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Research Progress on the Origin and Mechanism of Liver Cancer Stem Cells

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Abstract

Hepatocellular Carcinoma (HCC) is one of the most common malignancies in the world and it is highly fatal. Liver Cancer Stem Cells (LCSCs) are a kind of cell with resembling characteristics to normal stem cells in HCC. The self-renewal, differentiation, tumorigenic potential and chemical resistance of LCSCs may be responsible for the high recurrence rate and refractory of HCC. In recent years, an accumulation of studies has shown that LCSCs originate from Hepatic Progenitor Cells (HPCs), hepatocytes, liver cancer cells and extrahepatic stem cells, and find that these cells transform into LCSCs through signaling pathways, gene mutation and other mechanisms. This paper reviews the original cells of LCSCs and their transformation mechanisms discovered in the last decade. In addition, in the process of HPCs and hepatocytes transforming into LCSCs, there is a cross-connecting network between the signaling pathways, which will provide some valuable clues for the targeted therapy of LCSCs.

Keywords: Hepatocellular carcinoma; Liver cancer stem cells; Origin; Mechanism

Abbreviations

HCC: Hepatocellular Carcinoma; LCSCs: Liver Cancer Stem Cells; HPCs: Hepatic Progenitor Cells; EpCAM: The Epithelial Cell Adhesion Molecules; TGF-β: Transforming Growth Factor-β; HOCs: Hepatic Oval Cells; COH: The Canal of Hering; Ring 1: The Ring Finger Protein 1; HNF4α: Hepatocyte Nuclear Factor 4α; EMT: Epithelial-Mesenchymal Transition; TNF-α: Tumor Necrosis Factor-α; HBx: Hepatitis B Virus X; NF2: Neurofibromatosis Type 2; AFB: Aflatoxin B_1; DCLK1: Doublecortin-like Kinase 1; ESC-related: The Embryonic Stem Cell-related; lncRNA: Long non-coding RNA; THOR: Testis-Associated Highly Conserved Oncogenic lncRNA; LEF1: Lymphoid Enhancer-binding Factor-1; CHD1L: Chromodomain-Helicase-DNA-binding-protein 1-like; MSCs: Mesenchymal Stem Cells; IR-MSCs: Irradiated MSCs; CiSCs: Cancer-induced Stem Cells; BM-MSCs: Bone Marrow-Mesenchymal Stem Cells

Introduction

Primary liver cancer can be divided into Hepatocellular Carcinoma (HCC), intrahepatic cholangiocarcinoma, liver angiosarcoma, hepatoblastoma, and fibrolamellar carcinoma [1-3]. HCC is one of the most common malignancies in the world, accounting for 75% to 85% of primary liver cancer. It is the second leading cause of cancer-related death worldwide [3,4].

Liver Cancer Stem Cells (LCSCs) have the ability of self-renewal and differentiation, chemical drug resistance and tumorigenicity even after serial transplantation. Multiple cell surface markers, such as the Epithelial Cell Adhesion Molecules (EpCAM), CD133, CD44, CD13, CD90, CD47, CD44, CD24 and OV6, have been isolated to enrich corresponding LCSCs in HCC [1,5]. There are many signaling pathways regulating LCSCs, including Wnt/β-catenin, Notch, Transforming Growth Factor-β (TGF-β), Hedgehog [1] and IL-6/STAT3. Both RAS/RAF/MEK and PI3K/Akt/mTOR pathways are deregulated in LCSCs and they primarily involve in the regulation of cell growth, survival and differentiation of HCC [6].

Surgical resection of HCC has made great progress in the past years, but the 5-year survival rate of HCC patients is still unsatisfactory due to the frequent recurrence and chemotherapy resistance. More and more evidence has shown that LCSCs are critical for leading to chemotherapy resistance, recurrence, poor prognosis and incurable disease of HCC [7,8].

