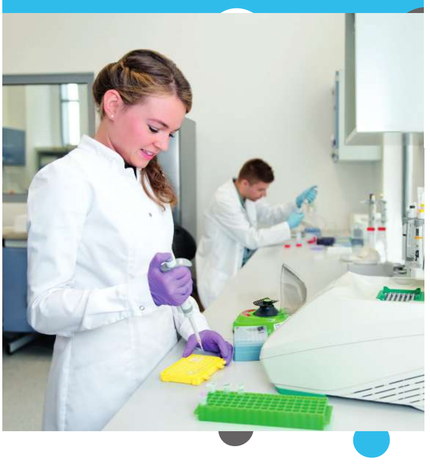

Phage Display and SELEX brought together - a bivalent aptamer-antibody conjugate for VEGFR1 specific targeting

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INTRODUCTION

Vascular Endothelial Growth Factor Receptor 1 (VEGFR1) is a validated and often studied target molecule that serves as an important factor for vascular system organization, angiogenesis and inflammation[1-3]. Bivalent protein-RNA conjugate was obtained to evaluate if it would exert improved binding affinity and specificity towards VEGFR1 in a cell-surface context, and possibly block its activation by the natural ligand.

GOALS

- Utilize aptamers and antibodies fusions as interchangeable effector and directing agents
- Conduct pilot experiment using scFv antibody fragment and aptamer conjugate and its suitability to interact with improved binding affinity and specificity with VEGFR1
- Assess independence of receptor binding sites
- Evaluate VEGF-induced phosphorylation of VEGFR1 in non-transfected cell lines

APTAMER

We have chosen to use an RNA aptamer named #38Jr (Fig. 1), selected towards ECD of VEGFR1 by Ohuchi et al.[4], that does not hinder VEGF binding to the receptor.

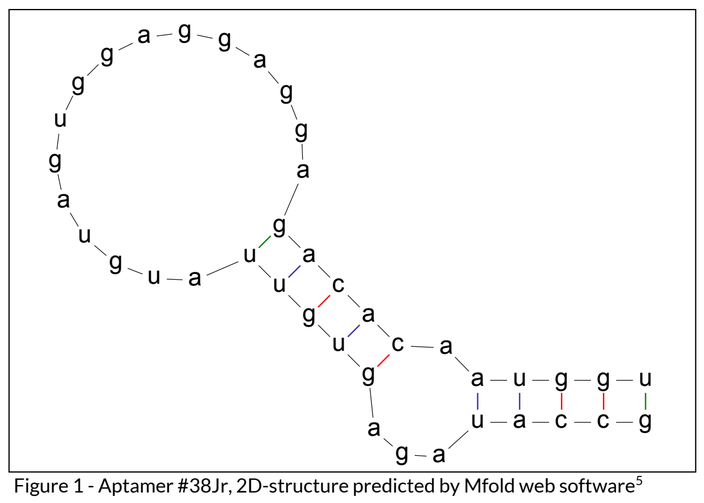

Figure 1 - Aptamer #38Jr, 2D-structure predicted by Mfold web software[5]

PHAGE DISPLAY SELECTION OF PB439 scFv ANTIBODY

For selection of binders against VEGFR1, phage displayed library of single chain antibody fragments (scFv) was panned against ECD of VEGFR1. Four rounds of selection, including two counter-selection rounds with VEGFR2, were performed. During the last round, the elution was performed with VEGF only, to select for variants binding to the ligand binding site of the antigen. ELISA results for PB439 clone are shown in Fig. 2.

