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Review

Generation of antibodies against membrane proteins ☆

Takao Hamakubo [a,*], Osamu Kusano-Arai [a,b], Hiroko Iwanari [a]

[a] Department of Quantitative Biology and Medicine, Research Center for Advanced Science and Technology, The University of Tokyo, 4-6-1 Komaba, Meguro, Tokyo 153–8904, Japan
[b] Institute of Immunology Co. Ltd, .1-1-10 Koraku, Bunkyo, Tokyo 112–0004, Japan

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ABSTRACT

The monoclonal antibody has become an important therapeutic in the treatment of both hematological malignancies and solid tumors. The recent success of antibody-drug conjugates (ADCs) has broadened the extent of the potential target molecules in cancer immunotherapy. As a result, even molecules of low abundance have become targets for cytotoxic reagents.
The multi-pass membrane proteins are an emerging target for the next generation antibody therapeutics. One outstanding challenge is the difficulty in preparing a sufficient amount of these membrane proteins so as to be able to generate the functional antibody. We have pursued the expression of various membrane proteins on the baculovirus particle and the utilization of displayed protein for immunization. The strong antigenicity of the virus acts either as a friend or foe in the making of an efficient antibody against an immunologically tolerant antigen. This article is part of a Special Issue entitled: Recent advances in molecular engineering of antibody.

1. Introduction

Monoclonal antibodies (mABs) have attracted considerable interest in the treatment of cancer and autoimmune disorders [1–4].

In cancer, the therapeutic antibody targets are cell surface molecules which are predominantly comprised of membrane proteins. The main protein targets in cancer immunotherapy are growth factor receptors or overexpressed differentiation antigens. These target proteins are not strictly tumor specific, but perform an important function in the targeting cancer drugs. Furthermore, recent studies have demonstrated the heterogeneity of cancer cells and the problem of cancer stem cells acting in their own microenvironment [5,6]. To help overcome these difficulties, both toxins and radionuclide-conjugated antibodies have been developed for the purpose of improved cytotoxic activity [7–9]. Antibody engineering has also evolved so as to allow the design of bispecific antibodies, which increase the cytotoxic efficiency by either the conjugation of immunoadoptive target molecules or the blockade of immune checkpoints [10–12].

Thus, one of the important issues for the next generation of therapeutic antibodies is to obtain a higher affinity for the purpose of targeting less abundant surface molecules. Although it is difficult to raise high affinity antibodies against membrane proteins, there have been many useful strategies put forth for therapeutic antibodies, such as the use of DNA immunization [13] or phage-display [14]. Each of these technologies has its own merits and demerits, and the selection of which to use in order to obtain an effective antibody is largely dependent on the characteristics of the target protein. We here introduce our baculovirus display technology for generating mABs against membrane proteins.

2. Membrane protein preparation

The major target membrane proteins used for cancer immunotherapy thus far have been either cell surface receptors or adhesion molecules (Table 1). There is one anti-G protein-coupled receptor (GPCR) mAB on the market for the treatment of leukemia. The others include single-pass membrane receptors, and there is no ion channel or transporter protein in clinical use or phase III trials. The blockade of receptor function other than antibody-dependent cell-mediated cytotoxicity (ADCC) has certain favorable aspects that make it a good target choice. However, as the armed antibody therapeutics such as ADCs, radioimmunoconjugates and bispecific antibodies with an immunoadaptive recognition site have come to market, it has come to be expected that the less abundant proteins, such as GPCRs or other multi-pass membrane proteins, would eventually be realized as a target for cancer therapeutics.

In this regard, there have been difficulties in generating high affinity mABs against multi-pass membrane proteins. These antibodies are needed to recognize the native state of membrane proteins on the cell surface. The most pressing problems include (1) the difficulty of the preparation of a large amount of the protein in the proper conformation [15] and (2) the immunological tolerance that occurs due to the high level of sequence homology between species in the case of many critically important proteins.

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* Corresponding author. Tel./fax: +81 3 5452 5231.
E-mail address: hamakubo@qbm.rcast.u-tokyo.ac.jp (T. Hamakubo).

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Table 1
Therapeutic target membrane proteins of monoclonal antibodies for cancer in Phase 3 trials or on the market.