Therefore, targeting LCSCs contributes to the treatment of HCC. Small-molecule inhibitors against dysregulation of signaling pathways may effectively suppress LCSC-mediated tumorigenesis,

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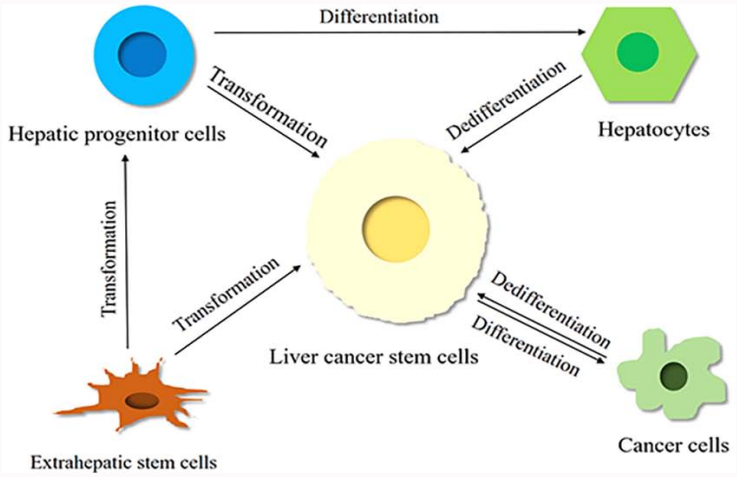

Figure 1: Potential origins of liver cancer stem cells.

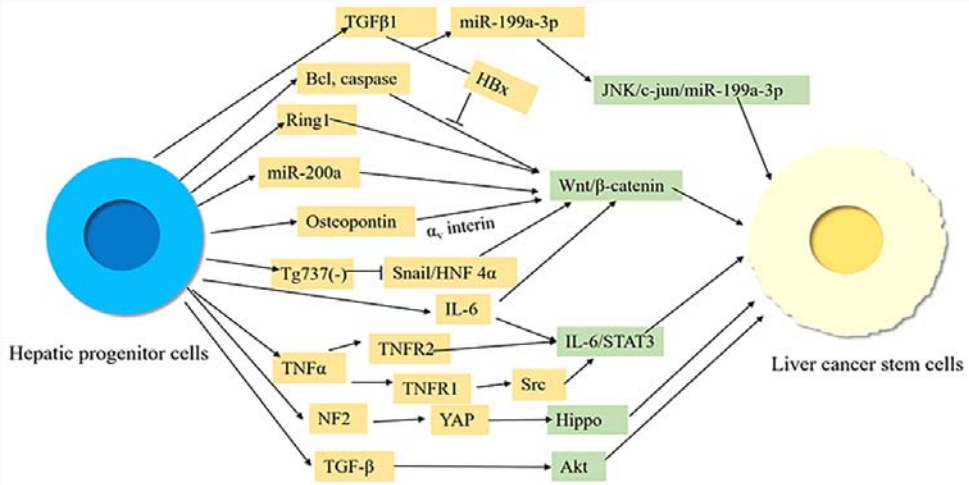

Figure 2: Hepatic progenitor cells transform into liver cancer stem cells through the imbalance of signal pathway. The promoting effect is represented by ⟶. The inhibition is represented by ⟞. The positive effect is represented by (+). The negative effect is represented by (-).

metastasis and self-renewal [1]. In addition, the targeted therapeutic strategies about the original cells of LCSCs and their transformation mechanisms can also achieve the purpose of reducing LCSCs and effectively inhibit the growth, metastasis and self-renewal of tumors. A large number of studies have shown that Hepatic Progenitor Cells (HPCs), hepatocytes, liver cancer cells and extrahepatic stem cells can transform into LCSCs [5,9] (Figure 1). In this review, the original cells and transformation mechanisms of LCSCs discovered in the last decade are comprehensively discussed.

LCSCs Originate from HPCs

HPCs, known as the Hepatic Oval Cells (HOCs), locate in the Canal of Hering (COH). In severe liver injury, the regenerative ability of hepatocytes is impaired, and liver stem cell chambers proliferate and activate. After HPCs amplifying and maturing, cells at the COH site showed the common morphology and immunophenotype of hepatocytes and bile duct cells [10]. An increasing number of experiments have shown that HPCs may transform into LCSCs through signaling pathways, gene mutation and other mechanisms, leading to the occurrence and development of HCC (Figure 2).

