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Research Progress of Base Reducing Enzyme Responsive Prodrug System and Its Potential in Cancer Treatment

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ABSTRACT

The core dilemma of cancer treatment is to achieve tumor targeted delivery of chemotherapy drugs, reduce toxic and side effects on normal tissues, and improve the enrichment and activation efficiency of drugs in tumor sites. As a kind of oxidoreductase with high specific expression in tumor tissues and low or no expression in normal tissues, it has become an ideal target for the design of prodrug systems. This paper systematically reviews the types, characteristics and expression mechanism of base reducing enzymes in tumor tissues, describes in detail the design principle, structural types and latest research progress of these prodrug systems, deeply analyzes their application advantages and potential in tumor treatment, and discusses the current research bottle neck, so as to provide comprehensive theoretical reference and practical guidance for follow-up research in this field.

KEYWORDS

Base reducing enzyme; Responsive prodrugs; Cancer treatment; Targeted delivery; Drug activation; Research progress

1. INTRODUCTION

Malignant tumors threaten human health, killing more than 10 million people every year and the incidence is rising. At present, the main means of tumor treatment are surgery, chemotherapy, radiation, immunotherapy and so on, and chemotherapy is indispensable in advanced and postoperative adjuvant therapy. However, traditional chemotherapy drugs lack tumor tissue specificity, damage normal proliferating tissues when killing tumor cells, cause toxic and side effects, and reduce patient compliance and quality of life. In addition, chemotherapeutic drugs have low enrichment efficiency, fast metabolism, difficult to reach effective concentration, easy to induce drug resistance and limit the effect of chemotherapy. In order to solve these problems, targeted drug delivery systems have emerged, and responsive prodrug systems have become a research focus because of "intelligent identification and precise activation". Prodrugs are non or low activity derivatives modified by drugs, which release active masterbatch under specific environment or enzymes in the body to achieve targeted delivery and controllable release. The characteristics of tumor microenvironment provide natural targets for the design of responsive prodrug systems [1].

Radical reducers are a class of oxidoreductases that rely on coenzymes such as NADH and NADPH. They can catalyze the reduction reaction of reduction sensitive groups such as nitro, azo and disulfide bonds to produce active products. Studies have shown that nitro reducing enzymes, azo reducing enzymes, thioredoxin reducing enzymes and other basic reducing enzymes are abnormally high expressed in malignant tissues such as lung cancer, breast cancer, colon cancer and liver cancer, but very low expressed in corresponding normal tissues. This difference is closely related to the hypoxia environment, oxidative stress and abnormal gene regulation of tumors. Based on this, researchers

have designed a series of base reducing enzyme responsive prodrug systems, which use highly active base reducing enzymes in tumor tissue to specifically activate prodrugs and release mother drugs to achieve precise killing of tumor cells and minimize damage to normal tissues [2].

In recent years, significant progress has been made in the research of base reducing enzyme responsive prodrug systems, and the structural design, activation mechanism, delivery vector optimization and in vitro and in vivo efficacy evaluation of prodrugs have been continuously improved. Combined with the latest research results at home and abroad, this paper comprehensively reviews the research progress of these prodrug systems and their potential in cancer treatment, so as to provide reference for their in-depth research and clinical transformation.

Biological characteristics of 2-base reducing enzymes and their expression mechanism in tumor tissues

2. TYPES AND CORE CHARACTERISTICS OF 1 BASE REDUCING ENZYMES

Radical reducers are a large family of enzymes, which are divided into many types according to substrate specificity, coenzyme dependence and structural characteristics. Nitro reducers (NTR), azo reducers (azr) and thioredoxin reducers (TrxR) are mainly related to tumor therapy and widely used. The core characteristics of various enzymes are different, but they have the common characteristics of high activity and specific catalytic reduction sensitive group cleavage in tumor tissues.

