Evaluation of MicroRNA-93 and miRNA-210 in Egyptian Patient with Post HCV Hepatocellular Carcinoma

Thesis

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List of Abbreviations

Abb.	Full term
3 UTR	3 Untranslated region
AAV	Adeno-associated virses
AF	Aflatoxin
AFP	Alpha feto protein
AFU	Alfa fucosidase
AMOs	Anti miRNA oligonucleotides
ANA	Antinuclear antibody
BCLC	Barcelona clinic liver cancer
CDKIs	Cyclin dependent kinases inhibitors
CDKs	Cyclin dependent kinases
CeRNA	Competing endogenous RNA
CEUS	Contrast enhanced ultrasound
CT	Computed axial tomography
CTP	Child-turcotte-pugh
DCP	Des-gamma carboxyprothrombin
Ds RBD	Double stranded RNA-binding domain protein
EGFR	Epithelial growth factor receptors
ERRFI-1	ERBB receptor feed back inhibtor1
FGFR	Fibroblost growth factor receptors
GP73	Golgi proten 73
GPC3	Glypican-3
HBV	Hepatitis B Virus
HCC	Hepatscellular carcinoma
HCV	Hepatitis C Virus
HDACS	Histone deacetylases
HIFU	High-intenisty focused ultrasound
HMGA2	High mobility group At-hook2
HSC	Hepatic stellaet cells

List of Abbreviations

Abb.	Full term
MAPK	Mitogen - activated protein kinase
MET	Met proto-onco-gene
mi RNP	mi RNA-containing ribonucleoprotion complex
MiRNA	Micro RNA
MOH	Egyptian ministry of health
MRI	Mgnetic resonance imaging
MyoD1	Myogenin and myoblast determination 1
NAFLD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis
PDCD4	Programmed cell death 4
PDGF	Platelat derived growth factor
PI3K	Phospatidyl insotiol 3-kinase
PIVKA-II	Protein-induced by vitamin K absenc or anagonist-II
PLK1	Polo like kinase 1
PTEN	Phosphatase and tensin homologue
RISC	RNA-induced silencing complex
Rt-qPCR	Quantitive real-time polymerase chain reaction
Sh RNA	Short haripain RNAs
SIRT1	Sirthuin 1
SNALPs	Stable nucleic acid lipid particles
SREBP2	Sterol regulatory element binding protein 2
ssRNA	Single stranded RNA
TACE	Tronsarterial chemoembolization
TCF/LEF	T-Cell-specific transcription factors
TGF	Transfroming growth factor
VEGFR	Vasoendothelial growth factor receptors
VMPI	Vacuole membrane protein 1
ZEB1/SIP1	Zinc-finger E Box binding protein

Abstract

High sensitivity and specificity was found in both miR-210 and miR-93a to distinguish between HCC and control group, the sensitivity for the miR-210 and miR-93a was 97%, 99% respectively and the specificity was 99.5%, 93%; respectively.

Also the studied miRNAs showed reasonable sensitivity and specificity to distinguish between BCLC-A /BCLC-Non A, The sensitivity for the miR-210 and miR-93a were 60%, 60% and the specificity 93%, 80%; respectively.

Furthermore we found positive correlation between genetic expression of miRNA 210, 93a and adjacent lymph node enlargement (p=0.000) and with BCLC staging (p=0.000).

As regard correlation between miRNA 210, miRNA 93a and liver function parameters, our results showed that there were no significant correlation between increased expression of miRNA93, 210 liver parameters (albumin, bilirubin, AST, ALT).

Keywords: Transfroming growth factor, Vasoendothelial growth factor receptors, Vacuole membrane protein 1, Zinc-finger E Box binding protein

INTRODUCTION

Hepatocellular carcinoma (HCC) is the major primary liver cancer, which is the fifth most common cause of cancer worldwide, with about 750,000 patients globally reported each year. An estimated 80–90% of all HCCs arise from the cirrhotic liver. Major risk factors are chronic viral hepatitis B (HBV) or C (HCV), which account for 80–90% of all HCCs worldwide, and alcoholic and non-alcoholic steatohepatitis-associated liver cirrhosis ***(Parkin, 2001).***