Dysregulation of the signaling pathways

Wnt/β-catenin signaling pathway: The activation of Wnt/β-catenin in HPCs is sufficient to cause the malignant transformation of HPCs and lead to the generation of HCC [11,12]. The Ring finger protein 1 (Ring1), a transcriptional repressor, displays tumorigenic activity. Overexpression of Ring1 activated the Wnt/β-catenin signaling pathway and upregulated the expression of Cyclin D1 and c-myc in HPCs, and may drive the transformation of HPCs into LCSCs [13]. In addition to overexpression of Ring1, downregulation of Tg737 and miR-200a can also activate the Wnt/β-catenin pathway. Knockout Tg737 inhibited the expression of snail controlled by Hepatocyte Nuclear Factor 4α (HNF4α), resulting in the imbalance of the Wnt/β-catenin/Snail-HNF4α negative feedback circuit, which made HPCs acquire LCSC-like features in the process of malignant transformation and promoted Epithelial-Mesenchymal Transition (EMT) [14]. However, downregulated miR-200a could directly target β-catenin of HPCs and activate the Wnt/β-catenin pathway, which led to the tumorigenicity of HPCs [15]. In addition, autocrine osteopontin induced the destruction of the E-cadherin/β-catenin complex *via* α_v integrin-Src signaling [11], therefore led to the activation of β-catenin and the malignant transformation process of HPCs.

TNFR /STAT3 signaling pathway: The activation of the TNFR /STAT3 signaling pathway closely relates to the occurrence of tumors. Tumor Necrosis Factor-α (TNF-α) is the most important

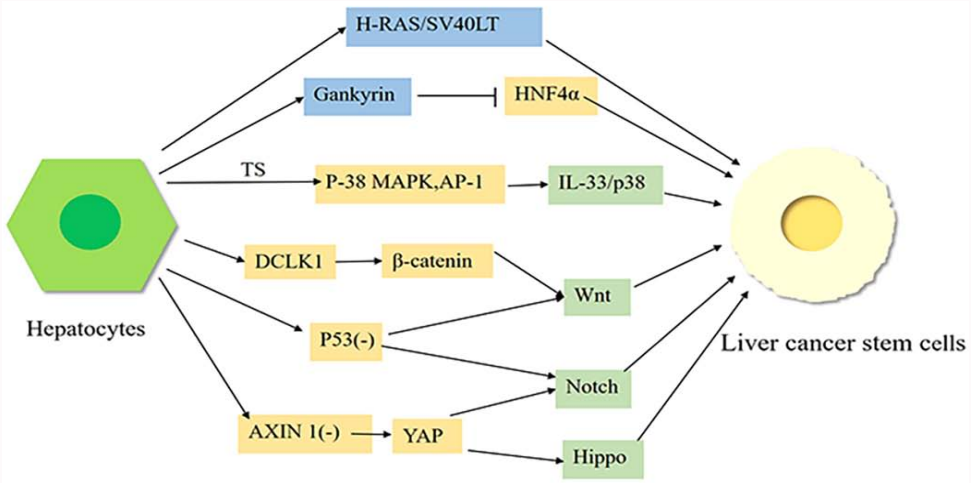

Figure 3: Hepatocytes transform into liver cancer stem cells through the imbalance of signal pathway and gene mutation. Signal pathway is green. Regulatory molecule is yellow. Gene is blue. The promoting effect is represented by ⟶. The inhibition is represented by ⊣. The negative effect is represented by (-).

cytokine in inflammation-associated tumorigenesis. Dysregulation of the TNFR-2/STAT3 signaling pathway can activate HPCs, and the activated HPCs abnormally differentiate into HCC, leading to the development of HCC [16]. TNF-α could also activate the TNFR 1/Src /STAT3 signaling pathway, up-regulate the expression of Nanog and Lin28, and promote the self-renewal of HPCs via the deregulation of ubiquitin D and checkpoint kinase 2 to trigger the chromosomal instability of HPCs. The transformation of HPCs to LCSCs is synergically promoted [17]. The experimental study found that there was a cross-linking network between IL-6/STAT3 and Wnt/β-catenin signaling pathway. The higher titers of IL-6/STAT3 and Wnt/β-catenin signaling pathway suggested that Hepatitis B virus X (HBx) may induce the intrinsic changes of HPCs through the way of the above signaling pathway. Thus, HPCs have tumorigenic potential [18].

