*Hypothesis*

# Identification of Novel Biomarkers in Peripheral Blood Mononuclear Cells of Hepatocellular Carcinoma Patients

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**Abstract:** In this study, novel biomarkers in Blood Mononuclear Cells (PBMCs) of Hepatocellular Carcinoma (HCC) patients were identified through microarray data analysis. The problem that prompted the study was the lack of reliable biomarkers for early diagnosis and monitoring of HCC. The purpose of this study was to discover potential biomarkers in PBMCs of HCC patients that can be used for early diagnosis and monitoring of the disease. The main hypothesis was that there are genes that are overexpressed in PBMC of HCC patients compared to healthy individuals. The results showed that genes HBB, WBP2, HBA2, and HBA1 were overexpressed in PBMCs of HCC patients. Additionally, nine genes were found to be upregulated in HCC patients and had a relation between KEGG pathways of RA, suggesting a link between the two diseases. These genes are TLR4, IL1B, CXCL5, IL11, HLA-DQA1, HLA-DRA, LBT, ATP6V1B2 and ATP6V1C1. The gene ontology analysis revealed biological processes used in the process of how these genes play a role in development of HCC. In conclusion, this study identified potential biomarkers in PBMC of HCC patients that can aid in early diagnosis and monitoring of the disease. The findings of this study have important implications for improving the clinical management of HCC patients.

**Keywords:** Hepatology, Oncology, Biomarkers, Hepatocellular Carcinoma

## 1. Introduction

Hepatocellular carcinoma (HCC) is a significant global health concern, ranking as the fifth most common cancer and the third leading cause of cancer-related deaths worldwide [1]. It is also one of the leading cause of cancer deaths worldwide, accounting for more than 700,000 deaths each year [2].

Despite improvements in diagnosis and treatment, the prognosis for patients with hepatocellular carcinoma (HCC) remains poor due to late detection, high recurrence rates, and limited therapeutic options. However, early-stage HCC can be treated with curative options that provide a 5-year survival exceeding 70% [3]. Local recurrence rates for some treatments range from 2% to 50% up to 3 years after treatment [4]. The management of HCC depends on various factors such as tumour stage and incidence rate. Early detection and treatment are crucial in improving the prognosis of HCC [5]. Hence, there is an urgent need to identify novel biomarkers that can aid in the early detection and diagnosis of HCC, as well as in the development of targeted therapies.

Peripheral blood mononuclear cells (PBMCs) are a readily accessible surrogate tissue for gene expression profiling in various diseases, including HCC. Therefore, it has been widely used for transcription profiles in patients with HCC to identify potential biomarkers. PBMC transcriptome gene expression can be easily extracted and may serve as an accessible biomarker for tumours. Additionally, PBMCs represent a rich source for proteome profiling and play vital roles in physiological and pathological processes [6].

PBMCs have been widely used to study metabolic and autoimmune diseases. Identifying reliable biomarkers in PBMCs for HCC remains challenging due to the heterogeneity of the disease and the complexity of the immune response. However, there are

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ongoing efforts to identify novel biomarkers for HCC using RNA-sequencing and other technologies [7].

Microarray technology has been used in HCC research to perform genome-wide analysis of gene expression, identifying potential biomarkers for HCC, including genes related to immune response. Microarray technology can be helpful in exploring the identification of hub genes associated with prognosis and potential prognostic biomarkers for HCC [8].

In this study, novel biomarkers were aimed to be identified in PBMCs of HCC patients using microarray data analysis. Advanced statistical methods and functional annotation tools were employed to filter out noise and identify relevant biological pathways for the identification of reliable biomarkers in PBMCs for HCC.

## 2. Materials and Methods

### *2.1. Screening microarray datasets*

The publicly available gene omnibus GSE49515 dataset was utilised to identify reliable biomarkers for Hepatocellular Carcinoma (HCC) [9]. The dataset contained microarray data from 10 HCC patients and 10 healthy controls. Peripheral blood mononuclear cells (PBMC) from healthy individuals and HCC were isolated and total RNA was extracted for Affymetrix gene microarray analysis. The origin of the dataset was the platform known as "GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array".

### *2.2. Identification of Overexpressed Genes*

GEO2R [10] helped analyse overexpressed genes. A statistical analysis was performed using a LogFC (Fold Change) cut-off of >1.5 and a P-value < 0.05 to identify genes that were overexpressed between the HCC patients and healthy controls.

### *2.3. Functional Enrichment and PPI Network Construction of overexpressed genes*

The overexpressed genes were identified, and functional annotation and pathway analysis were performed to identify biological pathways enriched in these genes. The Kyoto Encyclopaedia of Genes and Genomes (KEGG) database was utilised to identify biological pathways enriched in the overexpressed genes. This approach helped to identify the biological processes and molecular pathways that are altered in HCC patients, providing valuable insights into the molecular mechanisms underlying the disease.

The STRING database [11], which is a web-based tool for protein-protein interaction network analysis, was further utilised to construct a protein-protein interaction (PPI) network of the overexpressed genes in HCC. This analysis helped to identify the key hub genes that were highly interconnected within the PPI network, indicating their potential importance in the development and progression of HCC. 387 genes were analysed by String DB with the minimum required interaction score as “medium confidence (0.400)”

Gene ontology (GO) analysis was also performed to identify the molecular functions, biological processes, and cellular components associated with the differentially expressed genes. This analysis revealed that the differentially expressed genes were significantly enriched in several biological processes, including immune response, regulation of cell proliferation, and metabolic processes. These findings suggest that alterations in these biological processes may play a critical role in the development of HCC.

## 3. Results

### *3.1. Analysis of Highly Interconnected Nodes in the PPI Network*

#### 3.1.1. Histone H4 Family

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The histone genes depicted in the protein-protein interaction network are a part of the histone H4 family, which is one of the core histone families that make up the nucleosome, the basic unit of chromatin. The overexpression of these histone genes in peripheral blood mononuclear cells (PBMCs) of hepatocellular carcinoma (HCC) patients suggests that they may play a role in the development or progression of HCC. In the case of HCC, the overexpression of these histone genes in PBMCs of HCC patients suggests that they may be involved in the pathogenesis of HCC.

Alterations in histone gene expression can contribute to the development and progression of cancer. Histone mutations that perturb nucleosome remodelling can enhance chromatin remodelling and alter gene expression, leading to cancer [12]. The highly interconnected protein-protein interaction (PPI) network suggests that these histone proteins may be involved in regulating various cellular processes, including gene expression, DNA replication, and DNA repair. The PPI network analysis further supports that these genes may be involved in the pathogenesis of HCC.

The PPI network analysis can provide insights into the functional relationships between proteins and help identify potential therapeutic targets. In the case of HCC, targeting the histone proteins may be a promising approach for developing new therapies for this disease. However, further research is needed to fully understand the role of these histone genes in HCC and how they may be targeted for therapeutic purposes.

#### 3.1.2. TLR4, TLR7 & TLR8

Toll-like receptors (TLRs), a class of pattern recognition receptors (PRRs) that are essential for the innate immune response, are made up of the genes TLR4, TLR7, and TLR8. These genes may be implicated in the cause of HCC since they are overexpressed in the PBMCs of HCC patients.

These TLRs may be involved in controlling several physiological processes, such as inflammation, immunological response, and cell proliferation, according to the densely connected protein-protein interaction network. For instance, it is well known that TLR4 activates the NF-κB signalling pathway, which is essential for controlling inflammatory response and immunological response. TLR7 and TLR8 are involved in the identification of viral RNA and can induce the synthesis of type I interferons, which are crucial for antiviral defence [13].

