Circulating Long Noncoding RNAs CASC2 and TUG1 as Noninvasive Diagnostic Biomarkers for Hepatitis C–Related Hepatocellular Carcinoma

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Received: 28 October 2024, **Accepted:** 17 November 2024, **Published:** 20 November 2024

Abstract

Background: Hepatocellular carcinoma (HCC) is a leading cause of cancer-related mortality worldwide, with chronic hepatitis C virus (HCV) infection representing one of its most important etiological factors. Despite advances in surveillance strategies, early diagnosis of HCV-related HCC remains suboptimal. Conventional diagnostic tools, including imaging modalities and serum alpha-fetoprotein (AFP), exhibit limited sensitivity and specificity, particularly for early-stage disease and AFP-negative tumors. This diagnostic gap has driven increasing interest in non-invasive molecular biomarkers that better reflect the underlying pathobiology of hepatocarcinogenesis.

Long noncoding RNAs (lncRNAs) have emerged as key regulators of gene expression and cellular homeostasis. Their aberrant expression in HCC, coupled with remarkable stability in circulation, positions them as promising candidates for liquid biopsy-based diagnostics. Among these, Cancer Susceptibility Candidate 2 (CASC2) and Taurine Upregulated Gene 1 (TUG1) have demonstrated consistent deregulation in HCV-related HCC and increasing clinical relevance.

Aim: This review aims to critically evaluate the diagnostic utility of circulating lncRNAs CASC2 and TUG1 as non-invasive biomarkers for hepatitis C virus–related hepatocellular carcinoma. Emphasis is placed on their biological relevance, expression patterns in serum or plasma, diagnostic performance compared with conventional biomarkers, and potential integration into clinical pathology practice.

Conclusion: Accumulating evidence indicates that circulating CASC2 and TUG1 provide significant diagnostic value in HCV-related HCC. CASC2 is consistently downregulated, reflecting loss of tumor-suppressive mechanisms, whereas TUG1 is upregulated and associated with oncogenic signaling pathways. Individually, both lncRNAs demonstrate promising sensitivity and specificity for HCC detection, including in early-stage and AFP-negative cases. Notably, their combined assessment yields superior diagnostic accuracy compared with AFP alone, underscoring their complementary biological roles. Incorporation of CASC2 and TUG1 into non-invasive diagnostic panels may substantially enhance early detection of HCV-related HCC and improve patient outcomes. Further large-scale, multicenter validation studies are warranted to support their translation into routine clinical use.

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Keywords: *Long Noncoding RNAs, CASC2 , TUG1, Hepatitis C–Related Hepatocellular Carcinoma*

Introduction

Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver and represents a major global cause of cancer-related mortality. According to international cancer statistics, HCC accounts for more than 80% of primary liver cancers and is characterized by poor prognosis due to delayed diagnosis and limited therapeutic options at advanced stages. The disease burden is particularly high in regions with endemic viral hepatitis, where chronic liver injury and cirrhosis create a permissive environment for malignant transformation [1].

Chronic hepatitis C virus (HCV) infection is a well-established etiological factor in the development of

HCC. Persistent viral replication induces chronic hepatic inflammation, oxidative stress, and progressive fibrosis, ultimately leading to cirrhosis and hepatocarcinogenesis. Even after viral eradication with direct-acting antivirals, patients with advanced fibrosis remain at increased risk of HCC, highlighting the need for effective long-term surveillance and early diagnostic strategies in this population [2].

From a clinical pathology standpoint, early diagnosis of HCV-related HCC remains challenging. Current surveillance protocols rely mainly on abdominal ultrasonography, with or without serum alpha-fetoprotein (AFP) measurement, at six-month intervals in high-risk individuals. However, ultrasonography is operator-dependent and less sensitive in cirrhotic livers, while AFP demonstrates limited diagnostic accuracy due to low sensitivity in early-stage tumors and frequent false-positive elevations in chronic hepatitis and cirrhosis [3].

Multiple studies have shown that up to 40% of patients with early-stage HCC present with normal AFP levels, reducing its utility as a standalone diagnostic biomarker. Furthermore, AFP elevation is not specific to malignant transformation and may occur during active hepatic inflammation or regeneration. These limitations underscore the need for novel, non-invasive biomarkers that better reflect the molecular events underlying hepatocarcinogenesis rather than nonspecific liver injury [4].

Advances in molecular pathology have identified noncoding RNAs as critical regulators of gene expression in cancer. Long noncoding RNAs (lncRNAs), defined as transcripts longer than 200 nucleotides without protein-coding potential, regulate chromatin organization, transcriptional activity, and post-transcriptional gene regulation. In chronic HCV infection, viral proteins and inflammatory signaling pathways induce epigenetic alterations that lead to aberrant lncRNA expression during the transition from chronic liver disease to HCC [5].

