



ONCOLOGY

Dysregulation of selected lncRNAs and Hippo/EMT-associated genes in hepatocellular carcinoma patients

Ana Maria Ciurdorean^{1,2}, Paul Chiroi², Liviuta Budisan², Ekaterina Isachesku², Alexandra Trif², Stefan Strilciuc², Daniel Gabriel Cosma³, Ioana Rusu⁴, Andra Ciocan^{1,5}, Razvan Ciocan⁶, Ioana Berindan-Neagoe^{2,7,8}, Nadim Al Hajjar^{1,5}

1) 3rd Dept. of Surgery, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Department of Genomics, MEDFUTURE Institute for Biomedical Research, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

3) Faculty of Medicine, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

4) Department of Pathology, Octavian Fodor Regional Institute of Gastroenterology and Hepatology, Cluj-Napoca, Romania

5) Department of Surgery, Octavian Fodor Regional Institute of Gastroenterology and Hepatology, Cluj-Napoca, Romania

6) Department of Surgery-Practical Abilities, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

7) Doctoral School, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

8) Romanian Academy of Medical Sciences, Biomedical Sciences, Bucharest, Romania

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Address for correspondence:

Ioana Berindan-Neagoe
ioana.neagoe@umfcluj.ro

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Abstract

Background and aim. Liver cancer remains the sixth most common malignancy and the third leading cause of cancer-related death worldwide, with hepatocellular carcinoma accounting for most cases. Long non-coding RNAs regulate hepatocarcinogenesis by modulating proliferation, epithelial–mesenchymal transition, and invasion. This study aimed to characterize the expression of three such transcripts (MALAT1, NEAT1, NKILA) and two protein-coding genes of the Hippo/EMT axis (YAP1, SNAIL) in a single-center Romanian cohort of hepatocellular carcinoma patients and to interpret the resulting pattern in relation to the existing literature.

Methods. Tumoral and non-tumoral liver tissue samples were obtained from patients with non-metastatic hepatocellular carcinoma undergoing surgical resection. Total RNA was extracted using TRIzol, reverse-transcribed, and quantified by SYBR Green–based qRT-PCR on a QuantStudio 7 Flex platform. Relative expression was calculated by the $\Delta\Delta C_t$ method, normalized to B2M and 18S rRNA, and compared between the two groups using GraphPad Prism; $p < 0.05$ was considered significant.

Results. 39 tumoral and 39 non-tumoral tissue samples were analyzed. MALAT1 ($p = 0.0008$), YAP1 ($p = 0.0016$), and SNAIL ($p = 0.0365$) were significantly overexpressed in tumoral tissue, whereas NEAT1 was significantly reduced ($p = 0.0320$). NKILA showed no significant difference between groups ($p = 0.3592$). Effect sizes were graded, with the strongest separation for MALAT1 and YAP1 and more modest differences for SNAIL and NEAT1.

Conclusion. MALAT1, YAP1, and SNAIL overexpression in hepatocellular carcinoma was confirmed in a single-center cohort from Romania, including predominantly early-stage subjects. The reduction of NEAT1 diverges from most prior reports and may reflect isoform-specific detection or the molecular abnormality of the cirrhotic tissue comparator. Future work should incorporate isoform-specific assays, etiology-stratified cohorts, and outcome correlation.

Keywords: hepatocellular carcinoma, RNA, long noncoding, gene expression profiling, epithelial-mesenchymal transition, Hippo signaling pathway, real-time polymerase chain reaction

Background and aims

Primary liver cancer is still one of the most lethal malignancies worldwide. According to GLOBOCAN 2022 estimates, it ranked as the sixth most diagnosed cancer and the third leading cause of cancer death globally, with an age-standardized incidence of 12.7 per 100,000 in men and 4.8 per 100,000 in women. Hepatocellular carcinoma (HCC) accounts for most of these cases, and the global mortality-to-incidence ratio of roughly 0.86 reflects late presentation (diagnostic gap) and the limitations of available treatments [1].

The etiological landscape of HCC has shifted. Chronic hepatitis B and C virus infections, as well as alcohol-related liver disease, remain dominant drivers, but metabolic dysfunction-associated steatotic liver disease (MASLD) and steatohepatitis (MASH) now account for a growing proportion of cases in Europe and North America [2]. This heterogeneity matters because tumors arising from different etiological backgrounds differ in their mutational spectra, immune microenvironments, and the non-coding transcriptome [3,4].