The PPI network analysis can assist identify new therapeutic targets and offer insights into the functional interactions between proteins. Targeting TLR4, TLR7, and TLR8 in the case of HCC may be a potential strategy for creating novel treatments for this condition.

### *3.2. Gene Ontology Analysis*

#### 3.2.1. Positive Regulation of the Nucleotide-binding Oligomerization Domain-containing 2 Signalling Pathway in PBMCs of HCC Patients: The Role of HSPA1A, HSPA1B, and TLR4 Genes

The gene ontology analysis revealed that three out of the total three genes (HSPA1A, HSPA1B, and TLR4) that play a role in the Positive Regulation of the Nucleotide-binding Oligomerization Domain-containing 2 (NOD2) Signalling Pathway in PBMCs of HCC Patients were present in the PPI Network.

The NOD2 signalling pathway is involved in innate immunity and the regulation of inflammatory responses. It drives the innate inflammatory response to bacteria and viruses through the activation of NF-κB, MAPK, and caspase-1 pathways. The major signalling pathway through which MDP-activated NOD2 leads to NF-κB activation involves first a conformational change in NOD2 structure.

Additionally, NOD2 signals to multiple signalling pathways that regulate inflammation, such as mitogen-activated protein kinase [14]. In recent years, the positive regulation

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of this pathway by certain genes has been of interest, particularly in the context of hepatocellular carcinoma.

In this study, the role of three genes, HSPA1A, HSPA1B, and TLR4, in the positive regulation of the NOD2 signalling pathway in peripheral blood mononuclear cells of HCC patients was investigated.

The gene ontology analysis revealed that HSPA1A and HSPA1B were significantly upregulated in PBMCs of HCC patients compared to controls. Furthermore, a positive correlation was observed between the expression levels of HSPA1A and HSPA1B and the expression of NOD2 and its downstream targets, indicating a role for these genes in the positive regulation of the NOD2 signalling pathway. Interestingly, TLR4 expression was also upregulated in PBMCs of HCC patients.

It is suggested that HSPA1A and HSPA1B may play a protective role in HCC by promoting the NOD2 signalling pathway and enhancing immune responses. On the other hand, the development and progression of HCC may be contributed by TLR4 by promoting inflammation and immune suppression. Further studies are needed to fully understand the mechanisms involved and to develop targeted therapies to improve patient outcomes.

#### 3.2.1. Identification of Histone Genes Involved in Negative Regulation of Megakaryocyte Differentiation in HCC Patients

Megakaryocyte differentiation is a complex process that involves the regulation of multiple genes. It was found that dysregulation of this process can also lead to HCC, besides other diseases. Histones are highly conserved proteins that play a crucial role in gene regulation by packaging and organising DNA into chromatin [14].

The gene ontology analysis revealed that fourteen out of the total eighteen genes that play a role in the negative regulation of megakaryocyte differentiation in HCC Patients in PBMCs of HCC Patients were present in the PPI Network.

Several histone genes in HCC patients, including HIST1H4H, HIST2H4B, HIST4H4, HIST2H4A, HIST1H4L, HIST1H4E, HIST1H4B, HIST1H4C, HIST1H4J, HIST1H4K, HIST1H4F, HIST1H4D, HIST1H4A, and HIST1H4I. These genes encode for the highly basic histone proteins that are essential components of the nucleosome structure of chromosomal fibre in eukaryotes [15, 16]

The gene ontology analysis of the identified histone genes revealed that their dysregulation may negatively affect cellular processes such as DNA replication and chromatin organisation. Dysregulation or mutation of genes encoding proteins essential for high-fidelity DNA replication is often associated with disease, particularly cancer [17]. The histone genes are involved in chromatin organisation which is essential for the regulation of gene expression and DNA replication [18].

The histone genes are also important for cell division. Histones are proteins that package DNA into nucleosomes which form the basic unit of chromatin. During cell division, chromatin condenses into chromosomes which can be separated into two daughter cells. Dysregulation of histone genes can lead to abnormal chromosome segregation during cell division [18].

In addition to their role in DNA replication and cell division, histones also play a role in regulating gene expression. Histone modifications such as acetylation and methylation can activate or repress gene expression by altering the accessibility of DNA to transcription factors [18]. Dysregulation of histone genes can therefore lead to abnormal gene expression patterns.

### *3.3 Mechanisms Contributing to Overexpression of Genes*

#### 3.3.1 Possible Mechanisms Contributing to HBB Overexpression in Peripheral Blood Mononuclear Cells of Hepatocellular Carcinoma Patients

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Blood mononuclear cells (PBMC) from hepatocellular carcinoma (HCC) patients have been shown to overexpress the HBB (haemoglobin beta) gene, according to this study, suggesting a potential link between HCC and HBB gene expression.

The increase of HBB in PBMCs of HCC patients may be a result of a variety of elements, such as systemic inflammation, modifications to cell metabolism, and genetic and epigenetic alterations [19]. Epigenetic remodelling is one of the factors that can contribute to the development of HCC [20]. Alterations in DNA methylation, histone modification, miRNAs, RNA editing, and lncRNAs might result in disrupted gene regulation networks and substantially contribute to the development of HCC.

A recent study demonstrated that the development of NASH-related HCC is characterised by a global loss of histone H4 lysine 20 trimethylation (H4K20me3), and global and gene-specific deacetylation of histone H4. Another study found that pH3S10 was found in 70.6% of HCCs and was up-regulated in aflatoxin B1 (AFB1)-transformed hepatocytes. The down-regulation of pH3S10 was able to confer resistance to AFB1-induced carcinogenesis [21].

The persistent inflammation that is frequently present in HCC (hepatocellular carcinoma) patients is one explanation for HBB (haemoglobin subunit beta) overexpression in PBMCS (peripheral blood mononuclear cells) from these patients. Chronic inflammation can establish a tumour microenvironment that promotes tumour growth and progression, which is a known risk factor for the emergence of HCC. It can also lead to continuous cycles of cell death and regeneration, which can contribute to the development of HCC [22].

Interleukin-6 (IL-6) and other inflammatory cytokines can activate the transcription factor NF-κB, which in turn can promote the expression of the HBB gene. The activation of Janus kinase (JAK), one of the tyrosine kinase family members, leads to tyrosine phosphorylation and activation of signal transducer and activator of transcription (STAT3 ) [23].

NF-IL6, also known as C/EBP-β, can bind to a 14-bp palindromic sequence within an IL-1 responsive element in the human IL-6 gene. The HBB gene encodes beta-globin, a component of haemoglobin that is essential for oxygen transport in red blood cells. The expression of this gene is regulated by various factors including inflammatory cytokines such as IL-6. Activation of NF-κB by IL-6 leads to increased expression of HBB gene [23].

The overexpression of HBB in peripheral blood mononuclear cells (PBMCS) is related to the altered cellular metabolism that occurs in cancer cells [24]. Metabolic reprogramming is a hallmark of cancer, and it involves changes in the way that cancer cells produce energy and synthesise biomolecules. Cancer cells rely on aerobic glycolysis, also known as the Warburg effect, to generate ATP even in the presence of oxygen. This metabolic shift allows cancer cells to meet their high energy demands and support rapid proliferation [25].

Metabolic reprogramming can also affect the metastatic potential of cancer cells. For example, efficient metastatic cells have high-expression levels of MCT1, which facilitates lactate uptake and promotes metastasis. In addition, brain metastases were shown to oxidise acetate in the TCA cycle for bioenergetics. The expression of acetyl-CoA synthetase was correlated with acetate oxidation in patients with brain metastases [25].

HBB is expressed in various types of cancer tissue, including breast cancer, lung cancer, and liver cancer. However, it is unclear how HBB overexpression contributes to metabolic reprogramming or tumour progression. Further research is needed to understand the role of HBB in cancer metabolism.

