

Review

Transforming 'Junk' DNA into Cancer Warriors: The Role of Pseudogenes in Hepatocellular Carcinoma

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Abstract. *In the dynamic landscape of hepatocellular carcinoma (HCC) or the liver cancer research, pseudogenes have emerged from the shadows of genetic obscurity to become central figures, significantly influencing the disease molecular development and clinical trajectory. This review explores a transformative shift in perspective, recognizing pseudogenes not as genetic remnants without function, but as critical regulators in the molecular underpinnings of HCC. Engaging in complex interactions such as microRNA sponging, gene expression modulation, and signaling pathway disruptions, pseudogenes orchestrate a part of the molecular complexity driving tumor genesis, progression, and drug resistance in the liver cancer. Their unique expression patterns in hepatoma tissues herald new opportunities for early HCC detection, offering insights into patient prognosis, and identifying novel targets for therapeutic intervention of this disease. Such advancements underscore the importance of pseudogenes in enriching our understanding and management of HCC, paving the way for more effective diagnostic strategies and targeted therapies in the ongoing battle against this challenging malignancy.*

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Liver cancer, known as hepatocellular carcinoma (HCC), is a global menace, claiming countless lives (1, 2). This relentless disease is closely linked to several culprits—Hepatitis B and C viruses, excessive alcohol, obesity, and diabetes (1). Astonishingly, most HCC cases are attributed to Hepatitis B and C. These viruses ravage the liver, eventually unleashing HCC. A sex bias in HCC is evident, favoring men due to habits like heavy drinking, smoking, and higher cirrhosis rates among males (2). Regrettably, late-stage diagnosis limits treatment effectiveness (3). What's desperately needed are groundbreaking biomarkers for early detection and improved HCC management.

In the intricate genetic world, pseudogenes, once dismissed, have emerged as unexpected heroes, holding the key to HCC's mysteries. Recent research reveals their dynamic roles in human cancers, acting as both villains and heroes. They manipulate genes on multiple fronts, from DNA to RNA, even meddling with protein production (4). Pseudogenes are extraordinary; they're conserved across species, with unparalleled power to influence genes linked to cancer's intricate dance (5). Highlighting these unsung pseudogene heroes has revolutionized liver cancer detection and prognosis. As researchers navigate the challenging terrain of HCC, pseudogenes have emerged as game-changers, providing hope in the battle against this relentless adversary. They may hold the key to early diagnosis and innovative therapies, reshaping the future of HCC management.

Pseudogenes: The Genetic Mavericks

Pseudogenes, often dismissed as genetic relics, are the intriguing byproducts of gene duplication, retro transposition, and mutations. In most cases, these genetic events render pseudogenes incapable of encoding fully functional proteins (4). What sets them apart is their remarkable presence; pseudogene transcripts are expressed abundantly in tumor tissues or being exclusive to these cancerous regions, making

their expression cancer-specific (4, 5). Positioned within genomic regions prone to events like chromosomal deletion, mutation, duplication, and inversions, pseudogenes hold a secret genetic history.

Classified based on chromosomal rearrangements, pseudogenes fall into three intriguing categories: non-processed, processed, and unitary. Non-processed pseudogenes emerge when a parental gene spawns a clone on the same chromosome, lacking the necessary elements like promoter sequences, splicing sites, or marred by mutations, rendering them incapable of encoding functional proteins. Processed pseudogenes, however, take up residence on a different chromosome. Their origin story involves parental RNA undergoing reverse transcription to form cDNA, but this journey accumulates a plethora of mutations, ultimately altering the function of the encoded proteins. Within the vast human genome, over 18,000 pseudogenes are found, with roughly two-thirds belonging to the processed category (4-6). An example is ANXA2P2, a highly homologous processed pseudogene of the functional annexin A2 gene. It sports point mutations post retrotransposition and is linked to abnormal expression in various cancers, such as liver, pancreatic, prostate, gastric, and breast cancer (7).