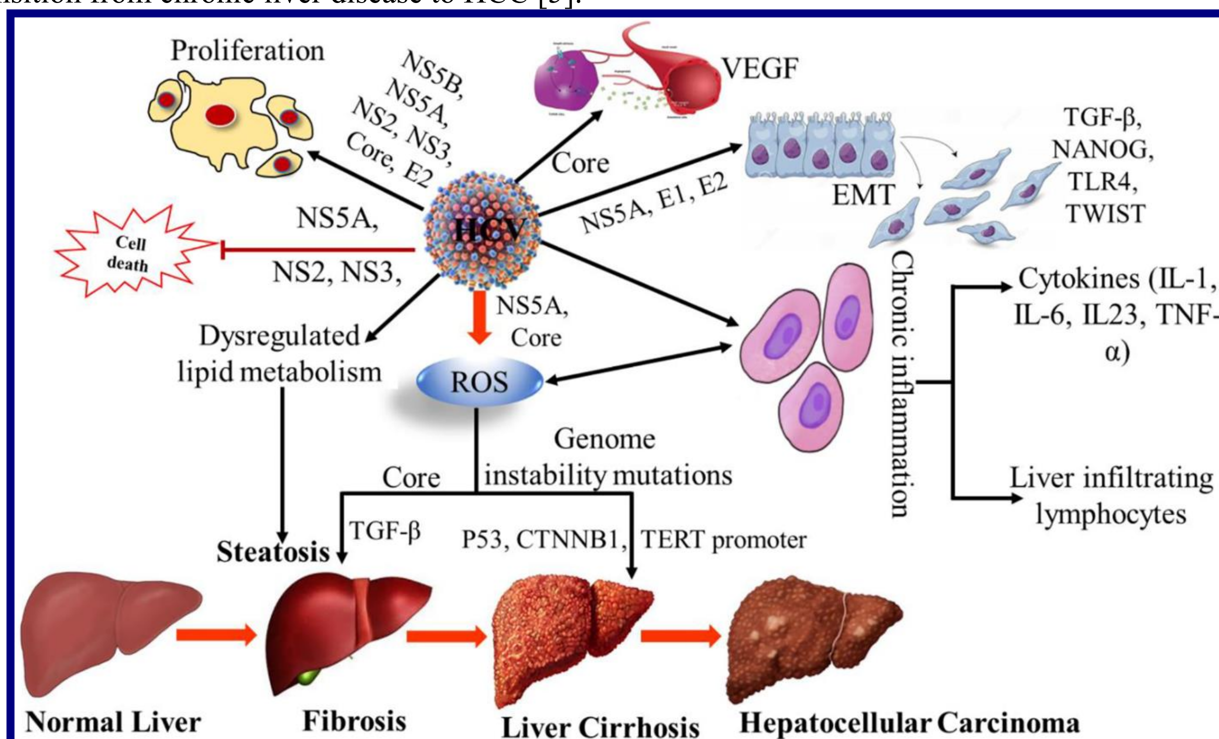

Figure (1): Mechanism of HCV induced hepatocellular carcinoma. VEGF, Vascular endothelial growth factor; EMT, Epithelial–mesenchymal transition; TGF-β, Transforming Growth Factor-β; TLR4, Toll-like receptor 4; TWIST, Twist-related protein; ROS, Reactive oxygen species; CTNNB1, Catenin Beta 1; TERT, Human telomerase reverse transcriptase; TNF-α, Tumor necrosis factor alpha; NS, non-structural; IL-1; Interleukin-1, IL-6; Interleukin 6, IL-23, Interleukin-23 [5].

Importantly, lncRNAs exhibit remarkable stability in circulation and can be detected in serum or plasma, either as free molecules or encapsulated within extracellular vesicles. This stability, combined with tissue- and disease-specific expression patterns, makes circulating lncRNAs attractive candidates for liquid biopsy-based diagnostics. From a pathology perspective, liquid biopsy approaches offer clear advantages, including minimal invasiveness, repeatability, and suitability for longitudinal monitoring of

high-risk patients [6].

Among the growing number of lncRNAs implicated in hepatocellular carcinoma, Cancer Susceptibility Candidate 2 (CASC2) and Taurine Upregulated Gene 1 (TUG1) have emerged as promising diagnostic biomarkers, particularly in HCV-related disease. CASC2 is generally downregulated and exhibits tumor-suppressive functions, whereas TUG1 is frequently upregulated and associated with oncogenic signaling pathways. Their inverse expression patterns suggest complementary diagnostic potential when evaluated together in circulation [7].

Despite increasing interest in circulating lncRNAs, significant gaps remain in the literature. Many studies evaluate heterogeneous HCC cohorts without stratification by viral etiology, limiting the applicability of results to HCV-related HCC. Moreover, the diagnostic roles of CASC2 and TUG1 have not been comprehensively reviewed from a clinical pathology perspective, particularly in comparison with established biomarkers such as AFP [8].

The aim of this review is to critically evaluate the evidence supporting circulating lncRNAs CASC2 and TUG1 as non-invasive diagnostic biomarkers for hepatitis C virus–related hepatocellular carcinoma. Emphasis is placed on their biological relevance, diagnostic performance, and potential integration into clinical pathology-based diagnostic algorithms for early HCC detection.

Pathobiology of HCV-Related Hepatocarcinogenesis and lncRNA Dysregulation

Chronic hepatitis C virus infection drives hepatocarcinogenesis through a multistep process involving persistent inflammation, oxidative stress, and progressive fibrotic remodeling of the liver. Continuous hepatocyte injury and regeneration promote genomic instability and accumulation of molecular alterations that predispose to malignant transformation. Unlike hepatitis B virus, HCV does not integrate into the host genome; instead, its oncogenic potential is mediated through indirect mechanisms involving chronic inflammation and direct interactions of viral proteins with host cellular pathways [9].

HCV core and nonstructural proteins, including NS3, NS4B, and NS5A, interfere with key regulatory pathways controlling cell cycle progression, apoptosis, and DNA repair. These viral proteins modulate signaling cascades such as MAPK, PI3K/AKT, Wnt/β-catenin, and TGF-β, leading to dysregulated hepatocyte proliferation and survival. Over time, these alterations facilitate clonal expansion of transformed cells and progression toward hepatocellular carcinoma [10].

Epigenetic dysregulation represents a central mechanism in HCV-related hepatocarcinogenesis. Chronic viral infection induces widespread changes in DNA methylation patterns, histone modifications, and chromatin remodeling. These epigenetic alterations result in aberrant gene expression profiles that persist even after viral clearance, contributing to the sustained risk of HCC observed in patients with advanced fibrosis following successful antiviral therapy [11].

Long noncoding RNAs have emerged as critical mediators of these epigenetic and transcriptional changes. LncRNAs act as scaffolds for chromatin-modifying complexes, guides for transcription factors, decoys for regulatory proteins, and sponges for microRNAs. In chronic HCV infection, inflammatory cytokines and viral protein–mediated signaling induce profound changes in lncRNA expression, thereby reshaping gene regulatory networks involved in hepatocyte differentiation, proliferation, and malignant transformation [12].