Target classes	Target and type	Name of mAB	Indicated diseases
Receptor (GPCR, 7TM)	CCR4	Mogamulizumab	Adult T-cell leukemia/lymphoma
Receptors (non-GPCR)	CD30 (TNFRSF8)	Brentuximab vedotin	Hodgkin's lymphoma
	EGFR	Cetuximab	Colorectal cancer
	EGFR	Panitumumab	Colorectal cancer
	EGFR	Necitumumab	NSCL cancer
	EGFR	Nimotuzumab	SCC(head neck), glioblastoma multiforme
	EGFR	Zalutumumab	Head and neck cancer
	HER2	Pertuzumab	Metastatic breast cancer
	HER2	Trastuzumab	Breast cancer
	IGF-1R	Dalotuzumab	Metastatic colorectal cancer
	Folate receptor a	Farletuzumab	Ovarian cancer
	CD80 (ligand for CD28, CTLA-4)	Galiximab	NHL
	CD20	Ibritumomab	NHL
	CD20	Obinutuzumab	Diffuse large B cell lymphoma, CLL, NHL
	CD20	Ofatumumab	Diffuse large B cell lymphoma, CLL, NHL
	CD20	Rituximab	NHL
	CD20	Tositumomab	Malignant lymphoma, CLL
	CD22 (SIGLEC family)	Inotuzumab ozogamicin	ALL, NHL
	CD22	Moxetumomab pasudotox	Hairy cell leukemia
	CD33 (SIGLEC family)	Gemtuzumab ozogamicin	Acute myeloid leukemia
	VEGFR2	Ramucirumab	Metastatic gastric or gastroesophageal junction adenocarcinoma; breast cancer; hepatocellular carcinoma
	CD4	Zanolimumab	Cutaneous T-cell lymphoma
	CD2	Elotuzumab	Multiple myeloma
	cMET (HGFR)	Onartuzumab	NSCL cancer, gastric cancer
	PD1	Nivolumab	NSCL cancer, renal cell carcinoma, melanoma
	CTLA-4	Ipilimumab	Advanced melanoma, sepsis
	CTLA-4	Tremelimumab	Metastatic melanoma
Adhesion molecule	EpCAM/CD3	Catumaxomab	Malignant ascites
Enzyme	Carbonic anhydrase ix (metalloenzyme)	Girentuximab	Non-metastatic renal cell carcinoma
Others (oncofetal or differentiation antigen)	CD52 (GPI anchor)	Alemtuzumab	B-cell chronic lymphocytic leukemia, GVH(graft versus host), Multiple Sclerosis
	5 T4	Naptumomab estafenatox	Advanced renal cell carcinoma

Based on the data from ref. [2]; mAB, monoclonal antibody; GPCR, G protein coupled receptor; TM, trans-membrane; CCR4, C-C chemokine receptor type 4; CD, cluster of differentiation; TNFRSF8, tumor necrosis factor receptor superfamily, member 8; EGFR, epidermal growth factor receptor; NSCL, non-small cell lung ; SCC, squamous cell carcinoma; HER2, human epidermal growth factor receptor 2; IGF-1R, insulin-like growth factor-1; CTLA-4, cytotoxic T-lymphocyte antigen 4; NHL, non-Hodgkin's lymphoma; CLL, chronic lymphocytic leukemia; ALL, acute lymphoblastic leukemia; VEGFR2, vascular endothelial growth factor receptor 2; cMET, met proto-oncogene; HGFR, hepatocyte growth factor receptor; PD-1, programmed death-1; EpCAM, epithelial cell adhesion molecule

The use of a peptide fragment or small domain as an immunogen is frequently unsuccessful in the effort to obtain specific antibodies which recognize cell surface antigens. This is due to the difference in conformation between the peptide and the protein. For single-pass membrane proteins, the use of entire extracellular portion of these proteins is reportedly largely successful. For example, the use of the Fc fusion protein [16] has been shown to be largely successful in the preparation of the large amount of protein necessary for immunization.

In recent years, the preparation has been sufficiently improved that several multi-pass membrane proteins, including GPCRs, have been obtained in an amount that allows crystallography [17,18]. The solubilized proteins are reconstituted in phospholipid vesicles in which the adjuvant molecule is also incorporated for immunization [19,20].This method is very useful in the generation of mABs for crystallization probe [21,22]. As the proteins are incorporated in a random orientation in the liposome, the antibodies generated by this method tend to recognize the cytosolic side of the membrane protein, probably due to the immunological tolerance of the exposed side.

We observed that a relatively large amount of membrane proteins are displayed on the budded baculovirus (BV) particles during the expression of membrane proteins of endoplasmic reticulum (ER) origin [23]. Upon further investigation we found this BV display useful for the generation of antibodies against the multi-pass membrane proteins that are typically difficult to obtain in sufficient amounts. In addition to the whole protein display method, there is also a BV display technique using a fragment peptide as a fusion protein with the viral membrane protein gp64 [24,25] (Fig. 1).

3. Baculovirus display of membrane proteins

3.1. Whole protein

The expression of functional membrane proteins on BV particles was first reported by Loisel et al. [26,27]. They attempted to recover the β2-adrenergic receptor (β2-AR) on the virus-like particles (Gag-particles) from Spodoptera frugiperda (Sf9) cells by infecting recombinant baculovirus harboring the human immunodeficiency virus type 1 (HIV-1) Pr55Gag protein gene. Contrary to their expectations, the β2-AR did not appear on the gag-particles, but on the BV particles. The receptor expressed on the virus was functionally coupled to both the Gs and adenylyl cyclase of host insect cell origin, and exhibited a higher level of activity than that recovered from the Sf9 cell membrane fraction. The membrane protein collected from the Sf9 cells often includes a substantial proportion of inactive protein that is difficult to separate and a major cause of the difficulty in raising the antibody. We encountered this phenomenon when expressing a membrane protein of endoplasmic reticulum origin [23]. We then examined GPCR expression on the BV using the leukotriene B4 (LTB4) receptor (BLT1) [28]. In this case, BLT1 couples to the Gi isoform of a trimeric G-protein which inhibits adenylyl cyclase. As the Gi isoform is not expressed in Sf9 cells, we were able to recover the highly sensitive LTB4 binding activity on the BV after the co-infection of recombinant baculoviruses which harbor trimeric Gi-protein subunit genes [28]. These show that BV has the capacity to display not only a single membrane protein by itself, but also the reconstituted functional protein complex. On this point, we have further demonstrated that the effector protein adenylyl cyclase,

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