

Advances in the Study of Liver Cancer Stem Cells

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Abstract. Liver cancer is one of the deadliest cancers in the world, and liver cancer stem cells (LCSCs) is a key factor in liver cancer pathogenesis, treatment resistance and disease recurrence. Despite significant advances in the understanding of LCSCs over the past few years, their role in liver cancer pathology remains a challenge. This review addresses the characterization of LCSCs in hepatocellular carcinoma, methods of identification, therapeutic strategies, and how they affect disease progression and patient prognosis. This paper pays special attention to recent studies on molecular markers, signaling pathways, and gene expression characterization of LCSCs. The paper also evaluates a variety of potential therapeutic approaches, including targeted drug therapies against specific signaling pathways, cell surface markers, etc., immunotherapeutic strategies such as CAR-T cell therapy, and tumor vaccines. Although most of these therapeutic strategies are still in the research phase, they offer new hope for the treatment of liver cancer. Finally, this paper discusses the challenges faced in translating these laboratory studies into clinical treatments, as well as potential directions for future research.

Keywords: LCSCs; hepatocarcinogenesis and progression; targeted drugs; immunotherapy; vaccines.

1. Introduction

Liver cancer is a highly malignant disease and one of the most lethal cancers. Meanwhile, Liver cancer is difficult to treat and poses a serious threat to patients' health. In the field of liver cancer research, the study of liver cancer stem cells has been attracting much attention. Due to the own characteristics and differentiation mechanism of tumor stem cells, liver cancer stem cells are considered to be the key factor in the occurrence and metastasis of liver cancer. Therefore, the study of liver cancer stem cells is a crucial part of liver cancer research [1].

Hepatocellular carcinoma stem cells, also known as hepatocellular carcinoma stem cell-like cells (CSCs), are a kind of hepatocellular carcinoma cells with stem cell properties, possessing the potential for self-renewal and multidirectional differentiation, and capable of forming tumors and becoming resistant to treatment. The presence and activity of hepatocellular carcinoma stem cells are closely related to hepatocellular carcinoma occurrence, development, recurrence and drug resistance. Therefore, the study of hepatocellular carcinoma stem cells not only contributes to an in-depth understanding of the pathophysiological process of hepatocellular carcinoma, but also provides new ideas and methods for the precise treatment of hepatocellular carcinoma [2].

In recent years, through in-depth research on tumor stem cells, scientists have discovered methods to identify as well as isolate tumor stem cells. These methods, including the use of cell surface markers and cell sorting technology are of great significance in understanding cancer development and finding therapeutic targets. Currently, targeted drug therapy, immunotherapy and gene therapy are more advanced and effective treatments. In addition, hepatocellular carcinoma stem cells are resistant to traditional therapies such as chemotherapy and radiotherapy, making the treatment of hepatocellular carcinoma further difficult. Therefore, in-depth research on liver cancer stem cells is crucial for exploring the pathogenesis of liver cancer, developing new therapeutic approaches, and improving the survival rate of liver cancer patients.

This paper will review the important progress of liver cancer stem cell research in recent years, aiming to explore the role of liver cancer stem cells in liver cancer pathogenesis, treatment resistance, etc., and to provide new ideas and methods for precise treatment and prevention of liver cancer. It is hoped that the review of this paper can provide certain references for the research and clinical practice

of liver cancer stem cells and further promote the development of the field of liver cancer stem cell research.

2. Stem Cells and HCC Stem Cells

2.1. Stem Cells

Stem cells are immature cells with self-renewal and differentiation potential in living organisms, and they are important in tissue development, repair and maintenance of physiological homeostasis.

Embryonic stem cells (ESCs) originate from the early stages of embryonic development. These cells can be harvested from the inner cell mass (ICM) during the blastocyst phase, which occurs approximately between 3 to 5 days post-fertilization [1]. They are considered totipotent, so they can differentiate into all types of cells in human body, including the various cell types formed by the three main germinal layers: ectoderm, mesoderm, and endoderm. Adult stem cells are found in mature tissues. These cells are usually pluripotent, and they can differentiate into specific types of cells to replace aging or damaged cells and maintain normal tissue function.

2.2. Liver Stem Cells

Normal stem cells have great potential for research and application, but liver cancer stem cells challenge the treatment and understanding of cancer.

