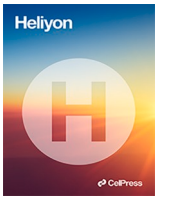
Heliyon 10 (2024) e27473

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Exploring the metastasis-related biomarker and carcinogenic mechanism in liver cancer based on single cell technology

Qiuxiang Zheng [a,1], Cuiping Lu [a,1], Lian Yu [b,**], Ying Zhan [a,***], Zhiyong Chen [a,*]

[a] Department of Oncology, Longyan First Hospital, Affiliated to Fujian Medical University, Longyan, 364000, China
[b] Department of Hematology, Longyan First Hospital, Affiliated to Fujian Medical University, Longyan, 364000, China

ARTICLE INFO

Keywords:
Hepatocellular carcinoma (HCC)
Single cell profile
Cancer metastasis
Transcription factor analysis
Drug sensitivity analysis

ABSTRACT

Background: Hepatocellular carcinoma (HCC) is a fatal primary malignancy characterized by high invasion and migration. We aimed to explore the underlying metastasis-related mechanism supporting the development of HCC.

Methods: The dataset of single cell RNA-seq (GSE149614) were collected for cell clustering by using the Seurat R package, the FindAllMarkers function was used to find the highly expression and defined the cell cluster. The WebGestaltR package was used for the GO and KEGG function analysis of shared genes, the Gene Set Enrichment Analysis (GSVA) was performed by clusterProfiler R package, the hTFtarget database was used to identify the crucial transcription factors (TFs), the Genomics of Drug Sensitivity in Cancer (GDSC) database was used for the drug sensitivity analysis. Finally, the overexpression and *trans*-well assay was used for gene function analysis.

Results: We obtained 9 cell clusters from the scRNA-seq data, including the nature killer (NK)/T cells, Myeloid cells, Hepatocytes, Epithelial cells, Endothelial cells, Plasma B cells, Smooth muscle cells, B cells, Liver bud hepatic cells. Further cell ecological analysis indicated that the Hepatocytes and Endothelial cell cluster were closely related to the cancer metastasis. Subsequently, the NDUFA4L2-Hepatocyte, GTSE1-Hepatocyte, ENTPD1-Endothelial and NDUFA4L2-Endothelial were defined as metastasis-supporting cell clusters, in which the NDUFA4L2-Hepatocyte cells was closely related to angiogenesis, while the NDUFA4L2-Endothelial was related with the inflammatory response and complement response. The overexpression and *trans*-well assay displayed that NDUFA4L2 exhibited clearly metastasis-promoting role in HCC progression.

Conclusion: We identified and defined 4 metastasis-supporting cell clusters by using the single cell technology, the specify shared gene was observed and played crucial role in promoting cancer progression, our findings were expected to provide new insight in control cancer metastasis.

1. Introduction

Hepatocellular carcinoma (HCC) is common solid primary liver malignancy encountered in the content of chronic liver disease and

* Corresponding author.
** Corresponding author.
*** Corresponding author.
E-mail addresses: yulian_ly@126.com (L. Yu), zhying200@163.com (Y. Zhan), Czy1360098@163.com (Z. Chen).
[1] Equal Contribution.

https://doi.org/10.1016/j.heliyon.2024.e27473
Received 3 December 2023; Received in revised form 16 January 2024; Accepted 29 February 2024
Available online 7 March 2024
2405-8440/© 2024 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

cirrhosis [1,2]. Cancer metastasis is the leading cause of death of liver cancer, many factors are involved in this process [3], such as the inhibitory immune microenvironment, metastasis associated with cell clusters and enhanced epithelial-mesenchymal transition (EMT) [4]. Globally, the hepatitis C virus (HCV) and hepatitis B virus (HBV) are the predominantly risk factors of cirrhosis and contributed 2–4% incidence of HCC per year [5]. However, due to the development of highly effective and available antiviral agents and the improved HBV vaccination rates, the alcoholic liver disease and nonalcoholic fatty liver disease causing metabolic disorders had become the major contributors for HCC development [6]. HCC may be the result of excessive regeneration of liver cells that caused by metabolic disorders, immune injury and debilitating disease [7]. According to the annual cancer statistics report, the estimated new cases of HCC are 41,210 and the estimated deaths are 29,380 in 2023 [8], the highly mortality rate still threatens people's lives and health. Diagnosis of HCC based on symptoms usually occur in the advanced or late-stage, leading to many patients lose the opportunity for optimal treatment [9]. Although tremendous progression occurred in the treatment of HCC, its prognosis remains poor, in which the 5- year survival rate is less than 20% [10] and the 1-year survival rate of terminal-stage HCC patients is less than 10% [11], blaming the recurrence (70% within 5 year) caused by metastasis largely [12,13]. Therefore, further study on the underlying mechanism of HCC metastasis is urgently needed.

The liver is an important hub for multiple physiological processes, such as macronutrient metabolism, immune system support [14], blood volume regulation, lipid and cholesterol homeostasis and endocrine control of growth signaling pathways [15], and depend on the abundant blood and lymphatic vasculatures (such as hepatic vein and portal vein) to achieve these functions [16]. In cancer patients, plenty of cancer cells were released in circulation daily, however, melanoma studies reported that $<0.01\%$ of these tumor cells contribute to metastasis [17] due to the cancer cells encountering the circulation, colonization and deadly immune killing stress in novel surroundings [18,19]. Hanahan et. Specify that "activating invasion" is the primary risk factor supporting cancer metastasis [20], invasion of adjacent tissue and seeding at distal position to form metastases maintains the feature of malignancy [21]. It is believed that the invasion and seeding requires the moving together of a cluster of tumor cells [22,23], which brings epithelial-mesenchymal transition (EMT) into the scenario [24,25]. Under normal physiological conditions, the epithelial cells are immotile and tightly combine with each other and the adjacent extracellular matrix (ECM) [26]. During EMT, a specific group epithelial cells lose their apico-basal polarity and intercellular adhesion traits, and develop the enhanced invasive, resist stress and disseminate capacities associated with the mesenchyme morphology, which are crucial for cancer metastasis and progression [27]. Nevertheless, studies on both animal models and human patients have demonstrated that the amount of circulating tumor clusters was more strongly associated with metastasis and relapse-free survival than that of single circulating tumor cells [28–30], indicating several specific-cell clusters are necessary for cancer metastasis. The cells with EMT signatures were characterized by the over-expression of the master EMT inducers, fibronectin, vascular endothelial (VE)-cadherin and the reduction of cell adhesion E-cadherin [31,32]. Hepatocytes are the predominant epithelial cell population of liver and constitutes the majority of liver volume and perform the many ascribed liver functions [15], contributing the basis of tumor metastasis. The studying of mouse model with breast cancer revealed that the asparagine synthetase is a determinant of metastatic potential [33], decreasing the levels of asparagine decreased the metastatic spread through L-asparaginase treatment or dietary restriction, due to the asparagine promoted EMT [33]. Epithelial endothelial transition (EET) is an EMT subtype, which involved the transformation of epithelial cells into endothelial cells [34], the endothelial cells are responsible for the vasculogenesis and angiogenesis supporting the metastasis and development of cancer [26]. However, not all cells originating from tumor site contributed to the advancement of metastasis through the EMT-dependent metastasis mechanisms, such as the hepatic stellate cells (HSCs) undergone the transition of "quiescent" to "activated" state [35] and secreted a multitude of chemokines (C–C motif ligand 5[CCL5]), cytokines (osteopontin, epimorphin) [36] and growth factors (hepatocyte growth factor [HGF]) through the paracrine manner to exert their pro-metastasis capability [37]. In addition, the single cell-RNA (scRNA) analysis is a useful tool for the studying of metastasis-related cell clusters. Wang el. Identified a cell cluster bridging the primary and metastatic group in gastric cancer (GC), elucidating the transition state of cancer metastatic process [38]. Another study revealed distinct malignant cell clusters shared by multiple tumors, including a single cluster associated with the metastasis and treatment resistance signatures by using the scRNA technology [39]. Therefore, the scRNA analysis were performed to explore the underlying metastasis-related molecular mechanism in HCC.

Metastatic cancer usually encompasses a variety of cells with different genetic and phenotypic characteristics, which underpin the metastasis and progression of cancer through the diverse mechanism [40,41]. Hundreds of genes determining invasion potential have been reported [42–44], such as the specific mutations of cyclin-dependent kinase inhibitor 2A (CDKN2A), phosphatase and tensi homolog (PTEN), retinoblastoma (RB1) and phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) [45,46], the L1 cell adhesion molecule (L1CAM) conferring metastasis-initiating abilities and chemoresistance to colorectal cancer cells [47]. Genetic expression profile of metastatic small cell lung cancer (SCLC) are differed from that in the profile of primary tumor [48], this might explain the difference of treatment response in some cancers. Therefore, understanding intertumoral heterogeneity can reveal the metastatic mechanisms of cancer progression and how the cell clusters contributes to tumor development. In this study, we performed a single cell RNA-seq analysis of HCC samples to explore the underlying mechanism of these metastatic cell clusters supporting cancer progression. First, the single cell clustering obtained nine cell clusters, in which the hepatocytes and endothelial cells had the largest proportion in metastatic samples compared with that in tumor and normal samples. Second, we further preformed the cell clustering analysis of these two type cells and defined the metastasis-supporting cells. Finally, we explore the metastasis signature of these cells including the potential upstream transcriptional regulation factor and drug sensitivity of different metastasis characteristic patients.

