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Circulating and Tissue lncRNAs CASC2 and TUG1 as Prognostic Biomarkers in HCV-Associated Hepatocellular Carcinoma

Nahla Ibrahim Elattar[1], Hanan samir Ahmed[1], Ola Elsayed Abdel Latif Elnaggar[1], Nahla Elsayed Elgammal [2]

1 Clinical Pathology Department, Faculty of Medicine, Zagazig University,
2 Hepatology, Gastroenterology and Infectious Diseases Department, Faculty of Medicine, Zagazig University,
Corresponding author: Ola Elsayed Abdel Latif Elnaggar

ABSTRACT

Background: Hepatocellular carcinoma (HCC) arising in the setting of chronic hepatitis C virus (HCV) infection is characterized by marked biological heterogeneity and highly variable clinical outcomes. Despite advances in antiviral therapy and improvements in locoregional and systemic treatments, prognosis remains poor for many patients due to high rates of tumor recurrence, vascular invasion, and disease progression. Traditional prognostic systems based on tumor size, number, histological grade, and liver function provide useful stratification but fail to fully capture the molecular diversity that drives outcome variability in HCV-associated HCC. Consequently, there is growing interest in molecular biomarkers that can refine prognostic assessment and guide risk-adapted clinical management.

Long noncoding RNAs (lncRNAs) have emerged as key regulators of cancer biology, influencing tumor growth, invasion, metastasis, and therapeutic resistance. Aberrant lncRNA expression is closely linked to epigenetic reprogramming and oncogenic signaling pathways activated during hepatocarcinogenesis. Importantly, several lncRNAs show stable and quantifiable expression in both tumor tissue and circulation, making them attractive candidates for prognostic biomarker development.

Aim: This review aims to critically evaluate the prognostic significance of the long noncoding RNAs CASC2 and TUG1 in hepatitis C virus–associated hepatocellular carcinoma. Emphasis is placed on evidence derived from tissue-based and circulating lncRNA studies, with particular focus on associations with overall survival, disease-free survival, recurrence risk, tumor aggressiveness, and clinicopathological features.

Conclusion: Evidence indicates that CASC2 and TUG1 hold significant prognostic value in HCV-associated HCC. Reduced expression of CASC2, a tumor-suppressive lncRNA, is consistently associated with aggressive tumor behavior, advanced disease stage, and poorer survival outcomes. In contrast, elevated expression of the oncogenic lncRNA TUG1 correlates with increased tumor invasiveness, higher recurrence rates, and unfavorable survival. Importantly, these prognostic associations are observed at both the tissue and circulating levels, supporting the biological relevance and clinical feasibility of these markers. Combined evaluation of CASC2 and TUG1 may further enhance prognostic stratification by capturing complementary aspects of tumor biology. Although additional large-scale and prospective validation studies are required, current data support the integration of CASC2 and TUG1 into molecular prognostic frameworks for HCV-related hepatocellular carcinoma, with potential implications for personalized risk assessment and clinical decision-making.

Keywords: lncRNAs, CASC2, TUG1 , HCV-Associated Hepatocellular Carcinoma

INTRODUCTION

Hepatocellular carcinoma (HCC) arising in the setting of chronic hepatitis C virus (HCV) infection remains a major cause of cancer-related mortality worldwide. Despite significant advances in antiviral therapy and improvements in oncologic management, prognosis remains poor for a substantial proportion of patients. This is largely attributable to late presentation, high recurrence rates, and marked biological heterogeneity that influences tumor behavior and patient survival [1].

Chronic HCV infection promotes hepatocarcinogenesis through sustained inflammation, oxidative stress, and progressive fibrotic remodeling, ultimately leading to cirrhosis and malignant transformation. Even after successful viral eradication with direct-acting antivirals, patients with advanced fibrosis remain at elevated risk for HCC development and progression, underscoring the persistent oncogenic imprint of HCV-related liver disease [2].

Current prognostic stratification of HCC relies primarily on clinicopathological parameters, including tumor size, number, vascular invasion, histological grade, and liver function status, as well as composite staging systems such as the Barcelona Clinic Liver Cancer classification. Although these models are useful for treatment allocation, they fail to adequately explain the wide variability in outcomes observed among patients with similar clinical stages and therapeutic interventions [3].

From a clinical pathology perspective, this limitation highlights the need for molecular prognostic biomarkers that capture intrinsic tumor biology rather than gross anatomical features alone. Traditional serum markers, such as alpha-fetoprotein, have shown inconsistent prognostic performance and are influenced by hepatic inflammation and regeneration, limiting their reliability for outcome prediction in HCV-associated HCC [4].

Long noncoding RNAs have emerged as important regulators of cancer progression and metastasis. These transcripts modulate chromatin structure, transcriptional programs, and post-transcriptional gene regulation, thereby influencing key oncogenic processes such as proliferation, apoptosis, epithelial–mesenchymal transition, and angiogenesis. In hepatocellular carcinoma, aberrant lncRNA expression has been repeatedly linked to aggressive tumor behavior and unfavorable survival outcomes [5].

Importantly, lncRNAs can be evaluated in both tumor tissue and circulation, providing complementary prognostic information. Tissue-based lncRNA expression reflects intrinsic tumor biology, while circulating lncRNAs may integrate signals related to tumor burden, invasiveness, and metastatic potential. This dual accessibility is particularly relevant in HCV-related HCC, where repeated tissue sampling is often limited by cirrhosis and bleeding risk [6].

Among the expanding repertoire of lncRNAs implicated in hepatocellular carcinoma, Cancer Susceptibility Candidate 2 (CASC2) and Taurine Upregulated Gene 1 (TUG1) have gained attention due to their opposing biological functions and consistent association with tumor progression. CASC2 generally acts as a tumor-suppressive lncRNA, whereas TUG1 functions predominantly as an oncogenic regulator. Dysregulation of both has been reported in HCC tissue and circulation, with emerging evidence linking their expression to survival, recurrence, and clinicopathological aggressiveness [7].

