

Mitotic quiescence in hepatic cancer stem cells: An incognito mode

Kandasamy Ashokachakkaravarthy, Biju Pottakkat

Department of Surgical Gastroenterology, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, India

Abstract

Hepatocellular carcinoma represents one of the most aggressive cancers with high recurrence rates. The high recurrence is a major problem in the management of this disease. Cancer stem cells (CSCs) are often regarded as the basis of cancer recurrence. The anti-proliferative therapy kills the proliferating cells but induces mitotic quiescence in CSCs which remain as residual dormant CSCs. Later on, withdrawal of treatment reactivates the residual CSCs from dormancy to produce new cancer cells. The proliferation of these newly formed cancer cells initiates new tumor formation in the liver leading to tumor recurrence. HCC cells evade the immune surveillance via modulating the key immune cells by alpha feto-protein (AFP) secreted from CSCs or hepatic progenitor cells. This AFP mediated immune evasion assists in establishing new tumors by cancer cells in the liver. In this review, we will summarise the CSC mechanisms of recurrence, mitotic quiescence, dormancy and reactivation of CSCs, metastasis and immune evasion of hepatocellular carcinoma.

Introduction

Hepatocellular carcinoma (HCC) accounts for 90% of the primary liver cancers and remains the sixth most common cancer globally. Among cancer related deaths, HCC represents a leading

cause worldwide.^{1,2} Specific risk factors for HCC include hepatitis B virus (HBV), hepatitis C virus (HCV), alcoholism, hemochromatosis, aflatoxin, diabetes mellitus, smoking, obesity and non-alcoholic fatty liver disease (NAFLD).¹ For early stage HCC, a partial hepatectomy is considered for curative resection in patients with preserved liver function. For intermediate stage HCC, transarterial embolization (TAE) (embolic particles without chemotherapeutic agents) or transarterial chemoembolization (TACE) (embolic particles with chemotherapeutic agents) offers advantage of tumor necrosis by selective cannulation of tumor feeding arteries with vaso-occlusive particles and/or chemotherapeutic agents. For advanced stage HCC, a systemic therapy of sorafenib, a multikinase inhibitor remains the most common treatment. In patients who fail sorafenib therapy, cytotoxic chemotherapy, immunotherapy and antiviral therapy are being considered as second line treatment depending on the etiology and nature of disease.³ Recurrence after curative treatment remains a major obstacle in the management of HCC. Recurrences after HCC curative treatment are common even in early stage HCC where the 1 year, 3 year and 5 year recurrence rates were approximately 20%, 50% and 75% respectively. Overall survival after treatment remains poor in HCC due to increased recurrence rates.^{4,5} Accumulating evidence shows that cancer stem cells (CSCs) are responsible for HCC recurrence. In HCC, a distinct subpopulation of cells called CSCs or tumor initiating cells (TICs) shows properties of stemness such as self-renewal and proliferation. A growing body of evidence shows that critical biological processes of tumors such as invasion, metastasis and therapeutic resistance towards chemo- and radiotherapy are largely determined by CSC population in a tumor.⁶⁻⁹

Correspondence: Biju Pottakkat, Department of Surgical Gastroenterology, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, 605006, India. E-mail: bijupottakkat@gmail.com

Key words: Hepatocellular carcinoma; cancer stem cells; mitotic quiescence; dormancy; recurrence.

Contributions: AK, retrieving information, reviewing and writing the manuscript; BP, revision and consolidation of manuscript.

Conflicts of interest: the authors declare no potential conflict of interest.

Received for publication: 27 August 2019.

Revision received: 10 October 2019.

Accepted for publication: 2 January 2020.

This work is licensed under a Creative Commons Attribution NonCommercial 4.0 License (CC BY-NC 4.0).

©Copyright: the Author(s), 2020
Licensee PAGEPress, Italy
Oncology Reviews 2020; 14:452
doi:10.4081/oncol.2020.452

Existence of cancer stem cells and revival of stemness

CSC theory hypothesises that a distinct subset of cancer cells called CSCs give rise to its descendants, which are more differentiated and are commonly known as cancer cells. It also states that CSCs govern the growth and spread of tumors. The progenitor cells and cancer cells which are derived from CSCs have limited ability to undergo mitotic division and are predestined to stop proliferation at a time point. This form of cellular hierarchy exists in normal tissues as well, where the normal stem cells (NSCs) produce progenitor cells which gives rise to mature cells.¹⁰⁻¹² This cellular hierarchy in cancer was first evidenced by Pierce and Wallace in 1971. They demonstrated that undifferentiated malignant cells give rise to the genesis of benign differentiated cells.¹³ Biologically, CSCs exists as a malignant equivalent of the NSCs and exhibit stemness to sustain cancer propagation, immune evasion and to interact with its environment for survival factors. Stemness is the ability to self-renew and give rise to differentiated cells by perpetuation of lineage in order to maintain the balance between quiescence, proliferation and regeneration.^{14,15} Recent

evidences show that progenitor cells in many cancers acquire the ability of self-renewal which give rise to CSCs. This process of transformation of progenitor cells back to its predecessor stem cell state is called *dedifferentiation*.¹⁶⁻¹⁹

