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Uncovering the Regulatory Role of Long Non-Coding RNAs in Colorectal Cancer Progression and Liver Metastasis: Implications for Therapeutic and Diagnostic Targeting

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ABSTRACT

Background: Colorectal cancer (CRC) is a leading cause of cancer-related deaths, largely due to metastasis, particularly to the liver, and the limited understanding of the molecular mechanisms underlying this process. In this study, we aimed to investigate the role of long non-coding

Methods: RNAs (lncRNAs) are key regulatory factors in CRC progression and metastasis to liver tissues. Using high-throughput sequencing and microarray approaches, we analyzed gene expression profiles from two independent lncRNA datasets to identify potential players involved in liver metastasis.

Results: Our findings revealed five lncRNAs—*PROX1-AS1*, *SOX9-AS1*, *LINC01594*, *LINC01555*, and *APOA1-AS*—previously known for their roles in CRC progression, now identified as being involved in the liver metastatic process. Additionally, 20 other lncRNAs, including *VCAN-AS1*, *SYP-AS1*, *SMIM2-IT1*, *NCOA7-AS1*, and *LINC01449*, were also identified as potential contributors to CRC liver metastasis. Notably, two lncRNAs—*SATB2-AS1* and *LINC01116*—emerged as common candidates across both datasets, suggesting their significant role in promoting CRC metastasis to the liver. These two lncRNAs hold promise as molecular targets for therapeutic and diagnostic development.

Conclusion: Our study uncovers a novel layer of regulatory mechanisms involving lncRNAs in CRC liver metastasis. These findings advance our understanding of the molecular behaviors that drive CRC progression and offer new avenues for targeted therapeutic strategies and diagnostic tools, particularly for liver metastasis in CRC.

Keywords: Colorectal cancer (CRC), Liver metastasis, Long non-coding RNAs (lncRNAs), Gene expression profiling, Therapeutic targets

Introduction

Colorectal cancer (CRC) is one of the most prevalent malignancies globally and a leading cause of cancer-related deaths. Despite advancements in treatment strategies, the prognosis for patients with advanced or metastatic CRC remains poor. A key challenge in combating CRC is the insufficient understanding of the molecular mechanisms that drive tumor initiation, progression, and metastasis, particularly at the genetic and regulatory levels. This lack of knowledge limits the discovery of novel therapeutic targets and impedes the development of effective diagnostic tools, making CRC an often-untreatable condition in its advanced stages (1, 2).

In this study, we aimed to fill this critical knowledge gap by investigating the molecular behaviors and pathways that contribute to CRC progression, with a particular focus on metastasis to the liver—one of the most common and life-threatening metastatic sites for CRC. Our primary goal was to identify potential therapeutic and diagnostic targets by delving into the molecular underpinnings of CRC at a high resolution. We employed cutting-edge high-throughput sequencing technologies, coupled with microarray-based gene expression profiling, to systematically analyze the vast landscape of genes implicated in CRC (3-5). Given the complexity of cancer biology and the sheer volume of gene expression data generated, we utilized systems biology approaches to streamline our analysis. By integrating and analyzing thousands of gene expression profiles, we aimed to uncover key molecular players—particularly those involved in regulatory networks that influence CRC metastasis.

A major focus of our research is on the role of regulatory factors, especially long non-coding RNAs (lncRNAs), which have emerged as important regulators of gene expression and cancer progression. Through upstream regulatory analysis, we sought to gain deeper insights into the function of lncRNAs and their potential as novel therapeutic or diagnostic targets in CRC (6-10). By shedding light on these critical molecular mechanisms, our study seeks to contribute to a better understanding of CRC at the molecular level, potentially paving the way for the development of more effective, targeted interventions for CRC patients. Our findings may offer new avenues for therapeutic strategies aimed at halting CRC progression and improving patient outcomes, particularly in cases of liver metastasis.

Materials and Methods

Expression Profile Analysis

We utilized the GSE148918 dataset from the Gene Expression Omnibus (GEO) to identify differentially expressed genes (DEGs). The analysis was conducted inR programming environment along with packages such as Limma and DESeq2. A threshold of ±1 for log fold change and a P-value ≤ 0.05 were set as the criteria for significant gene expression changes (Figure 1A). To validate these findings, we employed another dataset (GSE174602), obtained through microarray techniques. This dataset was also analyzed in R, using the same thresholds as in the initial analysis (11-13).

Upstream Analysis

Identifying DEGs helps to understand which genes are altered during the progression of metastatic colorectal cancer to liver tissue, but it is crucial to determine the factors regulating these genes. Several regulatory elements, such as transcription factors (TFs), microRNAs, and protein kinases, play pivotal roles in this process. While many of these regulators have been extensively studied in cancer, others, such as long non-coding RNAs (lncRNAs), remain less understood, particularly in metastatic cancers. We used the lncHUB_lncRNA_Co-Expression database from the EnrichR web tool (https://maayanlab.cloud/Enrichr/) (Figure 1E). This database revealed lncRNAs that are co-expressed with our candidate genes, providing insights into their potential regulatory roles (14).

