Understand the role of exosomal miRNA in the development of breast cancer in patients with chronic HBV infection

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Abstract

Background: Female chronic hepatitis B patients has shown a higher incidence of breast cancer with poorer prognosis, compared to the non-infected female patients. A significant difference in miRNAs expression in serum exosomes has been identified between female patients with chronic HBV infection and non-infected female. Whether this difference could contribute to the differential incidence and development of breast cancer in these female patients has not been fully understood.

Methods: Information of exosomal expression of miRNAs (expression profiles of GSE33857) were acquired from the Gene Expression Omnibus (GEO) database. Targeted genes of this miRNAs and the differentially expressed genes of breast cancer in GEPIA V2.0 database was used to obtain the differentially expressed genes (DEGs). Four analytic methods were performed, including functional annotation, protein-protein interaction (PPI) network and module construction, the clinical pathologic parameters analysis, alongsides prognosis analysis.

Results: In the serum exsomes, expression of miR-1237-3p and miR-363-3p were down-regulated whilst expression of miR-100-5p, miR-125b-5p, miR-1260a, miR-1287-5p, miR-148a-3p, miR-885-5p and miR-99a-5p were up-regulated in female chronic hepatitis B (CHB) patients. 228 up-regulated genes and 641 down-regulated genes were selected as DEGs. The DEGs involved in several biological processes ranging from cellular response to hormone stimulus. According to the main enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway was the pathways in cancer. Our analysis revealed that among the hub genes, CXCL11 was up-regulated and CXCL12, GNG2, LPAR1, GPR17, CXCL1, ADCY5, ADCY3, CX3CL1, S1PR1 were down-regulated. These genes expression levels were verified in UALCAN, Oncomine and HPA database. High expression level of CXCL11 and low expression levels of CXCL12, LPAR1, ADCY3 and CX3CL1 were positively correlated with some clinical pathological parameters that predicted worse prognosis. Kaplan-Meier (K-M) plotter analysis revealed that these genes that showed a low expression level were positively correlated with poor prognosis of breast cancer, and the high expression genes were negatively correlated with poor prognosis of breast cancer.

Conclusion: This bioinformatics analysis study explored the mechanisms of chronic HBV related occurrence and development of breast cancer by changing the expression of miRNAs of serum exosomes. It provided novel directions and therapeutic strategy for chronic HBV infection associated occurrence and development of breast cancer in female patients.

Introduction

Hepatitis B is a common infectious disease caused by the hepatitis B virus (HBV). It was estimated that there were 250 million population with chronic HBV infection worldwide in 2020^[1]. Approximately one million people died from liver conditions, including liver cancer and cirrhosis that were associated to HBV infection^[2]. In addition to liver cirrhosis and hepatocellular carcinoma, HBV infection could also contribute to the development of extrahepatic malignances, including lymphoma, cholangiocarcinoma and breast carcinoma^[3].

Breast cancer in a common malignant tumor in female, with an estimated number of 274,480 new cases and 42,170 death toll in 2020^[4]. Studies have found a significant higher incidence^[3], an earlier age-of-onset^[5] and poorer prognosis^[6] of breast cancer in female HBV patients in comparison to those without HBV infection. However, mechanism of such association between breast cancer and chronic HBV infection has not been elucidated. Evidence suggested damages of hepatocytes that were caused by chronic inflammation from HBV-infection^[7] could lead to

abnormal metabolism of sexual hormone, resulting in malignant changes of breast tissues^[8]. Another study showed that HBV could cause directly induces malignancy via viral replication within breast cells^[9].

Exosomes are small vesicles with a size of 50-150nm in diameter derived from endosomes^[10]. It is characterized with lipid bilayer and contains abundant RNA, DNA, proteins and lipids from cellular metabolism^[11, 12]. The lipid bilayer protects the exosomal contents from degradation and maintains the integrity of exosomes in serum^[13]. Exosomes travel within the circulation system and could interact with tissues, affecting biological functions^[14-16]. Roles of exosomes have been highlighted in several lines of cancer researches, including tumor growth, invasion and metastasis, immune escape, chemotherapy resistance and radiotherapy tolerance^[17-19]. Moreover, the accessibility of exosome detection from serum samples allows it to become an ideal candidate for clinical biomarkers.

Recent study has identified differential expression level of exosomal miRNAs between HBV-infected and normal individual^[20]. Moreover, alterations of miRNA levels in blood exosomes were found to be able to modify the progression of hepatocellular carcinoma^[21] and breast carcinoma^[22] respectively. However, whether or not HBV infection could promote breast carcinogenesis via the alteration of exosomal miRNAs in patient's serum remains unknown. Here, we performed a comprehensive statistical analysis to identify the common exosomal miRNAs showing a differential expression level in HBV-infected individual and investigate if these miRNAs contribute to the expression changes and clinical outcomes in patients with breast cancer. Through search of potential genetic links between HBV infection and breast cancer, our study could provide novel insight of the regulation mechanism underlying the more rapid progression and poorer prognosis of breast cancer in HBV infected patient, for genetic targets development for diagnosis, evaluation of disease therapy and prognosis.

In this study, identification of exosomal miRNAs showing differential expression level in HBV infected patients was performed using GEO2R analysis^[23], with potential target genes screened by the mirWALK^[24]. Intersection of these genes and breast cancer related genes identified based on the GEPIA V2.0 data^[25] was analysed, generating the differentially expressed genes (DEGs). The DEGs was then used for GO and KEGG analysis, constructing the PPI network analysis and the identification of hub genes for breast cancer. Expression of these hub genes were also verified using the Oncomine, UALCAN and HPA databases. Moreover, relationship between the identified hub genes with critical clinical pathological parameters and the prognosis of breast cancer were also investigated using the bc-GenExMiner and the K-M plotter databases.

Materials And Methods

Chip data

GSE33857 was generated from Agilent-029297 Human miRNA Microarray v14 Rev.2 data. These data contained miRNAs data from 100 samples, including four patients with chronic HBV infection, 64 patients with chronic hepatitis C infection, 7 patients with non-alcoholic hepatitis, 12 healthy individuals and 13 huh7 liver cancer cells^[23].

Identification of DEGs

GEO2R is an interactive web tool provided by the National Center for Biotechnology Information (NCBI). This tool was used to analyze the differences in exosomal miRNAs between female with and without chronic HBV infection. In this study, a significantly different expression level of the miRNAs was defined based on a fold change $|\log FC| > 1.5$ with $p < 0.01$. MirWalk database (<http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/index.html>) was used to analyze the predicted target genes for miRNAs. GEPIA (Gene Expression Profiling Interactive Analysis) V2.0 database

(<http://gepia.cancerpku.cn/>) was used to perform the screening to identify the significantly expressed genes in breast cancer tissues^[25]($|\log FC| > 1.0$ and $p < 0.05$). Venn diagram was used to present the intersection of up-regulated miRNAs target genes which showed a low expression level with the significantly low expressed genes in breast cancers, alongsides the down-regulated miRNAs target genes which showed a high expression level with the significantly high expressed genes in breast cancer.