Despite these observations, the prognostic significance of CASC2 and TUG1 in HCV-associated HCC has not been comprehensively reviewed in a unified framework. Many available studies evaluate mixed-etiology HCC cohorts, limiting disease-specific interpretation, and often assess tissue and circulating expression separately. A focused synthesis from a clinical pathology standpoint is therefore needed to clarify their prognostic value and translational potential [8].

The aim of this review is to critically evaluate the prognostic relevance of CASC2 and TUG1 in hepatitis C virus–associated hepatocellular carcinoma, with emphasis on associations with overall survival, disease-free survival, recurrence risk, and clinicopathological features. By integrating tissue-based and circulating evidence, this review seeks to define the potential role of these lncRNAs in molecular prognostication and personalized risk stratification for HCV-related HCC [9].

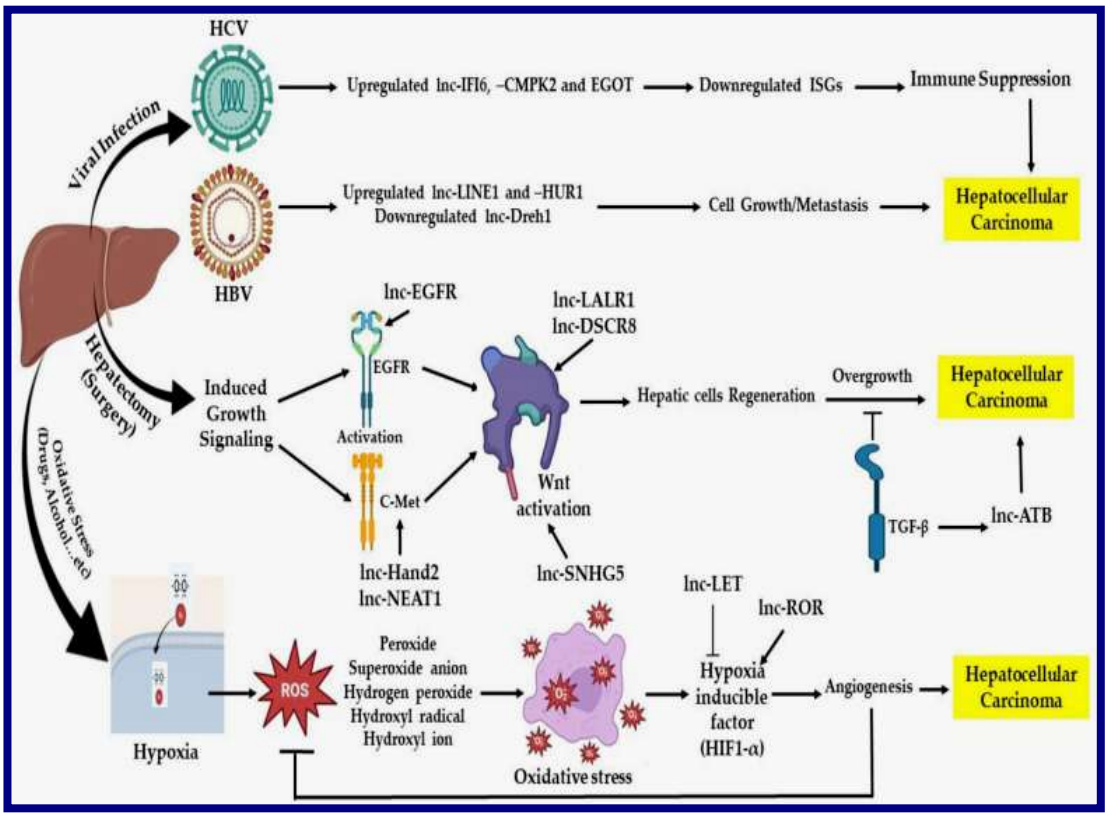

Figure (1): Major growth factor receptor and signaling pathways in HCC [4].

Biological Rationale for lncRNAs as Prognostic Biomarkers in HCV-Associated Hepatocellular Carcinoma

Hepatocellular carcinoma progression is driven by complex molecular alterations that extend beyond tumor initiation and directly influence invasion, metastasis, recurrence, and resistance to therapy. Prognostic biomarkers should therefore reflect biological processes governing tumor aggressiveness rather than merely tumor presence. Long noncoding RNAs are increasingly recognized as central regulators of these processes through their ability to modulate gene expression networks at epigenetic, transcriptional, and post-transcriptional levels, making them biologically well suited for prognostic assessment in HCC [10].

In the setting of chronic hepatitis C virus infection, persistent inflammatory signaling and oxidative stress induce durable epigenetic reprogramming within hepatocytes. These changes promote dysregulated expression of lncRNAs that control pathways involved in cell cycle progression, apoptosis, epithelial–mesenchymal transition, and angiogenesis. Importantly, many of these molecular alterations persist even after viral eradication, contributing to continued tumor aggressiveness and poor clinical outcomes in HCV-associated HCC [11].

Unlike diagnostic biomarkers, which aim to distinguish malignant from non-malignant disease, prognostic lncRNAs are expected to correlate with disease severity, progression rate, and survival outcomes. Experimental and clinical studies have shown that specific lncRNA expression patterns are associated with microvascular invasion, poor tumor differentiation, advanced stage, and metastatic potential in HCC. These associations support the concept that lncRNAs are not passive byproducts of malignancy but active drivers of tumor behavior [12].

From a mechanistic standpoint, lncRNAs exert prognostic influence by interacting with chromatin-modifying complexes such as Polycomb Repressive Complex 2, guiding histone modifications that silence tumor suppressor genes or activate oncogenic transcriptional programs. In addition, lncRNAs function as competing endogenous RNAs, sequestering tumor-suppressive microRNAs and thereby enhancing expression of oncogenes involved in proliferation and invasion. These mechanisms directly link lncRNA dysregulation to aggressive tumor phenotypes and adverse outcomes [13].