Dedifferentiation might be induced by various factors in biological systems. In 2006, two researchers from Japan named Takahashi and Yamanaka introduced a concept called induced pluripotency by using dedifferentiation as the principle behind this biological singularity. They demonstrated the transformation of adult cells into induced pluripotent stem cells (iPSCs) by introducing four molecular factors called Oct3/4, Sox2, c-Myc and Klf4 into the cells.²⁰ The factors used to create iPSCs are called Yamanaka factors (Oct3/4, Sox2, c-Myc and Klf4), named after the researcher Yamanaka. The Yamanaka factors were critically involved in the regulation of developmental molecular signalling network which controls pluripotency and stemness.²¹⁻²³ Recently researchers found that radiofrequency ablation (RFA) could induce dedifferentiation in human HCC tissues by heat shock response and also observed that rapid rise of temperature induced heat stress leads to phosphorylation of tyrosine and activation of protein kinase FA/Glycogen Synthase Kinase 3 (GSK) which are further involved in the neoplastic transformation and tumorigenesis.^{24,25}

Identification of hepatic cancer stem cells

The concept of CSCs was proposed in the 1970s, but it gained the first experimental support in 1994, by an animal experiment where a particular subset of cells (CD34+/CD38-) caused xenograft tumors when inoculated to severe combined immunodeficient (SCID) mice but the other subset of cells was unable to create any tumors. This raised the notion that only cells having tumorigenic potential could develop new tumors. By the subsequent years of research, the first evidence for CSCs was documented in 1997 by John Dick, the pioneer in CSC biology who identified CSCs from acute myeloid leukemia (AML).^{26,27} Since then, the CSC research has been accelerated in pursuit of evidence for the existence and identification of CSCs in various cancers including HCC. The properties of CSCs, including tumorigenicity, pluripotency, functional characters and immunogenicity in hosts have been investigated since the identification of CSCs in various cancers.²⁸ CD133 or prominin-1 was first identified as a marker of hematopoietic stem cell (HSC), which was then recognized as the first CSC marker of liver cancer in 2007 and the CSCs identified from liver were termed as hepatic cancer stem cells (HCSCs) or liver cancer stem cells (LCSCs).^{29,30} The discovery of HCSCs led to a drastic change in the etiological perspective of cancer pathophysiology. In the past few years of CSC research, it is reported that HCSCs possess few distinguishing markers such as surface markers (CD133, CD90, EpCAM, CD44, CD47, CD24, CD15, CD13), nuclear markers (Oct4, SOX2 and Nanog), enzymatic markers (ALDH1A1, PLSCR1) and cytoplasmic markers (CK19, OV6).³¹⁻³³ A wide range of research on CSCs in HCC reported that HCSCs are responsible for initiation, progression, therapy resistance, the development of intrahepatic metastases, distant organ metastases, tumor relapse and recurrences in HCC.³⁴⁻³⁸

Cancer stem cells mechanism of recurrence

As stated earlier, HCC has a post-resection recurrence rate as high as 70% at 5 years which affects the overall survival of patients.^{4,39,40} The basis of this high recurrence rate was a mystery

over the past few decades of research until researchers attempted to clarify the influence of surgical resection margins on post-operative recurrence. On the basis of a suspicion that cancer cells may remain in the peri-tumoral tissues of liver even after surgical removal of tumors, they wanted to investigate the effect of various surgical resection margins on recurrence free survival (RFS). As a result, they found that increasing the resection margin could actually decrease the recurrence and increase survival. But this could help only for patients having tumors of small size, single node and absence of macrovascular invasion.^{41,42} These results repeatedly raised a suspicion that surgical resection might not represent the exclusive factor which influencing the recurrence rates of HCC. Concurrently, various studies reported the presence of CSCs in HCC and the HCSC markers correlated with early recurrence of HCC. It is also found that HCSC-specific gene signature is associated with recurrence of HCC. Finally, experimental HCC also demonstrated the tumorigenic potential of HCSCs in SCID mice in which HCSCs produced new tumors suggesting that HCSCs play a critical role in initiating new tumors as well as recurrence of HCC.⁴³⁻⁴⁵ To explore the role of HCSCs in tumor recurrence, investigators created orthotopic HCC mouse models by inoculating human alpha-feto protein (hAFP) expressing HCC cells into immunodeficient mice. These mice were then treated with metronomic cyclophosphamide which caused complete tumor regression and increased the survival significantly but a fraction of circulating hAFP was found even after completion of cyclophosphamide treatment. This observation suggested that a proportion of residual tumor cells capable of producing hAFP may still remain in liver. Immunohistochemical analysis revealed that these residual cancer cells possess CD13 and hAFP but did not express proliferating cell nuclear antigen (PCNA) confirming that these are dormant CSCs with suspended proliferation. Finally, tumor was regrown in mice after withdrawal of treatment.⁴⁶ This experiment demonstrates that residual dormant CSCs revive its proliferative and tumorigenic nature, initiating tumor recurrence after discontinuation of treatment.

