Review Article

The Clinical Significance of Long Non-Coding RNA (Taurine Up-Regulated Gene1) in Hepatitis C Virus-Related Hepatocellular Carcinoma Patients.

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Abstract

Background: The biology of hepatocellular carcinoma remains undiscovered. A lack of early Diagnostic markers and the poor prognosis of HCC attributes to its high morbidity. HCC is the second most frequent cause of cancer incidence and mortality among men in Egypt, the fourth most prevalent cause of death from cancer. HCC is the ninth most frequently diagnosed cancer in femalesLong non- coding RNAs (lncRNAs) have been confirmed to be key regulators of most cell processes and cancer. lncRNA TUG1 has mainly been focused on cancer, and lncRNA TUG1 can regulate the development of cancers. TUG1 was used as a non-invasive, cost effective, and complementary biomarker in viral hepatitis C and viral hepatitis C-associated hepatocellular carcinoma. The present study aimed to evaluate the role of LncTUG1 in blood of HCC/HCV patients with the aim of identifying a diagnostic biomarker with a valid non- invasive technique. TUG1 could be new potential biomarkers with a valid non- invasive technique, cost effective, and complementary biomarker in viral hepatitis C and viral hepatitis C-associated hepatocellular carcinoma.

Keywords: TUG1, HCC. Long non coding, HCV, liver cirrhosis

Introduction

Liver cancer is one of the most frequently diagnosed cancers all over the world. Most of patients with liver cancer (~ 70% to 90%) are diagnosed as primary cancer which is known as hepatocellular carcinoma (HCC) (Torre et al, 2015). It is considered as the sixth most common cancer and the third leading cause of cancer-related deaths worldwide (Maluccio and Covey, 2012). Chronic liver disease and cirrhosis remain the most important risk factors for the development of HCC of which viral hepatitis and excessive alcohol intake are the leading risk factors worldwide (Julius et al, 2016). HCV infection globally is well known as one of the main risk factor for HCC development (Petruzziello et al, 2016).

HCV-induced HCC is a model of chronic inflammation driven cancer, where complex interactions between multiple cell types form a carcinogenic tissue microenvironment that fosters and promotes progression of neoplastic clones (Ruzic et al, 2019). The carcinogenic mechanism of HCV is not completely known (McGivern and Lemon et al, 2011). Therefore, further understanding of the molecular mechanism of HCC progression may improve the diagnosis and developing novel therapeutic targets.

Long noncoding RNAs (lncRNAs) have recently come to the forefront as functional non–protein-coding RNAs that are involved in a variety of cellular

14

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processes ranging from maintaining the structural integrity of chromosomes to gene expression regulation in a spatiotemporal manner, long non-coding RNAs present interaction domains for DNA, Messenger RNA (mRNA), MicroRNAs (miRNAs), and proteins depending on both sequence and secondary structure. LncRNAs have been implicated to regulate a range of biological functions and the disruption of some of these functions, such as genomic imprinting and transcriptional regulation, and playing a critical role in cancer development. The tumor expression of certain lncRNAs provides a source of regulatory regions that can be used to reduce the risk of affecting normal tissues during transgene-mediated treatment (Zhang et al, 2019). Long noncoding RNAs have been shown to interfere in the pathogenesis of development and progression of HCC. LncRNAs have both diagnostic and therapeutic potentials (Fernande et al, 2019).

Taurine up- regulated gene 1 (TUG1) is a 7.1 kb lncRNA. It was firstly recognized in 2005, it has been regarded as a critical element for retinal development in rodents (Young et al, 2005). TUG1is located in chromosome 22q12. Recent investigations have highlighted its role in the tumorigenesis process with controversial evidence pointing to either tumor suppressor roles or oncogenic roles (Wang et al, 2017; Li et al, 2018).

Review
Liver cirrhosis is a chronic liver disease, that is characterized by a process of necrosis and regeneration of hepatocytes, resulting in fibrosis and capillarization of the liver sinusoids (**Liberal & Grant., 2016**).

Epidemiology
liver cirrhosis is a leading cause of mortality and morbidity across the world. It is the 11th leading cause of death and 15th leading cause of morbidity, accounting for 2.2% of deaths and 1.5% of disability-adjusted life years worldwide in 2016 (**Global Health Estimates., 2016**). The prevalence of the disease is underestimated because it is asymp-tomatic, not diagnosed in the early stages, and usually progresses to the decompensated stage at a rate of 5 to 7% per year (**Geong et al., 2019**).

