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Review

A new approach for drug discovery from glycobiology and phage-displayed peptide library technology

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Abstract

Peptides which mimic functional activities of glycosphingolipids were prepared by a technology of phage-displayed peptide library using monoclonal antibodies against glycosphingolipids. These peptides were named glyco-replica peptides. Peptides prepared with anti-GD1α antibody by this technology were found to contain WHW as common motif, and they showed suppressive activity not only on adhesion between hepatic sinusoidal endothelial cells and lymphosarcoma RAW117-H10 cells, but also on metastasis of the tumor cell to the liver and lung. The WHW motif seems to be important to mimic the functional activity of the ganglioside GD1α. Next, we prepared GD3-replica peptides using a monoclonal antibody against GD3 (4F6). A peptide, GD3-P4 with highest affinity to 4F6 was used to immunize mice to examine if the mice show their immune response to raise antibodies against GD3. We confirmed the immune response and succeeded in the production of a monoclonal antibody (3D2) against GD3. The monoclonal antibody 3D2 showed specific binding to GD3 on a thin-layer chromatography plate and also melanoma tissues. Interestingly, the amino acid sequence of the CDR regions of light and heavy chains showed high similarity with those of the original GD3 monoclonal antibody (4F6) used for the preparation of GD3-replica peptide. The technology of the phage-displayed peptide library was applied to *in vivo* bio-panning study using an angiogenesis experimental model. The obtained peptides were found to show strong binding property to the neo-vasculature system and to be quite useful to carry an anti-tumor drug to the tumor tissue. Based on these experimental results, we discuss about some applications of this method to drug discovery.

Keywords: Phage displayed peptide library; Glyco-replica peptide; Glycolipid; Ganglioside; Angiogenesis; Melanoma; Drug discovery; Glycobiology; Metastasis; Molecular mimicry

Elucidation of the functional activities of glycoconjugates is the most important issue in the research field of glycoscience. However, the difficulties in the preparation of large amounts of each pure glycoconjugate molecule and the synthesis of the complex molecules are big hurdles to clear. Amplification technology, which is commonly used in DNA or RNA research field, is not applicable to the target glycoconjugate molecule. This amplification technology contributed to a great progress to the molecular biology, but the biotechnology of glycoconjugates is still at a very primitive stage as compared with that of molecular biology. In order to solve this problem, we have been preparing peptides which mimic glycosphingolipids, using a technology of phage-displayed peptide library with monoclonal antibodies against glycosphingolipids. The obtained peptides have functional activities similar to those of the target glycosphingolipid [1–3]. For this approach, we used a randomly arranged 15mer peptide library displayed on the envelope protein PIII of filamentous phages [2]. The size of the library was approximately 2.5×10^8 peptides. Here, we present some of our studies on the

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approach to drug discovery in combination with glycobiology and technology of the phage-displayed library. The obtained peptides by this technology showed functional similar activities to the antigen glycosphingolipids of the monoclonal antibodies. We named them glyco-replica peptides. The procedure of bio-panning is shown in Fig. 1.

1. Glycosphingolipid-replica peptides and functional activities

First, we used H11 mouse monoclonal antibody against paragloboside and AD117m against lactotetraosylceramide for this approach. These two glycosphingolipids are isomers of inner linkages of lactotetraose in the carbohydrate moiety, but their terminal β-galactose has the same linkage. We examined how similar the obtained peptides are, using these two different monoclonal antibodies. Interestingly, we could obtain an amino sequence with 9mer peptide VPPXFXXXY in the 15mer peptide expressed on the phage PIII protein as common sequence. These 9mer peptides showed a similar binding property for both monoclonal antibodies AD117m and H-11, and also for a β-galactose-recognizing lectin (*Ricinus communis* lectin). This binding property changed very much by substituting the X with other amino acids. The 15mer peptides with VPPXFTLMY suppressed the binding of Jack bean β-galactosidase to paragloboside, but interestingly, one 15mer paragloboside-replica peptide, ARFPKELRGSVRSAH and some 9mer peptides with VPPXFTLMY activated the β-galactosidase activity against paragloboside [3]. The results suggest that the molecular shapes obtained by this technology show an agonistic or also an antagonistic property to the glycosphingolipid counterparts.

