The Combination Therapy of Nanoparticle and Monoclonal Antibody

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Abstract: Coating the surface of nanoparticles with polyethylene glycol (PEG), or “PEGylation,” is a commonly used approach for improving the efficiency of drugs and gene delivery to target cells and tissues. Due to the properties of drugs, some of them are limited in clinical application. Attaching monoclonal antibodies on the surface of nanoparticles could be a potential strategy to achieve high treatment efficiency with a low side effect profile. However, the monoclonal antibody-conjugated nanoparticles showed higher efficacy compared to the normal drug-loaded nanoparticles in vitro. This review will highlight some bio-target that can be used on monoclonal antibody-conjugated nanoparticles, the result of in vivo tumor inhibition, and survival tests of those monoclonal antibody-conjugated nanoparticles.

1. Introduction

Therapeutic monoclonal antibodies (mAbs) have entered the clinic over 25 years ago and became one of the most efficient therapy for cancer over the 25 years. Due to their ability to target specific molecular components, a large amount of mAbs has been approved in different diseases including oncology, autoimmune disorders, chronic diseases [1]. There are more than 80 kinds of mAb therapies are proved in Europe and/or in the United States, and the sales of therapeutic antibodies were over 100$ billion in 2017 worldwide [1].

Cytotoxic drugs are known to kill certain types of cancer cells and prevent the spread of tumors. Most of the cytotoxic drugs are targeting pathways that control normal cell growth and malignant transformation [2]. Because cancer cells divide significantly faster than normal cells, so they are more sensitive to cytostatic. However, those cytotoxic drugs will accumulation in normal tissues and cells, which can cause serious adverse effects, such as hair loss, nausea, and damage to bone marrow. One way to improve the efficiency of this cancer therapy is improving the selectivity and efficacy that targeting the altered levels of expression on malignant cells. Antibody-drug conjugates (ADCs) is consisting of an antibody carrier and a drug-linker moiety that conjugate one or multiple drugs [2]. Linking the cytotoxic drugs with antibodies can achieve selective and sustained drug delivery to tumors. The drugs would be able to target cancer cells through specific antibody-antigen binding, which means the antibodies of ADCs can target antigens expressed on the surface of epithelial tumors [2]. Thus, this combination therapy can increase drug accumulation in tumors and reduce side effects.

Nanotechnology is a promising strategy to improve general cancer immunotherapies. The nanoparticle can guide the drug to specific sites in vivo via systemic application, tumor implants, or microneedle injection [3]. Table 1 showed several nanoplatforms for cancer targeting treatments. By using nanoparticles, the immune therapy will have an enhanced efficacy and induce a better anti-tumor response. Thus, the nanoparticle is one of the ideal carriers of combination therapy. Otherwise, nanoparticles can make solid tumors more accessible to T-cell and cancer cell-directed immunotherapy by targeting immune suppressive cell types in the TME (tumor microenvironment)[3].
<table>
<thead>
<tr>
<th>Nanoplatforms</th>
<th>Drug</th>
<th>Size(nm)</th>
<th>Target/ligand</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liposomes carbon nanotubes [13]</td>
<td>Paclitaxel</td>
<td>164</td>
<td>anti-HER2 mAb</td>
<td>The nanohorn was formulated with PEG and thermally stable and pH-sensitive phospholipid to prolong the release of paclitaxel.</td>
</tr>
<tr>
<td>Polymeric nanoparticles [14]</td>
<td>siRNA</td>
<td>80 × 320</td>
<td>-</td>
<td>Cationic lipid coated PLGA nanoparticles</td>
</tr>
<tr>
<td>Polymeric micelles [15]</td>
<td>1,2-Diaminocyclohexane - platinum (II)</td>
<td>30</td>
<td>-</td>
<td>Polymeric micelles can enhance tumor permeability with a TGFβ inhibitor and have a long-circulating time.</td>
</tr>
</tbody>
</table>
2. Current Monoclonal antibodies and nanoparticles used for cancer treatment

2.1 Clinical Data of Monoclonal Antibodies in different cancer treatments

Table 2. FDA or EMA approved mAb that targets PD-1.