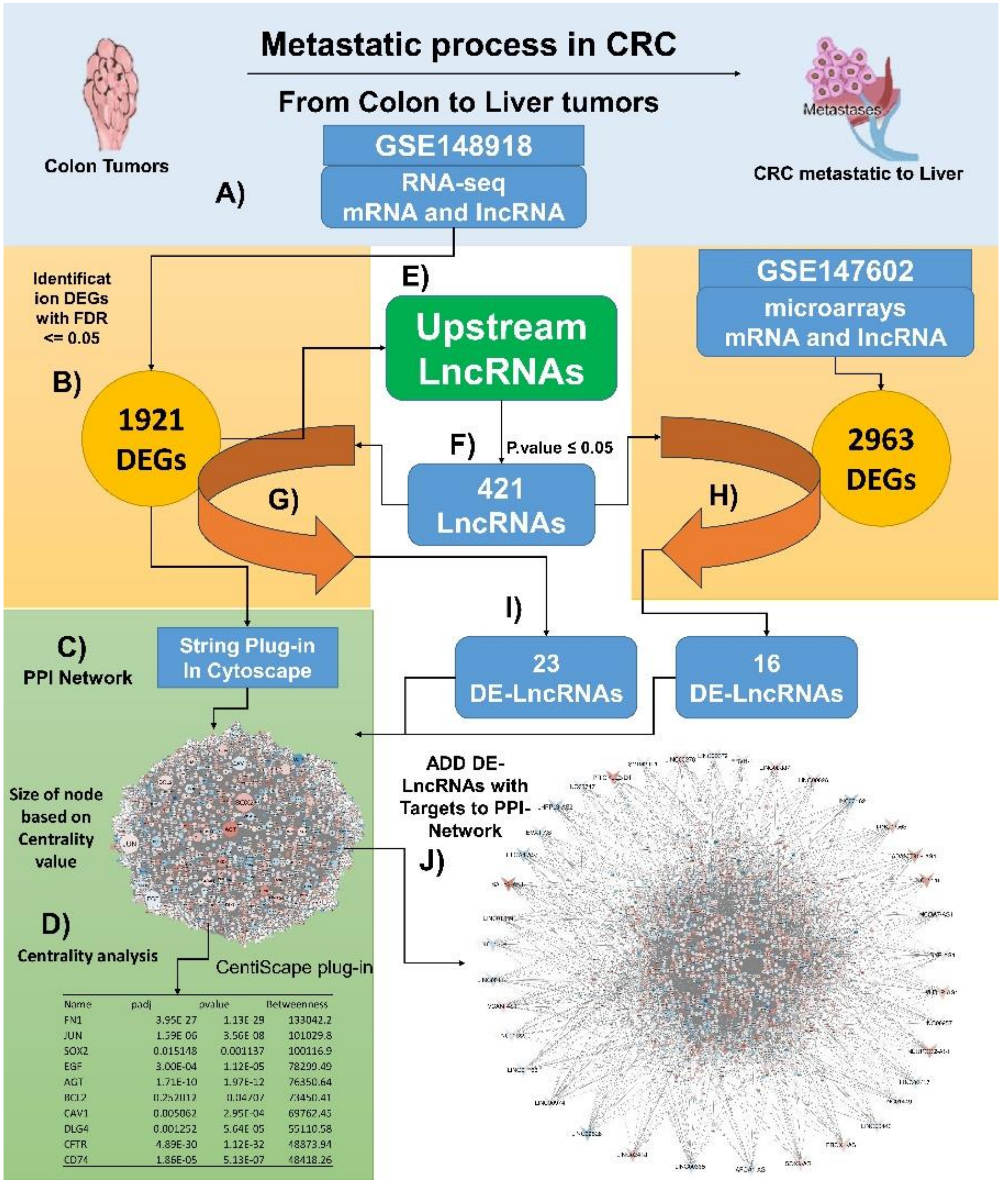

Figure 1: Overview of Analytical Workflow Leading to Current Study Results. This figure summarizes the steps taken in the study to analyze and interpret the data. A) The comparison between metastatic liver tumors and primary CRC tumors was performed using bulk RNA-seq techniques. B) Re-analysis of the data identified 1921 significantly differentially expressed genes (DEGs). C) These DEGs were input into Cytoscape software, using the STRING database to construct the protein-protein interaction (PPI) network. D) Centrality analysis was performed using the CentiScape plugin to determine the most influential genes within the PPI network, identifying key hub genes. E) Upstream analysis was conducted to predict which regulatory lncRNAs might regulate the identified DEGs. F) This upstream analysis highlighted 421 lncRNAs as potential regulators of the DEGs. G) The expression of these 421 lncRNAs was cross-referenced with the DEGs to filter relevant lncRNAs. H) To further refine this, an additional microarray dataset was re-analyzed and compared with the 421 candidate lncRNAs. I) From this analysis, 23 lncRNAs were identified as DE-lncRNAs in the first dataset, and 16 DE-lncRNAs were found in the second dataset. J) Integration of both DE-lncRNAs and their target DEGs was performed to map them to the PPI network.

Protein Network Construction

We utilized the STRING database to source protein-protein interaction data, and Cytoscape software was employed to visualize and analyze the network. The protein network was constructed using the default settings of the STRING-db package within Cytoscape (Figure 1C). Given the complexity of the network, we applied the CentiScape plug-in to calculate betweenness centrality (Figure 1D), which measures the importance of each node within the network. Nodes with the highest betweenness scores were identified as hub genes, likely playing significant roles in the metastatic progression from primary colorectal cancer to liver metastasis (15-18).

DE-LncRNAs

The results from the upstream analysis were verified by comparing them with the expression profiles obtained in the current study. Specifically, the lncRNA database results were cross-referenced with our identified DEGs (Figure 1G). A second dataset of lncRNAs derived from microarray techniques was identified and re-analyzed. The outcomes from this second dataset were then compared to the DEGs found in the current study, reinforcing the robustness of our results (Figure 1H).

Results

Differential Expression Genes

We analyzed 12 samples from the GSE148918 dataset, which includes 8 Organoid_CRC samples and 4 Organoid_Liver samples (Supplementary Figure 1). The dataset contains 64,099 genes, comprising both mRNA and LncRNA (Supplementary Table 1). Of these, only 1,921 genes met the significance criteria, with a fold change of less than -1 or more than 1, and a P-value of ≤ 0.05. Among the 1,921 genes, 1,118 were overexpressed in liver metastatic samples, while 805 genes were downregulated (Figure 2) (Supplementary Table 2). To validate our LncRNA results, we used an additional dataset, GSE147602, which consists of 20 samples: 10 CRC samples and 10 liver metastatic samples. Following Principal Component Analysis (PCA), 4 noisy CRC samples and 1 noisy liver sample were removed, leaving 15 samples for further analysis (Supplementary Figure 2). These samples contained 38,213 genes each (Supplementary Table 3). After filtration, we identified 2,963 significant genes. Of these, 1,468 showed overexpression, while 1,495 were downregulated in liver metastatic samples (Supplementary Figure 3) (Supplementary Table 4).