The prognostic relevance of lncRNAs is further supported by their association with therapeutic resistance. Several lncRNAs implicated in HCC progression have been shown to modulate sensitivity to chemotherapy, targeted therapy, and locoregional

treatments by influencing apoptosis, DNA repair, and drug efflux pathways. In HCV-related HCC, where treatment options may be limited by underlying liver dysfunction, such molecular determinants of resistance are particularly important for outcome prediction [14].

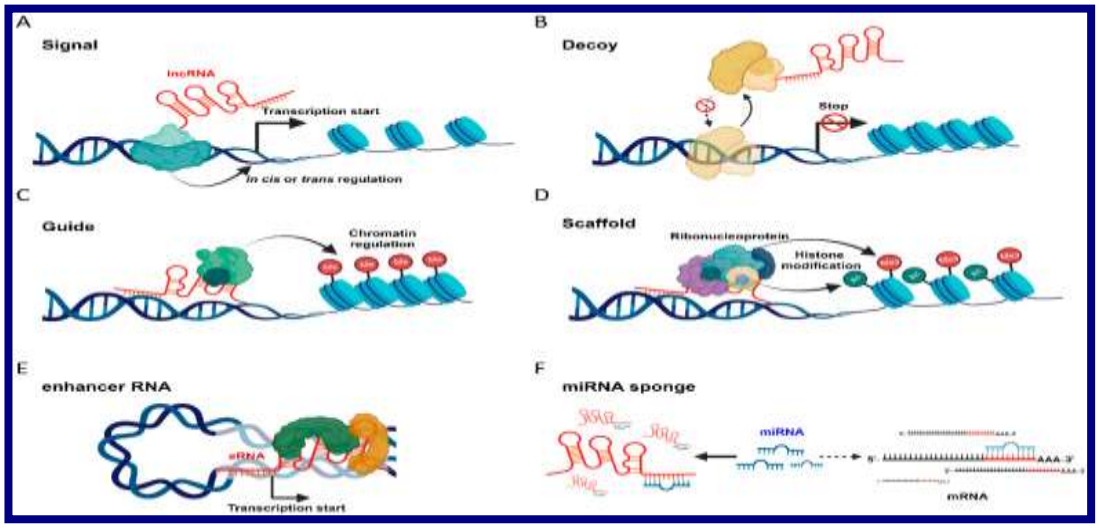

Figure (2): Diverse mechanisms of lncRNAs' functions in cellular regulation. (A) Signal. LncRNAs act as molecular indicators, responding to various cellular stimuli. (B) Decoy. LncRNAs can bind and sequester transcription factors or other proteins, preventing them from interacting with their target genomic loci. (C) Guide. LncRNAs direct chromatin-modifying enzymes to specific genomic regions, enabling targeted epigenetic modifications. (D) Scaffold. LncRNAs facilitate the formation of multi-protein complexes, providing a structural platform for these assemblies. (E) Enhancer RNA. LncRNAs can function as enhancers, looping DNA to bring distant regions into proximity for transcriptional activation. (F) miRNA Sponge. LncRNAs can act as sponges for miRNAs, sequestering them and preventing them from binding to their target mRNAs, thus inhibiting miRNA-mediated gene repression [12].

An additional advantage of lncRNAs as prognostic biomarkers is their detectability in both tumor tissue and circulation. Tissue expression reflects intrinsic tumor biology and clonal selection, while circulating lncRNAs may capture dynamic changes related to tumor burden, dissemination, and recurrence risk. This dual compartment assessment allows for baseline prognostic stratification as well as longitudinal monitoring, which is difficult to achieve with conventional clinicopathological parameters alone [15].

In chronic liver disease, interpretation of protein-based prognostic markers is often confounded by necroinflammatory activity and hepatic regeneration. In contrast, lncRNA expression appears to be more closely linked to malignant signaling pathways than to inflammatory fluctuations. This relative specificity enhances their potential utility in HCV-associated HCC, where background liver pathology is almost universally present and complicates prognostic evaluation [16].

Within this biological framework, CASC2 and TUG1 exemplify lncRNAs with opposing yet complementary prognostic roles. CASC2 downregulation is associated with loss of tumor-suppressive control and enhanced oncogenic signaling, whereas TUG1 upregulation promotes epigenetic silencing of tumor suppressor genes and facilitates invasion and metastasis. Their consistent association with aggressive clinicopathological features provides a strong biological rationale for evaluating them as prognostic biomarkers in HCV-related hepatocellular carcinoma [17].

Tissue CASC2 Expression and Prognostic Implications in HCV-Associated Hepatocellular Carcinoma

Tissue-based expression analysis has consistently demonstrated that CASC2 is significantly downregulated in hepatocellular carcinoma compared with adjacent non-tumorous liver tissue. This reduction is more pronounced in tumors exhibiting aggressive histopathological features, suggesting that loss of CASC2 expression is linked to malignant progression rather than tumor initiation alone. From a prognostic standpoint, reduced CASC2 expression in tumor tissue reflects impaired tumor-suppressive regulation and is therefore biologically plausible as a marker of unfavorable outcome [18].

Several studies evaluating CASC2 expression in resected HCC specimens have reported significant associations between low

CASC2 levels and adverse clinicopathological characteristics. These include larger tumor size, poor histological differentiation, presence of microvascular invasion, and advanced tumor stage. Such associations indicate that CASC2 downregulation accompanies phenotypic features known to predict recurrence and reduced survival in HCC patients, including those with underlying HCV infection [19].

Survival analyses further support the prognostic relevance of tissue CASC2 expression. Patients with low intratumoral CASC2 levels have been shown to experience significantly shorter overall survival and disease-free survival compared with those exhibiting higher expression. These associations remain significant in multivariate models that account for conventional prognostic variables, suggesting that CASC2 provides independent prognostic information beyond established clinicopathological factors [20].

