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LYTAC-mediated Degradation of Key Proteins in Hepatocellular Carcinoma and its Potential Clinical Application

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Abstract: Lysosome-Targeting Chimeras (LYTAC) technology, with its unique mechanisms and advantages, can efficiently and specifically degrade intracellular, extracellular, and membrane proteins, offering a novel approach for hepatocellular carcinoma (HCC) treatment. This article reviews LYTAC-mediated degradation strategies targeting key proteins in HCC and their potential clinical applications. LYTAC technology employs targeting ligands to bind critical HCC proteins and directs them to lysosomes for degradation. Research has shown that this technology can effectively degrade key proteins such as heparanase (HPA1) and the epidermal growth factor receptor (EGFR), thereby inhibiting tumor cell proliferation, invasion, and metastasis while enhancing immune cell recognition and cytotoxicity against tumors. Furthermore, LYTAC technology can be combined with immunotherapy, chemotherapy, and other treatments to improve drug targeting, modulate the tumor microenvironment, and facilitate personalized medicine approaches.

Keywords: Clinical Application; LYTAC; Key Proteins; Hepatocellular Carcinoma; HPA1; EGFR.

1. Introduction

The abnormal expression or aggregation of protein is often closely related to the occurrence and development of many diseases, among which liver cancer, as a common malignant tumor, involves the abnormal regulation of many key proteins in its pathogenesis. Traditional treatment methods for liver cancer, such as surgery, chemotherapy and radiotherapy, can delay the progress of the disease to a certain extent, but they are often accompanied by greater side effects and recurrence risks. Therefore, it is particularly important to explore safer and more effective treatment methods for liver cancer.

Targeted protein degradation (TPD) technology, as a new therapeutic strategy, has gradually attracted extensive attention in scientific research. This technology can restore the normal physiological balance of cells by specifically degrading pathogenic proteins, which provides a new idea for disease treatment. Among them, LYTAC (Lysosome Targeting Chimera) technology, as an important part of TPD technology, shows great potential in the field of liver cancer treatment with its unique mechanism and advantages. LYTAC technology realizes efficient and specific degradation of target protein by using the lysosomal degradation mechanism of cells themselves. Compared with other protein degradation technologies, LYTAC technology can not only degrade intracellular proteins, but also act on extracellular and membrane proteins, and has high safety and low toxic and side effects. Therefore, the application of LYTAC technology to the degradation of key proteins of liver cancer is expected to provide a new and more accurate means for the treatment of liver cancer.

As a malignant tumor with high mortality, traditional treatment methods have some problems, such as low resection rate, high chemotherapy resistance and large side effects of radiotherapy, which leads to a 5-year survival rate of patients below 20% and a postoperative recurrence rate of over 50%. TPD technology provides a new idea for treatment, especially LYTAC technology, which degrades specific proteins through endocytosis-lysosomal pathway mediated by cell membrane receptors and realizes accurate delivery by tissue-specific receptors. Because of the high expression of ASGPR in hepatocytes and almost no expression in other organs, LYTAC system based on ASGPR can effectively reduce the risk of off-target. In contrast, CI-M6PR is widely expressed in multiple tissues, which easily leads to nonspecific uptake, thus limiting its application. LYTAC technology can efficiently degrade liver cancer-related signaling pathway proteins in preclinical models, and its toxicity can be controlled, demonstrating its ability to target intracellular, extracellular and membrane proteins simultaneously. The current research focuses on optimizing receptor adaptability, improving lysosomal escape efficiency and developing bifunctional molecular library, aiming at solving the problems of insufficient stability in vivo and fluctuation of delivery efficiency.

This paper reviews the new strategy of LYTAC-mediated degradation of key proteins in liver cancer and its potential clinical application. By deeply analyzing the principle, development history, technical characteristics and application prospect of LYTAC technology in the treatment of liver cancer, it provides new ideas and directions for accurate treatment of liver cancer. At the same time, this paper will also discuss the challenges and future research direction of LYTAC technology before clinical application, and provide useful reference for the further development and application of LYTAC technology.

