) [21]. Domain antibodies are the smallest known antigen-binding fragments of antibodies, ranging from 11 to 15 kDa (the molecular weight of mAbs is generally ~150 kDa) [22]. The particular advantage for tumor targeting is that these antibody fragments may have increased tumor penetration and favorable plasma pharmacokinetics over whole mAbs, owing to their reduced molecular weight [4]. Nevertheless, the linker between domains, molecular weight, charge, valency and binding affinity of antibody fragments should be carefully manipulated to optimize the particular advantage [4]. MAb or antibody fragments have also been used widely to target different reagents (including nanoparticles, liposomes, cytotoxic drugs, cytokines, toxins or radionuclides) to tumor cells [1]. The purpose of this novel strategy, combining antibodies to those different reagents, is to increase specificity of the therapeutic effect and maximize desired effects, while minimizing side effects. The immunoconjugate gemtuzumab (Mylotarg[®]; Wyeth, PA, USA), consisting of a humanized anti-CD33 mAb linked to the cytotoxic antibiotic ozogamicin (*N*-acetyl- γ calicheamicin), has been approved for the treatment of elderly patients suffering from CD33-positive acute myeloid leukemia in their first relapse, who are not eligible for other chemotherapies [23].

Antibody engineering promotes cancer nanomedicine development

Nanomedicines have great potential in enhancing drug delivery for cancer diagnosis and therapeutics. In recent years and at present, nanomedicines are being widely investigated in the delivery of therapeutic drugs (including chemotherapeutics [12,24,25], protein [9,10] and gene drugs [11,26]) and imaging reagents (e.g., dye-doped silica nanoparticles [27], iron oxide [28], quantum dots [29,30] and gold nanoparticles [27]). The tumor targeting of nanomedicines is being propelled and highlighted in the forefront of cancer research, with gradually enhanced understanding of the characteristics of tumor biology [31]. Nanomedicines can be delivered to tumors by both passive and active targeting mechanisms [6,32]. In passive targeting, nanomedicines tend to accumulate preferentially within the tumors through the enhanced

permeability and retention effect. In active targeting, targeted nanomedicines decorated with ligands, such as folate, aptamer and antibodies, have enhanced nanomedicine uptake in cancer cells overexpressing specific tumor antigen. Antibodies, including mAbs or mAb fragments, are amongst the most frequently used ligands for targeted nanomedicines [6]. Antibodies have many unique advantages compared with other ligands. First, antibodies have much higher specificity and affinity than the small molecule ligands, such as folate or RGD peptides. Second, a huge amount of NH₂ and COOH groups, which provide conjugation sites for crosslinking of nanomedicines, exist in the antibodies. Third, several mAbs, such as rituximab and trastuzumab, are US FDA-approved drugs for cancer therapy, whereas none of the other ligands, such as folate or RGD peptides, are approved.

“...targeted nanomedicines decorated with antibodies of optimal binding valency, affinity and size should be developed in order to achieve rapid and complete tumor penetration.”

The contribution that antibody engineering has made to nanomedicine development can be measured on the following. First, targeting antibodies to internalizing antigens results in receptor-mediated internalization of targeted nanomedicines into the cytoplasm or nucleus, where the targeted nanomedicines are broken down to release free drug [6,33,34]. Obviously, targeting of nanomedicines to internalizing antigens results in delivery of much more drugs to the cytoplasm or nucleus compared with targeting to noninternalizing antigens [35]. Higher intracellular drug concentrations should lead to improved diagnostic or therapeutic outcomes for most nanomedicines, such as iron oxide nanoparticle [28] or liposomal drugs encapsulating chemotherapeutics [6]. However, receptor-mediated internalization is not required for nanomedicines for targeted radionuclide therapy, since radionuclide therapy can operate extracellularly. Similarly, the radioimmunoconjugates ibritumomab tiuxetan (Zevalin[®]) directed against CD20 (a noninternalizing antigen) show substantial antitumor activity and have entered standard clinical practice for lymphoma therapy [1]. Second, the binding valency, affinity and size of antibodies, which play very important roles in optimizing the physical properties that enable penetration of and retention by solid tumors, can be modulated flexibly by genetic engineering [4,21] and computer simulation [36].