Mechanistically, the prognostic impact of CASC2 loss is linked to its role in regulating oncogenic signaling pathways. Reduced CASC2 expression leads to derepression of oncogenic microRNAs and subsequent activation of downstream targets involved in cell cycle progression, invasion, and resistance to apoptosis. These molecular alterations promote tumor aggressiveness and may explain the observed associations between CASC2 downregulation and poor clinical outcomes [21].

In the context of HCV-associated HCC, chronic inflammatory signaling and epigenetic modifications may further exacerbate CASC2 suppression. Persistent viral-induced epigenetic reprogramming can reinforce silencing of tumor-suppressive lncRNAs, thereby sustaining aggressive tumor behavior even after viral eradication. This mechanism may contribute to the particularly poor prognosis observed in a subset of HCV-related HCC patients with markedly reduced CASC2 expression [22].

From a pathology workflow perspective, tissue CASC2 assessment can be performed using quantitative molecular techniques on formalin-fixed paraffin-embedded specimens, allowing integration into routine diagnostic and prognostic evaluation. Although standardization of assay platforms and cutoffs remains necessary, tissue-based CASC2 expression has shown reproducible associations with outcome across multiple cohorts, supporting its translational potential [23].

Collectively, these findings indicate that low tissue expression of CASC2 is a robust marker of aggressive tumor biology and unfavorable prognosis in hepatocellular carcinoma. In HCV-associated disease, CASC2 downregulation reflects the combined effects of viral-induced molecular dysregulation and tumor progression, underscoring its value as a prognostic biomarker. Integration of CASC2 tissue expression into prognostic models may enhance risk stratification and guide postoperative surveillance strategies [24].

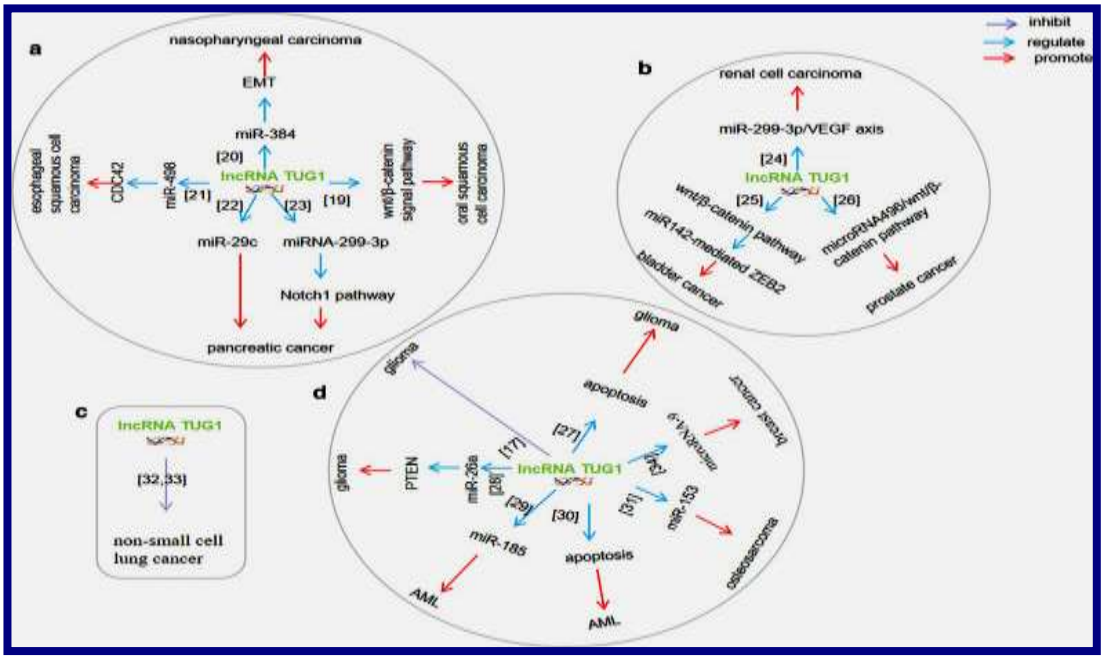

Figure (2): lncRNA TUG1 regulates cancer cells proliferation of a variety of cancers. A network map illustrating the regulation of proliferation by lncRNA TUG1 in a variety of cancers, including oral cancer, oesophageal cancer, and pancreatic cancer. The pie charts labelled A, B, C and D represent cancers of the digestive system, cancers of urinary system, non-small cell lung

cancer and cancers of other systems, respectively [24].

Tissue TUG1 Expression and Prognostic Implications in HCV-Associated Hepatocellular Carcinoma

TUG1 is among the best-characterized oncogenic long noncoding RNAs in hepatocellular carcinoma, with multiple studies demonstrating significant upregulation in tumor tissue compared with adjacent non-tumorous liver. This increase is clinically important because it is not merely an epiphenomenon of malignant transformation; rather, higher intratumoral TUG1 expression has been repeatedly linked to biological processes that drive progression, including proliferation, invasion, and metastatic potential. These features directly align with adverse outcomes in HCV-associated HCC, making tissue TUG1 a biologically credible prognostic biomarker [25].

Clinically, elevated TUG1 expression in HCC tissue has been associated with indicators of aggressive phenotype such as larger tumor size, advanced stage, and poorer differentiation. Importantly, these clinicopathological correlations suggest that TUG1 is enriched in tumors with high proliferative capacity and invasive behavior, which are also the tumors most likely to recur after curative-intent therapy. Such associations support the use of tissue TUG1 as a marker of high-risk disease biology rather than simply tumor presence [26].

Several investigations have reported that patients with high TUG1 expression have inferior overall survival and disease-free survival compared with patients with low TUG1 expression. In prognostic modeling, TUG1 has shown potential to stratify patients into distinct risk categories that remain clinically meaningful even when conventional factors such as stage and liver function are included. These findings are consistent with the concept that lncRNA-based risk estimation captures molecular aggressiveness that may not be fully reflected in radiologic staging alone [27].