Gene ontology and KEGG pathway analysis

Enrichr^[26, 27] database (<https://maayanlab.cloud/Enrichr/>) was used to analyze the biological process (BP), cell component (CC), molecular function (MF) and KEGG pathway of the DEGs.

Identification of top-level modules and hub genes in the PPI networks

The Search Tool for the Retrieval of Interacting Genes (STRING, v11.0) (<https://string-db.org/>) was used to analyze protein interactions between genes, and generate PPI network for visualization. Cytoscape software V3.7.1 (<http://www.cytoscape.org>) was used to screen the top-level modules in the PPI network based on the following criterial using the Molecular Complex Detection (MCODE): degree cut-off = 2, node score cut-off = 0.2, max depth = 100 and k-score = 2. A plug of the Cytoscape software, CytoHubba was installed in order to calculate the nodes of each protein, as well as the MCC algorithm to identify the top ten genes as hub genes.

Validation of relative mRNA expression levels of hub genes

To further validate the mRNA level of hub genes in breast cancer, we tested these differential expressions of genes from the UALCAN and Oncomine databases. UALCAN (<http://ualcan.path.uab.edu/>) contains the RNA sequence and the relative expression of genes related to normal and tumor tissues in 31 tumors^[28]. Oncomine v4.5(<https://www.oncomine.org/>) which provides cancer microarray data sets and data mining functions for multiple cancer type was used to validate the implication of the potential gene target we identified in the breast tumor development and progression^[29].

Explore the expression level of hub genes in the human protein atlas database (HPA)

HPA V20.0(<https://www.proteinatlas.org/>) was an online free database that can provide abundant transcriptome and proteome data analysis for human normal or pathological tissues through RNA sequence analysis and immunohistochemistry^[30]. This study explored the protein expression and distribution of hub genes in breast cancer tissues and normal tissues in HPA.

Explore the expression level of the hub genes to breast cancer clinicopathological parameters bc-GenExMiner v4.15^[31](<http://bcgenex.ico.unicancer.fr>) is a statistical mining tool which contains breast cancer transcriptomic data (DNA microarrays [n = 10,716] and RNA-seq [n = 4,712]. This database allows statistical analysis of gene expression, prognosis and correlation in breast cancer tissue. The data on this site was last updated in 06/2020. We analyzed the relationship between the hub genes and breast cancer clinicopathological parameters by using this database.

Survival analysis of hub genes

K-M plotter^[32](<https://kmplot.com/analysis/>) was an online platform that can evaluate the effects of 54,000 genes (mRNA, miRNA, proteins) on the survival of 21 cancers, including breast cancer (n = 6234), ovarian cancer (n = 2190), lung cancer (n = 3452) and gastric cancer (n = 1440). The sources of the database include GEO, the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) and TCGA. The main purpose of this tool was to

discover and verify survival-related biomarkers based on meta-analysis. The expression of genes was divided into high-expression group and low-expression group according to the median and $p < 0.05$ indicates that the difference of prognosis was statistically significant.

Results

Identification of exosomal miRNAs showing differential expression with HBV infection

Using GSE33857 database, our analysis identified 251 miRNAs expressed differentially in serum samples from female chronic hepatitis B (CHB) patients ($n = 4$), compared to controls ($n = 12$) (Fig. 1A). Among them, there were nine miRNAs showing significant changes in comparison to controls ($p < 0.01$), seven of which showed upregulation (fold change ≥ 1.5), including miR-100-5p, miR-125b-5p, miR-1260a, miR-1287-5p, miR-148a-3p, miR-885-5p and miR-99a-5p; two of which showed downregulation (fold change, ≤ -1.5), including miR-1237-3p and hsa-miR-363-3p. Basing on the mirWALK database, we identified 3293 target genes that were associated to these down-regulated miRNAs and 6260 genes associated to these up-regulated miRNAs (binding score > 0.95) (Supplementary Table 1).

Integration with genes showing differential expression in breast cancer

Using the GEPIA V2.0, 1416 up-regulated genes and 2134 down-regulated genes were identified in breast samples from patients with breast cancer in comparison to controls (Supplementary Table 2). To find genetic integration between HBV-related exosome and breast cancer, intersection analysis was performed on the target genes identified showing differential expression in serum samples from patients with HBV-infection and breast cancer respectively. This analysis identified 228 common genes showing an increased expression level in the breast cancer that were associated to the HBV-related downregulated miRNAs (Fig. 1B). On the other hand, there were 640 common genes showing a decreased expression level that were associated to the upregulated miRNAs (Fig. 1C)(Supplementary Table 3).

Pathway and network analysis of the common genes

To understand the biological meanings of the identified common genes, GO enrichment and KEGG analysis was performed for pathway and network analysis. The common genes showing differential expression with HBV-infection and breast cancer were enriched for regulation of cellular response to hormone stimulus, signal transduction, epithelial cell proliferation, aorta morphogenesis, extracellular matrix organization, MAPK cascade, regulation of endothelial cell proliferation, and cytoskeleton organization, positive regulation of intracellular signal transduction, negative regulation of signal transduction in biological process (BP)(Fig. 2A, Table 1). The main cell component (CC) involved included integral components of plasma membrane, cytoskeleton, actin cytoskeleton, contractile actin filament bundle, stress fiber, actomyosin, platelet alpha granule membrane, focal adhesion, alongside axon and spindle midzone(Fig. 2B, Table 1).In addition, the main molecular function (MF) involved were actin binding, phosphatidylinositol-4,5-bisphosphate 3-kinase activity, actin filament binding, phosphatidylinositol bisphosphate kinase activity, transmembrane receptor protein kinase activity, phosphatidylinositol 3-kinase activity, protein kinase binding and kinase binding, protein kinase activity, and integrin binding (Fig. 2C, Table 1). At last, the main enriched KEGG pathways of these genes included pathways in cancer, ras signaling pathway, axon guidance, PI3K-Akt signaling pathway, proteoglycans in cancer, fluid shear stress and atherosclerosis, leukocyte transendothelial migration, Rap1 signaling pathway, focal adhesion and melanoma (Fig. 2D, Table 2).