Based on this first study, we tried to prepare ganglioside-replica peptides to make sure that the replica peptides possess similar biological functions. Here we introduce three approaches for anti-tumor associated drug discovery by using the phage-displayed peptide library technology.

2. GD1α-replica peptides with anti-metastatic activity

Glycosphingolipids change their carbohydrate structure along with the process of tumor progression and also during cell differentiation. From the comparative study on glycosphingolipids of lymphosarcoma cell lines with different metastatic potentials, we found that GD1α is a characteristic ganglioside of a sub-cell line (RAW117 H10) with high rate of metastasis to the liver [4]. The ganglioside is not found in the parental non-metastatic tumor cell line RAW117. GD1α ganglioside was found to be involved in the adhesion between RAW117 H10 and hepatic sinusoidal endothelial (HSE) cells from the following three experiments: 1, HSE cells specifically adhered to the area coated with GD1α on a plastic culture plate, 2, GD1α strongly inhibited the adhesion of RAW117 H10 cells to HSE cells [4] and 3, the monoclonal antibody against GD1α suppressed the adhesion of tumor cells to HSE cells.

Then, we tried to prepare GD1α-replica peptides by using the phage peptide library technology with the anti-GD1α monoclonal antibody. Two of the obtained peptides, one (Q1P) is FRSDVRFWHWSTPFM and the other (Q3P) is WHWRHRIPLQLAAGR, were found to have a WHW motif. Then, we examined if they have suppressing activity in the adhesion between tumor cells and HSE cells. The two 15mer peptides showed inhibitory activity in the adhesion. Also, the peptide inhibited the onset of metastasis of the RAW117 H10 cells in the liver of mice. One shot of 200 μg Q3P per mouse suppressed the tumor metastasis to lung by 100% and to the liver by about 50% [5].

Based on this result, we determined the minimum sequence of the GD1α−replica peptide responsible for the biological function [6]. As shown in Fig. 2, the WHW sequence is necessary and sufficient to maintain the function. However, since the peptide linkage is quite flexible, we introduced a long alkyl chain to WHW (WHW-lipo-peptides) to stabilize the molecular shape (Fig. 2c) thus making possible the formation of a liposomal structure to

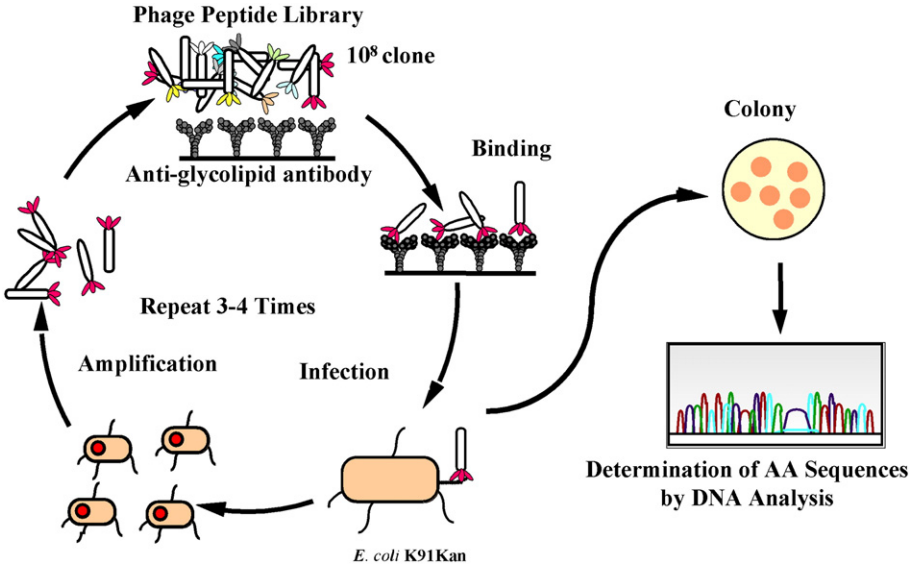

Fig. 1. Preparation of glyco-replica peptides with phage-displayed peptide library.