Despite staging and therapeutic improvements recognized in the 2022 update of the Barcelona Clinic Liver Cancer system [5], many patients are still diagnosed outside the curative window [6]. Systemic therapy has advanced significantly over the past five years. For example, Atezolizumab combined with bevacizumab showed a hazard ratio of 0.58 for death compared with sorafenib in IMbrave150 [7]; tremelimumab with durvalumab extended median overall survival to 16.4 months in HIMALAYA (HR 0.78); and nivolumab with ipilimumab achieved a median overall survival of 23.7 months in CheckMate 9DW (HR 0.79) [8,9]. Although these improvements are substantial, most responses are not durable, and no molecular biomarker currently guides first-line therapy. Because alpha-fetoprotein levels and ultrasound screening are inadequate for detecting early lesions, tissue-level molecular analysis is essential for understanding tumor biology and biomarker discovery.

Long non-coding RNAs (lncRNAs) are transcripts exceeding 200 nucleotides with limited coding potential that have emerged as a compelling class of candidates. They are more tissue- and tumor-restricted in their expression than protein-coding messenger RNAs, and they act via diverse mechanisms including chromatin scaffolding, transcriptional and post-transcriptional regulation [10], and sequestration of microRNAs [11,12].

MALAT1 is one of the most well-studied lncRNAs. Lai et al. found overexpression in 112 HCC cases and identified high MALAT1 levels as an independent predictor of recurrence after liver transplantation [13]. Similarly, Malakar et al. described a proto-oncogenic mechanism involving upregulation of SRSF1 and activation of mTORC1 [14]. NEAT1 shows a complex pattern: several primary studies associate its overexpression with vascular invasion and advanced stage [15–18], but a thorough analysis of 57 microarray and RNA-sequencing datasets revealed that

NEAT1 is significantly decreased in HCC [19]. NKILA, which acts as a cytoplasmic brake on NF-κB signaling, has been reported to be downregulated in a paired HCC series and to be associated with vascular invasion and poorer survival [20].

YAP1, the nuclear effector of the Hippo pathway, is overexpressed in most HCC cases and acts as an independent prognostic marker [21]. *SNAIL* encodes SNAIL, a key transcriptional repressor of E-cadherin, with its expression in HCC tissue linked to portal vein invasion and early recurrence after resection [22].

The aim of the present study was to quantify the expression of lncRNAs MALAT1, NEAT1, NKILA, and the protein-coding genes of the Hippo/EMT axis, *YAP1* and *SNAIL*, in tumor and non-tumor liver tissues from a single-center, non-metastatic and early-stage-dominated Romanian HCC cohort and to interpret the resulting expression patterns in light of the published literature, with attention to transcripts that exhibit discordant behavior.

Methods

Study cohort and sample processing

We obtained tumoral and non-tumoral HCC tissue biopsies from the patients described below (Table I). Only curative surgical resections were performed. The tissue was fresh-frozen within about 10 to 15 minutes after the specimen was removed. The sections were made from the middle of the tumor by the Pathologist and from the adjacent macroscopically non-tumorous liver parenchyma. We aliquoted these samples, suspended them in TRIzol Reagent, snap-froze them in liquid nitrogen, and homogenized them with a Polytron instrument (MICCRA MiniBatch D-1). Initially, we included 45 subjects diagnosed with HCC, but six did not meet the quality control criteria. Therefore, we further performed the analyses on tumor and normal tissue samples from a cohort of 39 HCC patients.

Table I. Clinical characteristics for the study cohort.

Characteristics	Number of cases (%)
Histology	Non-metastatic hepatocellular carcinoma
Total number	45 (100%)
Age range (median)	47-78 (62.6)
Gender	
Male	31 (68.89%)
Female	14 (31.11%)
Stage	
I-II	40 (88.88%)
III-IV	5 (11.11%)
pT category	
T1a	3 (6.67%)
T1b	7 (15.56%)
T2	30 (66.67%)
T3	4 (8.89%)
T4	1 (2.22%)
Vital status at last follow-up	
Alive	39 (86.67%)
Deceased	6 (13.33%)

Gene expression evaluation by qRT-PCR

For total RNA extraction, we used the TRIzol (TRI Reagent, Sigma-Aldrich) protocol and performed quantitative and qualitative controls using the NanoDrop 1000 spectrophotometer (Thermo Scientific). cDNA synthesis was performed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems™) from 1500 ng of total RNA, according to the manufacturer's recommendations. For qRT-PCR, we used the PowerUp SYBR Green Master Mix (Applied Biosystems™) and the QuantStudio 7 Flex instrument (Applied Biosystems™) according to the manufacturer's protocol. Three lncRNAs and two genes were analyzed, and B2M and 18S were used as housekeeping genes (Table II). Expression changes were evaluated using the $\Delta\Delta CT$ method from the obtained CT values.