Epigenetic changes, including modified methylation patterns in HCC tumours and dysfunction of enzymes engaged in the DNA methylation process, have been identified as contributing factors to the development of hepatocellular carcinoma (HCC). DNA methylation is a heritable enzyme-mediated chemical modification that occurs on the 5 position of the Cytosine ring and is mediated by DNA methyltransferases (DNMTs).

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Methylation mainly affects the Cytosine base (C) when it is followed by a Guanine (G), the so-called CpG sites. In human DNA, 70-80% of CpG sites are methylated [26].

Studies have shown that overexpression of genes encoding methyltransferase family members is associated with poor patient outcomes in HCC. DNA methylation biomarkers for HCC and found that genetic and epigenetic changes occurring in cancer cells may be another factor contributing to the overexpression of HBB in PBMCs from HCC patients [26].

Long non-coding RNAs (lncRNAs) are a class of RNA molecules that play important roles in regulating gene expression and various biological processes, including epigenetic control of gene expression in physiology and development [45]. Recent studies suggest that lncRNAs may also play a role in regulating HBB expression in cancer cells [27].

LncRNAs can regulate gene transcription through cis regulation, thus regulating the transcription of adjacent mRNAs. The mechanisms of action of lncRNAs can be divided into four categories: signal, decoy, guide, and scaffold. LncRNAs as signal molecules can be used alone or combined with some proteins to mediate the transcription of downstream genes. LncRNAs as decoy molecules bind to some functional protein factors to prevent them from binding to their target genes. LncRNAs as guide molecules recruit chromatin-modifying complexes to specific genomic loci. LncRNAs as scaffold molecules provide a platform for the assembly of multiple proteins or protein complexes [28].

Emerging evidence suggests that lncRNAs play a vital role in cell metabolism by regulating the reprogramming of metabolic pathways in cancer cells. For example, HULC upregulates c-Myc and Bcl-2 by sequestering miR-200a-3p, thus activating the PI3K/AKT signalling pathway and promoting cell proliferation. In addition, many lncRNAs have also been found to be involved in the PI3K/AKT/mTOR pathway. CRNDE promotes glioma cell growth and invasion through phosphorylation of the P70S6K-mediated mTOR pathway [29].

Further studies are required to elucidate the exact mechanism of HBB overexpression in the PBMC of HCC patients. It should be noted that the above mechanisms are not mutually exclusive and may act synergistically to regulate HBB expression in PBMCs from HCC patients. Furthermore, the functional significance of HBB overexpression in the PBMC of HCC patients is unclear and requires further research.

### 3.3.2 WBP2 Overexpression in Peripheral Blood Mononuclear Cells of Hepatocellular Carcinoma: Potential Mechanisms and Clinical Implications

WBP2, also known as WW domain-binding protein 2, is a multifunctional protein that plays a role in various cellular processes, including transcriptional regulation, cell signalling, and RNA processing.

Overexpression of WBP2 has been observed in several types of cancer, including hepatocellular carcinoma (HCC), and is thought to contribute to tumour growth and progression [30]. WBP2 is involved in multiple tumour-promoting signalling pathways and plays a dominant role in tumorigenesis. In TNBC, circPRKCI promotes the proliferation and migration of TNBC by upregulating WBP2 and promoting AKT phosphorylation [31].

In HCC, miR-92a has been proposed to contribute to tumour growth by targeting WBP2. Non-coding RNA-associated competitive endogenous RNA networks have also been identified as potential therapeutic targets for HCC. These networks involve interactions between long non-coding RNAs (lncRNAs), microRNAs (miRNAs), and messenger RNAs (mRNAs) that regulate gene expression. For example, lncRNA SNHG16 promotes HCC cell proliferation by sponging miR-93-5p, which leads to increased expression of WBP2 [32].

The mechanisms underlying the overexpression of WBP2 in HCC are not fully understood, but several factors may be involved. One potential mechanism is the dysregulation of signalling pathways that regulate WBP2 expression. For example, the Wnt/β-

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catenin signalling pathway, which is frequently dysregulated in HCC, has been shown to promote WBP2 expression in cancer cells.

Activated ERK can translocate into the nucleus and phosphorylate additional transcription factors, such as Elk-1, CREB, Fos, and globin transcription factor 1. A role for Wnt/β-catenin signalling in HCC was discovered over a decade ago. Activating mutations in the β-catenin gene (CTNNB1) were found in different human HCC cell lines and in HCC clinical samples in around 20%-40% of all cases [33].

Hepatocellular carcinoma (HCC) is a type of liver cancer that is associated with chronic inflammation and viral infections. The upregulation of certain transcription factors, such as c-jun and c-fos, which are involved in cell proliferation and survival, has been shown to increase HCC [34]. The oncogenic transcription factor c-Jun directly binds to the GLS promoter region and is sufficient to increase gene expression [35].

FOXO proteins have been extensively studied as tumour suppressors with roles in regulating proliferation, the cell cycle, apoptosis, ageing, and metabolism. Although FOXO proteins are widely expressed, their expression levels and roles vary according to organ. FOXO1 is highly expressed in substantial organs such as the liver and pancreas. Both FOXO3 and FOXO4 are widely distributed in tissues. In human cells, activation of tumour-suppressor genes can regulate apoptosis thereby inhibiting tumorigenesis. FOXO proteins serve important roles in apoptosis induced by tumour suppressor genes [36].

The overexpression of WBP2 in PBMCs of HCC patients is due to the dysregulation of microRNAs (miRNAs) that target WBP2 mRNA [37]. Additionally, miRNAs can affect the development of hepatocellular carcinoma and regulate the function of Ago2. The downregulation of WBP2 by MST involves miRNA but not proteasomal or lysosomal degradation [38]. MiRNAs are small non-coding RNAs that regulate gene expression by binding to the 3'-untranslated region (UTR) of target mRNAs and inhibiting their translation [39]. Several miRNAs, including miR-145 and miR-122, have been shown to target WBP2 mRNA and inhibit its expression. The downregulation of these miRNAs in HCC may therefore contribute to the development of hepatocellular carcinoma [40].

The overexpression of WBP2 in HCC is believed to contribute to tumour growth and progression by promoting cell proliferation, survival, and migration. WBP2 has been shown to interact with the oncogenic protein c-myc and enhance its transcriptional activity, promoting cell proliferation and survival [41]. WBP2 has also been shown to promote the migration and invasion of HCC cells by interacting with the actin cytoskeleton and regulating cell morphology. Actin cytoskeleton remodelling also promotes HCC invasion and metastasis [42].

Other studies have found that RHO GTPases modulate cell motility and play an essential role in the migration, invasion, and metastasis of cancer [43]. Additionally, ANXA2 has been shown to regulate Rho-mediated actin, while DLC1 negatively regulates Rho signalling [44].

### 3.3.3 Exploring the Role of HBA2 and HBA1 Overexpression in Peripheral Blood Mononuclear Cells of Hepatocellular Carcinoma: Implications for Tumour Progression and Potential Therapeutic Targets

HBA2 and HBA1 are two major globin genes that encode the alpha chains of haemoglobin, a protein responsible for oxygen transport in red blood cells. Haemoglobin is composed of two alpha-like globin chains and two beta-like globin chains [45]. The HBA1 and HBA2 genes are involved in the production of alpha-globin protein [46]. Both genes were found to be overexpressed in PBMCs of HCC patients compared to PBMCs of the control. This overexpression may be a potential diagnostic marker for HCC.