A subset of cells within hepatocellular carcinoma (HCC), known as Liver Cancer Stem Cells (LCSCs), exhibit properties akin to those of typical stem cells, such as the capabilities of self-renewal and differentiation. These cells are considered to be key drivers in liver cancer tumor cells and play a decisive role in tumorigenesis, progression, metastasis and treatment resistance. Studies have shown that LCSCs are more resistant to chemotherapy and radiotherapy than normal cancer cells [2], which makes tumors more likely to recur and metastasize after initial treatment, leading to a poor prognosis.

2.3. Formation and Molecular Mechanisms of HCC Stem Cells

Liver cancer stem cells may originate from two different processes. One is that they can originate directly from normal hepatic stem cells or hepatic precursor cells that have acquired malignant properties due to genetic and epigenetic alterations. The other possibility is that mature hepatocytes acquire stem cell-like properties through cellular reprogramming (dedifferentiation), resulting in the formation of LCSCs.

The molecular mechanisms of HCC stem cells are as follows:

1) Genetic mutations: The formation of LCSCs is usually associated with mutations in key tumor suppressor genes and proto-oncogenes. For example, inactivation of the p53 gene can lead to failure of cell cycle regulation and inhibition of apoptosis, thereby promoting tumor development [3]. Concurrently, the dysregulation of signaling cascades including Wnt/ β -catenin, Notch, and Hedgehog is crucial in modulating the self-renewal and differentiation processes of Liver Cancer Stem Cells (LCSCs) [4].

2) Epigenetic control mechanisms, including DNA methylation, alterations in histone structure, and the influence of non-coding RNAs (such as microRNAs), are integral in governing the characteristics of LCSCs [5,6]. They can alter gene expression without changing the DNA sequence, affecting cell fate decisions and tumorigenesis.

3) Microenvironmental factors: the microenvironment of LCSCs includes surrounding cells, extracellular matrix (ECM), secreted factors, and blood vessels, which are critical for their proliferation and survival [7].

4) Cell cycle regulation: LCSCs often exhibit abnormalities in cell cycle regulation; for example, imbalances in cyclins and cycle-dependent kinase inhibitors may lead to unlimited cell proliferation.

5) Immune escape: HCC stem cells can escape the surveillance of the host immune system by expressing certain cell surface molecules (e.g., PD-L1) or secreting immunosuppressive factors [8], which contributes to the growth and spread of the cancer.

The molecular mechanisms of HCC stem cells are complex and diverse, and therefore, therapeutic strategies for HCC are numerous. Understanding and treating the molecular mechanisms of liver cancer is an evolving field, and therapeutic strategies continue to progress as new biomarkers and therapeutic targets are discovered.

3. LCSCs in Hepatocarcinogenesis and Progression

3.1. Self-renewal Capacity

Self-renewal is a specialized process of cell division by which LCSCs can give rise to two daughter cells with the same capacity as the mother cell. During self-renewal, stem cells must maintain their undifferentiated state while maintaining the stem cell nature of the tumor [9].

Self-renewal of HCC stem cells can be carried out by two basic types of cell division:

1) Asymmetric division: Liver cancer stem cells divide to produce two distinct daughter cells. One is a stem cell that retains all of its stem cell properties. The other is a more differentiated cell that will contribute a more specialized cell type to the tumor [10].

2) Symmetric division: Liver cancer stem cells divide into two identical daughter stem cells, which allows for rapid expansion of the stem cell population.

The regulation of self-renewal is multifaceted, involving genetic, epigenetic, microenvironmental factors and internal signaling pathways.

Specific genetic mutations and chromosomal rearrangements in the regulatory mechanisms of self-renewal may lead to the activation of self-renewal signaling pathways in liver cancer stem cells. For example, c-Myc and β -catenin are well-studied oncogenes and tumor suppressor genes involved in the regulation of stem cell self-renewal [11]. Epigenetic mechanisms are important regulators of the self-renewal process. They regulate gene expression by altering chromatin structure rather than the DNA sequence itself. The aberrant activity of DNA methyltransferases and histone deacetylases can lead to silencing or activation of self-renewal-related genes. The self-renewal capacity of HCC stem cells is also influenced by the surrounding microenvironment [7].

Multiple signaling pathways play key roles in the control of self-renewal of HCC stem cells, including the Wnt/ β -catenin, Notch, Hedgehog, and the PI3K/Akt/mTOR pathway. Their mechanisms are as follows:

1) Wnt/ β -catenin: Wnt proteins engage with Frizzled receptors, leading to the stabilization and subsequent prevention of β -catenin degradation [4] and allowing it to accumulate and enter the nucleus, which then regulates the transcription of self-renewal-related genes.