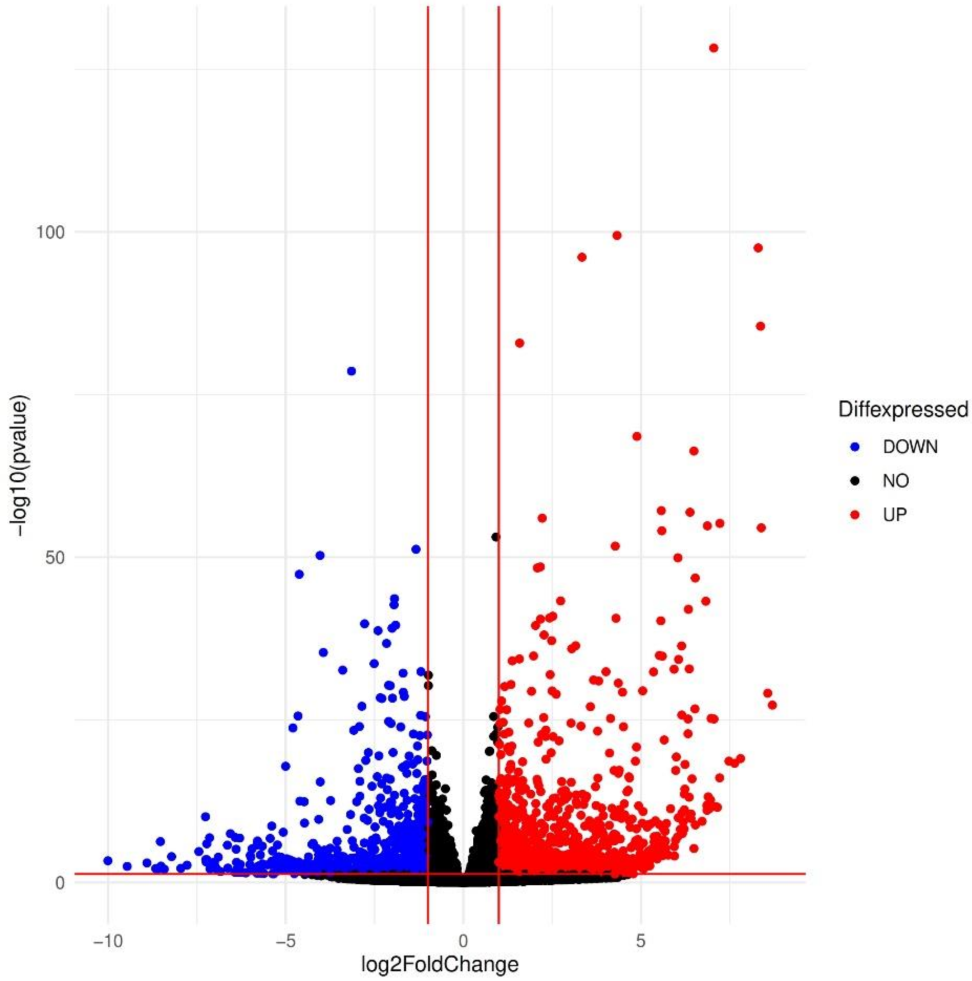

Figure 2: Dispersion of Expressed Genes from Primary CRC Tumors to Liver Metastatic Tumors. This figure illustrates the distribution of gene expression changes between primary colorectal cancer (CRC) tumors and liver metastatic tumors. Genes shown in red are overexpressed, and those in blue are downregulated. Black nodes represent non-significant genes. Significant genes are defined by a p-value ≤ 0.05, with upregulated genes having a log2 fold change (Log2FC) ≥ +1 and downregulated genes having a Log2FC ≤ -1.

Construction of PPI Network

Overall, 1,921 genes were used to construct the protein-protein interaction (PPI) network. The STRING database identified 1,607 proteins, with 159 proteins found to have no connections. While these nodes were identified in the database, their interactions with other genes in our dataset were not found. These unconnected nodes were excluded from further analysis. The final network consisted of 1,448 nodes and 8,748 edges (Figure 3). Given the complexity of the network, we needed a method to evaluate the interactions of each node and determine which nodes are more critical. To achieve this, we used the betweenness centrality criterion. The top 10 nodes, including *FN1*, *JUN, and SOX2*, were identified as hub genes, playing a significant role in our network. These genes are particularly important in the later stages of liver metastasis compared to the earlier stages in colon tissue (Table 1).

Table 1: Top 10 Hub Genes in the PPI Network

Name	***padj***	***P-value***	***Betweenness***
FN1	3.95E-27	1.13E-29	133042.2
JUN	1.59E-06	3.56E-08	101029.8
SOX2	0.015148	0.001137	100116.9
EGF	3.00E-04	1.12E-05	78299.49
AGT	1.71E-10	1.97E-12	76350.64
BCL2	0.252012	0.04707	73450.41
CAV1	0.005062	2.95E-04	69762.45
DLG4	0.001252	5.64E-05	55110.58
CFTR	4.89E-30	1.12E-32	48873.94
CD74	1.86E-05	5.13E-07	48418.26

Figure 3: Illustration of the Protein-Protein Interaction (PPI) Network. This figure depicts the PPI network consisting of 1,448 nodes and 8,748 edges. Nodes in red represent upregulated genes, while nodes in blue represent downregulated genes. The size of the nodes and their labels are proportional to their centrality scores, reflecting their significance within the network.

