

# Testing regulation of cyclin dependent kinase 6 (CDK6) by microRNA-885-3p (miR-885-3p) in hepatocellular carcinoma (HCC)



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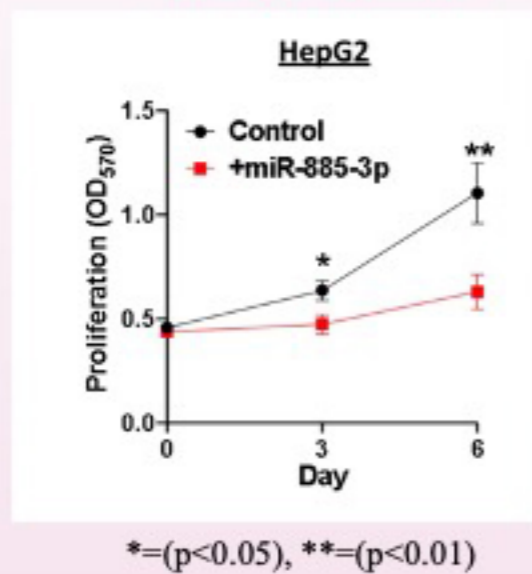
## ABSTRACT

The result of our preliminary experiment showed that miR-885-3p overexpression can significantly reduced the proliferation rate of the liver cells, but the mechanism still remains unknown. The aim of this research is to propose a mechanism of action of miR-885-3p by identifying its target. The CDK6 gene was selected as a potential target gene by using an online computational program.

In vitro interactions between miR-885-3p and CDK6 were determined by luciferase reporter assay. The luciferase activity was significantly reduced in cells containing miR-885-3p overexpression with CDK6 gene insertion. Therefore, CDK6 was confirmed to be the target of miR-885-3p.

## Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant liver cancers and its pathogenesis is still not well understood.<sup>1</sup> In search of an effective therapeutic method for HCC, scientists recently studied microRNA (miRNA) and found that alteration of miRNA expression can generate various significant therapeutic effects on tumor cells.<sup>2</sup> For instance, our preliminary experiment result showed that miR-885-3p overexpression can suppressed the proliferation rate of liver cancer cells. However, the mechanism of action and target gene of this miR-885-3p were not yet identified.



## Method

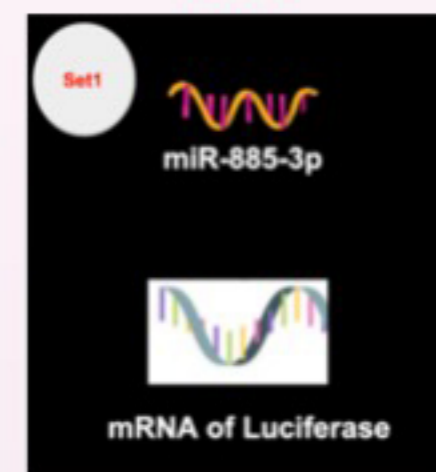
The potential target genes were screened from "miRDB" online database by using computational prediction system to calculate for interaction strength between miR-885-3p and genes in the database. Prediction results showed that CDK6 was likely to interact strongly with miR-885-3p and was selected as a suspected target.