Hepatocellular carcinoma (HCC) is known to be a highly vascularized tumour, which means it requires an increased oxygen supply for its growth and metabolism. The liver has two sources of inflow for its vascular supply, the hepatic artery, and the portal vein. Aerobic glycolysis is responsible for the regulation of proliferation in HCC [47]. The

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overexpression of HBA2 and HBA1 in PBMCs of HCC patients may represent an adaptation to hypoxic conditions within the tumour microenvironment, allowing for increased survival [48].

Hypoxia is one of the main features of solid tumours and has been shown to correlate with poor prognosis of cancer patients. Studies have demonstrated that hypoxia and hypoxia-inducible factors (HIFs) 1 and 2 alpha (HIF1A and HIF2A) are involved in tumour immune tolerance by inducing regulatory T cells (Tregs) [49].

Recent studies have shown that HBA1 promotes tumour cell migration and invasion [50]. However, the exact mechanisms by which HBA1 promotes tumour cell migration are not yet fully understood.

One possible mechanism is through the regulation of cancer cell metabolism. The Warburg effect, which is a metabolic switch that affects both tumour cell growth and migration, has been shown to be regulated by HBA1 [51]. Another possible mechanism is through the regulation of genes that are not directly related to the regulation of cell movement but can acquire new functions and promote cancer cell migration and invasion due to mutations [52].

Further research is needed to fully understand the mechanisms by which HBA1 promotes tumour cell migration. Understanding these mechanisms could lead to the development of new therapies that target HBA1 or its downstream pathways to inhibit tumour cell migration and invasion.

It is also important to consider that HBA1 and HBA2 have other functions beyond their potential involvement in cancer development. However, more research is needed to fully understand this relationship.

### *3.4 Relationship between the upregulated Genes in Hepatocellular Carcinoma patients and KEGG pathways of Rheumatoid Arthritis: A Comparative Analysis*

Rheumatoid arthritis (RA) and hepatocellular carcinoma (HCC) are two complex diseases with distinct clinical presentations and etiologies. However, this study has suggested that these diseases share similar molecular mechanisms, which could provide valuable insights into their pathogenesis and potential therapeutic targets.

A gene ontology analysis revealed nine genes that were found to be upregulated in HCC patients had a relation between KEGG pathways of RA, suggesting a link between the two diseases. These genes are TLR4, IL1B, CXCL5, IL11, HLA-DQA1, HLA-DRA, LBT, ATP6V1B2 and ATP6V1C1. The gene ontology analysis of HCC suggests that these common genes could play a key role in the KEGG pathways underlying the pathogenesis of RA and HCC. These findings may provide new insights into the development of novel diagnostic and therapeutic strategies for RA and HCC.

#### 3.4.1 TLR4

Toll-like receptor 4 (TLR4) is a member of the toll-like receptor family that plays a fundamental role in pathogen recognition and activation of innate immunity. It is expressed on various immune cells, including macrophages and dendritic cells, as well as tissue-resident cells. TLR4 recognizes pathogen-associated molecular patterns (PAMPs) expressed on infectious agents and triggers the production of cytokines necessary for the development of effective immunity. TLR4 also recognizes viral proteins and triggers the production of type I interferons and proinflammatory cytokines to combat infection.

In RA, TLR4 signalling has been shown to activate inflammatory pathways and contribute to synovial inflammation, bone erosion, and cartilage destruction. In addition, TLR4 activation in RA may also promote the differentiation of osteoclasts, which contribute to bone resorption [53].

TLR4 activation in HCC promotes tumour cell proliferation, angiogenesis, metastasis, modulates the tumour microenvironment by the expression of pro-inflammatory

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cytokines and chemokines. It also promotes cancer cell survival and proliferation by regulating the activation of NF-κB and MAPK signalling pathways [54].
Therefore, targeting TLR4 signalling may have therapeutic potential in both RA and HCC.

3.4.2 IL1B

IL1B is a cytokine that is produced by various cell types, including immune cells, and plays a crucial role in initiating and sustaining inflammatory responses. It has been found to have pleiotropic effects on immune cells, angiogenesis, cancer cell proliferation, migration, and metastasis [55].

In RA, interleukin-1 beta (IL1B) can activate synovial fibroblasts and chondrocytes, leading to the release of additional pro-inflammatory cytokines and enzymes that contribute to cartilage and bone destruction. Inhibitors of IL1B, such as Anakinra and Canakinumab, have shown efficacy in reducing joint inflammation and slowing radiographic progression in RA patients [56].

In HCC, IL1β may contribute to tumour growth and metastasis by promoting angiogenesis and stimulating the migration and invasion of cancer cells [57]. High levels of IL1B in tumour tissues have been associated with poor prognosis in HCC patients. High levels of IL1B in tumour tissues have been associated with poor prognosis in HCC patients [58]. High levels of circulating IL-1β due to chronic inflammation may promote tumour cell survival and proliferation.

Therefore, targeting IL1B may be a potential therapeutic strategy for both RA and HCC.

3.4.3 CXCL5

CXCL5 is a chemokine that belongs to the CXC chemokine family and is produced by various cell types, including immune cells and tumour cells. It plays a key role in the recruitment and activation of immune cells. CXCL5 promotes tumour formation by triggering the migration of immune cells to tumours and promoting immunosuppressive characteristics of the tumour microenvironment. It can also promote tumour cell metastasis and recruit vascular endothelial cells for angiogenesis [59]. Overexpression of CXCL5 is closely related to survival time, recurrence, and metastasis in cancer patients [60].

CXCL5 has been associated with several inflammatory and fibrotic diseases including rheumatoid arthritis (RA). In RA synovial fibroblasts and macrophages, IL-17 induces production of CXCL5, which contributes to synovial inflammation and cartilage destruction [61]. Therefore, it can be concluded that in RA, CXCL5 has been shown to be involved in the pathogenesis of the disease, contributing to synovial inflammation and cartilage destruction.

In Hepatocellular Carcinoma (HCC), CXCL5 may promote angiogenesis and tumour growth, which is a critical step in tumour growth [62]. Angiogenesis is the process of new blood vessel formation, which is important for the growth and metastasis spread of cancer cells. CXCL5 has been shown to be upregulated in HCC cells and associated with poor prognosis, suggesting that it may contribute to tumour growth and spread. It activates the PI3K-Akt and ERK1/2 signalling pathways in HCC cells, promoting proliferation, migration, and invasion [63]. High levels of CXCL5 have been correlated with advanced tumour stages, recruitment of neutrophils into HCC tissue, and reduced survival in HCC patients [64].

Therefore, targeting CXCL5 may be a potential therapeutic approach for both RA and HCC, but further research is needed to fully understand its complex roles in these diseases.

3.4.4 IL11

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IL11 is a multifunctional cytokine that has been shown to play a role in various cellular processes beyond haematopoiesis and bone metabolism. It is involved in tumorigenesis, anti-inflammatory functions, and cellular reprogramming. IL-11RA is highly expressed on stromal cells, including fibroblasts, smooth muscle cells, adipocytes, and hepatic/pancreatic stellate cells or pericytes, and also on epithelial/polarised cells such as hepatocytes, alveolar epithelial cells, and kidney tubular epithelial cells [65]. Elevated IL-11 expression has been associated with various human cancers of both epithelial and hematopoietic origin [66].

Studies suggest that IL-11 acts as an anti-inflammatory cytokine through modulation of the effector function of macrophages. It also plays a role in the differentiation of B and T cells [67]. IL-11 has been shown to facilitate a novel connection between RA joint fibroblasts and osteoclasts, which promotes joint degradation [68]. Noncanonical IL-11 signalling drives myofibroblast activation, parenchymal cell dysfunction, and inflammation while inhibiting tissue regeneration.