Unitary pseudogenes emerge when the parental gene's function fades into oblivion due to chromosomal mutations. In the intricate web of biology, pseudogenes have long played pivotal roles in fundamental processes like embryonic development, cell cycle regulation, cell proliferation, differentiation, and cell death (7, 8). In the realm of cancer, these enigmatic genetic elements become agents of change. They exchange DNA with parental genes through gene conversion, homologous recombination, insert themselves into coding or non-coding regions, and give rise to new gene product variants linked to cancer or the abnormal amplification of oncogenes (7). Beyond this, pseudogenes step into the spotlight as competitive endogenous RNAs. They engage in molecular duels, binding with miRNA, RNA binding proteins, or vying for the translational machinery. Emerging evidence hints at their potential roles in regulating wild-type genes, oncogenes, and tumor suppressor genes (9, 10). Pseudogenes, once sidelined, now emerge as genetic mavericks with an evolving and pivotal role in our understanding of cancer biology.

Pseudogenes in Hepatocellular Carcinoma: Deciphering the Molecular Links

The intricate world of HCC reveals a profound connection with pseudogenes, shedding light on their recurrent and significant dysregulation. Advancements in microarray technology and bioinformatics have unveiled the genome-wide expression of these pseudogenes (11). Among them, ANXA2P2, a processed pseudogene closely related to annexin A2, has drawn attention due to its heightened expression in

HCC, particularly in cases associated with metastasis and invasion (7). Conversely, pseudogenes like DUXAP10 have been found to be up-regulated in HCC, stimulating the proliferation of hepatic cells through the activation of the PI3K/AKT pathway. On the contrary, elevated levels of PDIA3P1 in HCC tissues hinder the p53 pathway, leading to apoptosis suppression and increased proliferation of liver cancer cells (12, 13).

Mechanistically, certain pseudogenes have been revealed as microRNA sponges, contributing to HCC pathogenesis. For instance, RACGAP1P, over-expressed in HCC tissues, functions as a decoy for miR-15a-5p, consequently promoting cell growth and migration through the activation of the Rho/ERK pathway (14). DUXAP8, found in tumor cells, acts as a sponge for miR-490-5p, resulting in increased BUB1 expression in HCC (15). Another player, OCT4-pg4, is over-expressed in HCC cells, regulating OCT4 expression by sequestering miR-145, thereby facilitating the growth and tumorigenicity of HCC cells (16). INTS6P1, in competition with its INTS6 cognate gene for miR-17-5p, accelerates tumorigenesis, with down-regulated expression observed in HCC tissues (17, 18). The microRNA cluster miR-142, miR-155, and miR-182 is modulated by Aurora kinase A pseudogene (AURKAPS1), driving the activation of the ERK pathway and promoting cell movement, migration, and invasion due to heightened AURKAPS1 levels in tumor cells (19). Pseudogene PPIAP22, a 99% identical counterpart of peptidylprolyl isomerase A (PPIA), is found in the cytoplasm, nucleus, and exosomes. It's up-regulated expression acts as a sponge for miR-197-3p, contributing to cancer cell metastasis and immune cell infiltration, particularly macrophage infiltration, through the CCL15-CCR1 or CXCL12-CXCR4/CXCR7 pathways (20).

Intriguingly, E2F3P1, an E2F3 pseudogene residing on chromosome 17, plays a pivotal role in regulating cell cycle control points and fostering cell proliferation. Genetic variants of E2F3P1, such as the rs9909601 allele A, confer a more robust prognosis. E2F3P1 also influences E2F3 expression by acting as a sponge for miR-24, miR-149, and miR-892b (21, 22). MSTO2P, highly expressed in HCC cells and tissues, exerts a multifaceted impact by enhancing E-cadherin expression, reducing N-cadherin levels, and modulating vimentin expression. Furthermore, it boosts protein expression in the PI3K/AKT/mTOR pathway, ultimately promoting hepatoma cell proliferation (23). The pseudogene HSPB1P1's abnormal expression emerges as a regulator of EZH2 gene expression in HCC, amplifying tumor cell proliferation rates (24).