Hepatitis C virus (HCV): is an infectious disease that primarily affects the liver, causing acute or chronic hepatitis (**Scaglione et al., 2015**). The World Health Organization (2018) estimates a global prevalence of infection by hepatitis C virus of 150 million patients, with 4 million new cases this year. Reports also estimate 500,000 deaths annually by HCV, with Europe, Africa and the eastern Mediterranean being the places with more prevalence (1.5% to 2.3%) (**Thrift et al., 2017) & (El- Serag et al., 2016**). In the rest of the world, the prevalence is 1% approximately. The form of transmission of HCV is through direct exposure to infected blood as in the case of blood transfusions contaminated with the virus, use of syringes of injecting drug users, use of equipment or medical material without adequate sterilization.

Prevalance of HCV in Egypt:
HCV is an enveloped single-stranded RNA virus which belongs to Flaviviridae family. The prevalent genotype in Egypt is type 4 (73%), the origin, evolution, and dynamics are difficult to determine (**Amer et al., 2015**). Hepatitis C virus (HCV) is a global public fitness trouble of the world. In 2016 the World Health Assembly set objectives for the removal of viral hepatitis C, such as diagnosis, remedy, and discount in the associated mortality aiming to achieve hepatitis C removal (**Herzer et al., 2017**). The highest prevalence of HCV in the world occurs in Egypt with estimates higher than 10% among the general population. There are approximately, 3.7 million persons in Egypt have chronic HCV infection in 2015 (**WHO, 2015 and El- Ghitany., 2019**).

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Epidemiology:

Hepatocellular carcinoma HCC is the sixth most commonly diagnosed cancer and the third leading cause of cancer death worldwide in 2020 **(Sung et al., 2021)**. The highest incidence rates in the world are found in Asia and Africa. In most countries, incidence rates of HCC among men are 2- to 4-fold higher than rates among women **(Petrick et al., 2019)**. The increasing incidence of HCC cases, possibly is due to the higher incidence of HCV and its complications with the increased survival rate in cirrhotic patients, which allows some patients to develop HCC **(Ferlay et al., 2018)**.

In Africa, there is a significant difference in median age at diagnosis between Egypt (58 years) and other African countries (46 years) **(Yang et al., 2017)**.

In Egypt, HCC represents the fourth common cancer **(Akinyemiju et al., 2017)**. It was reported that there is increasing the incidence of HCC in Egypt, the reason of which is attributed to improvement in screening programs and diagnostic tools **(Abd-Elsalam et al., 2018)**, increasing the survival rate of cirrhotic patients that increases the chance of developing HCC, and increasing the incidence and complications of hepatitis C virus which is the most important risk factor in developing liver cancer including HCC in Egypt **(Rashed et al., 2020)**.

HCC is an extremely complex condition and there are multiple factors involved in the etiology of HCC. In 80% of cases, HCC develops in a cirrhotic liver, and cirrhosis is the strongest independent risk factor for it, irrespective of etiology The major risk factors for HCC development include, hepatitis B virus (HBV) which is considered as an oncogenic virus, also hepatitis C virus (HCV), diabetes, obesity, alcoholic fatty liver disease (AFLD), and non-alcoholic fatty liver disease (NAFLD). A variety of risk factors for the development of HCC have been clearly identified. **(Yang et al., 2019)**.

Molecular pathogenesis of HCC:

HCC develops in ~80% of cases in the setting of a severely damaged cirrhotic liver that already gathers molecular alterations. In addition, several etiological (hepatitis C virus (HCV) and hepatitis B virus (HBV) infection, alcohol use disorder and non-alcoholic steatohepatitis (NASH)) and environmental (aflatoxin, aristolochic acid and tobacco) factors have been identified with distinct specific paths to cancer development **(Llovet et al., 2022)**. Progression from cirrhosis to HCC is mediated by a step-wise accumulation of somatic mutations and copy number variations in driver genes **(Brunner et al., 2019)**. The most frequent alteration is the reactivation of the telomerase reverse transcriptase (*TERT*), a key event observed in 20% of high-grade dysplastic lesions and up to 60% of early HCC. Ten pathways were found to be recurrently altered in HCC, including pathways involved in cell cycle control (*TP53, CDKNA2, CCND1*), oxidative stress (*NFE2L2, KEAP1*), and chromatin modification (*ARID1A, ARID2*), also the Wnt/β-catenin pathway (*CTNNB1, AXIN1*) and the RTK/RAS/PI3K pathway (*RPS6KA3, PIK3CA, KRAS, NRAS, FGF19, VEGFA*) .The TGFβ pathway is additionally involved in HCC progression, with some tumors presenting aberrant activation of this pathway, whereas others harboring inactivating mutations in genes required for TGFβ signal transduction e.g., the *SPTBN1* gene . Last, ~20% of HCC express markers of progenitor cells, e.g., epithelial cell adhesion molecule (EpCAM) and cytokeratin 19 (CK19) and arise from either progenitors or dedifferentiated hepatocytes **(Sas et al., 2022)**