Table 1

The GO function enrichment analysis of the overlapping genes with differential expression in breast cancer

GO	Category	Description	Count	%	P-value
GO:0032870	GO Biological Processes	cellular response to hormone stimulus	17	0.21	4.63E-08
GO:0009966	GO Biological Processes	regulation of signal transduction	28	0.12	1.15E-06
GO:0050678	GO Biological Processes	regulation of epithelial cell proliferation	14	0.19	2.20E-06
GO:0035909	GO Biological Processes	aorta morphogenesis	7	0.44	2.31E-06
GO:0030198	GO Biological Processes	extracellular matrix organization	27	0.12	2.56E-06
GO:0043408	GO Biological Processes	regulation of MAPK cascade	25	0.24	2.72E-06
GO:1902533	GO Biological Processes	positive regulation of intracellular signal transduction	43	0.09	5.93E-06
GO:0009968	GO Biological Processes	negative regulation of signal transduction	30	0.11	6.48E-06
GO:0001936	GO Biological Processes	regulation of endothelial cell proliferation	14	0.18	6.90E-06
GO:0007010	GO Biological Processes	cytoskeleton organization	18	0.14	8.60E-06
GO:0005887	GO Cell Component	integral component of plasma membrane	111	0.08	4.59E-09
GO:0005856	GO Cell Component	cytoskeleton	48	0.09	7.78E-07
GO:0015629	GO Cell Component	actin cytoskeleton	31	0.11	5.10E-06
GO:0097517	GO Cell Component	contractile actin filament bundle	9	0.23	4.30E-05
GO:0001725	GO Cell Component	stress fiber	9	0.23	4.30E-05
GO:0042641	GO Cell Component	actomyosin	9	0.19	1.93E-04
GO:0031092	GO Cell Component	platelet alpha granule membrane	5	0.29	6.12E-04
GO:0005925	GO Cell Component	focal adhesion	29	0.08	9.24E-04
GO:0030424	GO Cell Component	axon	15	0.11	0.001232225
GO:0051233	GO Cell Component	spindle midzone	6	0.20	0.001603682

GO	Category	Description	Count	%	P-value
GO:0003779	GO Molecular Functions	actin binding	32	0.13	6.66E-08
GO:0046934	GO Molecular Functions	phosphatidylinositol-4,5-bisphosphate 3-kinase activity	13	0.19	6.14E-06
GO:0051015	GO Molecular Functions	actin filament binding	18	0.14	9.62E-06
GO:0052813	GO Molecular Functions	phosphatidylinositol bisphosphate kinase activity	13	0.18	1.01E-05
GO:0019199	GO Molecular Functions	transmembrane receptor protein kinase activity	12	0.19	1.22E-05
GO:0035004	GO Molecular Functions	phosphatidylinositol 3-kinase activity	13	0.17	2.17E-05
GO:0019901	GO Molecular Functions	protein kinase binding	42	0.08	2.93E-05
GO:0019900	GO Molecular Functions	kinase binding	36	0.09	7.90E-05
GO:0004672	GO Molecular Functions	protein kinase activity	41	0.08	1.36E-04
GO:0005178	GO Molecular Functions	integrin binding	13	0.14	2.09E-04

Table 2
The KEGG function enrichment analysis of the overlapping genes with differential expression in breast cancer

Category	Description	Count	%	P-value
KEGG_PATHWAY	Pathways in cancer	47	0.090384615	3.07E-06
KEGG_PATHWAY	Ras signaling pathway	27	0.11637931	3.28E-06
KEGG_PATHWAY	Axon guidance	23	0.127071823	3.97E-06
KEGG_PATHWAY	PI3K-Akt signaling pathway	35	0.098870056	5.48E-06
KEGG_PATHWAY	Proteoglycans in cancer	24	0.119402985	7.33E-06
KEGG_PATHWAY	Fluid shear stress and atherosclerosis	19	0.136690647	9.38E-06
KEGG_PATHWAY	Leukocyte transendothelial migration	16	0.142857143	2.69E-05
KEGG_PATHWAY	Rap1 signaling pathway	23	0.111650485	3.35E-05
KEGG_PATHWAY	Focal adhesion	22	0.110552764	5.72E-05
KEGG_PATHWAY	Melanoma	12	0.166666667	5.83E-05

To further explore the relationship between the differentially expressed genes (DEGs), we performed network analysis STRING database on the common genes showing differential expression with HBV-infection and breast cancer

(Fig. 3A). Overall, the constructed PPI network was consisted of 868 nodes and 3285 edges. The top three modules within the network were identified via the MCODE analysis (Fig. 3B-D). Module 1 was composed of 16 nodes and 120 edges with the highest degree at 16.000. Module 2 was composed of 23 nodes and 102 edges with the highest degree at 9.273. Module 3 was composed of 35 nodes and 153 edges with the highest degree at 9.000.

Identification and validation of hub genes

The screening of hub genes related to the common genes showing differential expression with HBV-infection and breast cancer was performed using CytoHubba, and the top 10 genes which showed high correlation with the highest degree in the modules was identified (Fig. 3E). These hub genes included C-X-C Motif Chemokine Ligand 12(CXCL12), G-protein gamma 2 subunit(GNG2), lysophosphatidic acid receptor 1 (LPAR1), G protein-coupled receptor 17 (GPR17), C-X-C Motif Chemokine Ligand 1 (CXCL1), adenylate cyclase 5 (ADCY5), adenylate cyclase 3 (ADCY3), Fractalkine (FKN, CX3CL1), C-X-C Motif Chemokine Ligand 11 (CXCL11) and Sphingosine-1-Phosphate Receptor 1 (S1PR1).

Validation of these hub genes was firstly performed at a transcriptomic level using Oncomine and UALCAN. Overall, nine of the ten hub genes (CXCL12, GNG2, LPAR1, GPR17, CXCL1, ADCY5, ADCY3, CX3CL1, S1PR1) expressed decreasingly in the majority of cancer studies (Fold > 1.5), with the exception of CXCL11 which showed remarkable increases in multiple type of cancer, including breast cancer in Oncomine databas(Fold > 1.5)(Fig. 4, Table 3). In breast cancer in particular, expression of eight hub genes (CXCL12, GNG2, LPAR1, GPR17, ADCY5, ADCY3, CX3CL1, S1PR1) showed an significantly decrease ($p < 0.0001$) whilst the expression of CXCL11($p < 0.0001$) was significantly increased in breast cancer tissue, according to the UALCAN database (Fig. 5)

Table 3
Datasets of the hub genes in breast cancer (ONCOMINE database)

Gene	Types of cancer vs normal	Fold change	t-Test	P-value	Dataset
CXCL12	Invasive Ductal Breast Carcinoma	-5.734	-41.69	2.59E-110	Curtis et al ³⁰
	Medullary Breast Carcinoma	-6.063	-17.075	5.91E-23	
	Invasive Lobular Breast Carcinoma	-3.738	-17.01	1.19E-42	
	Invasive Ductal and Invasive Lobular Breast	-3.537	-15.234	2.63E-13	
	Tubular Breast Carcinoma	-3.033	-13.063	1.71E-23	
	Invasive Breast Carcinoma	-4.348	-6.78	4.97E-07	
	Breast Carcinoma	-4.016	-6.691	4.08E-06	
	Mucinous Breast Carcinoma	-5.087	-10.593	2.68E-15	
	Invasive Ductal Breast Carcinoma	-6.730	-25.411	3.41E-51	
	Invasive Breast Carcinoma	-3.976	-12.417	6.37E-24	TCGA Breast
	Invasive Lobular Breast Carcinoma	-3.032	-8.964	6.88E-13	
	Mixed Lobular and Ductal Breast Carcinoma	-3.846	-7.112	6.80E-05	
	Lobular Breast Carcinoma	-2.791	-6.306	4.95E-06	
	Invasive Ductal Breast Carcinoma	-5.095	-12.634	1.28E-08	
	Invasive Breast Carcinoma Stroma	18.556	16.597	6.99E-21	Finak et al ³²
GNG2	Mucinous Breast Carcinoma	-4.527	-8.923	5.41E-06	TCGA Breast
	Mixed Lobular and Ductal Breast Carcinoma	-3.254	-5.635	6.90E-05	
	Invasive Lobular Breast Carcinoma	-2.621	-6.814	4.37E-10	
	Invasive Breast Carcinoma	-2.975	-8.439	1.12E-13	
	Invasive Ductal Breast Carcinoma	-4.573	-13.287	4.15E-21	