Nitro reducing enzyme is the most deeply studied and widely used radical reducing enzyme, which belongs to flavin dependent oxidoreductase. With NADH or NADPH as electron donor, it catalyzes the gradual reduction of nitroaromatic compounds to amino aromatic compounds, and generates nitroso and hydroxylamine groups in the middle. Hydroxylamine derivatives are highly toxic, which is the key to the activation of prodrugs. It is widely found in bacteria, fungi and mammalian cells, but the activity of mammals is low, and the activity of tumor cells is significantly increased due to metabolic and microenvironment changes, which can be increased several times to dozens of times under hypoxia. In addition, Mycobacterium tuberculosis's unique azoflavin dependent nitroredoxin DDN can activate the prodrug pretomanid, which provides a new idea for the synergistic treatment of tumor and infection complications [3].

The common characteristics of various base reducing enzymes are: low activity in normal tissues, weak catalysis to prodrugs, and avoiding toxic and side effects caused by premature activation of prodrugs; Due to the changes of micro environment and gene expression, the activity of tumor tissue is significantly increased, which can efficiently catalyze the cleavage of prodrug modification groups, release active mother drugs, and achieve tumor targeted therapy.

The mechanism of high expression of 2.2-base reducing enzymes in tumor tissues

The abnormally high expression of base reducing enzyme in tumor tissue is the core basis for its use as a prodrug targeting target, and its regulatory mechanism is complex, which is closely related to the hypoxia micro environment, the level of oxidative stress and abnormal gene regulation, among which the hypoxia micro environment is the most critical factor.

Tumor cells proliferate faster than blood vessels, resulting in insufficient blood supply to tissues and the formation of a hypoxic micro environment with an oxygen partial pressure of less than 5%, which is a typical feature of malignant tumors. The hypoxic microenvironment activates hypoxia inducible factor - 1 alpha (HIF - 1 alpha) and regulates downstream gene expression, including the gene for gene reduction. Under normal oxygen, HIF - 1 alpha was hydroxylated by prolyl hydroxylase and degraded by ubiquitin proteasome system, with low expression; During hypoxia, the activity of prolyl hydroxylase is suppressed, and a large amount of HIF - 1 alpha accumulates into the nucleus, binds to the hypoxia response element (HRE), and starts the transcription of the gene. The promoter region

of nitro reducing enzyme, azo reducing enzyme and thioredoxin reducing enzyme contains HRE sequences, which are specifically bound by HIF-1 alpha under hypoxia to promote their expression and enhance the activity of tumor tissue base reducing enzyme. For example, under hypoxia, the expression of nitroredoxin mRNA in tumor cells increased by 3 to 5 times and the activity increased by 5 to 10 times, providing an enzyme source for the specific activation of prodrugs. In addition, hypoxia inhibits cellular oxidative metabolism, enhances the activity of base reducing enzymes, and forms a positive feedback cycle of "hypoxia base reducing enzyme high activity" [4].

Abnormal gene regulation can also lead to high expression of base reducing enzymes. Mutation or abnormal expression of many tumor related genes can directly or indirectly regulate their expression. For example, p53 tumor suppressor gene mutation has a very high incidence in tumors. After mutation, it not only loses its tumor suppressor function, but also indirectly promotes the transcription of base reducing enzyme gene by regulating the expression of HIF-1 alpha; MiR-146a, miR-21 and so on are highly expressed in tumor tissues, which can target the 3'UTR region of binding group reducing enzyme gene, inhibit its mRNA degradation and increase its expression. At the same time, epigenetic modifications such as DNA methylation and histone acetylation will also affect its expression, and the level of DNA methylation in the promoter region of the gene of base reducing enzyme in tumor tissue will decrease, which will release the suppression of transcription and lead to its high expression [5].

To sum up, the abnormal hypoxia microenvironment and gene regulation of tumors together lead to the abnormal high expression of base reducing enzymes, which provides a solid biological basis for the design of base reducing enzyme responsive prodrug system to ensure the specific activation of prodrugs at tumor sites and achieve precise treatment.

