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DDX55 promotes hepatocellular carcinoma progression by interacting with BRD4 and participating in exosome-mediated cell-cell communication

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Abstract

The involvement of DEAD-box helicase 55 (DDX55) in oncogenesis has been suggested, but its biological role in hepatocellular carcinoma (HCC) remains unknown. The present study verified the upregulation of DDX55 in HCC tissues compared with non-tumor controls. DDX55 displayed the highest prognostic values among the DEAD-box protein family for recurrence-free survival and overall survival of HCC patients. In addition, the effects of DDX55 in the promotion of HCC cell proliferation, migration, and invasion were determined ex vivo and in vivo. Mechanistically, we revealed that DDX55 could interact with BRD4 to form a transcriptional regulatory complex that positively regulated PIK3CA transcription. Following that, β-catenin signaling was activated in a PI3K/Akt/GSK-3β dependent manner, thus inducing cell cycle progression and epithelial–mesenchymal transition. Intriguingly, both DDX55 mRNA and protein were identified in the exosomes derived from HCC cells. Exosomal DDX55 was implicated in intercellular communication between HCC cells with high or low DDX55 levels and between HCC cells and endothelial cells, thereby promoting the malignant phenotype of HCC cells and angiogenesis. In conclusion, DDX55 may be a valuable prognostic biomarker and therapeutic target in HCC.

KEYWORDS
BRD4, DDX55, exosome, hepatocellular carcinoma, β-Catenin

1 | INTRODUCTION

Despite significant progress in cancer prevention and therapy, the morbidity and mortality of hepatocellular carcinoma (HCC) remain high worldwide.[1] In the era of precision medicine, the unresolved clinical dilemmas due to its distinct features (including insidious onset, frequent metastasis, and postoperative recurrence) emphasize the necessity to deeply explore the molecular mechanisms underlying HCC progression, thereby contributing to the molecular diagnosis and targeted therapy of HCC.[2]

Abbreviations: ANOVA, analysis of variance; ChIP, chromatin immunoprecipitation; co-IP, co-immunoprecipitation; DDX55, DEAD-box helicase 55; EFs, epigenetic factors; EMT, epithelial mesenchymal transformation; GEO, Gene Expression Omnibus; GSEA, gene set enrichment analysis; HCC, hepatocellular carcinoma; HE, hematoxylin and eosin; HPA, Human Protein Atlas; HR, hazard ratio; HUVEC, human umbilical vein endothelial cells; ICGC, International Cancer Genome Consortium; IF, immunofluorescence; IHC, immunohistochemistry; NPC, nasopharyngeal carcinoma; NTA, nanoparticle tracking analysis; OS, overall survival; PPI, protein–protein interaction; qRT-PCR, quantitative real-time polymerase chain reaction; RFS, recurrence-free survival; shRNA, short hairpin RNA; siRNA, small interfering RNA; TCGA, The Cancer Genome Atlas; TEM, transmission electron microscopy; TFs, transcription factors; WB, western blotting.

DEAD-box RNA helicases belong to helicase superfamily 2 and play crucial roles in all aspects of RNA metabolism.[3] Members of the superfamily are characterized by 12 conserved motifs, including the characteristic Asp(D)-Glu(E)-Ala(A)-Asp(D) motif.[4,5] Except for the classical ATP-dependent RNA helicase activities, emerging evidence has suggested that many DEAD-box proteins have diverse biological functions due to their distinct N- and C-terminal domains and their interactions with partner proteins.[4,6] In light of their key functions in various biological processes, the critical roles of the DEAD-box protein family in oncogenesis have been gradually revealed.[3,7–17] In our previous study, DDX55, a member of the DEAD-box RNA helicases, was preliminarily screened out as one of the core prognostic genes related to early recurrence of HCC after R0 resection.[18] In addition, Liu et al. and Cui et al. reported that DDX55 was a potential biomarker associated with liver metastasis in human colon carcinoma and a poor prognostic indicator in lung cancer.[3,19] The above findings undoubtedly indicate the involvement of DDX55 in tumorigenesis (particularly HCC). Nevertheless, the role of DDX55 in HCC and its underlying mechanisms remain to be deeply explored.

Tumor behavior is determined by not only the malignant phenotype of tumor cells themselves but also by their surrounding microenvironment.[20] Numerous studies have demonstrated that tumor cell-derived exosomes, nano-sized vesicles containing diverse active components (including DNA, RNA, and proteins), are pivotal in cell–cell communication during cancer progression.[21] Of particular note, Yuan et al. reported that Taxol-resistant nasopharyngeal carcinoma (NPC) cell-derived exosomes containing DDX53 could be internalized by normal NPC cells and impacted their Taxol-resistant capacity.[22] Thus, it is worth identifying whether DDX55 is present in HCC cell-derived exosomes and exploring their corresponding roles.

The present study identified that DDX55 upregulation was an independent prognostic indicator for recurrence-free survival (RFS) and overall survival (OS) of HCC patients. In addition, the effects of DDX55 in promoting HCC cell growth and metastasis were determined ex vivo and in vivo. Mechanistically, our data revealed that DDX55 could interact with BRD4 and activate the PI3K/Akt/GSK-3β/β-catenin pathway in HCC. Importantly, exosome-mediated intercellular transfer of DDX55 was observed in HCC, thereby promoting HCC progression.

2 | MATERIALS AND METHODS

2.1 | Clinical specimens and cell lines

Paired tumor and adjacent non-tumor tissues were obtained from HCC patients receiving resection in Zhongnan Hospital of Wuhan University, including 35 paraffin-embedded specimens and 40 frozen specimens. Human HCC cell lines (including HepG2, Huh7, Hep3B, Sk-hep1, and SNU387) were purchased from the Cell Bank of Shanghai Institutes for Biological Science, Chinese Academy of Science (Data S1 and S2). Human umbilical vein endothelial cells (HUVEC) were generously donated by Dr Shuangquan Wu (Wuhan University).

2.2 | Cell transfection

Short hairpin RNA (shRNA) lentiviral particles targeting DDX55 (shDDX55) and lentiviral vector containing human DDX55 cDNA (NM_020936-HA) were synthesized by Genechem (Shanghai, China), and stable transfection was performed based on the manufacturer's instructions. Small interfering RNA (siRNA) targeting β-catenin (CTNNB1) or BRD4 were synthesized by Tsingke, and transient transfection was conducted using Lipofectamine 2000 (#11668–019, Invitrogen). The shRNA target sequences and siRNA sequences are summarized in Supplementary Data S3.

2.3 | Transcriptome sequencing and enrichment analysis

Using the Huh7-shDDX55 cells and the Huh7-shNC cells, transcriptome sequencing was performed based on the Illumina Novaseq 6000 platform at Majorbio (Shanghai, China). After data quality control, the raw data of RNA-seq were normalized as previously described.[18] To explore the biological pathways associated with DDX55 dysregulation in HCC, gene set enrichment analysis (GSEA) was performed as previously described.[18]

2.4 | Western blotting (WB) and quantitative real-time polymerase chain reaction

Western blotting and quantitative RT-PCR (qRT-PCR) assays were performed as previously described.[23] The detailed methods and the primary antibodies/primers are summarized in Supplementary Data S3.

2.5 | Cell proliferation and cell cycle assays

Cell Counting Kit-8 (CCK8) (#CK04, Dojindo, Kumamoto, Japan) and Cell Cycle Kit (#CCS012, Multi Sciences, Hangzhou, China) were utilized to assess cell viability and cell cycle. The detailed methods are summarized in Supplementary Data S3.

2.6 | Cell migration and invasion assays

Cell migration ability was evaluated ex vivo by wound-healing assay or transwell migration assay. Cell invasion ability was evaluated ex vivo by transwell invasion assay. The detailed methods are summarized in Supplementary Data S3.

2.7 | Tube formation assay

Next, 4×10^4 indicated HUVEC per well were seeded into a Matrigel-coated 48-well plate. After 12 h incubation, the HUVEC were inspected and the tube number per field was calculated.

2.8 | In vivo xenograft assay

The subcutaneous tumor formation model and the lung metastasis model via tail vein injection were constructed in nude mice to examine the effects of DDX55 on HCC tumorigenicity and metastasis in vivo. The detailed methods are summarized in Supplementary Data S3.

2.9 | Immunohistochemistry and immunofluorescence staining

Immunohistochemistry (IHC) and immunofluorescence (IF) assays were performed as previously described.[23] The detailed methods are summarized in Supplementary Data S3.

2.10 | Co-immunoprecipitation

Co-immunoprecipitation (co-IP) assay was performed using an immunoprecipitation kit based on the manufacturer's instructions (#PK10008, Proteintech). The primary antibodies are summarized in Supplementary Data S3.

2.11 | Chromatin immunoprecipitation

Chromatin immunoprecipitation (ChIP) assay was performed using a ChIP assay kit based on the manufacturer's instructions (#P2078, Beyotime). The primary antibodies and primers are summarized in Supplementary Data S3.

2.12 | Exosome isolation, identification, and tracing

Indicated HCC cell-derived exosomes in cell culture supernatant were purified by differential centrifugation (Beckman optimal L-100XP). Size range of exosomes was assessed by nanoparticle tracking analysis (NTA) (Malvern, UK). Exosome morphology was observed by transmission electron microscopy (TEM) (Hitachi, Japan). Exosome biomarkers were detected by WB assays. Exosomes were labeled with a Cell Membrane Labeling Kit (PKH26) (#KM0044, BaiAo-Lai-Bo). The methods are summarized in Supplementary Data S3.

2.13 | Data mining of public databases

The intact mRNA-seq data and clinical information of 247 HCC tissues and 50 adjacent non-tumor tissues were retrieved from the Cancer Genome Atlas (TCGA) database.[24] In addition, multiple HCC cohorts containing transcriptome data and follow-up data were enrolled from the International Cancer Genome Consortium (ICGC) and Gene Expression Omnibus (GEO) databases. The IHC data of DDX55, BRD4, PIK3CA, and β-catenin in HCC tissues or non-tumor controls were downloaded from the Human Protein Atlas (HPA) database.[25] The hTFtarget database was used to explore the transcription factors (TF) and epigenetic factors (EF) associated with PIK3CA, which were supported by ChIP-sequencing (ChIP-seq) data.[26] The GSE123097 dataset containing the BRD4 protein ChIP-seq data in HepG2 cells was used to analyze the genome occupancy of BRD4.[27] The STRING database was used to perform protein–protein interaction (PPI) analysis.[28] Vesiclepedia, a comprehensive exosome database, was used to explore the identified contents in the exosomes derived from different cells.[29] Datasets containing DDX55 mRNA profiles in cells and their exosomes were downloaded from the GEO database. Detailed information is available in Supplementary Data S3.

2.14 | Statistical analysis

Following a normality test and homogeneity test of variances, statistical significance was determined by Student's *t* test for two groups or analysis of variance (ANOVA) for more than three groups. Survival analysis was performed by joint use of the Kaplan–Meier method and Cox regression analysis. Selection of an optimal cut-off value for defining high or low DDX55 expression was achieved using X-tile-3.6.1 software according to overall survival data in the TCGA dataset. The clinical implications of DDX55 dysregulation in HCC were assessed by Pearson's χ^2 test. Statistical analyses and graphing were conducted using SPSS 22.0 and GraphPad Prism 8, and a *P*-value <0.05 was considered statistically significant.