Gene	Types of cancer vs normal	Fole change	t-Test	P-value	Dataset
LPAR1	Ductal Breast Carcinoma	-3.074	-6.086	8.33E-07	Richardson et al ³³
	Invasive Ducatl Breast Carcinoma	-2.27	-14.405	5.98E-29	TCGA Breast
	Medullary Breast Carcinoma	-2.317	-7.909	1.27E-09	Curtis et al ³⁰
	Mucinous Breast Carcinoma	-2.101	-9.037	7.90E-13	
CXCL1	Lobular Breast Carcinoma	-5.281	-11.323	5.33E-06	Sorlie et al ³⁴
	Ductal Breast Carcinoma	-6.82	-12.515	5.27E-08	
	Lobular Breast Carcinoma	-4.4	-9.896	9.00E-05	Sorlie et al ³⁵
	Ductal Breast Carcinoma	-7.644	-11.854	6.77E-09	
	Invasive Ducatl Breast Carcinoma	-8.437	-5.111	4.66E-05	Ma et al ³⁶
	Ductal Breast Carcinoma	-6.64	-8.949	5.93E-05	Perou et al ³⁷
	Invasive Lobular Breast Carcinoma	-5.38	-8.731	3.62E-13	TCGA Breast
	Invasive Ductal Breast Carcinoma	-6.05	-14.245	9.30E-30	
	Invasive Ductal Breast Carcinoma	-4.106	-5.419	4.13E-06	Radvanyi et al ³⁸
	Invasive Ductal Breast Carcinoma	-2.306	-38.362	9.35E-100	Curtis et al ³⁰
ADCY3	Invasive Lobular Breast Carcinoma	-2.19	-21.264	6.51E-58	
	Invasive Ductal and Invasive Lobular Breast	-2.356	-20.889	2.46E-45	
	Tubular Breast Carcinoma	-2.406	-17.919	5.08E-32	
	Invasive Breast Carcinoma	-2.189	-8.887	4.73E-09	
	Breast Carcinoma	-2.269	-6.719	5.48E-06	
	Mixed Lobular and Ductal Breast Carcinoma	-2.008	-8.438	2.23E-12	TCGA Breast

Gene	Types of cancer vs normal	Fold change	t-Test	P-value	Dataset
	Male Breast Carcinoma	-3.108	-17.039	7.63E-17	
	Invasive Ductal Breast Carcinoma	-6.639	-21.53	6.08E-43	
	Invasive Breast Carcinoma	-4.574	-12.712	5.12E-25	
	Invasive Lobular Breast Carcinoma	-2.350	-9.43	6.60E-15	
	Invasive Ductal Breast Carcinoma	-3.714	-4.751	7.37E-05	Radvanyi et al ³⁸
	Ductal Breast Carcinoma	-9.607	-9.446	1.41E-05	Perou et al ³⁷
	Ductal Breast Carcinoma	-9.119	-10.194	4.25E-10	Richardson et al ³³
	Invasive Ductal Breast Carcinoma Epithelia	-25.610	-6.262	4.57E-05	Ma et al ³⁶
	Tubular Breast Carcinoma	-5.442	-16.773	2.06E-36	Curtis et al ³⁰
	Mucinous Breast Carcinoma	-5.875	-14.955	1.28E-25	
	Invasive Lobular Breast Carcinoma	-4.279	-16.479	8.34E-44	
	Invasive Ductal and Invasive Lobular Breast Carcinoma	-5.319	-15.410	1.15E-34	
	Invasive Ductal Breast Carcinoma	-4.960	-24.263	7.62E-58	
	Breast Carcinoma	-4.460	-6.528	4.48E-06	
	Medullary Breast Carcinoma	-2.845	-7.190	2.26E-09	
CXCL11	Invasive Ductal Breast Carcinoma	10.712	5.279	2.81E-05	Karnoub et al ³⁹
	Ductal Breast Carcinoma	12.741	7.494	1.52E-09	Richardson et al ³³
	Invasive Breast Carcinoma	8.811	13.055	1.69E-25	TCGA Breast
	Invasive Ductal Breast Carcinoma	5.867	15.594	2.08E-31	
	Invasive Lobular Breast Carcinoma	5.268	7.777	8.07E-11	

Gene	Types of cancer vs normal	Fole change	t-Test	P-value	Dataset
S1PR1	Invasive Ductal Breast Carcinoma Stroma	-2.958	-6.441	2.35E-06	Ma et al ³⁶
	Tubular Breast Carcinoma	-2.051	-14.313	1.67E-32	Curtis et al ³⁰
	Invasive Breast Carcinoma	-2.030	-7.920	4.64E-09	
	Medullary Breast Carcinoma	-2.138	-9.752	1.39E-13	
	Invasive Ductal Breast Carcinoma	-2.153	-19.997	2.33E-45	
	Invasive Ductal Breast Carcinoma	-4.289	-15.878	3.35E-26	TCGA Breast
	Invasive Breast Carcinoma	-2.743	-8.538	1.50E-14	
	Invasive Lobular Breast Carcinoma	-2.437	-6.583	2.41E-09	

Next, the immunohistochemical images of breast cancer acquired from the HPA database demonstrated the differential protein expression level of the ten hub genes between breast cancer tissue and normal breast tissue (Fig. 6). These showed that CXCL12 was low expressed in breast cancer tissue and normal breast tissue. GNG2 was low expressed in breast cancer tissue and medium expressed in normal breast tissue. GPR17 was not detected in breast cancer tissues and normal breast tissue. ADCY5 was low expressed in breast cancer tissue and medium expressed in normal breast tissue. ADCY3 was low expressed in breast cancer tissue and normal breast tissue. CX3CL1 was medium expressed in breast cancer tissues and highly expressed in normal breast tissue. CXCL11 was medium expressed in breast cancer tissues and low expressed in normal breast tissue. S1PR1 was low expression in breast cancer tissue and medium expressed in normal breast tissue. We did not manage to find images for LPAR1 and CXCL1 from the HPA dataset.

The relationship between the hub genes and the key clinical parameters of breast cancer

To provide clinical insights, we investigated the relationship between the hub genes identified with significant differential expression with HBV-infection and breast cancer, and critical clinical indicators that are routinely applied to estimate the prognosis of breast cancer, by using univariate Cox proportional hazards model in bc-GenExMiner database. These indicators included age, the estrogen receptor (ER), the progesterone receptor (PR) and the human epidermal growth factor receptor 2 (HER-2), alongside the lymph node metastasis.

