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Preparation scheme and research progress of GPCR antibody drugs

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Abstract. As a class of proteins that play crucial roles in physiological and pathological processes within the human body, G protein-coupled receptors (GPCRs) have emerged as prominent targets for drug development. However, there is still ample room for improvement and replacement of currently developed drugs due to their inherent toxicity and associated side effects. In recent years, the attention has shifted towards antibody-based therapeutics targeting GPCRs, owing to their exceptional specificity and low plasma clearance rate. This review provides an overview of the fundamental preparation process and existing challenges encountered in developing GPCR antibody drugs, along with an introduction to several strategies employed in their formulation.

Keywords: GPCR drugs, antibody, Glutazumab, antigen, erenumab.

1. Introduction

The GPCR is extensively present in various organs and tissues, including the central nervous system, immune system, cardiovascular system, retina, and other body parts. It plays a crucial role in the body's development and maintenance of normal physiological functions. If the intracellular signalling pathway is dysregulated, or if somatic cells are targeted by external pathogenic factors as receptors, a range of diseases can ensue. Scientists from European molecular biology laboratory-European Institute of Bioinformatics and other institutions have classified and summarized the FDA-approved drugs according to known targets. According to statistics, GPCR proteins account for 12% of the drug targets of proteins in the human body; among small molecule drugs, 33% are targeted at GPCR protein family. At the same time, they classified and summarized the approved drugs up to 2015 (without distinguishing small molecule drugs and biological drugs) for the four most popular targets of GPCR, nuclear receptor, ion channel and kinase, and found that the number of drugs developed based on GPCR targets was as many as 370, accounting for the largest proportion; and the ratio of the number of approved drugs in recent 5 years to the total number of approved drugs was also high, showing a rapid growth [1, 2]. The above data show that GPCR, as a class of proteins that play an important role in the physiological and pathological processes of the human body, has always been one of the main targets of drug development. However, there is still room for improvement and replacement of the developed drugs due to toxic side effects and other deficiencies. In recent years, antibody drugs targeting GPCR have begun to enter people's attention. With their excellent targeting and low plasma clearance rate, these drugs have rapidly

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won widespread attention in the pharmaceutical field. However, only two GPCR antibody drugs have been listed so far, which proves that there are still great difficulties in the preparation of GPCR antibody drugs. It is very important to find new preparation methods for GPCR antibody drugs.

2. The structure of GPCR and connection with disease

As a crucial protein within the human body, alterations in the structure and functionality of GPCR can impact the metabolic processes and contribute to the development of certain ailments. In the development of antibody drugs targeting GPCR, scientists primarily emphasize studying its extracellular region's structural characteristics. The GPCR consists of seven α-helix segments spanning the cell plasma membrane. The N-terminal and three loops are positioned externally, facilitating interaction between the receptor and its ligand. On the other hand, the C-terminal and three loops are located internally within the cell. Among these, both the C-terminal and third loop play a crucial role in mediating intracellular signal transduction by interacting with downstream G proteins. Consequently, specific ligands binding to GPCR trigger G protein activation, leading to the generation of second messengers like Ca^{2+} or cAMP. These messengers then transmit extracellular signals received by GPCR downstream. However, it is worth noting that apart from relying on G proteins for signal transduction, GPCR can also regulate downstream pathways through interactions with β-arrestin and other molecules without their involvement. The main mechanism of action of GPCR drugs is to use antibodies to specifically bind to different GPCR targets, thereby blocking the extracellular signal that is the activation of the ligand to the target, thereby regulating the cell signal transduction pathway, and then affecting the physiological state and metabolic mode of cells to treat different diseases [3].

Numerous research studies have provided evidence indicating the involvement of several GPCR family members in the initiation and progression of diverse cancer types. Hormone receptor GPCR proteins play a significant role in hormone-dependent cancers, including androgen receptor AR, which facilitates the proliferation of cells in prostate cancer upon activation [4]. Certain receptors, including PAR1, can be activated by MMP-1 and subsequently trigger signalling pathways that facilitate the invasion and progression of cancer cells. Additionally, chemokine receptors like CXCR2 and CXCR4 are frequently overexpressed in myeloma and lymphoma cells, as well as potentially solid tumour cells such as those found in pancreatic cancer. These receptors play a role in both cell migration and angiogenesis [5]. GPCR proteins are involved in many signalling pathways and cell functions, and their roles in various types of cancers are different. Moreover, GPCR is also closely related to inflammation. The chemokine family in the GPCR protein superfamily is mainly expressed on all kinds of cells in the immune system, and participates in the physiological and pathological processes of the development, migration, survival and immune function of immune cells. Among them, inflammation is a kind of pathological phenomenon mediated by the chemokine receptors on immune cells activated by chemokines to mediate the immune cells to exert the host immune function. For instance, neutrophils predominantly express CXCR1 and CXCR2. In the event of infection or injury in specific body regions, neutrophils migrate towards the affected site due to the chemotactic influence of corresponding chemotactic factors (CXCL1, CXCL2, CXCL8). Upon reaching the lesion site, they release inflammatory mediators that induce localized inflammation. In general, there are many types of cancer-related GPCRs with complex functions, but their expression distribution has a certain specificity. By detecting the types of GPCRs highly expressed on the surface of cancer cells, people can develop corresponding antibodies and use them for treatment. The role of GPCR proteins in inflammatory responses is mainly to chemotactic immune cells migrate to the lesion under the action of the corresponding chemokines, such as the chemotactic effect of CXCR1/2 on neutrophils. Developing antibody drugs targeting chemokines or chemokine receptors and blocking the binding of chemokines to their receptors will help reduce inflammatory responses caused by neutrophils.