Active targeting of solid tumors mediated by targeted nanomedicines decorated by antibodies is more difficult, as a number of barriers, such as survival in the bloodstream, extravasation into the tissue and binding specifically to the target cells, can impede the diffusion and distribution of targeted nanomedicines with the tumor parenchyma [4,6,34]. It is generally accepted that targeted nanomedicines reach tumors by the same passive targeting mechanism of the enhanced permeability and retention effect as nontargeted nanomedicines [6,30]. Furthermore, the binding of targeted nanomedicines to the first tumor cells they encounter will impede their diffusion through the tumor parenchyma. This phenomenon is referred to as the 'binding site barrier' [37]. As a result, several studies have failed to show advantages of targeted nanomedicines over nontargeted nanomedicines [38,39]. In fact, the existence of the binding site barrier was proposed by Weinstein *et al.* [40] and firmly demonstrated by Adams *et al.* [41]. They demonstrated that the binding site barrier impedes the penetration of antibodies into tumor masses because durable, high-affinity interactions between the antibodies and their targets block the diffusion of such antibodies throughout the tumor mass [40,41]. These observations intensified the importance of elaborate designation of appropriate antibodies used to be the ligands of targeted nanomedicines. Thus, for targeted nanomedicines to achieve a rapid and complete tumor penetration, a high-affinity binding interaction may be undesirable. Third, a variety of antibodies active against tumor antigens have been developed to target cancers. Tumor antigens are substances produced in tumor cells that trigger an immune response in the host. Tumor antigens are useful in identifying tumor cells and are potential candidates for use in cancer diagnosis and therapy [8]. Surveys of the molecular basis of tumorigenesis have identified aberrantly expressed tumor antigens, and a variety of antibodies against tumor antigens have been developed by genetic engineering, hybridoma technology and phage display technology [14,42,43]. Trastuzumab, a humanized mAb directed against HER2 (tumor antigen), was most often adopted for targeting ligands in the targeted nanomedicines [9,11,25]. Evidently, the production of novel antibodies against more specific tumor antigens will greatly enhance the diagnostic or therapeutic outcomes of targeted nanomedicines. Fourth, a combination of mAbs and chemotherapeutic drugs has spawned additional combination therapies, which had synergistic effects on cancer therapy and showed promising results [3]. In preclinical studies with cell culture

or animal models, the antitumor effects of these combination therapies were significantly greater compared with either therapy alone [3,44,45]. MABs can also increase the sensitivity of tumor cells to chemotherapeutic drugs and patients treated with mAbs first followed by chemotherapy after, showed higher clinical response rates than patients who received chemotherapy alone [3,46]. The mechanism underlying the synergistic effects of the two types of therapies was very complicated. One mechanism proposed is that mAbs can revert chemoresistance in tumor cells to chemosensitivity. For example, HER2 downregulation by trastuzumab increased tumor cell sensitivity to cisplatin by decreasing DNA repair activity, following cisplatin-induced DNA damage [47]. In conclusion, targeted nanoparticles delivering chemotherapeutic drugs will benefit from the synergistic effect of chemotherapeutic drugs and mAb combined therapy.

Difficulties & challenges

Although significant advances have been achieved in targeted nanomedicines decorated with antibodies, there are still many considerable challenges and issues remaining for cancer therapeutic applications [48]. First, antibody–nanomedicines conjugation strategies are far from perfect. Many conjugation procedures are covalent reactions, which appear to be effective ways to irreversibly fix antibodies to nanomedicines, but it should also be kept in mind that the covalent reactions can affect the biological activity of antibodies. Furthermore, a good orientation of the antibodies on the nanomedicines is not guaranteed [48]. However, in many cases, the effect of covalent reactions on the biological activity of antibodies and optimal orientation of the antibodies on the nanomedicines can not be well controlled, resulting in low antibody–nanomedicines conjugation efficiency or impaired biological activity of antibodies [28,48,49]. Efficient, directional and moderate antibody–nanomedicines conjugation strategies should be urgently developed. Second, the binding site barrier impedes the penetration of targeted nanomedicines into tumor masses, resulting in a failure to show advantages of targeted nanomedicines over nontargeted nanomedicines [38,39]. Thus, targeted nanomedicines decorated with antibodies of optimal binding valency, affinity and size should be developed in order to achieve rapid and complete tumor penetration. Third, although targeted nanomedicines can discriminate tumor tissues from normal tissues to some extent, targeted nanomedicines still show

moderate binding to normal tissues, leading to nonspecific toxicity and side effects [32]. To solve this problem, uncharged, small-sized (~100 nm) and sterically stabilized targeted nanomedicines should be prepared [50]. The development of novel antibodies targeting more specific tumor antigens is also equally extraordinary.

Future perspective

Looking forward to the bright future, for targeted nanomedicines decorated with antibodies, there are a number of research hot points that are particularly promising, but require sustained effort for success. These include:

- Urgent development of efficient, directional and moderate antibody–nanomedicines conjugation strategies;
- Development of targeted nanomedicines decorated with antibodies of optimal binding valency, affinity and size;

- Development of targeted nanomedicines to overcome nonspecific organ and reticulo-endothelial system uptake;
- Standardization and manufacturability. For clinical cancer therapy, targeted nanomedicines must be standardized and manufactured according to US FDA requirements.

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