BIO_06 - Selection of aptamers which binds human ACE-2 (angiotensin converting enzyme) for blocking RBD (receptor-binding domain) of SARS-CoV-2 protein S

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Introduction: The recent outbreak of Coronavirus disease caused by the SARS-CoV-2 virus has grown from a public health emergency to a major global pandemic. One way to inhibit the infectious process is to block the interaction between viral protein S with the human ACE-2 receptor, to prevent the internalization of virion in host cells. Recent studies show how the interaction between protein S and its receptor, a highly conserved protein in humans, ACE2. These studies indicate that the RBD binding site is mostly in a α-helix of human ACE-2, with essential amino acids making important close interactions. These characteristics make the receptor an attractive target for blocking the binding of SARS-CoV-2 protein S. Aptamers, which are short sequences of DNA or RNA molecules with defined structures that can specifically bind to a molecular target via 3-D structures, emerge as a promising alternative.

Objective: This study aims to select aptamers that specifically bind to the human ACE-2 receptor in order to block its binding to viral protein (RBD).

Methodology: Human ACE-2 protein was studied and a sequence containing the essential amino acids for viral RBD interactions was selected. A peptide containing these amino acids was synthesized and used for the selection of aptamers by Systematic Evolution of Ligands by Exponential Enrichment (SELEX) methodology. Sequencing was done by NGS technology. A screening was performed to select the sequences that presented frequency above 1000 repetitions of the same sequence. A competitive ELISA-like was developed in order to evaluate the inhibition of human ACE2/ RBD binding by aptamers interference. The Kd equilibrium constant) of the selected aptamers and recombinant RBD with recombinant ACE2 immobilized in a CM2 biosensor was determined by surface plasmon resonance technique, a SensiQ apparatus was utilized.

Results: Four aptamers were selected for the evaluations. All of them were able to reduces binding RBD-human ACE2; with a PI ranging 35 to 39%. Surface plasmon resonance showed that Kd for aptamers 2.2; 2.4, 2.7 and 2.9 were 45, 93, 270 and 188 nM, respectively. However, all of them were higher than RBD Kd (1.04 nM).

Conclusion: We were able to select aptamers which bind with the recombinant human ACE-2; however, the initial results show that none of the four selected aptamers was able to present a satisfactory inhibition of the ACE2-RBD binding. New aptamers selection against the recombinant ACE2 are being made in order to select best molecules.

Keywords: Aptamers; Human ACE2; SARS-CoV-2