At the mechanistic level, the prognostic impact of TUG1 is supported by its ability to regulate gene expression through epigenetic silencing of tumor suppressor pathways. TUG1 has been shown to interact with chromatin-modifying complexes and facilitate repression of anti-proliferative genes, thereby promoting tumor growth and survival. This epigenetic function provides a plausible explanation for the association between high TUG1 expression and poor patient outcomes, including recurrence and progression [28].

In addition to epigenetic regulation, TUG1 participates in competing endogenous RNA networks that promote malignant signaling. By sponging tumor-suppressive microRNAs, TUG1 can derepress oncogenic targets involved in epithelial–mesenchymal transition, migration, and invasion. These pathways are strongly linked with recurrence and metastatic dissemination, which are key determinants of prognosis in HCV-associated HCC, particularly in patients with cirrhosis where treatment options may be constrained [29].

From a clinical pathology standpoint, tissue TUG1 assessment is technically feasible using RT-qPCR in resected tumor samples, and its strong signal difference between high-risk and lower-risk tumors supports its potential for incorporation into molecular prognostic frameworks. However, pre-analytical standardization, selection of appropriate internal controls, and validation of cutoffs across etiologic subgroups remain essential for clinical implementation, especially when focusing specifically on HCV-associated HCC [30].

Overall, tissue-based evidence supports the conclusion that high intratumoral TUG1 expression is associated with aggressive clinicopathological features and unfavorable survival outcomes in hepatocellular carcinoma. For HCV-associated HCC, TUG1 likely reflects a convergence of viral-driven epigenetic dysregulation and tumor-intrinsic oncogenic programming, making it a strong candidate biomarker for identifying patients at increased risk of recurrence and reduced long-term survival after treatment [31].

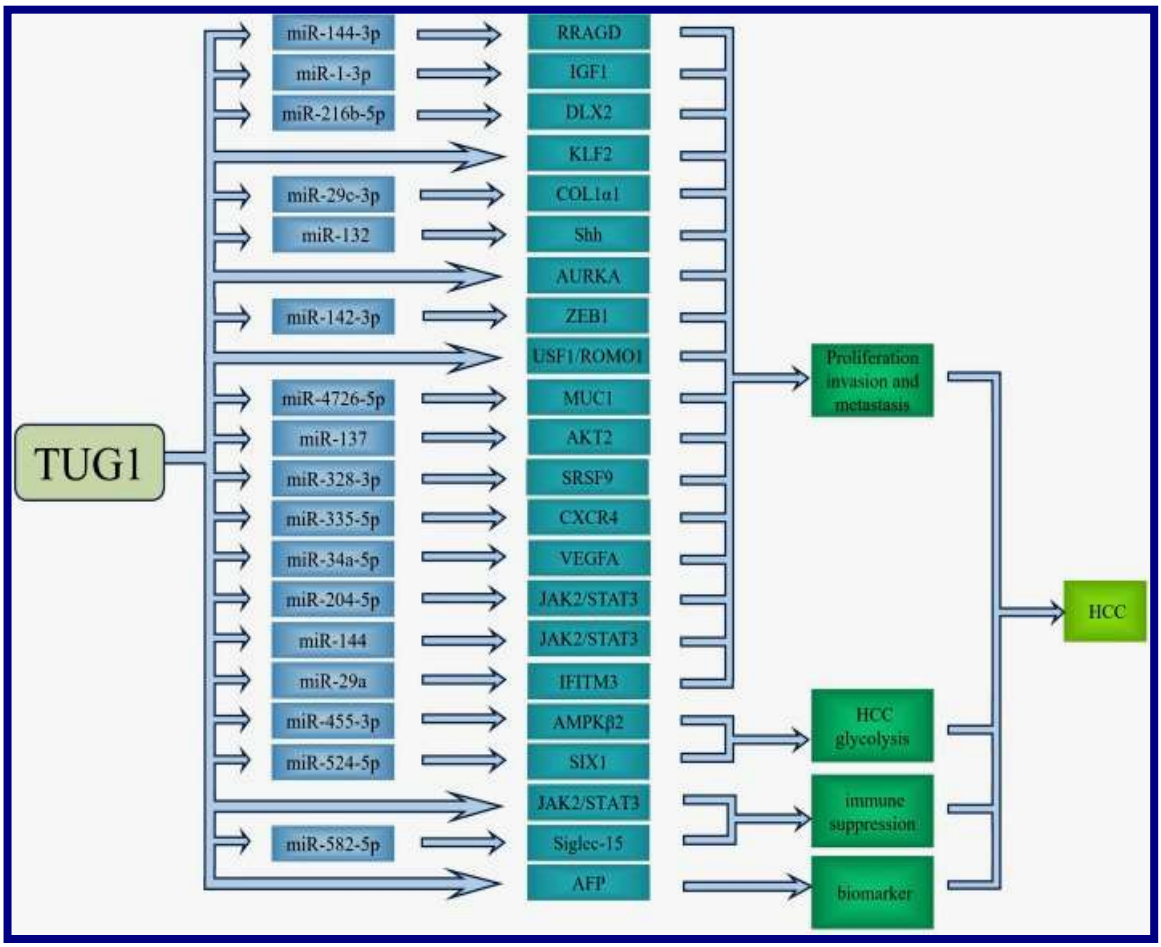

Fig. (3): TUG1 mediates mechanisms involved in HCC progression. [31].

Circulating CASC2 as a Prognostic Biomarker in HCV-Associated Hepatocellular Carcinoma

Circulating or whole-blood lncRNA assessment is conceptually attractive for prognostic evaluation in HCV-associated HCC because it can be repeated over time, requires minimal invasiveness, and may capture systemic tumor–host interactions that influence progression and recurrence. From a clinical pathology perspective, blood-based biomarkers are particularly valuable in cirrhotic patients who may not undergo resection or who have limited access to tumor tissue. Liquid-biopsy frameworks support the feasibility of using circulating nucleic acids to reflect tumor dynamics, but they also highlight the need for strict preanalytical and analytical standardization to ensure clinical reliability [32,33].