Conversely, up-regulation of pseudogene POU5F1B or OCT4-pg1 is linked to increased cell proliferation in HCC through the activation of the AKT pathway (25). Meanwhile, the down-regulation of ubiquitin-proteasome pathway associated pseudogene (UPAT) in HCC tissues fosters

Table I. *Pseudogenes and their implications in hepatocellular carcinoma (HCC) pathogenesis.*

Pseudogenes	Involvement in pathological processes
ANXA2P2	Impact on cell proliferation, motility, apoptosis, and regulation of microtubules expression (specifically β tubulin and F actin)
DUXAP10	Association with cell proliferation and modulation of cell-cycle
PDIA3P1	Influence on cell proliferation, migration, and invasive potential
RACGAP1P	Role in promoting cell growth and facilitating cell migration
DUXAP8	Contribution to cell proliferation and regulation of the cell cycle
OCT4-pg4	Influence on cell proliferation and promotion of colony formation
INTS6P1	Impact on cell growth, migration, and cell survival
AURKAPS1	Involvement in cell movement, migration, and promotion of invasion
PPIAP22	Contribution to tumor cell metastasis and infiltration by immune cells, particularly macrophages, via CCL15-CCR1 or CXCL12-CXCR4/CXCR7 pathways
E2F3P1	Role in cell proliferation and modulation of the cell cycle, genetic variants (<i>e.g.</i> , rs9909601 allele A) associated with a more favorable prognosis
MSTO2P	Multifaceted impact, including effects on cell proliferation, apoptosis, metastasis, and invasion
HSPB1P1	Influence on cell proliferation, apoptosis, and modulation of the cell cycle
POU5F1B	Association with enhanced cell proliferation
UPAT	Contribution to cell migration, invasion, epithelial-mesenchymal transition (EMT) process, and properties associated with cancer stem cells (CSCs)

cellular migration, invasion, epithelial-mesenchymal transition, and cancer stem cell characteristics. UPAT, through the ubiquitin-proteasome pathway, aids in ZEB1 degradation, while ZEB1, in turn, transcriptionally restrains UPAT expression (26). The comprehensive exploration and therapeutic targeting of pseudogenes represents a groundbreaking frontier in the early diagnosis and management of liver cancer. This innovative approach illuminates the intricate mechanisms that underlie HCC progression, offering new avenues for intervention and raising hope for improved clinical outcomes. For a detailed summary of the specific pseudogenes mentioned and their implications in HCC pathogenesis, refer to Table I.

Pseudogene Expression Studies for HCC Diagnosis

The primary goal of liver cancer research is to develop early diagnostic biomarkers because HCC is often diagnosed at an advanced stage, leaving patients with limited treatment options. Currently, available diagnostic markers include Alpha-Fetoprotein (AFP), ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI)s, but they have limitations such as AFP's low sensitivity (36-64%) and specificity (79-91%) due to fluctuating serum levels, ultrasonography's ability to detect only small nodules (<3 cm), and the high cost of CT and MRI scans (17). To improve early detection and screening for HCC, pseudogenes have emerged as potential diagnostic markers. Numerous studies have shown dysregulation of pseudogenes during HCC initiation and development. These studies often compare pseudogene expression in HCC with that in healthy liver or nearby peritumoral tissues.

Information about pseudogene mRNA expression levels was collected from The Cancer Genome Atlas (TCGA) RNA sequence database. To assess the prognostic and diagnostic value of these aberrantly expressed pseudogenes, researchers conducted Kaplan-Meier curve analyses and receiver operating characteristic (ROC) curve analyses. Statistical tests were performed, and *p*-values were calculated to identify pseudogenes with abnormal expressions in HCC. The results obtained from these analyses were validated using cell-based assays. Additionally, for some pseudogenes, researchers constructed protein-protein interaction networks using bioinformatics databases and conducted gene pathway analyses to understand the biological pathways involving these pseudogenes (12). Below is an overview of studies investigating pseudogene expression levels in primary tissues, serum, and plasma for HCC diagnosis.

