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Monoclonal Antibody Production Using Hybridoma Technology: Advances, Challenges and Applications

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Abstract

Hybridoma technology remains a fundamental approach in the production of monoclonal antibodies (mAbs), which are essential tools for diagnostics, therapeutics, and research due to their specificity and affinity for target antigens. First developed by Kohler and Milstein in 1975, hybridoma technology involves the fusion of antibody-producing B cells with myeloma cells, resulting in hybridoma cells capable of continuous antibody production. This review provides a detailed overview of the principles of hybridoma technology, the production protocol, recent advancements, and the challenges faced in monoclonal antibody production. Additionally, the applications of mAbs in virology, including rapid tests for COVID-19 detection and Differentiating Infected from Vaccinated Animals (DIVA) tests, are discussed. Advances in automation, genetic engineering, and bioreactor technology have improved the scalability, efficiency, and safety of mAb production, highlighting the ongoing evolution of this technology. The future prospects of hybridoma technology include further integration with machine learning and other innovative biotechnologies, which may enhance antibody discovery and development.

Keywords: Antibody Production, Hybridoma Technology, Monoclonal Antibodies (mAbs), Cell Fusion, Virology Applications.

Introduction

Monoclonal antibodies (mAbs) are crucial tools in modern biomedical research and therapeutic development due to their high specificity for target antigens. Hybridoma technology, established by Georges Kohler and Cesar Milstein in 1975[1], has become one of the most reliable and efficient methods for producing these antibodies. The primary aim of this review is to provide a detailed overview of hybridoma technology, including the principles underlying the technique, recent advancements, various applications, challenges, and future prospects.

Principle of Hybridoma Technology

Hybridoma technology involves the fusion of antibody-producing B cells with immortal myeloma cells to produce hybridomas capable of producing monoclonal antibodies indefinitely[2]. The B cells are derived from an animal, typically a mouse, that has been immunized with the target antigen, leading to a strong immune response. The myeloma cells, which are immortalized tumor cells, lack hypoxanthine-guanine phosphoribosyltransferase (HGPRT), which makes them dependent on specific culture media to survive [3]. The fusion process is often facilitated using polyethylene glycol (PEG), resulting in the formation of stable hybridoma cells capable of continuous monoclonal antibody production [2].The importance of hybridoma technology lies in its ability to maintain the natural pairing of heavy and light chains of antibodies, thus retaining high specificity and affinity for target antigens. This method has remained a gold standard due to its reliability and the quality of the antibodies produced [4].

Hybridoma Production Protocol

The production of hybridomas follows a series of well-established steps:

Immunization

Animals, typically mice, are injected with the target antigen multiple times to ensure a strong immune response. After sufficient antibody titers are achieved, the spleen cells, which contain antibody-producing B lymphocytes, are harvested [5].

Cell Fusion

The spleen cells are then fused with myeloma cells using PEG or similar agents.

a) Polyethylene Glycol (PEG) Fusion: PEG is a fusogenic agent that facilitates cell fusion by disrupting cell membranes, allowing them to come into close contact and eventually merge. PEG induces fusion by dehydrating the cell membranes and reducing the repulsion between them, which promotes membrane mixing and the formation of hybrid cells. Typically, PEG is added for a few minutes, followed by washing steps to remove excess PEG that could be toxic to the cells. The fusion process is delicate, as the optimal concentration of PEG, incubation time, and washing must be precisely controlled to ensure high fusion efficiency and cell viability[6,7].

b) Alternative Fusion Methods: Besides PEG, other methods can be used for cell fusion, such as electrofusion, which involves applying an electrical field to induce fusion. Electrofusion aligns the cells using an alternating current and then fuses them with a direct current pulse. This method is advantageous because it allows for more targeted and efficient fusion, reducing the randomness associated with PEG fusion. Electrofusion is often used in scenarios where higher fusion specificity is required, and it has shown to be more efficient in producing hybridomas with desired characteristics[8].

