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Design and construction of a genetic vector expressing a poly-miR-122 for gene therapy of hepatocarcinoma

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Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related death worldwide and there is still no effective treatment for this disease, so gene therapy is a promising therapeutic alternative for the treatment of HCC. The use of microRNA (miRNA) in gene therapy has become a powerful tool for the regulation of genes involved in acquired genetic diseases such as cancer. The miRNA-122 (miR-122) is specific and the most abundant in the liver, it has been shown to function as a tumor suppressor. The levels of miR-122 decrease significantly and specifically in HCC. The objective of this work was to construct a genetic vector that contains a poly-miR-122 governed by the α-fetoprotein (AFP) promoter, specific to HCC. A poly-miR-122 sequence containing three miR-122 precursors (pre-miR-122) was designed, this was analyzed for secondary structure prediction and thermodynamic stability. The result of the prediction analysis of the poly-miR-122 sequence showed that the primary transcript will have thermodynamic stability, indicating that it can function in the treatment against HCC cells. Subsequently, the recombinant plasmid pIRES2-AFP-poly-miR-122-EGFP was constructed. The identity of this recombinant plasmid was confirmed by enzymatic restriction. The presence of the AFP promoter in the recombinant plasmid was confirmed by PCR. With automated sequencing, an identity of 99.6% was found with the AFP promoter (NCBI: L34019.1). This genetic construction can express the active and stable miR-122, so it could be used for the specific treatment of HCC by gene therapy.

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