Computational Integration with Phage Library to Optimize Clones of NEU3 Antibodies for Cancer Diagnostics and Therapeutics

Poster #3105

Yichen Guo[1], Jina Yom[1], Andy (Xi) Han[1], Zhaoying, Guo[1], Eden Zewdu[1], Xiaomin Hu[2], Rachel Gonzalez[1], Tianli Qu[1], Bailey Gilmore [1], Xuan Liu[1], Wei Fu[1]

1) OriGene Technologies Inc.; 9620 Medical Center Drive, Suite 201, Rockville MD 20850
2) OriGene Wuxi Biotechnology Co., Ltd. No.168, Meiliang Road, Binhu District Wuxi, Jiangsu

Visit us at Booth #3322

Abstract

Sialidase 3 (NEU3) expression levels have been found to be dysregulated in several cancers, including breast, prostate, and colon cancers, implicating its involvement in tumorigenesis and metastasis. Research suggests that targeting NEU3 activity could potentially offer novel therapeutic strategies for combating cancer progression and metastatic spread. Understanding the molecular mechanisms underlying the involvement of NEU3 in cancer pathogenesis holds promise for the development of novel diagnostic and therapeutic strategies targeting this enzyme in the context of cancer treatment. This study aimed to develop a stable and high-affinity NEU3 antibody for clinical and pre-clinical use.

To achieve this, a combination of high-diversity phage library and computation-assisted analysis was employed to generate single-chain variable fragments (ScFv) clones specifically targeting NEU3. This research presents a robust approach for the generation of NEU3-targeting single-chain variable fragments (ScFv) from a phage library characterized by a high diversity of 10^7 unique clones and high titer of 10^{13} cfu/mL. Computational optimization will be then applied to enhance the affinity and stability of these candidates through rational design and virtual mutagenesis programs. The binding activity of the optimized mutants will be validated using biophysical methods such as surface plasmon resonance (SPR) and thermal stability assays.

This study highlights the synergy of high-diversity phage libraries and computational methods in crafting NEU3 antibodies with enhanced affinity and stability. The findings advance the field of antibody engineering, emphasizing the crucial integration of experimental and computational approaches. This work lays the groundwork for the development of high-performance biologics with diagnostic and prognostic applications across diverse cancer types. Furthermore, it demonstrates the possibility of integrating computational approaches in antibody development, offering the promise of expediting the process in the future

Introduction

Sialidase 3, also known as NEU3, has emerged as a significant player in cancer biology due to its involvement in various aspects of tumorigenesis and metastasis. NEU3 expression levels are frequently dysregulated in multiple cancer types, including breast, prostate, and colon cancer, where it has been implicated in promoting tumor growth, invasion, and metastasis[1]. Through its enzymatic activity in removing sialic acid residues from glycoproteins and glycolipids, NEU3 influences critical cellular processes such as cell adhesion, migration, and signaling pathways, thereby contributing to cancer progression. Targeting NEU3 activity presents a promising avenue for the development of novel therapeutic interventions aimed at combating cancer progression and metastatic spread.

A single-chain variable fragment (ScFv) phage library is a powerful tool in antibody engineering and discovery. Comprising a vast collection of ScFv sequences displayed on the surface of bacteriophage particles, this library offers a diverse repertoire of potential antibody candidates. Each ScFv consists of a variable heavy (VH) and variable light (VL) chain connected by a flexible linker, maintaining the antigen-binding specificity of full-length antibodies[2]. By screening the library against specific antigens using biopanning techniques, high-affinity ScFv binders can be rapidly identified and isolated..

Phage libraries, in conjunction with computational engineering techniques, represent a powerful approach for enhancing antibody affinity and stability. Phage libraries provide vast pools of diverse antibody variants, allowing for the selection of high-affinity clones through biopanning methods. Computational engineering complements this process by enabling the design and prediction of antibody mutations that can enhance binding affinity and stability. By utilizing computational algorithms and molecular modeling, researchers can identify key residues for mutagenesis and predict the impact of mutations on antibody structure and function[3]. These predictions guide the generation of focused libraries enriched with variants predicted to have improved properties. Through iterative rounds of selection and computational analysis, antibodies with enhanced affinity and stability can be developed for therapeutic applications, diagnostics, and biotechnological uses.

Design & Methods

Naïve Rabbit ScFv Phage Library Establishment

The phage library establishment is shown in Figure 1. It includes several time PCR for VL and VH, gel purification, band isolation, ligation, transformation and growth, phage rescue and package. The cDNA for VL and VH amplification are from rabbit bone marrow, spleen, and PBMC.

Diversity and Titration

The diversity of the established phage library was determined by single colony scanning (Figure 2) and NGS. NGS quality control is shown in Figure 3. The phage library titration calculation is shown in Figure 8.

Biopanning

Firstly, the NEU3 protein is immobilized onto a microplate, followed by blocking to prevent nonspecific binding. The phage display library is then added to the target-coated surface and allowed to incubate, facilitating the binding of phages displaying target-specific protein. After washing away unbound phages, the bound phages are eluted and amplified using bacteria. This process is repeated through three rounds of selection with increased stringency. Finally, enriched phage clones are characterized for target .The biopanning results are shown in Figure 7 and Table 1.