Screening and Cloning

The hybridomas are screened to identify those producing the desired antibody, often using techniques like enzyme-linked immunosorbent assay (ELISA). Positive hybridomas are cloned by limiting dilution to ensure monoclonality[9].

a. Limiting Dilution: The goal of limiting dilution is to isolate single hybridoma cells to ensure that each well contains a clone derived from a single cell, thus guaranteeing monoclonality. The process involves serially diluting the cell suspension to a concentration where, statistically, each well contains only one viable cell. This ensures that the resulting colonies are monoclonal, meaning that they all originate from a single hybridoma cell, and thus produce identical antibodies. Limiting dilution is critical for the consistency and reproducibility of monoclonal antibodies, as it eliminates the variability that could arise from mixed populations of cells [10].

Antibody Production

Once stable hybridoma clones are identified, they are expanded in culture to produce monoclonal antibodies in large quantities. The antibodies are purified using techniques such as affinity chromatography to ensure their quality and purity.

i. Antibody Purification: Affinity chromatography is the most commonly used method for purifying monoclonal antibodies. This technique takes advantage of the specific binding affinity between the antibody and an antigen or protein A/G immobilized on a chromatography matrix. The hybridoma culture supernatant is passed through the affinity column, allowing the antibodies to bind to the matrix. After washing away non-specifically bound proteins, the antibodies are eluted using a low-pH buffer. This process yields highly pure antibodies suitable for research or therapeutic applications[11].

Large-Scale Production

For large-scale production, hybridoma cells are typically cultured in bioreactors, which provide controlled environments that support optimal cell growth and antibody production. Bioreactors allow precise control of parameters such as temperature, pH, oxygen, and nutrient supply, which are crucial for maximizing yield. Batch, fed-batch, or continuous culture systems can be used depending on the scale and purpose of production. The use of bioreactors has significantly improved the scalability of monoclonal antibody production, making it feasible to produce sufficient quantities for clinical use[12,13].

Conservation of Hybridoma Cells

Hybridoma cells are valuable, and preserving their viability over long periods is crucial. Cryopreservation is the standard method for long-term storage of hybridoma cells. Cells are typically frozen in a medium containing a cryoprotectant, such as dimethyl sulfoxide (DMSO), which prevents the formation of ice crystals that could damage cell membranes. The cells are gradually cooled to -80°C before being transferred to liquid nitrogen for indefinite storage. Proper cryopreservation ensures that hybridoma cells retain their ability to produce monoclonal antibodies upon thawing, providing a reliable source for future antibody production [14,15].

Challenges in Hybridoma Technology

Despite its effectiveness, hybridoma technology has some inherent challenges. One significant issue is the labor-intensive nature of the process, which typically takes several months to produce a functional monoclonal antibody. Additionally, hybridoma cells can lose stability and productivity over time, affecting the consistency of antibody production.

Another challenge is the ethical and biological limitations of using animal models, which can present issues such as zoonotic infections and variable immune responses that are not always applicable to human biology. Moreover, the use of murine systems often results in the generation of human antimouse antibodies (HAMA) in clinical applications, which can lead to adverse immune reactions in patients. Advances in genetic engineering, such as the use of transgenic mice to produce fully human antibodies, have addressed some of these limitations.

Recent Advancements

Recent years have seen several advancements in hybridoma technology aimed at improving efficiency and addressing its limitations. The use of transgenic mice, capable of producing fully human antibodies, has significantly reduced the immunogenicity of monoclonal antibodies in clinical use[16]. Electrical pulse-based fusion methods have also been introduced to increase the efficiency of hybridoma formation by ensuring more targeted fusion of B lymphocytes and myeloma cells[17,18].

Moreover, the integration of phage display technology has complemented hybridoma technology. Phage display allows the screening of vast libraries of antibody fragments, which can then be expressed as full-length antibodies[19]. This combination has resulted in the development of highly specific monoclonal antibodies, especially for therapeutic purposes. The use of high-throughput screening techniques has also improved the selection process for high-affinity antibodies[20], reducing the time required for antibody development.