Several studies have demonstrated distinct lncRNA expression signatures across different stages of HCV-related liver disease, including chronic hepatitis, cirrhosis, and hepatocellular carcinoma. These stage-specific expression patterns suggest that lncRNAs are not merely bystanders but active participants in disease progression. Importantly, dysregulated lncRNAs associated with malignant transformation can be detected not only in liver tissue but also in circulation, reflecting underlying pathological processes [13].

The release of lncRNAs into the bloodstream occurs through multiple mechanisms, including passive release from apoptotic or necrotic cells and active secretion via extracellular vesicles such as exosomes. In HCC, increased tumor cell turnover and altered vesicle trafficking contribute to elevated levels of tumor-associated lncRNAs in serum or plasma. These circulating lncRNAs retain their stability due to protection from RNase degradation, enabling reliable detection using molecular assays [14].

From a clinical pathology perspective, the link between HCV-induced molecular alterations and circulating lncRNA profiles provides a strong biological rationale for their use as non-invasive diagnostic biomarkers. Unlike conventional serum markers that reflect hepatic injury or regeneration, lncRNAs more directly mirror oncogenic signaling and epigenetic reprogramming. This distinction is particularly important in cirrhotic patients, where inflammatory activity can confound traditional biomarkers [15].

Within this framework, CASC2 and TUG1 represent two biologically relevant lncRNAs with opposing roles in hepatocarcinogenesis. Their dysregulation reflects key pathogenic events in HCV-related HCC, including loss of tumor-suppressive control and activation of oncogenic pathways. Understanding their mechanistic involvement provides essential context for interpreting their diagnostic performance in circulation [16].

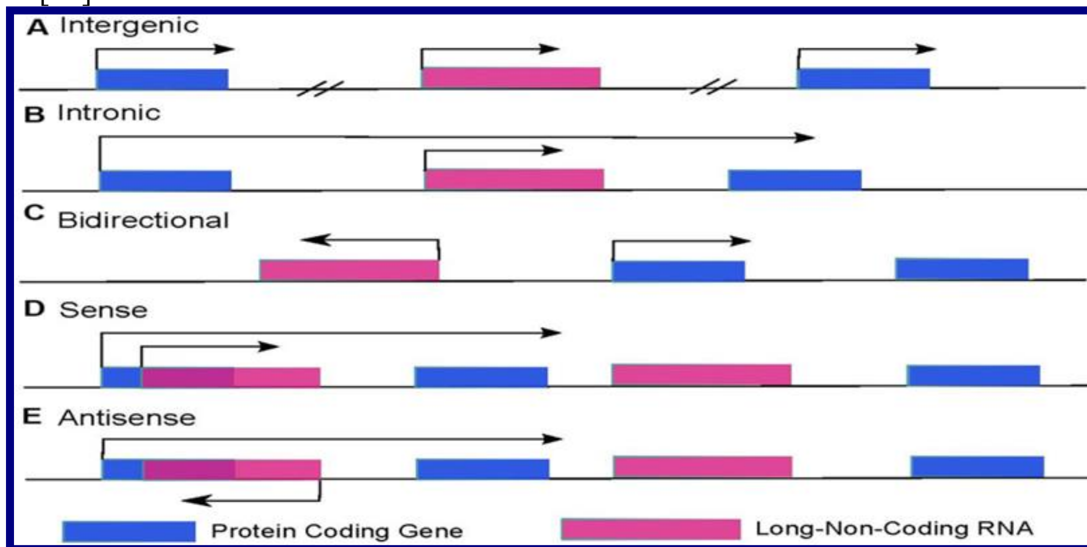

Figure (2): LncRNAs categories are decided by the location of lncRNAs and surrounding protein-coding genes. (A) Intergenic lncRNAs are autonomously transcribed non-coding RNAs longer than 200 nucleotides that do not overlap annotated coding genes. (B) Intronic lncRNAs are lncRNAs that locate inside of an intron of a protein-coding gene and can initiate in either direction. (C) Bidirectional lncRNAs are transcripts that are transcribed from the same promoter as a protein- coding gene but in the opposite direction. (D) Sense lncRNAs overlap with one or more introns and/or exons of a protein-coding gene in the sense direction. (E) Antisense lncRNAs are transcribed from the opposite strand of protein-coding genes [13].

Biological Characteristics and Functional Role of CASC2 in Hepatocellular Carcinoma

Cancer Susceptibility Candidate 2 (CASC2) is a long noncoding RNA originally identified as a tumor suppressor in several human malignancies. It is located on chromosome 10q26 and produces multiple transcript variants that participate in the regulation of gene expression at transcriptional and post-transcriptional levels. In normal hepatic tissue, CASC2 contributes to the maintenance of cellular homeostasis by restraining uncontrolled proliferation and supporting apoptotic pathways, functions that are progressively lost during hepatocarcinogenesis [17].

In hepatocellular carcinoma, CASC2 expression is consistently downregulated at both tissue and circulating levels. Mechanistically, reduced CASC2 expression has been linked to epigenetic silencing through promoter hypermethylation and altered chromatin accessibility, processes that are exacerbated by chronic inflammatory signaling in hepatitis C virus–infected livers. These alterations disrupt the tumor-suppressive functions of CASC2 and facilitate malignant transformation [18].

At the molecular level, CASC2 exerts its tumor-suppressive effects primarily through acting as a competing endogenous RNA. By sponging oncogenic microRNAs, such as miR-21 and miR-367, CASC2 indirectly regulates the expression of downstream tumor suppressor genes involved in cell cycle control and apoptosis. Loss of CASC2 therefore results in unchecked microRNA activity and activation of oncogenic pathways relevant to HCC development [19].

Experimental studies have demonstrated that restoration of CASC2 expression in HCC cell lines leads to inhibition of cell proliferation, induction of apoptosis, and suppression of migration and invasion. These effects are mediated through modulation of key signaling pathways, including PI3K/AKT and

MAPK cascades, which are commonly activated in HCV-related hepatocellular carcinoma. Such findings reinforce the biological plausibility of CASC2 as a marker of malignant transformation rather than nonspecific liver injury [20].

