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Transferrin Receptor Serves as a Potential Target for Cancer Therapy

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Abstract: Transferrin receptor (TfR) is a glycoprotein that transfers iron from the extracellular matrix to the intracellular environment. Due to the rapid proliferation need, the demand for iron in cancer cells is much greater than in normal cells, possibly explaining the upregulated TfR expression in cancer cells. The overexpression of TfR and its extracellular accessibility, ability to internalize, and central role in cancer cell make this receptor a potential target for antibody-mediated therapy. The TfR can be targeted indirectly by antibodies conjugated to anti-cancer agents or directly through the use of antibodies that disrupt the function of the receptor and induce Fc-mediated effector functions. This article reviews the developments of antibody-based cancer therapy targeting TfR.

Keywords: Transferrin receptor, Malignant cells, Iron transfer

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1. Introduction

The limitation of individualized dosing, systemic side effects, and multidrug resistance all limit the effectiveness of tumor treatment, thereby affecting the quality of life of patients and limiting the remission rate. It is evident that the existing cancer therapy strategies are unable to adequately treat drug-resistant tumors[1]. Therefore, targeted drug delivery that can enhance selective transportation of the drug and cellular uptake is introduced so as to reduce systemic toxicity and improve disease efficacy.

Transferrin receptor (TfR) is a homodimeric protein that serves as a key regulator of cellular iron homeostasis and cell proliferation[2,3]. TfR interacts with iron-loaded transferrin (Tf) to import iron into the cell. Iron is a cofactor of intracellular enzymes, including the ribonucleotide reductase (RNR), that are coupled to DNA synthesis and, thus, required for cellular proliferation[2,3]. A number of studies have pointed out that TfR expression in metastatic drug-resistant tumors is upregulated, highlighting the selectivity of this protein receptor for cancer[2,3]. Interestingly, TfR has also been shown to increase cancer cell survival and play pivotal roles in tumor growth[2,3].

TfR is considered for targeted therapy of cancer since it is upregulated on the cell surface of many cancer types and can be efficiently internalized[1]. This receptor can be targeted in two ways: (i) Delivery of therapeutic molecules into malignant cells and (ii) blockade of the natural function of the receptor leading directly to cancer cell death[1-3]. In this article, we review the targeting of TfR for delivery purposes and the use of this approach to anti-cancer therapy, with special emphasis on the developments in the use of antibodies targeting TfR.

2. TfR

TfR is 180 kDa in size. It is a type II transmembrane glycoprotein formed by two subunits with a size of about 90 kDa cross-linked by two disulfide bonds[4]. TfR plays an important role in the biological activities and metabolic processes[5,6]. The process of iron uptake by cells needs to be mediated by TfR. Processes such as DNA synthesis, periodic proliferation of cells, immune regulation, and electron transport chain in the cell require iron ions for normal and orderly procession[6].

The binding of Tf to TfR jointly assists the transport of iron ions into cells[7], indicating the important role of TfR in the intracellular iron balance and the regulation of cell growth[7]. Meanwhile, cellular iron also regulates the expression of TfR on the cell surface[7]. TFR is not usually expressed in normal vasculature, with the exception of normal brain vascular endothelium, where it allows transport of Tf and iron into tissues. This might suggest a similar function of TfR in DNA synthesis and proliferation of cancer cell[7,8]. Due to this, TfR has received great attention in cancer metabolism-related research[8].

3. TfR expression in tissues

TfR exists in normal tissues, but it is highly expressed in organs and tumors of the digestion, urinary, and reproductive systems[9]. It is generally believed that the expression of TfR is spontaneously increased in metabolically vigorous cells (such as tumor cells) to cater to the high metabolism needs of the cells, and thus, iron ions are actively endocytosed[9]. The expression level of TfR can also be used to indicate the prognosis of certain tumors[10]. The previous studies have found that the expression level of TfR in tissues is closely related to the TNM staging of tumors and the long-term prognosis of patients[10-13]. In a series of cancer cases, the correlation between the expression of TfR and the degree of malignancy has also been repeatedly demonstrated in transitional cell carcinoma of the bladder, breast cancer, glioma, and lung adenocarcinoma[10-13].