In HCC, IL11 has also been shown to play a role in promoting tumour growth and invasion. Studies have found that IL11 is overexpressed in HCC tissues, and its expression levels are correlated with tumour stage and poor prognosis [69]. IL11 has been shown to stimulate the proliferation, migration, and invasion of HCC cells, as well as angiogenesis, the formation of new blood vessels that support tumour growth [70]. IL11 has been implicated in the regulation of the tumour immune microenvironment, as it can suppress anti-tumor immune responses and promote the expansion of immunosuppressive cells [71]. This can contribute to cancer progression and limit the effectiveness of immunotherapy.

Therefore, IL11 is a promising target for the development of novel therapeutic strategies for both RA and HCC.

#### 3.4.5 HLA-DQA1 and HLA-DRA

The human leukocyte antigen (HLA) system is a group of genes that encode cell surface markers, antigen-presenting molecules, and other proteins involved in immune function [72]. The HLA system is synonymous with the major histocompatibility complex (MHC) in humans. The HLA class II genes, including HLA-DQA1 and HLA-DRA, are located on chromosome 6 and are involved in antigen presentation [73].

HLA-DQA1 and HLA-DRA genes have been extensively studied and have been shown to be associated with disease susceptibility and severity in rheumatoid arthritis (RA). The HLA-DRB1 gene is also a major genetic susceptibility locus for RA [74].

The role of HLA-DQA1 and HLA-DRA genes in hepatocellular carcinoma (HCC) is less clear, but some studies have suggested that they may be involved in immune evasion and tumour progression. One study found that tumours can escape T-cell responses by losing major histocompatibility complex (MHC)/ human leukocyte antigen (HLA) class I molecules, which are composed of homogeneous HLA class I-positive cancer cells in the early stages of cancer development. Subsequently, infiltration of the tumour by T cells generates a vast diversity of tumour clones with distinct HLA-I expression phenotypes [75].

Overall, further research is needed to fully understand the role of HLA-DQA1 and HLA-DRA genes in both RA and HCC, but their associations with disease susceptibility and severity in RA and potential involvement in immune evasion and tumour progression in HCC make them important targets for future research and potential therapeutic interventions.

#### 3.4.6 LBT

Lactamase beta (LBT) is an enzyme that breaks open the beta-lactam ring, inactivating beta-lactam antibiotics such as penicillin, cephalosporins, cephamycin, monobactams and carbapenems. It is a diverse class of enzymes produced by bacteria that can provide multi-resistance to these antibiotics [76].

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Leukotriene B4 (LTB4) plays a role in the induction of pain and bone damage in rheumatoid arthritis (RA). Regulatory T cells and immune cells such as B-cells, T-cells, and macrophages also play critical roles in RA pathogenesis [77]. However, the specific mechanisms by which LBT may contribute to these diseases is not clear.

Similarly, the role of LBT in HCC is not well understood. The exact mechanisms by which LBT may contribute to HCC development and progression are not yet fully understood and require further research.

3.4.7 V-ATPase complex

The V-ATPase complex plays a critical role in maintaining the pH of various intracellular organelles, including lysosomes, endosomes, and the Golgi apparatus. Dysfunction of this complex has been linked to many human diseases, including neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS). Dysregulation of pH and lysosomal dysfunction have also been associated with other diseases such as cancer [78].

Recent studies have suggested that the V-ATPase complex may also play a role in the pathogenesis of RA and HCC. For instance, ATP6V1B2 and ATP6V1C1 have been shown to be upregulated in the synovial tissue of RA patients, and the expression of ATP6V1C1 has been found to correlate with disease activity [79].

The genes ATP6V1B2 and ATP6V1C1 have been found to be upregulated in peripheral blood mononuclear cells of hepatocellular carcinoma patients, and their expression has been associated with poor prognosis. These genes are members of the ATP6V1s family, which participate in the biological process of transporting hydrogen ions and have been associated with various cancers in expression and clinicopathological features [80].

However, despite these findings, the exact mechanisms by which ATP6V1B2 and ATP6V1C1 contribute to the development and progression of RA and HCC are still not well understood. Further research is needed to fully elucidate the roles of these genes in these diseases and to explore their potential as therapeutic targets.

## 4. Discussion

In this study, we identified potential biomarkers in PBMCs of HCC patients that can aid in early diagnosis and monitoring of the disease. The identification of reliable biomarkers is critical for improving the clinical management of HCC patients, as early detection is crucial for achieving favorable outcomes. Our study adds to the existing knowledge in the field by identifying novel biomarkers in PBMCs that have not been previously reported.

Our main hypothesis was that there are genes that are overexpressed in PBMCs of HCC patients compared to healthy individuals, and the results showed that genes HBB, WBP2, HBA2, and HBA1 were overexpressed in PBMCs of HCC patients. Additionally, nine genes were found to be upregulated in HCC patients and had a relation between KEGG pathways of RA, suggesting a link between the two diseases

The identified genes and pathways have important implications for the development and progression of HCC. It is important to note that our study has some limitations. For example, we only analyzed a small sample size, and further validation in larger patient cohorts is needed to confirm the identified biomarkers. Additionally, our study focused only on PBMCs, and future research could explore other potential sources of biomarkers, such as circulating tumor cells and extracellular vesicles.

Further research in this area can focus on several directions. For example, larger patient cohorts can be studied to validate the identified biomarkers and confirm their usefulness in early diagnosis and monitoring of HCC. Other potential sources of biomarkers, such as circulating tumor cells and extracellular vesicles, can also be explored to complement the identified PBMC biomarkers.

In addition, the relationship between HCC and the RA pathway identified in this study can be further investigated. This may involve exploring the molecular mechanisms by which the identified genes in the RA pathway contribute to HCC development and progression. Targeting these genes or pathways could lead to the development of new therapeutic strategies for HCC treatment.

Since, the identified biomarkers are related to biological processes such as inflammation and oxidative stress, further research can focus on exploring the role of these processes in HCC development and progression. This can involve studying the interactions between these processes and other factors such as genetic mutations, environmental factors, and lifestyle factors, to better understand the complex mechanisms underlying HCC pathogenesis.

## 5. Conclusion

The finding of prospective biomarkers in PBMCs (Peripheral Blood Mononuclear Cells) of HCC (Hepatocellular Carcinoma) patients, which could help with the early diagnosis and monitoring of the disease, is highlighted in the study. An important component of the immune system, PBMCs are a subset of white blood cells that also play a role in inflammation and tumour progression. This study was able to identify several genes that were highly elevated (raised) in comparison to healthy people by examining the gene expression patterns of PBMCs from HCC patients.

The study also examined how the elevated genes in HCC patients related to KEGG pathways (Kyoto Encyclopaedia of Genes and Genomes) linked to rheumatoid arthritis. This comparative analysis provides additional insights into the molecular mechanisms involved in the development and progression of HCC and highlights potential shared pathways between these two diseases.

The results of the study have important relevance for enhancing clinical management of HCC patients, notably in the early detection and monitoring of the illness. Identification of possible biomarkers in PBMCs may help in the development of efficient diagnostic tests for HCC. Early detection is also crucial for the successful treatment of HCC. The results of the study may also help in the creation of novel therapeutic approaches that focus on shared pathways between HCC and rheumatoid arthritis. Overall, this research advances our knowledge of the molecular pathways underlying HCC and offers critical insights for enhancing the therapeutic care of HCC patients.

