Prediction analysis of B cell epitopes of human aquaporin 4

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Abstract: Objective: To analyze the advantageous B cell epitopes of human aquaporin-4 (hAQP4) for monoclonal antibodies preparation. **Method:** Multi-parameter including hydrophilic, secondary structure, flexibility, polarity, accessibility, charge distribution, and antigen index were used to analyze the B cell epitopes of hAQP4. **Result:** hAQP4 has six transmembrane fragments, which are amino residues of 38-57, 69-88,113-133,156-176 and 188-209. Antigenic peptides and the peptides of 11-19, 45-55, 65-81, 84-92, 105-111, 131-151, 158-175, 232-239, 246-258, 292-306, and 317-323 were selected by multi-parameter analysis. Ten peptides of hAQP4 included peptides of 1-11,10-19,45-56,58-69,112-119,130-151,158-175,178-185,253-275 and 300-317 were predicted to have high immunogenicity. Among the peptides of 10-19 and 58-69 had same immunogenicity. **Conclusion:** We have obtained B cell epitopes of hAQP4 using multi-parameter analysis prediction. It might be a potential molecular target for the development of vaccines.
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Key words: human aquaporin 4; B cell; epitopes; predict; vaccine target

1. Introduction

Cerebrospinal fluid (CSF),derived from free cells and lymphocytes, is a colorless and transparent liquid. CSF is mostly water, which contains some proteins and high concentrations of Na+, K+ and Cl^-.There is about 100 ml CSF circulating in the ventricles of brain, the central canal of spinal cord, subarachnoid and perivascular space. CSF is secreted by the choroid plexus epithelium and is absorbed by the arachnoids, thus completing the circulation. The water circulation is the main process of the CSF circulation.

It was thought that water balance in human body is achieved by the diffusion caused by a semi-permeable membrane osmotic pressure. However, water as a main component of human body, its daily exchange can reach up to 100 liters. Because cell membrane is composed of two layers of molecular lipids, it is impossible to exchange a large amount of water daily only through the lipids. Water channel proteins (aquaporin, AQP) were discovered involving the water transmembrane transport (Sui et al.,2011). They selectively transport water and some small solutes cross plasma membranes in mammals, plants, and lower organisms (Verkman 2002; Song and He 2012). According to their transport function the aquaporin family is divided into two subfamilies. One subfamily conducts pure water, the other, aquaglyceroporins conducts glycerol well at close to diffusion rates for glycerol or urea and have low conductance of wate (Maurel et al., 1994). Water channel proteins are a water-specific membrane proteins with physiological function mediating free water molecules across the membrane. In the existence of osmotic pressure gradient, the water molecules can rapidly diffuse across membrane via water channel proteins.

Since the first AQP protein was found in 1988, eleven types of AQPs have been identified in human. AQP-4 is the most subtype in the brain and is mainly expressed in the blood-brain barrier (BBB),ventricle ependymal perivascular astrocyte and the glial membrane of the blood-brain barrier(Bloch et al., 2005). AQP4 is also expressed in the cerebellum Prukinje layer, hypothalamic paraventricular nucleus and supraoptic nucleus. Additionally, AQP4 is less expressed in microvascular endothelial cells and no AQP4 expression is found in neurons, oligodendrocytes and microglia(Søren et al.,1997).

In the model of brain cytotoxic edema, AQP4 knockout (*AQP4-/-)* rats have lower incidence of cerebral edema and have less degree of edema, suggesting AQP4 promotes the occurrence of cytotoxic edema under no BBB injury. In the model of brain vasogenic edema, the edema is bigger than in *AQP4-/-* rats than in the normal rats, indicating AQP4 reduce vasogenic edema under BBB damage (Wingerchuk et al.,2006). Therefore, AQP4 plays a distinct roles in brain edema caused by different causes. Vasogenic edema is caused by the dysregulation of BBB permeability, which can be induced in a series of conditions including hypoxic, vascular disease, infection or tumors. In vasogenic edema, AQP4 is not required for water across the BBB into the brain, but the brain needs AQP4 to transport water out the BBB. Intracranial pressure is

higher and clinical damage is more severe in *AQP4-/-* rats.

AQP4 provides an effective way to remove water from vasogenic edema by allowing extracellular edema fluid pass through astrocyte membrane of the BBB into blood and CSF, thus reducing cerebral edema. Therefore investigations on AQP4 have important theoretical and clinical significance. Monoclonal antibodies against AQP4 are required for these further studies.

Here, we used bioinformatics to analyze secondary structure of AQP4 extracellular region and then use online B-cell epitope prediction to determine the advantagous B-cell epitopes for the development of specific monoclonal antibodies.