In HCV-related HCC, the most directly relevant clinical evidence for circulating CASC2 comes from studies measuring CASC2 expression in peripheral blood and relating it to clinically meaningful disease parameters. Refai and colleagues reported that CASC2 was downregulated in whole blood of HCC patients on top of HCV compared with HCV-only patients and healthy controls, and importantly, blood CASC2 levels correlated with Barcelona Clinic Liver Cancer stage and serum AFP. Because BCLC stage integrates tumor burden, liver function, and performance status, this association supports a prognostic linkage, even though the study was not primarily designed as a survival analysis [32].

The interpretation of circulating CASC2 as a prognostic marker should be framed as “risk stratification by tumor aggressiveness and stage” rather than immediate prediction of overall survival, because most available HCV-focused datasets emphasize cross-sectional clinicopathological correlations. Nevertheless, stage association is clinically meaningful: biomarkers that track with BCLC stage frequently align with recurrence risk and survival probability in real-world practice, especially when used alongside standard models rather than as standalone predictors. In this context, reduced circulating CASC2 may identify a subgroup with more advanced biological behavior and higher-risk disease phenotype [32,34].

A key technical consideration is that “circulating CASC2” is not a single uniform analyte across studies, since it can be measured

from plasma/serum, whole blood, peripheral blood mononuclear cells, or extracellular vesicle–enriched fractions. Whole-blood assays may reflect a composite signal derived from leukocytes plus tumor-associated nucleic acids, whereas plasma/serum assays may more directly reflect cell-free or vesicle-associated transcripts. These compartment differences can affect prognostic interpretation, and they underscore why harmonization of sample type and processing protocols is essential before broad clinical translation [33–35].

Preanalytical variables are especially critical for lncRNA quantification because RNA yield and stability are influenced by hemolysis, storage time, freeze–thaw cycles, and choice of extraction method. Hemolysis can markedly distort circulating RNA measurements by releasing intracellular RNAs, potentially confounding associations with tumor aggressiveness or stage if not controlled. For prognostic studies aiming to link CASC2 to recurrence or survival, rigorous reporting and control of these factors is not optional; it is necessary for reproducibility and clinical credibility [35,36].

Analytically, RT-qPCR remains the most common platform used for circulating lncRNA measurement, but results depend strongly on normalization strategy and reference gene selection. In prognostic biomarker studies, inappropriate normalization can create spurious associations with stage or outcomes, particularly in chronic liver disease where systemic inflammation alters leukocyte biology and RNA profiles. Therefore, adherence to validated normalization principles and transparent reporting of quantification methods are essential if circulating CASC2 is to move from an "associated marker" to a clinically deployable prognostic tool [37,38].

Overall, the current evidence supports circulating CASC2 as a biologically plausible prognostic indicator in HCV-associated HCC, mainly through its association with integrated clinical staging (BCLC) and established tumor marker behavior (AFP). However, robust outcome-driven validation (recurrence, disease-free survival, overall survival) in larger HCV-specific cohorts remains the key gap. Until such datasets mature, circulating CASC2 is best positioned as a candidate component of multimarker prognostic panels rather than a definitive standalone prognostic test [32,40].

Circulating TUG1 as a Prognostic Biomarker in HCV-Associated Hepatocellular Carcinoma

Circulating TUG1 has attracted growing attention as a prognostic biomarker candidate in HCV-associated HCC because it combines biological relevance (oncogenic behavior in liver cancer) with practical feasibility for longitudinal monitoring using peripheral blood. From a clinical pathology standpoint, circulating RNA biomarkers are particularly appealing in HCV-related cirrhosis, where repeated biopsies are often avoided and where relapse, recurrence, and progression risk remain clinically critical even after treatment interventions [41].

Among the most HCV-focused clinical datasets, Mohyeldeen and colleagues demonstrated that serum TUG1 is significantly dysregulated across the HCV disease spectrum and is markedly altered in HCV-associated HCC compared with chronic HCV without HCC. While the study's primary emphasis was diagnostic discrimination, the observed relationships between serum lncRNA expression and advanced disease phenotype support prognostic relevance, because circulating profiles that differentiate HCC from non-malignant HCV states frequently co-segregate with tumor burden and biological aggressiveness in routine clinical practice [41].

A key prognostic argument for circulating TUG1 is strengthened when blood-based findings are interpreted alongside tissue-based evidence linking high TUG1 expression to aggressive clinicopathological features and inferior outcomes. Tissue studies have shown that TUG1 promotes tumor growth and survival through epigenetic silencing mechanisms and oncogenic transcriptional programming. When the same lncRNA is detectable in circulation and is dysregulated in HCV-HCC, the most parsimonious interpretation is that circulating TUG1 can act as a surrogate of tumor-intrinsic biology, making it suitable for risk stratification approaches that extend beyond baseline diagnosis [42].

Additional clinical evidence from HCV-related HCC cohorts indicates that circulating or blood-associated TUG1 expression is clinically significant and relates to disease severity metrics used in prognostication. Abdelzaher and colleagues examined TUG1 in HCV-related HCC and reported clinically meaningful associations that support its potential application in patient stratification. Although survival endpoints require broader prospective validation, correlations with established markers of tumor activity and clinical stage are relevant because they map onto recurrence probability and survival expectations in standard HCC management pathways [43].

From a mechanistic standpoint, the plausibility of circulating TUG1 as a prognostic marker is supported by well-described

oncogenic functions in HCC, including effects on proliferation, invasion, epithelial–mesenchymal transition, and immune escape pathways. These mechanisms are directly aligned with high-risk phenotypes such as vascular invasion, metastatic potential, and early recurrence after therapy. Therefore, elevated circulating TUG1 would be expected to track with adverse biological behavior, even when imaging-defined tumor burden appears similar across patients [44,45].