Long non-coding RNAs regulation and function

In 1961, the central position of RNA in the flow of genetic information was revealed **(JACOB &MONOD., 1961)** and in the following 50 years, the emergence of whole-genome sequencing technology has greatly accelerated our understanding of

both coding and non-coding RNAs (ncRNAs) (**Carninci et al., 2005**). Many regulatory RNAs harboring various sizes have been discovered, especially long non-coding RNAs (lncRNAs) (**Ponting et al., 2009**).

Genomes are extensively transcribed and give rise to thousands of long non-coding RNAs (lncRNAs), which are defined as RNAs longer than 200 nucleotides that are not translated into functional proteins. This broad definition encompasses a large and highly heterogeneous collection of transcripts that differ in their biogenesis and genomic origin. The human genomes contain more than 16,000 lncRNA genes (**Uszczynska-Ratajczak et al., 2018**). These mainly include lncRNAs transcribed by RNA polymerase II (Pol II), but also by other RNA polymerases; and lncRNAs from intergenic regions (lincRNAs) as well as sense or antisense transcripts that overlap with other genes (**Statello et al., 2021**).

Several lncRNAs control the expression of nearby genes by affecting their transcription, and also affect other facets of chromatin biology, such as DNA replication or the response to DNA damage and repair. Other lncRNAs function away from their loci; their functions can be of a structural and/or regulatory nature and involve different stages of mRNA life, including splicing, turnover and translation, as well as signalling pathways. Consequently, lncRNAs affect several cellular functions that are of great physiological relevance, and alteration of their expression is inherent to numerous diseases. The specific expression patterns of these functional lncRNAs have the potential of being used as optimal disease biomarkers, and strategies are under development for their therapeutic targeting (**Statello et al., 2021**).

lncRNAs with cancer-relevant functions
The number of lncRNAs implicated in cancer initiation and progression is continuously growing, and can be found compiled in curated databases such as Lnc2Cancer or the Cancer LncRNA Census. lncRNAs have been implicated in the acquisition of every hallmark of cancer cells, from the intrinsic capacity of proliferation and survival, through increased metabolism, to the relationship with the tumour microenvironment. Early evidence of the involvement of lncRNAs in cancer came from their transcriptional regulation by key oncogenic or tumour-suppressive transcription factors such as p53, MYC, the oestrogen receptor or signalling cascades such as the Notch pathway (**Kim et al., 2015**). These lncRNAs contribute to the functional output of the oncogenic or tumour-suppressive responses. Some lncRNAs are activated by p53 following DNA damage. Mouse *lincRNA-p21* promotes apoptosis by contributing to p53-dependent transcription repression in *trans* and to activation in *cis* in a transcript-independent manner of cyclin-dependent kinase inhibitor 1. Human *PANDA* regulates p53-dependent apoptosis and cell cycle arrest; human *DINO* stabilizes p53 in the nucleus, thereby reinforcing its transcriptional activity; *GUARDIN* preserves genomic integrity through two independent cytoplasmic and nuclear mechanisms. Furthermore, lncRNAs such as *MEG3* participate in the p53 regulatory network without being transcriptional targets of p53. The imprinted *MEG3* is down-regulated in multiple cancers and contains an evolutionary conserved RNA structure that mediates p53 activation in *trans* (**Zhou et al., 2012**).