Tumor dormancy, mitotic quiescence and reversibility of G0

The concept of tumor dormancy was first introduced by Hadfield in 1954, who described *dormancy* as a state of temporary mitotic and growth arrest. Dormant cancer cells are the malignant cells having potential to proliferate but show no sign of multiplication.⁴⁷ It has been defined that cancer cell dormancy is a state of quiescence exhibited by cancer cells with no constant growth as well as absence of apoptotic and proliferative markers.⁴⁸ But these attributes of quiescence are also exhibited by few other adult cells such as skeletal muscle cells, adipocytes, cardiomyocytes and neurons which exits the cell cycle and enters G0 phase to irreversibly suspend proliferation. Although, these cells may undergo physiological senescence by stimulation of growth promoting pathways which enforces hypertrophy and hyperactivity but they fail to re-enter the cell cycle and replicate.⁴⁹ These cells were considered to be in a state of senescence where the proliferative potential is lost. Senescence is a physiological state of cell cycle arrest but not growth arrest.⁵⁰ In contrast to this, Dormancy is a reversible quiescent state in which the cells undergo both cell cycle arrest and growth arrest where there is no evidence of hyperplasia or hypertrophy.

In the early postulates of cell cycle, it was first generalized that G0 is an irreversible, inactive and non-cycling state by which cells undergo senescence. Later it was rediscovered by an experiment

which demonstrated that a temporary serum starvation could enforce the cells to undergo a reversible G0 phase called *Quiescence*. Since then, senescence was distinguished from quiescence which is a non-dividing state of the cell, achieved by reversible arrest of cell cycle, growth and proliferation. The reversibility of G0 and mitotic arrest marks the quiescence more advantageous than senescence for a cell.⁵¹⁻⁵⁵ In consequence, the dormant cancer cells exist in a state of mitotic quiescence where there is no sign of proliferation or growth. Among various distinguishing properties of CSCs, quiescence exists potentially as a regulatory trait for the maintenance of stemness which is one of the regulatory traits of NSCs.⁵⁶

Hibernating HCSCs

Among CSCs, a subset of cells remains dormant and exist in a quiescent state. These are called hibernating CSCs. Dormant tumors contain a few quiescent CSCs that can be identified by markers such as CD13 and lack of expression of PCNA. CD13 is also known as aminopeptidase N, which is Zn(2+) dependent enzyme. It is a membrane bound ectopeptidase which catalyses the degradation of N-terminal neutral amino acid containing proteins and peptides.^{47,57,58} Recently, the hibernating HCSCs such as quiescent and dormant states of HCSCs were documented by a human study which analyzed the existence of quiescent HCSCs in human HCC tissue samples by analyzing the expression of CD13. The authors found a high expression of CD13 in HCC tissues, significantly correlating with overall poor survival.⁵⁹ Similar results were reported by other studies on pancreatic and non-small cell lung cancers in which the high expression and serum levels of CD13 are strongly associated with poor survival.^{60,61} Another study analyzed the expression of CD13 and its correlation with tumor recurrence in patients with HCC. These authors observed that the expression of CD13 was associated with early recurrences in HCC.⁶² However, other report shows that low PCNA expression resulted in higher rates of disease-free survival after surgery when compared to high PCNA expressing HCC group.⁶³

