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Humanized Antibody Preparation Strategy

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ABSTRACT

The humanized antibody mainly refers to the antibody which is re-expressed by the mouse monoclonal antibody by gene cloning and DNA recombination technology, and most of the amino acid sequence is substituted by the human sequence, and the affinity and specificity of the parent mouse monoclonal antibody are substantially retained, and the antibody is reduced. Its heterogeneity is beneficial to the human body.

Chimeric antibody

The variable region of the mouse antibody gene is recombined with the constant region of the human antibody gene by DNA recombination technology, and the recombinant gene is introduced into the myeloma cell for expression. Depending on the vector plasmid used to label the gene product, an appropriate antibiotic or preparation is used for screening, and a cell line secreting the human mouse chimeric antibody is cloned in a similar manner to the conventional technique.

2. CDR-grafted humanized antibodies

The FR in the V region of the murine antibody still retains some immunogenicity. This antibody is far from being a true humanized antibody, and some can produce a strong anti-idiotype response. In order to reduce the composition of the mouse, people try to replace the FR of the mouse with human FR to form a more complete antibody humanization service antibody, that is to say, except that the three CDRs are murine sources, all of them are human structures, also known as CDR-grafted antibodies. Or modified antibodies.

3. Fully humanized antibody

A. Antibody library technology

The production of phage antibody library technology relies on the development of three experimental techniques: one is the development of PCR technology that allows one to clone a complete set of immunoglobulin variable regions from B lymphocyte total RNA by RP-TCR. Genes, making the construction of antibody libraries simple and easy to manipulate. One is the establishment of phage display technology to achieve the unification of genotype and phenotype, providing a highly efficient screening system, which is the core of phage antibody technology. The other is to secrete a fragment of an immunoglobulin molecule that expresses binding ability from Escherichia coli.

Phage antibody libraries unite phenotypes and genotypes. Combining the selection ability and the amplification ability, it has a powerful screening function, which can simulate the antibody production process in vivo in vitro, and make the antibody engineering technology enter a new era. The development of phage antibody library technology has made it possible to obtain antibodies without immunization in vitro. Because it occurs in vitro, it does not rely on the antibody recognition and presentation system in vivo, and can theoretically produce antibodies against any substance. At present, phage antibody library technology is also insufficient. For example, antibody affinity obtained from an antibody library of an immunized animal is high, limited by the conversion rate of the foreign gene, and the storage capacity of the antibody library is insufficient to cover the antibody diversity of some animals. Therefore, a large-capacity antibody library is the key to obtaining high-affinity antibodies and fully human monoclonal antibody against rare antigens.

B. Transgenic mouse technology

Through gene knockout technology, the mouse's own gene is inactivated and introduced into a new gene, creating a transgenic mouse carrying the human antibody heavy light chain gene cluster and inactivation of its own gene. The human DNA fragment carried by the transgenic antibody gene mouse has a complete function and can efficiently perform isotype gene transformation and affinity maturation.

Human antibodies prepared from transgenic mice are superior to anti-normal human protein monoclonal antibodies produced by other techniques. The antibody system that recognizes antigens and mobilizes antigens in mice remains intact, and it is easy to recognize human proteins as foreign bodies. In addition, since the antibody is produced in vivo, it undergoes normal equipment and maturation processes to ensure a high target binding affinity of the finished product. However, transgenic mice also have some drawbacks, that is, transgenes usually have in vivo cell mutations and other unique sequences leading to incomplete human sequences. Moreover, since the

antibodies are assembled in mice, the resulting monoclonal antibodies have a murine glycosylation pattern, so these monoclonal antibodies are ultimately not fully humanized.

EXTERNAL LINK

https://www.creativebiolabs.net/Anti-CD19-fully-human-monoclonal-antibody-MDX-1342-32078.htm