Primary Tissue Insights

The protein ANXA2P2 has been consistently reported to be present at higher levels in tissues affected by HCC when compared to non-cancerous tissue (7). In a comprehensive examination, the long non-coding RNA (lncRNA) known as DUXAP10 was found to be significantly over-expressed in HCC after assessing its presence in a range of six HCC cell lines and 46 matched pairs of HCC and adjacent normal liver tissues using quantitative reverse transcription PCR (qRT-PCR) (12). This increased expression of DUXAP10 in cancerous tissues was not only significant but also proved to be a determinant factor for patient prognosis, with high levels of this lncRNA associated with a decrease in overall survival, as determined by Kaplan-Meier survival analysis

(12). When the function of DUXAP10 was inhibited, a notable cell cycle arrest at the G1 checkpoint occurred, alongside a consequential reduction in the expression of the AKT protein, a key player in cell proliferation and survival pathways. These findings suggest that DUXAP10 could be a therapeutic target for interventions in HCC. Moreover, the strength of DUXAP10's expression as a reliable biomarker for HCC diagnosis was reinforced through receiver operating characteristic (ROC) curve analysis, which showed a high area under the curve (AUC) of 0.887, indicating a strong predictive value (12).

Parallel to these discoveries, the study conducted by Kong *et al.* unveiled an up-regulation of PDIA3P1 in both HCC cell lines and patient tissue samples. This was evidenced by the analysis of 90 paired samples of HCC tissues and adjacent non-cancerous liver tissues, where more than half exhibited a higher expression of PDIA3P1 in the tumor tissues (13). Similar to DUXAP10, the increased levels of PDIA3P1 were also associated with a negative impact on patient survival rates. Kaplan-Meier analysis revealed that patients with elevated PDIA3P1 expression had a significantly lower overall survival rate, suggesting that PDIA3P1, like DUXAP10, may serve as a potential prognostic marker and therapeutic target in HCC (13).

Analysis of the pseudogene RACGAP1P revealed significant up-regulation in cancer cells in cases of early recurrent disease (<2 years) compared to samples from patients with no evidence of recurrence within four years. Elevated RACGAP1P expression was associated with slightly shorter overall survival and disease-free intervals (14). Up-regulation of DUXAP8 in HCC tissues was linked to shorter overall survival and involvement in various cell cycle-related biological processes. Gene ontology analysis revealed that DUXAP8 regulated BUB1, a cell cycle regulatory gene, and promoted cell proliferation and metastasis in HCC. Bioinformatics and cell-based studies identified the interaction of miR-490-5p with BUB1 and DUXAP8. Specifically, DUXAP8 sequestered miR-490-5p, leading to enhanced BUB1 expression (15).

Immunohistochemical staining of OCT4-pg4 in 54 HCC tissues and 10 normal tissues showed up-regulation in 21 tumor tissues. Reporter gene assays revealed that OCT4-pg4 had binding sites for miR-145, contributing to the positive regulation of OCT4 by OCT4-pg4. Kaplan-Meier analysis demonstrated an inverse relationship between OCT4-pg4 expression and overall survival in HCC (16). The expression of INTS6P1 in HCC tissues was significantly down-regulated according to microarray analysis, a finding validated by qRT-PCR in 26/33 pairs of HCC tissues compared to normal tissues (17, 18). qRT-PCR detected up-regulated expression of AURKAPS1 in HCC tissues, and functional assays demonstrated its role in cancer cell migration and invasion. AURKAPS1 was found

to increase RAC1 protein expression by competitively binding to miRNAs (miR-142, miR-155, miR-182), promoting tumor development and progression (19). PPIAP22, a pseudogene, was significantly up-regulated in HCC tumor tissues compared to normal tissues. mRNA levels of both PPIAP22 and PPIA were considerably higher in tumor tissues (20).

Using the TCGA database and qRT-PCR, MSTO2P expression was found to be significantly higher in HCC tissues and cell lines compared to normal tissues and cell lines. Elevated MSTO2P levels were associated with poorer survival rates (23). Over-expression of pseudogene HSPB1P1 was observed in HCC tissues, leading to poorer overall survival in HCC patients. Gene analysis revealed that HSPB1P1 acted as a ceRNA, regulating EZH2 mRNA and protein levels, thereby enhancing cancer cell proliferation (24). POU5F1B expression was significantly higher in HCC tissues, as confirmed by TCGA data. Kaplan-Meier curve analysis demonstrated that up-regulation of POU5F1B correlated with shorter survival time in HCC patients. This was further validated using qRT-PCR and western blot assays (24).

