Selecting DNA aptamers for the open conformation of Transglutaminase-2 protein

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Introduction:

DNA Aptamers are short oligonucleic acid units (approximately 15-120 nucleotides) that bind to a specific target molecule, such as a protein. They are capable of forming complex structural interactions with the proposed target, to which they are capable of binding with both, a high degree of specificity and affinity.

Aptamers were selected for the target protein transglutaminase-2 (TG-2) locked in the open conformation with an irreversible inhibitor. TG-2 in human tissue is responsible for cross-linking proteins by their glutamine and lysine residues. The open conformation is 130 nanometers larger than the closed form. The open conformer of the enzyme is the active form and catalyzes the acyl-transfer reaction. The frequency of conformers observed in the human body is dynamic, but in equilibrium. TG-2 is responsive to calcium ions, which bind to the enzyme switching the conformation open; absence of calcium ions leads to the closure of the enzyme via a conformational shift. A physiologic block on one conformer may be possible using this method of aptamer binding conjugated with an inhibitor in order to provoke an equilibrium response (according to Le Chatelier’s Principle) by the body’s internal systems.

Selecting for TG-2 is essential for its elevated levels in cells have been recognized as a factor in the promotion of undesired autoimmune inflammation and degenerative diseases, including but not limited to metastatic cancer. The method employed is the Systematic Evolution of Ligands in Exponential Enrichment (SELEX). The incubated oligonucleotides with the locked open conformers are collected and subsequently incubated with the locked closed conformers. Only the desired doubly specific aptamers selected for the open conformation are collected and subject to amplification using Polymerase Chain Reaction (PCR). Thus, the evolved pool is used for the succeeding selection round.

The repetition of this procedure is what gives SELEX the potential to generate a pool of aptamers with the highest binding affinity to the targeted TG-2. In addition, the use of a fluorescence assay will be used to confirm the presence of a successful selection process.

Results:

Table 1: Key concentration and percent values from the final round of amplification of the open conformation aptamers.

<table>
<thead>
<tr>
<th>Band</th>
<th>Concentration (nM)</th>
<th>Primers Concentration (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA Standard</td>
<td>200</td>
<td>Average of positive and negative controls</td>
</tr>
<tr>
<td>Round 1</td>
<td>238</td>
<td>Average of the selection rounds</td>
</tr>
<tr>
<td>Round 2</td>
<td>270</td>
<td>Percent of primers purified from solution</td>
</tr>
<tr>
<td>Round 3</td>
<td>253</td>
<td>Average percent mass of the primers remaining in solution</td>
</tr>
<tr>
<td>Round 4</td>
<td>312</td>
<td>Mass of the primers remaining in solution</td>
</tr>
<tr>
<td>Round 5</td>
<td>396</td>
<td>Percentage of mass of the primers remaining in solution</td>
</tr>
</tbody>
</table>

Figure 1: Shown in the top left corner is the inhibited/opened form of TG-2, the top right corner displays the closed GDP-bound form of TG-2 (including the equilibrium reaction from the conformer change). II: SELEX procedure for selecting the open conformation of the TG-2 protein.

Figure 2: Picture of the gel electrophoresis from the final amplification (symmetric followed by asymmetric PCR) of the purified and evolved aptamer pools before analysis with the fluorescence assay.

Figure 3: Corrected (using DPBS fluorescence measurement) fluorescence units level for the open and closed TG-2 resin bound proteins incubated with the selected aptamers across the five rounds of the SELEX procedure. Greater fluorescence is indicative of higher concentrations of aptamers to target FAM-labelled aptamers bound to the TG-2 resin background signal ratio. The trend of the data indicates the slight increase of the SELEX selected open conformation aptamers affinity for the desired open TG-2 target compared to the closed unwanted target. This can be interpreted as a positive sign that the aptamers being selected demonstrate a higher binding affinity to the open TG-2 in comparison to non-specific binders.

Conclusion:

The purpose of this research was to select in vitro aptamers for the protein transglutaminase-2 from a FAM-labelled library aptamer pool. After the fifth rounds of selection, this objective was achieved, although more rounds of selection could be improved both, the statistical significance and quality of the results, for the potential of a highly evolved aptamer pool to have greater binding constants is practically certain.

This objective was achieved as the analysis of the fluorescence assay performed on the open and closed conformation beaded resins of the target TG-2 protein confirms the increasing trend of the detection of fluorescence levels after 5 SELEX rounds from the aptamers selected for the open TG-2, as opposed to the decreasing fluorescence level measurements observed in the assays including the closed form of the enzyme incubated with the open conformation selected aptamer pool (Figure 3).

As for the fluorescence assay, the result is a mixed blessing. For one, it can be interpreted that aptamers were selected for the targeted open conformation of the protein. Unfortunately, the validity of the specification of the aptamers is in question due to the wide range of fluorescence values recorded in a relatively unpredictable pattern (Figure 3).

While the selection did reduce the level at which aptamers were selected in the negative form (i.e.: fewer aptamers were generated capable of binding to the closed after being engineered positive), the selected aptamers have not exceeded the binding capacity of the FAM-library.

References:


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