3 | RESULTS

3.1 | DDX55 upregulation correlated with clinicopathological features of hepatocellular carcinoma patients

In line with the aberrant upregulation of DDX55 in multiple cancers revealed by pan-cancer analysis, DDX55 mRNA overexpression in HCC tissues compared with non-tumor controls has been verified in multiple large-scale HCC cohorts (Figure 1A; Figure S1A-C). Consistently, IHC and WB assays revealed enhanced DDX55 protein expression in HCC, which was predominantly located in the nucleus and cytoplasm (Figure 1B-C; Figure S1C). Of note, normal liver tissues had a lower baseline DDX55 level among human normal organs, which highlighted the significance of DDX55 upregulation in HCC progression (Figure S1A-C). WB analysis of DDX55 expression in HCC cell lines showed that DDX55 protein was relatively lower in SNU387 cells than in Huh7, HepG2, Sk-hep1, and Hep3B cells (Figure 1D). Mechanistically, DDX55 is located on chromosome 12q24.31, which frequently exhibits copy number alterations and abnormal methylation in several cancers.[30–33] From the perspective of genetic and epigenetic alterations of DDX55, we found that DNA copy gain and DNA hypomethylation of its specific CpG site (cg17182540, Pearson's $r = 0.2126$, $P < 0.0001$) might result in DDX55 upregulation in HCC (Figure S1D-E).

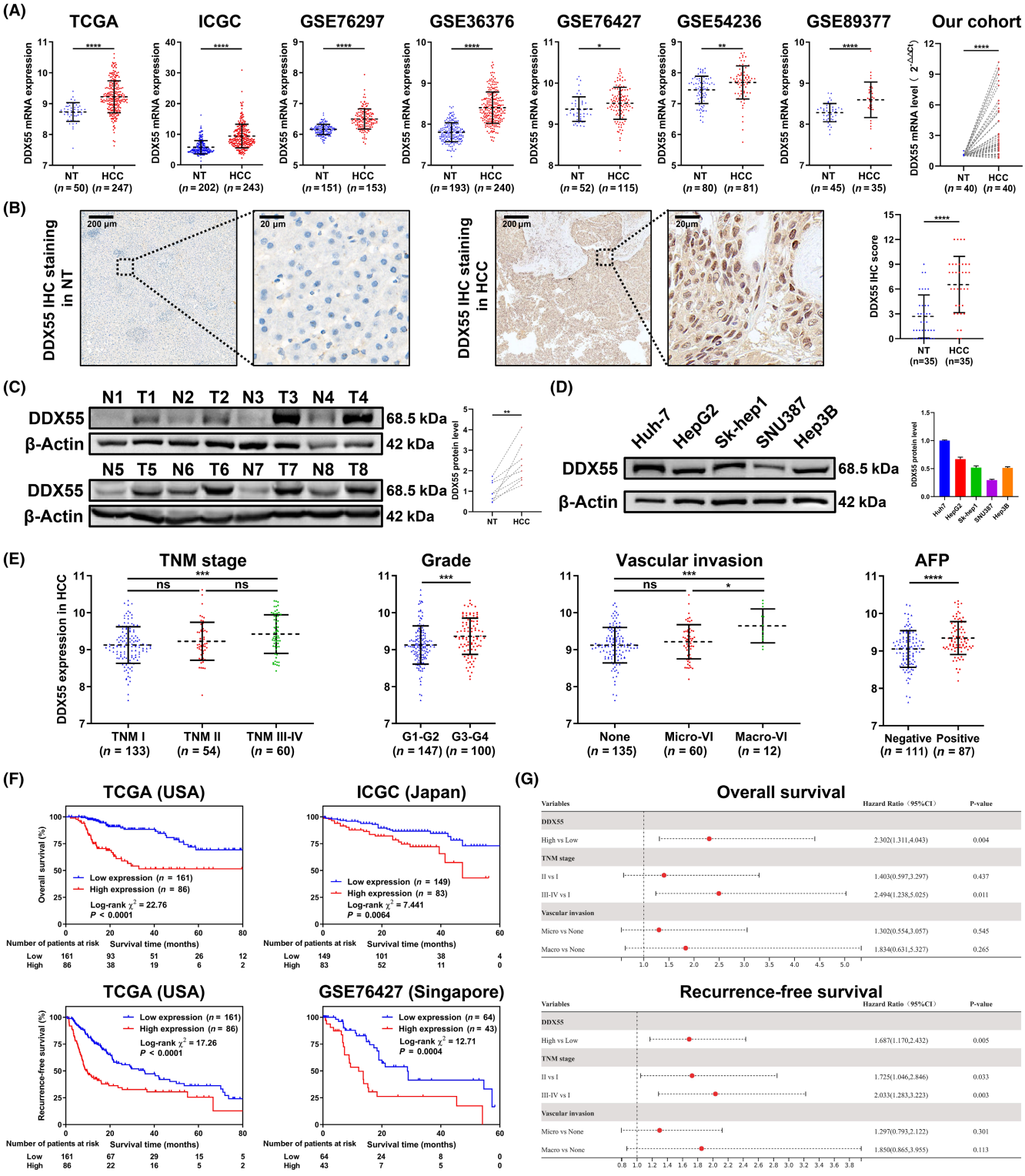

FIGURE 1 DEAD-box helicase 55 (DDX55) is upregulated in hepatocellular carcinoma (HCC) and serves as an unfavorable prognostic indicator. (A) Comparison of DDX55 mRNA levels between HCC tissues and non-tumor controls (NT) based on public cohorts and our cohort. (B) Representative Immunohistochemical (IHC) images of DDX55 protein expression in NT tissues and HCC tissues in our cohort and the quantification of IHC staining for DDX55. (C) DDX55 protein expression in HCC tissues and their matched NT tissues was measured by western blotting (WB). (D) WB analysis of DDX55 expression in HCC cell lines. Relative protein expression was normalized to β-actin level. (E) Correlation between DDX55 mRNA levels and key clinical characteristics of HCC patients (including TNM stage, histologic grade, vascular invasion, and serum alpha fetoprotein [AFP]) in the TCGA-LIHC cohort. AFP-Positive: >20 ng/mL; AFP-Negative: ≤20 ng/mL. (F) Kaplan–Meier analyses of overall survival (OS) and recurrence-free survival (RFS) of HCC patients with high or low DDX55 expression levels based on TCGA-LIHC cohort, ICGC cohort, and GSE76427 cohort. Statistical significance of Kaplan–Meier analyses was evaluated by log-rank test. (G) Multivariate Cox regression analyses of OS and RFS of HCC patients based on TCGA-LIHC cohort. Data are presented as mean ± SD. Statistical significance was evaluated by Student's *t* test for two groups or one-way ANOVA with Bonferroni multiple comparison test for three groups. *$P < 0.05$, **$p < 0.01$, ***$P < 0.001$, ****$P < 0.0001$. ns: no significance

Then, the clinical implications of DDX55 upregulation in HCC were studied. As shown in Figure 1E and Table S1, DDX55 upregulation was significantly correlated with elevated alpha fetoprotein, advanced TNM stage and histologic grade, and vascular invasion (all $P < 0.05$). Based on the TCGA cohort, Kaplan–Meier survival analyses suggested that HCC patients with DDX55 upregulation had worse prognosis (including OS and RFS) (all $P < 0.05$), which was confirmed by data from ICGC and GSE76427 cohorts (Figure 1F). Moreover, multivariate Cox regression analysis identified that DDX55 upregulation was an independent prognostic indicator for the unfavorable OS [Hazard ratio (HR) =2.302 (1.311, 4.043), $p = 0.004$] and RFS [HR =1.687(1.170, 2.432), $p = 0.005$] of HCC patients (Figure 1G; Table S2 and S3). Further subgroup analyses further supported that the prognostic value of DDX55 was independent of the TNM stage in HCC (all $P < 0.05$) (Figure S2). Of note, we systematically investigated the prognostic values of the DEAD-box helicase family, and DDX55 displayed the highest prognostic value for RFS and OS of HCC patients (Table S4; Figure S3).

3.2 | DDX55 promoted hepatocellular carcinoma cell proliferation and metastasis ex vivo and in vivo

To identify the oncogenic roles of DDX55 in HCC, we selected HCC cell lines according to endogenous DDX55 protein levels and separately established stable DDX55-knockdown cell lines (Huh7-shDDX55 and HepG2-shDDX55) and DDX55-overexpression cell lines (SNU387-oeDDX55) (Figure 2A). CCK8 and clone formation assays suggested that DDX55 silencing significantly weakened the proliferation of Huh7 cells and HepG2 cells, while DDX55 overexpression promoted SNU387 cell growth (Figure 2B-C). Cell cycle analysis indicated that DDX55 silencing brought about a significant increase of the G0/G1 fraction and a reduction of the S fraction in Huh7 cells and HepG2 cells, while DDX55 overexpression resulted in the opposite pattern in SNU387 cells (Figure 2D). The wound-healing and transwell experiments consistently revealed a significant suppression of HCC cell migration and invasion abilities after DDX55-knockdown, but DDX55 upregulation facilitated HCC cell migration and invasion (Figure 2E-F).

Then, in vivo experiments were performed for further examining effects of DDX55 on HCC tumorigenicity and metastasis. Huh7-shDDX55 cells and the corresponding controls (Huh7-shNC) were implanted into nude mice via subcutaneous injection. The tumor weight and volume in the Huh7-shNC group were considerably larger than those in the Huh7-shDDX55 group (Figure 3A-C). Hematoxylin and eosin (HE) and IHC staining data also demonstrated reduced tumor burden and lower Ki67 and PCNA levels in the Huh7-shDDX55 group (Figure 3D-E). Next, an in vivo lung metastasis model was constructed to assess the impact of DDX55 on HCC metastasis. Compared to the Huh7-shNC group, the DDX55 silencing group displayed a significantly lower number of metastatic foci and fluorescence signal intensities (Figure 3F-G). HE staining revealed a decreased size of metastatic foci in the Huh7-shDDX55 group (Figure 3H). Collectively, above findings identified that DDX55 upregulation could facilitate HCC cell growth and metastasis ex vivo and in vivo.

3.3 | DDX55 promoted hepatocellular carcinoma progression by activating the β-catenin pathway

To clarify the molecular mechanisms underlying the oncogenic roles of DDX55 in HCC, transcriptome sequencing was carried out using the Huh7-shDDX55 cells and the Huh7-shNC cells. GSEA analysis highlighted the Wnt pathway as one of the top ranked biological pathways associated with DDX55, which was initially supported by downregulation of several Wnt target genes, including CD44, VEGFA, FOXC1, MMP9, CCND1, MYC, ZEB1, and CDH2 (Figure 4A; Figure S4A). Accumulation of cytoplasmic β-catenin and subsequent translocation to the nucleus are the typical markers for the Wnt/β-catenin pathway activation.[34] WB and IF assays consistently verified the decreased cytoplasmic/nuclear β-catenin in Huh7-shDDX55 cells and the upregulated cytoplasmic/nuclear β-catenin in SNU387-oeDDX55 cells compared to the corresponding controls (Figure 4B-C).

