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MARKER INDUCED CLEAVAGE OF A TARGETED mRNA USING THE ANTISENSE OLIGONUCLEOTIDE-RELEASING CASSETTE (ASO-RC*)*

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In this study, we develop antisense oligonucleotide-releasing cassettes (ASO-RCs) for cancer treatment. The ASO-RCs were designed and assembled for cancer marker-dependent release of an antisense agent followed by the selective cleavage of the targeted mRNA.

Introduction.

Cancer is a term for a disease in which abnormal cells divide without control and can have the ability to invade nearby tissues. Cancer is a leading cause of death worldwide, and according to the World Health Organization, it causes nearly 10 million deaths in 2020. Currently, there are many ways to the treatment of cancer such as chemotherapy, radiation therapy, surgery, and many others. But most of these treatments are having long-lasting side effects. And unfortunately, its treatment can cost patients thousands of dollars. So to tackle this life-threatening disease ASO-RC has emerged as an exciting and promising strategy

Main Part.

Gene therapy based on antisense oligonucleotides (ASOs) is the light of hope that can be used for treating and fighting selectively cancer cells without having surgery and chemotherapy which may also reduce the cost of the treatment of this disease. ASO is a single-stranded deoxyribonucleotide, which is complementary to its mRNA target. The principle of the antisense approach is the hybridization of ASO to their target mRNA that leads to activation of an enzyme called RNase-H, which then causes cleavage of the targeted mRNA with a significant reduction of the targeted gene translation. But is also very crucial that conventional ASOs agents should not target a vital housekeeping gene due to the absence of selectivity for cancer cells. To increase the efficiency and selectivity of the antisense approach ASO-RCs were designed. These Light and Heavy cassettes consist of 2 and 3 DNA strands respectively held together by the protector which binds on one side of the cassettes. While on the other side there is an ASO that binds complementary to the DNA strands. The main advantage of the ASO-RCs is that they will release ASO only in the presence of a cancer marker that is expressed in the cancerous cells. So the cancer marker will bind to the protector of the cassettes and this binding will cause DNA strands to make loops separately and the ASO release from the cassettes which ultimately binds to the targeted mRNA and initiate the activity of the RNase-H followed by the downregulation of the targeted mRNA. Developed ASO-RCs Light and Heavy cassettes showed less selectivity because of the activation targeted mRNA cleavage in the absence of cancer marker. For the Light ASO-RC, the efficiency of cleavage in the absence of cancer marker is 41% while in its presence 45%. For the Heavy ASO-RC, the efficiency of cleavage in the absence of cancer marker is 27% while in its presence 35,3%.

Applications.

We conclude from the results that ASOs are also get released from the cassettes in the absence of cancer markers and both Light and Heavy cassettes showed low selectivity *in vitro* because of the weak assembly. Further optimization is needed to achieve the desired cancer marker-dependent ASOs release.