2. Overview of LYTAC Technology

The core principle of LYTAC technology is to use the lysosomal degradation mechanism of cells to achieve efficient and specific degradation of specific target proteins. Lysosome is an important organelle in cells, which contains many kinds of hydrolases and can decompose and digest various substances in cells. By ingenious design, LYTAC

molecules can specifically bind to the target protein and guide it into lysosomes, so that the target protein can be degraded into small molecules such as amino acids by hydrolases in lysosomes and finally excreted (Figure 1).

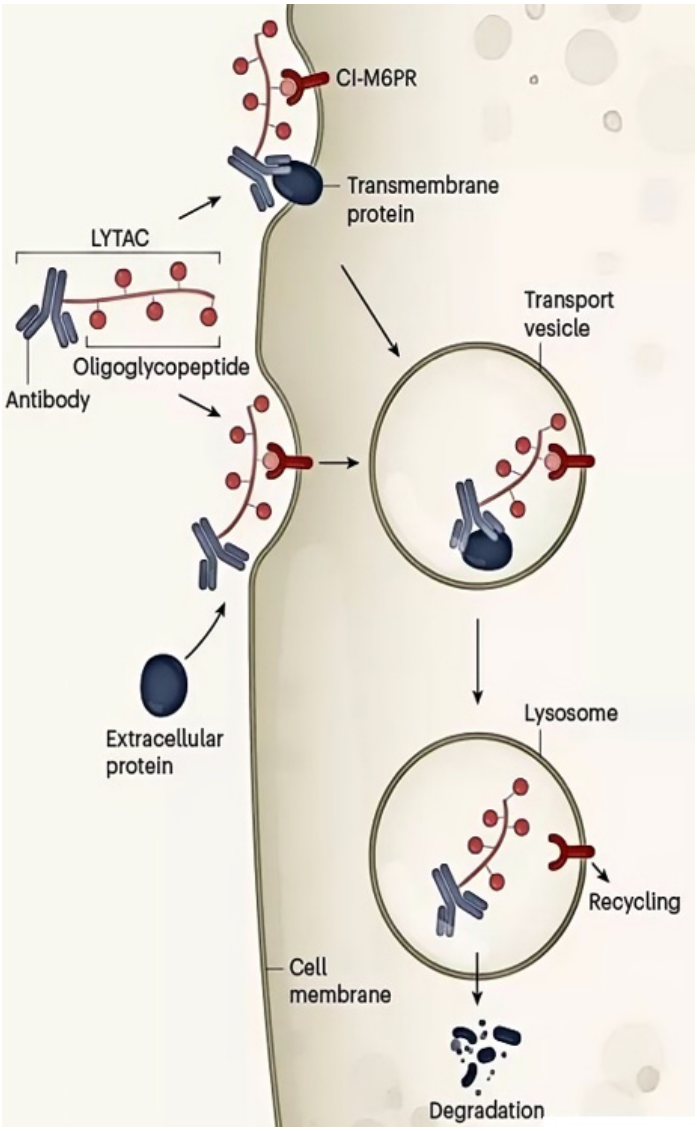

Figure 1. Mechanism of LYTAC (Source: Nature Reviews Drug Discovery)

The development of lysosomal degradation technology can be traced back to the discovery of lysosomes. Since 1950s, scientists have gradually realized the important role of lysosomes in the degradation of protein, and began to explore the methods of using lysosomes to degrade specific protein. With the continuous progress of biotechnology, LYTAC technology came into being, which combined the knowledge of biology, chemistry and pharmacy to form a brand-new protein degradation strategy.

LYTAC technology has obvious advantages and characteristics compared with other protein degradation technologies. LYTAC molecules can specifically bind to the target protein, achieving efficient and selective degradation of the target protein and avoiding the side effects caused by non-specific degradation. LYTAC technology can not only degrade intracellular proteins, but also act on extracellular and membrane proteins, expanding its application scope. LYTAC molecules usually have good stability and biocompatibility, which can maintain activity in vivo for a long time and reduce the damage to normal cells. As a new treatment strategy, LYTAC technology provides new ideas and means for disease treatment and has broad application prospects.