Upstream analysis

After identifying the alterations, the next important question was to understand how these alterations occurred. To address this, we performed upstream analysis within the molecular biology framework. Using 1,921 DEGs, we queried a lncRNA database to identify potential interactions, finding approximately 400 lncRNAs that met the significance threshold of a *P*-value ≤ 0.05. When comparing these with the 1,921 DEGs, only 23 lncRNAs were identified as differentially expressed lncRNAs (DE-lncRNAs). We also mapped their range of activity by constructing an interaction network (Figure 1I). Additionally, we utilized other databases to validate the influence of these lncRNAs on the 1,921 DEGs. From the second dataset, 2,963 DEGs were identified, and when compared to the ~400 lncRNAs, only 16 lncRNAs were found to be DE-lncRNAs. Interestingly, both datasets revealed two common lncRNAs, *LINC01116* and *SATB2-AS1*, which exhibited significant alterations in expression between primary and liver metastatic tumor samples. *LINC01116* showed a 5-fold increase in expression, while *SATB2-AS1* was upregulated by 7-fold (Table 2) (Figure 4).

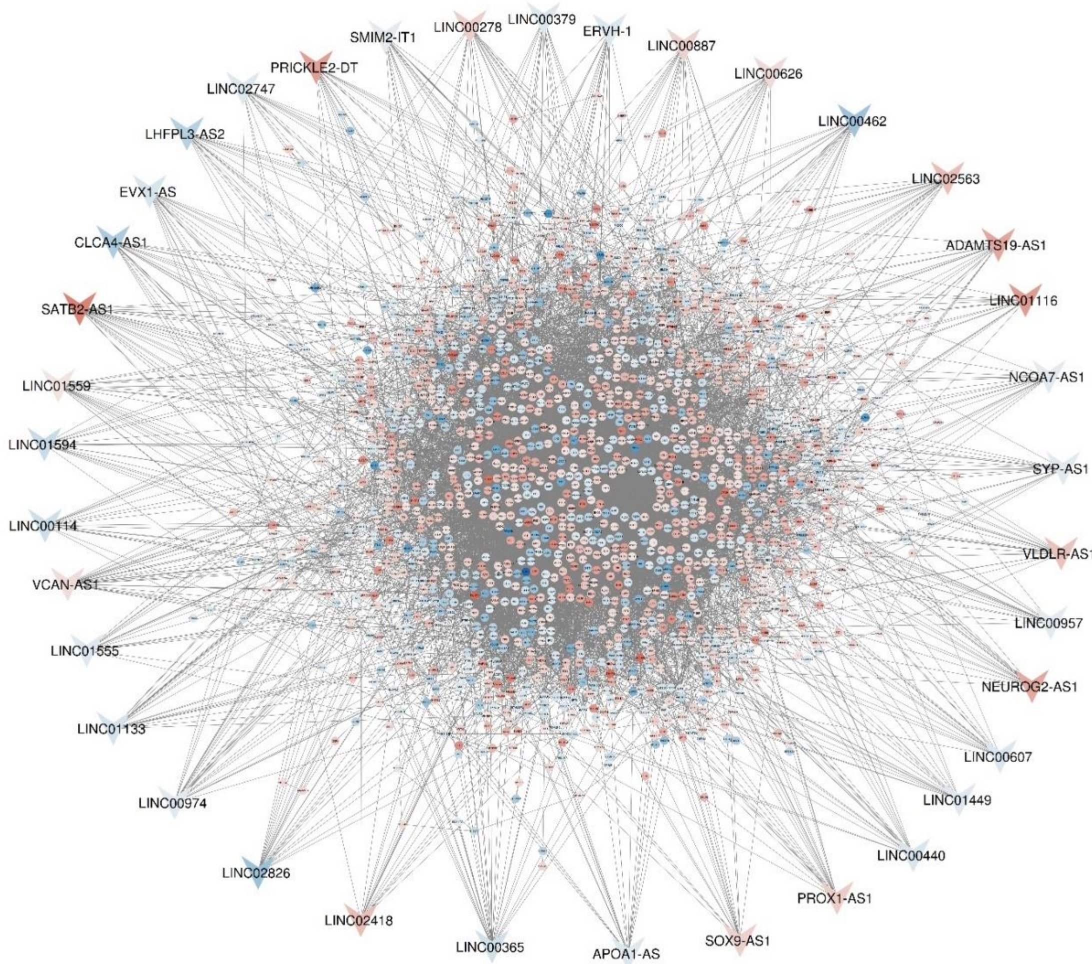

Figure 4: The integration of 37 differentially expressed lncRNAs (DE-lncRNAs) into the PPI network, representing 1,523 nodes and 9,450 edges. Red nodes indicate overexpressed genes, while blue nodes indicate down-expressed genes. DE-lncRNAs are represented by V-shaped nodes.

Table 2: Indicated DE-lncRNAs from first and second datasets.

First Dataset					***Second Dataset***				
Symbol	LogFC	P-value	Padj	Targets	Symbol	LogFC	P.Value	Padj	Targets
LINC00365	-1.96228	0.01873	0.13292	26	VCAN-AS1	1.6172	0.00036	0.00448	29
SATB2-AS1	1.3E-52	7.22302	6.2E-56	25	SATB2-AS1	-1.4441	0.04048	0.12922	25
LINC00278	2.477	0.0095	0.07997	22	LINC00974	-1.1489	0.00107	0.00973	24
LINC02826	-4.91475	0.00683	0.06268	21	SYP-AS1	-1.1536	7.57E-05	0.00148	19
LINC01559	1.14904	5.6E-11	3.9E-09	21	LINC00440	-1.4691	0.0008	0.00789	19
CLCA4-AS1	-3.87085	0.02089	0.14368	21	SMIM2-IT1	-1.03	0.00295	0.02051	18
LINC01133	-1.71347	1.9E-18	3.2E-16	21	LINC01555	-1.3152	0.007	0.03816	18
LINC02563	3.48567	0.00133	0.01714	20	LINC01449	-1.2126	2.05E-07	6.73E-05	18
LINC00462	-4.69382	0.01943	0.13672	20	LINC00114	-2.1923	0.00927	0.04644	18
VLDLR-AS1	3.19298	0.01845	0.13161	19	NCOA7-AS1	-1.0249	0.0004	0.00479	17
PRICKLE2-DT	5.4572	0.00024	0.00433	19	ERVH-1	-1.3516	0.00025	0.00347	17
PROX1-AS1	2.56183	3.6E-09	1.9E-07	19	APOA1-AS	-1.248	7.72E-07	0.00011	17
ADAMTS19-AS1	4.95854	4.1E-06	0.00012	18	LINC01116	1.9556	0.00066	0.00683	16
LINC00887	2.25373	0.0002	0.00369	18	LINC00626	1.7331	0.0161	0.06867	16
EVX1-AS	-1.64684	0.00097	0.01333	18	LINC00607	-1.411	0.00133	0.01147	16
NEUROG2-AS1	4.80579	1.6E-06	5.2E-05	17	LINC00379	-1.3688	0.01837	0.07516	16
LHFPL3-AS2	-3.44886	0.01825	0.13062	17					
LINC02418	3.48651	0.00144	0.01826	17					
LINC00957	-1.05286	0.01165	0.09339	17					
SOX9-AS1	2.3242	3.7E-24	8.9E-22	16					
LINC02747	-1.30917	0.00271	0.03019	16					
LINC01594	-2.46856	0.01107	0.09008	16					
LINC01116	6.3E-07	5.67987	1.3E-08	16					

