Mini Review
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Advances in monoclonal antibodies production and cancer therapy

Abstract

Monoclonal antibodies are highly specific class of antibodies which are produced by identical hybridoma cells. These antibodies are developed by various advanced and powerful technologies being used in the areas of cell biology, biochemistry, biotechnology, immunology and medical sciences. The techniques involved hybridoma technology, phage display technology, single B-cell amplification by polymerase chain reaction (PCR) and many other modified methods. The advancement in antibody therapeutics is an essential area of growth in the pharmaceutical manufacturing and more than 30 monoclonal antibodies have been approved on the US and Europe markets. Regardless of these advancements, certain significant target antigens remain intractable to therapeutic antibodies due to complexity of the target molecules. The present review describes recent advances in monoclonal antibody production and cancer therapy for better understanding the therapeutic efficacy.

Keywords: monoclonal antibody, hybridoma, phage display, b-cell amplification, therapeutics

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Abbreviations: PCR, polymerase chain reaction; mAbs, monoclonal antibodies; IP, intellectual property; HAMA, human anti-mouse antibodies; PEG, polyethylene glycol; CDRs, complementarity-determining regions; FDA, food and drug administration; VH, variable domains; Ig, immunoglobulins

Introduction

Monoclonal antibodies (mAbs) are the dominant group of recombinant proteins used in human therapy. These proteins were first successfully developed by Köhler and Milstein[1] in mid-1970s and published in Nature in 1975 with free of intellectual property (IP) rights, and produced a method for B-cell immortalization and the production of monoclonal antibodies. The mAbs have various applications in the fields of cell biology, immunology, biotechnology and medicines. They are also being used in vivo imaging techniques of different kinds of diseases.[1–4]

Antibodies are also called immunoglobulins (Ig), they play a vital role in internal defenses or immunity against a vast number of invading microorganisms and other disease causing agents or antigens. They are a major group of glycoproteins which are a part of neutralizing immune response present in blood or other body fluids of vertebrates. These antibodies have five common isotypes (IgG, IgE, IgA, IgM and IgD), among them, IgGs are permeable to extravascular spaces, have a longer half-life as they bind to neonatal Fc receptor (FcRn) as compared to other isotypes and have the most therapeutic potential. FcRn binding affinity, cross-reactivity, and the elimination pathways are comparable in nonhuman primates and humans. Therapeutic mAbs usually bind to nonhuman primate antigens than rodent antigens because of the greater sequence homology.[5–18]

Köhler and Milstein[1] developed mAbs by the isolation of primary B-cells which were later differentiated and matured in vivo and further fused with immortalized myeloma cells or lymphoblastoid cells. These myeloma cell lines were already being cultured since the 1960s and were used for differentiation of fused and unfused cells in hybridoma technology. Subsequently, the hybridoma technology destined for immortalized differentiated cells was soon recognized as the potential technique for the production of mAbs on a large scale in pharma industry as future blockbusters.[1,4]

Till now, the mAbs are being developed by a number of pharmaceutical industries on a large scale. It has been projected that more than 30 mAbs and their derivatives (fusion proteins and antibody fragments) have been developed; furthermore, more than 350 mAbs are in various phases of drug trials and patents. Significantly, antibodies are reflected to be individual products and can be patented to produce market exceptionality.[19–21]

Production, genetic manipulation and progress in molecular biology techniques enabled development of recombinant hybridoma cell lines instead of less-defined and undifferentiated cells. The next generation of mAb development cell lines was determined by the glitches of hybridoma instability. The production of chimeric antibodies with human Fc and mouse Fv regions helped the prohibition of human anti-mouse antibodies (HAMA) responses in human patients.[22–24] The chemistry, and advances in production technologies and therapeutic uses of mAbs have been discussed in the current study.

