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Oncogenic long noncoding RNA MALAT1 and HCV-related hepatocellular carcinoma

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ABSTRACT

Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related mortality worldwide. The oncogenic function of the long non-coding RNA; metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) in HCC remains unclear. We aimed to evaluate MALAT1 serum expression profile in HCC and explore its relation to the clinicopathological features. Quantitative Real Time-Polymerase Chain Reaction was applied in 70 cohorts (30 HCC, 20 HCV, 20 controls). Further meta-analysis of clinical studies and *in vitro* validated experiments was employed. Serum MALAT1 showed area under the curve of 0.79 and 0.70 to distinguish patients with cancer from normal and cirrhotic individuals at fold change of 1.0 and 1.26, respectively. Expression level was significantly higher in males ($P < 0.001$) and patients with massive ascites ($P = 0.005$). Correlation analysis showed positive correlation of MALAT1 with total bilirubin (r = 0.456, $P < 0.001$) and AST (r = 0.280, $P = 0.019$), and negative correlation with the hemoglobin level (r = 0.312, $P = 0.009$). Meta-analysis showed that the over-expressed MALAT1 was linked to tumor number [Cohen's d = 0.450, 95% CI (0.21 to 0.68)], clinical stage [Cohen's d = 0.048, 95% CI (-0.83 to 0.74)], and AFP level [Cohen's d = 0.354, 95% CI (0.1 to 0.57)]. *In silico* data analysis and systematic review confirmed MALAT1 oncogenic function in cancer development and progression.

In conclusion, circulatory MALAT1 might represent a putative non-invasive prognostic biomarker indicating worse liver failure score in HCV-related HCC patients with traditional markers. Large-scale verification is warranted in future studies.

1. Introduction

Hepatocellular Carcinoma (HCC) is considered the sixth most commonly diagnosed type of cancer worldwide [1] and the second leading cause of cancer-related mortality [2]. Hepatitis C virus (HCV) is proposed to be one of the major risk factors for HCC worldwide [3], about 1–4% per year of HCV-infected cirrhotic patients are expected to develop malignant transformation in hepatocytes [3,4]. In Egypt, the prevalence of chronic liver disease due to HCV infection represents 13.8% of the whole population, and up to 80% of liver cancer patients

Abbreviations: AFP, alpha fetoprotein; AUC, area under the ROC curve; cDNA, complementary DNA; DRAIC, Down-regulated RNA In Cancer; EMT, epithelial-mesenchymal transition; eRNAs, enhancer RNAs; EZH2, Enhancer Of Zeste 2 Polycomb Repressive Complex 2 Subunit; GAS5, growth arrest specific 5; HCC, Hepatocellular carcinoma; HCN, Hepcarcin; HCV, Hepatitis C virus; HEIH, Highly Expressed In Hepatocellular Carcinoma Long Non-Coding RNA; HULC, hepatocellular carcinoma up-regulated long non-coding RNA; ITGB1, Integrin β1; LINC00047, Long Intergenic Non-Protein Coding RNA 47; lncRNAs, long non-coding RNAs; LTBP3, latent transforming growth factor β-binding protein 3; MALAT1, metastasis-associated lung adenocarcinoma transcript 1; mascRNA, MALAT1-associated small cytoplasmic RNA; ncRNA-a, activating ncRNAs; NEAT2, Nuclear Enriched Abundant Transcript 2; OncoLncs, oncogene long non-coding RNAs; plncRNAs, promotor-associated long RNAs; PRC, polycomb repressive complex; ROC, Receiver operating characteristic; RT-PCR, Reverse transcription-Polymerase Chain Reaction; siRNA, small interfering RNA; TF, transcription factors; TS, thymidylate synthase; TSLncs, tumor suppressor long non-coding RNAs

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have underling hepatitis C [5].

HCV has seven major genotypes that vary according to the geographic area. Genotype-4, in particular, is confined to the Middle East region, accounting for up to 90% of HCV Egyptian patients and about 15% of total global HCV infection [5]. Despite numerous studies on HCC, there are no effective biomarkers for the early tumor detection and prognosis and most cases are diagnosed at an advanced or a non-resectable stage at presentation leading to lower survival rate [6]. Thus, it is extremely necessary to discover new biomarkers for precise diagnosis, prognosis, and treatment of HCV-related HCC.

Recently, long non-coding RNAs (lncRNAs) have been implicated in the pathogenesis of liver diseases [7]. They are non-coding transcripts longer than 200 nucleotides [8], that are transcribed by RNA polymerase II, followed by 5′ capping, 3′ polyadenylation, and splicing similar to protein-coding genes [9]. LncRNAs can be classified according to their genomic location. For instance, they can be found overlapping protein-coding genes or exist in distinct intergenic regions with separate transcriptional units from annotated coding region. Other types include promoter-associated long RNAs (plncRNAs), telomeric-repeat containing RNA and transcribed ultra-conserved RNAs [10,11]. They can be transcribed from sense, antisense or both strands relative to the promoter of a protein-coding gene. Additionally, lncRNAs can be categorized according to their function into enhancer RNAs (eRNAs), which interact with enhancer regions, and activating ncRNAs (ncRNA-a) which have an enhancer like effects. Finally, they can also be classified according to their structure into linear or circular, with the latter being generated by back-splicing of introns of mRNAs [11].