Discussion

Colorectal cancer is one of the major causes of mortality in the Iranian population. A key approach to addressing this issue is halting the progression of the disease. A comprehensive understanding of the molecular players involved in the progression is essential, with metastasis being the primary mechanism of concern. We aimed to address this problem by investigating the molecular mechanisms at play, particularly through upstream analysis focusing on lncRNA regulation, using high-throughput techniques such as RNA-seq and microarray datasets. *LINC00365* has been reported to function as a tumor suppressor by inhibiting HIF-1α-mediated glucose metabolism reprogramming in breast cancer (19). Additionally, *LINC00365* was overexpressed in gastric cancers, influencing the epithelial-mesenchymal transition (EMT) mechanism (20). Zhu et al. further identified *LINC00365* as a promoter of colorectal cancer progression (21). Collectively, these studies highlight the role of *LINC00365* across various cancer types, with its overexpression being linked to colorectal cancer progression. However, in the current study, *LINC00365* was downregulated in liver tissue, where it displayed 26 interactions the highest number of interactions among the lncRNAs identified in the PPI network. *LINC00365* appears to play a barrier role in preventing metastasis to liver tumors.

In addition, *LINC00278* has previously been reported to play a role in metastatic liver tumors in colorectal cancer (CRC) (22, 23). In our study, *LINC00278* was overexpressed and exhibited 22 interactions in the PPI network. Similarly, *LINC01559* was also overexpressed in liver metastatic tumors, with 21 regulatory interactions in the protein network (Table 2). *LINC01559* suppresses CRC progression by regulating miR-106b (24), and it has also been linked to triple-negative breast cancer (25). Therefore, *LINC01559* is proposed as a candidate lncRNA in liver metastatic cancers. Furthermore, *LINC01133* could inhibit the progression of CRC to metastatic liver by regulating miR-186 (26). In our study, *LINC01133* was identified as a DE-lncRNA and was downregulated in metastatic liver tumors (Table 2), which aligns with its role in inhibiting metastasis. This downregulation supports the findings of our methodology, highlighting *LINC01133*'s inhibitory role in liver metastasis from CRC. Furthermore, *LINC01133* could inhibit the progression of CRC to metastatic liver by regulating miR-186 (26) . In our study, *LINC01133* was identified as a DE-lncRNA and was downregulated in metastatic liver tumors, which aligns with its role in inhibiting metastasis. This downregulation supports the findings of our methodology, highlighting *LINC01133*'s inhibitory role in liver metastasis from CRC.

LINC02826 has been implicated in hepatocellular carcinoma (27), and in our study, it was highly downregulated during the metastatic process to the liver in CRC samples, with 21 interactions in the PPI network (Table 2). *LINC02826* may be involved in the metastatic process, given its significant alteration from primary to metastatic liver tumors and its interactions. Therefore, *LINC02826* is proposed as a candidate lncRNA for further study in CRC progression to liver metastasis.

Moreover, the relationship between *CLCA4-AS1* and head and neck squamous carcinoma was reported by Liao (28). In our study, *CLCA4-AS1* was identified as a DE-lncRNA, downregulated in liver metastatic tumors, with 21 connections in the PPI network (Table 2), suggesting its regulatory role in the metastatic process of CRC to the liver.

LINC00462, one of our DE-lncRNAs, may also be related to the progression network, as it displayed 20 interactions and was highly downregulated (Table 2). A previous study identified *LINC00462* as a biomarker for liver cancers (29). Therefore, we propose, for the first time, that *LINC00462* might be involved in the progression of CRC to liver metastasis.

Moghadami et al. previously reported *VLDLR-AS1* as a biomarker for CRC due to its downregulation in healthy samples (30). In our study, *VLDLR-AS1* was overexpressed in metastatic tumors compared to primary CRC tumors, with 19 interactions in the progression network (Table 2), suggesting its potential role in the metastatic progression to the liver and its value as a therapeutic target.

Additionally, *PROX1-AS1* was identified as a DE-lncRNA in our study, with 19 interactions in the progression network and overexpression in liver metastatic tumors compared to primary CRC tumors (Table 2). *PROX1-AS1* plays a role in CRC progression mechanisms (31). Our study extends this by demonstrating its role in CRC progression to liver metastasis. *PROX1-AS1* could be a potential molecular therapy target to reduce CRC progression to liver tissue.

The lncRNA *PRICKLE2-DT* was highly overexpressed (5-fold change) in liver metastatic tumors, with 19 interactions within the progression network (Table 2). *PRICKLE2-DT* could play a novel role in the progression of CRC metastasis to liver tissue, marking its potential importance for the first time in this context.

LINC00887 was previously reported to be involved in liver metastasis progression (32). This supports our methodology, which identified *LINC00887* as a DE-lncRNA influencing the PPI network through 18 interactions (Table 2), further validating its potential as a key player in metastatic progression. This finding encourages attention to other candidate lncRNAs discovered in this study.