2. Material and methods

2.1. Data acquisition and preprocessing

The dataset GSE149614 was downloaded from the Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/, GEO) [49], including the 10 primary tumor, 2 portal vein tumor thrombus (PVTT), 1 metastatic lymph node (MLN) and 8 non-tumor liver tissue (NTL) samples, in which the PVTT and MLN samples were merged as metastasis samples.

2.2. Single cell RNA-seq dimension reduction clustering

The single-cell transcriptomic dataset GSE149614 was performed the filtering, normalization and dimensionality reduction by using the Seurat R package (v4.3.1) [50]. First, the raw expression matrix for each cell was filtered according to the following criteria: gene counts $\geq$200, mitochondrial genes$\leq$10%, unique molecular identifiers (UMIs) $\geq$200 and the log10GenesPerUMI $>$0.8 (indicating the unit read counts ratio of the number of genes). Second, the screened genes were expressed in at least three cells. Subsequently, the SCTransform function was applied for data normalization [51], the principal components analysis (PCA) was performed for the detection of batch effect, then the harmony R package was applied for the elimination of batch effect (setting: max. iter.harmony = 50, lambda = 0.5, assay. use = "SCT") [52]. The first 30 principal component was applied for the dimensionality reduction of Uniform Manifold Approximation and Projection (UMAP) and the FindNeighbors function was used for the clustering visualization [50]. After that, the FindAllMarkers function was used to identify the differentially expressed genes (DEGs) among cell clusters (avg-logFold Change$>$0.25 and p-adj$<$0.05), these cell clusters were further re-clustered based on the expression of these annotated DEGs (marker genes) [50].

2.3. Gene function analysis and screening for transcription factor

The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis was used to explore the function of these shared genes through the WebGestaltR package [53], meanwhile, the Gene Set Enrichment Analysis (GSEA) was performed by using the clusterProfiler R package [54]. In addition, we identified the crucial upstream transcription factor by using the hTFtarget database (http://bioinfo.life.hust.edu.cn/hTFtarget) [55].

2.4. Drug sensitivity analysis

We downloaded approximately 1000 drug sensitivity data of different cancer cell lines from the Genomics of Drug Sensitivity in Cancer (GDSC, http://www.cancerrxgene.org) [56], and calculated the correlation between the anti-cancer response and the expression of target gene through the Spearman method (filtering $|$correlation coefficient(R)$|$ $>$0.6 and false discovery rate(FDR) $<$ 0.05). Meanwhile, the patients were divided into different groups based on the median value of target genes expression and the drug sensitivity difference was compared by the Wilcoxon test. In addition, the 1037 drug sensitivity data of different cancer cell lines were downloaded from the Cancer Cell Line Encyclopedia (CCLE, https://portals.broadinstitute.org/ccle) [57], in which the HCC cells including 504 cell lines treated by 24 drugs were applied for the drug sensitivity analysis with the ActArea response indictor.

2.5. Cell culture and the trans-well assay

The human being's liver cell strain L02 and the human hepatocellular carcinoma (HCC) cell strain Huh7 were commercially bought from American Type Culture Collection (ATCC, Manassas, USA) and cultured by using the Dulbecco's modified eagle medium (DEME) containing 10% fetal bovine serum (FBS, Thermo Fisher, USA) in a humidified incubator containing 5% CO2 atmosphere at 37 °C [58]. Then, the RNA extraction and cDNA synthesis were performed by using a TRIzol reagent and PrimeScript RT-PCR kit (Madison, USA) according to the product specification, the quantitative real-time PCR (qRT-RCR) was performed by using the FastStart Universal SYBR Green kit and the LightCycler 480 PCR System (Roche, USA), primer of NDUFA4L2 (forward: 5′- AAAAGACATCCGGGGATCAT; 5′- TCCGGGTTGTTCTTTCTGTC), primer of EBF1 (forward: 5′- GGTTTCCCGCATTCTTTAGG; 5′- GTGGCAACCGAAATGAGACT). We used the pCDH-CMV vector to construct NDUFA4L2 overexpression cell strain [59] and conducted a *trans*-well assay to verify the role of NDUFA4L2 in cancer metastasis [60]. Briefly, the clustered cells (5×10^4) were collected and inoculated into the upper chambers coated with Matrigel in 100 μL FBS-free medium, the lower chamber had 600 μL DMEM medium, after 24 h incubation, the 4% paraformaldehyde was used for cell fixation and 0.1% crystalline violet for cell staining, after that the cells was counted under a light microscope, three technical replicates were performed for all experiments.

2.6. Statistical analysis

All statistical analyses were completed using the R software(v3.6.3). The correlation analysis was performed by using the Spearman method, and the Wilcoxon test was used to detect the significant difference (p-value $<$0.05 was regard as statistical significance). The Sangerbox (http://sangerbox.com/home.html) provided several experimental and technical supporting.

3. Results

3.1. Single cell profile of different tissue samples

After filtering, merging and dimensionality reduction clustering, we obtained 9 cell clusters, including the nature killer (NK)/T cells, Myeloid cells, Hepatocytes, Epithelial cells, Endothelial cells, Plasma B cells, Smooth muscle cells, B cells, Liver bud hepatic cells (Fig. 1A). The expression of these marker genes in each cell cluster was displayed in (Fig. 1B), in which the phoenolpyruvate carboxy kinase 1 (PCK1) [61], hepatocyte nuclear factor alpha (HNF4A) [62] and carbamoyl phosphate synthetase 1 (CPS1) [63] were identified as the marker genes of Hepatocytes (Fig. 1C). We visualized the proportion of these cell cluster in varying tissue samples and found that the Hepatocytes and Endothelial cells in the metastatic sample had the largest proportion compared with that in normal and tumor samples, while the proportion of epithelial cells in the metastatic sample had no markedly increased compared with that in tumor samples (Fig. 1D).

3.2. Single cell landscape of hepatocytes

Notably, the metastasis sample had the highest number of hepatocytes, we separated the hepatocytes cluster and preformed further clustering analysis. These hepatocytes were classified into four sub-clusters based on the significant expression of differential genes, including the NDUFA4L2-Hepatocyte, SAA1-Hepatocyte, GTSE1-Hepatocyte and CD48-Hepatocyte based on the expression of significant genes (Fig. 2A and B). The SAA1-Hepatocyte and NDUFA4L2-Hepatocyte had the largest proportion in normal and metastatic samples respectively (Fig. 2C), in ecological analysis of cell sub-clusters, the abundance of NDUFA4L2-Hepatocyte and GTSE1-Hepatocyte in metastatic tissue was significantly higher than that in primary tumor samples (Fig. 2D), implying these two cell sub-clusters are the predominant risk factors involved in metastasis of HCC. The NADH dehydrogenase (ubiquinone) 1 alpha sub-complex 4-like 2 (NDUFA4L2) [64] and G2 and S phase-expressed-1 (GTSE1) [65] as marker genes were significantly expressed in the

