Epitope Mapping of Series of Monoclonal Antibodies Against the Hepatocellular Carcinoma-associated Antigen HAb18G/CD147

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Abstract

The hepatocellular carcinoma-associated antigen HAb18G/CD147, a member of CD147 family, could promote tumour invasion and metastasis via inducing the secretion of matrix metalloproteinases (MMP). Anti-CD147 monoclonal antibodies (MoAb) have exhibited obvious inhibitory effect on MMP induction. However, none of the epitopes of these MoAb has been reported. We previously prepared five MoAb against HAb18G/CD147, named HAb18, 3B3, 1B3, 5A5 and 4D2. To map the epitopes of these MoAb, a series of truncated fragments of extracellular region of HAb18G/CD147 was expressed in Escherichia coli and the MoAb-binding affinity to these fragments was examined with an enzyme-linked immunosorbent assay and Western blot. The residues 39LTCSLNDSATEV50, 36KILLTCS42 and 22AAGTVFTTVEDL33 were determined to be the epitopes of HAb18, 3B3 and 1B3, respectively, which were further proved by a dot-blot analysis with synthesized peptides and bioinformatics epitope prediction. The binding regions of MoAb 5A5 and 4D2 were located at residues E120–R203. Then we constructed and expressed full-length HAb18G/CD147 and truncated HAb18G/CD147 without residues A22–V50 in COS-7 cells. Gelatin zymography and Boyden chamber assay showed that the COS-7 cells expressing truncated HAb18G/CD147 failed to induce MMP production and enhance the cells’ invasive potential, compared with the cells expressing full-length HAb18G/CD147. Taken together with the obviously inhibitory effects of HAb18 on the function of full-length HAb18G/CD147, these findings suggest that residues 22AAGTVFTTVEDLGSKILLTCSLND-SATEV50 may play a critical role in the functions of HAb18G/CD147 on MMP secretion and tumour invasion. These key residues can be used as potential drug target in cancer therapy.

Introduction

HAb18G/CD147, a new hepatocellular carcinoma-associated antigen, was cloned from human hepatocellular carcinoma cDNA library by hepatoma MoAb HAb18 [1] and found abundantly expressed in human hepatoma tissues [2, 3]. Querying by NCBI BLAST showed that its coding gene was homologous to human CD147 molecule, so it was named HAb18G/CD147 as a new member belonging to CD147 family. CD147 is also named as extracellular matrix metalloproteinase inducer (EMM-PRIN) [4], basigin [5], neurothelin [6] in different tissues or cells. As an adhesion molecule, CD147 has been reported to bind to a variety of cells, such as endothelial cells and fibroblasts. Up-regulated CD147 expression is detected in numerous malignant tumours and correlated with tumour progression in pre-clinical and clinical research. CD147 could stimulate fibroblast cells to produce elevated levels of several kinds of MMP, including MMP-1, MMP-2 and MMP-9, which were well known for prompting the invasion of hepatoma cells [7–9].

CD147 is composed of an extracellular region containing two immunoglobulin (Ig)-like domains, a single transmembrane region and an intracellular region [10–12]. The first immunoglobulin domain in the N-terminal is required for counter receptor activity [13], involved in
MMP induction [14] and oligomerization. Peptides derived from the second 20 amino acids of this domain could block CD147 activity possibly by interfering with CD147 oligomerization [15]. The second immunoglobulin domain is required for association with caveolin-1 which leads to decreased self-association on the cell surface [16]. It is also reported that CD147 contains at least two bioactive domains, and the epitopes responsible for induction of cell aggregation are different from those regulating lymphocyte activation [17].

In our previous work, MoAb HAb18 was generated from mice immunized with hepatocellular carcinoma cell suspension, and the other four monoclonal antibodies (MoAb), named 3B3, 1B3, 5A5 and 4D2, were prepared from mice immunized with recombinant HAb18G/CD147 by hybridoma method [18]. These MoAb against different epitopes of HAb18G/CD147 had their distinct functions. Therefore, mapping the epitopes of these MoAb is very important to further understand the function of HAb18G/CD147.