In aged breast cancer patients (> 51 years old), ADCY5 (n = 2285, p < 0.0001), a significant higher mRNA expression levels whilst CXCL11 (n = 2812, p = 0.001), CXCL12 (n = 2844, p < 0.001), GNG2 (n = 2215, p < 0.01), CXCL1 (n = 2844, p < 0.001), ADCY3 (n = 2783, p < 0.0001), CX3CL1 (n = 2844, p < 0.0001) showed a significant lower mRNA levels (Table 4). ER positive status was significantly positively correlated with CXCL12 (n = 6597, p < 0.0001), LPAR1 (n = 6597, p < 0.0001), ADCY5 (n = 4652, p < 0.0001) expression levels and negatively correlated with CXCL11 (n = 6310, p <

0.0001), GNG2(n = 4872, p < 0.001), CXCL1(n = 6597, p < 0.0001), ADCY3(n = 6506, p < 0.0001), CX3CL1(n = 6597, p < 0.0001) expression levels. PR positive status was positively correlated with CXCL12(n = 3224, p < 0.0001), LPAR1(n = 3224, p < 0.0001) expression levels and negatively correlated with CXCL11(n = 3034, p < 0.0001), GNG2(n = 2761, p < 0.0002), GPR17(n = 3034, p = 0.02) CXCL1(n = 3224, p < 0.0001), ADCY5(n = 2461, p < 0.0001), ADCY3(n = 3224, p < 0.0001), CX3CL1(n = 3224, p < 0.0001), S1PR1(n = 3224, p < 0.01). Similarly, HER-2 positive status was positively correlated with CXCL11(n = 683, p < 0.0001), CXCL1(n = 683, p < 0.0001), ADCY5(n = 642, p = 0.019), CX3CL1(n = 683, p < 0.0001) expression levels and negatively correlated with CXCL12(n = 683, p = 0.02), LPAR1(n = 683, p < 0.0001), ADCY3 (n = 643, p < 0.0001). Moreover, Triple-negative (ER+, PR+, HER-2 negative) status was positively correlated with CXCL11(n = 848, p < 0.0001), GNG2(n = 671, p = 0.0043), GPR17(n = 848, p = 0.030), CXCL1(n = 848, p < 0.0001), ADCY3(n = 848, p < 0.0001), CX3CL1(n = 848, p < 0.0001) expression levels and negatively correlated with CXCL12(n = 848, p < 0.0001), LPAR1(n = 848, p < 0.0001), ADCY5(n = 671, p < 0.0001) expression levels (Table 4). Lymph node metastasis rate showed significant positive correlations with CXCL11 (n = 3458, p < 0.001), LPAR1 (n = 3429, p < 0.01) expression levels and negatively correlated with ADCY3 (n = 3429, p < 0.001), CX3CL1 (n = 3511, p < 0.01) expression levels (Table 4).

Table 4

The relationship between the hub genes and the clinicopathologic parameters of breast cancer (bc-GenExMiner v4.1)

Parameters	CXCL12		GNG2		LPAR1		GPR17		CXCL1	
	mRNA	P-value	mRNA	P-value	mRNA	P-value	mRNA	P-value	mRNA	P-value
Age										
≤ 51	ref	p = 0.0002	ref	p = 0.0092	-	p = 0.6050	-	p = 0.6108	ref	p < 0.0001
>51	↓		↓		-		-		↓	
Nodal status										
negative	-	p = 0.1709	-	p = 0.1230	ref	p = 0.0016	-		-	
positive	-		-		↑		-	p = 0.9069	-	p = 0.0664
ER(IHC)										
negative	ref	p < 0.0001	ref	p = 0.0003	ref		-		ref	p < 0.0001
positive	↑		↓		↑	p < 0.0001	-	p = 0.0558	↓	
PR(IHC)										
negative		p < 0.0001	ref		ref	p < 0.0001	ref		ref	
positive	↑		↓	p < 0.0001	↑		↓	p = 0.0200	↓	p < 0.0001
HER2(IHC)										
negative	ref	p = 0.0202	-		ref	p < 0.0001	-		ref	p < 0.0001
positive	↓		-	p = 0.1968	↓		-	p = 0.5521	↑	
Triple-negative status										
Not	ref	p < 0.0001	ref	p = 0.0043	ref	p < 0.0001	ref	p = 0.0299	ref	p < 0.0001
TNBC	↓		↑		↓		↑		↑	
Parameters	ADCY5		ADCY3		CX3CL1		CXCL11		S1PR1	
	mRNA	P-value	mRNA	P-value	mRNA	P-value	mRNA	P-value	mRNA	P-value
Age										
≤ 51	ref	p < 0.0001	ref	p < 0.0001	ref	p < 0.0001	ref	p = 0.0010	-	p = 0.9688

Parameters	CXCL12		GNG2		LPAR1		GPR17		CXCL1	
	mRNA	P-value	mRNA	P-value	mRNA	P-value	mRNA	P-value	mRNA	P-value
>51	↑		↓		↓		↓		-	
Nodal status										
negative	-		ref	p = 0.0009	ref	p = 0.0052	ref	p = 0.0002	-	p = 0.6140
positive	-	p = 0.1407	↓		↓		↑		-	
ER(IHC)										
negative	ref	p < 0.0001	ref	p < 0.0001	ref	p < 0.0001	ref	p < 0.0001	-	p = 0.3491
positive	↑		↓		↓		↓		-	
PR(IHC)										
negative	ref		ref	p < 0.0001	ref	p < 0.0001	ref	p < 0.0001	ref	p = 0.0011
positive	↓	p = 0.0480	↓		↓		↓		↓	
HER2(IHC)										
negative	ref		ref	p < 0.0001	ref	p < 0.0001	ref	p < 0.0001	-	p = 0.1189
positive	↑	p = 0.0193	↓		↑		↑		-	
Triple-negative status										
Not	ref	p < 0.0001	ref	p < 0.0001	ref	p < 0.0001	ref	p < 0.0001	-	p = 0.1986
TNBC	↓		↑		↑		↑		-	

Survival analysis of hub genes expression in patients with breast cancer

In addition, relationship between the ten hub genes and the survival rate of breast cancer has been analysed to aid the understanding of the prognostic value of the ten hub genes. The survival analysis was performed using K-M plotter. The relatively low expression of GNG2(n = 943,p < 0.0001), LPAR1(n = 1879, p < 0.01), ADCY3(n = 1879,p < 0.01) and high expression of CXCL12(n = 1879, p < 0.001) was significantly associated with the poor overall survival (OS) (**Fig. 7**). The relatively low expression expression of GNG2 (n = 2032, p < 0.001), LPAR1 (n = 4929, p < 0.001), GPR17 (n = 4929, p < 0.0001), CXCL1 (n = 4929,p < 0.001), ADCY5 (n = 2032,p < 0.0001), ADCY3(n = 4929, p < 0.0001),

CXCL11 (n = 4929, p < 0.001), S1PR1 (n = 4929, p < 0.0001) and high expression of CXCL12 (n = 4929, p < 0.0001) was associated with the poor recurrence free survival (RFS) (Fig. 8).