It is also important to recognize that "circulating TUG1" can be measured from different blood compartments (serum/plasma vs whole blood vs exosomal fractions), and the biological meaning may differ across matrices. Exosome-associated transcripts can better reflect tumor-derived RNA export, while whole-blood assays may include leukocyte-derived signals influenced by cirrhosis-associated inflammation. This distinction is not trivial for prognostic work, because confounding from systemic inflammation may inflate or obscure associations with tumor aggressiveness unless sample type and processing are standardized across cohorts [46].

Preanalytical and analytical rigor is therefore essential before circulating TUG1 can be positioned as a clinically deployable prognostic biomarker. Hemolysis, storage conditions, extraction efficiency, and normalization strategy can all alter measured lncRNA levels and can generate apparent "prognostic" differences that are actually artifacts. For outcome-linked studies (overall survival, recurrence-free survival), adherence to established qPCR quality frameworks and transparent reporting standards is necessary to ensure that any observed association between TUG1 and prognosis is reproducible and clinically credible [35,38].

Overall, current evidence supports circulating TUG1 as a biologically plausible prognostic biomarker candidate in HCV-associated HCC, with the strongest near-term role being risk enrichment when combined with clinical stage and conventional markers rather than standalone outcome prediction. The major research gap is the need for HCV-specific, prospective cohorts with standardized sampling and clearly defined endpoints (recurrence, survival), which would allow circulating TUG1 to be validated as an independent predictor and integrated into practical prognostic models [41,43].

Association of CASC2 and TUG1 with Clinicopathological Prognostic Parameters in HCV-Associated HCC

In clinical pathology, prognostic biomarkers are most useful when they correlate with, and add information beyond, established clinicopathological parameters that drive outcomes. In hepatocellular carcinoma, key prognostic determinants include tumor size and number, vascular invasion, tumor differentiation, extrahepatic spread, and integrated staging systems such as BCLC. Therefore, the value of CASC2 and TUG1 as prognostic biomarkers is strengthened when their expression levels align with these parameters in a biologically coherent manner and when they plausibly reflect tumor aggressiveness rather than nonspecific liver injury [47].

Across HCC studies, reduced CASC2 expression has been repeatedly associated with adverse tumor features, including increased invasive behavior and metastatic potential, reflecting its tumor-suppressive role. Mechanistically, CASC2 downregulation facilitates oncogenic signaling through multiple pathways, including MAPK and Wnt/β-catenin, and through ceRNA mechanisms that release oncogenic microRNAs from suppression. These biological effects map closely onto clinicopathological markers of poor prognosis, such as vascular invasion and advanced stage, supporting CASC2 as a molecular correlate of aggressive phenotype [48,49].

Conversely, high TUG1 expression aligns with clinicopathological features associated with poor outcomes, including larger tumor size and more advanced clinical stage in multiple HCC cohorts. TUG1's ability to epigenetically silence tumor suppressor programs and enhance migration and invasion supports its association with high-risk pathological phenotypes. In practice, such correlations are clinically meaningful because they indicate that TUG1 is not merely an "HCC marker," but one that tracks with tumor biology relevant to recurrence and survival [42,50].

When interpreted in the framework of integrated staging, associations between these lncRNAs and BCLC stage are particularly informative. BCLC incorporates tumor burden, liver functional reserve, and performance status, and it is routinely used to guide therapeutic decisions and estimate prognosis. Evidence that blood CASC2 levels correlate with BCLC stage suggests that CASC2 may function as a molecular reflection of global disease severity and tumor aggressiveness, supporting its use in risk stratification models that complement conventional staging [32,51].

Serum AFP is an imperfect prognostic marker, but it remains a commonly used indicator of tumor activity and is incorporated into some prognostic systems. Studies reporting correlations between CASC2/TUG1 expression and AFP suggest that these lncRNAs may capture aspects of tumor biology that overlap with AFP-defined tumor activity while also providing more direct

mechanistic linkage to invasion and progression pathways. In clinical pathology terms, concordance with AFP can support face validity, whereas discordance in select subgroups may indicate that lncRNAs add orthogonal prognostic information [32,52].

Beyond size and stage, prognostic pathology increasingly recognizes that molecular features may underlie aggressive behavior even in tumors that appear similar radiologically. Processes such as epithelial–mesenchymal transition, angiogenesis, and immune evasion contribute to recurrence risk and poor survival, and lncRNAs are central regulators of these processes. Evidence linking TUG1 to EMT-related signaling and immune checkpoint regulation provides a plausible mechanism for why high TUG1 expression could associate with vascular invasion, metastatic risk, and recurrence, even when standard pathology variables are controlled [45,53].

From a practical standpoint, the strongest clinical use case is to integrate CASC2 and TUG1 with established clinicopathological parameters rather than substituting for them. In HCV-associated HCC, where cirrhosis complicates tumor assessment and limits treatment tolerability, a molecular layer that better captures biological aggressiveness could enhance decisions about intensity of surveillance, candidacy for transplantation pathways, or prioritization for adjuvant strategies. However, this requires consistent evidence that lncRNA associations with clinicopathological parameters are robust across cohorts and are not driven by preanalytical artifacts [33,54].

In summary, available evidence indicates that reduced CASC2 and elevated TUG1 are aligned with adverse clinicopathological features and integrated staging parameters that are well established as prognostic determinants in HCC. These associations support their biological relevance and suggest practical utility in molecular risk stratification, particularly when tissue and circulating assessments are interpreted together within the clinical pathology framework of staging, histology, and liver functional reserve [47,55].