In HCC tissues, the expression of the pseudogene UPAT was down-regulated and associated with shorter recurrence-free survival (25). Integrating plasma-based pseudogene markers into clinical practice has the potential to improve early detection and monitoring of HCC, ultimately enhancing patient care and management. Further research in this direction may yield valuable insights into the development of non-invasive screening and monitoring strategies for HCC.

Serum Clues

Evidence acquired from the TCGA database of 49 HCC patients illustrated that ANXA2P2 expression in HCC tissue was substantially up-regulated. Moreover, Kaplan-Meier curves produced using TCGA data revealed that elevated levels of ANXA2P2 expression were associated with low overall survival of patients with HCC and these results were validated using qRT-PCR. Several studies have reported that serum ANXA2P2 can serve as an early detection biomarker for HCC. Additionally, transwell and wound healing assays were performed to study the tumorigenic properties of this pseudogene and the results showed that ANXA2P2 promotes HCC cell migration and invasion but not proliferation (7).

Plasma Studies

To explore the feasibility of using INTS6P1 as a novel biomarker for patients with HCC in peripheral blood or plasma, INTS6P1 expression levels were investigated using qRT-PCR in 50 HCC patient plasma samples (HbsAg

Table II. *Pseudogenes expression levels in primary tissues, serum, and plasma for hepatocellular carcinoma (HCC) diagnosis.*

Pseudogenes	Regulation	Source	Involvement in pathological processes	Diagnostic biomarkers
ANXA2P2	Up-regulated	Tissue, serum	Cell growth and division, motility, apoptosis, microtubules expression (β tubulin and F actin)	Differentiate patients with HCC from healthy control
DUXAP10	Up-regulated	Tissues	Cell growth and division, cell-cycle	Differentiate patients with HCC from healthy control
PDIA3P1	Up-regulated	Tissues	Cell growth and division, cell invasion and metastasis	Differentiate patients with HCC from healthy control
RACGAP1P	Up-regulated	Tissues	Cell growth and migration	Differentiate HCC and control
DUXAP8	Up-regulated	Tissues	Cell growth, division, and cell cycle	Differentiate patients with HCC from healthy control
OCT4-pg4	Up-regulated	Tissues	Cell growth, division, and colony formation	Differentiate patients with HCC from healthy control
INTS6P1	Down-regulated	Tissues, plasma	Cell growth, migration, survival	Differentiate HCC from healthy tissues in a primary tissue sample and plasma samples, Differentiate chronic HBV infection patients from normal controls
AURKAPS1	Up-regulated	Tissues	Cell movement, migration, invasion	Differentiate HCC and control
PPIAP22	Up-regulated	Tissues	Tumor cell metastasis, immune-cells infiltration	Differentiate patients with HCC from healthy control
MSTO2P	Up-regulated	Tissues	Cell growth, division, and cell-cycle	Differentiate patients with HCC from healthy control
HSPB1P1	Up-regulated	Tissues	Cell growth and division, programmed cell death, cell invasion and metastasis	Differentiate patients with HCC from healthy control
POU5F1B	Up-regulated	Tissues	Cell growth and division, programmed cell death, cell cycle	Differentiate HCC and healthy patients
UPAT	Down-regulated	Tissues	Cell growth and division Cell invasion and metastasis, Epithelial- Mesenchymal Transition process, and Cancer Stem Cells properties	Differentiate HBV-related HCC from healthy controls

positive with shown pathology), 20 HBV patient plasma samples that were HBsAg-positive, and 30 healthy controls (AFP <10 ng/ml, HBsAg-negative) (17). As a result, INTS6P1 was observed to be down-regulated in patients with HCC as compared with normal controls and non-HCC individuals. Currently, no other biomarker is capable of substituting serum AFP to diagnose HCC. Nevertheless, studies conducted on INTS6P1 indicated that this pseudogene could act as an efficient biomarker for HCC screening and detection (17).

Table II encapsulates the findings from various studies on pseudogene expression in HCC, highlighting their up-regulation or down-regulation, sources of detection, and their roles as potential diagnostic biomarkers. Overall, these results indicate the possibility of the use of pseudogenes in HCC as a diagnostic marker. Although further efforts are needed to incorporate these results into clinical practice, including the right sample to be used (plasma, serum, or other body fluid). Furthermore, systematic studies involving trials in several hospitals, clinics, or research institutions should be performed to determine the strength of pseudogenes as a new biomarker for diagnosis.