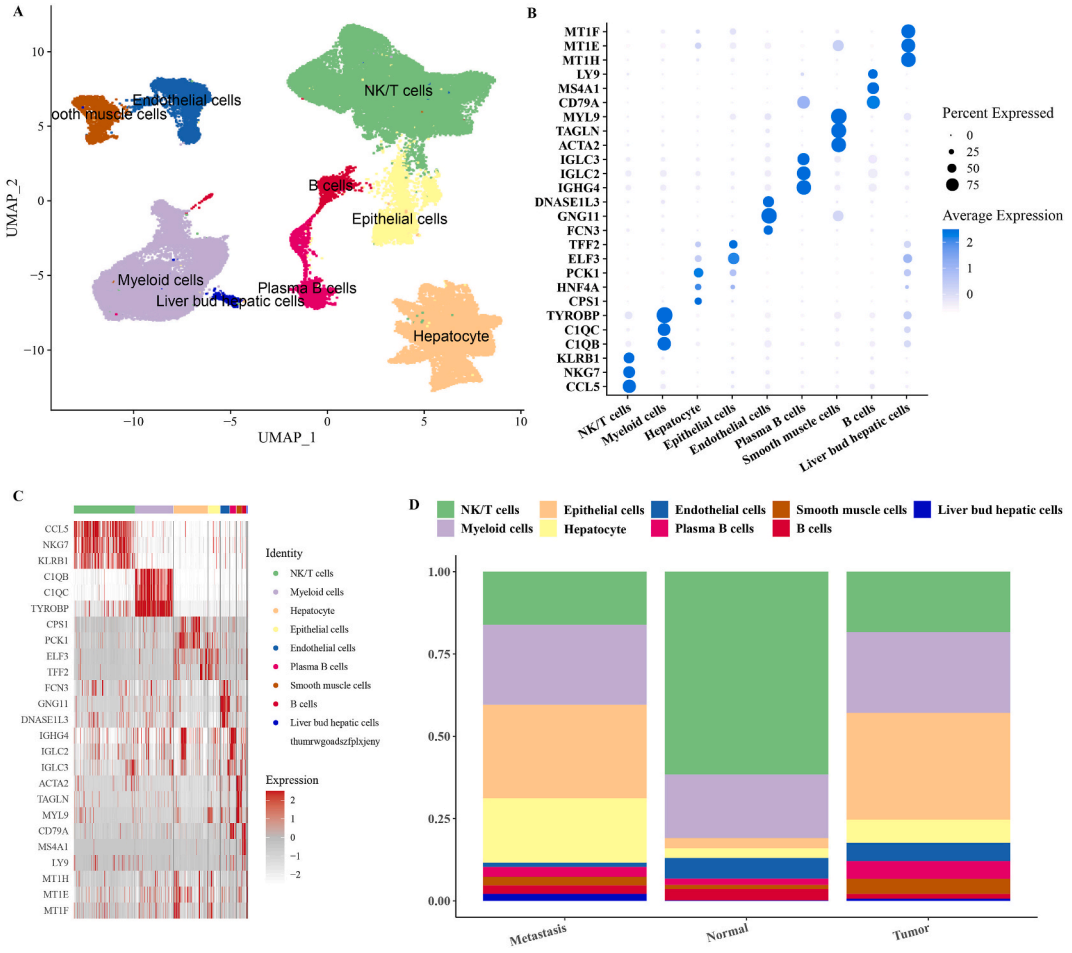


Fig. 1. Single cell profile *analysis*. (A) The umap plot of cell clustering. (B) Bubble plot of marker genes expression in varying cell clusters. (C) The heatmap of marker gene expression in each cell cluster. (D) The proportion of cell clusters in different tissue samples.

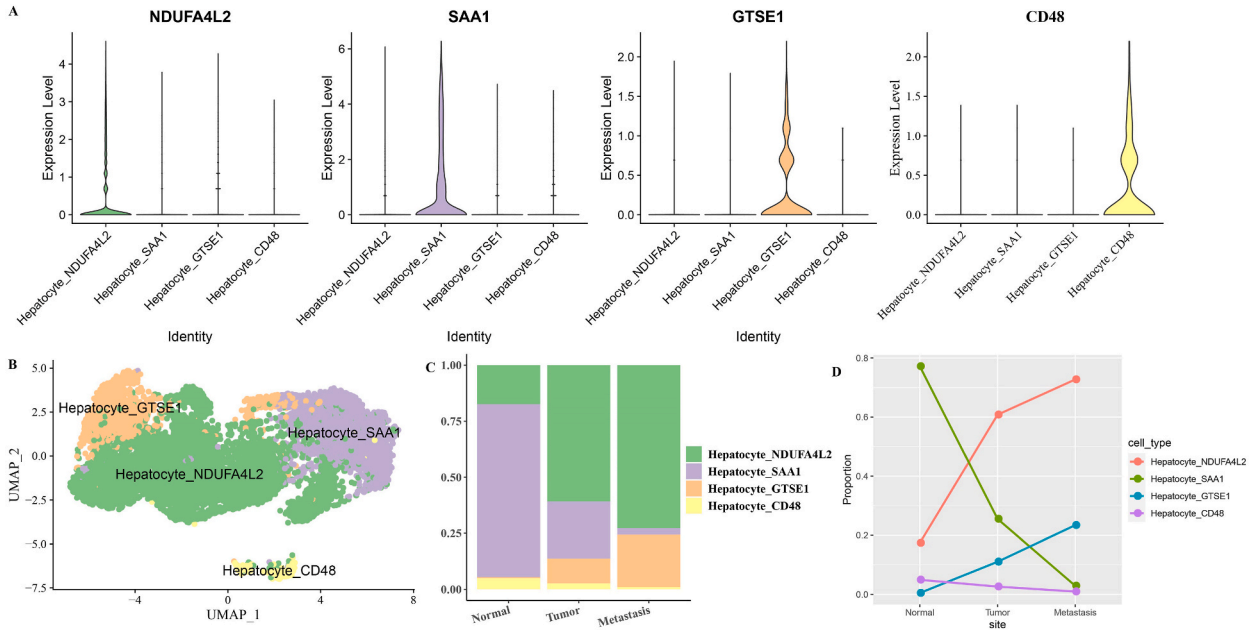


Fig. 2. Single cell landscape of *hepatocytes*. (A) The bubble plot of marker gene expression in different hepatocyte clusters. (B) The umap plot of hepatocytes clustering. (C) The proportion of hepatocyte clusters in different tissue samples. (D) The broken line plot of hepatocyte abundance changes in different tissue samples.

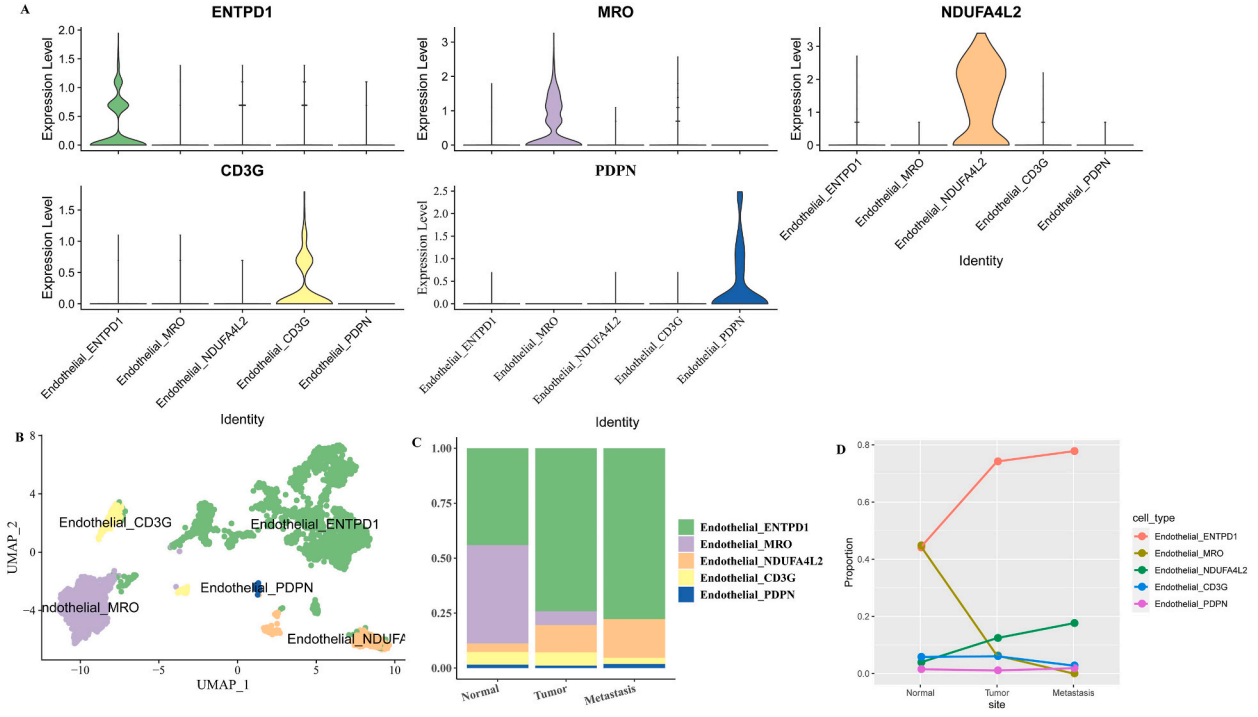


Fig. 3. Single cell landscape of endothelial cells. (A) The bubble plot of marker gene expression in different endothelial cell clusters. (B) The umap plot of endothelial cell clustering. (C) The proportion of endothelial cell clusters in different tissue samples. (D) The broken line plot of endothelial cell abundance changes in different tissue samples.

corresponding hepatocyte cell clusters, these two cell cluster were regard as metastasis-supporting hepatocytes.