Statistical analysis

Gene expression was compared between two independent groups comprising 39 tumoral (TT) and 39 non-tumoral (TN) liver tissue samples. We assessed differences between groups using an unpaired t-test. A p-value < 0.05 was considered statistically significant. We performed statistical analyses and graphical representations using GraphPad Prism version 8.0.2. (version 8.0.2; GraphPad Software, Inc., San Diego, CA, USA).

Results

Study cohort information

Forty-five patients with histologically confirmed non-metastatic hepatocellular carcinoma were enrolled, with clinical characteristics as summarized in table I. The cohort was predominantly male (31/45, 68.89%) with a median age of 62.6 years (range 47-78). Most patients presented with early-stage disease: 35 of 45 (77.78%) were stage I-II, and 10 of 45 (22.22%) were stage III-IV.

Following RNA quantity and quality control, 39 TT and 39 non-tumoral TN tissue samples were included in the expression analysis; the remaining six were excluded because of RNA integrity below the threshold. All expression analyses reported below therefore refer to 39 TT and TN tissues.

Differential expression of long non-coding RNAs

Relative expression of MALAT1, NEAT1 and NKILA was quantified by qRT-PCR in 39 TT and 39 TN tissues, normalized to B2M and 18S using the $\Delta\Delta CT$ method (Figure 1).

MALAT1 was markedly overexpressed in tumoral tissue relative to non-tumoral tissue. Median relative expression was 3.90 (IQR 1.89–8.10) in TT versus 0.58 (IQR 0.30–1.11) in TN, corresponding to an approximately 7-fold increase (mean 8.18 ± 12.42 vs 1.16 ± 1.81), and

Table II. Housekeeping genes, lncRNAs, and target genes sequences used

Target	Sequence
B2M	FW-CACCCCTGAAAGATGAG/RW-CCTCCATGATGCTGCTTACATG
18S	FW-CGCTCCACCACTAAGAACG/RW-CTCAACACGGGAAACCTCAC
NEAT1	FW-GAGAAAAGTCCAAAAGGAGCAC/RW-GGATGAGGCCTGGTCTTGT
MALAT1	FW-TGTCCTTATAGGCTGGCCATT/RW-AACTGCAGAGTTTGAGTGGTTTT
NKILA	FW-CTCAGGGAAGAGAAATTGGAGA/RW-AAACCAGGCAATGGGTGAT
YAP1	FW-ATCCAGCACAGCAAATTCT/RW-TGGATTTTGAGTCCCACCAT
SNAIL	FW-GCTGCAGGACTCTAATCCAGA/RW-ATCTCCGGAGGTGGATG

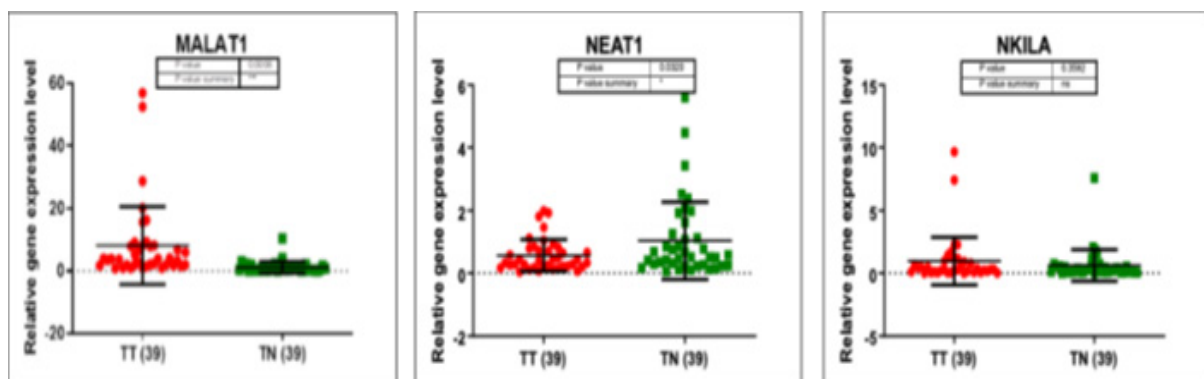


Figure 1. Differential lncRNA expression in HCC tumoral versus non-tumoral tissue measured by qRT-PCR. Relative expression of MALAT1, NEAT1 and NKILA in 39 TT and 39 TN tissue samples, normalized to B2M and 18S rRNA by the $\Delta\Delta CT$ method. Each point represents one sample; horizontal bars indicate mean $\pm$ SD. p-values as shown; ** $p < 0.001$, $p < 0.05$, ns = not significant.

the difference reached high statistical significance ($p = 0.0008$). The distribution in tumoral tissue was strongly right-skewed, with a subset of tumors showing relative expression values between 20 and 57, whereas non-tumoral tissue showed a narrow distribution clustered near unity.