Chemistry of mAbs

The mAbs exhibit biological effector functionality by interacting with complementary and Fc domains. A typical IgG has total molecular weight of approximately 150 kDa. It is a symmetrical Y-shaped globular molecule comprising of two identical heavy chains (~50 kDa each) and two identical light chains (~25 kDa each) respectively. The heavy chains are further consisted of one variable domain and three constant domains, and the light chains consist of one variable and one constant domain. The variable domain is moreover consisted of three small sections of peptide which is called complementarity-determining regions (CDRs) (Figure 1). CDRs are the hypervariable regions that determine the specific antibody binding.[25–28]

Advances in mAbs production technologies

The development and recognizing mAb by immortalized hybridoma technology has long been in used since 1977. Since then the rodent mAb technology has been rapidly and successfully applied

for the development of new and improved diagnostics and therapeutics to enhance the understanding of human biology.[29]

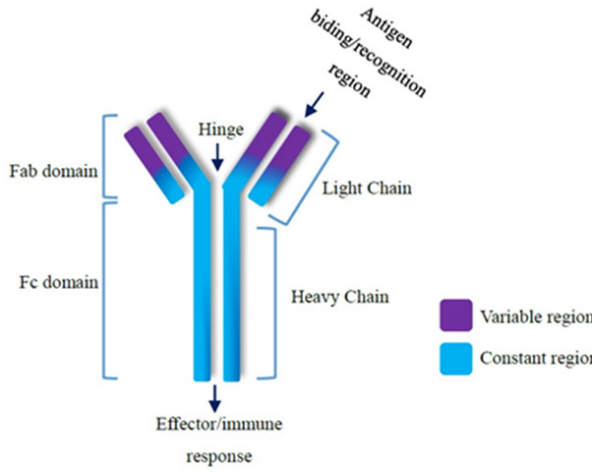

Figure 1 The structure of monoclonal antibody and its components.

Hybridoma technology

The mAbs have been developed by using mouse hybridoma technology for therapeutic applications. This technique uses innate functionality of B-lymphocytes and myeloma or immortalized cancerous cells for the effective production of immortal hybridoma cells secreting mAbs specific for antigen of interest. The B-cells are fused with myeloma cells in the presence of polyethylene glycol (PEG) as a fusing agent (Figure 2). The antibodies produced by this technique are specific in nature to the target antigen and has various application in therapeutics. However, immune responses are often weak in mice, resulting in low affinity and/or non-specific mAbs. Animals other than rodents have not been usually used to produce mAb due to the problems associated in the establishment of immortalized antibody-producing cell lines by hybridoma.[1,30,31]

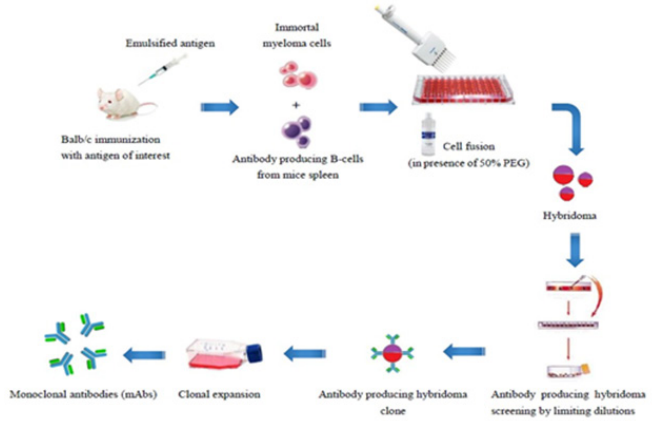

Figure 2 A schematic illustration of hybridoma technology.

Phage display

Phage display technology was developed by G. Smith in 1985 to display the peptides on the surface of lysogenic filamentous bacteriophages. Since then, this technology has become one of the leading method to develop large number of peptides, proteins and antibodies with the basis of physical linkage of phage phenotype (phage coat fusion protein expression) and genotype as single-strained DNA (ssDNA) of the related virion. This technique allows the development of phage display libraries containing up to 10^{10} phage variants, affinity assay of protein-ligand interactions, characterization, epitope recognition, enzyme substrate selection, antibody repertoire screening and favors the emergence of identical phage particles from *Escherichia coli* clone.[32–36]