Applications of Hybridoma-Derived Monoclonal Antibodies

Monoclonal antibodies produced using hybridoma technology have a wide range of applications in research, diagnostics, and therapy. In diagnostics, they are used in immunoassays for the detection of various biomarkers, including hormones, cytokines, and tumor markers[7]. For instance, mAbs are essential components of pregnancy tests, in-

fectious disease diagnostics, and cancer biomarker detection [21].In therapeutics, monoclonal antibodies are used for treating cancers, autoimmune disorders, and infectious diseases. The use of mAbs like Trastuzumab for HER2-positive breast cancer [22]and Rituximab for B-cell non-Hodgkin's lymphoma has transformed clinical treatment paradigms[23]. Hybridoma-derived mAbs are also indispensable in basic research, being used in techniques such as immunoprecipitation, flow cytometry, and Western blotting to detect specific proteins and study their interactions [21,24].

Use of Monoclonal Antibodies in Virology

Monoclonal antibodies have found significant use in the field of virology, particularly in the development of diagnostic tests and therapeutic tools. During the COVID-19 pandemic, monoclonal antibodies were integral to the rapid development of diagnostic tests, including rapid antigen tests for SARS-CoV-2 detection[25]. These tests utilize mAbs to specifically bind to viral antigens present in nasal or throat swabs, allowing for the rapid and accurate identification of infected individuals. Such tests have been essential for widespread screening and controlling the spread of the virus.

Another important application in the virological field is the use of monoclonal antibodies in Differentiating Infected from Vaccinated Animals (DIVA) tests. DIVA tests are crucial in managing viral outbreaks in livestock, as they allow for the differentiation between animals that have been vaccinated and those that have been naturally infected. This differentiation is possible because the vaccine used in a DIVA strategy lacks certain viral antigens, which are then targeted by specific monoclonal antibodies in diagnostic assays. The ability to distinguish between vaccinated and infected animals is vital for effective disease control and eradication programs, especially in cases such as foot-and-mouth disease or avian influenza[26,27].

Limitations of Hybridoma Technology

A primary limitation of hybridoma technology is the dependence on animal models, which can limit the translational potential of the generated antibodies for human use. The risk of generating immune responses against murine antibodies, such as HAMA, poses a significant problem in therapeutic contexts[28]. To address this, chimeric, humanized, and fully human monoclonal antibodies have been developed [29].These engineered antibodies reduce the risk of adverse immune reactions, making them safer for use in patients.

Furthermore, hybridoma cell lines are prone to genetic instability, which can lead to variability in antibody production. Recent advances in gene editing, such as CRISPR-Cas9, have been applied to stabilize hybridoma lines and improve their productivity[30]. Bioreactor technology has also improved the scalability of mAb production, providing consistent yields and better control over culture conditions[31,32].

Future Directions and Recommendations

The future of hybridoma technology will likely involve further integration with other biotechnological advances to enhance its efficiency and applicability. One promising direction is the use of fully automated high-throughput systems for hybridoma screening, which could significantly reduce the time and labor involved in selecting high-affinity clones [33].

Another potential development is the use of in vitro immunization and single B-cell sorting methods, which could replace animal immunization and provide a more ethical and rapid pathway to antibody generation[33]. In addition, machine learning and artificial intelligence (AI) are expected to play a role in analyzing the vast datasets generated during screening, helping to identify the most promising antibody candidates more efficiently [34,35].

Conclusion

Hybridoma technology remains a foundational method for monoclonal antibody production due to its reliability and the quality of antibodies it produces. While it faces challenges such as ethical concerns and labor-intensive procedures, recent advancements are making hybridoma technology more efficient and applicable to modern therapeutic needs. The integration of genetic engineering, automation, and AI will likely ensure that hybridoma technology continues to play a significant role in both research and therapeutic antibody development.

Conflict of Interest

The authors declare no conflict of interest.

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