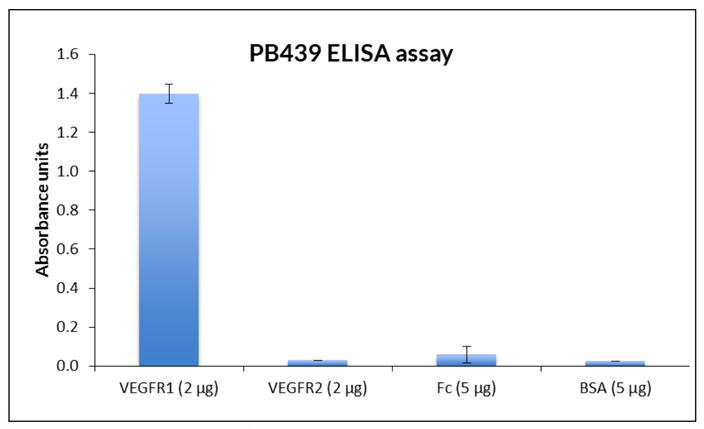

Figure 2 - ELISA showing PB439 specificity towards its target and non-target molecules

CONJUGATION

Bifunctional NHS/maleimide linker allows to modify protein's lysine residues with active maleimide groups. The chemistry is robust and quick, but not directional (Fig. 3).
Purified PB439 antibody was activated with excess of the linker residues and desalted. The aptamer was refolded, reduced and the buffer was exchanged by filtration . The conjugation of PB439 and #38Jr was optimized at molar ratio of 1:2 to increase the amount of conjugate over non-modified PB439. Unreacted maleimide groups on PB439 were blocked with the reductant. Next, the conjugate was separated from unconjugated aptamer using IMAC. Purified PB439-#38Jr was analyzed by SDS-PAGE showing predominant monosubstitution of scFv with aptamer (Fig. 4).

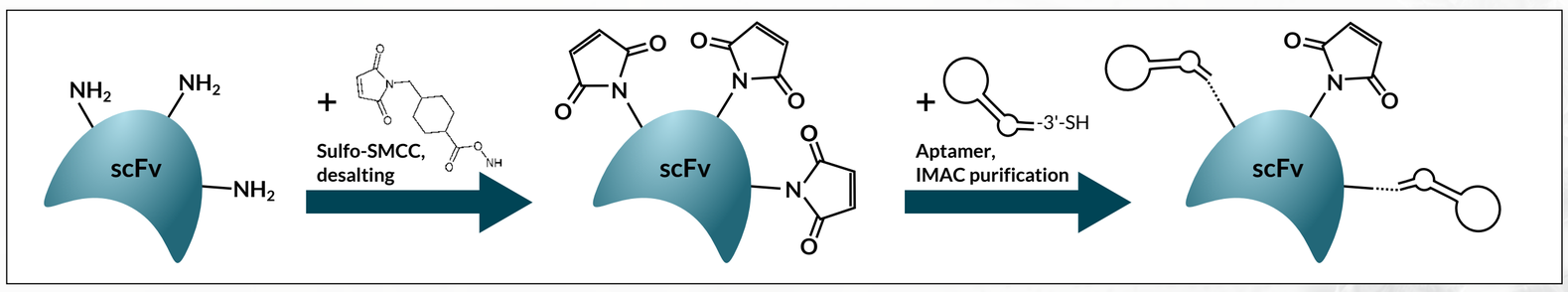

Figure 3 - Scheme depicting conjugation procedure of scFv and thiol-modified aptamer #38Jr

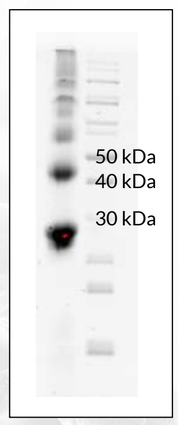

Figure 4 - SDS-PAGE analysis of aptamer-scFv conjugate

CELL BASED ASSAY

Five cell lines were tested for responsiveness to VEGF. Cells were starved and stimulated with VEGF (from 10 ng/ml to 500 ng/ml), lysed and analyzed using ELISA based assay with anti-phospho-VEGFR1 antibodies. Out of the tested natively VEGFR1 expressing cells, KG1 were used. PB439-#38Jr fusion and individual constituents were compared. Fusion conjugate proved to bind to the receptor leaving us with a model system for further investigations.

DISCUSSION

We have obtained an aptamer-antibody conjugate in a simple manner, using robust and non-directional chemistry, which proved not to hinder the constituents target binding ability. In the future, we are planning to use this technique to obtain full antibody-aptamer conjugates, which would exert both strong binding abilities and increased avidity, as well as be able to work as an opsogenic agent and induce immunogenic response in vivo.

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