To date, in human, 146,742 lncRNAs were identified and annotated in LNCipedia database version 4.1 (https://lncipedia.org/). Accumulating evidence indicates that lncRNAs can play crucial roles in various pathological states. They can regulate cancer-related genes at transcriptional, post-transcriptional and epigenetic levels; through modulating mRNA splicing, stability, degradation, and translation [8] and regulating epigenetic mechanisms and nuclear organization [11]. They can act as scaffolds for chromatin-protein complexes, recruit histone-modifying enzymes to chromatin, regulate DNA methylation, and induce chromatin modifications [12]. lncRNAs have been found to be deregulated in liver cancer [8]. Interestingly, they can behave like oncogenes (OncoLncs) or tumor suppressors (TSLncs) [9,13]. For example, over-expression of HULC (hepatocellular carcinoma up-regulated long non-coding RNA) and HEIH (Highly Expressed in Hepatocellular Carcinoma Long Non-Coding RNA) was correlated with HCC development or progression, whereas, DRAIC (Down-regulated RNA In Cancer) and GAS5 (growth arrest specific 5) down-regulation may act as a tumor suppressor in a variety of human cancers [14].

In the past decades, emerging evidence suggested the association of the lncRNA Metastasis-Associated Lung Adenocarcinoma Transcript 1 (MALAT1) with a wide variety of cancers; including lung cancer [15], osteosarcoma [16], glioblastoma multiforme [17], and hepatocellular carcinoma [18]. Human *MALAT1* gene (ENSG00000251562) is located along the long arm of chromosome 11q13.1 on the forward strand spanning 8.75 Kb long (11: 65,497,762-65,506,516; according to GRCh38). It has other synonyms; including Hepcarcin (HCN), Nuclear Enriched Abundant Transcript 2 (NEAT2), and Long Intergenic Non-Protein Coding RNA 47 (LINC00047). The precursor RNA of MALAT1 is first transcribed by RNA polymerase II (RNA Pol II) then cleaved by RNase P enzyme from its 3-prime end into two parts to yield a large nuclear-retained lncRNA and MALAT1-associated small cytoplasmic RNA (mascRNA) localized exclusively in the cytoplasm. The long ncRNA partly lacks poly(A) tail, but is stabilized by its U-rich motifs [19]. Due to alternative splicing, *MALAT1* gene can encode 17 different lncRNA transcripts ranging from 132 to 8708 bp in length (www.Ensembl.org) (Fig. 1). They are mainly accumulating in distinct zones called nuclear speckles that regulate post-transcriptional RNA processing machinery; namely pre-mRNA splicing and mRNA export. The long transcripts are highly abundant; about 3000 copies/cell [20]. They act as molecular scaffolds for ribonucleoprotein complexes, transcriptional regulators for numerous genes, including those involved in cancer metastasis and cell migration, and key modulators in cell cycle regulation. On the other hand, the small ncRNA originates from a highly conserved region. After processing, it folds into a tRNA-like cloverleaf, followed by the addition of a CCA motif by the RNase Z enzyme. Unlike the nuclear transcript, the mascRNA has a relatively short half-life. It is thought to act as a "sponge" for proteins, preventing them from reaching their natural destinations within the cell [21].

To assess the diagnostic and prognostic value of MALAT1 as a non-invasive biomarker in combination with alpha fetoprotein (AFP) in hepatocellular carcinoma, we examined the expression profile of the lncRNA MALAT1 in the circulation of liver cancer patients compared to normal and cirrhotic cohorts. Additionally, a comprehensive *in silico* analysis was employed to explore the biological features and the clinical implications of the OncoLnc MALAT1 in hepatic carcinogenesis aiming at providing insights into precision in diagnosis and treatment of HCC.

2. Materials and methods

2.1. In silico structural and functional analysis of MALAT1

Structural genomics analyses of *MALAT1* gene and transcripts were retrieved from online databases as Ensemble.org and GeneCards.com. COMPARTMENTS subcellular localization database was investigated to identify the subcellular localization of MALAT1 RNA (compartments.jensenlab.org/). Functional gene ontology was assessed by lncRNA2function (https://mlg.hit.edu.cn/lncrna2function/). Transcription factors binding sites were determined (Genecard.org). Protein-protein interaction was identified using STRING database version 10.0 (Search Tool for the Retrieval of Interacting Genes/Proteins). Starbase (http://starbase.sysu.edu.cn/), microRNA.org, and Diana-LncBase v2.0 (http://carolina.imis.athena-innovation.gr/) were used to identify protein and RNA inter-actors with MALAT1.

2.2. Expression profile analysis

2.2.1. Study participants

A total of 70 individuals were enrolled in the study (20 controls, 20 patients with liver cirrhosis (LC) caused by HCV infection, and 30 patients with hepatocellular carcinoma on top of HCV). The control group was obtained from healthy blood bank donors. They matched the other two groups in age, gender, and residence, and had no history of any concomitant infection or chronic illness. Patients were recruited from the General Surgery and Oncology Department, Suez Canal University Hospital, Egypt, during the period between January 2017 and July 2017.

2.2.2. Ethical approval

The study was conducted according to the ethical guidelines of the 1964 Declaration of Helsinki (2008 revision), and approved by the Medical Research Ethics Committee of Suez Canal University (Research ID 3137). Informed consent was obtained from participants prior to blood sampling.

2.2.3. Patient assessment

All the patients included in the study had clinical and radiological evidence suggestive of cirrhosis and confirmation of HCV infection by detection of HCV RNA in their serum. Patients with non-HCV induced HCC, other autoimmune or metabolic liver diseases were excluded. Liver cancer had typical imaging findings and elevated serum alpha fetoprotein (AFP). Patients with HCC underwent thorough clinical examination, abdominal ultrasonography, quantitative assessment of viral load by PCR, Barcelona-Clinic Liver Cancer (BCLC) staging, and prognostic assessment by Child-Turcotte-Pugh (CTP) scoring tool [22].