Assessment of LncRNA CASC2 and TUG1 expression in Hepatocellular Carcinoma

Thesis

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By

Noha Salah Refai Ibrahim

M.B.B.Ch

Faculty of medicine-Ain Shams University

Under Supervision of

Prof. Hany Youssef Halim

Professor of Medical Biochemistry and Molecular Biology

Faculty of Medicine - Ain Shams University

Assist. Prof. Manal Louis Louka

Assistant Professor of Medical Biochemistry and Molecular Biology

Faculty of Medicine - Ain Shams University

Assist. Prof. Iman Mohamed Fawzy Montasser

Assistant Professor of Tropical Medicine

Medical Biochemistry and Molecular Biology Department

Faculty of Medicine

Ain Shams University

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List of Abbreviations

Abb.	Full term
AASLD	American Association for the Study of Liver Diseases
AF	Aflatoxin
AFP	α-fetoprotein
ALP	Alkaline phosphatase
ALT	Alanine transaminase
ANOVA	Analysis of variance
ANRIL	antisense non-coding RNA in the INK4 locus
AST	Aspartate transaminase
AUC	Area under the curve
BCLC	Barcelona Clinic Liver Cancer
BUN	Blood urea nitrogen
CASC2	Cancer susceptibility candidate 2
cDNA	Complementary DNA
CHC	Chronic hepatitis C
CRNDE	Colorectal Neoplasia Differentially Expressed Long Non-Coding RNA
Ct	Threshold Cycle
CUDR	Cancer upregulated drug-resistant
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
dT	Deoxythymidine
EASL	European Association for the Study of the Liver
EDTA	Ethylenediaminetetraacetic acid
ERK	extracellular signal-regulated kinases
ESCC	Oesophageal squamous cell carcinoma
EZH2	Enhancer of zeste homolog 2
FN	False negative
FP	False positive

List of Abbreviations (Cont...)

Abb.	Full term
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GPC-3	Glypican-3
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HEIH	Highly Expressed In Hepatocellular Carcinoma Long Non-Coding RNA
HOTAIR	HOX transcript antisense RNA
HOXB7	Homeobox B7
hr	Hour
HULC	Hepatocellular Carcinoma Up-Regulated Long Non-Coding RNA
IFN	Interferon
IL-6	Interleukin 6
INR	International Normalised Ratio
JNK	c-Jun N-terminal kinases
kb	Kilobase
kDa	Kilodalton
lncRNA DBH-AS1	DBH (Dopamine Beta-Hydroxylase) Antisense RNA
lncRNA	Long non-coding ribonucleic acid
lncRNA-Dreh	down-regulated in hepatocellular carcinoma Long Non-Coding RNA
lncRNA-LET	lncRNA Low Expression in Tumor
MALAT-1	Metastasis Associated Lung Adenocarcinoma Transcript 1
MAPK	Mitogen-activated protein kinase
MEG3	maternally expressed Gene 3
min	Minute
miRNA	Microribonucleic acid

List of Abbreviations (Cont...)

Abb.	Full term
mRNA	Messenger ribonucleic acid
MVIH	Long noncoding RNA associated with microvascular invasion in hepatocellular
n	Number
NAFLD	Non alcoholic fatty liver disease
NaSe	Sodium selenite
NASH	Non alcoholic steatohepatitis
NASH	Nonalcoholic steatohepatitis
ncRNA	Non coding ribonucleic acid
NFE2L2	Nuclear Factor, Erythroid 2 Like 2
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NPV	Negative predictive value
NRF2	Nuclear factor-like 2
NSCLC	Non-squamous cell lung carcinoma
nt	Nucleotide
O.D	Optical density
ºC	Centigrade
p	Probability of being by chance value
PAMP	Pathogen-Associated Molecular Pattern
PCR	Polymerase chain reaction
PPV	Positive predictive value
PT	Prothrombin time
qRT-PCR	Quantitative reverse transcription polymerase chain reaction
r	Correlation coefficient
RNA	Ribonucleic acid
ROC	Receiver operating characteristic
rpm	Revolutions per minute

List of Abbreviations (Cont...)