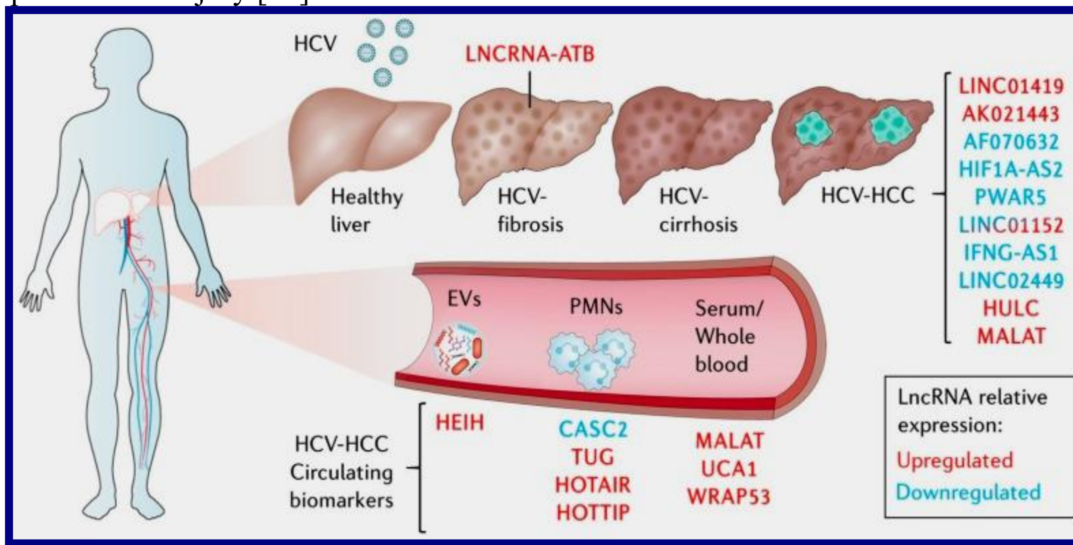

Figure (3): LncRNAs deregulated in HCV-hepatocellular carcinoma (HCC) in tumor samples and cell studies or as circulating biomarkers in liquid biopsies. EVs: extracellular vesicles, PMNs: polymorphonuclear leukocytes [19].

From a disease progression standpoint, CASC2 downregulation appears to occur early during hepatocarcinogenesis. Comparative analyses of chronic hepatitis, cirrhosis, and HCC tissues have shown a stepwise decline in CASC2 expression, with the most pronounced reduction observed in malignant lesions. This temporal pattern supports the relevance of CASC2 loss as an early molecular event associated with transition from cirrhosis to carcinoma [21].

Importantly for diagnostic applications, CASC2 is detectable in serum and plasma samples, where its expression levels reflect tissue deregulation. Circulating CASC2 is protected from degradation through association with ribonucleoprotein complexes or encapsulation within extracellular vesicles. This stability allows for reliable quantification using standardized reverse transcription quantitative PCR assays, a key requirement for clinical pathology implementation [22].

Clinical studies focusing on hepatitis C virus–related HCC have consistently demonstrated significantly lower circulating CASC2 levels compared with patients with chronic hepatitis C, cirrhosis, and healthy controls. These findings indicate that CASC2 downregulation is closely linked to malignant transformation rather than viral infection or fibrosis alone, enhancing its specificity as a diagnostic biomarker [23].

Taken together, the biological characteristics of CASC2, including its tumor-suppressive role, early deregulation during hepatocarcinogenesis, and detectability in circulation, provide a strong mechanistic foundation for its use as a non-invasive diagnostic biomarker in HCV-related hepatocellular carcinoma. These features distinguish CASC2 from conventional serum markers and support its further evaluation in diagnostic panels [24].

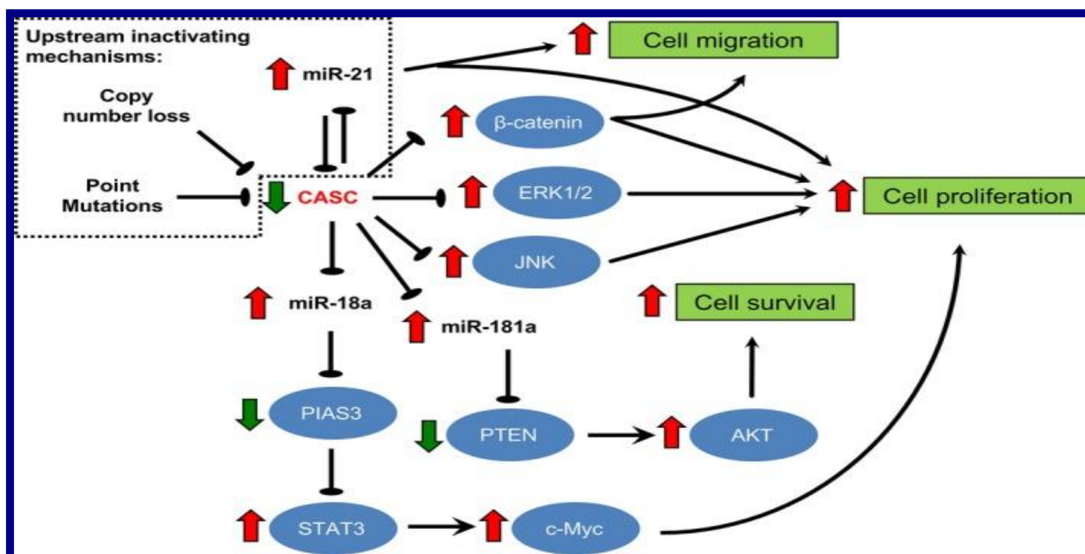

Figure (4): Upstream mechanisms underlying CASC2 downregulation and downstream effector pathways of CASC2 in human cancers. Genetic mechanisms (ie, point mutations and copy number loss) and aberrant upregulation of miR-21 contribute to loss-of-function or downregulation of CASC2 in human cancers. CASC2 exerts its tumour-suppressive effects through chelation of oncogenic microRNAs (eg, miR-18a, miR-21 and miR-181a) and inhibition of oncogenic pathways (eg, Wnt/β-catenin, ERK/MAPK and JNK) [24].