3.3. Single cell landscape of endothelial cells

Likewise, the endothelial cells also play an essential role in promoting cancer progression and had largest proportion in the metastatic samples, we used the same method and divided the endothelial cells into five sub-clusters, including the ENTPD1-Endothelial, MRO-Endothelial, NDUFA4L2-Endothelial, CD3G-Endothelial and PDPN-Endothelial (Fig. 3A and B), in which the ENTPD1-Endothelial and NDUFA4L2-Endothelial in the metastasis samples had the largest proportion (Fig. 3C) and their abundance also increased significantly from primary to metastatic tissues (Fig. 3D), implying these cells are crucial endothelial cell cluster supporting the metastasis of HCC. The ectonucleoside triphosphate diphosphohydrolase 1 (ENTPD1) [66] and NDUFA4L2 as marker genes were significantly expressed in the corresponding endothelial cell clusters, these two cell clusters were regard as metastasis-supporting endothelial cell.

3.4. The underlying role of the metastasis-supporting cells

After a comprehensive analysis between these metastasis-related cells, we found that the NDUFA4L2 as marker gene was expressed in both hepatocytes and endothelial cells. We calculated the correlation between NDUFA4L2 and others typically metastasis-related genes, including the TP53 [67], PIK3CA, PTEN [68], CDKN2A, VEGFA [69], MMP2, MMP9 and RB1 [70], the results showed that NDUFA4L2 had positive correlation with these genes (Fig. S1). After that, we analyzed the significant differentially expressed genes among these metastasis-related cells (Fig. 4A) and identified 11 shared marker genes in NDUFA4L2-Hepatocyte and NDUFA4L2-Endothelial clusters, including the AGT, SOX4, PKM, ALDOA, KRT18, MDK, NDUFA4L2, KRT8, SERPINA1, FN1 and APOE. The genomic mutation analysis revealed that the only FN1 had higher mutation frequency (4%) and other gene mutation frequency less than 0% (Fig. S2), implying the mutation are not the dominating risk factor for supporting HCC cell metastasis. The biological process of GO analysis indicated these genes were closely associated with the protein synthesis regulation, extracellular matrix and protein stability (Fig. 4B), KEGG enrichment analysis showed that these genes were significantly enriched in protein synthesis, cell adhesion and epithelial cell transformation pathway (Fig. 4C). The GSEA analysis displayed that the marker genes of NDUFA4L2-Hepatocyte cells were closely associated with the angiogenesis pathway (Fig. 4D), while the marker genes of NDUFA4L2-Endothelial cells were closely associated with the inflammatory response and complement pathway (Fig. 4E).

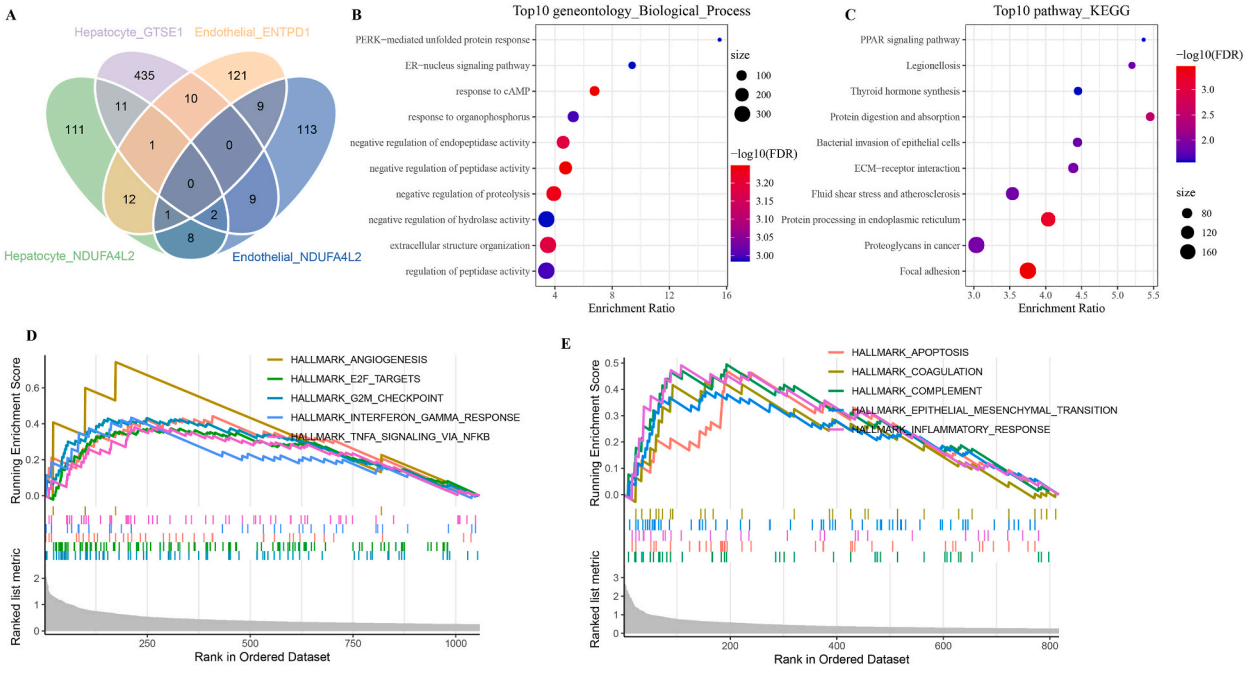


Fig. 4. The shared gene analysis among the metastasis-supporting cell clusters. (A) Veen plot of significantly expressed gene in four metastasis-supporting cell clusters. (B) GO enrichment analysis of the shared genes between the Hepatocyte_NDUFA4L2 and Endothelial_NDUFA4L2 cell clusters. (C) KEGG enrichment analysis of the shared genes between the Hepatocyte_NDUFA4L2 and Endothelial_NDUFA4L2 cell clusters. (D) GSEA of significantly expressed genes in Hepatocyte_NDUFA4L2 cluster. (E) GSEA of significantly expressed genes in Endothelial_NDUFA4L2 cluster.

3.5. Identifying upstream transcription factor of NDUFA4L2 gene

Based on the gene function analysis, we found that NDUFA4L2 is a key target gene in promoting liver cancer metastasis, it is worth to explore potential transcription factor regulating NDUFA4L2 expression to control HCC metastasis. Therefore, we utilized the