NEAT1 showed the opposite pattern. Median relative expression was lower in tumoral than in non-tumoral tissue, at 0.40 (IQR 0.22–0.76) in TT versus 0.52 (IQR 0.26–1.19) in TN (mean 0.57 ± 0.51 vs 1.04 ± 1.23), a difference that was statistically significant ($p = 0.0320$). Dispersion was also greater in the non-tumoral compartment, where individual samples reached relative expression values above 5, whereas tumoral values did not exceed approximately 2.2.

NKILA showed no statistically significant difference between cohorts ($p = 0.3592$). Median relative expression was 0.32 (IQR 0.12–0.96) in TT and 0.29 (IQR 0.10–0.56) in TN (mean 0.97 ± 1.90 vs 0.63 ± 1.26). Both groups contained isolated high-expressing outliers, reaching approximately 9.8 in the tumoral and approximately 7.6 in the non-tumoral group, against an otherwise tightly clustered distribution near unity.

Differential expression of protein-coding genes

We assessed YAP1 and SNAIL expression in the same TT and TN groups (Figure 2).

YAP1 was significantly overexpressed in tumoral tissue, with median relative expression of 2.26 (IQR 1.16–3.37) in TT versus 0.87 (IQR 0.37–1.51) in TN (mean 3.79 ± 5.30 vs 0.99 ± 0.72 ; $p = 0.0016$). As with MALAT1, tumoral distribution was right-skewed: most tumors clustered between 1 and 5, while individual cases reached relative expression values of approximately 11, 16, and 29, whereas non-tumoral tissue showed a compact distribution around unity.

SNAIL was also significantly overexpressed in tumoral tissue, though with a smaller effect size than the other significant targets. Median relative expression was

1.17 (IQR 0.55–2.50) in TT versus 0.80 (IQR 0.28–1.48) in TN (mean 1.75 ± 1.64 vs 1.07 ± 1.12), corresponding to an approximately 1.6-fold increase ($p = 0.0365$). Individual tumoral samples reached relative expression values of approximately 6.3, but the two distributions overlapped substantially, and a single non-tumoral sample reached approximately 6.1.

Relative expression of YAP1 and SNAIL in 39 TT and 39 non-tumoral TN tissue samples, normalized to B2M and 18S rRNA by the $\Delta\Delta C_t$ method. Each point represents one sample; horizontal bars indicate mean $\pm$ SD. * $p < 0.01$, $p < 0.05$.

Of the five targets assayed, four showed statistically significant differential expression between tumoral and non-tumoral liver tissue. MALAT1, YAP1, and SNAIL were significantly overexpressed in tumor, while NEAT1 was significantly reduced. NKILA showed no significant difference between groups. The effect sizes were graded: MALAT1 and YAP1 showed both the largest fold changes and the strongest statistical support, whereas SNAIL and NEAT1 showed modest separation between groups and borderline significance.

Discussion

In this tissue-based analysis of a single-center Romanian HCC cohort, we observed a coherent pattern of proliferative and invasion-associated dysregulation, with one clearly discordant result. MALAT1, YAP1, and SNAIL were significantly overexpressed in tumor tissue relative to non-tumor tissue; NKILA showed no differential expression; and NEAT1 was significantly reduced in tumor tissue, in contrast to the reported literature on HCC.