Biological Characteristics and Functional Role of TUG1 in Hepatocellular Carcinoma

Taurine Upregulated Gene 1 (TUG1) is a long noncoding RNA initially identified in taurine-treated retinal cells and later recognized as a multifunctional regulator of gene expression in various malignancies. TUG1 is located on chromosome 22q12.2 and is involved in transcriptional regulation, chromatin remodeling, and post-transcriptional control. In the liver, TUG1 expression is tightly regulated under physiological conditions but becomes aberrantly upregulated during hepatocarcinogenesis [25].

In hepatocellular carcinoma, TUG1 functions predominantly as an oncogenic lncRNA. Its overexpression has been associated with enhanced cell proliferation, resistance to apoptosis, and increased migratory and invasive capabilities. In the context of chronic hepatitis C virus infection, inflammatory cytokines and viral protein–mediated signaling contribute to sustained upregulation of TUG1, linking viral-induced molecular alterations to malignant transformation [26].

At the mechanistic level, TUG1 promotes hepatocarcinogenesis through multiple pathways. One of its principal functions involves acting as a competing endogenous RNA that sponges tumor-suppressive microRNAs, including miR-144, miR-132, and miR-29c. By sequestering these microRNAs, TUG1 leads to derepression of oncogenic target genes involved in cell cycle progression and survival signaling, thereby facilitating tumor development [27].

TUG1 also interacts with epigenetic regulators such as Polycomb Repressive Complex 2 (PRC2), guiding histone-modifying enzymes to specific genomic loci. This interaction results in transcriptional silencing of tumor suppressor genes through histone H3 lysine 27 trimethylation. Such epigenetic repression contributes to sustained oncogenic signaling and has been observed in hepatocellular carcinoma tissues, further supporting the role of TUG1 in malignant progression [28].

Evidence suggests that TUG1 upregulation occurs early in the course of hepatocarcinogenesis. Comparative expression analyses across chronic hepatitis, cirrhosis, and HCC samples have demonstrated progressively increasing TUG1 levels, with the highest expression observed in malignant tissues. This gradual increase indicates that TUG1 activation is closely linked to disease progression rather than merely reflecting advanced tumor burden [29].

From a diagnostic standpoint, TUG1 is readily detectable in serum and plasma samples, where it exhibits

high stability due to protection within extracellular vesicles or ribonucleoprotein complexes. Circulating TUG1 levels have been shown to mirror tissue expression patterns, enabling non-invasive assessment of its deregulation in patients with HCV-related HCC [30].

Clinical investigations focusing on hepatitis C virus–associated HCC have consistently reported significantly elevated circulating TUG1 levels compared with patients with chronic hepatitis C, cirrhosis, and healthy individuals. Importantly, increased TUG1 expression has been detected in patients with early-stage tumors and in those with normal AFP levels, highlighting its potential value as an adjunct diagnostic biomarker [31].

Collectively, the biological and functional properties of TUG1, including its oncogenic activity, early upregulation during hepatocarcinogenesis, and robust detectability in circulation, support its role as a promising non-invasive diagnostic biomarker for HCV-related hepatocellular carcinoma. These characteristics provide a strong foundation for evaluating TUG1 alongside other molecular markers in diagnostic panels [32].

Figure (5): Diagram summarises the roles of lncRNA TUG1 in the regulation of cancer cell proliferation, metastasis, angiogenesis, chemotherapeutic drug resistance, radiosensitivity, cell regulation, and cell glycolysis [32].

Circulating CASC2 as a Non-Invasive Diagnostic Biomarker in HCV-Related HCC

From a clinical pathology perspective, the diagnostic value of circulating CASC2 arises from its consistent downregulation in HCV-associated HCC compared with non-malignant HCV infection and healthy controls, suggesting that CASC2 reflects malignant transformation rather than simply necroinflammatory activity. In one of the earliest clinical studies specifically addressing HCC on top of HCV, whole-blood CASC2 expression was significantly reduced in HCC/HCV patients, supporting its relevance as a minimally invasive molecular marker that can be measured using routine RT-qPCR workflows in clinical laboratories [33].

More recent work has strengthened this diagnostic concept by directly comparing circulating CASC2 with AFP in an HCV-related HCC setting. In a case–control design including HCC/HCV, HCV without malignancy, and healthy controls, serum CASC2 was markedly downregulated in HCC/HCV, whereas it was higher in the non-malignant HCV group, indicating a disease-stage–dependent inversion. Importantly, ROC analysis in this study showed that CASC2 achieved higher diagnostic performance than AFP for identifying HCC among the studied population, supporting the proposition that CASC2 could complement or outperform conventional biomarkers under certain clinical conditions [34].

The clinical need for such biomarkers remains substantial because current surveillance tools are imperfect, especially in cirrhotic livers where ultrasound sensitivity is reduced and AFP lacks adequate sensitivity for early-stage tumors. Meta-analytic evidence indicates that ultrasound-based surveillance (with or without AFP) does not reliably detect all early HCC cases, reinforcing why pathology-oriented biomarker development is actively pursued. International clinical practice guidance continues to recommend ultrasound surveillance, reflecting feasibility and accessibility, but also implicitly acknowledging the need for improved non-invasive markers that can better capture early malignant biology [35,36].

Analytically, the feasibility of CASC2 testing depends on controlling key pre-analytical and analytical variables that are central to molecular pathology implementation. Sample type (serum, plasma, or whole blood), time to processing, hemolysis, and RNA extraction method can all influence circulating RNA yield and apparent expression levels. Most studies quantify CASC2 using RT-qPCR with relative quantification approaches, commonly applying the $2^{-\Delta\Delta Ct}$ method, which is widely used in translational biomarker studies but requires careful selection of internal controls and inter-run calibrators to avoid bias and enhance reproducibility [37,38].