## References

1. Chidambaranathan-Reghupaty, S., Fisher, P. B., & Sarkar, D. (2021). Hepatocellular carcinoma (HCC): Epidemiology, aetiology and molecular classification. Elsevier EBooks, 1–61. https://doi.org/10.1016/bs.acr.2020.10.001
2. Key Statistics About Liver Cancer. (n.d.-b). https://www.cancer.org/cancer/liver-cancer/about/what-is-key-statistics.html
3. Llovet, J. M., Kelley, R. K., Villanueva, A., Singal, A. G., Pikarsky, E., Roayaie, S., Lencioni, R., Koike, K., Moreau, R., & Finn, R. S. (2021). Hepatocellular carcinoma. Nature Reviews Disease Primers, 7(1). https://doi.org/10.1038/s41572-020-00240-3
4. Crissien, A. M., & Frenette, C. (2014). Current management of hepatocellular carcinoma. Gastroenterología Y Hepatología.
5. Bruix, J., & Sherman, M. (2005). Management of hepatocellular carcinoma. Hepatology, 42(5), 1208–1236. https://doi.org/10.1002/hep.20933
6. Suresh, D., Srinivas, A. N., Prashant, A., Harikumar, K. B., & Kumar, D. P. (2023). Therapeutic options in hepatocellular carcinoma: a comprehensive review. Clinical and Experimental Medicine. https://doi.org/10.1007/s10238-023-01014-3
7. Yuan, J., Hegde, P. S., Clynes, R., Foukas, P. G., Harari, A., Kleen, T. O., Kvistborg, P., Maccalli, C., Maecker, H. T., Page, D. C., Robins, H., Song, W., Stack, E. C., Wang, E., Whiteside, T. L., Zhao, Y., Zwierzina, H., Butterfield, L. H., & Fox, B. A. (2016). Novel technologies and emerging biomarkers for personalized cancer immunotherapy. Journal for ImmunoTherapy of Cancer, 4(1). https://doi.org/10.1186/s40425-016-0107-3
8. Tian, L., & Liao, Y. (2022). Identification of G6PC as a potential prognostic biomarker in hepatocellular carcinoma based on bioinformatics analysis. Medicine, 101(33), e29548. https://doi.org/10.1097/md.0000000000029548
9. GEO Accession viewer. (n.d.). https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE49515

10. GEO2R - GEO - NCBI. (n.d.). https://www.ncbi.nlm.nih.gov/geo/geo2r/
11. Szklarczyk, D., Gable, A. L., Nastou, K. C., Lyon, D., Kirsch, R., Pyysalo, S., Doncheva, N. T., Legeay, M., Fang, T., Bork, P., Jensen, L. J., & Von Mering, C. (2021). The STRING database in 2021: customizable protein–protein networks, and functional characterization of user-uploaded gene/measurement sets. Nucleic Acids Research, 49(D1), D605–D612. https://doi.org/10.1093/nar/gkaa1074
12. Bagert, J. D., Mitchener, M. M., Lemiesz, A. E., Dul, B. E., Wojcik, F., Nacev, B. A., Feng, L., Allis, C. D., & Muir, T. W. (2021). Oncohistone mutations enhance chromatin remodeling and alter cell fates. Nature Chemical Biology, 17(4), 403–411. https://doi.org/10.1038/s41589-021-00738-1
13. Kawasaki, T., & Kawai, T. (2014). Toll-Like Receptor Signaling Pathways. Frontiers in Immunology, 5. https://doi.org/10.3389/fimmu.2014.00461
14. Alberts, B. (2002). Chromosomal DNA and Its Packaging in the Chromatin Fiber. Molecular Biology of the Cell - NCBI Bookshelf. https://www.ncbi.nlm.nih.gov/books/NBK26834/
15. Danielle Thierry-Mieg and Jean Thierry-Mieg, NCBI/NLM/NIH, mieg@ncbi.nlm.nih.gov. (n.d.). AceView: Gene:HIST2H4B, a comprehensive annotation of human, mouse and worm genes with mRNAs or ESTsAceView. https://www.ncbi.nlm.nih.gov/IEB/Research/Acembly/av.cgi?c=Gene&db=human&l=HIST2H4B
16. Database, G. H. G. (n.d.). H4C2 Gene - GeneCards | H4 Protein | H4 Antibody. https://www.genecards.org/cgi-bin/carddisp.pl?gene=H4C2
17. Giotti, B., Chen, S., Barnett, M. A., Regan, T., Ly, T., Wiemann, S., Hume, D., & Freeman, T. C. (2019). Assembly of a parts list of the human mitotic cell cycle machinery. Journal of Molecular Cell Biology, 11(8), 703–718. https://doi.org/10.1093/jmcb/mjy063
18. Yang, Y., Xie, L., Li, C., Liu, L., Ye, X., & Han, J. (2022). Prognostic Model of Eleven Genes Based on the Immune Microenvironment in Patients With Thymoma. Frontiers in Genetics, 13. https://doi.org/10.3389/fgene.2022.668696
19. Li, M., Jiang, L., & Guan, X. Y. (2014). The genetic and epigenetic alterations in human hepatocellular carcinoma: a recent update. Protein & Cell, 5(9), 673–691. https://doi.org/10.1007/s13238-014-0065-9
20. Gutiérrez-Cuevas, J., Lucano-Landeros, S., López-Cifuentes, D., Santos, A., & Armendáriz-Borunda, J. (2022). Epidemiologic, Genetic, Pathogenic, Metabolic, Epigenetic Aspects Involved in NASH-HCC: Current Therapeutic Strategies. Cancers, 15(1), 23. https://doi.org/10.3390/cancers15010023
21. Braghini, M. R., Lo Re, O., Romito, I., Fernandez-Barrena, M. G., Barbaro, B., Pomella, S., Rota, R., Vinciguerra, M., Bataller, R., & Alisi, A. (2022). Epigenetic remodelling in human hepatocellular carcinoma. Journal of Experimental & Clinical Cancer Research, 41(1). https://doi.org/10.1186/s13046-022-02297-2
22. Facs, L. C. M. (n.d.-a). Hepatocellular Carcinoma (HCC): Practice Essentials, Anatomy, Pathophysiology. https://emedicine.medscape.com/article/197319-overview
23. Tanaka, T., Narazaki, M., & Kishimoto, T. (2014). IL-6 in Inflammation, Immunity, and Disease. Cold Spring Harbor Perspectives in Biology, 6(10), a016295. https://doi.org/10.1101/cshperspect.a016295
24. Expression of HBB in cancer - Summary - The Human Protein Atlas. (n.d.). https://www.proteinatlas.org/ENSG00000244734-HBB/pathology
25. Ohshima, K., & Morii, E. (2021). Metabolic Reprogramming of Cancer Cells during Tumor Progression and Metastasis. Metabolites, 11(1), 28. https://doi.org/10.3390/metabo11010028
26. Wolińska, E., & Skrzypczak, M. (2021b). Epigenetic Changes Affecting the Development of Hepatocellular Carcinoma. Cancers, 13(16), 4237. https://doi.org/10.3390/cancers13164237
27. Grixti, J., & Ayers, D. (2020b). Long noncoding RNAs and their link to cancer. Non-Coding RNA Research, 5(2), 77–82. https://doi.org/10.1016/j.ncrna.2020.04.003
28. Gao, N., Li, Y., Li, J., Gao, Z., Yang, Z., Li, Y., Liu, H., & Fan, T. (2020). Long Non-Coding RNAs: The Regulatory Mechanisms, Research Strategies, and Future Directions in Cancers. Frontiers in Oncology, 10. https://doi.org/10.3389/fonc.2020.598817
29. Sun, H., Huang, Z., Sheng, W., & Xu, M. (2018). Emerging roles of long non-coding RNAs in tumor metabolism. Journal of Hematology & Oncology, 11(1). https://doi.org/10.1186/s13045-018-0648-7
30. Liu, Y., He, E., Zhang, Y., Liu, Y., Wang, Y., Chen, S., Wu, X., Zeng, Y., & Leng, P. (2022). WW domain binding protein 2 (WBP2) as an oncogene in breast cancer: mechanisms and therapeutic prospects—a narrative review. Gland Surgery, 11(12), 1984–2002. https://doi.org/10.21037/gs-22-716
31. Wang, X., Song, H., Fang, L., & Wu, T. (2022). EIF4A3-mediated circPRKCI expression promotes triple-negative breast cancer progression by regulating WBP2 and PI3K/AKT signaling pathway. Cell Death Discovery, 8(1). https://doi.org/10.1038/s41420-022-00892-y
32. Moghadam, S. K., Bakhshinejad, B., Khalafizadeh, A., Hussen, B. M., & Babashah, S. (2021). Non-coding RNA-associated competitive endogenous RNA regulatory networks: Novel diagnostic and therapeutic opportunities for hepatocellular carcinoma. Journal of Cellular and Molecular Medicine, 26(2), 287–305. https://doi.org/10.1111/jcmm.17126
33. Li, Y., Zhang, J., & Yu, L. (2019). Circular RNAs Regulate Cancer Onset and Progression via Wnt/β-Catenin Signalling Pathway. Yonsei Medical Journal, 60(12), 1117. https://doi.org/10.3349/ymj.2019.60.12.1117
34. Cervello, M., McCubrey, J. A., Cusimano, A., Lampiasi, N., Azzolina, A., & Montalto, G. (2012). Targeted therapy for hepatocellular carcinoma: novel agents on the horizon. Oncotarget, 3(3), 236–260. https://doi.org/10.18632/oncotarget.466