Epitope mapping is the process of identification of the molecular determinants for antibody–antigen recognition. Identification of the epitope is a key step in the characterization of MoAb, especially those used in therapeutic strategies [19]. Several methods have been developed for mapping protein epitopes of MoAb, which involve competition assay, partial proteolysis, expressed fragments, peptide library, mass spectrometry (MS) and structure resolution (NMR and X-ray crystal diffraction). Based on the known gene sequence of HAb18G/CD147, constructing fragments of expressed is the most convenient and fastest strategy for epitope mapping.

In the present study, a series of truncated fragments of extracellular domain of HAb18G/CD147 were first constructed and expressed, and the epitopes of the above five MoAb were mapped by an enzyme-linked immunosorbent assay (ELISA) and Western blot, followed by a dot-blot analysis with synthesized peptides, bioinformatics epitope prediction and the final biological function analysis.

Materials and methods

Design of expressed fragments. CD147 is a single-chain type I transmembrane molecule containing 269 amino acids [4]. Excluding the N-terminal signal peptide (M\textsubscript{1}–G\textsubscript{21}), the transmembrane region (residues L\textsubscript{206}–Y\textsubscript{229}) and the intracellular region (residues E\textsubscript{230}–S\textsubscript{269}), the 184-residue-long extracellular region (residues A\textsubscript{22}–H\textsubscript{205}) that consisted of two Ig-like domains is the major exposure region recognized by MoAb. For primary screening, the fragments containing full length extracellular region and the two Ig-like domains were designed as F22 (residues A\textsubscript{22}–H\textsubscript{205}), D1(residues G\textsubscript{34}–P\textsubscript{105}) and D2 (residues E\textsubscript{120}–R\textsubscript{203}) respectively (Fig. 1). Considering that the first domain is

![Figure 1 Design of truncated fragments of HAb18G/CD147](image)

the critical activity region, protein prediction was performed with bioinformatics method. According to the results on hydrophilicity, hydrophobicity, antigenicity and secondary structure, we found several potential key residues, including residue K\textsuperscript{36}, H\textsuperscript{53}, L\textsuperscript{62}, G\textsuperscript{83}, etc. So, a series of fragments truncated from N-terminal in this domain were designed as F36, F39, F42, F45, F48, F51, F53, F62 and F83 (Fig. 1).

Gene expression and protein purification. Primers used to amplify the series of truncated HAb18G/CD147 genes by polymerase chain reaction (PCR) are listed in Table 1. Restriction sites NdeI and EcoRI were introduced in forward primer and XhoI in reverse primer for cloning-truncated fragments into expression vector pET32a(+) (Novagen, Madison, WI, USA). Cloning between NdeI and XhoI can lead to a non-fusion expression, while that between EcoRI and XhoI can lead a Trx-tag fusion expression. Furthermore, codegenerate codons were adopted in these primers for the codon preference in Escherichia coli systems. All these strategies could ensure a high-performance expression of these truncated protein fragments.

HAb18GEF/pET21a(+) is a recombinant vector constructed by our laboratory in previous work, containing the extracellular region of HAb18G/CD147 [20]. Taking this vector as template, PCR was performed in routine protocol. Purified PCR products were digested with restriction enzymes, XhoI and NdeI or EcoRI (TaKaRa, Otsu, Shiga, Japan) and inserted to vector pET32a(+). The recombinant vectors were transformed into E. coli JM109-DE3 competent cells. Cells were cultured in Luria–Bertani (LB) medium and induced by isopropyl-β-D-thiogalactopyranoside (IPTG) to overexpress proteins. The suspension cultures were harvested, resuspended in PBS, and then incubated on ice for 15 min. Proteins were isolated in 8 M urea, subsequently purified by
affinity chromatography using Ni-NTA resin columns (Invitrogen, Carlsbad, CA, USA). The protein concentration was determined using the Lowry method.