We further detected the levels of cell cycle-related and epithelial mesenchymal transformation (EMT)-related proteins downstream of the Wnt/β-catenin signaling. WB assays confirmed that DDX55 knockdown significantly inhibited the expression of N-cadherin, ZEB1, c-myc, and cyclin D1 and increased E-cadherin in Huh7 cells, while DDX55 overexpression led to an increase of N-cadherin, ZEB1, c-myc, and cyclin D1 and a decrease of E-cadherin in SNU387 cells (Figure 4D). To further determine whether DDX55 promoted HCC progression via β-catenin signaling, β-catenin was knocked down in SNU387-oeDDX55 cells and SNU387-oeNC cells. β-catenin silencing significantly eliminated DDX55-induced expression changes of EMT and cell cycle-related proteins (Figure 5A). Moreover, DDX55-enhanced growth and metastasis of HCC cells could be abolished after β-catenin silencing (Figure 5B-C). Taken together, these findings suggested that DDX55 could promote HCC cell growth and EMT by activating the β-catenin pathway.

3.4 | DDX55 regulated the PI3K/Akt/GSK-3β/β-catenin pathway by interacting with BRD4

How DDX55 activated the β-catenin signaling pathway was further explored. Sequencing data suggested that DDX55 silencing in Huh7 cells did not affect CTNNB1 (β-catenin) mRNA expression (Figure S4B). Intriguingly, GSEA analysis suggested that DDX55 was positively correlated with "pathways in cancer" (Figure 6A). In addition, the mRNA expression of PIK3CA, a core gene in regulating Akt and Wnt pathways, could be remarkably downregulated by silencing DDX55 in Huh7 cells (Figure 6B).[35] A positive correlation between DDX55 expression and PIK3CA expression in HCC

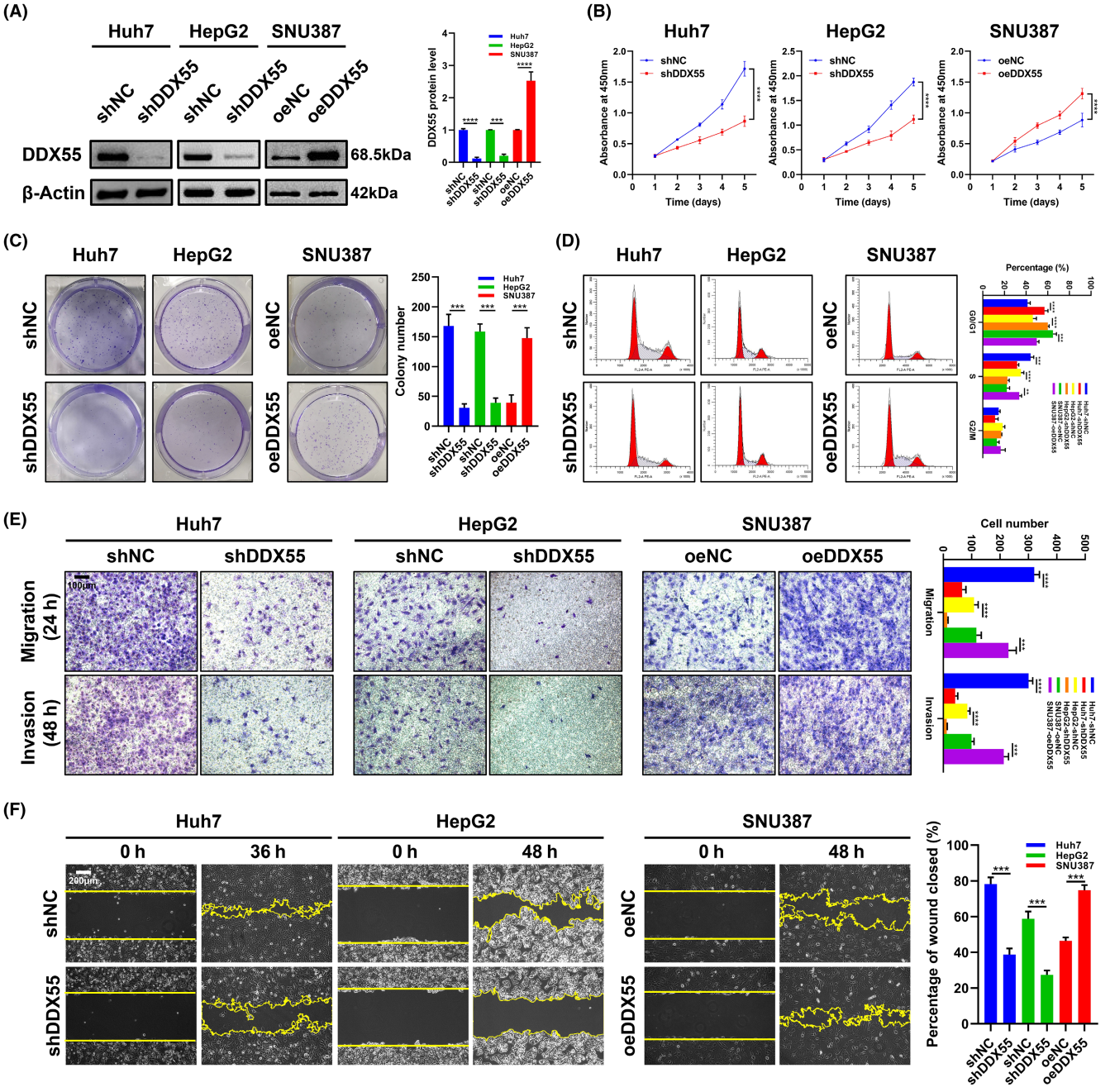

FIGURE 2 DEAD-box helicase 55 (DDX55) promotes hepatocellular carcinoma (HCC) cell growth and metastasis ex vivo. (A) Knockdown of DDX55 in Huh7 cells and HepG2 cells and overexpression of DDX55 in SNU387 cells, as validated by western blotting (WB). Relative protein expression was normalized to β-actin level. (B) CCK-8 assay, (C) clone formation assay, (D) cell cycle assay, (E) Transwell migration and Matrigel invasion assays, and (F) wound healing migration assay were performed in the indicated HCC cells. Data were presented as mean ± SD. Statistical significance was evaluated by Student's *t* test or two-way ANOVA. $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$

tissues was observed using the TCGA dataset and the HPA database (Figure S4C-D). Considering that PIK3CA regulated Akt phosphorylation to exert biological effects,[35] the positive correlation between DDX55 expression and p-Akt expression in HCC tissues was also determined by IHC (Pearson's $r = 0.7732$, $P < 0.001$) (Figure S4E). As we all know, phosphorylation and degradation of β-catenin mediated by active GSK-3β is a key regulatory approach for β-catenin signaling, and active PI3K/AKT signaling could inactivate GSK-3β.[34,35] To explore whether DDX55 activated β-catenin signaling in a PI3K/AKT/GSK-3β dependent manner, WB assays suggested that DDX55 knockdown significantly inhibited the levels of β-catenin, active β-catenin, PI3K(110α), p-Akt, and p-GSK-3β in Huh7 cells, while DDX55 overexpression significantly upregulated the expression of β-catenin, active β-catenin, PI3K(110α), p-Akt, and p-GSK-3β in SNU387 cells (Figure 6C).

To elucidate how DDX55 regulated PIK3CA expression levels, we comprehensively searched the potential TF and EF associated with PIK3CA transcription regulation in the hTFtarget database.

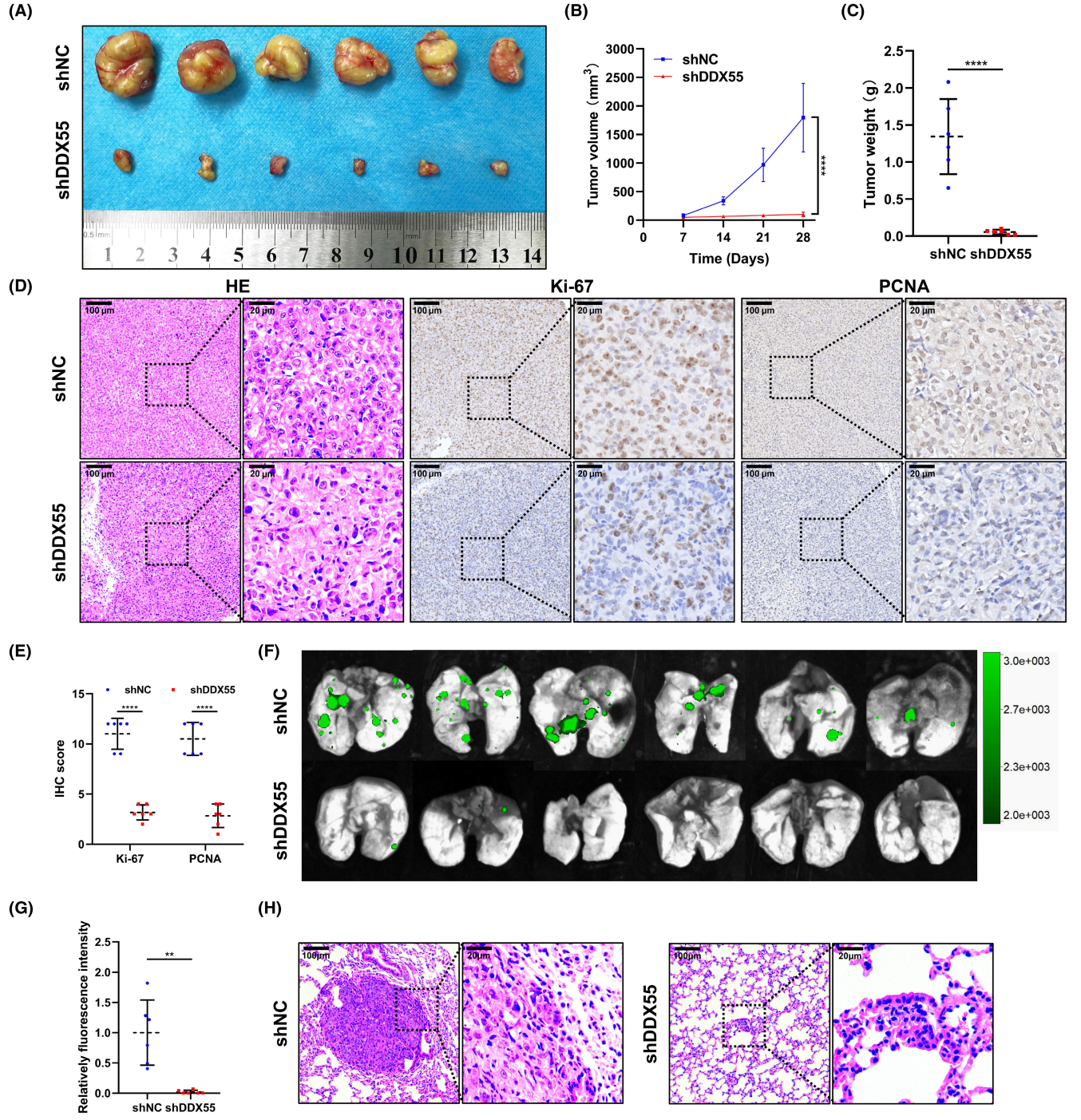

FIGURE 3 DEAD-box helicase 55 (DDX55) promotes hepatocellular carcinoma (HCC) tumorigenicity and metastasis in vivo. (A-C) Quantification of tumor volume and weight of subcutaneous xenografts derived from Huh7-shNC cells or Huh7-shDDX55 cells. (D) Representative images of hematoxylin and eosin (HE) staining and immunohistochemistry staining for Ki-67 and PCNA in the indicated xenografts. (E) The quantification of IHC staining for Ki-67 and PCNA. (F-G) Fluorescence images and quantification of lung metastatic foci derived from Huh7-shNC cells or Huh7-shDDX55 cells. (H) Representative images of HE staining in the indicated lung metastatic foci. Data are presented as mean ± SD. Statistical significance was evaluated by two-way ANOVA or Student's *t* test. $^{**}P < 0.01$, $^{****}P < 0.0001$

