Chemical biology-based strategies to stabilize the secondary structure of dynamic ceRNA molecules for detection and activation of p53 gene in colorectal cancer.

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The complementary endogenous RNA (ceRNA) molecules play a critical role in controlling the gene expression by modulating the availability of microRNA (miRNA) molecules in the cell. These ceRNA molecules are coded by the long intergenic non-coding (linc) DNA which was previously thought to be junk DNA. In general, the ceRNA molecules can act as sponges to bind all the available miRNA molecules that are otherwise responsible for gene silencing. In the context of Cancer, the TP53 gene, also known as the guardian of the genome, is very essential to trigger cellular apoptosis in time. Silencing of TP53 leads to cellular proliferation and Cancer eventually. In this study, a Chemical Biology approach has been designed to stabilize the ceRNA molecules that can bind the miRNA molecules that are responsible for silencing the TP53 gene in colorectal cancer. By using this strategy, one can design similar ways for pan-cancer applications in future.

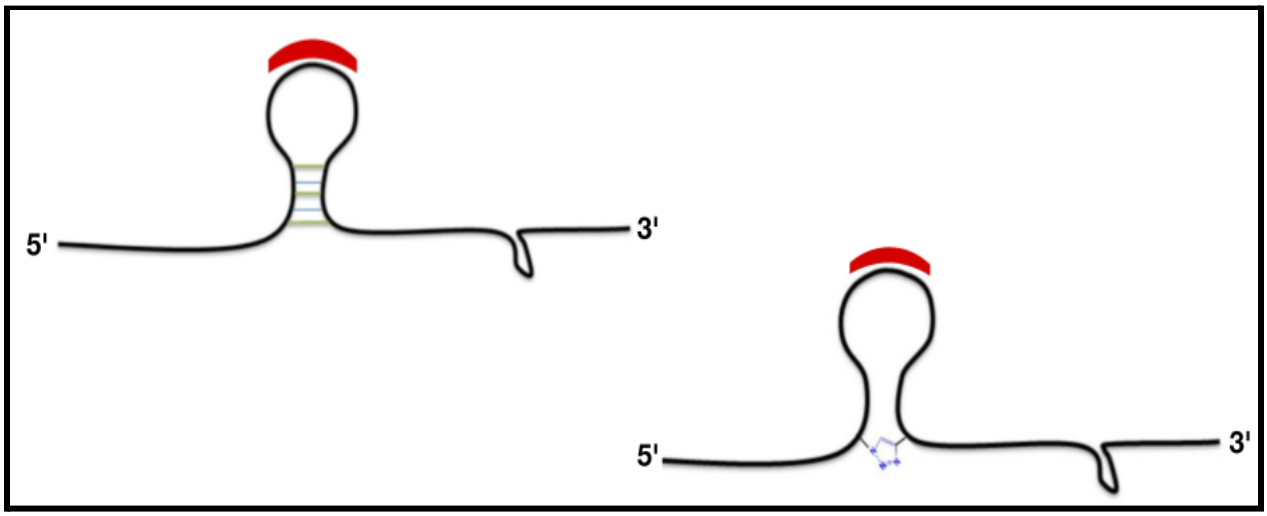

Figure 1. Proposed ceRNA stabilization strategies with photocrosslinking (top left) and click chemistry (bottom right).

Colorectal cancer (CRC) is the fourth most deadly cancer in the world according to the GLOBCAN 2020. In 2020, worldwide, there are estimated to have been 1,931,590 new cases (1). CRC is also known as colorectal adenocarcinoma as it emerges from the glandular epithelial cells of the large intestine at the colon and rectum region (2). The potential factors that cause CRC are changes in lifestyle. Individuals with a family history, genetic disorders and certain health conditions like Inflammatory bowel diseases (IBS) or type 2 diabetes. CRC gets metastasised once the condition gets severe and is not in a stage for further diagnosis (3, 4). In this communications, our strategy is to target the tumour suppressor gene (TP53) in CRC and activate it using the chemically stabilized competitive endogenous RNA (ceRNA) and

deliver it using a synthetic cell at the tumour suppressor site using Synthetic Biology. In this strategy, we believe that there is high specificity in this method where the tumour suppressor gene will be upregulated which will reduce the severity of CRC. By using Synthetic Biology the specificity increases and brings novelty to these strategies.

Competitive endogenous RNA (ceRNA) is a network of different RNA's that competes with mRNA for the same pool of microRNA's (miRNA). Previously, the ceRNA was considered junk but later by doing several studies, it was found that they code for long intergenic non-coding RNA (lincRNA). The ceRNA acts as miRNA sponges by miRNA binding sites to control the availability of endogenous miRNA from binding to their target mRNAs (5). This way they regulate the ability of miRNA to inhibit mRNA from being translated into proteins (5, 6). In colorectal cancer (CRC) we are mainly focussing on upregulating the p53 tumour suppressor gene using ceRNA by its sponging mechanism. Initially, we need to find where the p53 tumour suppressor gene is activated in CRC. Then the miRNA's will block the translational process by binding to the mRNA. Later the excess miRNA's will be sponged by chemically stabilized ceRNA. The ceRNA has a complex secondary structure and is dynamic in nature which results in constant change in the conformational changes. As ceRNA is a network of different RNAs it consists of thousands of nucleotide bases. The secondary structure having loops and stems and being dynamic it is not possible to do the sponging mechanism all by itself as it might not bind to the correct miRNA at the specific site on the sequence. To control the constant structural changes we are using two strategies.

The first strategy, the ceRNA will be designed using chemical biology. Here we will mainly focus on making the covalent bonds between nucleotides stronger. Initially, we need to design the artificial nucleotides in the stem of the loop and synthesize the ceRNA (Fig. 1). Next using the light source at a particular wavelength (nm) the bonds will be locked so that they will stay intact and don't change. This results in the formation of strong covalent bonds.

In the second strategy, the ceRNA used in this mechanism will be designed using the click chemistry reaction (7). We will use the azide-alkyne cycloaddition which contains triazoles. This is a 5-membered ring structure that is regioselective and stereospecific. The ceRNA as we know is dynamic in nature in its secondary structure. To stop these conformational changes we will use this heterocyclic ring structure and lock the stem region at our required site on the ceRNA (Fig. 1). The R_1 and R_2 groups on the heterocyclic compound will bind at specific bases and interlock them. After this process, we use synthetic biology to design a cell using genetic circuits to generate the ceRNA at the TP53 gene site and activate it.

Currently, we are in the process of finding and analyzing the ceRNA network that codes for lincRNA in CRC. We have to design the ceRNA using the strategies which are mentioned above and design the synthetic cell. Once we get the data generated we will later make it pan-cancer. The ongoing and upcoming research on MnScs will be published in the future issues of TCABSE-J.

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