The effect sizes varied rather than being uniform: MALAT1 and YAP1 clearly distinguished the two groups, while SNAIL and NEAT1 exhibited significant overlap and borderline significance. This gradation advises caution when interpreting the latter two. We will analyze each result individually, treating the NEAT1 finding as a

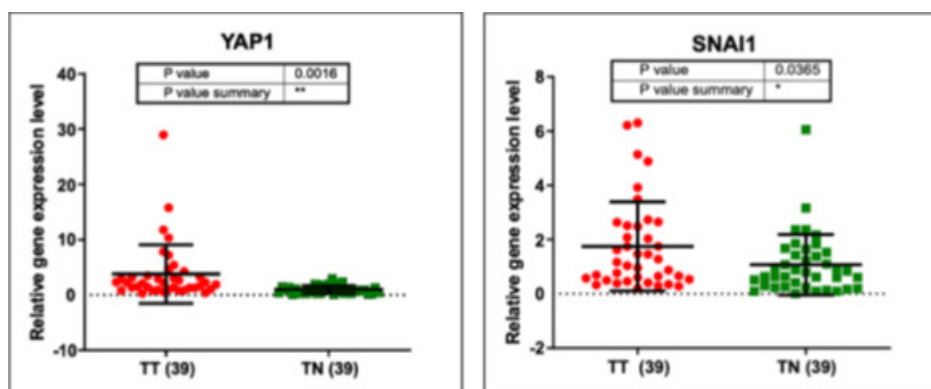


Figure 2. Differential gene expression in HCC tumoral versus non-tumoral tissue measured by qRT-PCR.

meaningful scientific inquiry prompted by this dataset, rather than dismissing it as an anomaly.

We investigated the expression of three long non-coding RNAs (MALAT1, NEAT1, and NKILA) together with two protein-coding genes, YAP1 and SNAIL, in HCC tumor tissue and non-tumor liver tissue. The main expression pattern was characterized by significant upregulation of MALAT1, YAP1, and SNAIL in tumor tissue, significant reduction of NEAT1, and no statistically significant difference in NKILA expression. Taken together, these findings suggest dysregulation of interconnected proliferative, mechanotransduction, and epithelial–mesenchymal transition (EMT)-associated networks. In particular, the concomitant increase in YAP1, MALAT1, and SNAIL supports the Hippo/YAP signaling pathway as a useful mechanistic framework for interpreting the present results.

The Hippo pathway is a highly conserved regulator of organ size, tissue regeneration, cell proliferation, and apoptosis and is particularly relevant to liver biology. Under physiological conditions, activation of the upstream MST1/2–LATS1/2 kinase cascade results in phosphorylation of YAP and TAZ, promoting their cytoplasmic retention and degradation. When Hippo-mediated growth restraint is attenuated, or when YAP/TAZ are activated through mechanical or microenvironmental signals, YAP can accumulate in the nucleus and interact predominantly with TEAD transcription factors, activating genes involved in proliferation, survival, stemness, tissue remodeling, and invasion [21]. Dysregulation of this pathway is well documented in hepatocarcinogenesis, and experimental inhibition of YAP has been shown to restore hepatocyte differentiation and induce regression in experimental HCC models. Importantly, YAP activation is not determined exclusively by YAP1 mRNA levels, because phosphorylation, protein stability, subcellular localization, extracellular matrix stiffness, cytoskeletal tension, and interactions with other signaling pathways all regulate YAP function. Therefore, the increased YAP1 transcript abundance detected in our study should be interpreted as evidence of altered YAP1 expression compatible with Hippo pathway dysregulation, rather than direct demonstration of YAP pathway activation.

Nevertheless, the significant increase in YAP1 observed in HCC tumor tissue is consistent with a substantial body of evidence implicating YAP in aggressive liver cancer biology. Increased YAP expression has been associated with larger tumors, vascular invasion, intrahepatic dissemination, stem-like HCC phenotypes, and unfavorable clinical outcomes [21]. This relationship is particularly relevant because YAP does not function only as a proliferative signal; it also acts as a mechanotransducer that converts changes in the tumor microenvironment into transcriptional programs that favor invasion. Yu et al. demonstrated that fluid shear

stress induces nuclear translocation of YAP in HCC cells and that activated YAP subsequently increases SNAIL expression, promoting an EMT-associated phenotype [23]. This observation provides an especially relevant mechanistic link to our data, in which both YAP1 and SNAIL were increased in tumor tissue.

The oncogenic importance of YAP1 is not restricted to HCC and appears to represent a recurring feature of malignancies arising in the upper gastrointestinal and hepatopancreatobiliary region. In gastric adenocarcinoma, YAP1 protein overexpression and nuclear accumulation have been associated with increased proliferation and invasion and with poorer clinical outcome [24]. Pancreatic cancer similarly exhibits increased YAP1 expression, and high YAP1 levels have been associated with poor prognosis and extensive extracellular matrix remodeling [25]. These observations are biologically relevant because both pancreatic ductal adenocarcinoma and HCC develop within highly remodeled, fibrotic, and mechanically abnormal microenvironments. Such conditions can promote YAP activity independently of canonical Hippo mutations through altered cell adhesion, cytoskeletal tension, and extracellular matrix stiffness. Thus, YAP1 overexpression in our HCC samples fits a broader model in which Hippo/YAP dysregulation integrates proliferative signaling with the mechanical properties of the tumor microenvironment across gastrointestinal malignancies.