3. RESEARCH PROGRESS OF BASE REDUCING ENZYME RESPONSIVE PRODRUG SYSTEM IN CANCER TREATMENT

In recent years, with the development of prodrug design, targeted delivery and molecular biology technology, significant progress has been made in the research of base reducing enzyme responsive prodrug system, from single prodrug molecular design to "prodrug+carrier" compound delivery system, from simple chemotherapy drug delivery to multimodal synergistic treatment, and the efficacy and safety in vitro and in vivo have been significantly improved. The following is detailed from both preclinical and clinical studies.

3.1. Preclinical Research Progress

Preclinical research is the basis for the clinical application of such prodrugs. At present, most systems are still in this stage, mainly focusing on cell and animal experiments of lung cancer, breast cancer, colon cancer, liver cancer, pancreatic cancer and other tumors, focusing on the activation efficiency, targeting, pharmacokinetics and efficacy safety of prodrugs.

In the treatment of lung cancer, nitro GEF, a nitro reducing enzyme responsive prodrug, can be specifically activated in lung cancer cells with high nitro reducing enzyme expression, releasing gefitinib to inhibit cell proliferation and migration, and IC_{50} is lower than that in the control group; Animal experiments can significantly inhibit the growth of lung cancer transplanted tumors in nude mice, with a tumor suppression rate of more than 70%, and do not affect liver and kidney function. Gemcitabine derivatives based on NQO1 response design, DHA and linoleic acid conjugate have selective toxicity to tumor cells, can induce apoptotic, ROS production and cycle arrest, but also retain antibacterial activity, providing new ideas for treatment. In terms of breast cancer treatment, azo reducing enzyme activated self monitoring prodrug developed by Kunming University of science and technology can monitor drug release in real time in 4T1 tumor bearing mouse model, significantly inhibit tumor growth, and mice have no significant weight loss and pathological damage to major

organs. Azo redoxin responsive prodrug azo doc loaded into liposomal can be enriched in breast cancer transplanted tumor sites in nude mice, with a tumor suppression rate of more than 75% and no significant damage to normal tissues, which is better than that in the control group. In the treatment of colon cancer, nitro-5 - Fu, a nitro redoxin responsive prodrug, has an activation efficiency of more than 85% in colon cancer cells, and the released 5 - fluorouracil can inhibit cell proliferation and induce cell death; Animal experiments can inhibit the growth of colon cancer transplanted tumors in nude mice, without gastrointestinal mucosal damage, and reduce toxic and side effects. Among the gemcitabine derivatives based on NQO1 responsiveness, 6 - HEPTENE derivatives have a significant effect on SW620 colon cancer cells, which can trigger G2/M phase arrest and provide candidate drugs for precise treatment. In the treatment of liver cancer, TrxR SOR, a thioredoxin redoxin responsive prodrug, loaded on liver cancer targeting nanoparticles, can actively target and enrich in the transplanted tumor site of liver cancer in nude mice, release sorafenib, with a tumor suppression rate of more than 80%, and reduce the damage to normal hepatocytes. In the treatment of pancreatic cancer, triptolide nano prodrugs activated by esterase and responsive to GSH target pancreatic tumors with the help of lactose acid. The release of drugs can reduce the tumor load by 99%, without obvious hepatorenal toxicity, and more than half of the treated animals achieve tumor free.

Preclinical studies have also found that such prodrug systems can enhance efficacy in conjunction with other treatments. For example, combined with PD-1/PD-L1 and other immune checkpoint inhibitors, prodrugs release tumor related antigens when killing tumor cells, activate the body's immune response, immune checkpoint inhibitors relieve the suppression of tumor cells on the immune system, and realize the synergy of chemotherapy and immunotherapy; Combined with PDT, prodrugs activate and release photosensitizers, produce ROS under light irradiation, and kill tumor cells in coordination with the parent drug; Exploring the combined targeting of base reducing enzymes and other targets such as CYP51 can enhance the efficacy and overcome drug resistance.