Biological plausibility further supports CASC2 as a tumor-associated marker: mechanistic studies in HCC models consistently frame CASC2 as a tumor suppressor lncRNA whose loss enhances oncogenic signaling, proliferation, and invasion. Reported pathways include regulation of MAPK signaling and competing endogenous RNA networks that modulate microRNA activity and downstream tumor-related targets. This mechanistic alignment between tissue biology and circulating downregulation strengthens the interpretation of CASC2 as a malignancy-associated molecular signature rather than a nonspecific inflammatory indicator [39–41].

In diagnostic biomarker strategy, CASC2 may be most clinically useful when interpreted in combination with standard markers and clinical context rather than in isolation. Circulating biomarker reviews emphasize that early HCC detection is likely to benefit from multi-marker models that integrate molecular markers with AFP and imaging, particularly in etiologically defined populations such as HCV-related cirrhosis where baseline liver injury confounds many serum tests. Within this framework, CASC2 is best positioned as part of a layered diagnostic algorithm in which molecular pathology adds discriminatory power for malignant transformation on top of chronic HCV-associated liver disease [42,43].

Circulating TUG1 as a Non-Invasive Diagnostic Biomarker in HCV-Related HCC

The clinical pathology relevance of circulating TUG1 stems from its consistent upregulation in patients

with hepatitis C virus–related hepatocellular carcinoma compared with individuals with chronic hepatitis C, cirrhosis, or healthy controls. Unlike biomarkers that reflect hepatocellular injury, elevated TUG1 levels appear to be closely linked to malignant transformation, making it a biologically plausible diagnostic indicator. Early translational studies demonstrated that serum TUG1 expression was significantly higher in HCC patients, supporting its potential utility as a blood-based molecular marker detectable through standard RT-qPCR techniques [44].

Subsequent clinical investigations focusing on HCV-related HCC further validated these observations by incorporating appropriate disease controls. In comparative analyses including HCC on top of HCV, non-malignant HCV infection, and healthy subjects, circulating TUG1 levels showed a stepwise increase across disease stages, with the highest expression observed in HCC patients. This gradient supports the concept that TUG1 upregulation reflects progression toward malignancy rather than inflammatory activity alone, a critical distinction in chronic liver disease diagnostics [45].

Diagnostic performance analyses using receiver operating characteristic curves have demonstrated that circulating TUG1 exhibits favorable sensitivity and specificity for distinguishing HCC from HCV-related cirrhosis. In several studies, the area under the curve for TUG1 approached or exceeded that of AFP, particularly in cohorts enriched for early-stage disease. Importantly, elevated TUG1 levels were detected in a substantial proportion of AFP-negative HCC cases, highlighting its potential role in addressing one of the major shortcomings of AFP-based surveillance [46].

From an analytical pathology perspective, circulating TUG1 demonstrates strong technical feasibility. TUG1 remains stable in serum and plasma samples under standard storage conditions, likely due to encapsulation within extracellular vesicles or association with RNA-binding proteins. This stability minimizes pre-analytical variability and supports its reproducibility across different laboratory settings, a key requirement for translation into routine diagnostic workflows [47].

The biological underpinnings of TUG1 upregulation further reinforce its diagnostic relevance. Mechanistic studies in hepatocellular carcinoma models have shown that TUG1 enhances oncogenic signaling, promotes cell cycle progression, and suppresses apoptosis through interactions with microRNAs and epigenetic regulators. These tumor-promoting functions align with its increased expression in circulation, strengthening the argument that circulating TUG1 reflects active malignant biology rather than passive RNA release from damaged hepatocytes [48].

Importantly, TUG1 expression appears to be less influenced by hepatic necroinflammatory activity compared with conventional serum markers. Studies comparing patients with active hepatitis, compensated cirrhosis, and HCC have reported minimal overlap between non-malignant disease and cancer-associated TUG1 levels. This relative specificity is particularly valuable in HCV-related liver disease, where fluctuating inflammatory activity frequently confounds interpretation of traditional biochemical markers [49].

Despite these promising findings, heterogeneity exists across published studies with respect to sample size, assay methodology, and normalization strategies. Differences in RNA extraction protocols, reference genes, and cutoff determination can influence reported diagnostic performance. From a clinical pathology standpoint, standardization of pre-analytical and analytical variables will be essential before TUG1 can be adopted as a routine diagnostic biomarker [50].

In summary, circulating TUG1 represents a robust and biologically meaningful non-invasive diagnostic biomarker for hepatitis C virus–related hepatocellular carcinoma. Its consistent upregulation in HCC, detectability in early-stage and AFP-negative disease, and technical stability in blood samples support its clinical relevance. When interpreted within an integrated diagnostic framework, TUG1 has the potential to significantly enhance early detection of HCV-related HCC [51].

Combined Diagnostic Performance of CASC2 and TUG1 in HCV-Related HCC

The combined assessment of circulating CASC2 and TUG1 has attracted increasing interest because these lncRNAs exhibit opposing expression patterns and complementary biological functions during hepatocarcinogenesis. CASC2 downregulation reflects loss of tumor-suppressive control, whereas TUG1 upregulation mirrors activation of oncogenic signaling pathways. From a diagnostic pathology

perspective, combining markers with inverse regulation increases discriminatory power and reduces the likelihood of false-negative or false-positive results in heterogeneous clinical settings such as HCV-related cirrhosis [52].

Clinical studies evaluating multi-marker models have demonstrated that the combination of CASC2 and TUG1 improves diagnostic accuracy compared with either lncRNA alone. In cohorts including patients with HCV-related HCC, cirrhosis, and chronic hepatitis, dual-marker panels achieved higher sensitivity and specificity for HCC detection, particularly in early-stage disease. Receiver operating characteristic analyses consistently showed improved area under the curve values when both lncRNAs were incorporated into a single diagnostic model, supporting their complementary diagnostic roles [53].