miRNA target prediction database - <http://www.mirdb.org/cgi-bin/search.cgi>

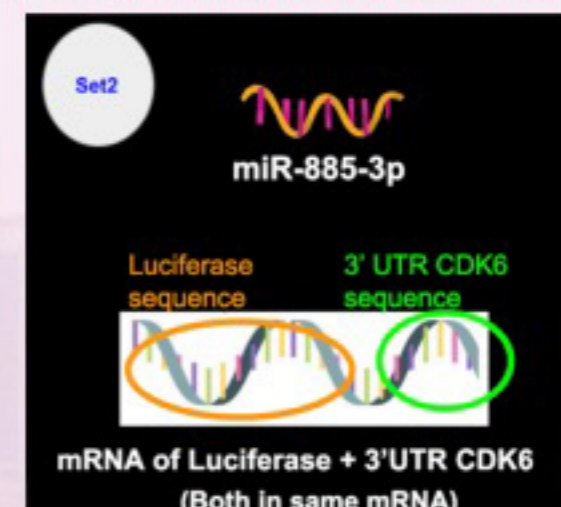
Target Rank	Target Score	miRNA Name	Gene Symbol	Gene Description
1	95	hsa-miR-885-3p	CDK6	regulator of G protein signaling 6
2	94	hsa-miR-885-3p	PTEN	phosphatidylinositol 3-kinase class I
3	93	hsa-miR-885-3p	PTEN	phosphatidylinositol 3-kinase class I
4	92	hsa-miR-885-3p	CDK6	regulator of G protein signaling 6
5	91	hsa-miR-885-3p	CDK6	regulator of G protein signaling 6
6	90	hsa-miR-885-3p	CDK6	regulator of G protein signaling 6
7	90	hsa-miR-885-3p	CDK6	regulator of G protein signaling 6
8	89	hsa-miR-885-3p	CDK6	regulator of G protein signaling 6
9	89	hsa-miR-885-3p	CDK6	regulator of G protein signaling 6
10	89	hsa-miR-885-3p	CDK6	regulator of G protein signaling 6
11	89	hsa-miR-885-3p	CDK6	regulator of G protein signaling 6
12	87	hsa-miR-885-3p	CDK6	regulator of G protein signaling 6
13	86	hsa-miR-885-3p	CDK6	regulator of G protein signaling 6
14	86	hsa-miR-885-3p	CDK6	regulator of G protein signaling 6
15	85	hsa-miR-885-3p	CDK6	regulator of G protein signaling 6
16	85	hsa-miR-885-3p	CDK6	regulator of G protein signaling 6
17	84	hsa-miR-885-3p	CDK6	regulator of G protein signaling 6
18	84	hsa-miR-885-3p	CDK6	regulator of G protein signaling 6

Luciferase reporter assay was performed to test for the interactions between miR-885-3p and CDK6 gene. In this experiment, two plasmids were co-transfected into each set of HEK293 cells as follow:

Set #1 (Control): plasmids of miR-885-3p + luciferase mRNA

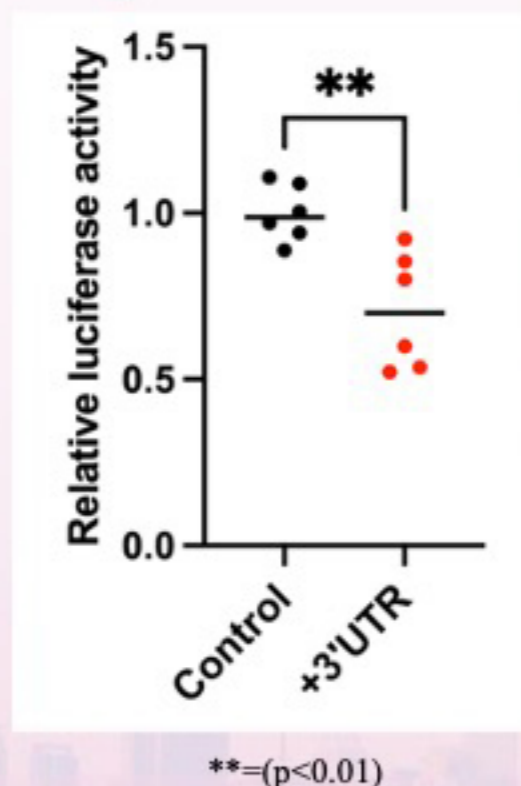


Set #2 (3'UTR CDK6): plasmids of miR-885-3p + luciferase mRNA with 3'UTR CDK6 gene insertion



## Result

The luciferase activity of cells that contain plasmid with 3'UTR CDK6 gene insertion was significantly reduced relative to the control. This result can confirm the interaction between miR-885-3p and CDK6 genes. In conclusion, miR-885-3p was a tumor suppressor gene that targeted CDK6 genes in liver cancer cells.



### Reference:

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- O'Brien J, Hayder H, Zayed Y, Peng C. Overview of MicroRNA Biogenesis, Mechanisms of Actions, and Circulation. *Frontiers in Endocrinology*. 2018; 9(402). doi:10.3389/fendo.2018.00402

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