Phage Titration

For phage titration, inoculate 50 mL of 2xYT with 500 µL of overnight culture and incubate at 37°C and 250 rpm until reaching an optical density (O.D.) of about 0.5. If O.D. reaches 0.5 prematurely, store the culture on ice to preserve F pili on the E. coli cells. Prepare serial dilutions of the phage suspension in PBS, infect bacteria with phage dilution, and incubate for 30 minutes at 37°C. Plate infected bacteria onto 2xYT-GA agar plates, then incubate overnight at 37°C. Count colonies formed and calculate colony-forming units (cfu) or cfu/mL titer based on the dilution factor.

Production of Soluble Monoclonal Antibody Fragments in Microtiter Plates

First, culture 92 clones from the third panning round agar plate with 2xYT-GA in a sealed 96-well U-bottom polypropylene microtiter plate overnight at 37°C. The next day, fill a new 96-well polypropylene microtiter plate with 2xYT-GA, add overnight cultures, and incubate until OD reaches 0.5. Pellet the bacteria by centrifugation, add 2xYT-SAI (containing saccharose, ampicillin, and 50 µM IPTG), and incubate overnight at 30°C. Finally, pellet the bacteria again by centrifugation and transfer the supernatant containing the antibody fragment to a new polypropylene microtiter plate for testing antibody binding directly.

ELISA of Soluble Monoclonal Antibody Fragments

The process involves coating microtiter plate wells with NEU3 protein overnight at 4°C, followed by blocking with buffer for 2 hours at room temperature. Antibody solution is then added and incubated at room temperature. After washing, secondary antibody is added and incubated at room temperature. Subsequently, PNPP substrate is added, and the plate is incubated for 20 minutes before measuring the extinction at 450 nm using an ELISA reader. In parallel, DNA sequencing of selected scFv clones is conducted for identification via nanopore sequencing. Expression steps include amplifying heavy and light chain sequences and ligating into expression vectors (Figure 5 and 6).

NEU3 protein generation and quality control

The human NEU3 gene (with a C-terminal Myc-DDK tag) in the form of the Origene vector RC216537 was transfected into HEK293TS cells. Later, the HEK cell pellet was harvested, lysed, and purified with column chromatography targeting the DDK affinity tag. The protein was eluted, the concentration of each fraction was assessed with the BCA assay, and an SDS-PAGE gel was run to verify the correct molecular weight of the expressed protein (Figure 4).

Results

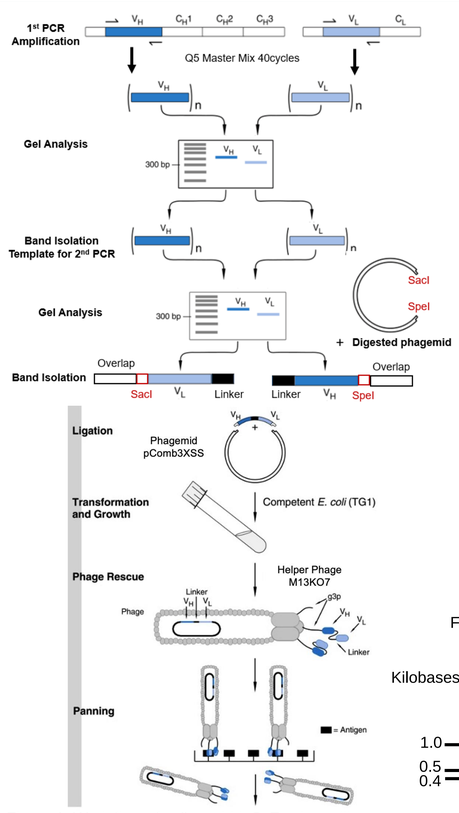


Figure 1. Work flow of naïve rabbit ScFv phage library establishment and biopanning

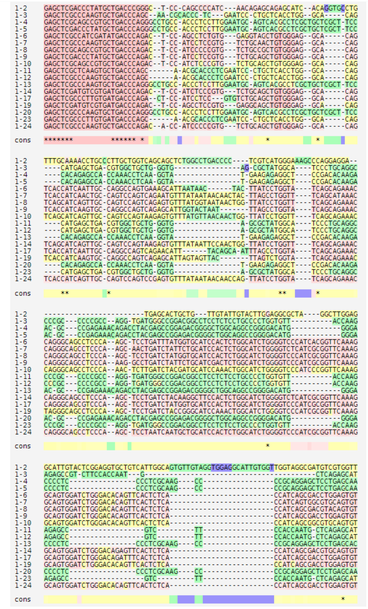
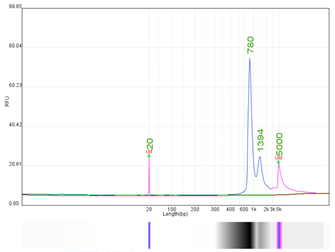


