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Review Article

Rabbit monoclonal antibody: potential application in cancer therapy

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Abstract: By targeting antigens specifically, monoclonal antibodies represent a new class of therapeutic agents for the clinical management of various diseases including cancers. Monoclonal antibody technology has been greatly developed by reducing murine content in antibodies to minimize side effects in clinical applications. However, several intrinsic disadvantages of antibodies with murine origin limit the clinical efficacy of monoclonal antibodies based targeted therapy. The development of rabbit monoclonal antibody technology provides an alternative source of monoclonal antibodies with higher specificity and less cost for the development of routine targeted therapy against cancers.

Keywords: Monoclonal antibody, rabbit monoclonal antibody, cancer therapy

Introduction

After the development of hybridoma technology by Köhler and Milstein in 1970s, monoclonal antibodies were becoming the major choice of targeted therapy for cancers. Antibodies were used clinically in naked or conjugated form [1, 2]. Naked antibodies alone could initiate multiple immunological responses to eliminate cancer cells. In the other hand, antibodies conjugated with toxins, radioactive particles, drug-activating enzymes, or liposomes carrying chemotherapeutic drugs could restrain the toxicity specifically to cancer cells and reduce systemic side effects, thus improving the efficacy of targeted therapy [3].

Throughout the development of monoclonal antibody, there have been four major types: murine, chimeric, humanized and fully human. In early 1980s, most of monoclonal antibodies were completely murine that could invoke an immune response resulting in their rapid removal from the blood and systemic inflammatory effects through the production of human anti-mouse antibodies (HAMA) when administrated in humans [4]. Since the late 1980s, several humanization strategies such as chimeric antibodies and humanized antibodies have been applied to reduce HAMA-mediated responses [5, 6]. Chimeric antibodies consists of variable regions from murine antibody and constant regions from human antibody while humanized antibodies were basically human origin except that complementarity-determining regions (CDRs) were derived from the mouse. Despite low incidence, chimeric and humanized monoclonal antibodies still have the potential to stimulate the production of HACA (human anti-chimeric antibody) or HAHA (human anti-human antibody) [7]. Recently, the development of phage display and transgenic mice technology made it is possible to produce fully humanized antibodies for clinical applications. However, it seems that immunogenicity is so complicated that even fully humanized antibodies like Vectibix and Humira, two antibodies recently launched for targeted therapy, were found to be highly immunogenic [8, 9].

Monoclonal antibodies in cancer therapy

In 1986, the US Food and Drug Administration (FDA) approved muromonab-CD3 (Orthoclone

Table 1. Monoclonal antibodies used for cancer therapy on the market

Antibody	Drug Name	Target	Antibody Type	Indication	First Approval Date and Country
Rituximab	Rituxan	CD20	Chimeric	Non-Hodgkin lymphoma (NHL)	1997 (United States)
Trastuzumab	Herceptin	HER2	Humanized	Brease cancer	1998 (United States)
Alemtuzumab	Campath	CD52	Humanized	Chronic lymphocytic leukemia (CLL)	2001 (United States)
Ibritumomab Tiuxetan*	Zevalin	CD20	Murine	Non-Hodgkin lymphoma (NHL)	2002 (United States)
Tositumomab*	Bexxar	CD20	Murine	Non-Hodgkin lymphoma (NHL)	2003 (United States)
Cetuximab	Erbitux	EGFR	Chimeric	Colorectal Cancer Head & neck cancers	2003 (Switzerland) 2006 (United States)
Bevacizumab	Avastin	VEGFA	Humanized	Colorectal cancer Non-small cell lung cancer (NSCLC) Breast cancer Kidney cancer Glioblastoma	2004 (United States) 2006 (United States) 2007 (European) 2007 (European) 2009 (United States)
Nimotuzumab	TheraCIM	EGFR	Humanized	Head & neck cancers	2005 (China)
Panituzumab	Vectibix	EGFR	Human	Colorectal cancer	2006 (United States)
Ofatumumab	Arzerra	CD20	Human	Chronic lymphocytic leukemia (CLL)	2009 (United States)

* Conjugated monoclonal antibodies

OKT3) as the first monoclonal antibody for clinical application. It could prevent acute organ rejection after transplantation by suppressing T-cell function [10]. From that time, many antibody drugs came to the market and benefited a large number of patients. It was a breakthrough in cancer research when rituximab was approved as the first monoclonal antibody for clinical application [11, 12]. At present, more than 24 therapeutic monoclonal antibodies were approved by the US FDA and 10 of them were used for cancer therapy. Most of them are unconjugated antibodies (Table 1) [2, 13, 14].

