Development of antibody fragments for targeted PET- and single EV-imaging

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Introduction

Extracellular vesicles (EVs) are a diverse group of lipid structures that almost all cells secrete. EVs can be found in many bodily fluids and they are involved cargo transportation and signaling (van Niel et al. 2018). Signaling between cells is a hot topic and EV-imaging could help the research significantly. The heterogeneous nature of EVs means that single-EV imaging techniques are a must. Fluorescently labelled Fabs binding to the membrane proteins (tetraspanin/TSPAN) of EVs could be used to study single EVs with possibly high resolution.

Fatty acid-binding protein 3 (FABP3) is a protein that is released during tissue damage (Goel et al. 2020). The release of the protein during tissue damage enables the use of FABP3 as a biomarker for different cardiovascular diseases. Radioactively labeled FABP3 antibodies can be used in positron emission topography (PET) -imaging to produce high quality images.

Aims

The aim of this project was to find high affinity binders for desired tetraspanin and FABP3 antigens that could be used in further research and diagnostics in different imaging techniques.

Materials and methods

1. Production of tetraspanin antigens

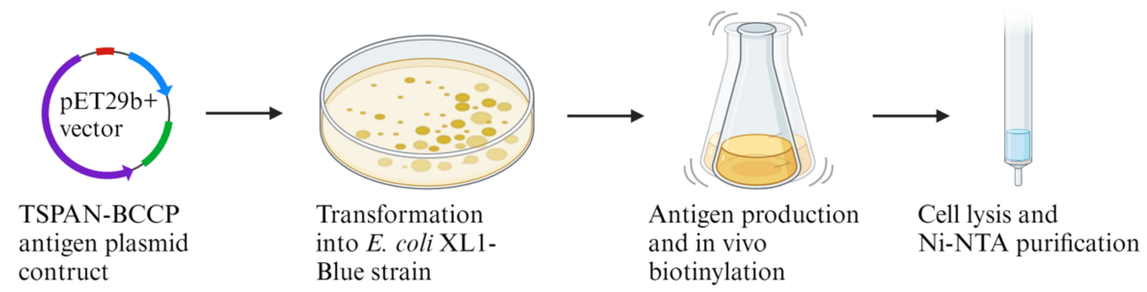

Figure 1. Production and purification of tetraspanin antigens.

2. Panning

Multiple different panning rounds were deployed with varying conditions. An in-house Fab phage display library FabE (University of Turku) was used to find antibodies. Tetraspanin antigens were panned against vesicles that contain the native tetraspanin membrane proteins in their surface while FABP3 was only panned against the antigen protein.

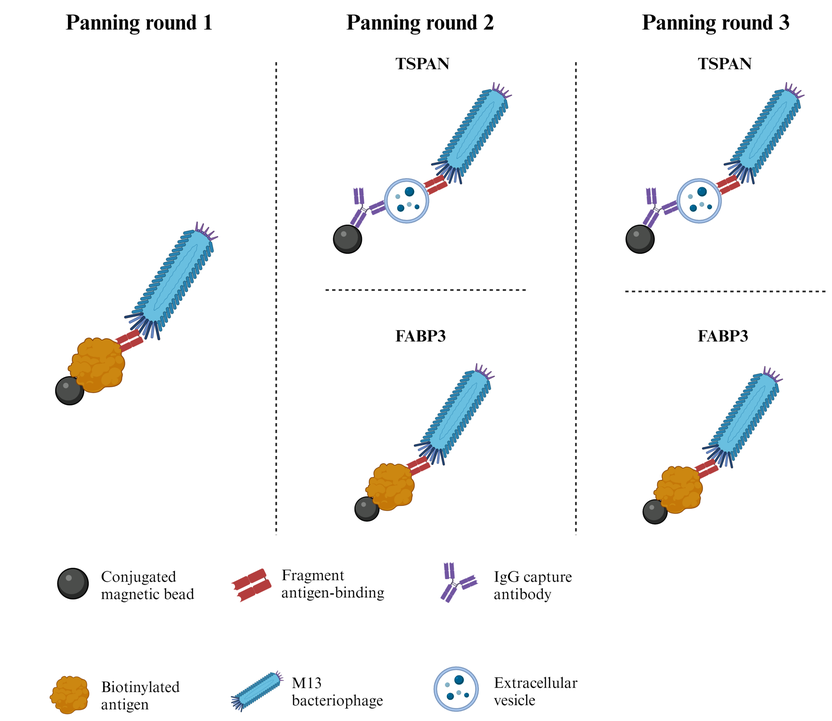

Figure 2. Enriching of phage libraries against target antigens with panning.