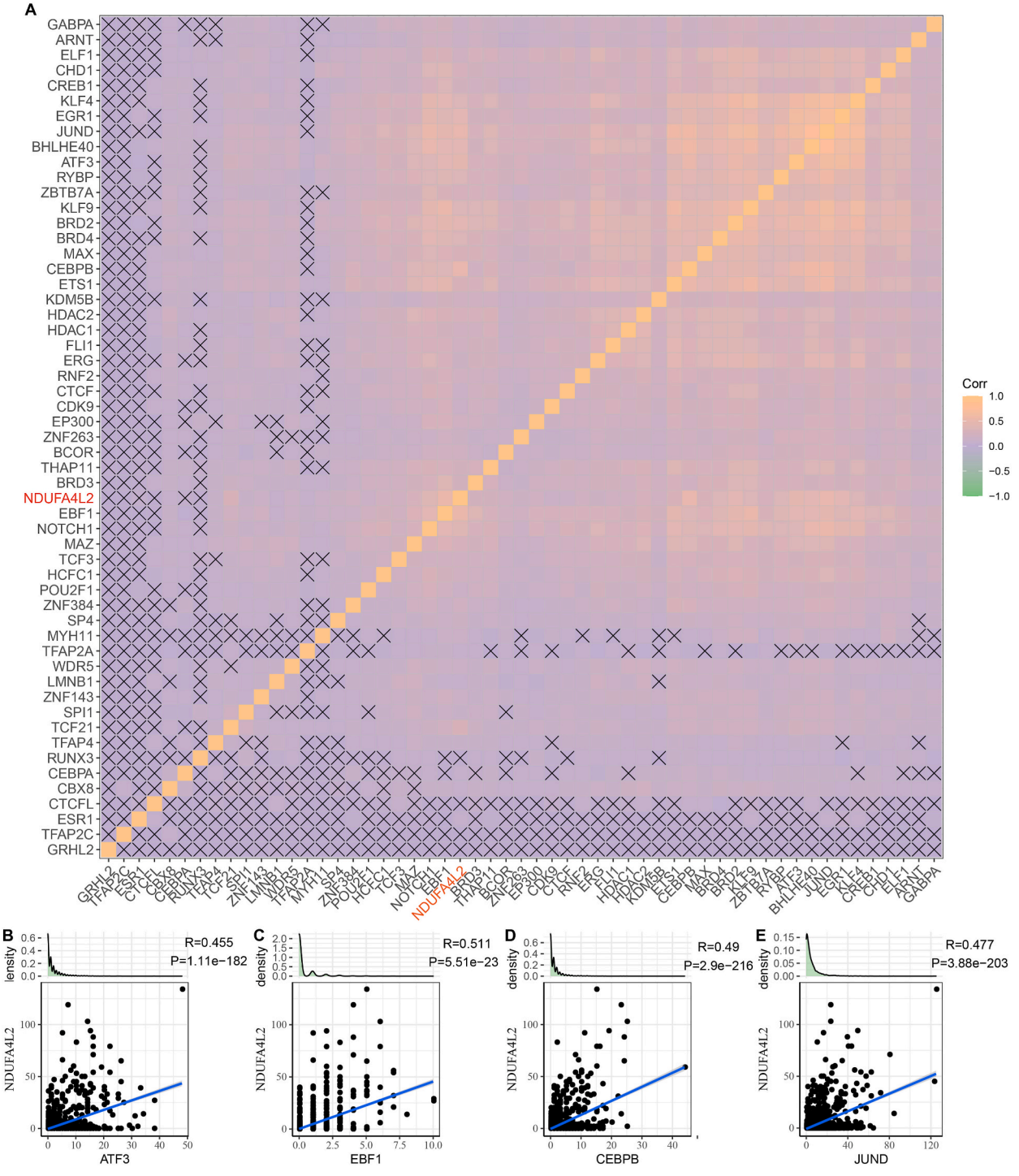


Fig. 5. Identifying the upstream transcription factor of NDUFA4L2. (A) The correlation analysis between the candidate transcription factor and the expression of NDUFA4L2. (B) Correlation analysis between ATF3 and NDUFA4L2. (C) Correlation analysis between EBF1 and NDUFA4L2. (D) Correlation analysis between CEBPB and NDUFA4L2. (E) Correlation analysis between JUND and NDUFA4L2.

hTFtarget database and obtained 55 candidate transcription factors (TFs), the correlation analysis between the TFs and NDUFA4L2 (Fig. 5A) indicated that the ATF3 (r = 0.455, Fig. 5B), EBF1 (r = 0.511, Fig. 5C), CEBPB (r = 0.49, Fig. 5D) and JUND (r = 0.477, Fig. 5E) were significant ($p < 0.05$) positive correlation with the expression of NDUFA4L2, in which the early B cell factor 1 (EBF1) gene had the largest correlation with the NDUFA4L2, implying that EBF1 is most likely the upstream TF of NDUFA4L2 gene.

3.6. The best drug targeting NDUFA4L2 and its transcription factor

We analyzed the drug sensitivity based on the GDSC database and the anti-cancer drug AUC response indictor in HCC lines. The correlation between anti-cancer response ratio and the expression of NDUFA4L2 displayed that four drugs were significantly correlated with the NDUFA4L2 (Fig. 6A). Based on the expression median of NDUFA4L2, the samples were divided into high- and low-level groups, the drug sensitivity analysis displayed that the patients with low-level NDUFA4L2 are more sensitive to Mirin and PLX-4720 ($p < 0.05$, Fig. 6B). Seven drugs were significantly correlated with the EBF1 (Fig. 6C), the patients with low-level EBF1 are more sensitive to THR-101 and Vorinostat ($p < 0.05$, Fig. 6D). In addition, we explored the drug sensitivity based on the CCLE database and the ActArea response indicator in HCC lines, five drugs were closely associated with the NDUFA4L2 expression (Fig. 6E), the patients with the low-level of NDUFA4L2 are more sensitive to AEW541, Irinotecan, Paclitaxel, PD-0332991 and Topotecan ($p < 0.05$, Fig. 6F), eight drugs were closely associated with the EBF1 (Fig. 6G), in which the patients with high-level EBF are more sensitive to AZD0530, AZD6244, Erlotinib, Lapatinib, PD-0325901 and ZD-6474 ($p < 0.05$, Fig. 6H) and might benefit from these drug treatment.

3.7. NDUFA4L2 as crucial metastasis-promoting factor in HCC progression

We measured the expression level of EBF1 and NDUFA4L2 by using a qRT-RCR kit. The results displayed that the expression of EBF1 in Hub7 cancer cells was significantly higher than that in the normal L02 liver cells ($p < 0.001$, Fig. 7A), meanwhile, the NDUFA4L2 was also higher expressed in Hub7 cells ($p < 0.001$, Fig. 7B), indicating the consistent expression patterns of these two genes. In addition, the overexpression results indicated that the level of EBF1 increased 21-fold in the overexpression (oe)-NDUFA4L2 cell strain (Fig. 7C), the significantly expression of NDUFA4L2 in the oe-NDUFA4L2 cells revealed the excellent overexpression efficiency (Fig. 7D), this result implied that there had a strong promoting relationship between EBF1 and NDUFA4L2. Subsequently, in the *trans*-well assay, we observed that the greatest number of oe-NDUFA4L2 cells occurred migration and invasion compared with oe-NC cells (Fig. 7E) and resulted in a larger blue area (Fig. 7F), indicating that the NDUFA4L2 acted as crucial metastasis-promoting factor in HCC progression.

4. Discussion

Hepatocellular carcinoma is a primary cause of cirrhosis death with poor prognosis, and its mortalities approximated incidence rates worldwide [71]. The metastasis is a hallmark of cancer that account for the largest number of cancer-related death, the continuous advancement of cancer biology studies and the appearance of new paradigms in metastasis study have revealed several molecular underpinnings of this dissemination process [72], such as gene mutations, soluble signals, cell-cell interactions,

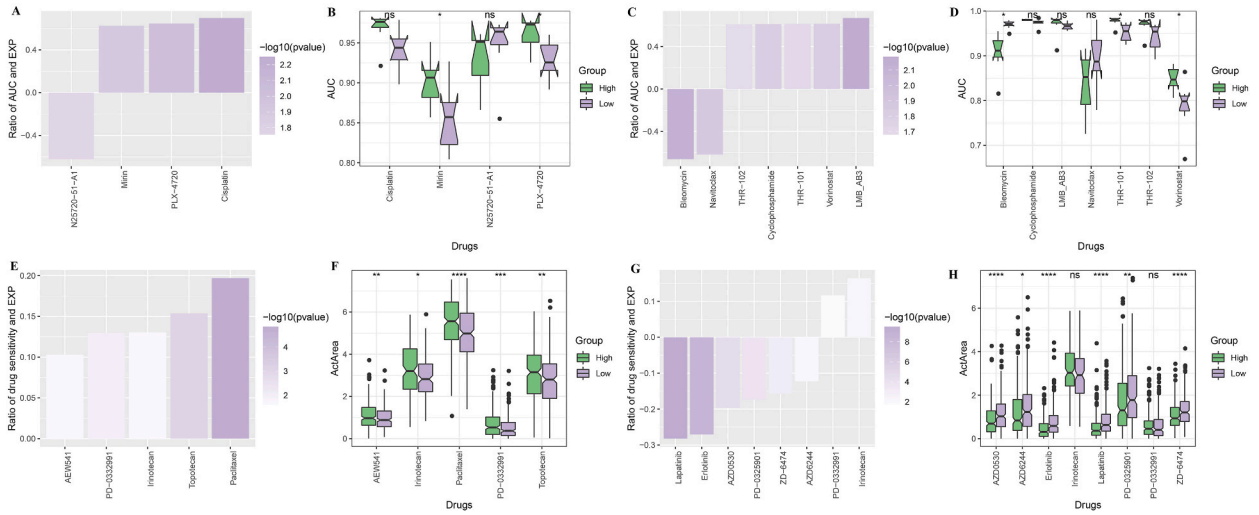


Fig. 6. The drug sensitivity analysis of NDUFA4L2 and EBF1. (A) Correlation analysis between candidate drug and NDUFA4L2 in GDSC. (B) Drug sensitivity difference of varying NDUFA4L2 level patients in GDSC. (C) Correlation analysis between candidate drug and EBF1 in GDSC. (D) Drug sensitivity difference of varying EBF1 level patients in GDSC. (E) Correlation analysis between candidate drug and NDUFA4L2 in CCLE. (F) Drug sensitivity difference of varying NDUFA4L2 level patients in CCLE. (G) Correlation analysis between candidate drug and EBF1 in CCLE. (H) Drug sensitivity difference of varying EBF1 level patients in GDSC in CCLE.