2. Materials and methods

2.1 Materials

hAQP4 amino acid sequence: full length amino acid sequence of human AQP4 has retrieved from Swissport database.

2.2 Methods

Online tool (http://www.imtech.res.in/raghava/bcepred/bcepred_submission.html) was used to analyze hQAP4. The Chou-Fasman and the Karplus-Schulz were used to predict protein secondary structure; the Kyte-Doolittle to predict the hydrophilic; the Emini to predict protein surface; the Jameson-Wolf to predict antigenic index; the Karplus to analyze protein flexibility; the Larsen JE to analyze the polarity of amino acids. hAQP4 linear epitopes were predicted according to the method by Ponomarenko et al (2007).

3.Results

3.1 Secondary structure of hAQP4

To predict the secondary structure, the full 323 amino acids of hAQP4 are analyzed (Figure 1). hAQP4 has six transmembrane fragments, which are amino residues of 38-57, 69-88,113-133,156-176 and 188-209. Therefore, these fragments should be not chosen as epitopes. Additionally, the amino-and carboxyl-terminals of hAQP4 localize in the cytoplasm.

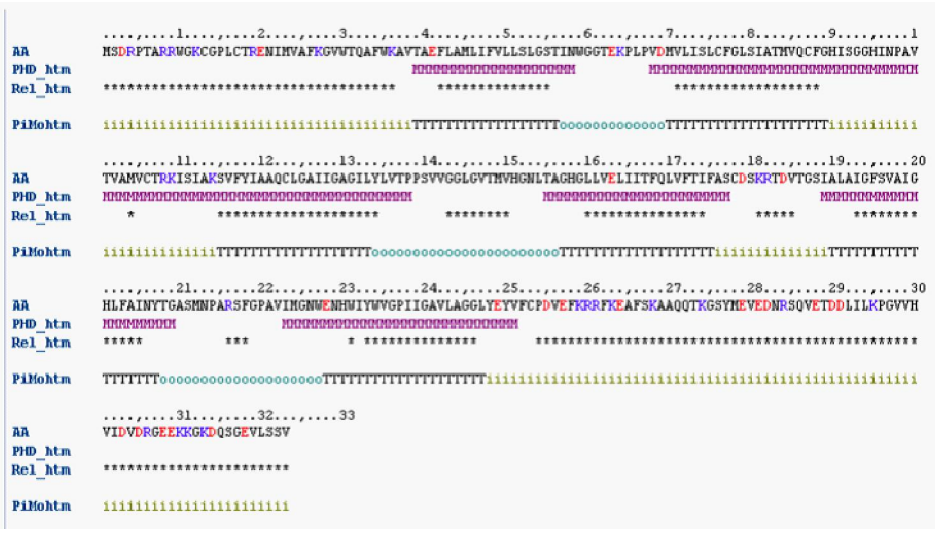

Figure 1. The secondary structure of hAQP4.

3.2 Multi-parameter analysis of hAQP4

As shown in Figure 2, we used the default threshold (1.9) for predicting hydrophilic regions. The fragments of 2-9, 176-188, 267-277, 279-291, and 303-319 were achieved. The fragments of 13-21, 207-221, and 311-319 were predicted for β-turn in the threshold of 2.3. Potential surface were predicted to be fragments of 2-8, 255-265, 268-274, and 304-316 using threshold of 2.3. The selected peptides were 1-7, 57-63, 174-183, 256-262, 258-274, 277-284, and 302-316 in the prediction of flexibility (threshold 2.0). The threshold of 1.8 was used to predict polarity, the peptides of 3-14, 108-114, 178-186, 254-267, 278-285, and 300-316 were obtained. The threshold of 1.9 was used in the prediction of solution and polarities, the peptides of 1-12, 58-69, 178-187, 211-217, 254-291, and 303-319 were collected. Finally, we used the threshold of 1.9 to predict antigenic peptides, the peptides of 11-19, 45-55, 65-81, 84-92, 105-111, 131-151, 158-175, 232-239, 246-258, 292-306, and 317-323 were selected.