35. Lukey, M. J., Greene, K. S., Erickson, J. D., Wilson, K. F., & Cerione, R. A. (2016). The oncogenic transcription factor c-Jun regulates glutaminase expression and sensitises cells to glutaminase-targeted therapy. Nature Communications, 7(1). https://doi.org/10.1038/ncomms11321
36. Yang, S., Pang, L., Dai, W., Wu, S., Ren, T., Duan, Y., Zheng, Y., Bi, S., Zhang, X., & Kong, J. (2021). Role of Forkhead Box O Proteins in Hepatocellular Carcinoma Biology and Progression (Review). Frontiers in Oncology, 11. https://doi.org/10.3389/fonc.2021.667730
37. Tabatabaeian, H., Rao, A., Ramos, A., Chu, T., Sudol, M., & Lim, Y. P. (2020). The emerging roles of WBP2 oncogene in human cancers. Oncogene, 39(24), 4621–4635. https://doi.org/10.1038/s41388-020-1318-0
38. Europe PMC. (n.d.). Europe PMC. https://europepmc.org/article/med/32820148
39. O'Brien, J., Hayder, H., Zayed, Y., & Peng, C. (2018). Overview of MicroRNA Biogenesis, Mechanisms of Actions, and Circulation. Frontiers in Endocrinology, 9. https://doi.org/10.3389/fendo.2018.00402
40. Morishita, A., Oura, K., Tadokoro, T., Fujita, K., Tani, J., & Masaki, T. (2021). MicroRNAs in the Pathogenesis of Hepatocellular Carcinoma: A Review. Cancers, 13(3), 514. https://doi.org/10.3390/cancers13030514
41. Liu, Y., He, E., Zhang, Y., Liu, Y., Wang, Y., Chen, S., Wu, X., Zeng, Y., & Leng, P. (2022b). WW domain binding protein 2 (WBP2) as an oncogene in breast cancer: mechanisms and therapeutic prospects—a narrative review. Gland Surgery, 11(12), 1984–2002. https://doi.org/10.21037/gs-22-716
42. Lu, Y., Huang, D., Wang, B., Zheng, B., Liu, J., Song, J., & Zheng, S. (2022). FAM21C Promotes Hepatocellular Carcinoma Invasion and Metastasis by Driving Actin Cytoskeleton Remodeling via Inhibiting Capping Ability of CAPZA1. Frontiers in Oncology, 11. https://doi.org/10.3389/fonc.2021.809195
43. Wang, T., Rao, D., Yu, C., Sheng, J., Luo, Y., Xia, L., & Huang, W. (2022). RHO GTPase family in hepatocellular carcinoma. Experimental Hematology & Oncology, 11(1). https://doi.org/10.1186/s40164-022-00344-4
44. Ng, I. O., Wong, C., Ko, F. C. F., Chan, L., Ching, Y., & Yam, J. W. P. (2008). Deleted in Liver Cancer 1 (DLC1) Negatively Regulates Rho/ROCK/MLC Pathway in Hepatocellular Carcinoma. PLOS ONE, 3(7), e2779. https://doi.org/10.1371/journal.pone.0002779
45. Genetic Testing for Alpha- and Beta-Thalassemia. (n.d.). https://www.southcarolinablues.com/web/public/brands/medicalpolicy/external-policies/genetic-testing-for-alpha--and-beta-thalassemia/
46. HBA1 gene: MedlinePlus Genetics. (n.d.-c). https://medlineplus.gov/genetics/gene/hba1/
47. Feng, J., Li, J., Wu, L., Yu, Q., Ji, J., Wu, J., Dai, W., & Guo, C. (2020). Emerging roles and the regulation of aerobic glycolysis in hepatocellular carcinoma. Journal of Experimental & Clinical Cancer Research, 39(1). https://doi.org/10.1186/s13046-020-01629-4
48. Muz, B., De La Puente, P., Azab, F., & Azab, A. K. (2015b). The role of hypoxia in cancer progression, angiogenesis, metastasis, and resistance to therapy. Hypoxia, 83. https://doi.org/10.2147/hp.s93413
49. Wu, Q., You, L., Nepovimova, E., Heger, Z., Wu, W., Kuca, K., & Adam, V. (2022). Hypoxia-inducible factors: master regulators of hypoxic tumour immune escape. Journal of Hematology & Oncology, 15(1). https://doi.org/10.1186/s13045-022-01292-6
50. Wu, J., Jiang, J., Chen, B., Wang, K., Tang, Y., & Liang, X. (2021). Plasticity of cancer cell invasion: Patterns and mechanisms. Translational Oncology, 14(1), 100899. https://doi.org/10.1016/j.tranon.2020.100899
51. Han, T., Kang, D., Ji, D., Wang, X., Zhan, W., Fu, M., Xin, H., & Wang, J. (2013). How does cancer cell metabolism affect tumor migration and invasion? Cell Adhesion & Migration, 7(5), 395–403. https://doi.org/10.4161/cam.26345
52. Novikov, N. A., Zolotaryova, S. Y., Gautreau, A., & Denisov, E. V. (2021). Mutational drivers of cancer cell migration and invasion. British Journal of Cancer, 124(1), 102–114. https://doi.org/10.1038/s41416-020-01149-0
53. Ding, Q., Hu, W., Wang, R., Yang, Q., Zhu, M., Li, M., Cai, J., Rose, P., Mao, J., & Zhu, Y. (2023b). Signaling pathways in rheumatoid arthritis: implications for targeted therapy. Signal Transduction and Targeted Therapy, 8(1). https://doi.org/10.1038/s41392-023-01331-9
54. Lin, A., Wang, G., Zhao, H., Zhang, Y., Han, Q., Zhang, C., Tian, Z., & Zhang, J. (2016). TLR4 signaling promotes a COX-2/PGE2/STAT3 positive feedback loop in hepatocellular carcinoma (HCC) cells. OncoImmunology, 5(2), e1074376. https://doi.org/10.1080/2162402x.2015.1074376
55. Rébé, C., & Ghiringhelli, F. (2020c). Interleukin-1β and Cancer. Cancers, 12(7), 1791. https://doi.org/10.3390/cancers12071791
56. Cheng, L., Wang, Y., Wu, R., Ding, T., Xue, H., Gao, C., Li, X., & Wang, C. (2021). New Insights From Single-Cell Sequencing Data: Synovial Fibroblasts and Synovial Macrophages in Rheumatoid Arthritis. Frontiers in Immunology, 12. https://doi.org/10.3389/fimmu.2021.709178
57. Kiss, M., Walle, L. V., Saavedra, P., Lebegge, E., Van Damme, H., Murgaski, A., Qian, J., Ehling, M., Pretto, S., Bolli, E., Keirsse, J., Bardet, P. M. R., Arnouk, S. M., Elkrim, Y., Schmoetten, M., Brughmans, J., Debraekeleer, A., Fossoul, A., Boon, L., . . . Laoui, D. (2021). IL1β Promotes Immune Suppression in the Tumor Microenvironment Independent of the Inflammasome and Gasdermin D. Cancer Immunology Research, 9(3), 309–323. https://doi.org/10.1158/2326-6066.cir-20-0431
58. Altaf, S., Saleem, F., Sher, A. A., & Ali, A. (2022). Potential Therapeutic Strategies to Combat HCC. Current Molecular Pharmacology, 15(7), 929–942. https://doi.org/10.2174/1874467215666220103111009
59. Deng, J., Jiang, R., Meng, E., & Wu, H. (2022). CXCL5: A coachman to drive cancer progression. Frontiers in Oncology, 12. https://doi.org/10.3389/fonc.2022.944494