<table>
<thead>
<tr>
<th>Monoclonal antibody INN and trade names</th>
<th>Target name</th>
<th>Warnings, risks, and safety concerns</th>
<th>Adverse events, serious and common</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nivolumab (Opdivo®)</td>
<td>PD-1</td>
<td>Immune-mediated adverse reactions, embryofetal toxicity</td>
<td>Systemic: increased ALT, AST, and AP; hyponatremia; hyper- and hypokalemia; hyper- and hypocalcemia; lymphopenia; fatigue; asthenia; musculoskeletal and abdominal pain; dyspnea; cough; GI. Cutaneous: rash, pruritus</td>
</tr>
<tr>
<td>Pembrolizumab (Keytruda®)</td>
<td>PD-1</td>
<td>Immune-mediated adverse reactions, embryofetal toxicity</td>
<td>Systemic: fatigue, peripheral edema, chills, pyrexia, renal failure, cellulitis, decreased appetite, dyspnea, arthralgia, nausea, diarrhea, cough. Cutaneous: rash, pruritus, vitiligo</td>
</tr>
<tr>
<td>Cetuximab (Erbitux®)</td>
<td>EGFR</td>
<td>Boxed warning: serious IR and cardiopulmonary arrest. Others: pulmonary toxicity, dermatologic toxicity, hypomagnesemia</td>
<td>Systemic: electrolyte imbalance, infection, GI, anaphylaxis, headache, diarrhea. Cutaneous: acneiform rash, nail changes, xeroderma, paronychial inflammation, pruritus</td>
</tr>
<tr>
<td>Panitumumab (Vectibix®)</td>
<td>EGFR</td>
<td>Boxed warning: dermatologic toxicity, IR. Others: increased toxicity with bevacizumab and chemotherapy, pulmonary toxicities, electrolyte depletion, ocular events</td>
<td>Systemic: pulmonary events, pulmonary embolism, GI, fatigue, abdominal pain, hypomagnesemia. Cutaneous: rash, dermatitis “acneiform,” erythema, exfoliation, paronychia, skin fissures, photosensitivity, xerosis, pruritus</td>
</tr>
</tbody>
</table>

More and more mAbs have been approved for the treatment of an increasing number of cancers. However, their range of adverse effects is still wide and varied from mild gastrointestinal symptoms and transient rashes to severe cytopenias. Here we used PD-1 and EGFR mAb as examples. Clinical data showed that severe reaction (grades 3 and 4) occurred in 2-5% of 1373 patients including...
symptoms of airway obstruction, hypotension, shock, cardiac arrest, myocardial infarction. About 90% of the severe happened in the first treatment. Moreover, 76%-88% of patients have a variety of dermatologic events, such as “acneiform” rash, xerosis and fissuring, paronychial inflammation, hypertrichosis, and infectious[6]. Those reactions are generally more serious with mAb than with small molecule drugs; they used to happen on seborrheic regions of the face, scalp, or neck[6]. Table 2 lists warnings, precautions, risks, and safety concerns related to their use and serious adverse events[6]. Pembrolizumab is a humanized IgG4κ mAb, which is targeting PD-1 and is used to treat patients with unresectable or metastatic melanoma. Although pembrolizumab is generally well tolerated, the immune-related adverse events of this mAb can be severe.

According to the data from an uncontrolled, open-label, multiple cohort trial involving 411 patients receiving the mAb, serious effects occurred in 36% of patients, such as renal failure, dyspnea, pneumonia, and cellulitis[6]. Another type of mAb used for targeting PD-1 is called Nivolumab, and it is also indicated for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilimumab[6]. According to adverse events that assessed in 574 patients with solid tumors, 41% of patients occurred grade 3 and 4 adverse reactions, which included abdominal pain, hyponatremia, and increases in aspartate transaminase and lipase reported in 2–5% of patients[6]. Other additional adverse events are ventricular arrhythmia, iridocyclitis, infusion-related reactions, and neuropathies. Thus, although therapies with mAb have generally been better tolerated than chemotherapy, they still have a wide range of adverse effects.

2.2 Mechanism of tumor targeting by nanoparticles

Most of the nanoparticles-based platforms designed for drug targeting to tumors are passive targeting [6]. Most of the tumors produce a large amount of various vascular permeability factors to ensure the sufficient supply of nutrients and oxygen to cancer cells, so that they have enhanced vascular permeability [7]. This characteristic allows extravasation of nanoparticles in tumor tissue and hard to eliminate extravasated nanomaterials. The effect is called the enhanced permeability and retention (EPR) effect and this effect-driven drug delivery does not occur in normal tissue, which makes it become one of the most important strategies to improve the delivery of drugs to tumors. Examples of nanoplatforms that using passively targeted are Doxil (Caelyx in Europe; pegylated liposomal doxorubicin), DaunoXome (non-PEGylated liposomal daunorubicin), DepoCyt (non-PEGylated liposomal cytarabine), Myocet (non-PEGylated liposomal doxorubicin), and Oncaspar (pegylated L-asparaginase) [6].