MALAT1 represents another component of this network. Its significant overexpression in our HCC tumor tissue agrees with previous studies describing MALAT1 as a tumor-associated lncRNA in HCC. Lai et al. reported increased MALAT1 expression in HCC and identified high MALAT1 levels as a predictor of tumor recurrence after liver transplantation [13]. Malakar et al. subsequently provided functional evidence that MALAT1 contributes to hepatocarcinogenesis by inducing the splicing factor SRSF1, altering oncogenic alternative splicing, and activating the mTOR pathway [14]. MALAT1 can therefore influence several fundamental aspects of malignant cell behavior, including proliferation, metabolism, apoptosis resistance, migration, and invasion.

Of relevance to the present study is the evidence directly connecting MALAT1 with YAP signaling. Wang et al. demonstrated in liver cancer models that YAP can increase MALAT1 expression at both transcriptional and post-transcriptional levels and described regulatory interactions among YAP, SRSF1, β -catenin/TCF signaling, and MALAT1 [26]. This provides a plausible biological context for the simultaneous increase in YAP1 and MALAT1 observed in our tumor samples. The present study does not establish a causal relationship between the two transcripts, but their concordant expression is compatible with activation of interconnected YAP-, Wnt-, SRSF1-, and MALAT1-dependent oncogenic programs.

Evidence from neighboring gastrointestinal cancers

strengthens this interpretation. MALAT1 is increased in pancreatic cancer tissues, where its suppression reduces cancer-cell proliferation, migration, and invasion [27]. Similarly, MALAT1 overexpression has been reported in gastric cancer and has been associated with metastatic behavior and poor prognosis [28]. Notably, experimental suppression of YAP1 in gastric cancer cells was reported to decrease MALAT1 expression together with changes in EMT-associated markers, while YAP1 overexpression promoted proliferation, migration, and invasion [29]. Thus, although the detailed regulatory architecture varies among tumor types, the association between YAP activity, MALAT1 expression, and invasive behavior appears to extend beyond HCC. Our finding of simultaneous MALAT1 and YAP1 overexpression therefore fits a broader gastrointestinal cancer phenotype rather than representing an isolated liver-specific phenomenon.

SNAIL provides a second important link between our findings and Hippo/YAP signaling. SNAIL encodes SNAIL, a transcriptional repressor that promotes EMT by suppressing epithelial differentiation programs, including E-cadherin expression, while facilitating cellular plasticity and invasion. Increased SNAIL expression has previously been associated with invasive behavior and poor prognosis in HCC [22]. In our cohort, SNAIL was significantly increased in tumor tissue, although the separation between tumor and non-tumor samples was less pronounced than for MALAT1 and YAP1. This relatively modest difference may be biologically plausible, particularly in a cohort containing a substantial proportion of surgically resectable disease, because EMT is a dynamic and heterogeneous process rather than a uniform property of all tumor cells.

The relationship between YAP and SNAIL may be particularly important in explaining this observation. Experimental data in HCC demonstrate that nuclear YAP can induce SNAIL expression and promote EMT under mechanical stress [23]. This suggests a potential sequence in which changes in the tumor microenvironment, including tissue stiffness, vascular forces, and cytoskeletal remodeling, increase YAP activity, which in turn facilitates SNAIL-dependent cellular plasticity. Similar YAP-driven invasive programs have been described in pancreatic and gastric cancer, although the precise downstream mediators differ among tumor types. Therefore, the parallel increase in YAP1 and SNAIL in our samples is consistent with, but does not by itself prove, engagement of a Hippo/YAP–EMT axis in HCC.

In contrast to MALAT1, YAP1, and SNAIL, NEAT1 expression was significantly lower in tumor tissue than in non-tumor liver. Several individual HCC studies have described increased NEAT1 expression and have linked NEAT1 to proliferation, invasion, vascular invasion, or advanced disease [15–18]. Experimental work has also suggested that NEAT1 can promote HCC-

cell proliferation and invasion through interactions with RNA-binding proteins and other regulatory pathways [30,31]. On this basis, reduced NEAT1 expression might initially appear inconsistent with the prevailing oncogenic model.