EVX1-AS was reported as a molecular driver in colon cancer progression (33), and our study confirmed its role as a DE-lncRNA in CRC liver metastasis with 18 connections in the current PPI network analysis (Table 2). Similarly, *LINC02418*, previously identified as associated with CRC progression (34), was a DE-lncRNA in this study with 17 interactions, highlighting its relevance to the metastatic process in liver tissue. Additionally, *LINC00957* was linked to CRC progression (35), further supporting its potential role in this context.

SOX9-AS1, known for its role in regulating *SOX8* and promoting proliferation in hepatocellular cancer cells (HCC) via miR-5590-3p, was reported already (36). In our study, *SOX9-AS1* was identified as a DE-lncRNA driving progression from CRC to liver metastasis through 16 interactions in the PPI network (Table 2).

LINC01594 was reported as a CRC metastatic biomarker (37). In our analysis, *LINC01594* was downregulated in liver metastatic tumors, with 16 interactions in the metastatic PPI network (Table 2), making it a promising target for suppressing metastasis or serving as a diagnostic biomarker for liver involvement.

Three *lncRNAs—LHFPL3-AS2*, *NEUROG2-AS1*, and *ADAMTS19-AS1*—were identified as DE-lncRNAs involved in CRC progression from primary to liver metastatic tumors, interacting with the PPI network through 17, 17, and 18 connections, respectively (Table 2).

Our analysis also identified 16 DE-lncRNAs from the second dataset, with 14 being unique. Among these, *LINC00607* was found to be downregulated in liver metastatic tumors, with 16 interactions in the progression network (Table 2). Its role in tumor progression was previously reported by Dong in hepatocellular carcinoma cells (38). Similarly, *LINC00114* was downregulated in metastatic CRC liver tissue, with 18 interactions in the PPI network (Table 2), aligning with Qin et al.'s findings that *LINC00114* plays a role in liver tumor progression via *EZH2* (39).

Additionally, *LINC01555* and *APOA1-AS* were identified as DE-lncRNAs in our study, with 18 and 17 interactions in the CRC progression network (Table 2), respectively. Both have been suggested as potential therapeutic targets to prevent metastasis from primary CRC tumors based on previous studies (40, 41).

Among the 10 other DE-lncRNAs identified in our analysis—such as *VCAN-AS1* and *LINC00626* (upregulated), and *SYP-AS1*, *SMIM2-IT1*, *NCOA7-AS1*, *LINC01449*, *LINC00974*, *LINC00440*, *LINC00379*, and *ERVH-1* (downregulated)—no previous reports link them directly to CRC liver metastasis (Table 2). Given their significant differential expression and their direct interactions within the progression network, these lncRNAs may play roles in CRC metastasis to the liver. Further experimental validation, such as through RT-PCR, is recommended to confirm their involvement.

SATB2-AS1, a known suppressor of metastasis in CRC and other cancers, was downregulated in our study, with 25 direct connections in the progression network. Although *SATB2-AS1* typically inhibits metastasis, its minimal downregulation (-1 logFC) in this study suggests it might play a nuanced role in the early stages of CRC metastasis to the liver. Further investigation using experimental approaches is warranted to clarify *SATB2-AS1's* exact function.

We identified two shared lncRNAs that influenced the PPI network during the transition from primary colon tumors to metastatic liver tumors. One of them, *SATB2-AS1*, is a known suppressor of metastasis in colorectal cancer, where its overexpression has been associated with reduced tumor aggression and metastatic potential (42, 43). Additionally, in hepatocellular carcinoma, *SATB2-AS1* was reported to play a developmental role via the miR3678-3b pathway (44). In our study, *SATB2-AS1* was one of the two shared DE-lncRNAs, interacting with 25 direct connections in the progression network (Table 2), linking primary CRC tumors to liver metastases. Interestingly, *SATB2-AS1* was downregulated in our analysis (Table 2), which might suggest its involvement in the metastatic process, possibly indicating a shift in its regulatory function as the tumor progresses from the primary site to the liver. The slight downregulation (-1 logFC) hints at a nuanced role in this transition. These findings suggest the need for further assessment of *SATB2-AS1*'s exact role in the progression of colorectal cancer to liver metastasis, with future studies utilizing laboratory techniques like RT-PCR to better understand its function.

Long intergenic non-protein coding RNA 1116 (*LINC01116)* is a long non-coding RNA (lncRNA), a type of RNA over 200 nucleotides in length that is not translated into proteins. Aberrant expression of *LINC01116* has been associated with several cancers, including lung cancer, gastric cancer, colorectal cancer (CRC), glioma, and osteosarcoma. It plays a critical role in promoting cell proliferation, invasion, migration, and apoptosis (45). Inhibiting *LINC01116* can reduce tumor progression in CRCs (46). Furthermore, *LINC01116*'s role in hepatocellular carcinoma (HCC) was highlighted by Kun Wang (47). In our study, *LINC01116* was found to be overexpressed and involved in 16 interactions within the progression network from CRC to liver metastasis. This positions *LINC01116* as a strong candidate for further investigation into its role in metastatic CRC to liver tumors. Future experimental studies should focus on inhibiting its expression and monitoring its effects on tumor progression in liver metastasis.

This study provides a comprehensive analysis of lncRNAs involved in the progression of colorectal cancer (CRC) to liver metastasis. By integrating data from multiple datasets, we identified several differentially expressed lncRNAs (DE-lncRNAs) with significant roles in the metastatic process, such as *LINC01116* and *SATB2-AS1*. These lncRNAs not only interact within key protein-protein interaction (PPI) networks but also present potential as diagnostic biomarkers and therapeutic targets. These lncRNAs play vital roles in regulating CRC progression, making them promising candidates for future research aimed at disrupting the metastatic process.