Single B cell amplification

Currently, for the isolation of high affinity mAbs by somatic hypermutation and affinity maturation technique, the direct molecular cloning of identical pairs of antibody light chain lambda variable (VLλ), heavy chain (IgH) variable (VH) and light chain kappa variable (VLκ) genes from single antigen-specific plasma/plasmablast cells (ASPCs) with the help of polymerase chain reaction (PCR) is an alternative technique being used for the development of mAb from immunized animals.[37–42] Additionally, there are some drawbacks such as expensive equipment and technical skills which are required to isolate ASPCs. The VH and VL genes from the cells are manually amplified and construction of immunoglobin heavy and light (IgH and IgL) chains is also limiting steps. For the development of rapid and scalable generation of mAbs from single cell, a high-throughput single-cell-based immunoglobulin gene cloning technique was used by developing a non-contact magnetic power transmission system (MAGrahd) for automated single-cell-based cDNA synthesis, 3' end homopolymer tailing and a target-selective joint PCR (TS-jPCR) for IgH and IgL gene expression.[43–53]

Advances in cancer therapy by mAbs

The advent of hybridoma technology revolutionized the areas of cell biology, immunology, biotechnology and medical research. Before the introduction of hybridoma technology, antibodies were produced by recurrent animal immunization with antigen of interest and the sera were used for the therapy. There were allergic responses and no clinical benefits to the patients of the administration of the crude sera. Therefore, by using the hybridoma technology, a large number of mAbs were produced against the target antigens with the help of mice splenocytes and immortal myeloma cells.[1,54–56]

Until now, the mAbs have extensively been used for diagnostics and cancer therapy. However, the administrations of mouse mAbs produces HAMA in cancer patients, cause rapid clearance and are highly immunogenic. To overcome these limitations, the recent biomedical and technological advances in genetic engineering helped reduction of immunogenicity, enhanced production of antibody-drug conjugates, smaller antibody fragments in cancer imaging.[57,58]

The recent advances such as use of

i. Transgenic mice (first generation, 1970s),

ii. Chimeric antibody (second generation, 1980s),

iii. Humanized, fully humanized and

iv. Bispecific antibody (third generation, 1990s and 2000s) aided in the increase of mAbs half-life and therapeutic potential.

a. The transgenic mouse antibody contains two identical heavy and light chains (CH, CL), and variable domains (VH, VL) further containing antigen binding site (CDRs), immunogenicity is generated by the constant (Fc) region of an antibody which is linked by disulphide (-S-S-) bonds.

b. Chimeric mAbs are constructed by substituting constant region of mouse antibody with constant region of human IgG antibody.

c. Humanized antibody contains >90% of human sequences and produced by the fusion of DNA for three CDRs of mouse variable domain into human IgG structure. Fully humanized antibodies are constructed with the use of transgenic mice having human Ig or phage display technique. It consisted of 100% human and therefore less immunogenic than its mouse counterparts, chimeric and humanized.

d. Bispecific antibodies are comprised of two distinct antigen-binding regions and binds to two distinct antigens consecutively. Shorter antibody fragments have been developed such as monovalent scFv (30 kDa) and divalent (scFv) (60 kDa) with antigen binding specificity for the respective antibody.[59–64]

A large number of mAbs have been patented for therapeutic use by the US Food and Drug Administration (FDA) and/or the European Union Health Authorities. These antibodies are administered against various fatal diseases such as autoimmunity, inflammatory responses, hematological malignancies, organ transplant rejection and human cancers respectively. Additionally, many other therapeutic mAbs have been in various phases of clinical trials against deadly infections such as HIV/AIDS, Ebola and Zika viral disease.[64–69]

Conclusion

Since last 60 years, there has been a great deal of progress and advancements in mAb development. Various powerful techniques such as hybridoma technology, phage display and B-cell amplification with the help of PCR have been developed for the construction of numerous types of antibodies and antibody fragments with widespread structural modifications. The mAbs are really significant as they are highly specific and play a key role in specific immunity against target antigens. They have various applications in areas of cell biology, biochemistry, biotechnology, immunology and medicines. This study will help in understanding the current advancements of mAbs development and will benefit in further progress in future.

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Conflicts of interest

There are no financial conflicts of interest.

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