There are several mechanisms for monoclonal antibodies to treat cancers. First, antibodies can bind to signaling molecules mainly growth factor receptors or their ligands, thus blocking the activation of signaling pathways important to the proliferation and survival of tumor cells. For example, Cetuximab is an anti-EGFR (Epidermal growth factor receptor) antibody while Bevacizumab binds to EVGF (vascular endothelial growth factor) and inhibit its interaction with VEGF receptor. Second, antibodies could kill tumor cell through the activation of human immune system. Once their Fab (Fragment of antigen binding region) specifically binds to antigens in tumor cells, the Fc (fragment of crystallizable region) could activate compliment cascade or Fc receptor containing immune cells such as natural killer cells, monocytes and macrophages so as to eliminate tumor cells as pathogens. This was termed as complement-dependent cytotoxicity (CDC) or antibody-dependent cell cytotoxicity (ADCC). Third, monoclonal antibodies can also be served as immunogens for cancer vaccines through the anti-idiotype-network cascade. Briefly, anti-idiotypic antibodies bind to the antigen-binding sites of antibodies, thus mimicking the three-dimensional structure of antigens to effectively induce human antibody that will react with the tumor antigen [2, 15].

Rabbit, an alternative source for antibody production?

Most of monoclonal antibodies approved for clinical application are mouse origin. However, the mouse system is limited by a small spleen and the mice used are usually inbred, thus offering a less diversity of immune responses. In

contrast, as the original and still reliable model system to produce antibodies for laboratory use, the rabbit has a robust immune system and bigger spleen to generate antibodies with high affinity and specificity. Recently, a stable rabbit hybridoma fusion partner cell line 240E-W was developed, making it possible to generate large amount of rabbit monoclonal antibodies (RabMAb) [16, 17]. In additional to challenge the prevalence of monoclonal antibodies with mouse origin in laboratory use, RabMAbs are demonstrating their potential for clinical applications by offering many advantages over mouse monoclonal antibodies [18]. Compared with antibodies from other sources, RabMAb has at least following advantages: wider repertoire, simpler structure, higher binding affinity, robust reproduction and easy to be humanized.

Wider repertoire of RabMAbs

Rabbits are known to elicit a strong immune response against foreign antigens by applying a mechanism different from the mice and human to generate antibody repertoire. It is very useful when the antigens with weak immunogenicity have to be used [19, 20].

Human and mouse create their primary antibody repertoire through combinational joining of multiple VH, D, and JH gene segments for heavy chains, and multiple V (λ) and J (λ) gene segments for light chains. The resulting VJ and VDJ gene rearrangements can be further diversified by somatic hypermutations that develop the secondary antibody repertoire which leads to the diversity in antibody affinity. Although the rabbit generates primary antibody repertoire in a manner same as the mice and human, it can develop secondary antibody repertoires with a gene conversion-like mechanism in addition to somatic hypermutations [21].

Simpler structure of rabbit immunoglobulin

Rabbit antibodies seem to be simpler compared with mouse and human antibodies that are known to have five classes of immunoglobulin (Ig), defined by their type of heavy chain: Cγ for IgG; Cμ for IgM; Cα for IgA; Cε for IgE and Cδ for IgD. No rabbit IgD has been found so far [24]. The most abundant class in rabbit serum is IgG with serum concentration of 5-20 mg/ml. Unlike IgG from other animal species, the rabbit IgG has no subclass. Compared with mouse and human IgGs, rabbit IgG tends to have fewer amino acids at the N terminus and in the D-E loop, and have extra disulphide bonds in the variable region of the heavy chain. For light chain, Majority (90-95%) of the light chains is derived from Cκ1 (isotype κ1), only 5-10% of total IgG light chains are isotypel. Several different allotypes of rabbit light chains have a disulphide bond between Vκ position 80 and Cκ position 171. This extra disulphide bond is not found in κ2 and l light chains. It was suspected that the greater stability of rabbit antibodies is in part a result of stabilization of the κ1 light chain by this extra disulphide bond [22, 23]. The simpler features of rabbit IgG make it easy for the molecular cloning and engineering of antibodies that are very critical in antibody drugs development.

Higher binding affinity

Antibody drugs cause side effects through nonspecific interactions. The high affinity is therefore very important to make antibodies good drugs. While most therapeutic antibodies have the dissociation constant (Kd) at nanomolar or sub-nanmolar level, RabMAbs have very high affinity with the Kd typically at the picomolar level [23]. As a result, RabMAbs offered increased sensitivity with no loss of specificity to the detection of various proteins playing important roles in human carcinogenesis, such as estrogen receptor, progesterone receptor and cyclin D1 [24]. It would be reasonable to predict that RabMAbs should have better clinical efficacy through the specific interaction with the targets.

Robust generation of RabMAbs for drug development

Generally, it is desirable to screen a large compound library to identify sufficient drug leads. This principle also applies to screen monoclonal antibodies for therapeutic antibody drugs. The rabbit has an advantage over the mouse because the spleen in the rabbit contains as much as 50 times more of lymphocytes than the mouse spleen. Hundreds of hybridomas can be generated from each immunized spleen, providing a much greater number of independent monoclonal antibodies that recognize different epitopes. Thus, a panel of bioactive RabMAbs could be easily obtained for further selection of antibody drug leads. The robustness in the gen-

eration of RabMAbs offers a much higher success rate to get the most desirable drug leads in a relatively shorter period of time.