3. Application of LYTAC in Degradation of Key Proteins in Hepatocellular Carcinoma

3.1. Key Protein Selection

Heparanase (HPA1) and the epidermal growth factor receptor (EGFR) both play crucial roles in hepatocellular carcinoma (HCC). HPA1, acting as a β-D-glucuronidase, cleaves the side chains of heparan sulfate proteoglycans, facilitating tumor cell infiltration, metastasis, and angiogenesis. EGFR, as a transmembrane protein, stimulates cell proliferation, differentiation, and survival by activating downstream signaling pathways. The overexpression of these proteins in HCC enhances the physical invasiveness and proliferative processes of the tumor. Therefore, HPA1 and EGFR are ideal candidates for targeted degradation using LYTAC technology, which could effectively suppress the progression of HCC, reduce its invasiveness, and enhance the recognition and cytotoxic efficiency of immune cells against the tumor [1].

LYTAC technology is a new protein degradation method

specially used to degrade cell surface proteins. In the field of liver cancer treatment, this technology is used to specifically eliminate protein, such as HPA1 and EGFR, which are crucial to the cancer process. The JW-9 compounds developed by a research group can degrade HPA1 through LYTAC mechanism, which not only inhibits the invasion and metastasis of liver cancer cells, but also enhances the immune response by enhancing the activity of natural killer cells [2]. For EGFR, although the existing research is limited, the targeted degradation pathway provided by LYTAC technology may effectively curb the progress of HCC, reduce the side effects of traditional inhibitors, and improve the safety and efficacy of treatment. Therefore, LYTAC technology provides an innovative and potential new strategy for the treatment of liver cancer.

3.2. Degradation Strategy

(1) Design of LYTAC molecule

LYTAC molecules consist of two components: a targeting ligand and a lysosome-targeting peptide (LTP). The targeting ligand is designed based on the structural and functional characteristics of key proteins in liver cancer, enabling it to specifically recognize and bind to the target protein. LTP is responsible for guiding the target protein to lysosomes. The selection of targeting ligands is the key to the molecular design of LYTAC. For the key proteins of liver cancer, researchers usually choose appropriate antibody fragments, small molecular compounds or peptide fragments as targeting ligands according to the characteristics of their cell surface receptors or intracellular binding sites. For example, an antibody fragment with high affinity to a specific receptor protein on the surface of liver cancer cells can be designed as a targeting ligand [3]. LTP usually contains some amino acid sequences that can be recognized by specific receptors on lysosomal membranes. These sequences can bind to the receptor on lysosomal membrane in cells, thus guiding the target protein to lysosomes. By optimizing the amino acid sequence of LTP, the researchers improved its binding affinity and specificity with lysosomal membrane receptors.

(2) Synthesis of LYTAC molecule

LYTAC molecules are usually synthesized by chemical coupling. Firstly, the targeting ligand and LTP were synthesized, and then they were connected by chemical bonds. Commonly used chemical bonds include covalent bonds and amide bonds. In the synthesis process, it is necessary to strictly control the reaction conditions to ensure the efficiency and stability of the connection between the targeted ligand and LTP.

Besides chemical synthesis, biosynthetic technology can also be used to prepare LYTAC molecules. For example, the target ligand and the gene sequence of LTP are linked together by genetic engineering technology, and then expressed and synthesized in cells. Biosynthesis method has higher synthesis efficiency and better biocompatibility, but it needs to solve the problems of gene expression regulation and post-processing.

(3) Binding mechanism with target protein

When LYTAC molecules enter cells, the targeting ligand first binds specifically to the key proteins of hepatocellular carcinoma. The binding between the target ligand and the target protein is mainly achieved by non-covalent interactions such as hydrogen bonding, electrostatic interaction and hydrophobic interaction. This specific binding ensures that LYTAC molecules can accurately recognize and bind key proteins of liver cancer without cross-reaction with other normal proteins. After the target ligand binds to the target protein, LTP begins to play its role. LTP can bind to a specific receptor on lysosomal membrane, such as mannose -6-phosphate receptor (M6PR). LTP leads the target protein-targeting ligand complex to lysosomes by binding to M6PR. In lysosomes, the target protein is degraded into small peptides by hydrolases in lysosomes, and finally eliminated by cell metabolism.