By performing PPI analysis between DDX55 protein and those proteins (i.e., the potential TF and EF) based on the STRING database, we found that DDX55 could physically interact with bromodomain (BRD)-containing proteins (particularly BRD4 protein) (Figure 6D). A Co-IP assay with WB in reducing and non-reducing conditions confirmed the interaction between BRD4 protein and DDX55 protein (Figure 6D). Interestingly, the positive correlation between BRD4 expression and PIK3CA expression was also observed using the TCGA dataset and the HPA database (Figure S4D and S5A). ChIP-seq analysis suggested that BRD4 occupancy

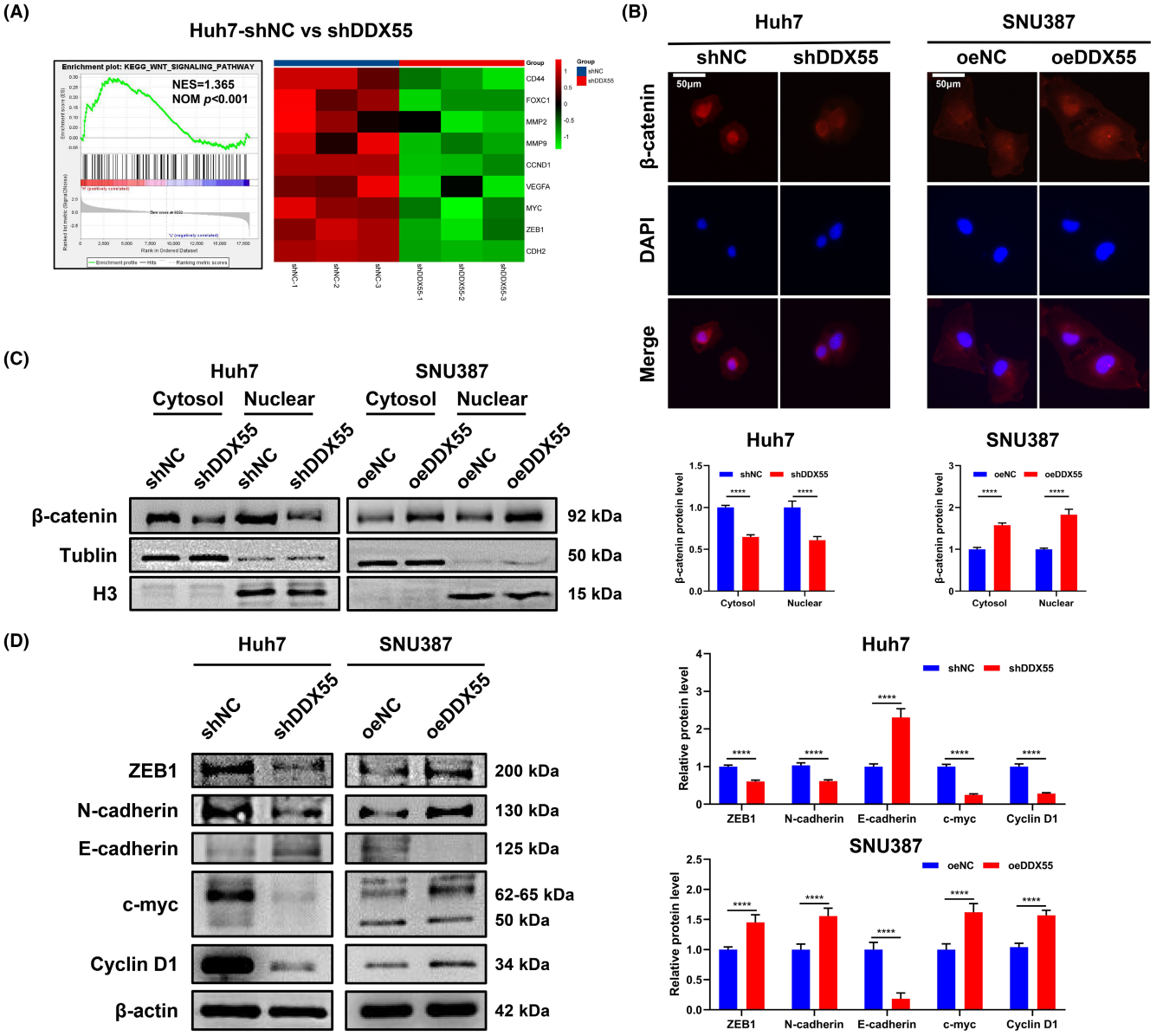

FIGURE 4 DEAD-box helicase 55 (DDX55) activates β-catenin pathway. (A) Transcriptome sequencing analyses of Huh7-NC cells and Huh7-shDDX55 cells were performed. Gene set enrichment analysis highlighted the Wnt pathway as one of the top ranked biological pathways associated with DDX55. Heatmap displayed the expression changes of several Wnt target genes following DDX55 knockdown. NES, normalized enrichment scores; NOM *p*, nominal *P*-value. (B) Immunofluorescence analysis of cytoplasmic and nuclear β-catenin expression in the indicated cells. (C) Western blotting (WB) analysis of cytoplasmic and nuclear β-catenin expression in the indicated cells, and the relative protein expression was normalized to Tublin level (cytoplasmic β-catenin) or H3 level (nuclear β-catenin). (D) WB analysis of ZEB1, N-cadherin, E-cadherin, c-myc, and Cyclin D1 expression in the indicated hepatocellular carcinoma (HCC) cells. Relative protein expression was normalized to β-actin level. Data are presented as mean ± SD. Statistical significance was evaluated by Student's *t* test. ****$P < 0.0001$

was enriched within the promoter of PIK3CA (Figure S5B). ChIP-qPCR analysis suggested that BRD4 and DDX55 co-occupy the PIK3CA promoter, and the enrichment of BRD4 on the promoter of PIK3CA could be significantly reduced by DDX55 knockdown (Figure 6E-G). By exploring the PPI between BRD4 and the potential TFs of PIK3CA, we found that several classical TFs (including CDK9) were both the potential TFs of PIK3CA and the potential interacting proteins of BRD4 (Figure S5C). To further determine whether DDX55 regulated PIK3CA expression by interacting with BRD4, we knocked down BRD4 in SNU387-oeDDX55 and SNU387-oeNC cells. BRD4 silencing could significantly eliminate DDX55-induced upregulation of PIK3CA protein and mRNA levels, as well as its downstream protein levels (including p-Akt, p-GSK-3β, β-catenin, and active β-catenin) (Figure 7A-B). Moreover, the DDX55-enhanced growth and metastasis of HCC cells could be abolished after BRD4 silencing (Figure 7C-D). Taken together, DDX55 might activate β-catenin in a BRD4/PI3K/Akt/GSK-3β-dependent manner.

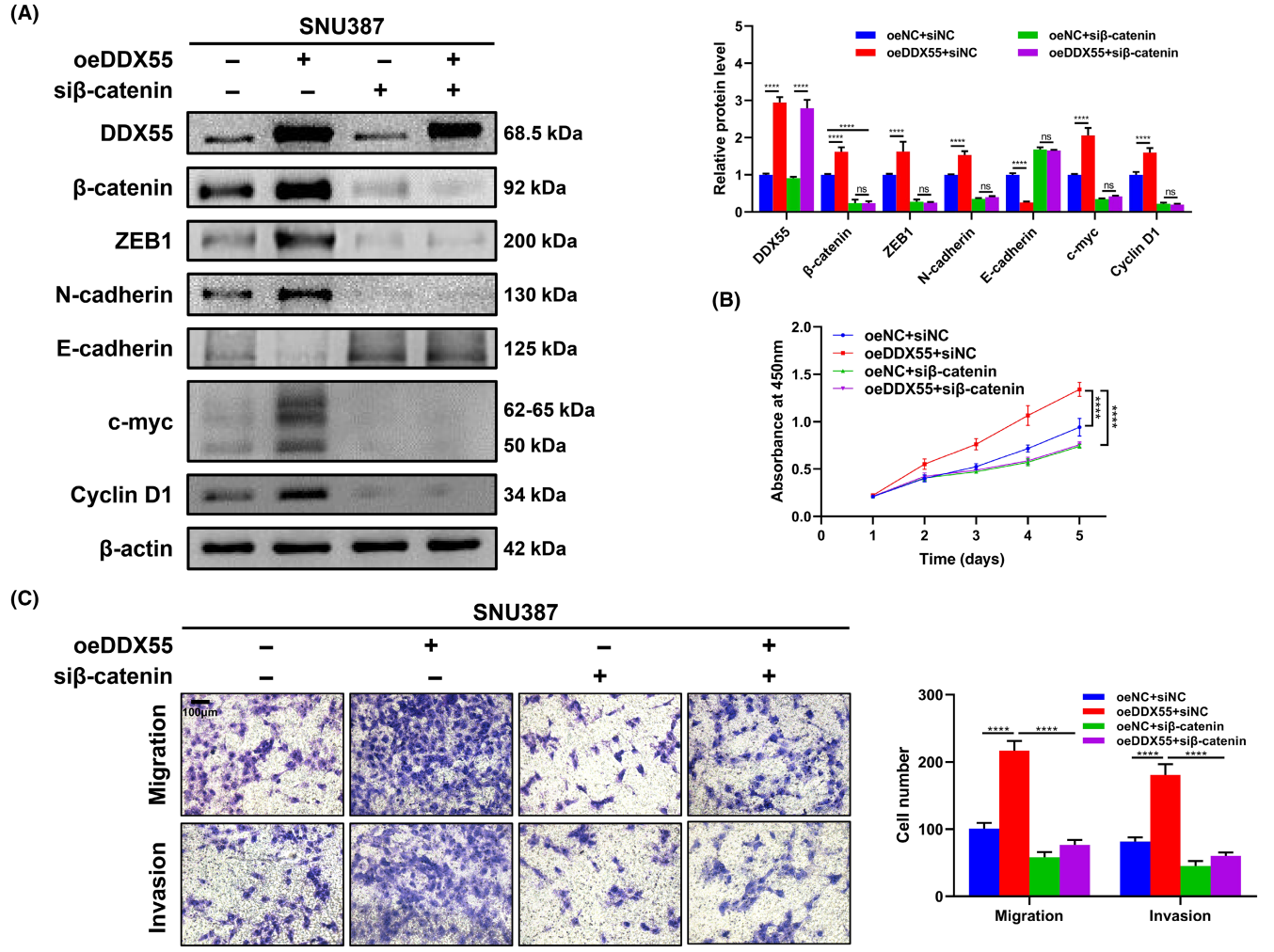

FIGURE 5 DEAD-box helicase 55 (DDX55) promotes hepatocellular carcinoma (HCC) progression by activating the β-catenin pathway. (A) Western blotting (WB) analysis of DDX55, β-catenin, ZEB1, N-cadherin, E-cadherin, Cyclin D1, and c-myc expression in the indicated SNU387 cells co-transfected with or without siβ-catenin. Relative protein expression was normalized to β-actin level. (B) Cell viability analysis using the CCK-8 assay in the indicated SNU387 cells co-transfected with or without siβ-catenin. (C) Cell migration and invasion analyses using Transwell assays in the indicated SNU387 cells co-transfected with or without siβ-catenin. Data are presented as mean ± SD. Statistical significance was evaluated by Student's *t* test for two groups or one-way ANOVA with Tukey's multiple comparison test for three groups (A and C) or two-way ANOVA (B). ****$P < 0.0001$. ns, no significance