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3. Preparation difficulties and solutions of GPCR antibody drugs

3.1. General preparation and difficulties of GPCR antibody drugs

As an emerging drug, antibody has many characteristics that are not available in traditional small molecule chemical drugs, such as target specificity, very low plasma clearance rate and fewer side effects. Generally speaking, there are two viable approaches for screening humanized therapeutic antibodies. The first method involves genetically modifying the antibody to increase its human component and subsequently selecting hybridoma cells from immunized animals. Alternatively, hybridoma cells can be selected after immunizing transgenic animals with the antigen XenoMouseTM in order to achieve humanization. [6]. Screening methods are divided into in vivo immunization and display screening. In vivo immunization method is to immunize animals with antigen, and then isolate B cells from the spleen of animals, prepare hybridomas, and screen monoclonal antibodies. This method was developed in the 1970s, and is still the main method for pharmaceutical companies to obtain candidate antibodies. The main methods of antibody display are: phage display, yeast display, ribosome display, etc [7]. The advantages of phage display are large library capacity and simple operation, and the disadvantage is that the protein expressed by prokaryotic system lacks the modification of eukaryotic system; the advantage of yeast display is the combination of screening and detection. No matter what antibody screening technology is used, the preparation of pure GPCR antigen with natural conformation in the human body is crucial for the successful screening of humanized anti-GPCR antibodies with therapeutic function. However, only two GPCR antibody drugs are currently on the market, namely Amgen has developed erenumab, a therapy that specifically targets the calcitonin gene-related receptor (CGRPR) to treat migraines [8]. Also, Mogamulizumab, a monoclonal antibody against CC chemokine receptor 4 (CCR4) developed by Kyowa Hakko Kirin [9].

The technical difficulty of GPCR antibody preparation lies in the key step of preparing humanized GPCR antigen with natural conformation, which is due to the following reasons: (1) The purification of GPCR, which consists of 7 transmembrane proteins, poses challenges due to its low expression level in cells. Consequently, obtaining purified samples for subsequent animal experiments and antibody preparation becomes a difficult task. (2) Identifying appropriate targets is a complex endeavor within the structure of GPCR as its extracellular region offers a vast surface area. This makes it challenging to pinpoint suitable targets for different antibodies.

3.2. Preparation scheme of GPCR antibody drug

3.2.1. Using the GPCR extracellular fusion protein as new antigen. In order to solve the problem of the difficulty in purification of large volume and large area GPCR protein antigens, researchers have attempted to use the coupling of the extracellular domain of GPCR protein and carrier protein as a new GPCR antigen, which makes the volume of GPCR antigens smaller and easier to bind to antibodies. The researchers designed a simple method for making a new antigen by chemically coupling a peptide containing the extracellular domain of GPCR to a carrier protein [10].

For example, the conjugation of the bovine serum albumin(BSA) or keyhole limpet hemocyanin (KLH) to two distinct carrier. In this way, one of the proteins is used for immunization, the other one is used for screening antisera for immunoreactivity [11]. The screening of therapeutic antibodies against GPCR extracellular domains of immunized animals can be accomplished using this new antigen. Although the extracellular structure prepared by this method differs greatly from the conformation of the natural GPCR extracellular structure, the GPCR protein prepared by this method can express some glycosylated GPCR extracellular domain peptides in some cells. [12].

Several GPCR antibody medications have been developed using a similar approach, including erenumab mentioned earlier. Erenumab is an antibody for the treatment of chronic migraine. Its mechanism of action is to regulate the activity of calcitonin receptor-like receptor (CLR), in addition, it can interact with RAMP protein. Researchers found a specific binding site on the extracellular domain of RAMP1, and designed a heterodimer fusion protein consisting of the extracellular domain of CLR

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and the extracellular domain of RAMP as an antigen for the preparation of antibodies, thus obtaining Erenumab [13]. The prepared heterodimer Fc fusion was immunized into transgenic mice XenoMouse to produce erenumab, and then hybridoma screening was performed to obtain a large number of therapeutic antibodies. Mogamulizumab is an antibody that has been humanized and specifically designed to target CC chemokine receptor 4 (CCR4), a protein commonly found in various types of blood cancers such as cutaneous T-cell lymphoma (CTCL). Keyhole leukoprotein (KLH) was fused with the N-terminal extracellular domain to isolate anti-CCR4 antibodies, which were then injected into mice for immunization and antibody production [14].