Taurine up regulated gene 1 (TUG1):
Taurine up regulated gene 1 (*TUG1*) is a lncRNA, also known as TI-227H, LINC00080 and NCRNA00080, is located on the human chromosome 22 autosomal long arm 1 region 2 sub-band (22 q12.2), with a total length of about 7.1 kb (**Tang et al., 2018**). This lncRNA plays a role in the epigenetic regulation of transcription. lncRNA *TUG1* is differentially expressed in cancers, and can affect the proliferation and apoptosis of cancer cells. The

expression of lncRNA *TUG1* is closely related to the prognosis of cancer patients (**Ding et al., 2020**).

lncRNA *TUG1* is thought to be involved in carcinogenesis and development mainly through competitive binding with miRNAs, regulation of cyclin-dependent kinase inhibitors, and effects on cancer proliferation and apoptosis (**Xiong et al., 2018**) miRNAs are short RNAs that regulate a variety of physiological and biological processes in eukaryotic cells (**Xia et al., 2020**). Abnormal expression of lncRNA *TUG1* affected the proliferation, apoptosis, and invasion of a variety of cancers, including bladder urothelial carcinoma, osteosarcoma, non-small cell lung cancer, HCC, bladder cancer and esophageal squamous cell carcinoma, suggesting that lncRNA *TUG1* may be used as a diagnostic marker or therapeutic target (**Qun et al., 2020**).

Function of lncRNA *TUG1* in cancers:
I - TUG1 in cancer cell proliferation and apoptosis:
It has been demonstrated that TUG1 is capable of promoting both the proliferation and apoptosis of cells). Furthermore, TUG1 can exert the same biological function through the regulation of different target genes in different cell types (**Ma et al., 2017**).

II- lncRNA *TUG1* regulates cancer invasion and metastasis:
Up-regulation of lncRNA *TUG1* expression exerts a carcinogenic role by promoting the migration, and invasion of laryngeal cancer cells, and by inhibiting apoptosis (**Zhang et al., 2018**).
lncRNA *TUG1* participated in the development of laryngeal carcinoma via inhibiting the activation of RhoA/rho associated coiled- coil containing protein kinase (ROCK)/matrix metalloproteinase (MMPs) signalling pathway by miR-145-5p (**Shenfa et al. 2019**). Up-regulation of lncRNA TUG1 promoted the proliferation and migration of esophageal squamous cell carcinoma (**Xu et al. 2015**).

III- TUG1 in cancer drug resistance
Drug resistance is one of the most important reasons for therapeutic failure in patients with cancer and is a persistent issue that requires continued investigation. It was demonstrated that the expression of miR-197 is elevated in breast cancer tumor and cell, while the levels of TUG1 and nemo-like kinase (NLK) are decreased. miR-197 confers cisplatin resistance in breast cance by inhibiting NLK. However, TUG1 functions as an endogenous sponge of miR-9-5p. Increased expression of TUG1 sensitizes breast cancecells to very low concentrations of cisplatin. Therefore, the TUG1/miR-197/NLK signaling pathway is likely to be a promising therapeutic target for those patients (**Tang et al., 2018**).

Regulation and dysregulation of TUG1
The expression level of TUG1 and the association between expression and survival probability in several common tumors were searched using The Cancer Genome Atlas database, including HCC, melanoma, osteosarcoma, renal cell carcinoma, bladder cancer, Colorectal Cancer (CRC), and breast cancer. According to The Cancer Genome Atlas database, there is a marked difference in the expression of TUG1 between normal and tumor tissues. TUG1 serves both oncogenic and tumor suppressive functions depending on the type of cancer. As an oncogene, aberrant upregulation of this lncRNA in different cancer types compared with their noncancerous counterparts has been observed in HCC, melanoma, osteosarcoma, renal cell carcinoma, bladder cancer, CRC and Gastric Carcinoma. By contrast, other reports have observed downregulation of *TUG1* in Breast Cancer. However, it has been demonstrated that high TUG1 expression may indicate a poor prognosis in the majority of cancer types (**Zhou et al., 2019**).

Conclusions
Cancer relevant lncRNAs are gradually becoming one of the hottest issues in the RNA biology and oncology. lncRNA

TUG1 detected by qRT-PCR could be used as promising potential biomarkers for the early detection of HCC developing on top of HCV with a valid non-invasive technique, cost effective, and complementary biomarker in viral hepatitis C and viral hepatitis C-associated hepatocellular carcinoma.

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The Clinical Significance of Long Non-Coding RNA (Taurine Up-Regulated Gene1) in Hepatitis C Virus-Related Hepatocellular Carcinoma Patients

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