3.2. Clinical Research Progress

At present, some base reducing enzyme responsive prodrugs have entered the clinical research stage, mainly focusing on phase I and II, focusing on safety, tolerance and preliminary efficacy.

CB1954 is the first nitroredoxin responsive prodrug to enter clinical research, and has completed a number of phase I and phase II clinical studies. In the phase I study of patients with pancreatic cancer, CB1954 was administered after nitroredoxin gene was introduced into tumor tissue. The treatment regimen was safe and tolerable, with no serious adverse reactions. Some patients had shrunk tumors and a disease control rate of more than 40%; In the phase II study of patients with liver cancer, its combination with nitroredoxin gene therapy can prolong the progression free survival and overall survival of patients, with mild adverse reactions and good tolerance.

Th-302 is another nitro reducing enzyme responsive prodrug. The parent drug is bromoisoxazoles, which release the parent drug under tumor hypoxia environment and nitro reducing enzyme catalysis. It has completed phase I and phase II clinical studies of a variety of solid tumors. In the phase II study of patients with non-small cell lung cancer, th-302 combined with paclitaxel can improve the objective remission rate and disease control rate, prolong the progression free survival period, have good safety, and there is no significant difference in adverse reactions between th-302 and paclitaxel monotherapy.

Gs-4774 is a thioredoxin reducing enzyme responsive prodrug. The parent drug is a nucleoside compound with antiviral and anti-tumor activities. The prodrug catalyzes the cleavage of disulfide bonds by thioredoxin reducing enzyme to release the parent drug. Phase I clinical studies on melanoma patients show that the prodrug is safe and tolerable, and some patients have shrunk their tumors, showing preliminary therapeutic effects. Some of the reduction responsive polymer prodrugs designed based on glutathione reducing enzyme have entered phase II clinical research, laying the foundation for clinical transformation [6].

At present, there are still some problems in clinical research, such as insufficient activation efficiency of prodrugs, targeted need to be improved, and some patients are not sensitive to treatment, which need to be solved by optimizing the structure of prodrugs, improving targeted delivery technology, screening suitable patient populations and so on.

4. ADVANTAGES AND POTENTIAL OF BASE REDUCING ENZYME RESPONSIVE PRODRUG SYSTEM IN CANCER TREATMENT

4.1. Core Strengths

Compared with traditional chemotherapy drugs and other targeted delivery systems, the base reducing enzyme responsive prodrug system has five core advantages and significant application value.

First, it has strong targeting and low off target effect. Based on the difference in the expression of base reducing enzyme between tumor and normal tissue, the specific activation of prodrugs in tumor site is realized, the damage of normal tissue is avoided, and the problem of missed target of traditional chemotherapy is solved. For example, the activation efficiency of CB1954 in tumor tissue exceeds 80%, and the prodrug activated by azo reducing enzyme can avoid toxic and side effects. Second, controllable drug release can be achieved. The activation of prodrugs depends on the activity of base reducing enzyme in tumor tissue, which is positively correlated with tumor malignancy and hypoxia, and can achieve "on-demand release" of drugs to avoid toxic and side effects. For example, in the hypoxia environment of advanced tumors, the activation efficiency of prodrugs is improved. Thirdly, the pharmacokinetic characteristics are excellent and the bioavailability is high. Through chemical modification, the water soluble of prodrugs is optimized to prolong the circulation time in the body and avoid rapid metabolic clearance. No or low activity of prodrugs can reduce nonspecific distribution and improve bioavailability. Nitroheterocyclic compounds confirm their advantages. Fourth, it has a wide range of applications for a variety of tumor treatment. Many kinds of base reducing enzymes are highly expressed in malignant tumor tissues, and can be combined with a variety of mother drugs to design diversified prodrug systems. For example, nitro reducing enzyme response type is suitable for solid tumors, azo reducing enzyme response type is suitable for breast cancer and so on. Fifth, it can cooperate with other treatment methods to enhance the efficacy. It can cooperate with immunity, optodynamics and radiation to form a multimodal treatment strategy to overcome tumor drug resistance. If it cooperates with immune checkpoint inhibitors to activate immunity, the combination of base reducing enzymes and other targets can overcome drug resistance.

