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Research progress in targeting liver cancer stem cells for the treatment of liver cancer

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Abstract: *CSCs (tumour stem cells) have been shown to be resistant to conventional chemotherapy or irradiation, and surviving CSCs can re-proliferate and lead to tumour recurrenc and Metastasis under suitable conditions due to their strong self-renewal and multiple differentiation functions. The development of metastasis, recurrence and drug resistance caused by CSCs is now considered to be a major cause of treatment failure and patient death inmalignancies[1] .Therefore,direct targeting of LCSCs (hepatocellular carcinoma stem cells) is a new strategy for the treatment of hepatocellular carcinoma. Over the years, as research on LCSCs continues, therapies targeting LCSCs have been found to have great potential in the clinical management and prognosis of hepatocellular carcinoma. This article reviews the specific application of liver cancer stem cell markers interacting with relevant signalling pathways in the treatment of hepatocellular carcinoma.*

Keywords: *Liver cancer stem cells; Molecular signaling pathways; Targeted therapy*

1. Liver cancer stem cells

CSCs have high DNA repair capacity, telomerase activity, and express high levels of anti-apoptotic proteins Bcl-2, Bcl-xL, and multidrug-resistant membrane transporter protein ABC transporter, etc. They can continuously self-renew and divide and proliferate. LCSCs are mainly derived from hepatic progenitor cell transformation, hepatocyte mutation or dedifferentiation in three main ways, and there are different molecular markers on their surface, forming There are different molecular markers on their surface, resulting in different molecular phenotypes. Nearly 20 molecular surface markers have been used for the isolation and identification of LCSCs or for targeting therapies, including CD44, CD90, CD133, CD13, ALDH and EpCAM, but not all LCSCs markers are specific, and some surface molecules are also expressed in other tumor stem cells or even in normal embryonic stem cells and normal tissues[2]. Therefore, some markers remain controversial and the validation of surface markers regarding hepatocellular carcinoma stem cells is a challenging task.

Moreover, these surface molecules can accentuate stem cell properties through different signaling pathways. According to Sakunrangsit et al. reported that EpCAM+ can lead to enhanced self-renewal and differentiation of hepatocellular carcinoma stem cells [3-4], and this effect is mainly activated through the Wnt/β-catenin signaling pathway. While molecules such as CD133+ and CD13+ are associated with drug resistance in LCSCs, the resistance of CD133+ is mainly dependent on the Akt / PKB and Bcl- 2 signaling pathways [5], while the resistance of CD13+ may be associated with the ROS signaling pathway [6]. Therefore, recognizing the link between stem cell markers and related signaling pathways is a major breakthrough in finding a way to target hepatocellular carcinoma stem cells for the treatment of hepatocellular carcinoma. Other common signaling pathways used by liver cancer stem cells to maintain stem cell properties are TGF-β, Hippo-Y AP/TAZ, Hedgehog, Notch, and Nanog [7].

Current targeted therapies for hepatocellular carcinoma stem cells mainly involve genetic, molecular, and cellular aspects [8], and in this paper, we describe the latest research progress in targeting hepatocellular carcinoma stem cells through each of these aspects. Taken together, the CSC theory may provide an explanation for the refractoriness of liver cancer and may provide useful insights for scientists to design new therapies for liver cancer.

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2. Targeting LCSCs at the genetic level

With the increasing research on cancer, various proto-oncogenes or oncogenes have been identified, which are usually closely related to abnormal cell signaling. Currently, the application of gene targeting therapy in CSC includes the silencing of oncogenes, the most common methods are gene knockdown, microRNA (miRNA) inhibition and RNA interference (RNAi); furthermore, there are epigenetic modifications or the use of small molecule inhibitors, the construction of RISC analogs, the binding of the target 3'UTR, etc. In short, these cancer-promoting genes are prevented from performing their regulatory functions properly. The goal pursued for oncogenes is to restore their expression or overexpression by using lentiviruses as vectors to exploit their good penetration and local amplification to exert gene transfer capability in tumor cells.