3.3. Experimental Verification

(1) In vitro experiment

Researchers have verified the degradation effect of LYTAC technology on key proteins in hepatocellular carcinoma cell lines [4]. By co-incubating LYTAC molecules with hepatocellular carcinoma cells, it was found that the expression level of target protein decreased significantly. For example, in a study, the overexpression of protein X in liver cancer cells was reduced by about 70% within 24 hours after treatment with specific LYTAC molecules [5]. This shows that LYTAC molecules can effectively guide the target protein to lysosomes for degradation.

In vitro experiments, the researchers also verified the degradation mechanism of LYTAC molecules through a series of experiments [6]. For example, after cells were treated with lysosomal inhibitors, it was found that the degradation of target protein was inhibited, which further proved that LYTAC molecules degraded target protein through lysosomal pathway. In addition, by means of immunoprecipitation experiments, the researchers also found that there was a specific binding between LTP and lysosomal membrane receptors, thus confirming the key role of LTP in the degradation of target proteins.

(2) In vivo experiment

In the animal model, the researchers injected LYTAC molecules into mice with liver cancer to observe its inhibitory effect on tumor growth [7]. The experimental results showed that the tumor volume of mice treated with LYTAC molecule was significantly smaller than that of the control group, and the expression level of target protein in tumor tissue was significantly reduced. For example, in one study, the tumor volume of liver cancer mice treated with LYTAC molecules decreased by about 50% within 3 weeks after treatment, while the tumor volume of control mice continued to increase. This shows that LYTAC technology can effectively degrade the key proteins of liver cancer in vivo, thus inhibiting tumor growth.

In vivo experiments, the researchers also evaluated the safety of LYTAC molecules [8]. By observing the weight changes and blood indexes of mice, it was found that LYTAC molecules had no obvious effect on the normal physiological functions of mice at the therapeutic dose and did not cause obvious toxic reactions. This provides a preliminary safety basis for the clinical application of LYTAC technology.

4. Potential Clinical Application of LYTAC-mediated Degradation of Key Proteins in Hepatocellular Carcinoma

4.1. Enhance the Effect of Immunotherapy

HPA1 is an enzyme overexpressed in hepatocellular carcinoma cells, which promotes tumor invasion, metastasis

and immune escape by degrading heparin sulfate in extracellular matrix. Using LYTAC technology, scientists have designed specific bifunctional molecules, which can connect HPA1 with lysosomal target receptors, thus guiding it into lysosomes for degradation. This process not only reduces the activity of HPA1, but also increases the abundance of HSPGs on the surface of liver cancer cells, activates natural cytotoxic receptors (NCRs) on NK cells, and enhances the ability of NK cells to recognize and kill liver cancer cells.

In addition to HPA1, other immunosuppressive molecules such as PD-L1 and CTLA-4 also play an important role in the process of liver cancer. LYTAC technology can also be used to specifically degrade these molecules, relieve their inhibitory effect on immune cells, improve the tumor microenvironment, and change from an immunosuppressive state to a state more conducive to immune attack. This improved microenvironment can enhance the effect of other immunotherapy, including immune checkpoint inhibitors and CAR-T cell therapy. The combined use of LYTAC technology and existing immunotherapy methods may form a comprehensive treatment plan, further improving the survival rate and quality of life of patients with liver cancer.

4.2. Improve Drug Targeting

To improve drug targeting, scientists have utilized LYTAC technology to design molecules that target specific receptors on the surface of liver cells, such as the asialoglycoprotein receptor (ASGPR), achieving efficient degradation of specific proteins in the liver while minimizing effects on other tissues (Figure 2). The study conjugated a binder for ASGPR, trivalent N-acetylgalactosamine (tri-GalNAc), to a binder for the target protein via a linker. Research has confirmed that using GalNAc-LYTACs to degrade EGFR attenuates EGFR signaling.