2) Notch: Upon activation of Notch receptor, Notch internal domain (NICD) is released into the nucleus and directly affects downstream genes, including self-renewal-related genes.

3) Hedgehog: Hedgehog ligand binds to its receptor Patched and derepresses Smoothed, which in turn activates Gli transcription factors. They are involved in the regulation of self-renewal gene expression.

4) PI3K/Akt/mTOR: Activation of this pathway promotes cell survival and proliferation, providing favorable conditions for self-renewal of LCSCs [12].

Understanding and targeting the self-renewal mechanism of LCSCs provides new avenues for treating HCC, stopping its recurrence and resisting drug therapy. However, developing effective strategies to target LCSCs remains a major challenge due to the heterogeneity of the tumors and the complex interactions with normal liver stem cells.

3.2. Ability to Differentiate in Multiple Directions

The multidirectional differentiation capacity of LCSCs means that they have the potential to differentiate to different types of hepatocytes in addition to being able to self-renew to maintain their stem cell pool. This means that LCSCs are capable of giving rise to a wide variety of cells in liver cancer tissues, including cancer cells with different degrees of differentiation and other cell types that may be involved in the formation of the tumor microenvironment. This property is one of the central

aspects of the definition of stem cells and is responsible for much of the heterogeneity and complexity of tumors.

Multidirectional differentiation is a process consisting of multiple stages. During the first stage, which is the stage that determines the fate of differentiation, liver cancer stem cells receive signals from the microenvironment. These signals trigger specific transcriptional programs that determine the type of cell the cell will differentiate into. Once a differentiation fate has been decided, specific transcription factors are activated. They will turn on or off a series of genes in order to direct the cell to differentiate toward the specified cell type. With the action of transcription factors, the expression pattern of genes within the cell changes. The products of these genes are responsible for the formation and maintenance of structures and functions required for specific cell types. During differentiation, cells may undergo a series of dividing and proliferating events that culminate in the formation of mature cells. The final stage is cell maturation. At this stage the cell fully acquires the properties of its target cell type and serves the appropriate physiological function.

4. Therapeutic Strategy

4.1. Drug Therapy Targeting LCSCs

Targeted drug therapy for LCSCs is an active area of research, with the aim of discovering drugs that specifically kill or inhibit LCSCs to prevent liver cancer progression, recurrence, and drug resistance. For different mechanisms of action and therapeutic strategies, these targeted drugs can be discussed in several categories.

4.1.1 Drugs to cell surface markers

Monoclonal antibodies that are markers for LCSCs specifically bind to specific molecules on the surface of LCSCs. Through this binding, they can directly inhibit tumor cell signaling or recruit immune system-mediated cell destruction. For example, Anti-EpCAM Antibody. EpCAM is a molecule that is highly expressed in many HCC stem cells, and anti-EpCAM antibodies (e.g., Catumaxomab and Panitumumab) bind to EpCAM to inhibit its signaling and may activate antibody-dependent cell-mediated cellular cytotoxicity (ADCC) to kill tumor cells.

In addition, targeted drugs against immune checkpoint inhibitors (ICIs), although not directly targeting cell surface markers, can enhance the immune response against tumor cells, including LCSCs, by unlocking the "brakes" of the immune system. LCSCs sometimes express PD-L1 to suppress immune attack. The use of inhibitors against PD-1 or PD-L1 can undo this inhibition and activate T-cells to target LCSCs. Antibody-Drug Conjugates, (ADCs) are also a way of using toxins to attack tumor cells. These drugs combine chemotherapeutic drugs with antibodies targeting surface markers on LCSCs [13] and use the antibodies to navigate and deliver the toxins directly to the tumor cells, reducing toxicity to normal cells.

Bispecific Antibodies have two binding sites and can target two different antigens at the same time. This design improves drug selectivity and potency. For example, BiTEs (Bispecific T-cell Energizers) can link T-cells (via CD3 binding sites) to tumor cells (via binding sites targeting LCSCs markers), enhancing the killing effect of T-cells on tumor cells.

4.1.2 Signaling pathway oriented drugs

Targeted drugs are designed to align and interfere with specific signaling pathways. These pathways are more active or abnormal in cancer cells, including cancer stem cells, than in normal cells. This specific action helps to reduce damage to normal cells, minimize side effects, and improve treatment efficacy. Moreover, by targeting those key pathways responsible for the survival and proliferation of cancer cells, the ability of cancer cells to develop drug-resistant mechanisms can be curtailed. Targeting signaling pathways in these cells could reduce the rate of recurrence, prolong patient survival, and hopefully increase the likelihood of a complete cure.