Pseudogenes as HCC Prognostic Predictors

Pseudogenes also hold promise as prognostic indicators in HCC, complementing their diagnostic utility. Key clinical parameters, such as TNM stage (comprising tumor size, nodal and distant metastasis), tumor invasion, recurrence, and overall survival have shown significant associations with changes in pseudogene expression patterns. For example, Wang *et al.* demonstrated that heightened ANXA2P2 expression is correlated with shortened overall survival in HCC patients, with a notable association with the tumor node metastasis (TNM) stage (7). Elevated DUXAP10 levels in HCC specimens are linked to tumor invasion and advanced disease stages (Stage III + IV) (12). Similarly, investigations into PDIA3P1 revealed alignment with clinical parameters, including tumor size, metastasis, and TNM stage expression (13). Increased RACGAP1P expression is associated with advanced tumor stages (T3-T4), higher clinical stages (III-IV), and abnormal AFP levels. Elevated RACGAP1P levels also correlate with shorter overall survival and progression-free survival (14). The up-regulation of DUXAP8 and OCT4-pg4 in cancerous tissues

is associated with Stage II and Stage III disease, leading to poorer overall survival in patients with HCC (15, 16). Conversely, a significantly reduced expression level of INTS6P1 serves as an early indicator for patients with HBV who require HCC screening (18).

Furthermore, a higher AURKAPS1 expression level is positively linked with tumor size and TNM stage (19). Individuals with HCC who exhibit high levels of PPIAP22 expression experience significantly shorter disease-free survival (DFS) and overall survival (OS). The expression of PPIA and PPIAP22 also correlates favorably with the clinical stage of patients with HCC. Notably, only the number of tumors showed a significant correlation with mRNA expression levels of PPIAP22 and PPIA among the clinical indicators examined (20). Studies on the E2F3P1 pseudogene have predicted that a genetic variant, specifically the A allele of rs9909601, is significantly associated with improved prognosis and can serve as a genetic marker for HCC prognosis (21, 22). These studies also suggest that single nucleotide polymorphisms in pseudogenes may influence their expression or the expression of their parent protein-coding genes, playing a substantial role in human cancer growth and progression (21, 22).

In contrast, over-expression of the MSTO2P pseudogene, compared to patients with reduced MSTO2P expression, is associated with lower 3-year and 5-year survival rates (23). Additionally, the up-regulation of EZH2, regulated by the pseudogene HSPB1P1 in HCC tissues, is associated with worst overall survival of patients with HCC (24). Furthermore, the findings indicate that POU5F1B expression is elevated in both HCC cells and tissues and is associated with poorer overall survival outcomes (25). In the context of HBV-related HCC, down-regulation of UPAT indicates a poor prognosis for HCC related to HBV (26). Statistical analyses, including Chi-Square tests and *t*-tests, have shown correlations between low UPAT expression and various clinical factors, such as the presence of HBV-DNA in venous blood, tumor lesions, larger tumor size (≥ 5 cm), microvascular invasion (MVI), portal vein tumor thrombosis (PVTT), progressive TNM staging, and Barcelona Clinic Liver Cancer (BCLC) staging. Additionally, patients with low UPAT expression experienced shorter recurrence-free survival times (26).

Pseudogenes such as CTB-63M22.1 might play a role in modulating cancer stemness and thereby influence the progression of HCC. Although the exact mechanisms and effects on patient prognosis are still not fully understood, this suggests the possibility of CTB-63M22.1 as an emerging therapeutic target and a potential prognostic biomarker for HCC (27). In hepatocellular carcinoma tissues and their corresponding cell lines, PLGLA was consistently found to be expressed at lower levels. This diminished expression of PLGLA was linked with the progression of the tumor and a

decline in patient survival rates. Reintroducing PLGLA notably hindered the proliferation, migration, and invasion of cancer cells. At the molecular level, PLGLA potentially binds with miR-324-3p, serving as a molecular sponge, thereby indirectly boosting the expression of GLYATL1 (28).