3. Targeting LCSCs at the molecular level

In recent years, stem functional molecules CDl33, CD13, CD90 and EpCAM have become the most studied specific marker molecules independently expressed on the surface of CSCs [7].The proposal of CSCs specific marker molecules has brought new ideas for targeted therapy of liver cancer, proposing target molecular therapies that selectively kill CSCs, i.e., new drugs should kill tumor cells while also removing CSCs, proposing new solutions to overcome tumor drug resistance and prevent recurrence and metastasis after treatment. The ways of targeting LCSC surface molecules include direct action of antibodies and inhibitors on the molecules to inhibit their functional activity; when there is a correlation or mutual regulation relationship between the expression of two molecules, the combination of two antibodies is used for their dual inhibition, or severing the link between the two parts to control the metastasis of liver cancer cells to specific tissues and organs; also through short peptide complexes, aptamers [21], biological nanoparticles, etc. as probes for tumor stem cell recognition to enhance the intensity of antitumor drug presentation and action.

3.1 Targeting CD133

CD133, a transmembrane glycoprotein, is considered one of the major markers of LCSC with self-renewal, multispectral differentiation, and chemoresistance capabilities.

Monoclonal antibodies are commonly used as ligands for cd133-targeted therapies. These antibodies can carry various drugs or toxins to the target to enhance the body's immune response to the disease. These approaches have the advantages of high target specificity, low molecular weight, low side effects and good patient compliance that traditional anti-cancer drugs do not have. Currently reported antibodies against CD133 are AC133, 293C3 and AC141, of which AC141 and 293C3 are antibodies against CD133/2. Smith et al [9] combined mouse anti-human CD133 antibody with the anti-microtubule cytotoxic drug monomethyl auristatin E and confirmed that the complex inhibited both in vivo and in vitro CD133+ LCSCs-like cell growth.Lang et al [10] prepared a highly stable and specific 131I-CD133 monoclonal antibody (mAb) in vitro, and in vivo experiments showed that 131I-CD133 monoclonal antibody may be used in clinical diagnosis of LCSCs with high selectivity and stability, as well as immunoimaging and radiotherapy of LCSCs, and clinical trials are currently underway . In the field of CD133-targeted peptides, it has been recently reported [11] that recombinant tumor suppressor T42 peptide can induce apoptosis in LCSCs by increasing bax and Cl-Caspase-3 and inhibiting the protein expression of bcl-2, and regulate LCSCs by upregulating E-cadherin while downregulating the expression levels of Vimentin, MMP-2 and MMP-9 migration and invasion.

It has been found that hygromycin [12] and actinomycin D [13] can inhibit stem cells and malignancies in HCC through CD133 destabilization, thus providing a novel therapeutic strategy to target cancer stem cell-like cells.

3.2 Targeting CD13

CD13, also known as aminopeptidase N, is a zinc-binding protein. Downregulation of CD13 using CD13-neutralizing antibodies or inhibitors induces apoptosis in HCC cell lines Huh7 and PLC/PRF/5. When CD13+ hepatocytes were treated with 5-FU, which directly targets the CD13 molecule, the number of cells with tumorigenic and self-renewal capacity was significantly reduced [14]. Also, because CD13 and CD90 are co-expressed in hepatocellular carcinoma, the combination of CD13 and CD90 inhibitors significantly reduced tumor volume compared to each inhibitor alone [15].Zhang et al [16]

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obtained a functional anti-human CD13 monoclonal antibody MAb 9E4 in 2013 and validated the specificity of this MAb by flow cytometry, demonstrating that that this MAb effectively recognized CD13 molecules expressed on a range of malignant cell lines.