3.5 | Intercellular transfer of DDX55 by exosomes promoted hepatocellular carcinoma progression

By data mining the Vesiclepedia and GEO databases, we revealed that both DDX55 mRNA and protein resides in the exosomes derived from human cancer cells and non-cancer cells (Figure S6A-B; Figure S7A-G). Interestingly, the DDX55 mRNA level in non-cancer cells and their exosomes was relatively lower than that in cancer cells and their exosomes (Figure S7A). Strikingly, the DDX55 mRNA level in non-cancer cells could be obviously upregulated after cancer cell-derived exosome treatment (Figure S7F-G). These findings suggested that DDX55 could be packaged into exosomes, and tumor cell-derived exosomes containing DDX55 might participate in tumor progression.

To determine whether exosomes mediated intercellular transfer of DDX55 in HCC, we first identified that DDX55 mRNA and protein indeed existed in Huh7-derived exosomes and DDX55 levels in exosomes could be upregulated following lentiviral oeDDX55 transfection in Huh7 cells (Huh7-oeDDX55) (Figure 8A-F). Furthermore, IF assays showed that SNU387 cells exhibited high uptake efficiency following Huh7-derived exosomes treatment (Figure 8G). Upregulation of DDX55 mRNA and protein in SNU387 cells were detected following treatment of Huh7-oeNC cell- or Huh7-oeDDX55 cell-derived exosomes (Figure 8H-I). The growth, migration, and invasion abilities of the exosome-treated SNU387 cells could be accordingly promoted (Figure 8J-K). Considering that DDX55-containing microvesicles derived from colorectal cancer cell could stimulate HUVEC proliferation,[36] we identified that exosomal DDX55 derived from HCC cells could be transferred to HUVEC and enhance the proliferation, migration, and tube formation abilities of HUVEC (Figure 8L-P). The regulatory landscape of DDX55 in facilitating HCC growth and metastasis is summarized in Figure 9.

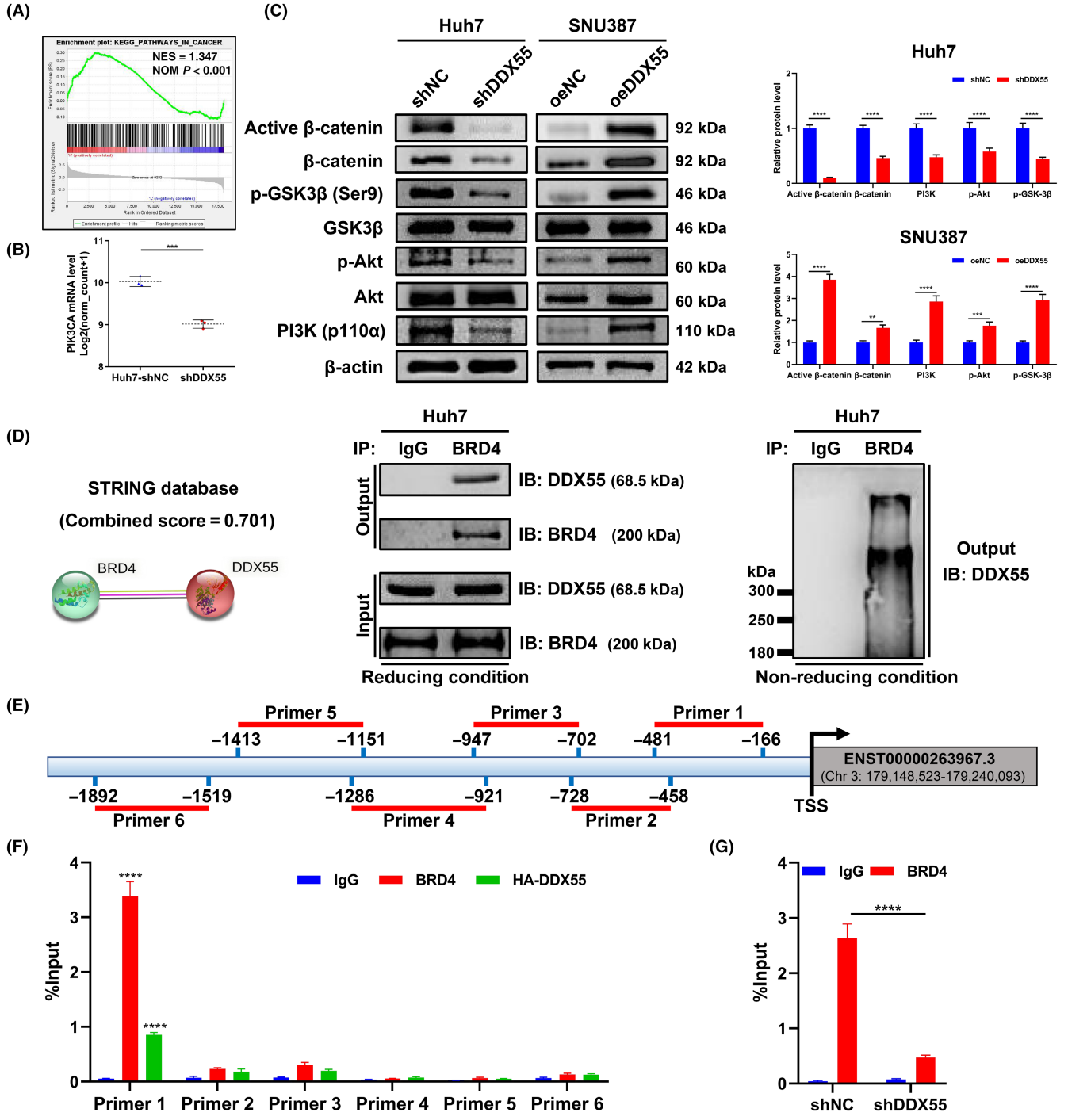

FIGURE 6 DEAD-box helicase 55 (DDX55) regulates the PI3K/Akt/GSK-3β/β-catenin pathway by interacting with BRD4. (A) Gene set enrichment analysis highlighted "pathway in cancer" as one of the top ranked biological pathways associated with DDX55. NES, normalized enrichment scores; NOM *p*, nominal *P*-value. (B) Transcriptome sequencing analysis of PIK3CA mRNA levels in the indicated Huh7 cells. (C) Western blotting (WB) analysis of active β-catenin, β-catenin, phosphorylated GSK-3β (ser9), GSK-3β, phosphorylated AKT (Ser473), total AKT, and PI3K (p110α) expression in the indicated hepatocellular carcinoma (HCC) cells. Relative protein expression was normalized to β-actin level, and phosphorylated proteins (p-Akt and p-GSK-3β) were normalized to corresponding total protein (Akt and GSK-3β). (D) The interaction between DDX55 protein and BRD4 protein was revealed by the STRING database and identified by co-immunoprecipitation assay with WB in reducing or non-reducing conditions. (E) Six pairs of primers were designed within the PIK3CA promoter. (F) ChIP-qPCR analysis suggested that BRD4 and DDX55 co-occupy the promoter region of PIK3CA (Primer 1: −481 ~ −166) in Huh7-oeDDX55-HA cells. (G) ChIP-qPCR analysis suggested the enrichment of BRD4 on the promoter of PIK3CA (Primer 1: −481 ~ −166) could be significantly reduced by DDX55 knockdown in Huh7 cells. Data are presented as mean ± SD. Statistical significance was evaluated by Student's *t* test. $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$