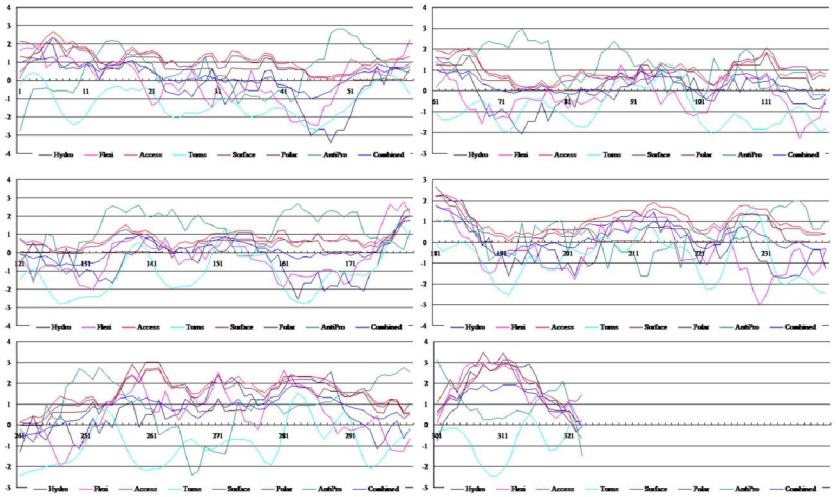

Figure 2 Multi-parameter analysis hAQP4. The Black lines for the hydrophilic; the red line for the corner of the secondary structure; the brown lines for the possibility of surface; the blue lines for flexible; the blue lake for polarity; the green lines for the solvent accessibility, the yellow line for antigen, the purple line for hydrophilic, secondary structure, possiblility of surface and flexibility, the pink line is the average propensity factor, mapping at a width of 60 amino acids.

3.3 Antigen index

Yu et al [(1994) has analyzed 944 amino acid residues from 98 epitopes in 30 types of virus and performed a statistics of the proportion of each amino acid residue. The proportion of each amino acid was compared with the proportion of each amino acid in a total of 314 common proteins. The ratio for each amino acid in the comparison was defined as antigen index. We have predicted multiple B cell epitopes and calculated their antigen index and the top 10 were shown in Table 1.

Table 1 The B-cell epitope index of hAQP4

Position	Peptides	Antigen index
1-11	MSDRPTARRWG	0.029
10-19	GKCGPLCTR	0.031
45-56	MLIFVLLSLGST	0.027
58-69	INWGGTEKPLPV	0.031
112-119	TRKISIAK	0.022
130-151	GILYLVTPPSVVGGLGVTMVH	0.018
158-175	HGLLVELIITFQLVFTIF	0.012
178-185	CDSKRTDV	0.023
253-275	PDVEFKRRFKEAFSKAAQQTKGS	0.030
300-317	HVIDVDRGEEKKGKDQSG	0.041

4. Discussion

B-cell epitope is either a linear peptide segment or conformational structure of protein, which are specifically recognized by B cell antigen receptor or antibodies. Epitopes have been classified as linear (continuous) or conformational (discontinuous). The accurate prediction of B cell epitopes is very helpful for the development of vaccines. The linear epitopes can be directly used for vaccine design and immune diagnosis. directly used for vaccine design and immune diagnosis(1Langeveld et al.,2001). Though B-cell epitopes can be directly identified using many biochemical or physical experiments, such as X-ray crystallography of antibody-antigen complexes, these experiments are usually costly, time-consuming and are not always successful (Gershoni et al., 2007).Computational methods to predict B-cell epitope are much more efficient and cost-effective (Huang et al., 2008). The following parameters have been currently accepted and have a good prediction for B cell epitopes. Hydrophilic is the most important parameter to predict B-cell epitopes. The widely used method is proposed by Hopp & Woods in 1980s. Generally, hydrophobic amino acid residues localize in the interior, while hydrophilic amino acid residues expose to the surface. Therefore, antibody-binding sites are usually localized in the hydrophilic regions. Another parameter is accessibility, which is the probability of rolling contact of the surface amino acid residues by single solvent molecule. The reference of accessibility is usually the radius of a H_2O molecule. Flexibility is also important parameter. Flexibility means the backbone of polypeptide has some degrees of activity. The more active amino acid residues indicate sites of high flexibility and are easy to form epitopes. The secondary structure is an important parameter. β turn mostly appears on surface and is easy to bind to antibody thus being regarded as epitopes. Polarity is also parameter. Most polar amino acid residues localize on the surface of globular proteins while non-polar ones in interior. Therefore, antibody mainly binds to high polarity

regions on surface. Other parameters include antigen and charge distribution. Based on the above parameters, some protein epitopes were successfully predicted using single-parameter model. However, the majority of methods are only able to predict continuous linear B cell epitopes.

In this study, we predicted the B cell epitopes of hAQP4 by comprehensive comparison using parameters including secondary structure, transmembrane region, hydrophilic, flexible, polarity and antigens. We further calculated antigen index of these epitopes and found that linear epitope peptides of 10-19 (GKCGPLCTR) and 300-317 (HVIDVDRGEEKKGKDQSG) could be targets. The hAQP4 can be involved in the transport of water, ion transport, on the body has an important role, inhibition of SJAQP-3 may seriously affect the body 's physiological function, so SJAQP-3 has very good vaccine target application prospect.

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