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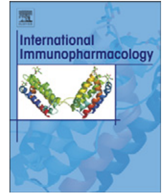

Review

Mammalian cell display technology coupling with AID induced SHM in vitro: An ideal approach to the production of therapeutic antibodies

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ABSTRACT

Traditional antibody production technology within non-mammalian cell expression systems has shown many unsatisfactory properties for the development of therapeutic antibodies. Nevertheless, mammalian cell display technology reaps the benefits of producing full-length all human antibodies. Together with the developed cytidine deaminase induced in vitro somatic hypermutation technology, mammalian cell display technology provides the opportunity to produce high affinity antibodies that might be ideal for therapeutic application. This review was concentrated on the development of the mammalian cell display technology as well as the activation-induced cytidine deaminase induced in vitro somatic hypermutation technology and their applications for the production of therapeutic antibodies.

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1. Introduction

The immune system of humans functions as defense power to resist invasion of bacteria, viruses and other microbes as well as protect the human body from the dangers of endogenous harmful substances. As the most important and effective weapon of the immune system, antibodies take advantage of their millions of potential antigen binding sites to identify antigen of almost all kinds and further eliminate these hazards when causing the in vivo cleanup effect. Thus, for decades, scientists have been investigating methods to recreate systems to build immunoglobulin-based binding sites in vitro.

The fundamental breakthrough should be the hybridoma technology [1–3], which is now most commonly used to screen and select antibodies for a wide spectrum of research, diagnostic, and even therapeutic applications [4]. Immunization of wild-type animals had been applied to produce the initial therapeutic antibodies. The anti-CD3 monoclonal antibodies (mAbs) marooned [5]. However, mAbs generated by traditional hybridoma technology comprise nonhuman sequences that make the rodent antibody imported proteins for human. When

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used as therapeutic agents in patients, mAbs tend to induce the human anti-mouse antibody (HAMA) response [6,7] as the human immune system could mount its own antibody response to these mAbs. This HAMA response might lead to rapid clearance, reduced efficacy, and anaphylaxis, which sometimes might be fatal [8,9]. In addition, generation and screening of hybridoma cells from an immunized animal are time-consuming and sample only a fraction of the antibodies generated during the adaptive immune response. Also, affinity levels of antibodies obtained from hybridoma cells are frequently not sufficient as effective agents in the clinic due to in vivo affinity ceiling [10]. These limitations on the use of murine mAbs in clinical applications paired with the medical and commercial success of mAbs in the management of human disease accelerated the progress of a variety of alternative methods to generate human or humanized antibodies [11–16].

The further advance of genetic engineering technology enabled it to replace constant regions and some or all of the non-specificity determining residues of murine antibodies with corresponding human antibody sequences [17]. On the other hand, immunization of transgenic mice in which the endogenous immunoglobulin loci have been replaced with a repertoire of human heavy and light chain germ line transgenes has recently emerged as an effective way to generate human antibodies against a great deal of antigens [18–21]. Humanization of mouse monoclonal antibodies (mAbs) and direct selection of fully human antibodies from transgenic mice dramatically advanced the progress of therapeutics antibody [22–24]. Nevertheless, for this immunization-based generation of antibodies, immunodominant epitopes may be preferentially selected, making it difficult to identify functional antibodies. Also, immune tolerance may lead to the generation of neutralizing antibodies when antigens are well conserved or toxic upon administration to animals.

Another breakthrough came with an innovative technology that enabled the cloning of antibody genes [25] and thus significantly improved in vitro antibody display systems. By inserting such genes into expression vectors, a variety of antibody libraries have been developed to screen for antigen-specific human antibody genes, including phage library [26–28], bacterial library [29] ribosomal library and yeast library [30], and researchers have successfully identified many antigen-specific monoclonal antibodies from these libraries [31,32].