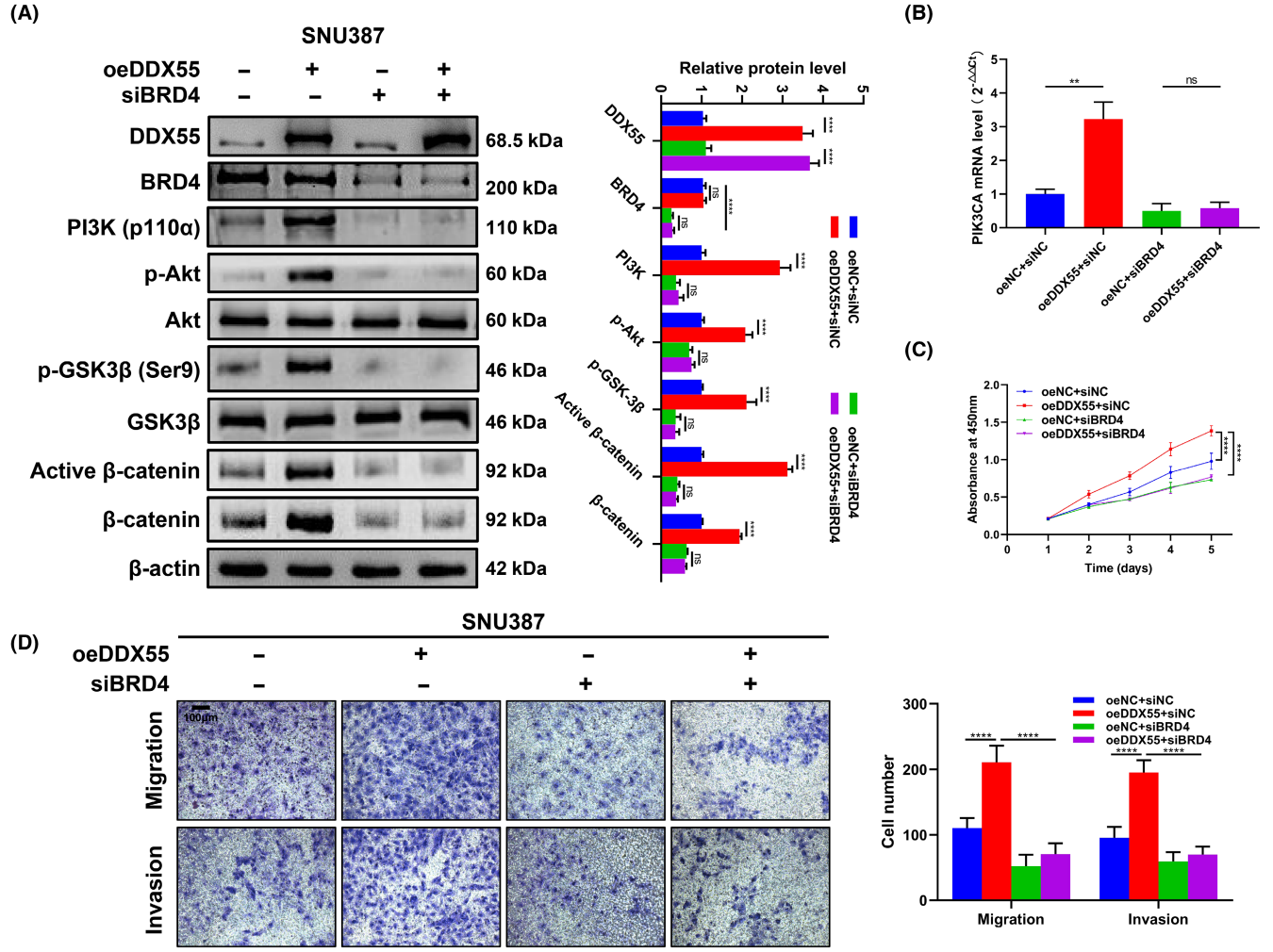

FIGURE 7 DEAD-box helicase 55 (DDX55) promotes hepatocellular carcinoma (HCC) progression via interacting with BRD4. (A) Western blotting (WB) analysis of DDX55, BRD4, active β-catenin, β-catenin, phosphorylated GSK-3β (ser9), GSK-3β, phosphorylated AKT (Ser473), total AKT, and PI3K (p110α) in the indicated SNU387 cells co-transfected with or without siBRD4. Relative protein expression was normalized to β-actin level, and phosphorylated proteins (p-Akt and p-GSK-3β) were normalized to corresponding total protein (Akt and GSK-3β). (B) Quantitative real-time PCR analysis of PIK3CA mRNA levels in the indicated SNU387 cells co-transfected with or without siBRD4. (C) Cell viability analysis using CCK-8 assay in the indicated SNU387 cells co-transfected with or without siBRD4. (D) Cell migration and invasion analyses using Transwell assays in the indicated SNU387 cells co-transfected with or without siBRD4. Data are presented as mean ± SD. Statistical significance was evaluated by Student's *t* test for two groups or one-way ANOVA with Tukey's multiple comparison test for three groups (A, B and D) or two-way ANOVA (C). $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. ns, no significance

4 | DISCUSSION

Facing the great threat of HCC to human health, our present study focused on the clinical implications and biological roles of DDX55 in HCC progression. By fully utilizing the multiple large-scale HCC cohorts, upregulation of DDX55 in HCC was verified at both the protein and mRNA levels. From the perspective of the primary risk factors of HCC, we found that DDX55 was significantly upregulated in alcohol consumption-related HCC, hepatitis B/C-related HCC, alcohol+hepatitis B/C-related HCC, and HCC without history of primary risk factors compared to non-tumor controls, while there was no significant difference in DDX55 expression between non-alcoholic fatty liver disease-related HCC and non-tumor controls (Figure S8). Previous research suggested that members of the DEAD-box protein family were involved in the response to ethanol stress and the regulation of antiviral innate immunity.[37–42] Thus, the roles and mechanisms of DDX55 in the development of HCC induced by the known and unknown risk factors are worthy of future studies. Consistent with the involvement of DDX55 in colorectal cancer liver metastasis and in the prognosis of lung cancer patients, we confirmed that DDX55 upregulation correlated with tumor progression and served as an independent prognostic indicator of unfavorable RFS and OS of HCC patients regardless of ethnicity, which represented the most prominent prognostic value among the DEAD-box protein family.[3,19] To our knowledge, the present study is the first to reveal the oncogenic roles of DDX55 in HCC via ex vivo and in vivo experiments, which highlights DDX55 as a potential therapeutic target.

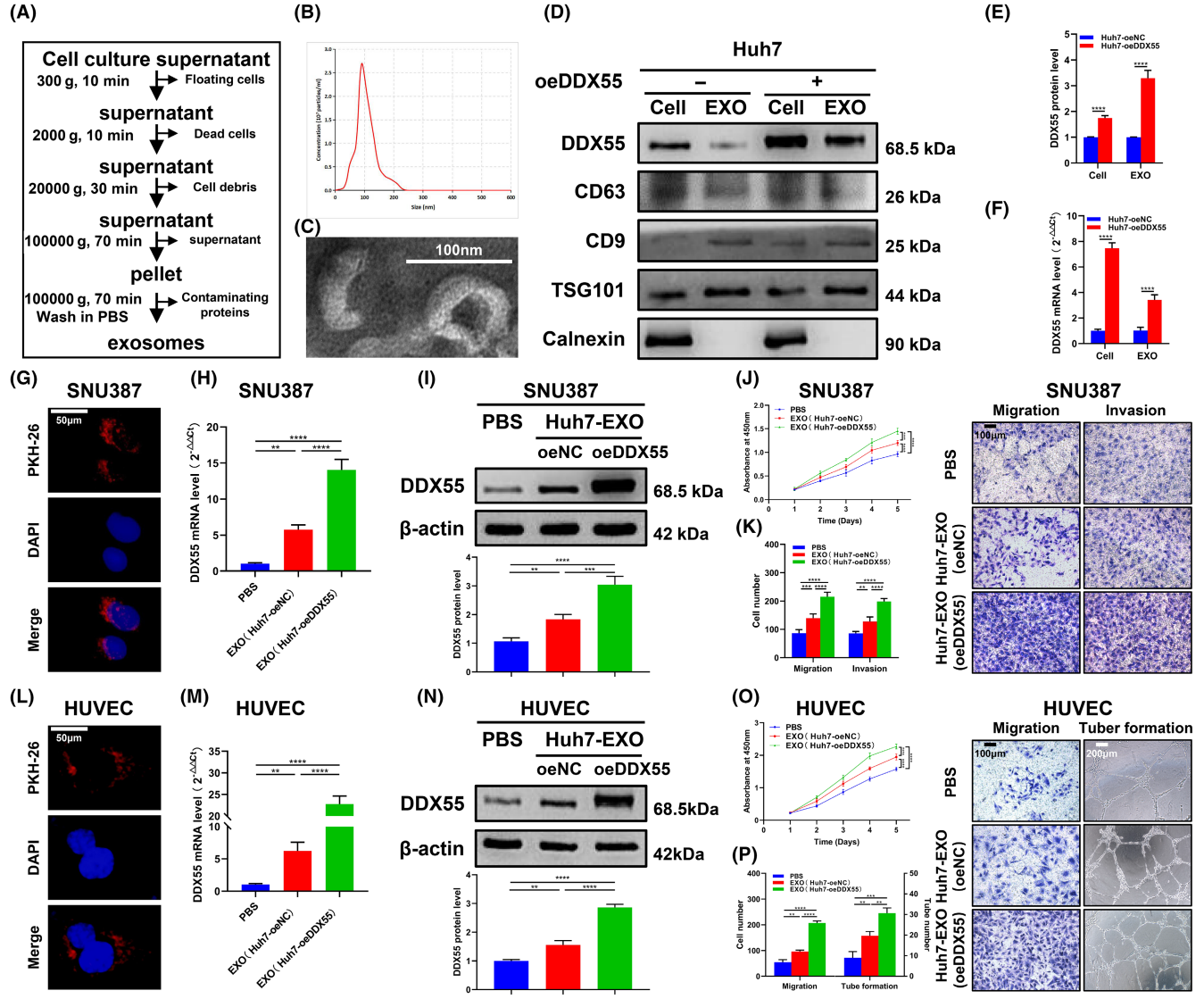

FIGURE 8 Intercellular transfer of DEAD-box helicase 55 (DDX55) by exosomes promotes hepatocellular carcinoma (HCC) progression. (A) Schematic description of the exosome purification process by differential ultracentrifugation. (B) Nanoparticle tracking analysis of Huh7-derived exosomes. (C) Visualization of Huh7-derived exosomes by transmission electron microscopy. (D-E) Western blotting (WB) analysis of exosome markers (including TSG101, CD63, and CD9), non-exosome marker (Calnexin), and DDX55 in the indicated Huh7 cells and their exosomes (EXO). (F) Quantitative real-time PCR (qRT-PCR) analysis of DDX55 mRNA levels in the indicated Huh7 cells and their exosomes. (G) SNU387 cells were incubated with PKH26-labeled exosomes derived from the Huh7 cells for 4 h, and exosomes were incorporated into SNU387 cells. After incubated with PBS, Huh7-oeNC-derived exosomes (100 μg/mL), or Huh7-oeDDX55-derived exosomes (100 μg/mL) for 48 h, (H) DDX55 mRNA level was assessed by qRT-PCR in the indicated SNU387 cells; (I) DDX55 protein expression was assessed by WB in the indicated SNU387 cells; (J) cell viability was assessed by CCK-8 in the indicated SNU387 cells; (K) cell migration and invasion abilities were assessed by Transwell assays in the indicated SNU387 cells. (L) Human umbilical vein endothelial cells (HUVEC) were incubated with PKH26-labeled exosomes derived from the Huh7 cells for 4 h, and exosomes were incorporated into HUVEC. After incubation with PBS, Huh7-oeNC-derived exosomes (100 μg/mL), or Huh7-oeDDX55-derived exosomes (100 μg/mL) for 48 h, (M) the DDX55 mRNA level was assessed by qRT-PCR in the indicated HUVEC; (N) DDX55 protein expression was assessed by WB in the indicated HUVEC cells; (O) cell viability was assessed by CCK8 in the indicated HUVEC; (P) cell migration and tuber formation abilities were assessed in the indicated HUVEC. Relative protein expression was normalized to β-actin level (I, N). Data are presented as mean ± SD. Statistical significance was evaluated by Student's *t* test (E, F) or one-way ANOVA with Tukey's multiple comparison test (H, K, M, P) or two-way ANOVA (J, O). $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$

Aberrant Wnt/β-catenin signaling activation plays a vital role in promoting HCC progression.[43,44] Accumulating evidence has reported that the DEAD-box protein family is implicated in regulating the β-catenin pathway through different mechanisms.[8,14,15,45–50] Here, we first observed that DDX55 upregulation could stabilize cytoplasmic and nuclear β-catenin protein and further induce transcriptional alteration of β-catenin downstream genes, thereby promoting cell cycle progression and EMT. As for the regulatory