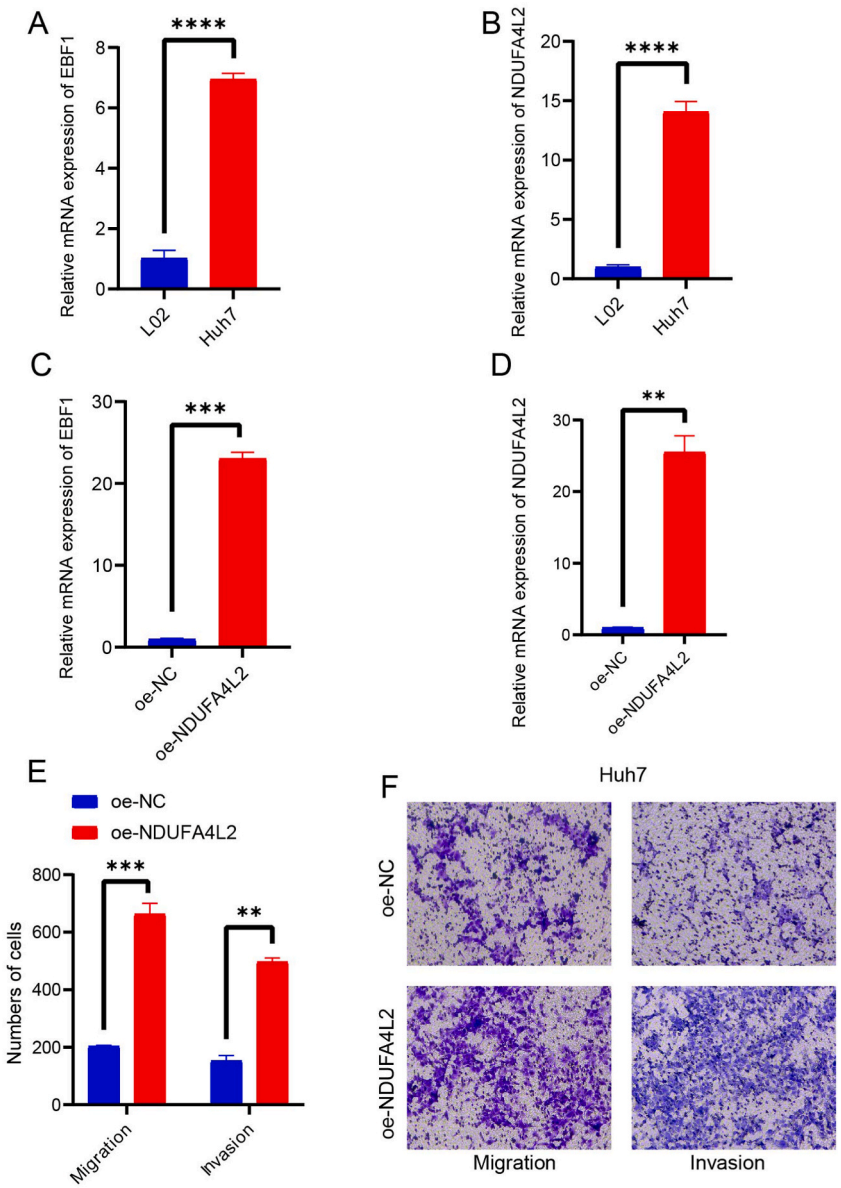


Fig. 7. The function analysis of NDUFA4L2. (A) The relative expression of EBF1 in tumor and normal liver cells. (B) The relative expression of NDUFA4L2 in tumor and normal liver cells. (C) The relative expression of EBF1 in NDUFA4L2 overexpression strain. (D) The relative expression of NDUFA4L2 in NDUFA4L2 overexpression strain. (E) The numbers of cell involved in migration and invasion. (F) The cellular staining in *trans*-well assay.

microbiome, the adhesive signals from extracellular matrix components (EMC), EMC mechanical pressures and epigenetic factors. In this study, we performed a single cell RNA-seq analysis to explore the cell clusters supporting HCC-metastasis. The results displayed that the cell clusters of NDUFA4L2-Hepatocyte, GTSE1-Hepatocyte, ENTPD1-Endothelial and NDUFA4L2-Endothelial, were closely associated with the cancer metastasis, GSVA indicated that these cell clusters played varying role to promote cancer progression. The NDUFA4L2 as shared gene were highly expressed in hepatocyte and endothelial cells and the its upstream transcription factor EBF1 involved in the NDUFA4L2 transcriptional regulation. We expected our finding can provide several new insights to benefit the groundbreaking of HHC metastasis.

Studies have shown that the metastatic properties with respect to growth and therapy response were determined by the nature of primary cancer cells [73] and some specific cell clusters acquired growth advantages to dominant cancer metastasis through altering metabolism. Yeo el. revealed that the cancer tissue comprised multiple cell clusters in breast cancer (BC) [28], and its subtype composition showed fluctuate under chemotherapy stress [74]. A cancer-associated fibroblasts (CAFs) sub-cluster characterized by the overexpression of urea transporter SLC14A1, confers stemness to BC cells through the WNT5A paracrine pathway, the inhibition of

CAF-SLC14A1 formation can suppress the interferon production and enhance the chemosensitivity [75]. In our study, the hepatocyte and endothelial cells are primary metastasis-supporting factors through activating different signaling pathway. NDUFA4L2 is a subunit of the mitochondrial respiratory chain complex I and involved in metabolic reprogramming and oxidative stress. The NDUFA4L2 had been reported to be overexpressed in multiple malignant tumor and associated with poor prognosis, such as the NDUFA4L2 was highly expressed in clear cell renal cell carcinoma (ccRCC) and knockdown of NDUFA4L2 had antiproliferative effect [76], hypoxia-induced the upregulation of NDUFA4L2 promote the colon adenocarcinoma progression through activating the ROS-mediated PI3K/AKT pathway [77], high levels of NDUFA4L2 was correlated with enhanced cell proliferation and anti-apoptosis in promoting glioblastoma progression [78]. GTSE1 is a microtubule plus-end tracking protein, its high expression increased the invasive potential in breast cancer [79], knockdown of GTSE1 resulted in the exhaustion of migrate and invade capabilities and the weakness of EMT in liver cancer [80]. In addition, the GTSE1 depletion could upregulate the tumor suppressor KLF4 [81] and activate the apoptosis signal to inhibit the proliferation, migration, and invasion of cancer cells [82]. The phosphohydrolase ENTPD1 were highly expressed in T cells and increased the extracellular adenosine diphosphate (ADP) concentration to inhibit the DNA damage repair to promote skin carcinogenesis and metastasis [66], the local overexpression of ENTPD1 can directly promote tumor growth through extracellular ATP and targeted inhibition of ENTPD1 enzymatic activity could be as an adjunct therapy [83]. Overall, our finding indicating that these cell clusters are crucial in cancer metastasis-supporting and we hypothesized that the cell cluster with NDUFA4L2 marker could be enhance the anti-apoptotic ability under hypoxic condition to underpin tumor metastasis, the cell cluster with GTSE1 marker could be enhance the EMT ability to promote tumor metastasis, while the cell cluster with the ENTPD1 marker disturbed the DNA damage repair through regulating the purinergic signaling to promote the metastasis.

EBF1 was reported as a B cell differentiation regulatory factor, and the EBF1 can interact with the transcription factor JAK2 to inhibit the transcription of PAX5 affecting B cell functional integrality [84], the EBF1 acted as a negative regulator of AKR1B1, the induced EBF1 expression suppressed the AKR1B1 expression resulting in attenuated gastric cancer growth and invasiveness [85], meanwhile, the EBF1 also acts as a powerful repressor of B lymphocyte-induced maturation protein-1 (Blimp-1) to block B cell maturation [86], the high expression of EBF1 in triple-negative breast cancer (TNBC) has a pivotal role in tumorigenicity, while depletion of EBF1 induces significantly tumor cell mitophagy and growth inhibition [87]. Our study indicated that the EBF1 as the upstream regulator was positively correlated with NDUFA4L2 expression, suggesting the drug combination targeted EBF1 and NDUFA4L2 are benefit to cancer therapy [88], such as the combination of Mirin and Vorinostat, PLX-4720 and THR-101. The over-expression of NDUFA4L2 significantly upregulated the EBF1, which as a potential transcription factor may be promote the NDUFA4L2 expression through the positive feedback regulation mechanism, while the NDUFA4L2 as the actual metastasis-promoting factor involved in HCC metastasis. However, the interaction between NDUFA4L2 and EBF1 needed further experimental verification, the huge heterogeneity of HCC doomed that a bias might be inevitable, our findings still need to be validated in larger datasets and clinical practice. This study performed a comprehensive workflow to explore potential cancer metastasis-supporting in HCC, four key cell clusters acted as potential basis to underpin this cancer metastasis process through different mechanism, the NDUFA4L2 as a key gene, is expected to be developed as a new target for HCC therapy.

5. Conclusion

We defined four cell clusters, including the NDUFA4L2-Hepatocyte, GTSE1-Hepatocyte, ENTPD1-Endothelial and NDUFA4L2-Endothelial that closely associated with the cancer metastasis. Overall, metastasis is the final frontier and involved in complex physiological process, our findings provide several molecular clues to overcome the cancer metastasis and some guidance of combination therapy to counter the genomic and phenotypic alteration in metastatic cancer cells through targeting multiple pathways simultaneously.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical statement

Informed consent was not required for this study because it is not involved any human experiments.

Data availability statement

The datasets generated and/or analyzed during the current study are available in the [GSE149614] repository, [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc= GSE149614].

