Apoptosis Inducing Novel microRNA for Breast cancer and Hepatocellular Carcinoma

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ABSTRACT

MicroRNAs (miRNAs) are one of the most prevalent small (~22 nucleotide [nt]) regulatory RNA classes in animals. These miRNAs constitute nearly 1 percent of genes in human genome, making miRNA genes one of the more abundant types of regulatory molecules. MiRNAs have been shown to play important roles in cell development, apoptosis, and other fundamental biological processes. MiRNAs exert their influence through complementary base-pairing with specific target mRNAs, leading in turn to degradation or translational repression of the targeted mRNA. Cancer stem cells (CSC) live indefinitely and become seed for new tumors. They are not easily killed by most current therapies. We have identified and tested a novel microRNA (miR-491) and demonstrated increased apoptosis in hepatocellular carcinoma (HCC) and in breast cancer cells in vitro. We prepared fluorescent labeled miR-491 by covalent or streptavidin/biotin coupling to test the apoptosis ability in cancer cells specifically for targeted therapy. The targeting and apoptosis inducing ability was also tested by microscopy using streptavidin gold nanoparticles conjugation. Further studies are ongoing. The preliminary results and current development could be possible to provide a new class of molecules for imaging and therapy of both HCC and breast cancer.

Summary:

MicroRNA provides a mechanism to target cancer stem cells and restraint tumors cells permanently; this could lead to promising cancer therapeutics and imaging agents. Apoptosis inducing microRNA was identified to target HCC and breast cancer cells for imaging and therapy.

INTRODUCTION

Breast cancer is the second most common cancer among women, estimated at 719,000 cases worldwide. The observations of miRNAs expressed in human breast cancer are stimulating broad interest in the possibility that miRNA profiles represent a promising new class of cancer therapeutics. MicroRNAs have been shown to be capable of distinguishing the different tissue developmental lineages and differentiation states of various human malignancies (1) including breast cancer (1a).

MicroRNAs (miRNAs) are a noncoding family of 21-23-nucleotide RNAs that regulate gene expression by targeting mRNAs in a sequence-specific manner, inducing translational repression or mRNA degradation, depending on the degree of complementarity between miRNAs and their targets(2). There have been approximately 450 miRNAs already identified. They appear to employ several mechanisms to repress gene expression and regulate cellular activities, such as development(3, 4), cell proliferation(5), apoptosis(6, 7), and cancer(8-10). An example of an miRNA that is abundant in the liver, and appears to affect hepatic function, is miRNA-122 (miR-122)(11). When miR-122 was silenced using antagomir-122, a cholesterol-conjugated inhibitory molecule of miR-122, there was a 44% decrease in cholesterol synthesis in hepatocytes(11). The mechanism of this effect appears to be that inhibition of miR-122 caused the activation of a transcriptional repressor protein involved in cholesterol biosynthesis(11). Another study reported that inhibition of miR-122 in the liver caused a marked loss of hepatitis C viral RNAs, and that miR-122 may represent a target for antiviral intervention(12). Our intent in this proposal is to explore the role of miRNAs in modulating proliferation and apoptosis in *in vitro* and *in vivo* models. We believe that these findings may help to identify novel approaches to intervene in the process of liver injury and failure in man.

The molecular mechanisms by which these miRNAs control gene expression in breast cancer are not well defined. Hence, widespread evaluation of miRNAs as potential cancer therapeutics is needed. However, to date no data is available regarding the function of these miRNAs in HBT3477 human breast cancer cells. In this study we have evaluated the ability of miR-491 induce apoptosis in normal, HBT3477 human breast cancer cells and comparison with Hep G2 cells using electron microscopy.

AIM

Study of apoptosis inducing ability of miR-491 (microRNA) in HBT3477 breast and HCC cancer cells

MATERIALS AND METHODS

Hep G2 and HBT 3477 cell culture

Over-expression of miRNA-491 in Breast and HCC cells.

miRNA-491 levels in cells was measured by stem-loop real time RT-PCR technique (SLqPCR)

Cells were sensitized with TNF-α (1-5 ng/ml) for 4 hours

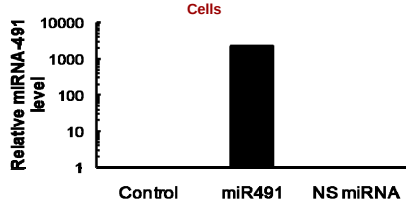
Cells were analyzed for apoptosis by

Caspase-3 activity, TUNEL assay , DAPI staining and morphological structure by Electron Microscopy