Akt signaling pathway: HPCs can induce the activation of Akt to produce LCSCs *via* TGF-β, then result in the occurrence of HCC [19,20]. TGF-β, as a hepatic profibrogenic cytokine, is mainly produced by activated mesenchymal cells upon chronic liver injury. TGF-β-induced activation of Akt and transformation of HPCs were mediated by microRNA-216a-modulated phosphatase and tensin homolog deleted on chromosome 10 suppression, leading to the production of LCSCs in the liver. Inactivation of FOXO3a in HPCs is also associated with the generation of LCSCs [20].

Hippo signaling pathway: It has been found that in addition to the activation of Wnt/β-catenin and TNFR/STAT3 signaling pathways, the dysregulation of the Hippo signaling pathway also gives rise to the malignant transformation of HPCs. Neurofibromatosis type 2 (NF2) gene is a tumor suppressor gene that encodes the protein, Merlin. As the only gene of the Hippo signaling pathway that mutates and inactivates in cancer, the expression of Merlin in tumor cells shows a significant negative correlation with the expression of YAP. Epidermal growth factor receptor can drive the excessive proliferation of NF2-/- progenitor cells inactivated by NF2 mutation, thus leading to the occurrence of tumors [21,22].

JNK/c-jun/miR-199a-3p signaling pathway: Viral infection, inflammation and other pathological causes can cause abnormal proliferation of HPCs, then lead to the transformation of HPCs into LCSCs with self-renewal ability and differentiation potential, and result in the occurrence of HCC [3,23]. HBx, a multifunctional HBx protein encoded by HBV, plays an important role in viral replication and HBV-induced carcinogenesis. HBx and TGF-β1 can induce the transformation of HPCs to LCSCs. TGF-β1 up-regulated miR-199a-3p, and cooperated with HBx to promote the malignant transformation of HPCs through JNK/c-jun/miR-199a-3p signal pathway [23]. JNK/c-jun/miR-199a-3p and Wnt/β-catenin pathways cross-link each other through HBx. HBx inhibited HPCs apoptosis by interfering with the balanced expression of Bcl2 family-related proteins and the caspase protein through activating Wnt/β-catenin signaling pathway, thus induced malignant transformation of HPCs [24].

Gene mutation

Tumorigenesis comprises multiple processes with an accumulation of genetic mutations that drive the progressive transformation of HPCs into malignant tumors [25]. Genetically engineered fetal liver progenitor cells lacking the function of tumor suppressor genes are the origin of HCC. Activation-induced cytidine deaminase may promote the malignant transformation of HPCs through its mutagenic activity [25]. The gene, deleted in malignant brain tumors 1 at chromosome 10q25.3-26.1, is a candidate tumor suppressor gene *via* virtue of frequent deletions and lack of expression, taking part in the malignant process of HPCs [26].

Some researchers have used Aflatoxin B_1 (AFB_1) to treat HOCs transfected with HBx gene and find that the synergistic action of HBx gene and AFB_1 made HOCs have the ability to form HCC. Moreover, p53 null transformed HOCs give rise to HCC [27].

Thorgeirsson's study speculated that oncogenic H-RAS and SV40LT reprogrammed HPCs and mature hepatocytes into cancer stem cells. EMT-related pathways were activated by different liver cell lines during oncogenic reprogramming, and H-RAS/SV40LT-mediated oncogenic reprogramming of adult hepatocytes requiring myc [28].

LCSCs Originate from Hepatocytes

The liver consists of two major cell types, parenchyma and non-parenchyma cells. Hepatocytes are the most abundant hepatic parenchyma cells, accounting for 92.5% of the liver [29]. It has been found that hepatocytes are highly plastically and can dedifferentiate

into immature progenitor cells under the condition of activation of the Hippo signal pathway [30]. Further experiments showed that HCC stemmed from hepatocytes. Different pathophysiological diseases can cause permanent hepatocyte injury, regeneration and inflammation, which promote hepatocytes to dedifferentiate into LCSCs and lead to HCC (Figure 3) [31,32].