Conclusion

Our analysis identified five lncRNAs—*PROX1-AS1*, *SOX9-AS1*, *LINC01594*, *LINC01555*, and *APOA1-AS*—previously known for their roles in CRC progression but, for the first time, were shown to affect the liver metastatic process. Additionally, from two lncRNA expression datasets, we identified 20 novel lncRNAs *(VCAN-AS1*, *SYP-AS1*, *SMIM2-IT1*, *NCOA7-AS1*, *LINC01449*, *LINC00974*, *LINC00626*, *LINC00440*, *LINC00379*, *ERVH-1*, *VLDLR-AS1*, *PRICKLE2-DT*, *ADAMTS19-AS1*, *NEUROG2-AS1*, *LHFPL3-AS2*, *LINC00365*, *LINC02826*, *LINC01559*, *CLCA4-AS1*, *LINC02563*, and *LINC00462*) that may play a role in CRC progression and liver metastasis.

Moreover, two lncRNAs—*SATB2-AS1* and *LINC01116*—emerged as the only common candidates across both datasets, suggesting a potential role in the metastatic process from primary CRC tumors to liver tissues. This makes them promising targets for molecular therapy and diagnostic applications. These findings highlighted a new dimension of regulatory mechanisms driven by lncRNAs in the metastatic network of CRC to liver tissues. Several novel molecular players involved in this progression process from primary CRC to liver metastasis were uncovered, contributing to our understanding of molecular behaviors. These insights pave the way for designing new therapeutic or diagnostic strategies focused on CRC progression, particularly in liver metastasis.

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Data Availability Statement

The data presented in this study are available on request from the corresponding author.

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Conflict of interest

The authors declare that there is no conflict of interests.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71:209-249.
2. Van Cutsem E, Van Cutsem E, Cervantes A, Adam R, Sobrero A, Van Krieken JH, Aderka D, et al. ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. Ann Oncol. 2016;27:1386-1422.
3. Manfredi S, Lepage C, Hatem C, Coatmeur O, Faivre J, Bouvier AM. Epidemiology and management of liver metastases from

colorectal cancer. Ann Surg. 2006;244:254-259.
4. Mardis ER. Next-generation sequencing platforms. Annu Rev Anal Chem (Palo Alto Calif). 2013;6:287-303.
5. Lockhart DJ, Dong H, Byrne MC, Follettie MT, Gallo MV, Chee MS, et al. Expression monitoring by hybridization to high-density oligonucleotide arrays. Nat Biotechnol. 1996;14:1675-1680.
6. Peng WX, Koirala P, Mo YY. Lncrna-mediated regulation of cell signaling in cancer. Oncogene. 2017;36:5661-5667.
7. Schmitt AM, Chang HY. Long noncoding rnas in cancer pathways. Cancer Cell. 2016;29:452-463.
8. Kitano H. Systems biology: A brief overview. Science. 2002;295:1662-1664.
9. Hozhabri H, Ghasemi Dehkohneh RS, Razavi SM, Salarian F, Rasouli A, Azami J, et al. (2022). Comparative analysis of protein-protein interaction networks in metastatic breast cancer. PLOS ONE, 17, e0260584.
10. Rasti A, Abazari O, Dayati P, Kardan Z, Salari A, Khalili M, et al. Identification of potential key genes linked to gender differences in bladder cancer based on gene expression omnibus (GEO) database. Adv Biomed Res. 2023;12:157.
11. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. 2015;43:e47-e47.
12. Love M, Anders S, Huber W. Differential analysis of count data–the deseq2 package. Genome Biol. 2014;15:10-1186.
13. Huang P, Deng W, Bao H, Lin Z, Liu M, Wu J, et al. Sox4 facilitates PGR protein stability and FOXO1 expression conducive for human endometrial decidualization. Elife. 2022;11.
14. Chen EY, Tan CM, Kou Y, Duan Q, Wang Z, Meirelles GV, et al. Enrichr: Interactive and collaborative HTML5 gene list enrichment analysis tool. BMC Bioinformatics. 2013;14:128.
15. Mering Cv, Huynen M, Jaeggi D, Schmidt S, Bork P, Snel B. String: A database of predicted functional associations between proteins. Nucleic Acids Res. 2003;31:258-261.
16. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: A software environment for integrated models of biomolecular interaction networks.Genome Res.2003;13:2498-2504.
17. Szklarczyk D, Gable AL, Nastou KC, Lyon D, Kirsch R, Pyysalo S, et al. The STRING database in 2021: Customizable protein–protein networks, and functional characterization of user-uploaded gene/measurement sets. Nucleic Acids Res. 2021;49:D605-D612.
18. Scardoni G, Petterlini M, Laudanna C. Analyzing biological network parameters with centiscape. Bioinformatics. 2009;25:2857-2859.
19. Liu B, Qu X, Wang J, Xu L, Zhang L, Xu B, et al. Linc00365 functions as a tumor suppressor by inhibiting HIF-1α-mediated glucose metabolism reprogramming in breast cancer. Exp Cell Res. 2023;425:113514.
20. Taghehchian N, Farshchian M, Mahmoudian RA, Asoodeh A, Abbaszadegan MR. The expression of long non-coding rna linc01389, linc00365, rp11-138j23. 1, and rp11-354k4. 2 in gastric cancer and their impacts on emt. Mol Cell Probes. 2022;66:101869.
21. Zhu Y, Bian Y, Zhang Q, Hu J, Li L, Yang M, et al. Linc00365 promotes colorectal cancer cell progression through the Wnt/β-catenin signaling pathway. J Cell Biochem. 2020;121:1260-1272.
22. He Y, Du X, Chen M, Han L, Sun J. Novel insight into the functions of n 6-methyladenosine modified lncrnas in cancers. Int J Oncol. International journal of oncology. 2022;61:1-16.
23. Yao L, Man C-F, He R, He L, Huang J-B, Xiang S-Y, et al. The interaction between N6-methyladenosine modification and non-coding RNAs in gastrointestinal tract cancers. Front Oncol. 2022;11:784127.
24. Shi K, Yang S, Chen C, Shao B, Guo Y, Wu X, et al. RNA methylation-mediated linc01559 suppresses colorectal cancer progression by regulating the miR-106b-5p/PTEN axis. Int J Biol Sci. 2022;18:3048.
25. Yang X, Yang Y, Qian X, Xu X, Lv P. Long non-coding rna linc01559 serves as a competing endogenous rna accelerating