Liver cancer is the third cancer-related cause of death, with an annual `mortality of about 700,000 persons globally. Low survival is attributed to late diagnosis, resistance to treatment, tumor recurrence, and metastasis, hence stressing the need for novel diagnostics and therapeutics ***(Bosch et al., 2005).***

MiRNA are small, interfering, non-coding RNA that are 21–30 nucleotides in length, and it has been predicted that there are approximately 1000 of these sequences in the human genome. Each miRNA negatively regulates its target genes by binding to multiple mRNA ***(Zamore and Haley, 2005).***

Interestingly, more than half of all genes that encode miRNA are located at fragile sites or in cancer-associated regions of the genome, suggesting that miRNA may serve as diagnostic markers or therapeutic targets ***(Calin et al., 2004).***

MIRNA Have been reported to influence the critical functions of cellular differentiation, proliferation, apoptosis, invasion and metastasis. The miRNA expression profiles in tumors are different from those in normal tissues and also vary according to the type of tumor. Interestingly, the direct targets of miRNA are also protein-coding genes of the cell cycle, apoptosis and metastasis in Hepatocellular carcinom. Recently, microarray analysis has revealed that a subset of miRNA are up- and downregulated during the development of HCC ***(Liu et al., 2012).***

Reductions in the expression of miRNA are frequently observed in HCC, and the targets of these downregulated miRNA may be putative oncogenes. Conversely, some of the upregulated miRNA act as oncogenic miRNA in HCC and may be targets of tumor suppressor genes ***(Sun et al., 2013).***

MiR-93 expressed over 10-fold in HCC cell lines compared to normal liver cells, It was demonstrated to interfere with the PTEN expression through binding of specific 3′ UTR regions. miR-93 oncogenic effects were shown to regulate phosphorylation of Akt by suppressing PTEN. MiR-93 was silencing tumor-related pathway genes, whereby its function is controlling c-Met/PI3K/Akt signal transduction ***(Katsuya, et al. 2014).***

Katsuya, et al. (2014), also confirmed that inhibiting miR-93 expression (anti-miR) would suppress proliferation,

migration, and invasion of HCC cells. Anti-miR treatment of HCC cell lines significantly enhanced chemosensitivity against kinase inhibitors, Sorafenib and Tivantinib**.**

Among Several miRNAs implicated in HCC, it was reported that miRNA-210 level was observed in patients with HCC compared with control subjects, Also a positive correlation presents between baseline miR-210 level and tumor size, vascular invasion, tumor differentiation and Barcelona Clinic Liver Cancer stage ***(Zamore and Haley, 2014)*.**

Asahiro and Masaki (2015), confirmed that Pololike kinase 1 (PLK1) was a candidate target of miR210, which is a critical regulator of cell cycle transmission at multiple levels. It was demonstrated that miR210 reduced the levels of PLK1 protein by binding the 3' untranslated region of its mRNA. Elevated baseline miR-210 level also served as an independent prognostic fact or predicting poor overall survival, Patients who did not respond to transarterial chemoembolization had higher baseline miR-210 levels than patients who did respond to treatment.

AIM OF THE WORK

This study was designed to clarify the significance of circulting miRNA 93a and miRNA 210 as markers for early detection of hepatocellular carcinoma.

Chapter (I)

HEPATOCELLULAR CARCINOMA

Hepatocellular carcinoma is the fifth most common cancer, the third most common cause for cancer death in the world, and a major cause of death in patients with chronic hepatitis C virus infection ***(Sun et al., 2013).*** The incidence of HCC is expected to increase in the next two decades, largely due to hepatitis C infection and secondary cirrhosis ***(Willaltt et al., 2008).***

The detection of HCC at an early stage is critical for a favorable clinical outcome. Potential preventive strategies in the development of HCC are being recognized. Novel molecular markers identified may aid in the diagnosis of early HCC. The natural history of HCC is highly variable and the clinical management choices for HCC can be complex, hence patient assessment and treatment planning have to take the severity of the nonmalignant liver disease into account ***(Walzer and kulik, 2008).***

Epidemiology

In **2000**, there were 564,000 new cases and 549,000 deaths from HCC worldwide, indicating the devastating prognosis of this tumor. The incidence of **HCC** is rising across