The Wnt/ β -catenin signaling pathway is abnormally active in many types of hepatocellular carcinoma, especially in LCSCs. This pathway is commonly associated with cell fate, proliferation and self-renewal. There are two main drugs that target this pathway for inhibition, LGK974 and PRI-724. LGK974 is an oral Wnt signaling release inhibitor that indirectly reduces β -catenin signaling by inhibiting the PORCNase enzyme, which prevents palmitoylation of the Wnt ligand and its subsequent activity. PRI-724 is a selective inhibitor specifically targeting the β -catenin and CREB binding protein (CBP) interactions, thereby inhibiting Wnt signaling pathway activation.

The Hedgehog (Hh) signaling pathway is essential in hepatocarcinogenesis, cell proliferation and maintenance of LCSCs. Vismodegib (GDC-0449) is an inhibitor that blocks the Hh signaling pathway, specifically by inhibiting its activity through binding to the Smoothed (SMO) receptor, which is a key signaling molecule in the pathway. Alternatively, Sonidegib (LDE225), similar to Vismodegib, is a selective SMO inhibitor for inhibiting the Hh signaling pathway. Meanwhile, the Notch pathway is important in cell differentiation, proliferation, and stem cell self-renewal in hepatocellular carcinoma. γ -secretase inhibitor is a key enzyme responsible for the activation of Notch receptors [4]. Inhibitors such as DAPT and MK-0752 prevent the cleavage and activation of Notch receptors, thereby inhibiting the Notch signaling pathway.

The PI3K/AKT/mTOR signaling pathway participate in cell survival, proliferation, metabolism, and protein synthesis, and it plays a critical role in the progression of hepatocellular carcinoma and the function of LCSCs. Rapamycin (Sirolimus) and its analogs are mTOR inhibitors that inhibit the activity of the mTORC1 complex, thereby decreasing protein synthesis and cellular proliferation. PI3K inhibitors like Buparlisib (BKM120) inhibit the PI3K signaling pathway and have potential inhibitory effects on LCSCs [14].

Drugs targeting these signaling pathways may alter the behavior of LCSCs, reduce liver cancer recurrence and drug resistance, and improve treatment efficacy. However, drug therapies must be precisely designed to maximize inhibition of LCSCs while minimizing effects on normal liver cells. The application of these drugs also requires large-scale clinical trials to evaluate their safety, efficacy, and role in the complex tumor microenvironment. In addition, given the heterogeneity of hepatocellular carcinoma, combinatorial therapeutic strategies that simultaneously target multiple signaling pathways may be required to effectively inhibit LCSCs.

4.1.3 Drugs towards epigenetic regulation

The process of epigenetic control is vital for the establishment and preservation of LCSCs. Drugs that target these epigenetic features can alter the gene expression patterns of cancer cells, thereby inhibiting tumor growth and spread.

DNA methylation is a process of adding methyl groups to DNA molecules, which often contributes to gene silencing. In many cancers, tumor suppressor genes are often inactivated by hypermethylation, so DNA methylation inhibitors are also a means to an end, including two of the following:

1) Azacitidine (Vidaza): This drug is a nucleoside analog that integrates into DNA and blocks the DNA methyltransferase enzyme, reducing DNA methylation and thus potentially reactivating silenced oncogenes [15].

2) Decitabine (Dacogen): Decitabine is also a nucleoside analog that inhibits DNA methylation and reactivates silenced genes.

Histone deacetylation is the process of removing the acetyl groups from histone tails, which can lead to chromatin condensation and thus inhibit gene expression. Histone deacetylase inhibitors (HDAC inhibitors) block this process, resulting in a more open chromatin structure and increased gene expression. Vorinostat is an HDAC inhibitor that increases histone acetylation and alters gene expression patterns to affect tumor cell growth and survival. And Romidepsin (Istodax) is another potent HDAC inhibitor that affects histone acetylation, thereby inhibiting cancer cell proliferation and inducing cell death. In the meanwhile, histone methylation is another epigenetic modification that can affect gene expression. Histone methyltransferase inhibitors can alter chromatin structure and modulate gene activity. Among them, EZH2 inhibitors are histone methyltransferases responsible

for adding methyl groups to specific histones. EZH2 inhibitors can reduce the silencing state of certain genes, which may lead to differentiation of cancer cells and limit their proliferation.