Easy to be humanized

To reduce the immunogenecity of antibody drugs, several humanization methods have been developed to minimize the animal contents in antibody drugs. The widely used one is CDR grafting [6]. This method is majorly accomplished by inserting the appropriate CDR coding segments into a human antibody framework. In addition, some structurally critical residues in the framework regions are mutated back to the parental residues in order to reconstitute the original antigen binding affinity and specificity. Although 90% of the sequence is human origin after CDR grafting, the unchanged non-human CDRs have been found to be immunogenic in human [25]. Moreover, it is difficult to predict the role a particular residue in antibody activity. As a result, multiple humanized versions have to be tested in labor-intensive in vitro and in vivo assays. In addition, the CDR grafted antibodies showed a reduced affinity to their antigens and in vitro based affinity maturation is required to regain the affinity in the humanized antibodies.

A novel humanization technology termed Mutational Lineage-Guided (MLG) humanization was recently developed to humanize RabMAb much easier [26]. MLG humanization is both conceptually and technically different from the CDR-grafting method. A panel of bioactive antibodies will be needed for MLG humanization, which is actually the advantage of RabMAbs.

Amino acid sequences of the variable regions of the heavy and light chains (VH and VL) from the collection of IgG sequence are aligned to form a phylogenetic tree. Related antibodies are grouped according to their sequences similarity to each other. Conserved sequences in a lineage-related group represent residues critical to the structure and function of IgG while unconserved residues have less even no effects on the biological activities. Since these variable positions were obtained from a group of antibodies usually from one parental B cell, they must have been effectively examined by an animal immune system. Thus, substitution of amino acids at these positions to humanize antibodies should be well tolerated without sacrificing antibody specificity and affinity. More importantly, such variations are found not only in the framework regions, but also in the CDRs. Therefore, MLG humanization can be applied to the humanization of the framework regions as well as the CDRs. Due to the large numbers of lymphocytes of rabbit spleen, enough bioactive RabMAbs will be available to generate humanized RabMAb by MLG humanization.

The present of RabMAb

The rabbit is well known to produce diverse antibodies against many antigens including phospho-peptides, carbohydrates and immunogens that are not immunogenic in the mouse. Polyclonal antibody from the rabbit has been proven to be very useful in the laboratory. However, the inconsistency during immunization and limited amount of antibody production impeded its clinical application until rabbit monoclonal antibodies could be generated. Rabbit and mouse chimeric hybridomas were first generated with rabbit lymphocytes and mouse fusion partner SP20 [20]. However, these hybridomas were not stable enough and the fusion rate was very low. In 1995, Katherine Knight and her colleagues established the double transgenic rabbit overexpressing v-abl and c-myc under the control of the immunoglobulin heavy and light chain enhancers which developed myeloma-like tumors, allowing the establishment of a plasmacytoma cell line named 240E-1 [16]. Hybridomas generated by the fusion of 240E-1 cells with rabbit lymphocytes can consistently secret rabbit monoclonal antibodies. However, like the early mouse myeloma lines developed in 1970s, 240E-1 derived hybridomas were not genetically stable to produce antibodies consistently. A better fusion partner cell line named 240E-W was established by repeated subcloning and medium optimization of 240E-1. By yielding greater number of stable hybridomas, 240E-W has enabled large-scale development of rabbit monoclonal antibodies. As the third generation of rabbit fusion partner cell line, 240E-W2 was developed in 2006 to improve antibody activity and specificity by minimizing the interference of endogenous heavy chain [17]. Although 240E-W2 derived hybridomas were generally stable and no compromised sensitivity and specificity were observed, endogenous light chain was still found in a subset of hybridoma clones [23]. To mitigate this potential concern, Epitomics Inc. is currently in the process of developing a fourth

generation of the rabbit fusion partner line, in which both endogenous heavy chain and light chain genes are removed or made inactive.

Recently, numerous RabMAbs have been widely used in various applications in life science researches. RabMAbs have been recognized as superior reagents in immunohistochecmistry (IHC) and in detection of post-translational modification of proteins such as phosphorylation and actylation. A number of RabMAbs were also developed for the detection of biomarkers in targeted therapy against cancer [27, 28].

To evaluate the therapeutic potential of RabMAbs, Yanlan Yu and co-workers generated a panel of neutralizing RabMAbs against human vascular endothelial growth factor-A (VEGFA) in 2010. They humanized the lead candidate with MLG humanization which retained its parental biological properties. More potent efficacy on inhibiting the growth of H460 lung carcinoma and A673 rhabdomyosarcoma xenografts in mice was observed when compared with Bevacizumab which is the marketed VEGFA antibody produced by Genetech [18]. These studies demonstrated the potential for developing humanized RabMAbs as therapeutics. Further research and more efforts are needed to develop RabMAbs as therapeutics in cancer and other diseases.

Conclusions and perspectives

RabMAb has been demonstrated to be superior reagent for many applications in lab including Western blots, immunohistochemistry and flowcytometry. Due to its special antibody repertoire, high affinity and easiness for generation and genetic engineering, RabMAbs are becoming an attractive source of anti-cancer therapeutics. A number of RabMAbs will be used in targeted therapy against cancers in near future.

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