A major advantage of combining CASC2 and TUG1 lies in their performance in AFP-negative HCC cases. AFP-negative tumors represent a substantial diagnostic challenge, as they often escape detection using conventional surveillance tools. Studies have shown that while CASC2 and TUG1 individually identify subsets of AFP-negative patients, their combined evaluation captures a larger proportion of these cases, thereby enhancing overall diagnostic yield and addressing a critical unmet need in clinical practice [54].

From a biological standpoint, the combined lncRNA signature reflects multiple layers of tumor biology. CASC2 loss is associated with deregulation of tumor-suppressive microRNA networks and increased oncogenic signaling, whereas TUG1 upregulation promotes epigenetic silencing of tumor suppressor genes and enhanced cellular proliferation. This multidimensional representation of hepatocarcinogenesis increases the likelihood that circulating changes accurately reflect malignant transformation rather than background liver injury [55].

In comparison with AFP-based strategies, lncRNA combinations appear less influenced by necroinflammatory activity and hepatic regeneration. AFP levels frequently fluctuate in patients with active hepatitis or cirrhosis, leading to diagnostic ambiguity. In contrast, CASC2 and TUG1 expression patterns demonstrate clearer separation between malignant and non-malignant disease states, particularly when assessed together. This distinction is of particular importance in HCV-infected populations with ongoing or residual hepatic inflammation [56].

Analytical considerations are central to the clinical translation of combined lncRNA testing. Studies assessing dual-marker panels typically employ standardized RT-qPCR assays with predefined cutoff values derived from training cohorts. While promising, inter-study variability in normalization strategies and assay platforms highlights the need for methodological harmonization. From a pathology laboratory standpoint, development of validated, reproducible protocols will be essential for reliable implementation [57].

Integration of CASC2 and TUG1 into diagnostic algorithms should be viewed as complementary to, rather than replacement for, existing surveillance tools. Current evidence supports their use alongside AFP and imaging modalities to improve early detection rates in high-risk HCV patients. Such integrative approaches align with contemporary trends in molecular pathology, where layered diagnostics combine clinical, imaging, and molecular data to enhance accuracy and clinical decision-making [58].

In summary, the combined evaluation of circulating CASC2 and TUG1 offers superior diagnostic performance for hepatitis C virus–related hepatocellular carcinoma compared with single-marker approaches. Their inverse expression patterns, biological complementarity, and enhanced accuracy in AFP-negative and early-stage disease underscore their potential value as components of non-invasive diagnostic panels. Continued validation in larger, well-characterized cohorts is warranted to support their routine clinical application [59].

Comparison of CASC2 and TUG1 with Conventional Diagnostic Biomarkers in HCV-Related HCC

Conventional diagnostic biomarkers for hepatocellular carcinoma, most notably alpha-fetoprotein, have long been used in clinical practice but remain limited by suboptimal sensitivity and specificity, particularly in patients with chronic hepatitis C virus infection. AFP levels may remain within normal ranges in a substantial proportion of early-stage HCC cases, while false-positive elevations frequently

occur in chronic hepatitis and cirrhosis due to hepatic inflammation and regeneration. These limitations significantly reduce the reliability of AFP as a standalone diagnostic marker in HCV-related HCC [60].
Several studies comparing circulating lncRNAs with AFP have demonstrated that CASC2 and TUG1 offer distinct diagnostic advantages. CASC2 downregulation shows stronger specificity for malignant transformation than AFP elevation, as CASC2 levels remain relatively stable in non-malignant chronic HCV infection and cirrhosis. This contrasts with AFP, whose expression is influenced by multiple non-neoplastic hepatic processes, thereby limiting its discriminatory capacity in high-risk populations [61].
TUG1, on the other hand, exhibits high diagnostic sensitivity and has been shown to identify HCC cases that are missed by AFP-based screening. Importantly, elevated circulating TUG1 levels have been detected in patients with early-stage tumors and normal AFP concentrations, highlighting its added value in AFP-negative HCC. This feature addresses a critical diagnostic gap in current surveillance strategies for HCV-related liver disease [62].
Beyond AFP, other conventional biomarkers such as AFP-L3 and des-γ-carboxy prothrombin have been evaluated for HCC diagnosis. However, these markers are limited by restricted availability, higher cost, and variable performance in early disease stages. Moreover, their diagnostic accuracy may be influenced by factors such as vitamin K deficiency or underlying liver dysfunction, further complicating their interpretation in HCV-infected patients [63].
From a clinical pathology standpoint, circulating lncRNAs offer several analytical advantages over protein-based biomarkers. CASC2 and TUG1 can be quantified using highly sensitive molecular techniques, allowing detection of subtle changes associated with early malignant transformation. Their expression patterns appear less affected by inflammatory activity, making them potentially more reliable indicators of cancer-specific molecular events than traditional serum markers [64].
Comparative diagnostic models integrating lncRNAs with AFP have consistently outperformed AFP alone. Studies incorporating CASC2 and TUG1 into multi-marker panels demonstrated improved sensitivity, specificity, and overall diagnostic accuracy for HCV-related HCC. Such integrative approaches align with current trends in molecular diagnostics, which emphasize combining complementary biomarkers to capture the complexity of tumor biology [65].
Despite these promising findings, conventional biomarkers remain deeply embedded in clinical practice due to their accessibility and long-standing use. Therefore, CASC2 and TUG1 should be viewed as complementary rather than replacement markers at this stage. Their greatest diagnostic value lies in augmenting existing surveillance frameworks, particularly for early detection and AFP-negative disease in high-risk HCV populations [66].
In summary, compared with conventional diagnostic biomarkers, circulating CASC2 and TUG1 demonstrate superior specificity for malignant transformation, enhanced sensitivity for early-stage and AFP-negative HCC, and reduced susceptibility to confounding by hepatic inflammation. These attributes position lncRNAs as valuable additions to the diagnostic armamentarium for HCV-related hepatocellular carcinoma, warranting further validation and standardization before widespread clinical implementation [67].