Combined Prognostic Models Incorporating CASC2 and TUG1 in HCV-Associated HCC

Single biomarkers rarely provide sufficient prognostic precision in hepatocellular carcinoma because outcome is determined by multiple interacting domains: tumor aggressiveness, tumor burden, liver functional reserve, and host immune–inflammatory status. For this reason, contemporary prognostic strategies increasingly favor multivariable models that combine molecular markers with clinicopathological staging. Within this framework, pairing CASC2 (tumor-suppressive, typically decreased) with TUG1 (oncogenic, typically increased) is conceptually attractive because the two lncRNAs represent opposing biological programs that together may better capture “net malignant potential” than either marker alone [56].

A combined CASC2–TUG1 approach can be justified on biological grounds as well as empirical observations. CASC2 downregulation is associated with activation of pro-oncogenic signaling pathways and enhanced invasion and metastasis potential, whereas TUG1 upregulation promotes epigenetic repression of tumor suppressor programs and supports migration, EMT-related behavior, and immune escape. In clinical pathology terms, these processes map onto high-risk features such as vascular invasion, early recurrence after curative-intent therapy, and reduced survival, suggesting that using both markers together could enrich prognostic discrimination beyond what either provides independently [57,58].

Evidence supporting combined lncRNA modeling is strengthened by studies that demonstrate clinically meaningful associations between blood or tissue levels of these lncRNAs and integrated measures of disease severity such as BCLC stage and AFP behavior. For example, blood-based work evaluating CASC2 and TUG1 in HCC on top of HCV demonstrates that these transcripts are not only dysregulated but also correlate with clinically relevant stratifiers used in routine prognostic assessment. Although many available datasets are cross-sectional, such associations are still valuable because BCLC stage and related parameters are well established surrogates for recurrence risk and survival probability in real-world HCC management [32,56].

In practice, a clinically implementable combined model would most plausibly take one of two forms: (1) a “molecular risk score” derived from standardized expression values (e.g., low CASC2 + high TUG1 defining high-risk disease), or (2) integration of CASC2 and TUG1 as covariates in a multivariable prognostic framework together with stage, liver function, and AFP. Either approach aligns with the direction of modern biomarker development, where molecular markers are used to refine, not replace, established staging systems. Importantly, combining markers with opposite directions of effect can reduce the chance that a single-marker anomaly or technical artifact drives clinical classification [59,60].

For HCV-associated HCC specifically, combined models may be especially useful for post-treatment risk stratification. Patients may present with similar imaging features but markedly different recurrence trajectories after resection, ablation, or locoregional

therapy, particularly in cirrhotic livers where tumor biology and microenvironmental pressures are complex. In this setting, a "CASC2-low/TUG1-high" pattern could plausibly identify patients who warrant more intensive surveillance intervals or earlier consideration of escalation pathways, provided that the model is validated against hard endpoints such as recurrence-free survival and overall survival in HCV-defined cohorts [41,43].

However, translation of combined CASC2–TUG1 prognostic models requires careful attention to analytical harmonization. Differences in specimen type (whole blood vs serum/plasma), RNA extraction workflows, and normalization strategies can significantly shift measured expression values and thereby alter risk classification thresholds. In clinical pathology, this means that combined models cannot be safely exported across studies without method-matched validation, and any scoring approach must be linked to explicit preanalytical SOPs and assay performance characteristics to avoid "false-risk" assignment driven by technical rather than biological variation [35,38].

Finally, combined prognostic modeling must meet accepted reporting and validation standards to ensure reproducibility and clinical credibility. This includes transparent definition of the endpoint (overall survival, disease-free survival, recurrence), prespecified cutoffs or modeling strategy, adequate sample size, and multivariable adjustment for core clinical confounders. Without this rigor, a combined lncRNA score may appear promising but fail to generalize across centers or etiologic subgroups. Therefore, the next step for CASC2 and TUG1 is not simply additional association studies, but well-designed HCV-focused prognostic validation cohorts built to support model calibration and clinical utility assessment [61,62].

Conclusion

Hepatitis C virus–associated hepatocellular carcinoma exhibits substantial heterogeneity in clinical behavior and patient outcomes, underscoring the limitations of prognostic assessment based solely on clinicopathological staging systems. Although tumor size, stage, and liver functional reserve remain central to risk stratification, they do not fully capture the molecular drivers of tumor aggressiveness, recurrence, and survival variability observed among patients with HCV-related HCC.

Long noncoding RNAs have emerged as biologically informative markers that reflect key oncogenic and tumor-suppressive processes underlying disease progression. Within this context, CASC2 and TUG1 represent two mechanistically distinct but complementary lncRNAs with consistent associations to adverse outcomes. Reduced CASC2 expression reflects loss of tumor-suppressive control and enhanced oncogenic signaling, whereas elevated TUG1 expression mirrors activation of epigenetic and transcriptional programs that promote invasion, metastasis, and recurrence.

Evidence from tissue-based analyses indicates that CASC2 and TUG1 expression levels correlate with aggressive histopathological features and unfavorable survival outcomes, supporting their role as indicators of intrinsic tumor biology. Circulating assessments further extend their prognostic potential by enabling minimally invasive risk stratification and the possibility of longitudinal monitoring, which is particularly valuable in patients with cirrhosis where repeated tissue sampling is limited.

The greatest clinical value of CASC2 and TUG1 lies in their integration into multivariable prognostic frameworks rather than their use as standalone markers. Combined evaluation of low CASC2 and high TUG1 expression captures opposing biological pathways that jointly define high-risk disease, offering a more nuanced assessment of malignant potential than conventional markers alone. Such molecular layering has the potential to refine prognostic accuracy, inform surveillance intensity, and support personalized clinical decision-making in HCV-associated HCC.

Despite encouraging findings, translation into routine clinical practice requires further standardization of analytical methods, harmonization of tissue and circulating assays, and validation in large, HCV-specific cohorts with clearly defined survival and recurrence endpoints. As these challenges are addressed, CASC2 and TUG1 may become valuable components of molecular prognostication strategies that complement existing clinical pathology frameworks and ultimately improve outcome prediction for patients with HCV-related hepatocellular carcinoma.

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