However, the published evidence concerning NEAT1 in HCC is not uniform. A comprehensive analysis by Xiong et al. incorporating 57 microarray and RNA-sequencing datasets found that NEAT1 was significantly reduced in HCC overall [19]. Therefore, our result is not without precedent and may reflect genuine biological heterogeneity in NEAT1 regulation. Interestingly, the same multi-dataset analysis showed different directions of NEAT1 dysregulation among digestive-system malignancies, emphasizing that NEAT1 expression is highly dependent on cellular and tissue context. In pancreatic cancer, NEAT1 has generally been reported to be increased and to promote proliferation, migration, and invasion through mechanisms including the miR-335-5p/c-MET axis [32]. Gastric cancer studies have likewise reported increased NEAT1 expression, with proposed mechanisms involving chromatin regulation, cell-cycle control, and interactions with pathways such as PI3K/AKT/mTOR [33]. The reduction observed in our HCC samples therefore distinguishes HCC from the more consistently increased NEAT1 pattern reported in pancreatic and gastric cancers.

NEAT1 generates distinct transcripts, including NEAT1_1 and the longer NEAT1_2 isoform, and different PCR assays may not necessarily capture their relative abundance in the same manner. Consequently, apparently contradictory findings across studies may partly reflect differences in primer location and isoform coverage [34,35]. Second, the non-tumor liver in patients with HCC should not be considered equivalent to healthy liver. Chronic inflammation, fibrosis, cirrhosis, metabolic liver injury, or viral hepatitis may alter lncRNA expression in the surrounding parenchyma. An apparent reduction of NEAT1 in tumor relative to liver could therefore result from decreased tumor expression, increased expression in chronically diseased tissue, or a combination of both. These are particularly relevant for NEAT1 because of its involvement in cellular stress responses and nuclear paraspeckle formation. Isoform-specific measurement and comparison with histologically normal liver tissue would help distinguish these in future studies [36].

NKILA displayed a different pattern, with no statistically significant difference between HCC tumor and non-tumor tissue. NKILA was originally characterized as a negative regulator of NF- κ B signaling, and previous HCC work has linked its loss to metastatic behavior. Chen et al. reported decreased NKILA expression in HCC tissues and demonstrated that low NKILA expression was associated with larger tumor size, vascular invasion, and poorer survival. Mechanistically, NKILA suppressed

phosphorylation of I κ B α , nuclear translocation of NF- κ B p65, and activation of the NF- κ B/Slug EMT pathway, thereby reducing migration and invasion [20]. Our inability to detect significant differential NKILA expression therefore differs from that study but should not be interpreted as evidence that NKILA lacks biological relevance in HCC.

One possible explanation is that NKILA may reflect a different component of tumor biology from the YAP1–MALAT1–SNAI1 pattern identified in our cohort. Whereas YAP acts as a mechanotransduction and growth-regulatory effector, NKILA primarily modulates inflammatory NF- κ B signaling and the NF- κ B/Slug branch of EMT. These pathways ultimately converge on related biological outcomes, including invasion and cellular plasticity, but their activity need not change in parallel at the transcript level. Furthermore, NKILA expression may be particularly sensitive to inflammatory cell composition and the condition of the surrounding liver. Thus, the absence of a significant tumor–non-tumor difference in our cohort may reflect biological heterogeneity rather than absence of involvement in HCC progression. Larger cohorts stratified by tumor stage, vascular invasion, inflammatory background, and underlying liver disease would be required to assess this possibility.

Our results can be interpreted using a model centered on Hippo/YAP signaling but incorporating complementary lncRNA and EMT pathways. Increased YAP1 may identify a tumor environment permissive for proliferative and mechanotransduction-dependent signaling. MALAT1 overexpression may reinforce oncogenic programs involving alternative splicing, Wnt/ β -catenin signaling, mTOR activity, and YAP-associated transcriptional regulation, whereas increased SNAI1 may link to EMT and invasive plasticity. In contrast, NEAT1 and NKILA show that lncRNA regulation in HCC cannot be reduced to a simple pattern in which all cancer-associated lncRNAs are uniformly increased or decreased. Their behavior is likely dependent on transcript isoform, inflammatory and fibrotic context, tumor stage, and the biological properties of the comparator liver tissue.