Dysregulation of the signaling pathway

Abnormal activation of the Wnt/β-catenin signaling pathway can bring about reprogramming of hepatocytes. Reprogramming of hepatocellular plasticity was mediated by Doublecortin-Like Kinase 1 (DCLK1). Overexpression of DCLK1 induced spheroid growth in untransformed primary human hepatocytes in suspension culture, which promoted the clonality and tumorigenicity of hepatic epithelial cells by activating β-catenin [33]. Loss of p53 promoted mature hepatocytes to dedifferentiate into nestin-positive progenitor-like cells, which may differentiate into HCC or cholangiocarcinoma after lineage-specific mutations via targeting Wnt and Notch signals [34]. The imbalance of Notch and YAP/Hippo signaling pathways mediated hepatocellular reprogramming, and there was a link between HCC initiation and hepatocellular reprogramming [32,35]. Besides, studies have shown that deletion of AXIN1 induced Notch and YAP signal transduction and led to HCC, but did not depend on the activation of Wnt/β-catenin [36]. P-38 MAPK and AP-1 in the IL-33/p38 signal pathway were activated during exposure to long-term tobacco smoking, and induced liver cancer stem cell-like characteristics of hepatocytes [37].

Gene mutation

When co-transduced with H-RAS and SV40LT, the Embryonic Stem Cell-related (ESC-related) gene in hepatocytes was activated and the expression of myc was significantly up-regulated by 21 times. However, knockout c-myc in hepatocytes expressing H-RAS/SV40LT decreased the production of LCSCs, which verified that c-myc was the key element for the activation of ESC-related genes in hepatocellular carcinogenic reprogramming into LCSCs [28]. Gankyrin, also known as 26S proteasome non-ATPase regulatory subunit, is an oncoprotein that is mainly overexpressed in HCC. It was found that the overexpression of Gankyrin could attenuate the hepatic function of primary hepatocytes. Furthermore, Gankyrin bound to HNF4α to promote proteasome-dependent HNF4α degradation in liver cancer cells, which indicated that Gankyrin mediated dedifferentiation of hepatocytes through down-regulating HNF4α, and then promoted the production of LCSCs and the development of HCC [38].

LCSCs Originate from Liver Cancer Cells

There are most differentiated liver cancer cells and a small part of LCSCs in HCC tissue [39]. During the development of liver cancer cells, the reactivation of gene expression signals makes them show similar phenotypes of their lineage precursor cells, which promotes the malignant transformation of tumors to a great extent [40]. Increasing evidence showed that liver cancer cells could dedifferentiate through signal pathways, gene mutation and other mechanisms, leading to the production of LCSCs [41,42].

Dysregulation of the signaling pathway

Wnt/β-catenin signaling pathway: Experiments have shown that the Wnt pathway played an important role in the activation of HCC-LCSCs and the transformation from differentiation to dedifferentiation [43]. The spheres of liver cancer cells with overexpression of DCLK1 expressed highly active β-catenin, α-Fetoprotein and SOX9, indicating that overexpression of DCLK1 could induce clonality and dedifferentiation phenotype of liver cancer cells [33]. The stimulation of TGF-β1 led to the overexpression of CD147. The signal of TGF-β1-CD147 in highly differentiated liver cancer cells is activated through β-catenin and matrix metalloproteinase, and the expression of differentiation marker HNF4 mRNA is inhibited, thus inducing the dedifferentiation of liver cancer cells [41]. The cytoplasmic CDC73 in liver cancer cells was phosphorylated and bound to β-catenin under the action of Shp2, which promoted the nuclear translocation of β-catenin. Activation of the Wnt/β-catenin signal pathway promoted the dedifferentiation of liver cancer cells [42]. In addition, various long non-coding RNA (lncRNA) have been shown to play a role in the occurrence and development of HCC [44]. A new LncRNA, THOR (Testis-associated Highly conserved Oncogenic lncRNA) promoted the dedifferentiation of liver cancer cells and expansion of LCSCs by targeting the β-catenin signaling pathway [45].