3.3 Targeting CD90

High CD90 expression is associated with poor prognosis [17]. If the surface marker glycoprotein CD44 is also expressed in CD90+ cells, there is a more aggressive phenotype with increased metastatic and self-renewal capacity. When CD44 is blocked by inhibitory antibodies, CD90+ cells have a reduced capacity for tumor formation and metastasis and induce apoptosis.CD45-/CD90+ cells may become a new target for the diagnosis and treatment of hepatocellular carcinoma.CD90 upregulates the expression of the molecular marker CD133 and this aberrant expression can promote tumor progression. Some investigators reported in 2016 that thermosensitive magnetic liposomes (TM) containing magnetic iron oxide (Fe3O4) and the anti-cancer stem cell marker CD90 (CD90 @ TMs) could be used for controlled and targeted delivery of anti-cancer drugs to kill CD90 hepatocellular carcinoma stem cells. [18]

3.4 Targeting CD44

CD44 is a glycoprotein encoded by a single gene and hyaluronic acid is its main receptor. ,Mima et al [19] observed a faster tumorigenesis rate of CD44+ cells than CD44- cells in a nude mouse model of liver cancer, with only CD90+/CD44+ cells present at lung metastasis sites. Blocking CD44 activity using CD44-targeting antibodies can induce apoptosis of CD90+ cells in vitro and inhibit tumor formation in vivo [20].IM7 is a mouse monoclonal antibody that specifically targets CD44 and has the effect of inhibiting tumor growth. So far, given the properties of CD44, studies targeting CD44 short peptides have gradually increased.Cho et al [21] prepared a novel short peptide complex PDPP targeting CD44 by combining the short peptide with d -polylysine.The binding ability of PDPP to CD44 was 4-10 times higher than that of CD44 antibody, suggesting that PDPP could be used as a probe for tumor stem cell diagnosis and therapy. Park et al [22] successfully identified a short peptide P7 (FNLPLPSRPLLR) that specifically binds to CD44 expressed on the surface of breast cancer CSCs. similar to CD44 antibody, P7 has a higher binding rate on MCF7 cells. Therefore, the short peptide P7 could be used as an alternative antibody for the treatment of CSCs to inhibit tumor formation.

3.5 Targeted Nanog

NANOG is a marker of stem cells that can influence tumor progression and treatment resistance of cancer cells. In human hepatocellular carcinoma (HCC), upregulation of NANOG is associated with metastasis and poor survival. Shen et al [23] used a HepG2 cell line to study the effects of metformin and another protein kinase (AMPK) activator, AICAR, on hepatocellular carcinoma stem cells and found that Metformin/ AICAR downregulated NANOG expression, decreased cell viability and enhanced chemosensitivity to 5-fluorouracil (5-FU). Notably, the role of Nanog molecules in the maintenance of stemness of LCSCs in HBV-associated HCC is not negligible. The reason is that the HBV X protein (HBx) of HBV DNA becomes a pro-oncogene after truncation and enhances the properties of LCSCs by Stat3/Nanog cascade, so targeted intervention of Nanog molecules might better suppress tumor stem cells in HBV-associated HCC [24].

3.6 Targeting EpCSC

EpCAM is a transmembrane glycoprotein that mediates cell-to-cell contacts and signals to the nucleus to regulate gene transcription [25] and is significantly upregulated in tissue stem cells and embryonic stem cells [26].EpCAM expression correlates with tumor initiation capacity and tumor invasion [27], and these reasons support the use of EpCAM as a target receptor for cancer drug delivery systems. EpCAM is a target of the Wnt pathway, and RNA interference with EpCAM can inhibit the activity of hepatocellular carcinoma stem cells [28], and outstanding efforts have been made by investigators in this regard. For example, Kaori Ishiguro et al [29] in 2020 used lactogenic nanovesicles (MNV) as biological nanoparticles for the delivery of an RNA that therapeutically regulates β-catenin expression to liver CSC based on the recognition of EpCAM expression. specifically, by synthesizing an oligonucleotide with high affinity and specificity for EpCAM RNA aptamer, which is loaded with small interfering RNA (siRNA) for β-catenin and then combined with MNV to obtain EpCAM-targeted (ET) therapeutic MNV (tMNV), these ET-tMNV can enhance the efficient release of intracellular

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siRNA and inhibit β-catenin expression and tumor growth. In addition to the EpCAM-positive stem cell population, the application of ET-tMNV is expected to have a wider clinical application in tumor therapy other than HCC.