3.2.2. Using cells that express PRCR or Membrane component as antigen. Cells harbouring undamaged and associated proteins that exhibit GPCR or its membrane fragment as an antigen, which encounters challenges in expressing on the cellular membrane owing to diverse impurities present in the membrane. Therefore, this method is currently subject to many limitations. On the other hand, membrane fragments extracted from cells expressing the target GPCR are also used as an antigen. The advantage of using cell fragments and cells with overexpressed proteins as antigens is that the proteins are in their natural conformation. The disadvantage is that the expression level of GPCR protein is very low relative to the total protein expression level on the membrane, and the antibody is more likely to bind to the membrane and other components on the membrane, which may lead to many non-specific results in the screening. Recombinant membrane proteins with accurate tertiary structure are considered optimal antigens for generating monoclonal antibodies targeting membrane proteins. However, cell-based expression systems have limitations such as low expression levels and protein aggregation. To address these challenges effectively, the utilization of double-layer dialysis has shown promising potential in achieving high yields and success rates. Notably, a recent study successfully employed double-layer dialysis to produce 25 different types of GPCR proteins and subsequently screened numerous anti-GPCR antibodies against four specific GPCRs using cell-free synthesis as antigen sources. In addition, this study successfully generated over 40 monoclonal antibodies targeting DRD1 with exceptional affinity and specificity for natural DRD1. These antibodies have broad applications in various research areas, including IHC [15].

3.2.3. Using the whole purified GPCR protein as antigen. Various approaches have been attempted to generate synthetic GPCR proteins that mimic the natural GPCRs' structure. These methods involve using suitable detergents to eliminate impurities from the cell membrane and extract the complete GPCR protein. Although several techniques have proven successful in extracting intact GPCR proteins, their preparation protocols differ depending on the specific type of GPCR. Moreover, it is important to note that purified GPCRs may exhibit structural variations compared to their natural counterparts, potentially affecting their affinity with cellular GPCRs. Nevertheless, utilizing purified whole GPCR proteins as antigens can effectively remove unrelated components present on the cell membrane, thereby enhancing the isolation of target-specific antibodies against GPCRs. Heptares Therapeutics has developed a StaR technology based on amino acid point mutation which introduces a limited number of site-directed mutations into GPCRs. This approach aims to enhance the thermal stability of purified GPCR proteins without compromising their pharmacological properties [16]. Additionally, by employing mutagenesis and other strategies to stabilize specific inactive or active states of GPCRs, it becomes possible to screen for antibodies specifically targeting these activated or inhibited states. Researchers are currently focusing on addressing two key challenges in efficiently producing functional native-like GPCRs: firstly, improving expression levels of these receptors to enhance preparation efficiency; secondly, overcoming issues related to poor stability during purification processes required for subsequent antibody development purposes. In general, this method still has many limitations and is difficult to be directly used in the preparation of GPCR antibody drugs, so more research is needed.

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4. Conclusion

Compared to small molecule medications, monoclonal antibodies offer the benefits of precise targeting, reduced side effects, extended presence in the bloodstream, and the ability to utilize various immune functions mediated by leukocytes. These unique characteristics have contributed to their rapid growth in the field of drug development. GPCR plays a vital role in regulating tumour cell behaviour such as growth, movement, proliferation, progression, and metastasis. Despite several small molecule drugs available for targeting GPCR being on the market, only two therapeutic antibody drugs against GPCR have received FDA approval as of September 16th, 2020. One of the main reasons for the slow development of therapeutic antibodies targeting attractive drug targets is the difficulty in preparing functional (natural or natural-like) GPCR antigens. Clearly, due to their complex structure, GPCRs are extremely challenging antigens from which to isolate therapeutic antibodies. However, as noted above, several solutions have been proposed. With the diversification of preparation methods for GPCR antigens, various cutting-edge antibody isolation platforms have emerged. As a class of proteins that play an important role in physiological and pathological processes of human body, GPCR has been one of the main targets of drug development. However, there is still room for improvement and replacement of the developed drugs due to their toxicity and side effects. Among the 370 disease-related GPCR proteins, many have not yet been marketed as effective drugs. With the gradual maturity of the related technologies of monoclonal antibody drugs, coupled with the huge potential of GPCR protein as a target, it is believed that scientific researchers and pharmaceutical companies will invest more energy in the development of monoclonal antibody drugs for GPCR proteins.

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