Suppressing GBAP1 expression in HCC cells was found to significantly reduce their proliferation and trigger apoptosis due to the inactivation of the PI3K/AKT signaling pathway, a phenomenon consistent in both controlled environments and animal models (29). This evidence, coupled with the fact that elevated METTL3 levels have been associated with adverse HCC prognosis (30, 31), underscores GBAP1's oncogenic influence. METTL3's role in up-regulating GBAP1 involves the methylation of GBAP1 RNA, marking it for recognition by IGF2BP2. The clinical implications of increased GBAP1 are substantial, including its correlation with more extensive tumor growth, venous infiltration, advanced TNM staging, and overall poorer patient outcomes. GBAP1's facilitation of HCC progression is further manifested by its ability to act as a competitive endogenous RNA, sequestering miR-22-3p to up-regulate BMPR1A expression and activate the BMP/SMAD signaling pathway in HCC cells (32). Collectively, these interactions position GBAP1 as a critical player in HCC pathogenesis and a potential target for prognostic assessment and therapeutic intervention.

The pseudogene KR1B10P1, an isoform of the oncogenic gene AKR1B10, is typically inactive in normal liver cells but found to be expressed in HCC tissues and cell lines, where it aligns with the activity of its parental gene. Elevated AKR1B10P1 (33) levels are associated with poor clinical outcomes in HCC, such as larger tumors, higher serum AFP, advanced TNM stages, and vascular invasion. Experiments altering KR1B10P1 expression confirmed its role in HCC cell growth. The transcription factor SOX4 has been identified as a key activator of AKR1B10P1 and a target of the tumor-suppressive miR-138. A positive feedback loop involving AKR1B10P1 and miR-138 enhances SOX4's effects on tumor proliferation. This newly discovered AKR1B10P1/miR-138/SOX4 regulatory circuit offers promising targets for HCC treatment strategies (33).

The pseudogene SNRPFP1, a counterpart of SNRPF, has been linked to high levels of a specific long non-coding RNA in HCC. Its up-regulation in HCC tissues and cell lines correlates with a negative prognosis. Inhibition of SNRPFP1 leads to reduced proliferation and increased apoptosis in HCC cells, as well as diminished cell motility. An inverse relationship exists between SNRPFP1 (34) and the tumor-suppressing miR-126-5p, with SNRPFP1 binding to miR-126-5p as a molecular sponge. Neutralizing miR-126-5p counteracts the growth and apoptosis effects of SNRPFP1 depletion, indicating that SNRPFP1 contributes to HCC progression by sponging miR-126-5p (34). UBE2MP1 is

Table III. *Pseudogenes as prognostic indicators in hepatocellular carcinoma (HCC).*

Pseudogenes	Regulation	Source	Prognostic biomarkers
ANXA2P2	Up-regulated	Tissue, serum	Poor prognosis and TNM stage
DUXAP10	Up-regulated	Tissues	Reduced overall survival, higher pathologic stage, vascular invasion
PDIA3P1	Up-regulated	Tissues	Worse overall survival, larger tumor size, metastasis, advanced TNM stage
RACGAP1P	Up-regulated	Tissues	Shortened survival time, larger tumor size, elevated AFP levels, advanced clinical stage
DUXAP8	Up-regulated	Tissues	Diminished overall survival time, increased mutation burden, higher tumor grade and stage
OCT4-pg4	Up-regulated	Tissues	Poor prognosis, associated with HCC stage
INTS6P1	Up-regulated	Tissues, plasma	Indicates an alarming state
AURKAPS1	Up-regulated	Tissues	Poor prognosis, linked to TNM stage and tumor size
PPIAP22	Up-regulated	Tissues	Reduced overall and disease-free survival, associated with clinical-stage and number of tumors
E2F3P1 (A allele of genetic variant rs9909601)	Up-regulated	Tissues	Improved prognosis and extended survival
MSTO2P	Up-regulated	Tissues	Lower survival rates and unfavorable prognosis
HSPB1P1	Up-regulated	Tissues	Adverse overall survival outcomes
POU5F1B	Up-regulated	Tissues	Poor prognosis
UPAT	Down-regulated	Tissues	Decreased recurrence-free survival and unfavorable prognosis
PLGLA	Down-regulated	Tissues	Associated with tumor progression and poor prognosis
METTL3	Up-regulated	Tissues	Poor HCC prognosis
GBAP1	Up-regulated	Tissues	Linked to tumor size, venous infiltration, TNM stage, and poor prognosis; promotes metastasis and growth
AKR1B10P1	Up-regulated	Tissues	Correlates with large tumor size, high serum AFP, advanced TNM stage
SNRFP1	Up-regulated	Tissues	Correlated with poor outcomes, promotes cell proliferation and apoptosis resistance
UBE2MP1	Up-regulated	Tissues	Adverse correlation with patient outcomes, influences cell proliferation and apoptosis resistance