Discussion

Breast cancer was a common malignant tumor in female population, with an increasing prevalence in the past decade[33]. It becomes a major health threaten to females and the public health worldwide, though clinical therapies including surgery, radiotherapy, chemotherapy and targeted therapy for breast cancer have been rapidly developing. Several epidemiological studies had highlighted an increased incidence[3] and bad prognosis[34] of breast cancer in female patients with chronic HBV infection. Therefore, this study investigated the mechanism that lies behind such association by analysing the involvement of HBV-related exosomal miRNA in the changes of gene expression with breast cancer. Via the identification of the key genetic targets that mediated the effects of HBV infection on the progression breast carcinoma, our results provided novel targets for the development of therapeutic strategy and prognostic biomarkers for breast cancer.

Firstly, our results found that miRNAs from the serum exosomes of female CHB patients, expression of hsa-miR-363 and hsa-miR-1237 showed significant down-regulation hsa-miR-1260, hsa-miR-100, hsa-miR-1287, hsa-miR-148a, hsa-miR-99a, hsa-miR-125b, hsa-miR-885-5p was significant up-regulation, in compared to uninfected individuals. Exosomes played critical roles in signal transmission within the human body through endocytosis and absorbance of organs, and has been implicated in occurrence and development of carcinoma^[19]. Our hypothesis was that the changes of exosomal miRNAs that were induced by the chronic HBV infection affects breast cells via the endocytosis of exosomes in the circulatory system. This could lead to changes of related gene expression in the breast cancer cells, triggering the process of cainogenesis.

To prove this hypothesis, we identified the potential target genes of those miRNAs that showed a differential expression in CHB patients, which were then cross-analysed with genes showing differentially expression in breast cancer.

Through GO analysis, we found that CC was mainly located in the cytoplasmic membrane components, cytoskeleton and actin skeleton. These components were associated with tumor cell invasion and metastasis, cell adhesion^[35, 36] and tumor cell immune tolerance^[37]. BP were mainly concentrated in cellular response to hormone stimulus, regulation of signal transduction^[38] and regulation of epithelial cell proliferation^[39]. These biological process were closely related to the occurrence and development of breast cancer. MF cluster analysis showed that the DEGs functions mainly include a variety of actins^[40], integrins^[41], and kinase anchoring and activity^[42]. These functions were also closely related to the occurrence of tumors. The results of KEGG analysis showed that many pathways in these gene-enriched pathways were closely related to cancer progression, such as pathways in cancer, Ras signaling pathway^[43], PI3K-Akt signaling pathway^[44]. It shows that these DEGs can regulate the occurrence, development, invasion and metastasis of breast cancer through a variety of signaling pathways. We constructed a PPI network in the STRING database, and used the MCC algorithm in Cytoscape to get the top ten hub genes. Then we used UALCAN and ONCOMINE database to verify the relative expression levels of these hub genes in breast cancer tissues and found that all the hub genes were dysregulated in breast cancer tissue and the result was consistent. HPA database was used to verify the protein expression level of hub genes. We found statistically different results in the protein expression level of GNG2, ADCY5, CX3CL1, CXCL11, S1PR1. Therefore, female CHB patients may affect the occurrence and development of breast cancer by regulating the expression of miRNAs in exosomes to manipulate the expression of hub genes in breast tissue.

Chemokines were secreted proteins with a molecular weight of 8-14kDa in the cytokine family^[45]. Both immune and tumor cells can express multiple chemokines. According to the number of conserved cysteines in the N-terminal domain of these proteins, they were divided into four families: CC, CXC, CX3C and XC families^[46]. Chemokines played an important role in tumorigenesis and development. CX3CL1 was a chemokine with a cysteine signature motif -Cys-X-X-X-Cys at the N-terminal end^[47] named neurotactin^[48] or fractalkine^[49] which was highly expressed in brain^[50], blood vessels^[51], bone^[52] and other tissues. Increased expression of CX3CL1 in tumor tissues will cause anti-cancer CD4 + T cells, CD8 + T cells, dendritic cells and NK cells to accumulate around the tumor to kill tumor cells^[53, 54]. High expression of CX3CL1 in breast cancer^[55], gastrointestinal cancer^[56], lung adenocarcinoma^[57] was closely related to good prognosis. On the contrary, advanced tumors will secrete TGF- β to reduce the expression of intracellular CX3CL1^[58]. It will inhibit the aggregation of anti-tumor cells to tumor tissues and promote tumor development. By bioinformatics analysis, we found that miR-885-5p was highly expressed in serum exosomes of female CHB patients which would inhibit CX3CL1 expression in breast cells. And the lower expression of CX3CL1 was correlated with higher lymph node metastasis rate in breast cancer patients. Patients with higher lymph node metastasis rate had significantly worse prognosis^[59], which may be associated with a worse prognosis for patients with lower expression of CX3CL1. CXCL12 was originally considered to be a growth factor of pre-B cells and was closely related to lymphocyte survival and embryonic development^[60]. At present, a large number of basic studies had found that CXCL12 promoted the occurrence and development of multiple tumors, invasion and metastasis^[61, 62] and resistance to chemotherapeutics^[63, 64] through multiple signaling pathways by combining with CXCR4^[65, 66]. And some clinical studies had found that breast cancer patients with high expression of CXCL12 had a poor prognosis^[67, 68]. But there were many basics^[69], clinical researches and meta-analysis^[70, 71] found that high expression of CXCL12 will prolong the survival of patients. By bioinformatics analysis, we found that high expression of miR-125b-5p and miR-1260a in serum exosomes suppressed CXCL12 expression in breast tissue. The bc-GenExMiner database showed that low CXCL12 expression was positively correlated with HER-2 positive status. HER-2 positive status was associated with poor prognosis^[72]. This may be related to the poor prognosis of patients with low CXCL12 expression in the K-M plotter database. In addition, the main metastatic feature of CXCL12 comes from the biological functions of CXCR4. By inhibiting the expression of CXCR4, the effect of breast cancer immunotherapy was enhanced^[73]. We searched CXCR4 expression of breast cancer in UALCAN and K-M plotter found that CXCR4 expression was highly expressed in breast cancer tissues (Fig. 5), and the RFS was poor when the expression of CXCR4 was low (Fig. 8). This result indicated that it may not be the increase in endogenous CXCL12 expression, but the high expression of CXCR4 caused by other reasons, that leads to the poor prognosis of breast cancer patients. This had also been confirmed by some researches that decreasing expression of endogenous CXCL12 will enhance the expression of CXCR4 to accept external CXCL12 and enhance mitotic ability^[69]. Interestingly, we also observed that miR-1237-3p which can inhibit CXCL12 expression was lowly expressed in the serum exosomes of female CHB patients. Whether this change will affect the overall expression of CXCL12 in breast tissue needs to be further investigated. CXCL1, a small chemokine belonging to the CXC chemokine family, was closely related to inflammation^[74], angiogenesis^[75], tumor formation^[76] and so on. CXCL1 can promote the invasion and metastasis of breast cancer cells by activating ERK to promote the expression of MMP2/9^[77]. Breast cancer cells promote CXCL1/S100A8/9 survival axis through high expression of TNF- α to promote breast cancer cells to resist chemotherapy^[78]. In addition, a large number of clinical studies have found that high expression of CXCL1 was associated with the poor prognosis of a large number of tumors^[79]. However, we have obtained the opposite result in breast cancer through bioinformatics technology. In this research, we found that high expression of miR-885-5p in serum exosomes of female CHB patients suppressed CXCL1 expression in breast tissue. The low expression of CXCL1 in breast cancer tissue was positively correlated with poor prognosis of breast cancer. Some researchers had found that the expression of CXCL1 in breast cancer