CRediT authorship contribution statement

Qiuxiang Zheng: Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration. **Cuiping Lu:** Writing – review & editing, Writing – original draft, Resources, Formal analysis, Data curation. **Lian Yu:** Software, Resources, Project administration, Methodology, Investigation. **Ying Zhan:** Writing – original draft, Validation, Software, Conceptualization. **Zhiyong Chen:** Writing – review & editing, Visualization, Project administration, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

None.

Abbreviations

HCC	Hepatocellular carcinoma
GSVA	Gene Set Enrichment Analysis
TFs	transcription factors
GDSC	Genomics of Drug Sensitivity in Cancer
CCLE	Cancer Cell Line Encyclopedia
HBV	hepatitis B virus
EMT	epithelial-mesenchymal transition
HSCs	hepatic stellate cells
HGF	hepatocyte growth factor
TAMs	tumor-associated macrophages
M-CSF	macrophage colony-stimulating factor
CDKN2A	cyclin-dependent kinase inhibitor 2A
PTEN	phosphatase and tensi homolog
PIK3CA	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha
L1CAM	L1 cell adhesion molecule
SCLC	small cell lung cancer
GEO	Gene Expression Omnibus
PVTT	portal vein tumor thrombus
MLN	metastatic lymph node
UMIs	unique molecular identifiers
PCA	principal components analysis
UMAP	Uniform Manifold Approximation and Projection
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
GSEA	Gene Set Enrichment Analysis

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27473.

References

[1] J.A. Marrero, et al., Diagnosis, staging, and management of hepatocellular carcinoma: 2018 practice guidance by the American association for the study of liver diseases, Hepatology 68 (2) (2018) 723–750.
[2] M. Xu, et al., Emerging nanobiotechnology for precise theranostics of hepatocellular carcinoma, J. Nanobiotechnol. 20 (1) (2022) 427.
[3] K. Stoletov, P.H. Beatty, J.D. Lewis, Novel therapeutic targets for cancer metastasis, Expert Rev. Anticancer Ther. 20 (2) (2020) 97–109.
[4] Y. Zhang, R.A. Weinberg, Epithelial-to-mesenchymal transition in cancer: complexity and opportunities, Front. Med. 12 (4) (2018) 361–373.
[5] J.K. Heimbach, et al., AASLD guidelines for the treatment of hepatocellular carcinoma, Hepatology 67 (1) (2018) 358–380.
[6] S. Chidambaranathan-Reghupaty, P.B. Fisher, D. Sarkar, Hepatocellular carcinoma (HCC): epidemiology, etiology and molecular classification, Adv. Cancer Res. 149 (2021) 1–61.
[7] Y. Li, et al., Potential of compounds originating from the nature to act in hepatocellular carcinoma therapy by targeting the tumor immunosuppressive microenvironment: a review, Molecules 28 (1) (2022).
[8] R.L. Siegel, et al., Cancer statistics, 2023, CA A Cancer J. Clin. 73 (1) (2023) 17–48.
[9] H. Gilles, T. Garbutt, J. Landrum, Hepatocellular carcinoma, Crit. Care Nurs. Clin. 34 (3) (2022) 289–301.
[10] D. Feng, et al., Prognostic value of the albumin-bilirubin grade in patients with hepatocellular carcinoma and other liver diseases, Ann. Transl. Med. 8 (8) (2020) 553.
[11] J. Bruix, J.M. Llovet, Major achievements in hepatocellular carcinoma, Lancet 373 (9664) (2009) 614–616.
[12] L. Rajendran, et al., The management of post-transplantation recurrence of hepatocellular carcinoma, Clin. Mol. Hepatol. 28 (1) (2022) 1–16.
[13] J. Hartke, M. Johnson, M. Ghabril, The diagnosis and treatment of hepatocellular carcinoma, Semin. Diagn. Pathol. 34 (2) (2017) 153–159.
[14] S.H. Ewe, J.L. Tan, Hepatotocellular carcinoma—a rare complication post fontan operation, Congenit. Heart Dis. 4 (2) (2009) 103–106.
[15] E. Trefts, M. Gannon, D.H. Wasserman, The liver, Curr. Biol. 27 (21) (2017) R1147–r1151.