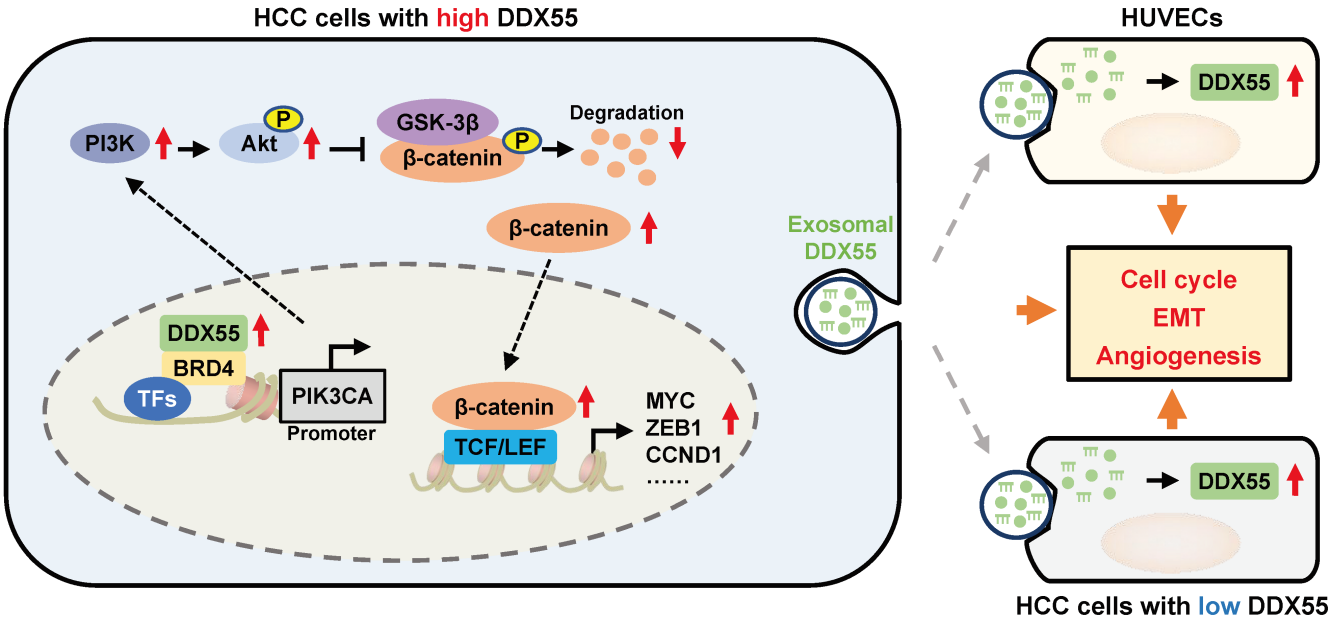

FIGURE 9 Schematic description of the oncogenic roles of DEAD-box helicase 55 (DDX55) in hepatocellular carcinoma (HCC)

mechanisms underlying the impact of DDX55 on β-catenin signaling, we concentrated on the correlation between DDX55 and PIK3CA, an oncogene encoding PI3Kα catalytic subunit and acting as a well-known activator of AKT in tumorigenesis, under the guidance of the sequencing analysis.[51] Consistent with the existing evidence with regard to the associations between DEAD-box proteins and the PI3K/Akt pathway, our data demonstrated that DDX55 could significantly enhance PIK3CA expression both at the mRNA and protein levels, and further reinforced β-catenin protein stability dependent on PI3K/Akt/GSK-3β pathway activation.[46,52–54] A high percentage of aberrant activation of the Wnt/β-catenin signaling (30%–40%) and the PI3K/Akt signaling (nearly 50%) have been reported in human HCC samples, which highlights the potential of DDX55 as a valuable therapeutic target in those HCC.[34,55]

Emerging evidence has suggested that DEAD-box proteins generally function as components of multi-protein complexes and act as a transcriptional co-activator in gene expression regulation.[4,6] BRD4 has been reported as an important transcriptional activator in tumorigenesis and usually regulates transcriptional activation of oncogenes by binding to acetylated lysines and recruiting specific transcription factors (such as CDK9).[27,56–59] Previous studies have suggested that BRD4 was closely implicated in regulating PI3K/Akt signaling activation in oncogenesis, but the specific mechanisms remain unclear.[60,61] Our study for the first time revealed that DDX55 might interact with BRD4 to form a transcriptional regulatory complex, which was responsible for the transcriptional activation of PIK3CA as well as corresponding phenotypic changes of HCC cells. Importantly, the presence of multiple bands of high molecular weight (≥300 kDa) in the Co-IP assay indicated the complexity of transcription activation complex containing BRD4 and DDX55. Thus, the specific TF recruited by DDX55-BRD4 complex for PIK3CA transcription activation remain to be explored. Of note, the high basal expression level of BRD4 was observed in HCC tissues, but BRD4 lacked significant prognostic value, in contrast to the excellent prognostic value of DDX55 in HCC (Figure S9A-D; Figure S4E). Intriguingly, we observed that the prognosis of HCC patients with high BRD4 expression and simultaneously high DDX55 expression was significantly worse than that of patients with high BRD4 but low DDX55 expression (Figure S9C). These findings undoubtedly underline the significance of DDX55 upregulation in HCC tumorigenesis, as well as the critical role of DDX55-BRD4 interaction in HCC progression. Currently, BRD4 inhibitor-oriented therapy is very promising in cancer research, and combined PI3K and BRD4 inhibitions could enhance tumor destruction.[62–64] From this perspective, our findings may provide a new therapeutic strategy in HCC, namely intervention of DDX55-BRD4 complex. In the future, the effective intervention strategies targeting DDX55, BRD4, or DDX55-BRD4 complex are worthy of exploring to promote clinical translation, such as exosomal miRNA-based intervention and design of relevant molecular targeted drugs.

Exosome-mediated communication in the HCC microenvironment plays a vital role in HCC progression (including tumor growth, metastasis, and angiogenesis) by regulating the biological behavior of target cells.[65] The contents and roles of tumor cell-derived exosomes are hot research topics and are yet to be clarified.[21] Of note, emerging evidence suggested that several members of the DEAD-box helicase family (including DDX53 and DDX55) existed in cancer cell-derived exosomes, and exosome-mediated transfer of them participated in behavior regulation of recipient cells (e.g., cancer cells and endothelial cells).[22,36] In addition, positive regulation of β-catenin signaling activation by DDX55 was identified in this study. It has been reported that activation of β-catenin signaling and its target genes are closely involved in angiogenesis regulation in endothelial cells.[66–68] Given the above evidence, this study further

verified that both DDX55 mRNA and protein indeed resided in the HCC cell-derived exosomes. In addition, HCC cell-derived exosomes mediated intercellular transfer of DDX55 to surrounding HCC cells and endothelial cells, thereby enhancing the malignant phenotype of cancer cells and promoting angiogenesis. Remodeling of immune microenvironment mediated by tumor cell derived-exosome plays a vital role in tumor immune escape and progression.[65,69] Interestingly, this study found that tumor cell-derived exosome containing DDX55 could be taken up by dendritic cells (Figure S7F). In the future, the impact of exosomal DDX55 on other types of cells (particularly immune cells) in HCC microenvironment will be worthy of future study.

In summary, we confirmed that DDX55 upregulation was associated with tumor progression and worse clinical outcomes in HCC. Mechanistically, we revealed that DDX55 could induce cell cycle progression and EMT by activating β-catenin in a BRD4/PI3K/Akt/GSK-3β-dependent manner, thereby promoting HCC tumorigenicity and metastasis. Importantly, we provided important evidence that exosomal DDX55 were involved in intercellular communication in HCC. These data indicate that DDX55 may be a valuable prognostic biomarker and therapeutic target in HCC.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare. None of the authors of this manuscript is a current Editor or Editorial Board Member of Cancer Science.

ETHICS STATEMENT

This study has been approved by the ethics committee of the Zhongnan Hospital of Wuhan University (No. 2021134K) (Data S4). The animal experiments were approved by the Experimental Animal Welfare Ethics Committee of Zhongnan Hospital of Wuhan University (No.ZN2021205) (Data S5). This study was performed in accordance with the Declaration of Helsinki.

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REFERENCES

1. Sung H, Ferlay J, Siegel RL, et al. 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. Llovet JM, Kelley RK, Villanueva A, et al. Hepatocellular carcinoma. *Nat Rev Dis Primers*. 2021;7:6.
3. Cui Y, Hunt A, Li Z, et al. Lead DEAD/H box helicase biomarkers with the therapeutic potential identified by integrated bioinformatic approaches in lung cancer. *Comput Struct Biotechnol J*. 2021;19:261-278.
4. Sarkar M, Ghosh MK. DEAD box RNA helicases: crucial regulators of gene expression and oncogenesis. *Front Biosci (Landmark ed)*. 2016;21:225-250.
5. Zhang L, Li X. DEAD-box RNA helicases in cell cycle control and clinical therapy. *Cells*. 2021;10:1540.
6. Fuller-Pace FV. DEAD box RNA helicase functions in cancer. *RNA Biol*. 2013;10:121-132.
7. Zhang H, Zhang Y, Chen C, et al. A double-negative feedback loop between DEAD-box protein DDX21 and snail regulates epithelial-mesenchymal transition and metastasis in breast cancer. *Cancer Lett*. 2018;437:67-78.
8. Yang F, Fang E, Mei H, et al. Cis-acting circ-CTNNB1 promotes beta-catenin signaling and cancer progression via DDX3-mediated transactivation of YY1. *Cancer Res*. 2019;79:557-571.
9. Xu Z, Li X, Li H, et al. Suppression of DDX39B sensitizes ovarian cancer cells to DNA-damaging chemotherapeutic agents via destabilizing BRCA1 mRNA. *Oncogene*. 2020;39:7051-7062.
10. Putra V, Hulme AJ, Tee AE, et al. The RNA-helicase DDX21 upregulates CEP55 expression and promotes neuroblastoma. *Mol Oncol*. 2021;15:1162-1179.
11. Gong C, Krupka JA, Gao J, et al. Sequential inverse dysregulation of the RNA helicases DDX3X and DDX3Y facilitates MYC-driven lymphomagenesis. *Mol Cell*. 2021;81:4059-4075.e11.
12. Heerma van Voss MR, Vesuna F, Bol GM, et al. Targeting mitochondrial translation by inhibiting DDX3: a novel radiosensitization strategy for cancer treatment. *Oncogene*. 2018;37:63-74.
13. Kao SH, Cheng WC, Wang YT, et al. Regulation of miRNA biogenesis and histone modification by K63-polyubiquitinated DDX17 controls cancer stem-like features. *Cancer Res*. 2019;79:2549-2563.
14. Zhang T, Yang X, Xu W, et al. Heat shock protein 90 promotes RNA helicase DDX5 accumulation and exacerbates hepatocellular carcinoma by inhibiting autophagy. *Cancer Biol Med*. 2021;18:693-704.
15. Zhang T, Ma Z, Liu L, et al. DDX39 promotes hepatocellular carcinoma growth and metastasis through activating Wnt/beta-catenin pathway. *Cell Death Dis*. 2018;9:675.
16. Zhang H, Xing Z, Mani SK, et al. RNA helicase DEAD box protein 5 regulates Polycomb repressive complex 2/Hox transcript antisense intergenic RNA function in hepatitis B virus infection and hepatocarcinogenesis. *Hepatology*. 2016;64:1033-1048.
17. Xue Y, Jia X, Li C, et al. DDX17 promotes hepatocellular carcinoma progression via inhibiting Klf4 transcriptional activity. *Cell Death Dis*. 2019;10:814.
18. Yu B, Liang H, Ye Q, Wang Y. Establishment of a genomic-clinicopathologic nomogram for predicting early recurrence of hepatocellular carcinoma after R0 resection. *J Gastrointest Surg*. 2021;25:112-124.
19. Liu J, Wang D, Zhang C, et al. Identification of liver metastasis-associated genes in human colon carcinoma by mRNA profiling. *Chin J Cancer Res*. 2018;30:633-646.
20. Wang S, Xu M, Li X, et al. Exosomes released by hepatocarcinoma cells endow adipocytes with tumor-promoting properties. *J Hematol Oncol*. 2018;11:82.
21. Li X, Li C, Zhang L, et al. The significance of exosomes in the development and treatment of hepatocellular carcinoma. *Mol Cancer*. 2020;19:1.
22. Yuan F, Zhou ZF. Exosomes derived from Taxol-resistant nasopharyngeal carcinoma (NPC) cells transferred DDX53 to NPC cells and promoted cancer resistance to Taxol. *Eur Rev Med Pharmacol Sci*. 2021;25:127-138.
23. Zhou W, Zhong Z, Lin D, et al. Correction: hypothermic oxygenated perfusion inhibits HECTD3-mediated TRAF3 polyubiquitination to alleviate DCD liver ischemia-reperfusion injury. *Cell Death Dis*. 2021;12:567.
24. Cancer Genome Atlas Research Network. Comprehensive and integrative genomic characterization of hepatocellular carcinoma. *Cell*. 2017;169:1327-1341.e23.
25. Uhlen M, Zhang C, Lee S, et al. A pathology atlas of the human cancer transcriptome. *Science*. 2017;357:eaan2507.