The active targeting strategy of nanoparticles indicates the use of targeting ligands such as antibodies and peptides to bind a specific receptor expressed on the target site[6]. In this strategy, nanoparticles and antibodies need to be accumulated in tumor tissue first by the EPR effect. Most of the targeting ligands for this kind of actively targeting strategy are playing a significant role in cellular internalization, such as folate [8], galactosamine [9], and EGF [10]. However, the physicochemical of active target nanoparticles are unstable in blood circulation in vivo, and they are hard to accumulate in the tumor due to the size of monoclonal antibodies [11]. Those problems are necessary to overcome in the future study.

3. PD-L1 monoclonal antibody-conjugated nanoparticles

3.1 Background of the nanoparticles (NPs) conjugated by PD-L1 mAb

The nanoparticles (NPs) were conjugated with antibodies by an amination reaction, mAb was conjugated on the NP surface. These NPs are generally spherical in shape, and the particle size observed from these FESEM images was in good agreement with that determined by DLS[4]. Researchers modified PEG-PCL NPs with PD-L1 mAb and loaded with docetaxel (DOC). PD-1 is a cell surface receptor, which plays a significant role in the downregulation of the immune system; it promotes self-tolerance by suppressing T cell activity. The binding between PD-1 and PD-L1 will reduce the proliferation of antigen-specific T cells in lymph nodes. PD-L1 expression has been
detected in more than 40% of human gastric cancer (GC) samples[4]. So that it is a good biotarget for GC treatment; PD-L1 mAb can significantly block the interaction between PD-1 and its ligands.

3.2 Structure and characteristics of the NPs

The NPs comprise a PCL core with DOC loaded; hydrophilic PEG shell was on the surface of DOC core, and mAbs were coating on the surface of NPs. The size distribution of these DOC-PEC-PCL NPs was in the range of 170-200nm, with a polydispersity of 0.203–0.358. Zeta potentials were ranging from −8.89 to −15.45 mV. The EE and LC of the NPs were 48.8%±1.3% and 46.5%±3.4%[4]. FESEM imaging showed that compared to the control group, DOC-PEG-PCL-mAb NPs have an enhanced permeability and retention (EPR) effect in tumor tissue. In vivo drug release test showed that 35% of drugs were released from the carriers in the first 8 hours. Afterward, a steady release was observed in the next 10 days, which provided a sustainable drug concentration in vivo.

The result of the cellular uptake analysis in vitro proved that PD-L1 mAb on the surface of DOC-PEC-PCL NPs contributes to the enhancement of the uptake of DOC encapsulated in the NPs[4]. Compared with the isotype IgG control-modified NPs, mAb NPs exhibited increased cytotoxicity in GC cell lines, while the NP exhibited little cell mortality at low drug concentrations without the PD-L1 expression[4]. Moreover, western blot analysis showed that the GC cell line treated with DOC-PEG-PCL-mAb NPs has the highest expression of caspase-3, caspase-8, and caspase-9. Researchers used PEG-PCL, PEG-PCL-IgG, PEG-PCL-mAb, DOC, DOC-PEG-PCL-IgG as control groups[4]. All of those results showed that the copolymer NPs combined with PD-L1 mAb could induce more apoptosis than other NPs.

DOC-PEG-PCL-mAb NPs also enhanced G2-M arrest. Researchers found that cyclin A and B proteins were increased in cancer cells after being treated with DOC-PEG-PCL-mAb NPs, those proteins are cell cycle markers[4]. Cells arrested in the G2/M phase will become more sensitive to the damaging from the cytotoxic agent. This result demonstrated that mAb conjugated NPs can allow cancer cells to be more easily killed by radiation.