The upregulation of TfR expression in LIHC tissue and cancer stem-like cells (CSCs) derived from LIHC cell lines prompts us to investigate the roles of TfR1 in the regulation of CSCs[12]. TfR levels in renal cell carcinoma, particularly clear cell renal cell carcinoma, were significantly associated with adverse clinical prognostic features (i.e., anemia, lower body mass index, and smoking), worse tumor pathology (i.e., tumor size, stage, grade, multifocality, and sarcomatoid dedifferentiation), and worse post-adjustment survival outcomes[12,13]. So far, the connection between the expression of TfR and cancer severity has been established.

Therefore, although TfR1 is expressed at a low level in a broad variety of cells, it is expressed at higher levels in rapidly proliferating cells, including malignant cells in which overexpression has been associated with poor prognosis[1,2].

4. The value of TfR in the early diagnosis of tumors

TfR has features such as increased expression and strong specificity in specific tissues, and is not prone to allosteric modulation or shedding. Thus, it is considered to be an effective tumor marker[14]. In a previous study, the use of anti-TfR monoclonal antibody to scan the pancreatic cancer mouse model revealed that the immune response of TfR in the animal model was significant, suggesting that high level of TfR expression is present in the pancreatic cancer model[15]. Isotope-labeled TfR reacts strongly with Tf on the tumor cell membranes with antigen-antibody reaction[15]. Therefore, isotope-labeled TfR is expected to be used in auxiliary method for non-invasive diagnosis of cancer in the early stage.

5. TfR cytotoxicity and related mechanisms

TfR does not only influence cell growth and proliferation but also may affect apoptosis and autophagy[16]. A previous study demonstrated that silencing TfR through small interfering RNA knockdown approach can significantly enhance cancer cell apoptosis and reduce cell proliferation[16]. It is currently believed that TfR may be related to the two major pathways of apoptosis, both exogenous and endogenous. TfR may interact with classic apoptosis factors such as caspase-3, BAX, and BCL-2[17]. From the results, the toxic effects caused by the silencing of TfR on the cancer cells cannot be completely explained by the mechanisms of necrosis, apoptosis, or autophagy; therefore, the mechanism of TfR onset requires further investigations.

6. Related applications of targeted drug administration in anti-tumor therapy targeting TfR

Multidrug resistance after chemotherapy is a common phenomenon in cancer progression. There are two common drug resistance mechanisms: (i) The impaired transmission of anticancer drugs to tumor cells and (ii) genetic or epigenetic changes in malignant cells that affect drug sensitivity[18]. Tf-targeted cancer therapy has been shown to have the ability to overcome the above-mentioned resistance mechanisms.

TfR has an important function in iron transportation. Monoclonal antibodies that specifically antagonize TfR can inhibit receptor activity and interfere with tumor cell growth[19]. Anti-Tf antibodies are actively used in targeted drug delivery strategies. The overexpressed TfR in the brain endothelial cells delivered therapeutic drugs through the blood–brain barrier to the targeted part of the brain[19,20]. Compared with non-targeted carriers, the binding of Tf significantly improves the stability and drug accumulation of nanoparticles in brain tissue. In another study, a Tf-modified PEG-PLA nanoparticle was used to encapsulate

Adriamycin[21]. Compared with free Adriamycin and non-targeted carriers, Tf-modified nanoparticles have significantly better biodistribution *in vivo* and tumor growth inhibition[22]. The rat anti-TfR monoclonal antibody (Ox26) is one of the important drug transport carriers. Ox26 carrying the drug recognizes and binds to TfR on the brain capillary endothelial cell membrane, and the drug passes through the blood–brain barrier through the receptor[23].

Gene mutations and epigenetic changes that lead to tumor insensitivity to chemotherapy drugs are the main limitations of current cancer treatment strategies. Active targeting using Tf has been shown to be a very effective treatment strategy to reverse multidrug resistance[24,25]. Scientists have been actively developing ligands targeting TfR, including Tf, antibodies, and aptamers, and the chemotherapy drugs were loaded into these ligands to construct a targeted delivery system for targeted therapy of tumors that can improve the therapeutic effect. At the same time, the targeted system reduces the damage to normal cells[26].