The protein targets of miRNA-491 were determined by both bioinformatics and biochemical approaches

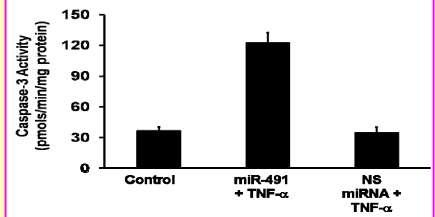
RESULTS

Table 1. Effect of HX/XO & HER on ROS Release in HepG2 Cells



Hep G2 cells were transduced with either control LV-EGFP or LV-CuZnSOD and treated with ROS-generating systems. The transduced Hep G2 cells were incubated with MCLA (O_2^-), H2DCF-DA (H2O2) or Mitosox (mitochondrial O_2^-). The mean fluorescent intensity (MCLA & H_2DCF-DA) was calculated in a fluorescent plate reader. Percent of positive cells were counted with mitosox staining. (n=3; # p<0.05; * p<0.01; ** p<0.001)

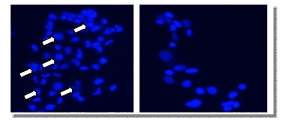
Fig 2. Effect of HX/XO on LDH Leakage in HepG2 Cells



Hep G2 cells were transduced with either control LV-EGFP or LV-CuZnSOD and treated with HX/XO and cell death was assessed by LDH leakage. (n=3; # p<0.01, compared with untreated cells; * p<0.01 compared with HX/XO treatment)

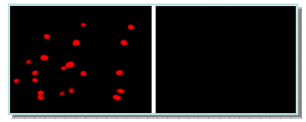
RESULTS

Fig 3. Effect of HER on LDH Leakage in HepG2 Cells



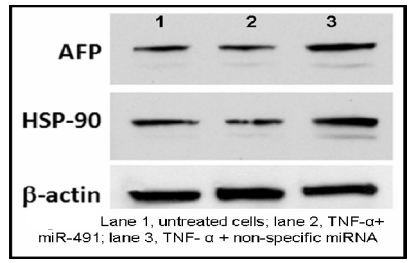
Hep G2 cells were transduced with either control LV-EGFP or LV-CuZnSOD and treated with HX/XO and cell death was assessed by LDH leakage. (n=3; # p<0.01, compared with untreated cells; * p<0.01 compared with HER treatment)

Fig 4. Activation of Caspase by HX/XO & HER in HepG2 Cells



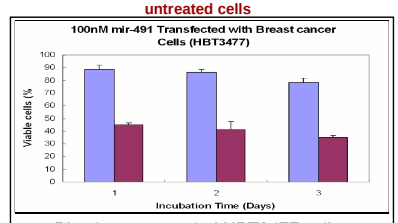
Hep G2 cells were incubated with either HX/XO or HER systems for 4 hours. Cells were collected and analyzed for caspase activation using Oncogene's caspase activity kit by flow cytometry. (n=3; #p<0.05, compared with untreated cells; *p<0.05 compared with treatment)

Fig 5. Liver Histology



Lane 1, untreated cells; lane 2, TNF-α+ miR-491; lane 3, TNF- α + non-specific miRNA

Fig 7. Apoptosis study in BT3477 cells using miRNA-491+TNF-a assayed by trypan blue compared with untreated cells



Blue bar: un treaded HBT3477 cells ; Brown: HBT3477 +100nM mir-491

Cell Morphological Study by TEM

Transmission Electron Microscopy (TEM) analysis has been considered a milestone of the research in the field of apoptosis

Mir491 was conjugated to Streptavidin Gold nano particles (GNP) transfected into the HBT 3477 and Hep G2 cells to evaluate the morphological aspects of apoptosis in the cells to asses the extent of the apoptosis level with targeted GNP (+ miR491) and nontargeted GNP (-miR491) targeted genes

CONCLUSIONS

Over-expression of miRNA-491 causes apoptosis in HBT3477 (breast cancer) and Hep G2 (HCC) cancer cells; this could be a novel class of targeting agent for imaging and therapy

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