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Optimization of antisense oligonucleotides potential for alternative splicing regulation in cancer cell lines

Alternative splicing is a process which allows for the production of more than one protein from one gene. It is known that 95% of human genes undergo alternative splicing. This process is based on removing noncoding sequences from pre-mRNA and joining together fragments that encode the specific isoform. Alternative splicing is strictly controlled by many regulatory factors, including splicing proteins, regulatory sequences in the pre-mRNA, pre-mRNA secondary structures and the transcription kinetics. Every change or dysregulation that appears in these regulatory factors can result in alterations of alternative splicing pattern. In consequence, the formed transcripts encode the proteins that are easily degraded, inactive or play aberrant functions being a direct cause of various diseases development, including cancers.

In recent years, two types of molecular tools that are able to redirect alternative splicing have been developed by many research groups. Bifunctional antisense oligonucleotides are composed of two functional parts. Antisense part is responsible for hybridization of oligonucleotide to the target pre-mRNA sequence, whereas regulatory fragment contains the nucleotide sequence that is recognized by splicing proteins in cells. Therefore, the main function of regulatory part is to recruit splicing proteins to the target pre-mRNA sequence. On the other hand, splice switching oligonucleotides (SSOs) are designed to hybridize with regulatory sequences in the pre-mRNA and block their interactions with splicing proteins.

In the presented studies the attempts were made to optimize the sequence and structure of BASOs as well as the sequence of SSOs. The model gene for the regulation of alternative splicing by oligonucleotides was the *PKM* gene, which contains two mutually exclusive exons, *i.e.* exon 9 (present in PKM1 transcript) and exon 10 (present in PKM2 transcript). The increased production of PKM2 has been found in many cancers. Additionally, the influence of PKM2 on cancer progression is well documented. Therefore, the molecular tools were designed to redirect alternative splicing of *PKM* gene.

In the first part of the studies, the optimization of BASOs sequence and structure was performed. The regulatory part has been designed based on literature data, binding affinity determination and thermodynamic studies. Next, the experiments in HeLa cell line have been carried out to select the optimal hybridization position in the *PKM* pre-mRNA. Additionally, the efficiency of different number of repeats of regulatory binding motifs has been assessed. Finally, the regulatory properties of BASOs have been tested in SKOV-3 cell line. What is more, the influence of BASOs on SKOV-3 cells ability to migrate and invade was assessed.

The aim of the second part of the studies was to optimize the regulatory efficiency of splice switching oligonucleotide which interacts with sequence in exon 10 and influence on *PKM* alternative splicing pattern. The authors suggest that the effect is partially caused by simultaneous hybridization with the similar sequence in intron 9. In the presented studies, the modified nucleotides have been introduced into the oligonucleotide to modulate its preferences to hybridize with full complementary sequence (exon 10) and the sequence of intron 9 that forms mismatches with SSO. To assess the impact of modified nucleotide residues on duplex stability and mismatch discrimination, the thermodynamic studies have been performed. In the final step, the newly designed SSOs have been transfected to HeLa cell line and their regulatory effect on splicing was evaluated.

The main result of performed studies is the optimization of two different molecular tools that can be used to regulate alternative splicing of *PKM* gene. It has been proved that designed BASOs are able not only to redirect splicing of target gene but also to influence on migration and invasion properties of SKOV-3 cell line. Additionally, it has been presented that the two types of modified nucleotides significantly change the hybridization properties of SSOs what have strong impact on the *PKM* gene alternative splicing regulation. The presented results can contribute to development of new oligonucleotide based tools that can be used in the therapy of diseases that are caused by aberrant alternative splicing.