tissue specimens was significantly lower than that in normal tissues^[80]. Unfortunately, they had not made a reasonable explanation for this. This conflict may require further research. CXCL11 was induced by IFN- γ , IFN- β and IFN- α ^[81], mainly through the combination of CXCR3^[82] or CXCR7 to play biological function^[83]. CXCL11 promoted the self-renewal ability, tumorigenicity and anti-chemotherapeutic ability of liver cancer cells through the CXCR3/ERK1/2 signaling pathway^[84]. Inhibition of CXCL11 expression can inhibit tumor cell proliferation^[85] and epithelial-mesenchymal transition^[86]. Our results found that compared with the expression level of miR-363-3p in serum exosomes of normal women, the expression of miR-363-3p was lower in serum exosomes of female CHB patients. This difference will lead to high expression of CXCL11 in breast tissue. High expression of CXCL11 was positively associated with nodal status rate, which may predict a poor prognosis. In addition we obtained similar results in the K-M plotter database, that high expression of CXCL11 was associated with poor prognosis of breast cancer patients.

Current studies have found that heterotrimers G proteins were involved in various biological activities such as cell proliferation, differentiation^[87] and angiogenesis^[88]. G protein $\alpha 2$ subunit (GNG2) was one of the subunits of G protein β dimer. It was expressed in fetal tissue, adrenal gland and brain. It regulated a series of intracellular effects, including ion channels and adenylate cyclase^[89] by activating Phospholipase $\alpha 2$ (PLC $\alpha 2$) neurotransmitter delivery^[90]. Reducing the expression of GNG2 can activate c-SRC and promote AKT signaling pathway, which were closely related to breast cancer cell proliferation^[91], invasion and metastasis^[92]. Our study found that elevated miR-125b-5p expression in serum exosomes of female CHB patients may lead to the low expression of GNG2 in breast cancer tissue, and breast cancer patients with low expression of GNG2 will lead to a poor prognosis.

LPAR1 was the earliest discovered lysophosphatidic acid receptor^[93] which was expressed in brain, heart, colon, small intestine and placenta^[94]. In combination with Gai/o, G α q/11 and G α 12/13, LPAR1 can activate AKT, rho, MAPK and PLC to affect cell survival, differentiation, adhesion and migration^[95]. The role of LPAR1 in breast cancer was still unclear. Some studies had found that LPAR1 inhibitor can inhibit breast cancer cell metastasis to the lung and liver^[96]. Nevertheless, other studies had found that inhibiting LPAR1 expression can promote the proliferation, invasion and metastasis of breast cancer cells^[97]. Our study found that elevated miR-125b-5p expression in serum exosomes of female CHB patients may lead to LPAR1 down-regulated in breast cancer tissues. Low expression of LPAR1 was positively associated with HER-2 positive status in breast cancer. HER-2 positive status was associated with poor prognosis^[72], which may explain patients with low expression of LPAR1 had poor prognosis. However, we also observed that miR-1237-3p, which can inhibit LPAR1 expression, was decreased in serum exosomes of such patients. Whether this change will affect the expression of LPAR1 in breast cancer needs to be confirmed by more studies.

Protein Coupled Receptors (GPCR) were the unique family of transmembrane receptors and interaction with a diversity set of ligands such as lipids, peptides, chemokines, odorants, hormones and pheromones to achieve its function^[98]. At present, it was found that GPR17 was mainly confined to oligodendrocyte cell lines and was very important for myelin formation in oligodendrocytes^[99]. It was considered to be closely related to multiple sclerosis^[100]. GPR17 agonists can inhibit glioma cell proliferation^[101]. At present, there was still no relevant research about GPR17 in breast cancer. According to our results, the expression of GPR17 in breast cancer was reduced by the high expression of miR-1260a, miR-1287-5p and miR-885-5p in serum exosomes of female CHB patients. And the low expression of the gene was correlated with the poor prognosis. This was similar to the results of GPR17 in glioma but it needs to be further confirmed in breast cancer. We also observed that miR-1237-3p and miR-363-3p, which inhibited the expression of GPR17, were lowly expressed in serum exosomes of female CHB patients. More basic and clinical studies should be undertaken to confirm whether this change affected GPR17 expression in breast cancer.

Adenylate cyclase can convert ATP into the second messenger cAMP, and influence sugar, lipid and protein metabolism through signal transmission. ADCY5 belongs to the adenylate cyclase family and consists of twelve transmembrane domains, which catalyze the conversion of ATP. It was a cyclic AMP, which was the second messenger of G protein-coupled receptors^[102]. The low expression of ADCY5 after DNA methylation was related to the low prognosis of lung cancer^[103]. The decrease of ADCY5 promoted the occurrence of prostate cancer^[104]. Using bioinformatics technology, we found that highly expressed miR-1260a in serum exosomes of female CHB patients will suppress ADCY5 expression in breast tissue and low expression of ADCY5 was associated with a poor prognosis. At present, the relationship between ADCY5 and malignant tumors was not clear. Studies have found that ADCY5 decline can promote mouse cell autophagy to promote mouse cell survival and resist apoptosis^[105], whether this can explain the relationship between ADCY5 decline and the malignancy remained to be further research. ADCY3 was widely expressed in tissues throughout the body, and its role in tumors had not yet been determined. Studies had found that its high expression can promote the occurrence and development of gastric cancer through over-expression of CREB^[106], and inhibiting ADCY3 expression by miR-335 can inhibit gastric cancer cells invasion and metastasis^[107]. However, other studies had found that promoting the expression of ADCY3 can inhibit the invasion and metastasis of pancreatic cancer^[108]. Through bioinformatics technology, researchers found that the expression of ADCY3 in malignant glioma was lower than that in normal tissues, and the low expression of ADCY3 was related to the poor prognosis^[109]. Our research speculated that female CHB patients may inhibit the expression of ADCY3 in breast tissue through high expression of miR-125b-5p in serum exosomes. This alteration would promote breast cancer and was positively correlated with HER-2 positive status. This change can explain the association of low expression of ADCY3 with poor prognosis in the K-M plotter.