STAT3 signaling pathway: LncARSR regulated the transduction of the STAT3 signal in liver cancer cells, and targeted STAT3 signal pathway to promote dedifferentiation of liver cancer cells and LCSCs proliferation [46]. Oct4 and Nanog are essential transcription factors to maintain the phenotype of stem cells. Studies have shown that ectopic co-expression of Oct4 and Nanog could regulate the signal transduction and the activation of the STAT3, which made MHCC97-L cells (liver cancer cells) with the characteristics of LCSCs, and promoted EMT by activating STAT3/Snail signal [47].

Notch signaling pathway: Studies have shown that the abnormal activation of the Notch signal pathway promoted the dedifferentiation of liver cancer cells. Lymphoid Enhancer-binding Factor-1 (LEF1) is a member of the LEF1/T-cell factor family. LEF1 could activate key members of the Notch signal pathway (such as NOTCH1 and NOTCH2) by directly binding to their promoter regions. LEF1 promotes tumor stemness and poor differentiation of HCC by activating the Notch signal pathway [48]. The high expression of HNF-1β not only promoted the dedifferentiation of liver cancer cells to LCSCs by activating the Notch pathway, but also enhanced the invasive potential of liver cancer cells, and promoted the occurrence of endoderm metastasis of liver cancer cells, and thus promoted the migration and invasion of liver cancer cells [49].

Other signaling pathways: The down-regulation of miR-613 promoted the expansion of LCSCs by promoting dedifferentiation of liver cancer cells and self-renewal of LCSCs, while overexpressed miR-613 inhibited the dedifferentiation of liver cancer cells by targeting the SOX9 signal [50]. Up-regulation of miR-365 inhibited the expansion of LCSCs through RAS-related C3 botulinum toxin substrate 1 signal inhibiting dedifferentiation of liver cancer cells and reducing the self-renewal ability of LCSCs [51]. In addition, KLF4 could directly activate the expression of HNF-6 by binding to its promoter. KLF4 promoted the differentiation of HCC by inducing the expression of HNF-6, and the imbalance of the KLF4/HNF-6 pathway promoted the dedifferentiation of HCC [52]. Transcriptionally activated p73β activated YAP, but did not activate the expression of Bax, which may promote malignant dedifferentiation of liver cancer cells [53].

Gene mutation

Knockout tumor suppressor atonal homolog 8 gene could mark potential CSCs in HCC and induce CD133-cells to differentiate into CD133+ cells, making them with CSCs characteristics such as self-renewal, differentiation and chemotherapy resistance [54]. Transcription factors, including Snail, Slug and Twist, mediated

dedifferentiation and acquired characteristics of stem cell under certain conditions [41]. Transcription factor KLF4 could directly bind to the EpCAM promoter, up-regulate the expression of EpCAM and E-CAD, induce partial reprogramming of Huh7 cells, lead to dedifferentiation of Huh7 cells, and increase the number of EPCAM+/CD133+LCSCs in Huh7 liver cancer cell lines with the increase of dryness and tumorigenicity [55].

HBx not only induces the transformation of HPCs into LCSCs, but also produces LCSCs by promoting the dedifferentiation of liver cancer cells. HBx could activate Oct-4, Nanog, KLF4, β-catenin and EpCAM *in vivo* and *in vitro*. HBx promoted the stemness of liver cancer cells by activating β-catenin and epigenetic up-regulation of miR-181. HBx could also stimulate cell migration, soft agar growth and spheroid formation. HBx promoted the occurrence of HCC by promoting changes in the expression of genes that were characteristics of LCSCs [56].

The oncoprotein Gankyrin promoted the development of HCC by down-regulating the dedifferentiation of liver cancer cells mediated by HNF4α and promoting the production of LCSCs [38]. Chromodomain-Helicase-DNA-binding-protein 1-like (CHD1L) belongs to the chromodomain-helicase-DNA-binding domain-containing chromatin remodeling family. The overexpression of CHD1L could maintain the "open chromatin" structure of the activity of estrogen-related receptor-β and transcription factor 4 promoter, thus endow liver cancer cells with progenitor cell-like characteristics [57].