7. Targeted administration of Tf combined with dihydroartemisinin

Dihydroartemisinine (DHA), which is an important derivative of artemisinin, can achieve anti-tumor effects by affecting iron metabolism and reactive oxygen species. Dihydroartemisinin contains a peroxide group that acts like hydrogen peroxide, which elicits an oxygen radical reaction. With the help of the peroxide group, the DHA reacts with divalent iron through oxygen radical reaction to turn it into trivalent iron, which complexes with Tf before cell entry. When the iron complex in the cell culture medium increases, the ability of DHA to kill tumor cells is enhanced[27]. By forming a covalent complex with TfR, iron-dependent DHA can achieve targeted killing of tumor cells[28]. DHA was assumed to promote cell death through apoptosis or autophagy[28,29]. It is currently believed that DHA can promote the programmed death of tumor cells through apoptosis[30]. Other studies have suggested that the combined effect of TfR and DHA can induce autophagy (non-apoptosis) in malignant glioma cells and inhibit the autophagy signaling pathway, which significantly affects the anti-tumor effect of DHA[31]. The anti-tumor effect of TfR combined with DHA is characterized by the inhibition of tumor cell growth and the induction of apoptosis. The toxic effect of DHA coupled with TfR on the tumor cells is much greater than that on normal cells, indicating the selective cytotoxicity of this combination on cancer cells[31].

8. Prospect of TFR-mediated targeted cancer therapy

As a cofactor, iron affects RNR, which is involved in DNA synthesis. In the process of iron transport, Tf first binds to Fe^{3+} ion. Then, Tf-Fe^{3+} complex binds to TfR, and vesicles are formed at the same time. The latter are endocytosed by the cells, which obtain iron ions from the endocytic vesicles. Subsequently, the cell excretes the Tf-TfR complex, thereby completing a round of iron transport[32]. Treatment of glioblastoma multiforme (GBM) is a predominant challenge in chemotherapy due to the presence of blood–brain barrier that restricts the delivery of chemotherapeutic agents to the brain along with the problem of drug delivery through hard parenchyma of the GBM[32,33]. However, overexpression of TfRs on the GBM cell surface coincidentally provides a solution to overcome these problems so as to deliver chemotherapeutic agents to within the tumor[32,33]. Therefore, TfR-mediated targeted drug delivery could counteract drug delivery issues in GBM to effectively deliver ligand-conjugated drug complexes across the blood–brain barrier by means of receptor-mediated transcytosis[32,33].

At present, TfR has become a new research target in the field of gene therapy[32,33]. It was proposed that TfR can be compounded with chemotherapeutic drugs[33]. As cancer cells internalize a large amount of Fe, chemotherapeutic drugs can be targeted on tumor cells[33]. Studies have reported that the TfR combined with artemisinin (artemisinin-TfR) can significantly reduce drug resistance and resistance of chemotherapeutics in small cell lung cancer[34].

At the same time, research findings show that TfR has the function of immune regulation. TfR is also found to be highly expressed in human glomerular mesangium and as a receptor of IgA, sparking speculation that TfR may be related to IgA deposition[11]. Therefore, TfR may regulate immunity, cell apoptosis, and proliferation by binding to IgA in the mesangial region. In addition, TfR also regulates T-cell immune activation[35].

Due to the increased expression of TfR in brain gliomas, the TfR-coupled drug delivery system that is able to successfully deliver anticancer compounds to the tumor site and cross the blood–brain barrier has proven to be an important approach[11,35]. Direct binding and immunotoxin studies with Tf and anti-TfR antibodies as targets indicate that they are of great significance in the development of tumor-specific treatments[11,35]. This conjugate enhances cellular uptake through a Tf-mediated mechanism and increases the selective cytotoxicity of many cancer cell lines and tumor xenograft animal models[11,35]. In addition, it has been demonstrated that culturing drug-resistant cell lines with TfR targeting conjugates *in vitro* led to the reversal of the drug resistance[11,35]. The immunotoxin of TfR also displayed anti-cancer effects[11,35]. The diphtheria toxin mutant that covalently binds to Tf (Tf-CRM107) for the treatment of glioblastoma is currently under clinical trials[11,35]. Nevertheless, since the preliminary research findings cannot be directly translated for use in the clinical settings, new targeting strategies, including the use of nanoparticle in the design of drug delivery systems, have to be explored.

TfR has an instrumental role in the transport of iron, which is required for electron transport chain, DNA

synthesis, cell cycle, and immune regulation[36]. At present, it is hypothesized that TfR may eliminate autologous tumor self-seeding and metastasis, affect passive cell immunity, and facilitate drug entry into cells through endocytosis mediated by TfR[37]. Therefore, the application of TfR as part of the targeted anti-cancer therapy deserves further investigations.

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Conflicts of interest

We declared no conflicts of interest.

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