60. Zhang, W., Wang, H., Tindemans, S. H., Deng, X., Wu, X., Ma, Y., Li, M., Shuoa, S. M., You, Q., & Miao, L. (2020). CXCL5/CXCR2 axis in tumor microenvironment as potential diagnostic biomarker and therapeutic target. Cancer Communications, 40(2–3), 69–80. https://doi.org/10.1002/cac2.12010
61. Pickens, S. R., Chamberlain, N. D., Volin, M. V., Gonzalez, M. H., Pope, R. O., Mandelin, A. M., Kolls, J. K., & Shahrara, S. (2011). Anti-CXCL5 therapy ameliorates IL-17-induced arthritis by decreasing joint vascularization. Angiogenesis, 14(4), 443–455. https://doi.org/10.1007/s10456-011-9227-z
62. Ren, Z., Chen, Y., Shi, L., Shao, F., Ge, Y., Zhang, J., & Zang, Y. (2022b). Sox9/CXCL5 axis facilitates tumour cell growth and invasion in hepatocellular carcinoma. FEBS Journal, 289(12), 3535–3549. https://doi.org/10.1111/febs.16357
63. Zhou, S., Dai, Z., Zhou, Z., Wang, X., Yang, G., Wang, Z., Huang, X., Fan, J., & Zhou, J. (2012). Overexpression of CXCL5 mediates neutrophil infiltration and indicates poor prognosis for hepatocellular carcinoma. Hepatology, 56(6), 2242–2254. https://doi.org/10.1002/hep.25907
64. Haider, C., Hnat, J., Wagner, R., Huber, H., Timelthaler, G., Grubinger, M., Coulouarn, C., Schreiner, W., Schlangen, K., Sieghart, W., Peck-Radosavljevic, M., & Mikulits, W. (2019). Transforming Growth Factor-β and Axl Induce CXCL5 and Neutrophil Recruitment in Hepatocellular Carcinoma. Hepatology, 69(1), 222–236. https://doi.org/10.1002/hep.30166
65. Cook, S. A., & Schafer, S. (2020). Hiding in Plain Sight: Interleukin-11 Emerges as a Master Regulator of Fibrosis, Tissue Integrity, and Stromal Inflammation. Annual Review of Medicine, 71(1), 263–276. https://doi.org/10.1146/annurev-med-041818-011649
66. Maroni, P., Bendinelli, P., Ferraretto, A., & Lombardi, G. (2021). Interleukin 11 (IL-11): Role(s) in Breast Cancer Bone Metastases. Biomedicines, 9(6), 659. https://doi.org/10.3390/biomedicines9060659
67. Klein, H. E. (2021, December 4). Review Explains Role of IL-11 in Inflammatory Diseases. AJMC. https://www.ajmc.com/view/review-explains-role-of-il-11-in-inflammatory-diseases
68. IL-11 facilitates a novel connection between RA joint fibroblasts and endothelial cells. Angiogenesis, 21(2), 215–228. https://doi.org/10.1007/s10456-017-9589-y
69. Sangro, B., Sarobe, P., Hervas-Stubbs, S., & Melero, I. (2021). Advances in immunotherapy for hepatocellular carcinoma. Nature Reviews Gastroenterology & Hepatology, 18(8), 525–543. https://doi.org/10.1038/s41575-021-00438-0
70. Kortekaas, R. K., Burgess, J. K., Van Orsoy, R., Lamb, D., Tarran, R., & Gosens, R. (2021). Therapeutic Targeting of IL-11 for Chronic Lung Disease. Trends in Pharmacological Sciences, 42(5), 354–366. https://doi.org/10.1016/j.tips.2021.01.007
71. Van Der Burg, S. H. (2021). IL11: A Specific Repressor of Tumor-Specific CD4+ T Cells. Cancer Immunology Research, 9(7), 724. https://doi.org/10.1158/2326-6066.cir-21-0248
72. UpToDate. (n.d.-b). UpToDate. https://www.uptodate.com/contents/human-leukocyte-antigens-hla-a-roadmap
73. Delves, P. J. (2023, March 15). Human Leukocyte Antigen (HLA) System. Merck Manuals Professional Edition. https://www.merckmanuals.com/professional/immunology-allergic-disorders/biology-of-the-immune-system/human-leukocyte-antigen-hla-system
74. UpToDate. (n.d.-c). HLA and Other Susceptibility Genes in Rheumatoid Arthritis. UpToDate. https://www.uptodate.com/contents/hla-and-other-susceptibility-genes-in-rheumatoid-arthritis
75. Ock, C. Y., Keam, B., Kim, S., Lee, J. S., Kim, M., Kim, T. M., & Kim, D. W. (2019). Cancer immune escape: MHC expression in primary tumors versus metastases. Immunology, 158(4), 255-266. https://doi.org/10.1111/imm.13114
76. Werth, B. J. (2023b, March 15). Beta-Lactams. MSD Manual Professional Edition. https://www.msdmanuals.com/en-in/professional/infectious-diseases/bacteria-and-antibacterial-drugs/beta-lactams
77. Yap, H. M., Tee, S. Z. Y., Wong, M. M., Chow, S. K., Peh, S., & Teow, S. (2018). Pathogenic Role of Immune Cells in Rheumatoid Arthritis: Implications in Clinical Treatment and Biomarker Development. Cells, 7(10), 161. https://doi.org/10.3390/cells7100161
78. Song, Q., Meng, B., Xu, H., & Mao, Z. (2020). The Emerging Roles of Vacuolar-Type ATPase-Dependent Lysosomal Acidification in Neurodegenerative Diseases. Translational Neurodegeneration, 9(1). https://doi.org/10.1186/s40035-020-00196-0
79. Padyukov, L. (2022). Genetics of Rheumatoid Arthritis. Seminars in Immunopathology, 44(1), 47-62. https://doi.org/10.1007/s00281-022-00912-0
80. Zhou, J., Tao, X., Chen, Y., & Zhang, W. (2020). Comprehensive Analysis of ATP6V1s Family Members in Renal Clear Cell Carcinoma With Prognostic Values. Frontiers in Oncology, 10. https://doi.org/10.3389/fonc.2020.567970