**ELISA.** High-binding 96-well flat-bottomed plates (Costar, New York, NY, USA) were coated overnight at 4°C with 100 µl of different truncated fragments of HAb18G/CD147 at a concentration of 1 µg/ml. The plates were then blocked with 100 µl of different truncated fragments of HAb18G/CD147 were heated at 100°C for 5 min in loading buffer (50 mM Tris–HCl, pH 6.8, 100 mM dithiothreitol, 2% SDS, 0.1% bromophenol blue, 10% glycerol), and separated by 12% SDS-PAGE and transferred to PVDF membranes (Millipore, Billellica, MA, USA) in transfer buffer (25 mM Tris, 192 mM glycine, 10% methanol) at 100 V for 1 h. Blocked with PBS-T buffer containing 5% non-fat dry milk at 37°C for 1 h, PVDF membranes were rinsed in PBS-T and incubated overnight with different MoAb diluted at 1:200 in PBS-T at 4°C. Finally, the membranes were washed in PBS-T and incubated with 0.4 µg/ml goat anti-mouse IgG conjugated with HRP at 37°C for 1 h. Finally, washed in PBS-T, proteins were detected with ECL-plus Western Blotting System (GE Amersham, Little Chalfont, UK) and the images were exposed to films (KODAK, Rochester, NY, USA).

**Table 1** Primers for the construction of truncated fragments of HAb18G/CD147.

| Fragment | Sequence | Location | Size (bp) |
|----------|----------|----------|----------|
| F22a (22–205) | 5'-GGCAATACGACTAGTGCTGGCCTGACCTTTGCACTACCTACCGTGAAAGACCTT-3' | 37–55 | 579 |
| D1b (34–87) | 5'-CTAGATTGATTGTCGGCGCAGGCGGAG-3' | 600–615 | |
| D2a (120–205) | 5'-GGCAATACGACTAGTGCTGGCCTGACCTTTGCACTACCTACCGTGAAAGACCTT-3' | 312–294 | |
| F36 (36–205) | 5'-GTCAATCGAGGCTGGCTGCAGCAGCGGAG-3' | 600–615 | |
| F39 (39–205) | 5'-GTCAATCGAGGCTGGCTGCAGCAGCGGAG-3' | 114–132 | 502 |
| F42 (42–205) | 5'-GTCAATCGAGGCTGGCTGCAGCAGCGGAG-3' | 600–615 | |
| F45 (45–205) | 5'-GTCAATCGAGGCTGGCTGCAGCAGCGGAG-3' | 132–150 | 484 |
| F48 (48–205) | 5'-GTCAATCGAGGCTGGCTGCAGCAGCGGAG-3' | 600–615 | |
| F51 (51–205) | 5'-GTCAATCGAGGCTGGCTGCAGCAGCGGAG-3' | 150–168 | 466 |
| F53 (53–205) | 5'-GTCAATCGAGGCTGGCTGCAGCAGCGGAG-3' | 600–615 | |
| F62 (62–205) | 5'-GTCAATCGAGGCTGGCTGCAGCAGCGGAG-3' | 156–174 | 500 |
| F83 (83–205) | 5'-GTCAATCGAGGCTGGCTGCAGCAGCGGAG-3' | 600–615 | |

*Sequence refers to CD147: Homo sapiens basigin (GenBank accession No. D45131).*

*Non-fusion protein.

*Fusion protein.

Underlines indicate restriction sites NdeI, EcoRI and XhoI.
Epitope prediction with bioinformatics method. To verify the mapping results, epitope prediction with bioinformatics method was also performed. Using PsiPred (ver2.1, a method for protein secondary structure prediction) [22], the secondary structure of HAb18G/CD147 extracellular region was predicted. The fold recognition was analysed by GenTHREADER (a method for genomic fold recognition) [23], which provided a result of structural classification of proteins (SCOP) to classify the structure of proteins [24]. Then hydrophilicity and antigenicity were calculated by the HoopWoods method [25], the final linear profile would indicate the potential epitope.