triple-negative breast cancer progression. Biomed J. 2022;45:512-521.
26. Yao Y, Zhang F, Liu F, Xia D. Propofol-induced linc01133 inhibits the progression of colorectal cancer via mir-186-5p/nr3c2 axis. environ Toxicol. 2024;39:2265-2284.
27. Zhang P, Chen L, Wu S, Ye B, Chen C, Shi L. Construction of a metabolism-related long non-coding rnas-based risk score model of hepatocellular carcinoma for prognosis and personalized treatment prediction. Pathol Oncol Res. 2022;28:1610066.
28. Liao H, He B. Predictive value of cuproptosis and disulfidptosis-related lncrna in head and neck squamous cell carcinoma prognosis and treatment. Heliyon. 2024
29. Zhang H, Liu R, Sun L, Hu X. An lncrna model for predicting the prognosis of hepatocellular carcinoma patients and cerna mechanism. Front Mol Biosci. 2021;8:749313.
30. Moqadami A, Ahmadi A, Khalaj-Kondori M. Lncrna vldlr-as1 gene expression in colorectal cancer in patients from east azerbaijan province, iran. Jentashapir J Cell Mol Biol. 2023;14:e141522.
31. Liu J, Zhan W, Chen G, Yan S, Chen W, Li R. Sp1-induced prox1-as1 contributes to tumor progression by regulating mir-326/fbxl20 axis in colorectal cancer. Cellular Signalling. 2023;101:110503.
32. Liao M, Zheng W, Wang Y, Li M, Sun X, Liu N, et al. Linc00887 promotes GCN5-dependent H3K27cr level and CRC metastasis via recruitment of YEATS2 and enhancing ETS1 expression. Cell Death Dis. 2024;15:711.
33. Gao M, Guo Y, Xiao Y, Shang X. Comprehensive analyses of correlation and survival reveal informative lncrna prognostic signatures in colon cancer. World J Surg Oncol. 2021;19:1-15.
34. Tian J, Cui P, Li Y, Yao X, Wu X, Wang Z, et al. Linc02418 promotes colon cancer progression by suppressing apoptosis via interaction with miR-34b-5p/BCL2 axis. Cancer Cell Int. 2020;20:460.
35. Zhang LH, Li LH, Zhang PF, Cai YF, Hua D. Linc00957 acted as prognostic marker was associated with fluorouracil resistance in human colorectal cancer. Front Oncol. 2019;9
36. Khashkhashi Moghadam S, Bakhshinejad B, Khalafizadeh A, Mahmud Hussen B, Babashah S. Non-coding rna-associated competitive endogenous rna regulatory networks: Novel diagnostic and therapeutic opportunities for hepatocellular carcinoma. J Cell Mol Med. 2022;26:287-305.
37. Liu B, Song A, Gui P, Wang J, Pan Y, Li C, et al. Long noncoding RNA LINC01594 inhibits the CELF6-mediated splicing of oncogenic CD44 variants to promote colorectal cancer metastasis. Cell Death Dis. 2023;14:427.
38. Dong S, Wang W, Liao Z, Fan Y, Wang Q, Zhang L. Myc-activated linc00607 promotes hepatocellular carcinoma progression by regulating the mir-584-3p/rock1 axis. J Gene Med.2023;25:e3477.
39. Qin J, Li Y, Li Z, Qin X, Zhou X, Zhang H, et al. Linc00114 stimulates growth and glycolysis of esophageal cancer cells by recruiting EZH2 to enhance H3K27me3 of DLC1. Clin Epigenetics. 2022;14:51.
40. Feng Z, Liu Z, Peng K, Wu W. A prognostic model based on nine DNA methylation-driven genes predicts overall survival for colorectal cancer. Front Genet. 2022;12
41. Rasool M, Karim S, Haque A, Alharthi M, Chaudhary AG, Natesan Pushparaj P. Deciphering gene expression signatures in liver metastasized colorectal cancer in stage iv colorectal cancer patients. J King Saud Univ Sci. 2024;36:103415.
42. Xu M, Xu X, Pan B, Chen X, Lin K, Zeng K, et al. LncRNA SATB2-AS1 inhibits tumor metastasis and affects the tumor immune cell microenvironment in colorectal cancer by regulating SATB2. Mol Cancer. 2019;18:1-16.
43. Wang Y-Q, Jiang D-M, Hu S-S, Zhao L, Wang L, Yang M-H, et al. Satb2-as1 suppresses colorectal carcinoma aggressiveness by inhibiting SATB2-dependent Snail transcription and epithelial–mesenchymal transition. Cancer Res. 2019;79:3542-3556.
44. Huang J, Yang Y, Zhao F, Zhang Z, Deng J, Lu W, Jiang X. LncRNA SATB2-AS1

overexpression represses the development of hepatocellular carcinoma through regulating the miR-3678-3p/GRIM-19 axis. Cancer Cell Int. 2023;23:82.

45. Xu Y, Yu X, Zhang M, Zheng Q, Sun Z, He Y, et al. Promising advances in linc01116 related to cancer. Front Cell Dev Biol. 2021;9:736927.

46. Ghasemian A, Omear HA, Mansoori Y, Mansouri P, Deng X, Darbeheshti F, et al. Long non-coding rnas and jak/stat signaling pathway regulation in colorectal cancer development. Front Genet. 2023;14.

47. Wang X-K, Zhang X-D, Luo K, Yu L, Huang S, Liu Z-Y, et al. Comprehensive analysis of candidate signatures of long non-coding rna linc01116 and related protein-coding genes in patients with hepatocellular carcinoma. BMC Gastroenterol. 2023;23:216.