4.2. Application Potential

Based on the above advantages, such prodrug systems have broad application potential in cancer treatment, and are expected to become an important means of precision treatment in the future, mainly reflected in five aspects.

First, for the treatment of advanced tumors. Advanced tumors have high malignancy, severe hypoxia, strong drug resistance, poor efficacy and side effects of traditional chemotherapy. This kind of prodrug system takes advantage of the characteristics of high activity of advanced tumor base reducing enzyme, efficiently activates prodrugs, releases a large number of mother drugs, reduces toxic and side effects, improves tolerance, and provides new choices for advanced patients. For example, th - 302 has a good effect in the clinical study of advanced non-small cell lung cancer and pancreatic cancer; Azoredoxin activated diagnostic and therapeutic prodrugs provide a new strategy for chemotherapy of advanced breast cancer.

Second, it is used for adjuvant therapy after tumor surgery. Postoperative tumor recurrence affects the prognosis of patients, and traditional adjuvant chemotherapy has great toxic and side effects. The prodrug system is targeted for delivery, specifically activates at the residual part of the tumor, kills

the residual cells, reduces the risk of recurrence, avoids normal tissue damage and promotes patient recovery. For example, CB1954 combined with nitroredoxin gene therapy can be used for postoperative adjuvant therapy of liver cancer and pancreatic cancer.

Third, it is used for personalized treatment of tumors. There are differences in the expression and activity of base reducing enzyme in tumor tissues of different patients. Detecting its expression level can screen suitable patients and avoid ineffective treatment and unnecessary side effects. It can also personalize the prodrug molecules and design an appropriate prodrug system to enhance the efficacy. Efficient nitroaryl groups provide support for the development of personalized fluorescent probes and prodrugs.

Fourth, it is used for combined treatment of tumors. As the core of combination therapy, it can cooperate with a variety of treatment methods to provide more effective solutions. For example, it cooperates with PD - 1 inhibitors to achieve synergy between chemotherapy and immunotherapy, and with PDT to improve the cure rate; Multifunctional prodrugs provide the possibility of personalized treatment for patients with cancer and infection.

Fifth, promote the clinical transformation of cancer treatment. Some prodrugs have entered clinical research, and with the optimization of technology, more prodrugs will achieve clinical transformation. For example, th - 302 is expected to be approved for listing, and CB1954 combined gene therapy is expected to become an important means of precision therapy; The glutathione reducing enzyme responsive polymer prodrug has entered the clinical phase II, laying the foundation for clinical transformation.

5. OUTLOOK

In view of the current research bottleneck, combined with the latest trends at home and abroad, the field will focus on five key points in the future to optimize the performance of prodrugs, enhance efficacy and promote clinical transformation. First, optimize the structure of prodrugs, screen new reduction sensitive groups, optimize the connecting chain, modify the mother drug molecules, study enzyme drug interactions, and improve the activation efficiency. The second is to build a "active targeting+passive response" dual delivery system, which combines prodrugs with carriers to achieve tumor site enrichment and activation and reduce the off target effect. Third, optimize the pharmacokinetic characteristics, solve the problems of poor water soluble and short circulation of prodrugs by modification, pay attention to the metabolism of polymer prodrugs, and reduce organ accumulation. Fourth, explore multimodal synergistic therapy, study the synergistic mechanism between prodrugs and other therapies, and design dual target prodrugs to overcome drug resistance. Fifth, we should improve clinical research and testing technology, carry out phase III research, clarify patient screening criteria, develop noninvasive testing technology, strengthen large-scale production and research and development, and promote clinical transformation.

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