The regulation of gene expression by non-coding RNAs is also part of epigenetics. They can be used as drug targets or as therapeutic tools. microRNA mimics are synthetic microRNA molecules that complement microRNAs that have been reduced in cancer cells, thereby suppressing the expression of tumor-promoting genes. microRNA inhibitors suppress overexpressed microRNAs, restoring the expression of tumor-suppressor genes that have been inhibited by these microRNAs. suppressor genes.

Targeting epigenetic regulatory drugs in LCSCs is of great importance in cancer therapy because they can alter the gene expression patterns of tumor cells, restore normal cellular function, and potentially lead to a shift to a more differentiated state of tumor cells, reducing their malignancy. In addition, such drugs are often thought to be useful in combination with conventional chemotherapy and radiotherapy to enhance efficacy and overcome drug resistance. However, since epigenetic regulation also plays a key role in the functioning of normal cells, potential side effects and effects on normal tissues need to be considered when using such drugs.

4.1.4 Metabolic pathway-oriented drugs

LCSCs, like other cancer cells, often exhibit altered metabolic properties that support their proliferation, survival and drug resistance. Drugs in metabolic pathways target these changes in an attempt to inhibit the viability and survival of cancer cells by blocking key energy and biosynthetic pathways required for their survival. Cancer cells often break down sugar efficiently through the glycolytic metabolic pathway, even in the presence of sufficient oxygen. Glycolysis inhibitors can impair the energy supply of cancer cells. 2-Deoxy-D-glucose (2-DG) is a glycolysis inhibitor that interferes with the first step of glycolysis, thereby limiting energy production in cancer cells. And 3-Bromopyruvate (3-BP) works in a different location. This drug inhibits key enzymes of glycolysis, such as lactate dehydrogenase, slowing the process of sugar breakdown in cancer cells.

The primary source of energy for cells is the oxidative phosphorylation process in mitochondria. Cancer cells may have abnormalities in this pathway, leading to changes in energy production and biosynthetic pathways. Therefore, inhibitors of oxidative phosphorylation can control this aberrant process, thus achieving control. Metformin, although first used as a blood sugar lowering drug, has also been found to inhibit mitochondrial complex I, thus potentially slowing down the metabolic rate of cancer cells. Cancer cells require large amounts of amino acids for protein synthesis and other metabolic activities, and inhibiting amino acid metabolism may block their growth. Inhibitors of amino acid metabolic pathways can also play a role. For example, L-Asparaginase destroys asparagine in the blood. Because cancer cells cannot synthesize it, they are very dependent on exogenous asparagine, and this dependence can be exploited by drugs.

Drugs targeting metabolic pathways in LCSCs could provide a novel therapeutic tool, especially when conventional therapies such as chemotherapy and radiotherapy are ineffective. However, drug development for metabolic pathways faces challenges. Since the liver is the major organ controlling metabolic processes in the body, the selectivity and toxicity profile of these drugs need to be carefully considered. In addition, since metabolic pathways are also critical in normal cells, researchers must identify metabolic features specific to cancer cells or cancer stem cells to minimize negative effects on normal cells.

4.2. Immunotherapy

4.2.1 ICIs

ICIs are a class of immunotherapeutic drugs whose mechanism of action is to block the ability of tumor cells to use immune checkpoints to suppress the immune system, particularly T-cell activity. Immune checkpoints are a group of inhibitory receptors found on the surface of immune cells, which help prevent autoimmune responses and maintain immune self-tolerance under normal physiological conditions. However, many tumor cells are able to pass these checkpoints to avoid recognition and

clearance by the immune system. Thus, LCSCs may also express mechanisms to evade the immune system, making them more difficult to remove. This article focuses on two ICIs, PD-1/PD-L1 inhibitors and CTLA-4 inhibitors.

PD-1 is an inhibitory receptor on the surface of T cells. When PD-1 binds to its ligands, PD-L1 or PD-L2, it can transmit a signal to inhibit T-cell activity, reduce cytokine production, and promote T-cell depletion. PD-L1 is a protein on the surface of many tumor cells that binds to PD-1 and helps tumor cells resist immune system attack. However, inhibitors targeting PD-1/PD-L1 can disrupt the engagement between the PD-1 receptor and its ligand PD-L1, which activates T-cells, enabling them to recognize and attack tumor cells, including LCSCs. CTLA-4 (Cytotoxic T-Lymphocyte-Associated Protein 4) is another inhibitory receptor that surfaces on T cells. Its main role is to inhibit T-cell activity in the early stages of the immune response. It binds to B7 molecules (CD80/CD86), which normally enhances T cell activity, but CTLA-4 binding has the opposite effect. CTLA-4 inhibitors reduce the inhibition of T-cell activation by stopping the binding of CTLA-4 to B7 molecules, which enhances the immune response and allows the T-cells to attack tumor cells more effectively.