Abb.	Full term
RQ	Relative quantification
RT	Reverse Transcription
SD	Standard deviation
TN	True negative
TNFα	Tumor necrosis factor α
TNM	Tumor, node, metastasis
TLR3	Toll-like receptor 3
TP	True positive
TUG1	Taurine Up-regulated Gene 1
ΔCt	Delta Threshold Cycle

INTRODUCTION

Hepatocellular carcinoma (HCC) is the dominant histological type of primary liver cancer which accounts for 70–85 % of primary malignancies in liver.

HCC represents the sixth most common neoplasm in humans**,** and it is now the third leading cause of cancer deaths worldwide. Its incidence is increasing, ranging between 3% and 9% annually ***(Allan et al., 2014).***

HCC is the most common cancer among males in Egypt, where it is responsible for 33.63% and 13.54% of all cancers in males and females respectively ***(Anwar et al., 2008).*** Its prevalence is 10% and it represents about 45.3% of the new cases of the digestive system cancers ***(Miller and Abu-Raddad, 2010).***

Unfortunately, treatment options for HCC patients are limited, and the disease is often diagnosed at an advanced stage. The usual outcome is poor, because only 10–20% of hepatocellular carcinomas can be removed completely using surgery ***(Lehman et al., 2008).***

If the cancer cannot be completely removed, the disease is usually killing within 3 to 6 months ***(Venook et al., 2010).***

Generally, hepatocarcinogenesis is a multistep process involving a number of genetic or epigenetic alterations that

eventually result in the malignant transformation of hepatocytes. There have been significant advances in diagnosis and management of HCC and lots of therapeutic strategies have been improved. However, the 5-year overall survival rate remains very low and the biology of HCC remains poorly understood. Therefore, the identification of the new biomarkers for HCC and a detailed understanding of the molecular mechanisms underlying hepatic carcinogenesis will supply an arm for improving diagnosis and management ***(Wang et al., 2002).***

With the completion of the human genome work, it was realized that more than 90 % of the genomic DNA could transcript into noncoding RNAs (ncRNAs) ***(van Bakel et al., 2010).***

Long ncRNAs (lncRNAs)**,** which are defined as being longer than 200 nucleotides, emerge as essential regulators in almost all aspects of biology, via regulation of chromatin organization, at transcriptional and post- transcriptional level. Additionally, a number of studies demonstrated that lncRNAs play an important role in tumorigenesis, and their mis-expression confers tumor initiation, cancer cell growth and metastasis.

Mounting evidence has shown that lncRNAs are capable of influencing various cellular processes such as proliferation, cell cycle progression, cell growth, and apoptosis, and thus play important roles in carcinogenesis and cancer metastasis ***(Jeffares et al., 1998).***

Taurine Up-regulated Gene 1 (TUG1), a 7.1-kb lncRNA, was originally identified as a contributor in the formation of photoreceptors and in retinal development in mice. Recently, TUG1 was found to be generally down-regulated in NSCLC (non-squamous cell lung carcinoma) ***(Zhang et al., 2014).*** On the contrary, some studies showed that TUG1 can promote the cell proliferation of ESCC (oesophageal squamous cell carcinoma) ***(Xu et al., 2014),*** urothelial carcinoma of the bladder ***(Han et al., 2013)*** and osteosarcoma ***(Zhang et al., 2013).***

A new lncRNA, cancer susceptibility candidate 2 (CASC2), located at chromosome 10q26, was originally identified as a down-regulated gene in endometrial cancer and acted as a tumor suppressor gene ***(Baldinu et al., 2007).*** It was also reported that CASC2 suppresses malignancy in human gliomas by miR-21 ***(Wang et al., 2015)*** and was down-regulated in non-small cell lung carcinoma as well ***(Han et al., 2016).*** But little is known about CASC2 and HCC, and also its relation with TUG1 remains unclear ***(Huang et al., 2015).***

AIM OF THE WORK

1. To evaluate both LncRNA TUG1 and CASC2 gene expression in whole blood and/or sera of HCC patients, as well as normal healthy subjects.
2. To relate LncRNA TUG1 and CASC2 to each other and to the different clinicopathological factors in HCC.
3. Explore new potential biomarkers with a valid non-invasive technique.