Eukaryotic vector construction. The DNA fragment encoding full-length HAb18G/CD147 (amino acid 1–269) was amplified by PCR with primers A and D (Table 2). Another truncated fragment named E51, which was a recombinant protein consisting of amino acid residues M30–G50 and amino acid residues T31–S269, was amplified by overlapping PCR with primers A and D and overlapping primers B and C (Table 2). E51 is just a full-length HAb18G/CD147-deleted amino acid residues A22–V50.

Table 2 Primers for the construction of eukaryotic vectors of HAb18G/CD147.

| Primer          | Sequence                          | Location |
|-----------------|-----------------------------------|----------|
| A (forward)     | 5’-GTCACCTGAGATGCGCGCGCTGCTGTTGCT-3’ | 30–50    |
| B (overlapping reverse) | 5’-CAGCCCGGTCGGCGCTGCTGCGCCGGGACGGCGGCTGCTG-3’ | 180–197  |
| C (overlapping forward)  | 5’-ACCGCGGGCCCGGACCGGCGGCTGCTG-3’ | 75–92    |
| D (reverse)     | 5’-GATCCTGATCCCAGGAAGGTTCTCTCTG-3’ | 822–839  |

Sequence refers to Homo sapiens basigin (GenBank accession No. D45131); Underlines indicate restriction sites of XhoI and BamHI. The overlapping forward and reverse primers (B and C) are composed of two oligos specific to sequence 75–92 and 180–197, respectively. The overlapping PCR products will lose the sequence 93–179, which encodes amino acid residues 22–50.

Expression of truncated HAb18G/CD147 fragments
Truncated HAb18G/CD147 DNA fragments were amplified by PCR, cut through two different groups of restrict

37 °C, 5% CO₂. A cell fluorescence assay was performed to detect green fluorescence after 12 and 24 h.

Gelatin zymography. HAb18G/CD147/pEGFP-N1/COS-7 and E51/pEGFP-N1/COS-7, as cells transfected with HAb18G/CD147/pEGFP-N1 and E51/pEGFP-N1, were cocultured with human dermal fibroblasts (conserved by our laboratory) in RPMI-1640 medium (5 × 10⁴ cells per 300 μl) in a 96-well plate at 37 °C for 24 h respectively. Then the supernatant was harvested and separated in 10% SDS-PAGE containing 1 mg/ml gelatin. After electrophoresis, gels were washed twice with 2.5% Triton X-100 at room temperature and incubated at 37 °C for 12 h with 40 mM Tris–HCl (pH 7.5), 10 mM CaCl₂ and 1 μM ZnCl₂, then stained with 0.5% Coomassie blue for 1 h. After destaining, MMP were detected as clear bands against blue background.

Borden chamber assay. The Borden chamber assay was performed using 24-well transwells units (MilliPore), each with an 8-μm pore size polycarbonate filter coated with 5 μg/ml of Matrigel (BD, Bedford, MA, USA) in a cold medium to form a continuous thin layer. Then the cell suspension of full-length HAb18G/CD147 or E51 transfected cells and human dermal fibroblasts (3 × 10⁵ cells) in a serum-free medium in the presence or absence of MoAb HAb18 were added into coated wells. COS-7 cells were used as negative control. The dried layer of Matrigel matrix was rehydrated with a 450-μl medium without fetal bovine serum (FBS). The cells were then cultured at 37 °C, 5% CO₂ for 24 h. The cells remaining in the upper compartment were completely removed by gently swabbing. The number of cells invaded through the filter into the lower compartment was determined using a colorimetric HE assay.