[16] Y. Lin, et al., [An update of understanding of the hepatic vascular system and new research strategies], Nan Fang Yi Ke Da Xue Xue Bao 42 (12) (2022) 1907–1911.
[17] K.J. Luzzi, et al., Multistep nature of metastatic inefficiency: dormancy of solitary cells after successful extravasation and limited survival of early micrometastases, Am. J. Pathol. 153 (3) (1998) 865–873.
[18] A. Maitra, Molecular envoys pave the way for pancreatic cancer to invade the liver, Nature 567 (7747) (2019) 181–182.
[19] J. Massagué, A.C. Obenauf, Metastatic colonization by circulating tumour cells, Nature 529 (7586) (2016) 298–306.
[20] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: the next generation, Cell 144 (5) (2011) 646–674.
[21] I. Carr, F.W. Orr, Invasion and metastasis, Can. Med. Assoc. J. 128 (10) (1983) 1164–1167.
[22] D.F. Dwyer, et al., Human airway mast cells proliferate and acquire distinct inflammation-driven phenotypes during type 2 inflammation, Sci Immunol 6 (56) (2021).
[23] A.A. Hamidi, et al., MicroRNAs as the critical regulators of cell migration and invasion in thyroid cancer, Biomark. Res. 10 (1) (2022) 40.
[24] S. Liu, J. Ren, L. Zhang, Ras homolog family member J (RHOJ): a key regulator of chemoresistance associated with epithelial-mesenchymal transition, Signal Transduct. Targeted Ther. 8 (1) (2023) 376.
[25] W. Jia, et al., Purinergic signalling in cancer therapeutic resistance: from mechanisms to targeting strategies, Drug Resist. Updates 70 (2023) 100988.
[26] D. Ribatti, Epithelial-endothelial transition and endothelial-mesenchymal transition, Int. J. Dev. Biol. 66 (4–5-6) (2022) 311–316.
[27] X. Chen, et al., Broadening horizons: the role of ferroptosis in cancer, Nat. Rev. Clin. Oncol. 18 (5) (2021) 280–296.
[28] N. Aceto, et al., Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis, Cell 158 (5) (2014) 1110–1122.
[29] M. Balic, et al., Circulating tumor cells: from bench to bedside, Annu. Rev. Med. 64 (2013) 31–44.
[30] J.M. Hou, et al., Clinical significance and molecular characteristics of circulating tumor cells and circulating tumor microemboli in patients with small-cell lung cancer, J. Clin. Oncol. 30 (5) (2012) 525–532.
[31] X. Cui, et al., Molecular mechanisms and clinical applications of exosomes in prostate cancer, Biomark. Res. 10 (1) (2022) 56.
[32] X. Chen, et al., Augmented EPR effect post IRFA to enhance the therapeutic efficacy of arsenic loaded ZIF-8 nanoparticles on residual HCC progression, J. Nanobiotechnol. 20 (1) (2022) 34.
[33] S.R.V. Knott, et al., Asparagine bioavailability governs metastasis in a model of breast cancer, Nature 554 (7692) (2018) 378–381.
[34] B. Sun, et al., Epithelial-to-endothelial transition and cancer stem cells: two cornerstones of vasculogenic mimicry in malignant tumors, Oncotarget 8 (18) (2017) 30502–30510.
[35] S.L. Friedman, Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver, Physiol. Rev. 88 (1) (2008) 125–172.
[36] Y.L. Jia, et al., Epimorphin promotes human hepatocellular carcinoma invasion and metastasis through activation of focal adhesion kinase/extracellular signal-regulated kinase/matrix metalloproteinase-9 axis, Hepatology 54 (5) (2011) 1808–1818.
[37] L. Dubuisson, et al., Expression and cellular localization of fibrillin-1 in normal and pathological human liver, J. Hepatol. 34 (4) (2001) 514–522.
[38] B. Wang, et al., Comprehensive analysis of metastatic gastric cancer tumour cells using single-cell RNA-seq, Sci. Rep. 11 (1) (2021) 1141.
[39] M. Karaayvaz, et al., Unravelling subclonal heterogeneity and aggressive disease states in TNBC through single-cell RNA-seq, Nat. Commun. 9 (1) (2018) 3588.
[40] D.A. Lawson, et al., Tumour heterogeneity and metastasis at single-cell resolution, Nat. Cell Biol. 20 (12) (2018) 1349–1360.
[41] J. Shi, et al., Advances in targeted therapy against driver mutations and epigenetic alterations in non-small cell lung cancer, Oncologie 24 (4) (2022).
[42] R. Taftaf, et al., ICAM1 initiates CTC cluster formation and trans-endothelial migration in lung metastasis of breast cancer, Nat. Commun. 12 (1) (2021) 4867.
[43] S. Wen, et al., Cancer-associated fibroblast (CAF)-derived IL32 promotes breast cancer cell invasion and metastasis via integrin β3-p38 MAPK signalling, Cancer Lett. 442 (2019) 320–332.
[44] C. Nadal, J. Maurel, P. Gascon, Is there a genetic signature for liver metastasis in colorectal cancer? World J. Gastroenterol. 13 (44) (2007) 5832–5844.
[45] N.J. Birkbak, N. McGranahan, Cancer genome evolutionary trajectories in metastasis, Cancer Cell 37 (1) (2020) 8–19.
[46] D.R. Robinson, et al., Integrative clinical genomics of metastatic cancer, Nature 548 (7667) (2017) 297–303.
[47] K. Ganesh, et al., L1CAM defines the regenerative origin of metastasis-initiating cells in colorectal cancer, Nat. Can. (Ott.) 1 (1) (2020) 28–45.
[48] D. Yang, et al., Intertumoral heterogeneity in SCLC is influenced by the cell type of origin, Cancer Discov. 8 (10) (2018) 1316–1331.
[49] Z. Tao, J. Huang, J. Li, Comprehensive intratumoral heterogeneity landscaping of liver hepatocellular carcinoma and discerning of APLP2 in cancer progression, Environ Toxicol. 39 (2) (2024) 612–625, https://doi.org/10.1002/tox.23904. Epub 2023 Jul 29. PMID: 37515494.
[50] L. Yu, et al., Characterization of cancer-related fibroblasts (CAF) in hepatocellular carcinoma and construction of CAF-based risk signature based on single-cell RNA-seq and bulk RNA-seq data, Front. Immunol. 13 (2022) 1009789.
[51] C. Hafemeister, R. Satija, Normalization and variance stabilization of single-cell RNA-seq data using regularized negative binomial regression, Genome Biol. 20 (1) (2019) 296.
[52] N. Chen, et al., Identification of HBEGF+ fibroblasts in the remission of rheumatoid arthritis by integrating single-cell RNA sequencing datasets and bulk RNA sequencing datasets, Arthritis Res. Ther. 24 (1) (2022) 215.
[53] H. Guo, et al., Molecular typing of gastric cancer based on invasion-related genes and prognosis-related features, Front. Oncol. 12 (2022) 848163.
[54] G. Yu, et al., clusterProfiler: an R package for comparing biological themes among gene clusters, OMICS 16 (5) (2012) 284–287.
[55] Q. Zhang, et al., hTFtarget: a comprehensive database for regulations of human transcription factors and their targets, Dev. Reprod. Biol. 18 (2) (2020) 120–128.
[56] W. Yang, et al., Genomics of Drug Sensitivity in Cancer (GDSC): a resource for therapeutic biomarker discovery in cancer cells, Nucleic Acids Res. 41 (2013) D955–D961. Database issue.
[57] J. Barretina, et al., The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity, Nature 483 (7391) (2012) 603–607.
[58] Z. Tao, et al., MiR-4465 directly targets PTEN to inhibit AKT/mTOR pathway-mediated autophagy, Cell Stress Chaperones 24 (1) (2019) 105–113.
[59] B. Ye, et al., The role of ZNF143 overexpression in rat liver cell proliferation, BMC Genom. 23 (1) (2022) 483.
[60] A. Flores-Pérez, et al., Angiogenesis analysis by in vitro coculture assays in transwell chambers in ovarian cancer, Methods Mol. Biol. 1699 (2018) 179–186.
[61] D. Xu, et al., The gluconeogenic enzyme PCK1 phosphorylates INSIG1/2 for lipogenesis, Nature 580 (7804) (2020) 530–535.
[62] T. Yang, et al., Therapeutic HNF4A mRNA attenuates liver fibrosis in a preclinical model, J. Hepatol. 75 (6) (2021) 1420–1433.
[63] G. Wu, et al., CPS1 expression and its prognostic significance in lung adenocarcinoma, Ann. Transl. Med. 8 (6) (2020) 341.
[64] J.M. Kubala, et al., NDUFA4L2 reduces mitochondrial respiration resulting in defective lysosomal trafficking in clear cell renal cell carcinoma, Cancer Biol. Ther. 24 (1) (2023) 2170669.
[65] W. Guo, et al., G2 and S phase-expressed-1 acts as a putative tumor promoter in cervical cancer by enhancing Wnt/β-catenin signaling via modulation of GSK-3β, Environ. Toxicol. 36 (8) (2021) 1628–1639.
[66] M.J. Whitley, et al., ENTPD1 (CD39) expression inhibits UVR-induced DNA damage repair through purinergic signaling and is associated with metastasis in human cutaneous squamous cell carcinoma, J. Invest. Dermatol. 141 (10) (2021) 2509–2520.
[67] A.J. Levine, M. Oren, The first 30 years of p53: growing ever more complex, Nat. Rev. Cancer 9 (10) (2009) 749–758.
[68] L. Salmena, A. Carracedo, P.P. Pandolfi, Tenets of PTEN tumor suppression, Cell 133 (3) (2008) 403–414.
[69] J. Bainbridge, B. Sivakumar, E. Paleolog, Angiogenesis as a therapeutic target in arthritis: lessons from oncology, Curr. Pharmaceut. Des. 12 (21) (2006) 2631–2644.
[70] M. Egeblad, Z. Werb, New functions for the matrix metalloproteinases in cancer progression, Nat. Rev. Cancer 2 (3) (2002) 161–174.
[71] Y. Lu, et al., CRISPR/Cas9 screens unravel miR-3689a-3p regulating sorafenib resistance in hepatocellular carcinoma via suppressing CCS/SOD1-dependent mitochondrial oxidative stress, Drug Resist. Updates 71 (2023) 101015.
[72] J. Fares, et al., Molecular principles of metastasis: a hallmark of cancer revisited, Signal Transduct. Targeted Ther. 5 (1) (2020) 28.
[73] D.P. Tabassum, K. Polyak, Tumorigenesis: it takes a village, Nat. Rev. Cancer 15 (8) (2015) 473–483.
[74] C. Kim, et al., Chemoresistance evolution in triple-negative breast cancer delineated by single-cell sequencing, Cell 173 (4) (2018) 879–893.e13.

[75] Z. Ma, et al., Interferon-dependent SLC14A1(+) cancer-associated fibroblasts promote cancer stemness via WNT5A in bladder cancer, Cancer Cell 40 (12) (2022) 1550–1565.e7.
[76] D.R. Minton, et al., Role of NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 4-like 2 in clear cell renal cell carcinoma, Clin. Cancer Res. 22 (11) (2016) 2791–2801.
[77] N. Ye, et al., Hypoxia-induced the upregulation of NDUFA4L2 promoted colon adenocarcinoma progression through ROS-mediated PI3K/AKT pathway, Cytotechnology 75 (6) (2023) 461–472.
[78] Z. Chen, et al., NDUFA4L2 promotes glioblastoma progression, is associated with poor survival, and can be effectively targeted by apatinib, Cell Death Dis. 12 (4) (2021) 377.
[79] M. Scolz, et al., GTSE1 is a microtubule plus-end tracking protein that regulates EB1-dependent cell migration, PLoS One 7 (12) (2012) e51259.
[80] X. Wu, et al., GTSE1 promotes cell migration and invasion by regulating EMT in hepatocellular carcinoma and is associated with poor prognosis, Sci. Rep. 7 (1) (2017) 5129.
[81] W. Chen, et al., GTSE1 promotes tumor growth and metastasis by attenuating of KLF4 expression in clear cell renal cell carcinoma, Lab. Invest. 102 (9) (2022) 1011–1022.
[82] V.V. Subhash, et al., GTSE1 expression represses apoptotic signaling and confers cisplatin resistance in gastric cancer cells, BMC Cancer 15 (2015) 550.
[83] L. Feng, et al., Vascular CD39/ENTPD1 directly promotes tumor cell growth by scavenging extracellular adenosine triphosphate, Neoplasia 13 (3) (2011) 206–216.
[84] M. Mesuraca, et al., ZNF423 and ZNF521: EBF1 antagonists of potential relevance in B-lymphoid malignancies, BioMed Res. Int. 2015 (2015) 165238.
[85] Y. Cheng, Y.J. Ni, L.M. Tang, ZNF521/EBF1 axis regulates AKR1B1 to promote the proliferation, migration, and invasion of gastric cancer cells, Kaohsiung J. Med. Sci. 39 (3) (2023) 244–253.
[86] H. Kikuchi, et al., EBF1 acts as a powerful repressor of Blimp-1 gene expression in immature B cells, Biochem. Biophys. Res. Commun. 422 (4) (2012) 780–785.
[87] Z. Qiu, et al., EBF1 promotes triple-negative breast cancer progression by surveillance of the HIF1α pathway, Proc. Natl. Acad. Sci. U. S. A. 119 (28) (2022) e2119518119.
[88] D. Yan, et al., Exploration of combinational therapeutic strategies for HCC based on TCGA HCC database, Oncologie 24 (1) (2022) 101–111.