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DEVELOPMENT AND OPTIMIZATION OF HIGH AFFINITY ONCOGENE-SPECIFIC DNA APTAMERS FOR HUMAN CANCER CELLS BINDING

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Introduction: Cancer is one of leading cause of death worldwide according to the world health organization [1]. Aptamer oligonucleotides appear to be a good alternative, since other treatments such as surgery, chemotherapy, and radiotherapy lead to side effects, toxicity and are invasive. Nevertheless, available aptamers suffer from high K-off (dissociation rate constant), which represent the proportion of dissociate complex ligan-receptor in unit time and this in absence of free ligand. This leads that the aptamers will get dissociate even with high affinity from his target. We propose in this study:

1) Selection of DNA aptamers with high affinity toward cancer cell surface proteins
2) Optimization to reduce the K-off by implementing hydrophobic modification in the selected DNA aptamers and implementing a scaffold that protect the complex from aqueous environments
3) Utilizing the acquired aptamer to assist in delivering DNA nanostructures within cancer cells

Main parts: Aptamers are a class of small nucleic acids with a great specificity and affinity for their targets. They consist of a random central region that is flanked between two constant regions. Aptamers fold in three-dimensional structure (such as bugles, stem-loops and hairpins … etc.) in order to bind their targets. Aptamers are chosen using a technique known as systematic evolution of ligand by exponential enrichment (SELEX), which entails numerous rounds of incubating aptamers with targets, discarding unbound aptamers, amplifying bound sequences, and moving on to the next round. After finding the best sequence for aptamers, the obtained aptamers are cloned and sequenced [2]. Aptamers' binding affinities which are determine by the dissociation constants (Kd) are typically in the low nanomolar to picomolar range, but they can go as low as femtomolar [3]. As a result, they can bind to a wide range of targets like proteins, bacteria, cells, virions, different small organic and inorganic molecules, using spatial complementarity that is closely matched, as well as Van der Waals' force, hydrophobic interactions, electrostatic/ionic interaction, and hydrogen bonding [4]. Always compared to monoclonal antibodies (mAbs), aptamers have the advantage over mAbs because not immunogenic, more stable and less expensive.

The major drawback of the aptamers binding to his target is due to aqueous environment that complex aptamers/proteins are under non-equilibrium (e.g., in vivo) condition which make the k-off high and the complex unstable. By implementing chemical modification to aptamers nucleotides and by rational design of a structural lock that protects the aptamers/protein complex, we can reduce the K-off, leading to a tight binding to the targeted cancer cell proteins. After achieving the tight binding, we aim to conjugate our obtained aptamers to DNA nanoconstructs to deliver into cancer cells, since the aptamers have the ability to internalize in the cells with different endocytic pathways.

Conclusion: Aptamers can be a good oligonucleotide gene therapy, by reduce of k-off using oligonucleotide modification for hydrophobicity and a scaffold that will protect from the aqueous surrounding environment we may achieve a super-aptamer with a tight binding to his target.

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