Results

Expression of truncated HAb18G/CD147 fragments
Truncated HAb18G/CD147 DNA fragments were amplified by PCR, cut through two different groups of restrict
endonucleases of NdeI and XhoI or EcoRI and XhoI and inserted into a prokaryotic vector pET32a(+) respectively. The results of a restriction endonuclease analysis and sequencing showed that the PCR products were inserted into the vector correctly. Twelve recombinant truncations of HAb18G/CD147 were overexpressed in E. coli JM109-DE3 strain (Fig. 1). Among them, seven truncations (D1, D2, F22, F36, F42, F48 and F83) were non-fusion pro-

teins, and the other five (F39, F45, F51, F53 and F62) were fusion proteins with Trx-tag (Figs. 2A and 3A).

Epitope mapping of monoclonal antibodies

Five MoAb, HAb18, 3B3, 1B3, 5A5 and 4D2, were prepared as described previously [19] and used for epitope mapping by an ELISA and Western blot analysis. The results showed that MoAb HAb18 and 3B3 could bind to both D1 and F22 (whole extracellular region of HAb18G/CD147) (Fig. 2A–C), MoAb 5A5 and 4D2 could bind to both D2 and F22 (Fig. 3A–C) and MoAb 1B3 could only bind to F22 (Fig. 2D).

To further map the epitopes of MoAb HAb18, 1B3 and 3B3, nine truncations, F36, F39, F42, F45, F48, F51, F53, F62 and F83, were used for a Western blot and ELISA analysis. Results showed that, for MoAb HAb18 binding, the truncations, F36 and F83, retained

Table 3 Reactivity of MoAb, HAb18, 3B3, 1B3, 5A5 and 4D2 with truncated HAb18G/CD147 fragments in an ELISA and western blot.

|       | Hab18 | 3B3 | 1B3 | 5A5 | 4D2 |
|-------|-------|-----|-----|-----|-----|
| F22   | ++    | ++  | ++  | ++  | ++  |
| D1    | ++    | ++  | ++  | ++  | ++  |
| D2    | ++    | ++  | ++  | ++  | ++  |
| F36   | ++    | ++  | ++  | ++  | ++  |
| F39   | ++    | ++  | ++  | ++  | ++  |
| F42   | ++    | ++  | ++  | ++  | ++  |
| F45   | ++    | ++  | ++  | ++  | ++  |
| F48   | ++    | ++  | ++  | ++  | ++  |
| F51   | ++    | ++  | ++  | ++  | ++  |
| F53   | ++    | ++  | ++  | ++  | ++  |
| F53   | ++    | ++  | ++  | ++  | ++  |
| F62   | ++    | ++  | ++  | ++  | ++  |
| F83   | ++    | ++  | ++  | ++  | ++  |
| pET32a(+) | +    | +   | +   | +   | +   |
| E, ELISA; W, Western blot; ++, strongly positive; +, positive; --, negative; Neg, negative control. |
full binding reactivity, while F42, F45 and F48 had weak binding reactivity and F51 had no binding reactivity (Table 3 and Fig. 2A,B), demonstrating that 39LTCSLNDSATEV50 was the critical amino acid residues of the epitope of MoAb HAb18.

For MoAb 3B3 binding, the truncations, F36 and F39, presented full binding reactivity, while F42 had no binding reactivity (Table 3 and Fig. 2A,C), suggesting that the critical amino acid residues of HAb18G/CD147 for interaction with MoAb 3B3 was 36KILLTCS42.

As to MoAb 1B3, none of the nine used truncations exhibited binding reactivity, indicating that the region of HAb18G/CD147 for interaction with MoAb 1B3 was located at amino acid residues 22AAGTVFTTVEDL33 (Table 3 and Fig. 2A,D).