By blocking these immune escape mechanisms used by tumor cells, ICIs restore and enhance the ability of body to identify and destroy tumor cells. This enhanced immune response helps to control tumor growth and spread and may even lead to complete tumor regression. However, because ICIs activate the immune system, they can lead to side effects. These side effects may affect any organ system, including the skin, gastrointestinal tract, liver, and lungs. Therefore, patient response needs to be carefully monitored when using these drugs.

4.2.2 CAR-T cell therapy

Chimeric antigen receptor (CAR) T cell therapy represents an innovative cancer treatment approach that merges cellular therapy with genetic engineering techniques. The goal of this therapy is to enable the patient's T-cells to more effectively recognize and destroy LCSCs, thereby improving treatment outcomes.

CAR-T cells recognize and bind to specific antigens on the surface of LCSCs, such as Glypican-3 (GPC3) [16], via CARs on their surface. This binding activates the T cells, causing them to release killer molecules. These killer molecules can directly kill the target cells. In addition, CAR-T cells can also secrete cytokines, which can further enhance the immune response and help destroy cancer cells. CAR-T cells may persist in the body after destroying cancer cells and form an immune memory. If the cancer cells recur in the future or new ones appear, these memory CAR-T cells can respond quickly to recognize and destroy them again.

Despite the remarkable success of CAR-T cell therapy in certain hematologic cancers, its application in solid tumors is still in the early stages of research. Therefore, more research is needed to target specific antigens on the surface of LCSCs. In addition, CAR-T cell therapy may be accompanied by serious side effects, and thus patient responses need to be closely monitored during treatment.

4.2.3 Tumor-specific vaccine

Vaccines targeting specific tumors function as a form of immunotherapy, engineered to stimulate the patient's immune response to identify and eliminate cancer cells. This therapy relies on specific antigens on the surface of tumor cells, which are usually not or poorly expressed in normal cells.

Researchers first need to identify antigens specific to tumor cells, which should be proteins or polysaccharides that are recognized by the immune system and cause less cross-reactivity to healthy cells. In LCSCs, there may be specific tumor-associated antigens that could be targeted by a vaccine. After a tumor-specific vaccine is injected into a patient, the antigens in the vaccine are taken up by immune cells. These immune cells process the antigen and express it on the cell surface in a form that binds to major histocompatibility complex (MHC) molecules.

Dendritic cells will present the processed antigen to T cells via MHC molecules. This process will activate the T-cells, especially CD8+ cells and CD4+ cells. Activated CD8+ T cells can directly

recognize and kill tumor cells expressing the same tumor antigen, while CD4+ T cells help strengthen the overall immune response by secreting cytokines. In this process, activated T cells expand, differentiate into effector cells, and track to the location of the tumor to attack. Tumor-specific vaccines may also induce the formation of memory T cells. These cells remain in the body for a long period of time and are able to quickly activate and attack cancer cells if the same tumor antigen reappears. This helps prevent tumor recurrence.

5. Conclusion

LCSCs are crucial in HCC genesis, progression, metastasis, and treatment resistance. In recent years, with the deepening of stem cell biology and oncology research, the understanding of LCSCs has significantly improved. These findings not only deepen the understanding of the complex biology of liver cancer, but also offer the possibility of developing new therapeutic strategies.

In the field of targeted drug therapy, the process of LCSCs growth and development is utilized to target signaling pathways and cell surface markers. However, precise targeting and avoiding damage to normal liver cells remain challenges. In terms of immunotherapy, despite the breakthrough of CAR-T cell therapy in hematological tumors, its application in solid tumors, especially hepatocellular carcinoma. The tumor microenvironment, tumor heterogeneity, and the occult nature of LCSCs are all important factors affecting treatment efficacy. Vaccine therapy, especially vaccines based on specific tumor-associated antigens, demonstrates the potential to activate immune system to target LCSCs. Although this field is still in its early stages, vaccine strategies offer a novel perspective for the treatment of LCSCs.

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