3. Screening, production and characterization of selected clones

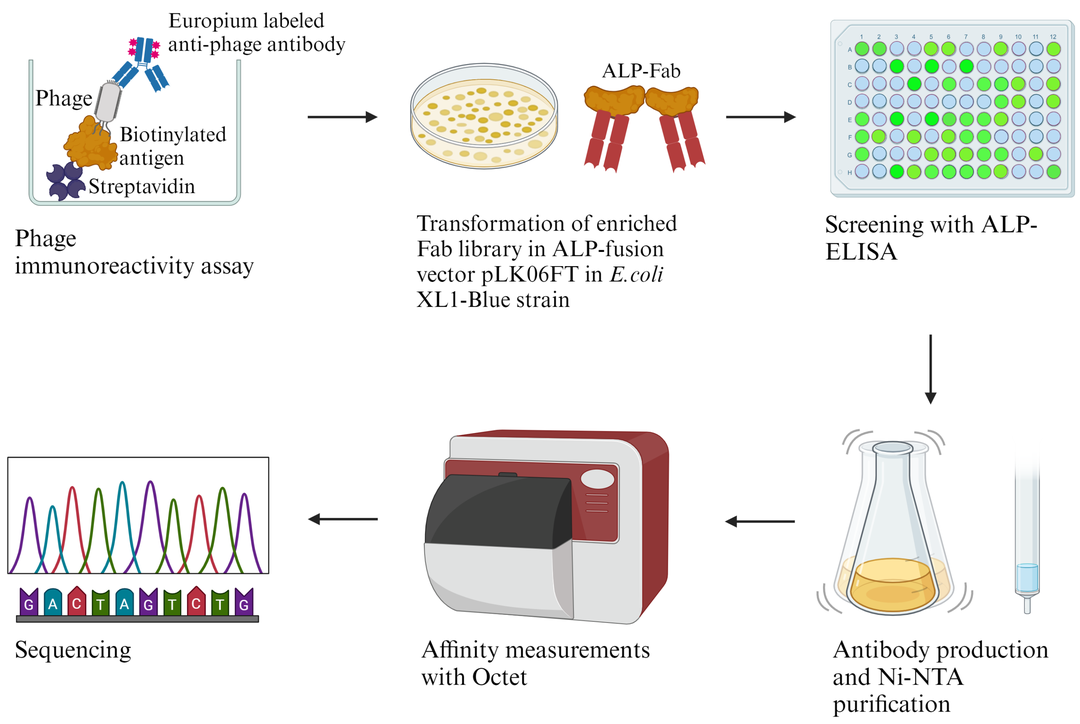

Figure 3. Steps following panning.

Results

Panning rounds 2 and 3 were analyzed with phage immunoreactivity assay. Based on the results, the enriched phage library from panning round 3 was used in the following screening.

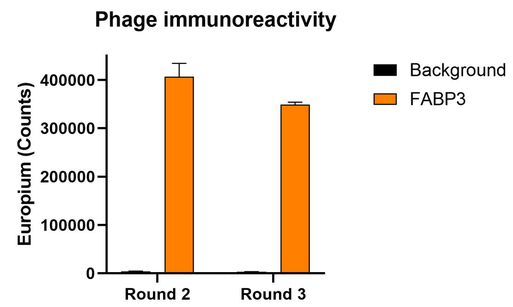

Figure 4. Phage immunoreactivity of panning rounds 2 and 3.

48 clones were picked after transformation to pLK06FT-vector and then screened with ALP-ELISA. Using pLK06FT adds an ALP-fusion to the Fab which then can be used to produce a signal with an enzyme that can be measured in absorbance (A) at 405 nm.

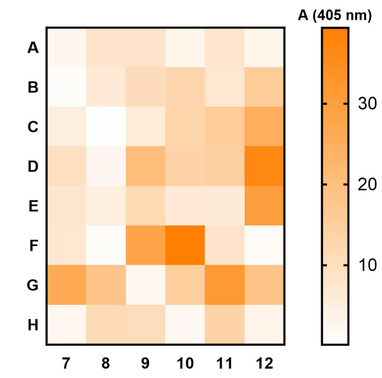

Figure 5. Screening results of 48 FABP3 clones.

2 clones, FABP3 F10 and FABP3 D12 were selected to be produced and purified for further chracterization. 7 clones were selected for sequencing (D9, F9, F10, G11, C12, D12 and E12). FABP3 F10 and D12 were analyzed with Bio-Layer-Interferometry (BLI, Octet). Both were fitted 1:1 and F10 had 5,05 nM KD while D12 had 0,93 nM KD.

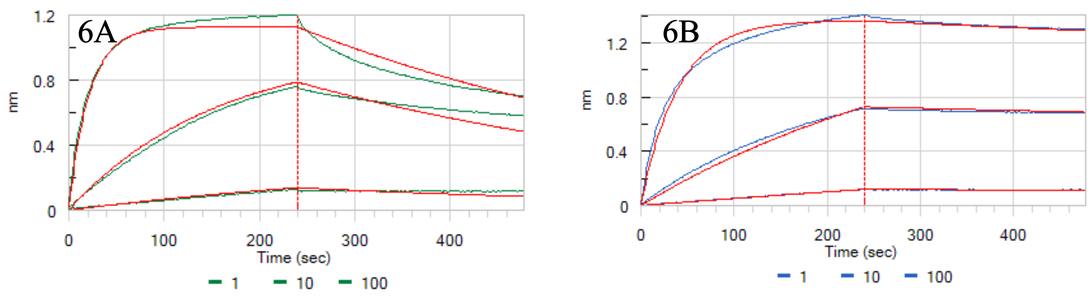

Figure 6A and 6B. Binding kinetics of FABP3 F10 (6A) and D12 (6B).

Conclusions

During the study two possible FABP3 antibodies were found with promising kinetics data with also 7 clones awaiting further sequencing analysis.

Further studies could be done by labelling the antibodies with fluorescent labels and then using them in flow cytometry studies.

Acknowledgements

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References:

van Niel, G., D'Angelo, G. & Raposo, G. (2018) Shedding light on the cell biology of extracellular vesicles. Nat Rev Mol Cell Biol 19:213–228

Goel, H., Melot, J., Krinock, M. D., Kumar, A. Nadar, S. K., & Lip, G. Y. H. (2020). Heart-type fatty cid-binding protein: an overlooked cardiac biomarker. Annals of medicine, 52(8), 444-461.

Illustrations done in BioRender.com

Figures done in GraphPad