The above findings were further confirmed by a dot-blot assay with a synthesized peptide. Two 12-amino acid peptides, peptide 1 (39LTCSLNDSATEV50) and peptide 2 (22AAGTVFTTVEDL33) were synthesized in our laboratory. Peptide 1 contained the possible epitopes of both HAb18 and 3B3, while peptide 2 was designed specifically for 1B3. A dot-blot assay showed that MoAb HAb18 and 3B3 could interact with peptide 1 and MoAb 1B3 with peptide 2 (results not shown).

Epitope prediction with bioinformatics method

The above-identified epitopes were compared with the results of epitope prediction with bioinformatics method. The extracellular region sequence of HAb18G/CD147 mature chain, from A22 to A207, was chosen for a secondary structure prediction and fold recognition analysis.

Using the mGenTHREADER method, an N-terminal fragment of axonin-1 from chicken (PDB entry ID is 1CS6) was hit, whose second structure (including corner and β-sheet) was highly similar to that of HAb18G/CD147 extracellular region (Fig. 4). According to the secondary structure of 1CS6, a chain whose SCOP codes were b.1.1.4, two structure domains of the extracellular region of HAb18G/CD147 were predicted to both belong to immunoglobulin superfamily and have a type-fold of immunoglobulin-like beta-sandwich which contained seven strands in two sheets. The second structure of HAb18G/CD147 extracellular region was also confirmed by PsiPred method.

Then the hydrophilicity and antigenicity of the extracellular region sequence of HAb18G/CD147 were obtained with HoopWoods method and the peak value indicated the possible binding site for antigen–antibody complex.
interaction (Fig. 5). As a result, S^{42}, N^{44}, D^{45} and E^{49} might be the key residues of antigen-binding site. These results of epitope prediction further proved the experimental results described above.

**Functional study of the recognized epitopes**

Eukaryotic vectors of HAb18G/CD147/pEGFP-N1 and E51/pEGFP-N1 (truncated HAb18G/CD147 deleted amino acid residues A^{22}–V^{50}) were constructed and identified by a sequencing and restriction endonuclease analysis. The vectors were transfected into COS-7 cells to express HAb18G/CD147-GFP and E51-GFP fusion proteins. The correct expression of recombinant proteins was identified by the detection of green fluorescence. The functions of the fusion proteins were evaluated with a gelatin zymography and Borden chamber assay. Gelatin zymography showed that HAb18G/CD147/pEGFP-N1/COS-7 cells, expressing full-length HAb18G/CD147, succeeded to induce the production of MMP-2 and MMP-9 by a coculture with human dermal fibroblasts; however, E51/pEGFP-N1/COS-7 cells, expressing E51, failed in the induction of MMP (Fig. 6). Borden chamber assay showed that the amounts of HAb18G/CD147/pEGFP-N1/COS-7 cells invaded through Matrigel coated on the filter increased obviously after 24 h culture compared with the control cells and E51/pEGFP-N1/COS-7 cells (Fig. 7). MoAb HAb18 exhibited obvious inhibitory effects on the production of MMP and the invasive potential of HAb18G/CD147/pEGFP-N1/COS-7 cells, which were induced by full-length HAb18G/CD147 (Figs. 6 and 7). These results indicated that amino acid residues A^{22}–V^{50} were essential for the functioning of HAb18G/CD147 and could be used as a promising drug target.

**Discussion**

Matrix metalloproteinases are believed to be a critical group of enzymes that affect tumour angiogenesis, tumour growth, local invasion and subsequent distant metastasis [26, 27]. The expression of MMP could be attributed to tumour stromal cells and is partially regulated by tumour–stroma interactions via tumour cell-associated extracellular matrix metalloproteinase inducer (EMMPRIN/CD147). Our previous studies have implicated that HAb18G/CD147, a hepatocellular carcinoma-associated antigen, is a member of CD147 family and plays an important role in the adhesion, invasion and metastasis of hepatocellular carcinoma via MMP involvement. Thus, an epitope mapping of functional HAb18G/CD147 extracellular region is very important for investigating the molecular mechanisms about invasion and metastasis of human hepatocellular carcinoma.