notably over-expressed and linked with poor patient prognosis. Inhibiting UBE2MP1 expression in HCC cells significantly hampers their proliferation and survival, suggesting its role in cancer progression. UBE2MP1 also appears to regulate the miR-145-5p/RGS3 pathway, potentially impacting HCC cell growth, pointing to its potential as a therapeutic target (35).

Understanding the prognostic impact of pseudogenes on HCC can significantly enhance patient management strategies, offering insights into tumor behavior, potential for recurrence, and overall survival. Table III summarizes these pseudogenes, providing a clear overview of their prognostic significance.

Pseudogenes Potential in HCC Therapy Monitoring

The unique biological mechanisms through which pseudogenes regulate cell growth and gene expression during liver cancer progression present both distinct therapeutic opportunities and challenges for novel drug development. When various doses of cancer-targeted drugs (sorafenib, regorafenib, and lenvatinib) were administered, there was no significant difference in relative cell proliferation between the si-ANXA2P2 group and the siRNA negative control group. This suggests that ANXA2P2 may not be primarily

involved in the signaling pathways associated with these targeted drugs or may not occupy a central position within these pathways (7). In simpler terms, the effectiveness of sorafenib, regorafenib, and lenvatinib in HCC cells was not compromised by ANXA2P2 (7). Currently, there is limited documentation regarding the potential of pseudogenes as therapeutic targets in HCC. Consequently, it remains challenging to determine the practical application of pseudogenes in monitoring therapeutic responses.

Future of Pseudogenes in HCC

While harnessing pseudogenes as robust biomarkers for HCC detection, prognosis, and therapeutic tracking has shown significant promise, several pivotal considerations demand comprehensive exploration. Among these considerations, standardizing techniques such as quantitative real-time PCR and other cell-based assays, which are pivotal for assessing pseudogene expression, remains a critical step. Achieving this standardization is essential to ensure consistent and reliable results across diverse laboratories and clinical settings. Furthermore, one of the ongoing challenges is determining the optimal specimen type—whether it be tissue, serum, or plasma—for obtaining precise HCC biomarker data. Primary tumor samples offer valuable insights into the

true dynamics of tumor growth and invasion. However, the inherent complexity of obtaining entirely pure cancerous tissue, given the presence of other circulating cells, has prompted researchers to consider specimens from plasma or serum as a pragmatic choice. Thus, significant research involves focusing on pseudogene expression within these samples. Nonetheless, the formidable cost associated with whole-genome sequencing technologies presents an obstacle that requires careful consideration. To solidify the role of pseudogenes as clinical biomarkers, multifaceted studies encompassing diverse pseudogene panels and spanning different clinical stages of patients with HCC are imperative. Robust, large-scale investigations involving substantial clinical populations will serve as the litmus test to validate research findings and assess their clinical applicability.

Moreover, as we navigate the path forward for pseudogenes in HCC, addressing these challenges and expanding our knowledge base will be instrumental in fully realizing their potential within clinical practice. Ultimately, this advancement will significantly enhance our ability to detect, prognosticate, and effectively manage hepatocellular carcinoma, a promising development in the field of oncology.

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Conflicts of Interest

As the sole Author of this manuscript, I, Kritika Chugh, hereby declare that there are no conflicts of interest related to the content of this review article. These encompass any financial, personal, or professional interests that might potentially influence the presented work. In accordance with the policy of the journal, I also confirm that no member of the journal's Editorial Leadership has co-authored or contributed to this manuscript, ensuring editorial independence and adherence to the journal's ethical standards. This statement is provided to ensure transparency and maintain the high ethical standards set by the *International Institute of Anticancer Research*.

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