4. EGFR Targeted Cetuximab-Valine-Citrulline (vc)-Doxorubicin Immunoconjugates- Loaded Bovine Serum Albumin (BSA) Nanoparticles

4.1 Background of cetuximab-vc-DOX-BSA-NPs

EGFR is a cell-surface receptor tyrosine kinase, and it is involved in the cell cycle and the regulation of cell survival, such as angiogenesis, cell movement, and cell invasion[5]. Many malignancies, including breast, ovarian, or non-small cell lung cancers overexpress EGFR on the surface. Thus, EGFR become one of the ideal targets for cytotoxic drugs for selective chemotherapy. Cetuximab is a chimeric monoclonal immunoglobulin G1 (IgG1) antibody, which is approved by the FDA in 2004, resulting in the induction of apoptosis and inhibition of proliferation of cancer cells[5].

This NP used MC-Val-Cit-PAB-PNP as an ADC peptide linker, which is cathepsin-cleavable. This Peptide linker can be selectively cleaved specifically by lysosomal proteases, so that it could rapidly release the drug in target cells. In previous research, DOX was loaded in BSA (bovine serum albumin), and it resulted in low drug-loading efficiency and non-responsive drug release on the target site[5]. To overcome those problems, researchers constructed a novel ADC coupling BSA, which has a structure of cetuximab-vc-DOX attached to the surface of BSA nanoparticles.

4.2 The Characterization of Cetuximab-vc-DOX NP

The size of BSA NP is 141.5±2.3 nm, and zeta potential is −39.20±1.04 mV. Researchers used RKO(EGFR-overexpressing) and LS174T (EGFR-weakly expressing) cell lines to assess cytotoxicity. In fact, the RKO cell line showed a strong reduction of viability when treated with cetuximab-vc-DOX-NPs. On the other hand, LS174T has no change in viability after 48 h incubation with cetuximab-vc-DOX-NPs and IgG-vc-DOX-NPs[5]. This result proved that cetuximab-vc-DOX-NPs could specifically bind to the EGFR-overexpressed cells.
4.3 The distribution and cellular uptake of NP

Cellular binding of NPs was analyzed by flow cytometric. The free doxorubicin showed the strongest fluorescence after 4 h incubation with the RKO cell line, the second is cetuximab-vc-DOX-NPs and the third is IgG-vc-DOX-NPs. The percentage of positive cells between cetuximab-vc-DOX-NPs and IgG-vc-DOX-NPs showed a significant difference, which is 92.2±14.2% and 69.7±7.8%[5]. Otherwise, after 24 h incubation with RKO cell, fluorescence intensity declined in all groups, especially for the free doxorubicin, which is drop from 1207 to 224. However, the fluorescence intensity of cetuximab-vc-DOX-NPs only dropped 446, from 758 to 312[5]. This result suggested the modification of cetuximab may inhibit the efflux pumps that transported the free doxorubicin out of the cell, and preserved doxorubicin.

4.4 The in vivo tumor inhibition test

In order to investigate the tumor inhabitation efficacy of NPs, RKO tumor-bearing nude mice were treated with different formulations via the tail vein[5]. The mass of the tumor has been checked every other day. The result showed that the cetuximab group showed obvious tumor inhibition after the first dose, but then the tumor volume increased rapidly. The tumor volume of the group administrated with cetuximab-vc-DOX-NPs did not show sustainable growth, which means that cetuximab-vc-DOX-NPs has higher inhibition efficacy compared to cetuximab[5]. Although the tumor volume of the group treated with doxorubicin is similar to the cetuximab-vc-DOX-NPs group, the mice treated with free doxorubicin underwent a significant loss of weight and 20% of mice were dead after three administrations[5]. On the other hand, mice treated with cetuximab modified NPs only lost a small amount of weight, and they could survive for 20 days after administration[5]. Thus, this in vivo test suggested that cetuximab-vc-DOX-NPs improved both the inhibition efficacy and the anticancer drug safety.

5. Conclusion

As the largest class of therapeutic proteins and a key driver in biopharmaceutical growth, monoclonal antibodies have many attractive advantages. With the combination of nanoparticles, it can provide powerful and long-lasting anti-cancer responses. This paper showed two strategies that link mAb with NPs and those NPs systems for drug delivery are multifunctional, with a reduced side effect and promoted synergistic therapeutic effects. Although NP linking with mAb showed great potential, there are still some disadvantages with this therapy. For example, the tumor accumulation is only marginally improved with positive targeting. Future studies may focus on using ultrasound-activated nanoparticles or other positive-activated nanoparticles to link with mAb to improve tumor accumulation. To sum up, the combination of ADC and NP delivery system has great potential and the convergence of these two disciplines will surely generate substantial momentum for improving cancer treatment.

References


