Modular platform for modified aptamers

a selection case study

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Introduction

Systematic Evolution of Ligands by Exponential Enrichment (SELEX) is a powerful technique introduced in 1990 [1] for selection of aptamers to a wide range of molecular targets. We have recently developed a modular platform named PureApta™ based on SELEX method for an easy introduction of nonstandard nucleotides into DNA aptamers structure. By modifying a deoxythymidine triphosphate analogue with various chemical groups using "click-chemistry"-based approach [2] we arrived at an easy method for the generation of fully substituted oligonucleotides (Figure 1). Here we provide an example of a selection directed towards a therapeutically important recombinant human receptor protein, which proves the successful integration of modified nucleotides into the SELEX process and thus – the feasibility of the approach.

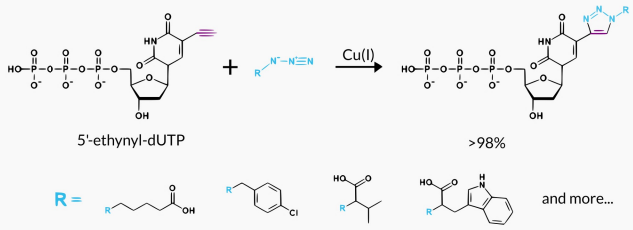

Figure 1. Modified nucleotides synthesis reaction scheme.

Selection

Aptamers were selected via the SELEX procedure, in which successive rounds of selection and amplification enriched an oligonucleotide library in aptamers binding the target with high affinity (Figure 2). As the SELEX target we chose a recombinant human transmembrane receptor with therapeutic potential. Protein construct comprised the mature receptor's extracellular domain linked to the IgG Fc fragment. To increase the binding affinity and reduce susceptibility to nuclease digestion we introduced to the selection a nonstandard nucleotide bearing a hydrophobic moiety at the nucleobase, which is nonetheless fully compatible with our adjusted SELEX methodology.

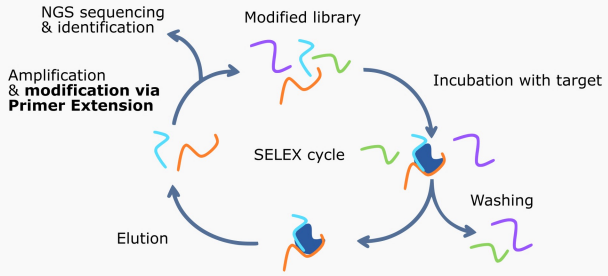

Figure 2. A selection cycle scheme with the SELEX method

Materials & methods

Modified nucleotides synthesis

Using a commercially available compound - 5'-ethynyl deoxyuridine triphosphate (EdUTP) as a substrate, we performed the copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction with selected azides to obtain chemically modified deoxyuracil analogues (Figure 1). The products were purified via RP-HPLC.

Aptamers selection

DNA aptamer candidates were selected within 7 iterative rounds of SELEX. Chosen modified nucleotide fully replacing thymidine was introduced during material amplification. For the selection, the target was immoilized onto Protein A magnetic beads via its Fc domain. Starting from the second round onwards, each round was accompanied by a counter selection using either the support, the Fc fragment, or a non-target receptor.

Beads-assisted binding

Biotinylated modified polyclonal aptamer pools, unselected modified library and unmodified library were immobilized on streptavidin-coated magnetic beads. The aptamer beads were then exposed to 2 µg of the target protein in 5% FBS in the selection buffer. Bound proteins were eluted and vacuum-blotted onto a nitrocellulose membrane, and the signal was developed using target-specific HRP-conjugated antibody.

ELONA

ELONA was performed on 96-well plates. The wells were coated with 2 µg of target protein and incubated for in the selection buffer containing 5% FBS and increasing concentrations of biotinylated modified polyclonal aptamer pools or unselected modified library. A non-target receptor (counter target used during the counter selection) was also used as an additional specificity control.

Results

Beads-assisted binding

With the increase of selection cycle number the polyclonal aptamer pools showed stronger binding to the target, as shown by the increasing signal intensity (Figure 3). Also the controls, which were the unselected libraries as well as empty beads showed negligible binding of the target. The results were consistent for a number of independent experiments.

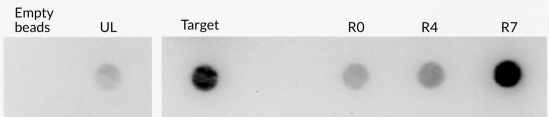

Figure 3. Beads-bound aptamers binding of the target in 5% FBS. Target - positive control blotted directly onto the membrane, R4, R7 - modified polyclonal aptamers pools from chosen selection rounds, R0 - unselected modified library, UL - unselected unmodified library, empty beads - no aptamers were attached to the magnetic beads.

ELONA

The aptamer population obtained after the 4th and the 7th round showed specific binding to the target rather than to the non-target protein and presented a dose-response signal increase. Moreover, the aptamer population after the 7th round was able to bind the target with the highest affinity (Figure 3). These results showed that the aptamer population after the 7th round of selection was indeed enriched in target-binding species comparing to non-selected sequences (library), or even aptamers selected after the 4th round (Figure 2). The results were consistent for a number of independent experiments.

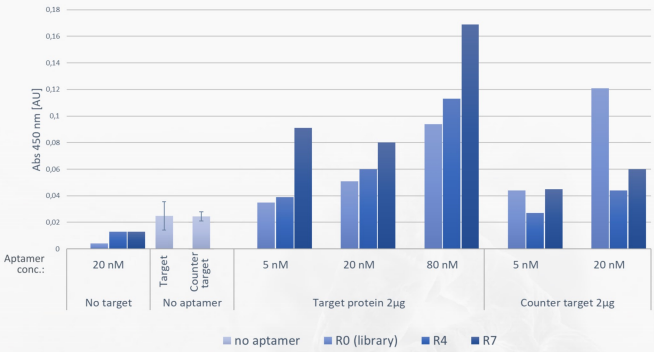

Figure 4. ELONA with modified polyclonal aptamer pools from rounds 4 and 7 or with modified unselected library (R0) showing binding to a target protein or a counter target.

Conclusions

Using the modular PureApta™ technology we selected a polyclonal pool of aptamers binding specifically to the recombinant human extracellular receptor protein used as the selection target. Selected aptamers showed a strong binding affinity with high specificity even in a complex media (5% FBS). Following research will comprise NGS sequencing of aptamers from all the selection rounds and identification of enriched sequence families. Identified representative sequences will be characterized and tested for their binding affinity and kinetics to the target protein.

Acknowledgments

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References

[1] Tuerk, C., Gold, L., Science 249, 505-510 (1990).
[2] Gierlich, J., Burley, G.A., Gramlich, P.M.E., Hammond, D.M., Carell, T., Organic Letters 8, 3639-3642 (2006).

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