LCSCs Originate from Extrahepatic Stem Cells

Stem cells are undifferentiated cells that exist in the embryonic, fetal and adult stages of life and produce differentiated cells that form tissues and organs [58]. Mesenchymal Stem Cells (MSCs) are a kind of stromal cells with the ability of self-renewal and multi-directional differentiation, which are initially identified from bone marrow. Bone marrow is the most abundant and characteristic source of MSCs [59]. The long-life span and self-renewal ability of MSCs make them survive long enough, accumulate DNA damage, produce cancer cells, and exert the function of CSCs [60]. Studies have shown that extrahepatic stem cells transformed into LCSCs through a variety of mechanisms.

Dysregulation of the signaling pathway

Irradiated MSCs (IR-MSCs) can also maintain the stemness of LCSCs through the Wnt/β-catenin signal pathway [61]. It has been found that CSCs were isolated from tumor cell lines transformed from MSCs. So it is speculated that the mutated MSCs may be the origin of CSCs [62,63]. Bone marrow-derived MSCs may also differentiate into LCSCs and promote the occurrence and development of HCC [60,64].

MSCs are heavily recruited into the tumor microenvironment. After exposure to factors secreted by cancer cells, it may show cellular plasticity, obtain more stem cell-like states, and obtain some characteristics of CSCs. These acquired characteristics may contribute to the progression, survival and metastasis of the tumor. We call these cells Cancer-induced Stem Cells (CiSCs), which are directly produced by human Bone Marrow-Mesenchymal Stem Cells (BM-MSCs) exposing to cancer cell lines. Wnt/β-catenin signal may play an important role in the transformation from BM-MSCs to CiSCs. These data suggested that soluble factors produced by cancer cells contributing to the transformation of normal human BM-MSCs into cells with the characteristics of cancer stem cells [60].

Cell fusion

Cell fusion is a strictly regulated process, including initiation, chemotaxis, adhesion, fusion and fusion after five steps [65]. It has been observed in experimental animals and some human subjects that the fusion of two cells induced malignant transformation of cells [66]. SK cells represented the transformation mechanism from normal MSCs to enhanced self-renewing CSCs with metastatic ability. SK cells and their xenograft cells represented the relative homogeneity of CSCs with substantial metastatic ability. Therefore, it represents a new mechanism of non-epithelial and endothelial CSCs in tumorigenesis, development and metastasis [64]. CD34+ LCSCs are formed by the fusion of HPCs and myeloid intermediates (granulocytic monocytes) derived from CD34+ hematopoietic progenitor cells. Circulating bone marrow-derived precursors can become the origin of tumorigenesis and carcinogenesis by fusion, transdifferentiation or reprogramming after homing to damage organs under pathological conditions [67].

Conclusion

At present, a large number of studies have shown that LCSCs can be derived from a variety of cells. This paper reviews the transformation of HPCs, hepatocytes, liver cancer cells, and extrahepatic stem cells into LCSCs, through signal pathways and gene mutations and the relationship between these transformation mechanisms. From these transformation mechanisms, we can find that there are cross-connected networks in the signal pathways of cell transformation, which enables us to further understand the complexity of cell transformation into LCSCs. In the process of transformation from HPCs to LCSCs, HBx acts as a cross-linking regulatory molecule to connect Wnt/β-catenin and JNK/c-jun/miR-199a-3p pathways, while Wnt/β-catenin signaling pathways are interconnected with IL-6/STAT3 pathways through IL-6. In the transformed mechanisms of hepatocytes, Wnt/β-catenin, Notch and Hippo pathways are cross-linked through p53 and YAP. It is also explaining whether MSCs produce LCSCs through fusion with HPCs or directly convert them into LCSCs through contact with soluble factors secreted by cancer cells.

Although experimental studies have described the origin of LCSCs and their mechanisms and studied the relationship between signal pathways, the relationship between various traceable cells into LCSCs is still unclear, and the cross-linking relationship among the Akt signal pathway, Wnt/β-catenin, Notch, Hippo and other pathways needs to be further verified. In this crossed network, the specific molecular mechanism of the interaction between signaling pathways and gene mutations and how they accurately participate in the regulation of LCSCs is still unclear. Signal pathways, gene mutation and the cross-linking relationship inter them provide us with a new direction of treatment. Looking forward to the future, the combined targeted therapy for the origin of LCSCs or the network connection of signal pathways may be a new therapeutic method to prolong the survival time and reduce the recurrence of HCC.

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