In the present study, we mapped the epitopes of a series of MoAb against antigen HAb18G/CD147. Epitope mapping is important for the characterization of MoAb, while an epitope mapping of MoAb can provide useful information about the bioactive domains of target molecules. There are several feasible methods for epitope mapping.
The usual technique hitherto expressed fragments based on the known gene sequence, has been used for epitope mapping of many antigens including ribonucleoproteins designated as Ro [28], La [29], ICA512/IA2 [30], avain reovirus [31], N53 protein [32], SARS-CoV [33], etc.

Two key problems about expressed fragment method should be considered. One is fragment design. A good fragment design could accelerate the progress of the experiment, reduce the workload and help result analysis. We first divided the extracellular region of HAb18G/CD147 into two domains. Based on bioinformatics analysis, the first domain was further designed as nine truncated fragments. All fragments worked well for epitope mapping. The other problem is high performance expression. A non-fusion protein has higher specificity, but a fusion protein has higher yield and is easier to be expressed. So the fragments were constructed with two kinds of insert manner for non-fusion and Trx-tag fusion expressions respectively. This strategy ensured that all fragments could be expressed with a high specificity and a high yield.

There are two kinds of epitopes: linear epitope and conformational epitope. In this study, all MoAb showed different reactivity with full-length protein and N-terminal-truncated fragments, which indicated that their epitopes were linear epitope, also called conformational-independent epitope.

CD147 is a highly glycosylated protein. Glycosylation was shown to determine MMP-stimulating activity of CD147 [14, 34]. The purified deglycosylated EMMPRIN/CD147 not only failed to induce the activity of MMP but also antagonized the activity of the native molecule [14]. To determine the role of epitope in HAb18G/CD147 function and to confirm whether the identified epitope above would be shielded or reconstructed by glycosylation, we constructed eukaryotic vectors of full-length HAb18G/CD147 and E51 (deleted amino acid residues A_{22}–V_{50}), and expressed in eukaryotic system to obtain glycosylation protein. Gelatin zymography E51/pEGFP-N1/COS-7 cells had lost the MMP induction activity compared with HAb18G/CD147/pEGFP-N1/COS-7 cells, which indicated the potential function of amino acid residues A_{22}–V_{50}. MoAb HAb18 exhibited inhibitory effects on the increased production of MMP and the enhanced invasive potential of HAb18G/CD147-GFP/COS-7 cells, which implied that the epitope still worked in eukaryotic protein and did not obviously interfere by glycosylation.

Several research works into epitope mapping of CD147 MoAb have been reported in recent years. Koch et al. reported T-cell activation-associated epitopes of CD147. They first established 15 specific MoAb against CD147, then mapped epitopes of these MoAb by cross-blocking analysis using real-time biospecific interaction analysis (BLAcore). Finally, the 15 CD147 MoAb were clustered into seven major groups defining seven putative epitopes [35]. Tayapiwatana et al. displayed the external domain of CD147 on phage, and the epitopes of CD147 were further mapped by a competitive inhibition ELISA. Certain clusters of MoAb recognition areas were identified [36]. Another result from this research group indicate that CD147 contains at least two bioactive domains: epitopes responsible for the induction of cell aggregation are different from those regulating lymphocyte activation [17]. In conclusion, all the results above were based on competition assay method, without epitope sequence in detail. However, in our study, the epitope regions were located at amino acid residues A_{22}–V_{50}, which contain different epitopes of three specific MoAb against HAb18G/CD147.

The pivotal role of HAb18G/CD147 in the regulation of MMP production suggests the key function of HAb18G/CD147 in tumour invasion and metastasis. Our study suggested that amino acid residues A_{22}–V_{50} may be critical residues for MMP induction activity of HAb18G/CD147. These amino acid residues would be used as potential targets for therapeutic antibody or antagonist peptide in cancer therapy.

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