S1P receptor (S1PR1) was a biologically active sphingolipid metabolite that mediated S1P activity and promoted cell proliferation and survival^[110, 111]. S1PR1 was widely expressed in vascular endothelium and it was very important for embryonic blood vessel formation and maturation^[112]. The role of S1PR1 in tumors was unclear currently. Some studies had found that S1PR1 and STAT3 stimulated and activated each other to enhance tumor growth^[113]. In addition, S1PR1 can also promote tumor development by activating multiple signaling pathways^[114–116]. S1PR1 can promote breast cancer cell survival by down-regulating the pro-apoptotic protein Bim and up-regulating the anti-apoptotic protein Mcl-1^[117]. S1PR1 inhibitors can enhance the toxicity and anti-proliferative effects of carboplatin on breast cancer cells^[118]. However, some researchers had found that inhibiting the expression of S1PR1 can promote breast cancer and improve survival^[119]. S1PR1 regulates the activation of RhoA and inhibits the mimic angiogenesis of breast cancer cells to inhibit tumor growth and distant metastasis^[120]. Some researchers had found that the expression of S1PR1 in breast cancer tissues was significantly lower than that in normal tissues by integrating TCGA data, and lower expression of S1PR1 indicated a worse prognosis^[121]. By analyzing relevant data, we also found that miR-1287-5p in serum exosomes of female CHB patients may inhibit the expression of S1PR1 in breast tissue. Low expression of S1PR1 was positively associated with poor prognosis. This is consistent with some other scholars.

By comparing the expression of miRNAs in serum exosomes of chronic HBV-infected female with uninfected female, we clarified the reasons why female with chronic HBV infection were more likely to develop breast cancer and had a worse prognosis. Since the data used for the analysis were obtained from multiple online bioinformatics resources, there may be some bias caused by confounding factors. And these theories need to be confirmed in more basic and clinical research.

Conclusion

In conclusion, female patients with chronic HBV infection may affect the expression of miRNAs in serum exosomes to regulate the target genes in breast tissue, this mechanism will improve the occurrence, invasion and metastasis of breast cancer and lead to a worse prognosis.

Abbreviations

HBV: Hepatitis b virus; GEO: Gene expression omnibus; DEGs: Differentially expressed genes; PPI: Protein-protein interaction; BP: Biological processes; KEGG: Kyoto Encyclopedia of Genes and Genomes; CXCL11: C-X-C motif chemokine ligand 11; CXCL12: C-X-C motif chemokine ligand 12; GNG2: G-protein gamma 2 subunit; LPAR1: Lysophosphatidic acid receptor 1; GPR17: G protein-coupled receptor 17; CXCL1: C-X-C motif chemokine ligand 1; ADCY5: Adenylate cyclase 5; ADCY3: Adenylate cyclase 3; CX3CL1: C-X-C motif chemokine ligand 3; S1PR1: Sphingosine-1-Phosphate Receptor 1; MCDE: Molecular Complex Detection; CXCR4: C-X-C chemokine receptor type 4; CXCR3: C-X-C chemokine receptor type 3; CXCR7: C-X-C chemokine receptor type 7; CREB: cAMP response element binding protein; STAT3: Signal Transducer And Activator Of Transcription 3.

Declarations

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Authors' contributions

This article was done in collaboration with all the following authors. LQ and LHS determined the research theme and formulated the main research plan. XWJ and CC analyzed the data, explained the results, and wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

In this study, miRNAs microarray datasets were downloaded from the GEO (<http://www.ncbi.nlm.nih.gov/geo>) and mRNA datasets were downloaded from GEPIA V2.0 database(Gene Expression Profiling Interactive Analysis; <http://gepia.cancerpku.cn/>) .

Ethics approval and consent to participate

None

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interests.

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Figures

Figure 1

Identification of differentially expressed genes in the expression omnibus (GEO) datasets. (A) Volcano plot of differentially expressed miRNAs in GSE33857. Red and blue color represent relatively high and low expression of miRNAs in the corresponding group, respectively. $p < 0.01$ and $|\log(\text{FC})| > 1.5$ were set as the threshold. (B-C) Venn diagrams showing the proportion of overlapping genes that were associated to down-regulated miRNAs and showed an increased expression level in breast cancer (C), alongside the proportion of genes that were associated to up-regulated miRNAs and showed a decreased expression level (D). Blue-targeted genes of differentially expressed miRNAs; Red-targeted genes of differentially expressed miRNA of breast cancer in GEPIA database.

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Figure 2

GO analysis and KEGG pathway analysis of DEGs. (A) Top 10 of biological process(BP). (B) Top ten of cellular component(CC). (C) Top ten of molecular function(MF). (D) Top ten of KEGG pathway enrichment

Figure 3

PPI network, module analysis, and hub genes identification. (A) PPI network of overlapping differentially expressed genes was constructed in STRING database. (B-D) Top three modules screened using MCODE in Cytoscape software. (E) Top ten hub genes selected by the CytoHubba in Cytoscape based on the degree of each protein node.

Analysis Type by Cancer	Cancer vs. Normal CXCL12	Cancer vs. Normal GNG2	Cancer vs. Normal LPAR1	Cancer vs. Normal GPR17	Cancer vs. Normal CXCL1	Cancer vs. Normal ADCY5	Cancer vs. Normal ADCY3	Cancer vs. Normal CX3CL1	Cancer vs. Normal CXCL11	Cancer vs. Normal S1PR1	Cancer vs. Normal CXCR4
Bladder Cancer		3		3				4		1	1
Brain and CNS Cancer		1	1	3		2		3		1	6
Breast Cancer	1	14	5	4		8	1	6	17	5	8
Cervical Cancer		1			3				2	1	
Colorectal Cancer		7	14	15	1	20	5	6	1	12	2
Esophageal Cancer	1				5					1	
Gastric Cancer		3	1		2		1	2			1
Head and Neck Cancer		1			7	1			1	4	5
Kidney Cancer		4				2		1	1	1	
Leukemia	1	3	1			9				1	3
Liver Cancer	1	4				1			3		1
Lung Cancer		3	1	1		1			7	13	
Lymphoma	9		3	9				3	7	1	
Melanoma				1				1		2	
Myeloma		1				1					
Other Cancer			2	1	1			1		1	1
Ovarian Cancer		1		2		1		1	1	1	1
Pancreatic Cancer		2					1	1			2
Prostat Cancer				1					1		
Sarcoma	1		3	1		1		1	4	2	1
Significant Unique Analyses	14	48	1	27	12	32	1	37	23	11	10
Total Unique Analyses	458	316	426	407	447	274	372	446	392	450	439

Figure 4

An overview of mRNA levels of hub genes and CXCR4 in a variety of cancers based on Oncomine. The transcription levels of hub genes and CXCR4 in different types of cancer in Oncomine. The numbers in colored cells show the quantities of datasets with statistically significant mRNA overexpression (red) or underexpression (blue) of target genes. Cell color was determined by the best gene rank percentile for the analysis within the cells. The threshold was set as gene rank percentile p-value ≤ 0.05 , and fold change ≥ 1.5 or ≤ 1.5 .

Figure 5

Relative expression of hub genes and CXCR4 in breast cancer samples in UALCAN database. (Student's t-test, ***p < 0.001) Blue: breast cancer sample; Red: control sample. Image adapted from HPA database.

Figure 6

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Figure 7

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Figure 8

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