Figure 2. Partial alignment for phage library diversity assessment

Figure 3. NGS quality control



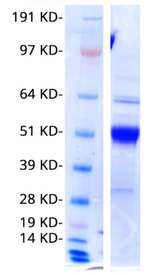


Figure 4.
SDS-PAGE of NEU3 protein quality control
Lane 1: Marker
Lane 2: NEU3 protein (48kDa)

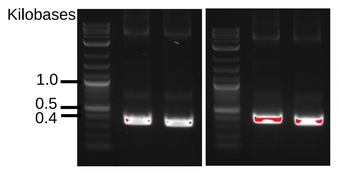


Figure 6.
Amplification of heavy chain and light chain for antibody expression

Figure 5. Plasmid map of positive clone after biopanning

Results

Figure 7. Three rounds of biopanning ELISA and titration

First Biopanning

NEU3			Negative
0.9122	0.9069	1.1323	0.0396
0.9308	0.911	1.1307	0.0373

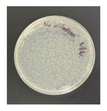
Second Biopanning

NEU3			Negative
0.1083	0.1036	0.1565	0.0845
0.1225	0.1223	0.2418	0.056

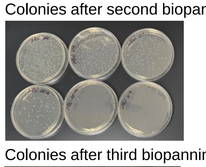
Third Biopanning

NEU3			Negative
0.159	0.047	0.156	0.0392
0.191	0.180	0.172	0.0448

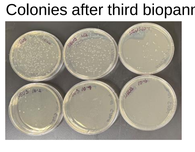
Colonies after first biopanning



Colonies after second biopanning



Colonies after third biopanning

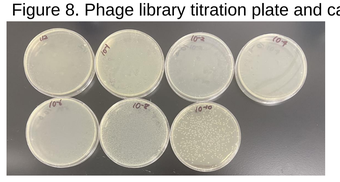


NEU3 ELISA results

0.1103	0.1115	0.1081	0.1015	0.1037	0.1017	0.1017	0.1017	0.103	0.1016	0.1036	0.102
0.1017	0.1044	0.1033	0.1027	0.106	0.1018	0.1069	0.1033	0.1066	0.104	0.1084	0.108
0.1037	0.1121	0.104	0.1035	0.1127	0.1025	0.104	0.1047	0.1096	0.0997	0.1038	0.1028
0.1106	0.1072	0.1016	0.1046	0.1082	0.1081	0.108	0.1007	0.0959	0.1065	0.1034	0.1033
0.1103	0.101	0.1111	0.1014	0.1053	0.1139	0.1128	0.1002	0.101	0.1059	0.1054	0.1037
0.1102	0.1165	0.1075	0.1074	0.1086	0.1113	0.103	0.1231	0.1043	0.1307	0.104	0.1219
0.502	0.1075	0.1038	0.1144	0.1056	0.1291	0.103	0.1356	0.1171	0.1159	0.1054	0.1158
0.5697	0.1061	0.1075	0.1044	0.1185	0.1186	0.1064	0.6284	0.1215	0.1273	0.1392	0.1243

Table 1. ELISA result of NEU3 after production of soluble monoclonal antibody fragments

Figure 8. Phage library titration plate and calculation



Determining the Phage Titer

$$\text{CFU/ml} = \text{number of colonies on plate} \times 1/\text{dilution} \times 1/\text{fraction plated} \times 2.$$

650 colonies on 10^{-10} dilution plate

Phage titer is 1.3×10^{13} CFU/mL

Conclusion and Discussion

1. The rabbit naïve ScFv phage library is established with the diversity of 10^7 and enrichment of 1.3×10^{13} cfu/mL.
2. After three rounds of biopanning, two positive clones were selected for antibody production.
3. The software, BioLuminate, will be applied for affinity maturation and stability improvement
4. The binding activity and stability of the optimized mutants will be validated using biophysical methods such as surface plasmon resonance (SPR) and thermal stability assays respectively
5. The NEU3 antibody expression will be optimized by software, which will be assessed by IHC.

Reference and Acknowledgement

[1] Miyagi T, Yamamoto K. Sialidase NEU3 and its pathological significance. Glycoconj J. 2022 Oct;39(5):677-683. doi: 10.1007/s10719-022-10067-7. Epub 2022 Jun 8. Erratum in: Glycoconj J. 2022 Aug

[2] Jaroszewicz W, Morcinek-Orłowska J, Pierzynowska K, Gaffke L, Węgrzyn G. Phage display and other peptide display technologies. FEMS Microbiol Rev. 2022 Mar 3

[3] Tiller KE, Chowdhury R, Li T, Ludwig SD, Sen S, Maranas CD, Tessier PM. Facile Affinity Maturation of Antibody Variable Domains Using Natural Diversity Mutagenesis. Front Immunol. 2017 Sep 4

➢ We acknowledge Schrodinger software (BioLuminate) for affinity and stability improvement in the future work
➢ We acknowledge Wuxi team to provide work flow chart
➢ We acknowledge Alex Strom for NEU3 protein production