In vitro display systems have several advantages for antibody discovery and optimization [33]. They can be considerably faster and might be performed in a high throughput mode. Besides, in vitro display systems provide an opportunity to overcome issues associated with in vivo immunization-based approaches for the generation of antibodies such as tolerance to conserve antigens, poor immunogenicity, toxicity, and immunological epitopes. However, these display systems based on the microbial expression have a number of features that might not be ideal for displaying and selecting antibodies as potential therapeutic agents. For example, currently, phage display technology is the most developed and commonly used technology for screening and selecting therapeutic antibody. However, phage display has the obvious disadvantages of lacking of post-translational modification as well as the inability to work with the native glycoprotein IgG and carry out precise functional screening [34]. In addition, the quantity and quality of the phage display library might not yet meet current needs for more efficient development of antibody drugs [35]. Therefore, it seems difficult to identify full-length antibodies with properties that are compatible with economic manufacture and therapeutic utility directly from the phage display library [36,37].

In natural conditions, naive B cells display the membrane antibodies that might bind to antigens after the rearrangement of V, D and J genes and thus constitute the naive antibody library. When stimulated by specific binding antigens, these naive B cells become matured B cells. These matured B cells can then secrete specific antibodies after the rearranged V, D and J genes within cells going through the class switch and somatic hypermutation (SHM). Mimicking this process, researchers finally turn to mammalian expression systems for antibody display that might be an ideal display system for screening therapeutic antibodies in vitro.

In the last decade, scientists have exercised considerable effort to develop mammalian cell surface display technology. The use of transient mammalian expression systems for the production of complex proteins has increased [38] and boosted by the availability of efficient transaction protocols and cell lines that grow at high density. It is then desirable to develop technology that can display full-length human antibodies on the surface of mammalian cells [39,40], and there is a great chance to identify ideal therapeutic antibodies in vitro when this mammalian cell display technology was coupled with further developed in vitro SHM technology.

2. Mammalian cell display technology

Basically, the cell surface display technology provides a platform for the direct interaction between antigen and specific antibodies that expressed on the surface of mammalian cells. In this approach, it might enrich those cells that display specific antibodies and further screen genes encoding these antibodies directly according to the genotype–phenotype correlation inside the display systems. Commonly, we are required to transport those encoding genes into cells and make sure their correct transcription and translation in order to display proteins on the surface of mammalian cells. Those encoding genes can then be inserted into some definite expression vectors and transported to suitable host cells in which it might transcript and translate into our interesting proteins.

Specifically, those encode genes can either be introduced into the genome of host cells by heterogeneous integration, which might result in stable and long-last time expression (stable expression systems), or they can directly transcribe for mRNAs using the promoters located on the expression vectors resulting in rapid, short, but the efficient protein expression (transient expression systems). Transient expression systems have been shown to be quite satisfactory ways for selection and analysis of antibody expression [39,40]. Either way, it requires desirable host mammalian cell lines and suitable eukaryotic expression vectors for antibody display.

2.1. Cell lines and vectors for mammalian cell display

A noticeable advantage for mammalian cell display might be its more similarity with the crude antibody production approach compared to those microbial display technologies. But the proliferation rate of mammalian cells would be much slower than microbial cells in vitro. Thus, to enable rapid and convenient construction of mammalian cell surface display systems, cells chosen for antibody display should be robust, able to grow rapidly and competent for extraordinary efficiency transaction. For these reasons, common laboratory cell lines, such as HEK-293, COS and CHO cells have been extensively used. Among those, the human embryonic kidney 293 cell lines HEK-293 [41] had been used most extensively for mammalian cell surface display because of its ease of transport in, high expression yield and native human glycosylation. At the same time, vectors that are suitable for eukaryotic expression systems including pcDNA series, pDGB and some virus based vectors have been chosen for mammalian cell display. And a large number of transient ion methods ranging from liposuction, nullification, electroporation, calcium phosphate transaction, and other methods [42,43] have been used and radically improved transaction efficiency. Also, further developed methods and tools have been applied to complete this technology. These methods and tools were implicated mainly to increase the specificity and diversity of antibodies displaying on the mammalian cell surface.

2.2. Amplification of variable gene pools

The variable area of antibodies acts as the functional area to identify the antigen and make a combination with it. In natural conditions, rearrangement of naive V/D/J genes in naive B cells determines the diversity

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