The comparison with pancreatic and gastric cancers further suggests that some elements of this regulatory architecture are shared across gastrointestinal malignancies. YAP1 activation, MALAT1 overexpression, EMT-associated changes, extracellular matrix remodeling, and interactions with PI3K/AKT/mTOR and Wnt/ β -catenin signaling recur in HCC, pancreatic cancer, and gastric cancer [23–29,32,33,37]. However, the NEAT1 findings also demonstrate important tumor-specific differences. These observations favor a model in which Hippo/YAP functions as a central signaling hub that communicates with several cancer-associated transcriptional and non-coding RNA networks, while the specific configuration of those networks depends on tissue origin and microenvironment.

Limitations

Several limitations require explicit statement. The cohort comprised 39 evaluable HCC cases, limiting the power for subgroup analysis and precluding meaningful stratification by etiology, stage, or relapse status; the discrepancy between the 45 patients recruited and the 39 analyzed reflects RNA quality and should be interpreted accordingly.

The number of analyzed samples limits robust stratification according to etiology, tumor stage, vascular invasion, or recurrence. Furthermore, RT-qPCR measures transcript abundance and does not establish functional pathway activation. This distinction is particularly important for YAP1, for which nuclear localization and phosphorylation status are major determinants of biological activity. Evaluation of total YAP1 protein, phosphorylated YAP1, nuclear YAP localization, and canonical YAP/TEAD transcriptional targets such as CTGF and CYR61 would provide stronger evidence of Hippo pathway activation. Similarly, assessment of E-cadherin, vimentin, SNAI1 protein, and other EMT markers would help determine whether the expression pattern identified here corresponds to a functional EMT phenotype. Using non-tumor liver as the comparator is another limitation because this tissue may contain fibrosis, cirrhosis, inflammation, or metabolic alterations and therefore should not be regarded as histologically or molecularly normal liver. Finally, the present analysis examines a limited number of prespecified transcripts and cannot establish causal relationships among MALAT1, NEAT1, NKILA, YAP1, and SNAI1.

Despite these limitations, the combined expression pattern provides a biologically coherent basis for further investigation. The strong increase in MALAT1 and YAP1, together with the more moderate increase in SNAI1, is compatible with coordinated dysregulation of proliferative and EMT-associated programs in HCC and supports further investigation of the MALAT1–Hippo/YAP–SNAI1 network. In contrast, reduced NEAT1 expression highlights the context-dependent behavior of lncRNAs in liver cancer and warrants validation with isoform-specific assays and better-characterized liver controls. The lack of significant NKILA dysregulation further emphasizes that inflammatory and EMT-regulatory pathways may not be uniformly altered across HCC cases. Future studies integrating transcript expression with YAP protein localization, Hippo pathway activity, EMT markers, background liver histology, and clinical outcomes will be necessary to determine whether these molecules represent interconnected components of a common regulatory program or biologically distinct HCC subgroups.

Conclusions

In a Romanian surgical cohort comprising mainly early-stage HCC cases, MALAT1, YAP1, and SNAI1

were notably overexpressed in TT compared with TN liver tissue. This confirms three well-established associations within a population often underrepresented in existing research. The differences were clear for MALAT1 and YAP1 and more subtle for SNAI1, aligning with the predominantly early-stage disease in this group. NKILA showed no difference in expression levels, a null finding likely due to its low abundance and inflammation-driven regulation rather than a lack of biological significance.

The reduction of NEAT1 in tumoral tissue is discordant with most primary HCC reports but consistent with pooled multi-dataset analyses, and it generates a specific, falsifiable proposition: that the direction of NEAT1 change reported in HCC depends on which isoform the assay detects and on the fibrotic state of the comparator tissue. This prediction can be tested directly by isoform-specific quantification of NEAT1_2 alongside total NEAT1 in the same RNA sample, and by including histologically normal liver as an additional comparator. Until such data are available, we suggest that reports of NEAT1 dysregulation in HCC should routinely specify isoform coverage and comparator histology, since neither is currently standardized and both plausibly account for the conflicting directions in the published record.

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The authors' contributions

A.M.C. contributed to conceptualization, methodology, investigation, and writing of the original draft, as well as review and editing of the manuscript. P.C. contributed to conceptualization, writing of the original draft, and review and editing of the manuscript. L.B. and E.I. contributed to methodology, validation, formal analysis, and investigation. A.T. contributed to investigation. S.S. contributed to review and editing of the manuscript. D.G.C. contributed to writing of the original draft. I.R. contributed to investigation and resources. A.C. contributed to resources and to review and editing of the manuscript. R.C. contributed to review and editing of the manuscript. I.B.-N. contributed to conceptualization, review and editing of the manuscript, and supervision. N.A.H. contributed to resources and supervision. All authors have read and agreed to the published version of the manuscript.

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