Pre-Analytical and Analytical Considerations for Circulating lncRNA Testing in Clinical Pathology

Successful translation of circulating lncRNAs into routine clinical diagnostics depends on controlling the pre-analytical phase, because circulating RNA measurements are highly sensitive to sample handling. In liquid biopsy workflows, variability introduced before analysis can rival or exceed true biological differences, particularly when separating HCV-related cirrhosis from early HCC where expression differences may be modest. Therefore, clinical pathology laboratories must define standardized operating procedures for specimen collection, transport, processing time, storage conditions, and freeze–thaw limits before interpreting CASC2 or TUG1 results clinically. [68]
Specimen type is a major determinant of analytical comparability, since serum and plasma may yield

different RNA profiles due to coagulation-related release of cellular components and vesicles. In addition, whole blood assays may reflect leukocyte-associated transcripts rather than tumor-derived circulating RNA, which may complicate interpretation if the goal is tumor-specific detection. For HCV-related HCC diagnostics, studies have used serum, plasma, and whole blood across different designs, so careful attention to the chosen matrix is essential to avoid incorrect cross-study comparisons and to ensure that locally validated cutoffs are matrix-specific. [69]

Hemolysis and cellular contamination represent particularly important confounders for circulating RNA assays. Even mild hemolysis can release intracellular nucleic acids into the specimen and distort apparent lncRNA abundance, leading to false elevation or masking of true tumor-associated signals. In chronic liver disease, where thrombocytopenia and fragile blood cells may coexist, rigorous sample inspection, rejection criteria, and documentation should be considered part of the pathology quality system when implementing lncRNA-based testing. [70]

Analytically, RNA extraction method and yield assessment directly influence sensitivity and reproducibility, especially for low-abundance lncRNAs. Because circulating lncRNAs may be present as free RNA, protein-bound RNA, or packaged within extracellular vesicles, extraction kits and protocols differ in their ability to recover the clinically relevant fraction. This is particularly important for diagnostic applications where inter-individual differences are interpreted at the patient level, meaning that extraction efficiency must be stable and monitored across batches. [71]

Quantification is most commonly performed using RT-qPCR, and many translational studies rely on relative quantification. While this approach is practical and familiar in clinical molecular laboratories, it requires strict control of normalization strategy, reference selection, and run-to-run calibration. The widely used $2^{-\Delta\Delta Ct}$ method is appropriate when amplification efficiencies are similar and reference targets are stable, but it can introduce bias when these assumptions are violated, making validation of assay performance characteristics essential before clinical reporting. [72]

Selection of internal controls is a key unresolved issue in circulating lncRNA diagnostics, particularly in chronic liver disease where housekeeping transcripts may vary with inflammation, fibrosis, or metabolic status. In HCV-related cohorts, the “best” normalizer can differ depending on whether the specimen is serum, plasma, or whole blood, and whether the comparator group is chronic hepatitis, cirrhosis, or healthy controls. Consequently, clinical pathology implementation should include internal control verification within the intended-use population, not only in healthy samples. [73]

From a diagnostic assay design perspective, defining clinically meaningful cutoffs requires separation of analytical validity from clinical validity. Analytical validity includes precision, linearity, limit of detection, and robustness to pre-analytical variation, whereas clinical validity requires demonstrating reliable discrimination between HCV-related HCC and non-malignant HCV disease controls. Published serum lncRNA studies in HCC illustrate that diagnostic performance can appear strong in case–control settings but may shift when tested in real-world surveillance populations, emphasizing the need for careful validation design. [74]

Finally, reporting standards should align with clinical decision-making rather than purely research outputs. For diagnostic deployment, pathology reports should specify specimen type, extraction method, assay platform, normalization approach, and interpretive comments indicating that results are supportive evidence within a broader diagnostic pathway including imaging and established biomarkers. This structured reporting model is consistent with the direction of circulating biomarker research in early HCC detection and supports safer integration of CASC2 and TUG1 into diagnostic algorithms for HCV-related liver disease. [75]

Conclusion

Hepatitis C virus–related hepatocellular carcinoma remains a major diagnostic challenge, particularly at early stages when curative interventions are most effective. Conventional surveillance tools, including ultrasonography and serum alpha-fetoprotein, are limited by suboptimal sensitivity and specificity in cirrhotic and chronically inflamed livers. These limitations underscore the need for non-invasive molecular biomarkers that more accurately reflect the underlying biology of malignant transformation

rather than nonspecific hepatic injury.

Accumulating evidence supports a central role for circulating long noncoding RNAs as promising diagnostic tools in this setting. CASC2 and TUG1 represent two biologically and clinically relevant lncRNAs with complementary expression patterns and mechanistic roles in hepatocarcinogenesis. Downregulation of CASC2 reflects loss of tumor-suppressive regulatory networks, whereas upregulation of TUG1 mirrors activation of oncogenic and epigenetic pathways. Their detectability in serum or plasma, combined with stability and reproducibility using molecular assays, makes them well suited for liquid biopsy–based diagnostics.

From a clinical pathology perspective, the greatest diagnostic value of CASC2 and TUG1 lies in their ability to improve detection of early-stage and AFP-negative hepatocellular carcinoma in patients with chronic hepatitis C. When evaluated together, these lncRNAs demonstrate superior discriminatory performance compared with single-marker approaches and offer meaningful advantages over conventional serum biomarkers that are influenced by inflammation and hepatic regeneration. Their integration into multi-marker diagnostic panels has the potential to enhance risk stratification and diagnostic accuracy in high-risk populations.

Despite promising results, translation into routine clinical practice requires further standardization of pre-analytical and analytical procedures, validation in large multicenter cohorts, and integration into existing surveillance algorithms. Future studies should focus on assay harmonization, establishment of clinically actionable cutoffs, and evaluation in real-world surveillance settings.

In conclusion, circulating CASC2 and TUG1 represent robust and biologically grounded non-invasive diagnostic biomarkers for hepatitis C virus–related hepatocellular carcinoma. Their incorporation into clinical pathology–driven diagnostic strategies holds significant promise for improving early detection and ultimately patient outcomes.

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