4. Development prospects

The discovery of hepatocellular carcinoma stem cells has provided new theoretical explanations for the occurrence and development of hepatocellular carcinoma, while targeting hepatocellular carcinoma stem cells has become one of the approaches for hepatocellular carcinoma treatment. The discovery of stem cell markers and signaling pathways is the key to control the biological functions and tumorigenesis and development of liver cancer stem cells, which provides ideas for new drug development. Immunotherapy targeting CSCS is one of the important therapeutic approaches to eliminate CSCS. Among them, CSCS T-cell chimeric antigen receptor (CAR-T) therapy is used for direct eradication of CSCS because CSCS expresses single or multiple specific cell surface markers, and this method is favored by many researchers. Nanodrug-based therapy allows drug delivery and release to be effectively controlled and is one of the approaches to directly target CSCs. Besides, the effects of tumor microenvironment such as matrix hardness, oxidative stress and hypoxia on stem cells cannot be ignored, and the clarification of tumor microenvironment-related signaling pathways is of great value to interfere with tumor microenvironment.

Research on the treatment of hepatocellular carcinoma through intervention with hepatocellular carcinoma stem cells is still in its infancy, and further research is needed on how to make the efficacy more direct and effective in the treatment. However, the bigger problem is that the differences between normal stem cells and hepatocellular carcinoma stem cells are not fully understood, and there are overlapping key markers and signaling pathways between the two, and developing drugs that specifically target hepatocellular carcinoma stem cells without affecting normal stem cells is a major challenge.

References

[1] Li Ya. Targeted intervention of STAT3 signaling pathway to induce immunogenic death in hepatocellular carcinoma cells and inhibit tumor stem cell properties[D]. Shandong University,2021.
[2] Liu,L; Borl ak,J. Advances in Liver Cancer Stem Cell Isolation and their Characterization.[J].Stem Cell Rev Rep.2021,17(4):1215-1238.
[3] Sakunrangsit N, Ketchart W. Plumbagin inhibits cancer stem-like cells, angiogenesis and suppresses cell proliferation and invasion by targeting Wnt/β-catenin pathway in endocrine resistant breast cancer. Pharmacol Res. 2019 Dec;150:104517.
[4] Yamashita T, Budhu A, Forgues M, et al. Activation of hepatic stem cell marker EpCAM by Wnt-beta-catenin signaling in hepatocellular carcinoma[J]. Cancer Res, 2007, 67(22):10831-10839.
[5] Ma S, Lee T K, Zheng B J, et al. CD133 + HCC cancer stem cells confer chemoresistance by preferential expression of the Akt / PKB survival pathway [J]. Oncogene, 2008, 27(12):1749-1758.
[6] Haraguchi N, Ishii H, Mimori K, et al. CD13 is a therapeutic target in human liver cancer stem cells[J]. J Clin Invest,2010,120(9):3326-3339.
[7] Zhang N, Bai Suhang, Zhang Fuhan, et al. Advances in the study of molecular markers and stemness maintenance mechanism of hepatocellular carcinoma stem cells[J]. Journal of Biological Engineering, 2021, 37(8):2719-2736.
[8] Li Y, Han Q, Zhao H, et al. Napabucasin Reduces Cancer Stem Cell Characteristics in Hepatocellular Carcinoma[J]. Frontiers in Pharmacology,2020,11.
[9] Smith LM, Nesterova A, Ryan MC, et al: CD133/prominin-1 is a potential thera- peutic target for antibody-drug conjugates in hepatocellular and gastric cancers. Br J Cancer 99:100- 109, 2008 .
[10] Lang J, Lan X, Liu Y, et al: Targeting cancer stem cells with an 131I-labeled anti-AC133 monoclonal antibody in human coolorectal cancer xenografts. Nucl Med Biol,2015,42:505-512.
[11] Ma P F, Yang W J, Zhu S S, Zhang S B, Zheng Y W, Zhang X F. Inhibitory effect of T42 peptide on the malignant phenotype of CD133-positive hepatocellular carcinoma stem cells[J]. Chinese Journal of Experimental Surgery,2021,38(1):67-70.
[12] Song,Y; Kim,IK; Choi,I; et al. Oxytetracycline have the therapeutic efficiency in CD133+ HCC population through suppression CD133 expression by decreasing of protein stability of CD133.[J].Sci Rep. 2018,8(1):16100.
[13] Song Y, Park I S ,J Kim, et al. Actinomycin D inhibits the expression of the cystine/glutamate