26. Zhang Q, Liu W, Zhang HM, et al. hTFtarget: A comprehensive database for regulations of human transcription factors and their targets. *Genomics Proteomics Bioinformatics*. 2020;18:120-128.
27. Xiong L, Wu F, Wu Q, et al. Aberrant enhancer hypomethylation contributes to hepatic carcinogenesis through global transcriptional reprogramming. *Nat Commun*. 2019;10:335.
28. Szklarczyk D, Gable AL, Nastou KC, 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.
29. Pathan M, Fonseka P, Chitti SV, et al. Vesiclepedia 2019: a compendium of RNA, proteins, lipids and metabolites in extracellular vesicles. *Nucleic Acids Res*. 2019;47:D516-D519.
30. Gao Y, He Y, Xu J, et al. Genetic variants at 4q21, 4q23 and 12q24 are associated with esophageal squamous cell carcinoma risk in a Chinese population. *Hum Genet*. 2013;132:649-656.
31. Son JW, Jeong KJ, Jean WS, et al. Genome-wide combination profiling of DNA copy number and methylation for deciphering biomarkers in non-small cell lung cancer patients. *Cancer Lett*. 2011;311:29-37.
32. Wei Q, Yu D, Liu M, et al. Genome-wide association study identifies three susceptibility loci for laryngeal squamous cell carcinoma in the Chinese population. *Nat Genet*. 2014;46:1110-1114.
33. Wolf M, Korja M, Karhu R, et al. Array-based gene expression, CGH and tissue data defines a 12q24 gain in neuroblastic tumors with prognostic implication. *BMC Cancer*. 2010;10:181.
34. Xu C, Xu Z, Zhang Y, Evert M, Calvisi DF, Chen X. Beta-catenin signaling in hepatocellular carcinoma. *J Clin Invest*. 2022;132:e154515.
35. He Y, Sun MM, Zhang GG, et al. Targeting PI3K/Akt signal transduction for cancer therapy. *Signal Transduct Target Ther*. 2021;6:425.
36. Hong BS, Cho JH, Kim H, et al. Colorectal cancer cell-derived microvesicles are enriched in cell cycle-related mRNAs that promote proliferation of endothelial cells. *BMC Genomics*. 2009;10:556.
37. Markkula A, Lindstrom M, Johansson P, Bjorkroth J, Korkeala H. Roles of four putative DEAD-box RNA helicase genes in growth of listeria monocytogenes EGD-e under heat, pH, osmotic, ethanol, and oxidative stress conditions. *Appl Environ Microbiol*. 2012;78:6875-6882.
38. Takemura R, Inoue Y, Izawa S. Stress response in yeast mRNA export factor: reversible changes in Rat8p localization are caused by ethanol stress but not heat shock. *J Cell Sci*. 2004;117:4189-4197.
39. Rollenhagen C, Hodge CA, Cole CN. The nuclear pore complex and the DEAD box protein Rat8p/Dbp5p have nonessential features which appear to facilitate mRNA export following heat shock. *Mol Cell Biol*. 2004;24:4869-4879.
40. Su C, Tang YD, Zheng C. DExD/H-box helicases: multifunctional regulators in antiviral innate immunity. *Cell Mol Life Sci*. 2021;79:2.
41. Sun J, Wu G, Pastor F, et al. RNA helicase DDX5 enables STAT1 mRNA translation and interferon signalling in hepatitis B virus replicating hepatocytes. *Gut*. 2022;71:991-1005.
42. Chang PC, Chi CW, Chau GY, et al. DDX3, a DEAD box RNA helicase, is deregulated in hepatitis virus-associated hepatocellular carcinoma and is involved in cell growth control. *Oncogene*. 2006;25:1991-2003.
43. Li H, Lan T, Xu L, et al. NCSTN promotes hepatocellular carcinoma cell growth and metastasis via beta-catenin activation in a Notch1/AKT dependent manner. *J Exp Clin Cancer Res*. 2020;39:128.
44. Zhang Y, Wang X. Targeting the Wnt/beta-catenin signaling pathway in cancer. *J Hematol Oncol*. 2020;13:165.
45. Chen HH, Yu HI, Cho WC, Tarn WY. DDX3 modulates cell adhesion and motility and cancer cell metastasis via Rac1-mediated signaling pathway. *Oncogene*. 2015;34:2790-2800.
46. Chen L, Xu M, Zhong W, Hu Y, Wang G. Knockdown of DDX46 suppresses the proliferation and invasion of gastric cancer through inactivating Akt/GSK-3beta/beta-catenin pathway. *Exp Cell Res*. 2021;399:112448.
47. Li K, Mo C, Gong D, et al. DDX17 nucleocytoplasmic shuttling promotes acquired gefitinib resistance in non-small cell lung cancer cells via activation of beta-catenin. *Cancer Lett*. 2017;400:194-202.
48. Liu C, Wang L, Jiang Q, et al. Hepatoma-derived growth factor and DDX5 promote carcinogenesis and progression of endometrial cancer by activating beta-catenin. *Front Oncol*. 2019;9:211.
49. Wang Z, Luo Z, Zhou L, Li X, Jiang T, Fu E. DDX5 promotes proliferation and tumorigenesis of non-small-cell lung cancer cells by activating beta-catenin signaling pathway. *Cancer Sci*. 2015;106:1303-1312.
50. Zhang M, Weng W, Zhang Q, et al. The lncRNA NEAT1 activates Wnt/beta-catenin signaling and promotes colorectal cancer progression via interacting with DDX5. *J Hematol Oncol*. 2018;11:113.
51. Arafeh R, Samuels Y. PIK3CA in cancer: The past 30 years. *Semin Cancer Biol*. 2019;59:36-49.
52. Sarkar M, Khare V, Guturi KK, Das N, Ghosh MK. The DEAD box protein p68: a crucial regulator of AKT/FOXO3a signaling axis in oncogenesis. *Oncogene*. 2015;34:5843-5856.
53. Yu Y, Wang JL, Meng LL, et al. DDX54 plays a cancerous role through activating P65 and AKT signaling pathway in colorectal cancer. *Front Oncol*. 2021;11:650360.
54. Yu Y, Zhao D, Li K, et al. E2F1 mediated DDX11 transcriptional activation promotes hepatocellular carcinoma progression through PI3K/AKT/mTOR pathway. *Cell Death Dis*. 2020;11:273.
55. Dimri M, Satyanarayana A. Molecular signaling pathways and therapeutic targets in hepatocellular carcinoma. *Cancers (Basel)*. 2020;12:491.
56. Wu Q, Liu F, Ge M, et al. BRD4 drives esophageal squamous cell carcinoma growth by promoting RCC2 expression. *Oncogene*. 2022;41:347-360.
57. Tsang FH, Law CT, Tang TC, et al. Aberrant super-enhancer landscape in human hepatocellular carcinoma. *Hepatology*. 2019;69:2502-2517.
58. Donati B, Lorenzini E, Ciarrocchi A. BRD4 and cancer: Going beyond transcriptional regulation. *Mol Cancer*. 2018;17:164.
59. White ME, Fenger JM, Carson WE 3rd. Emerging roles of and therapeutic strategies targeting BRD4 in cancer. *Cell Immunol*. 2019;337:48-53.
60. Hao J, Yang Z, Wang L, et al. Downregulation of BRD4 inhibits gallbladder cancer proliferation and metastasis and induces apoptosis via PI3K/AKT pathway. *Int J Oncol*. 2017;51:823-831.
61. Wang Y, Lin Q, Song C, Ma R, Li X. Circ_0007841 promotes the progression of multiple myeloma through targeting miR-338-3p/BRD4 signaling cascade. *Cancer Cell Int*. 2020;20:383.
62. Andrews FH, Singh AR, Joshi S, et al. Dual-activity PI3K-BRD4 inhibitor for the orthogonal inhibition of MYC to block tumor growth and metastasis. *Proc Natl Acad Sci U S A*. 2017;114:E1072-E1080.
63. Singh AR, Joshi S, Burgoyne AM, et al. Single agent and synergistic activity of the "first-in-class" dual PI3K/BRD4 inhibitor SF1126 with sorafenib in hepatocellular carcinoma. *Mol Cancer Ther*. 2016;15:2553-2562.
64. Stratikopoulos EE, Dendy M, Szabolcs M, et al. Kinase and BET inhibitors together clamp inhibition of PI3K signaling and overcome resistance to therapy. *Cancer Cell*. 2015;27:837-851.
65. Wu Q, Zhou L, Lv D, Zhu X, Tang H. Exosome-mediated communication in the tumor microenvironment contributes to hepatocellular carcinoma development and progression. *J Hematol Oncol*. 2019;12:53.
66. Zeng L, Xiao Q, Chen M, et al. Vascular endothelial cell growth-activated XBP1 splicing in endothelial cells is crucial for angiogenesis. *Circulation*. 2013;127:1712-1722.
67. Jiang L, Yin M, Wei X, et al. Bach1 represses Wnt/beta-catenin signaling and angiogenesis. *Circ Res*. 2015;117:364-375.
68. Skurk C, Maatz H, Rocnik E, Bialik A, Force T, Walsh K. Glycogen-synthase Kinase3beta/beta-catenin axis promotes angiogenesis

through activation of vascular endothelial growth factor signaling in endothelial cells. *Circ Res*. 2005;96:308-318.

69. Hosseini R, Asef-Kabiri L, Yousefi H, et al. The roles of tumor-derived exosomes in altered differentiation, maturation and function of dendritic cells. *Mol Cancer*. 2021;20:83.

SUPPORTING INFORMATION

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