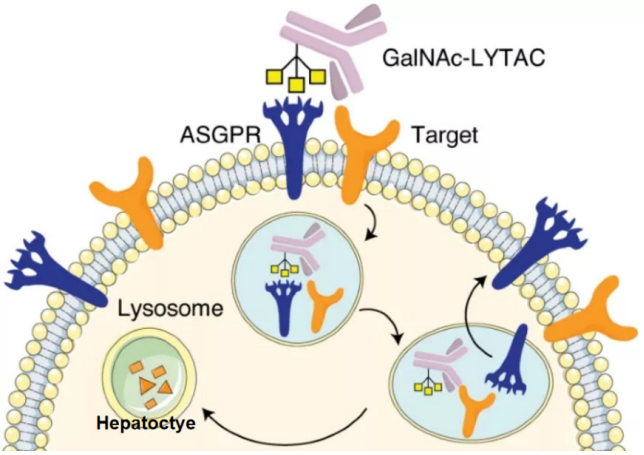

Figure 2. GalNAc-LYTAC (source: Nature Chemical Biology)

The combination of LYTAC technology and traditional therapy, such as restoring the sensitivity of chemotherapy drugs by degrading drug-resistant proteins, further improved the therapeutic effect. In clinical application, LYTAC technology is expected to support personalized medicine and customize the treatment plan for patients' specific tumor markers; Combined with surgery, radiotherapy, immunotherapy, etc., a multi-mode treatment strategy is formed; As a new drug development platform, accelerate the research and development process of anticancer drugs.

4.3. Individualized Therapy

LYTAC technology has shown great potential in individualized treatment of liver cancer. According to the specific antigens of patients' liver cancer cells, such as AFP and GPC3, high affinity antibody fragments were screened and designed by genomics and protein genomics, and then LYTAC-T cells were constructed. These specially designed T cells can be infused into patients, specifically bind to liver cancer cells and activate the killing effect of T cells on cancer cells. This method not only improves the pertinence and effectiveness of treatment, but also reduces the influence on normal tissues and side effects because of its high specificity. It also inhibits the proliferation of tumor cells by continuously degrading key proteins, which is helpful to overcome the drug resistance problem.

5. Challenges and Future Prospects

Although LYTAC technology shows great potential in targeted protein degradation, its practical application still faces many challenges. These include ensuring the stability and specificity of LYTAC molecules in complex environment in vivo to reduce non-specific degradation and side effects; Overcome the mechanism of target protein escaping lysosomal degradation, optimize the molecular structure of LYTAC to improve the fusion efficiency and degradation rate with lysosomes; And before clinical application, strictly evaluate the safety and effectiveness of LYTAC technology to ensure that it is non-toxic or low toxic to normal cells, and verify its efficacy through clinical trials, which requires a lot of time and resources.

Optimizing the molecular design of LYTAC by structural biology and computational chemistry can improve its stability and specificity, and develop molecules with higher degradation efficiency and lower side effects. With the in-depth study of disease mechanism, the constantly discovered new targets provide more application opportunities for LYTAC technology, which is expected to open up new therapeutic approaches. The combination of LYTAC technology with immunotherapy, chemotherapy and other therapies can improve the therapeutic effect and provide new ideas for refractory diseases. Strengthening cooperation with clinicians and verifying its safety and effectiveness through clinical trials will promote the transformation of LYTAC technology into clinical application and bring new hope to patients.

6. Conclusion

LYTAC technology shows the effective degradation ability of key proteins of liver cancer such as HPA1 and EGFR by specifically guiding the target protein into lysosomes for degradation. In vitro and in vivo experiments have proved that LYTAC molecule can significantly reduce the expression level of key proteins in hepatocellular carcinoma cells, and then inhibit tumor growth. The application of LYTAC technology is not only limited to the direct degradation of tumor-related proteins, but also has the potential to enhance the effect of immunotherapy, improve drug targeting and support personalized treatment. By combining with existing immunotherapy methods such as immune checkpoint inhibitors and CAR-T cell therapy, LYTAC technology is expected to improve tumor microenvironment and improve the efficiency of immune cells in identifying and killing tumors. In addition, this technology can also be used to design molecules targeting specific hepatocyte surface receptors, so as to achieve efficient degradation of specific proteins in the

liver and reduce the impact on other tissues. Although LYTAC technology has shown great potential in targeted protein degradation, its practical application still faces challenges, including ensuring the stability and specificity of molecules in vivo, optimizing molecular structure to improve degradation efficiency, and evaluating the safety and effectiveness before clinical application. In the future, optimizing the molecular design of LYTAC by means of structural biology and computational chemistry, combined with the discovery of new targets, is expected to further expand its application scope and provide more accurate and effective strategies for the treatment of liver cancer.

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