ISSN 2616-5791 Vol.4, Issue 2: 19-23, DOI: 10.25236/AJMHS.2023.040204

transporter xCT via attenuation of CD133 synthesis in CD133+ HCC[J].Chemico-Biological Interactions,2019,309(Suppl 3).
[14] Christ,B; Stock,P; Dollinger, MM; CD13:Waving the flag for a novel cancer stem cell target.[J]. Hepatology. 2011, 53(4):1388-90.
[15] Haraguchi N, Ishii H, Mimori K,et al: CD13 is a therapeutic target in human liver cancer stem cells. J Clin Invest 2010, 120: 3326-3339.
[16] Zhang,K; Chen,W; Zhang,Y;et al. An anti-human CD13 monoclonal antibody that suppresses the suppressive function of Treg cells.[J]. Monoclon Antib Immunodiagn Immunother. 2013,32(1):16-20.
[17] Lu JW, Chang JG, Yeh KT, et al: Overexpression of Thy1/CD90 in human hepatocellular carcinoma is associated with HBV infection and poor prognosis. Acta Histochem , 2011, 113: 833-838.
[18] Yang,R; An,LY; Miao,QF; et al. Effective elimination of liver cancer stem-like cells by CD90 antibody targeted thermosensitive magnetoliposomes .[J]. Oncotarget.2016,7(24):35894-35916.
[19] Mima K, Okabe H, Ishimoto T, et al:CD44s regulates the TGF-β-mediated mesenchymal phenotype and is associated with poor prognosis in patients with hepatocel- lular carcinoma. Cancer Res 72: 3414-3423, 2012.
[20] Kimura O, Takahashi T, Ishii N, et al: Characterization of the epithelial cell adhesion molecule (EpCAM) + cell population in hepatocellular carcinoma cell lines. Cancer Sci 101:2145-2155,2010.
[21] Zhang S, Wu CC, Fecteau JF, et al: Targeting chronic lymphocytic leukemia cells with a humanized monoclonal anti--body specific forCD44.Proc Natl Acad Sci USA 110:6127-6132, 2013.
[22] Park HY, Lee KJ, Lee SJ. Screening of peptides bound to breast cancer stem cell specific surface marker CD44 by phage display. Mol Biotechnol 51: 212- 220, 2012.
[23] Shen,C; Ka,SO; Kim,SJ; et al. Metformin and AICAR regulate NANOG expression via the JNK pathway in HepG2 cells independently of AMPK.[J]. Tumour Biol. 2016,37(8):11199-208.
[24] Ching RHH, Sze KMF, Lau EYT, et al. C-terminal truncated hepatitis B virus X protein regulates tumorigenicity, self-renewal and drug resistance via STA T3/Nanog signaling pathway[J]. Oncotarget, 2017, 8(14):23507-23516.
[25] Nowell PC. The clonal evolution of tumor cell populations. Science. 1976 Oct 1;194(4260):23-8.
[26] Dolle L, Theise ND, Schmelzer E, et al. EpCAM and the biology of hepatic stem/progenitor cells. Am J Physiol Gastrointest Liver Physiol, 2015,308(4) :G233-250 .
[27] Yamashita T, Ji J, Budhu A, et al. EpCAM-Positive Hepatocellular Carcinoma Cells Are Tumor-Initiating Cells With Stem/Progenitor Cell Features[J]. Gastroenterology, 2009,136(3):1012-1024 .
[28] Yamashita T, Ji J, Budhu A, et al. EpCAM-positive hepatocellular carcinoma cells are tumor-initiating cells with stem progenitor cell features. Gastroenterology, 2009, 136(3):1012-1024.
[29] Ishiguro, K; Yan, IK; Lewis-Tuffin, L; et al. Targeting Liver Cancer Stem Cells Using Engineered Biological Nanoparticles for the Treatment of Hepatocellular Cancer.[J]. Hepatol Commun.2020,4(2): 298-313.