As said earlier, serum starvation lets the cell to enter into quiescent state. This observation is further supported by an experiment demonstrating the effect of ischemia on HCC. Gade *et al.*, demonstrated that over 79% of HCC cells survived under severe ischemia via undergoing a quiescent state by means of residing in Go/G1 phase of cell cycle after severe ischemic stress. These cells died after treatment with autophagy inhibitor, which confirms that ischemia causes quiescence and autophagy dependence in HCC.⁶⁴ To evaluate the antiproliferative effect, Carr *et al.*, examined the effect of regorafenib (fluoro-sorafenib) on HCC cells. They observed that, as a consequence of sustained exposure of regorafenib, HCC cells acquired a quiescent state and they were able to proliferate after removal of regorafenib treatment. This experiment supports the theory of drug induced quiescence and demonstrated the proliferative potential of the quiescent HCC cells after drug removal.⁶⁵ Another study evaluated the effect of low doses of sorafenib and regorafenib on AFP producing HCC cells, in which the authors found HCC cells being redundant in their AFP producing activity but remained alive during drug exposure.⁶⁶ These observations collectively indicate that all HCC cells were not killed during the exposure of antiproliferative drugs. Some of the cells become quiescent, remain alive and retain proliferative potential which forms the basis of cancer recurrence even after treatment, which is common in HCC.

Dissemination of cancer cells and metastasis

Some cancer cells are disseminated from tumors into nearby areas of the organ leading to intra-organ metastasis and into the circulation causing distant organ metastasis. These are called disseminated tumor cells (DTCs). DTCs can be present and detected in both tumor and blood. These cells could act as a potential source for micrometastasis, tumor spread and distant organ metastasis through hematological dissemination.⁶⁷⁻⁶⁹ Initially a study reported that postoperative dissemination of AFP expressing tumor cells cause metastasis and could be the potential source for recurrence in HCC patients. Moreover, the authors reported that patients with persistently high AFP expression died due to metastases and recurrence, whereby 75% of survivors who had no AFP expression, evidenced no metastases or recurrence.⁷⁰ Subsequently, a study detected DTCs in blood and bone marrow of HCC patients. These DTCs were positive for AFP expression.⁷¹ A case of rapid intra-hepatic dissemination of HCC was reported and the dissemination in lungs occurred after TACE and RFA combination therapy in less than a month caused pulmonary metastases.⁷² Recently, a pilot study attempted to detect the DTCs in HCC. Results revealed that, DTCs were detected in 65% of HCC patients and these patients had high levels of preoperative AFP. Furthermore, DTC positive HCC patients were positive for portal vein invasion and had distant metastases. The distant metastasis-free survival was more in DTC negative HCC but there was no significant difference in liver specific recurrence free survival and overall survival between DTC negative and DTC positive HCC, which indicates that DTC positive patients either have intrahepatic metastases or distant organ metastases leading to recurrence and poor survival.⁷³

Homing DTCs acquire dormancy and stemness

To detect the source of recurrence, an animal study was conducted in which HCC was induced in male rats by diethylnitrosamine (DEN). These male rat HCC livers were then replaced by the livers of healthy female rats by orthotopic liver transplantation. Subsequently, after recurrence, the transplanted female rat livers were analyzed for Y chromosome using in situ hybridization technique. By detection of Y chromosome from DTCs, they found that DTCs from male HCC liver were metastasized to the transplanted female liver. This experiment revealed that DTCs were homing into the new liver after transplantation, leading to tumor recurrence.⁷⁴ These results provide direct evidence for the tumor initiating potential of residual DTCs. A recent study reported the results of an animal experiment on DTCs and CSCs in which it was found that more than 20% of detected DTCs were CSCs and the CSC population expanded after reaching bone marrow. The bone marrow enriched the non-CSCs to transform into CSCs.⁷⁵ Similar observations were obtained from breast cancer, where DTCs in the early stage which were detected in bone marrow exhibited phenotypic features of stemness.⁷⁶⁻⁷⁹

Acquisition of dormancy in residual cancer cells

The mechanisms of DTC acquisition of dormancy and stemness have been proposed previously. DTCs were reported to acquire dormancy after intra-organ metastasis or distant organ metastasis. In addition, they initiate intrinsic dormant programs and constrains self-renewal to sustain their survival.⁷⁶⁻⁷⁸ It is reported that growth arrest-specific-6 (GAS6) regulates the transi-

tion between residual DTCs into CSCs. Emerging evidence shows that microenvironment niche-derive GAS6 regulates a crucial role in the process of homing, survival and conversion of DTCs to CSCs.^{80,81} AXL, the receptor of GAS6 was reported to be implicated in HCC progression and metastasis. A study found that high expression of AXL in human HCC tissues correlate with high recurrence rates in HCC and the GAS6/AXL axis plays significant role in the tumor recurrence. It is also shown that GAS6/AXL axis not only involved in cancer progression and metastasis, but is also associated with recurrence and treatment resistance.^{82,83} Emerging evidences support the notion that homing DTCs deploy dormancy as a survival mechanism during treatment by inhibiting proliferation and self-renewal. Most clinical evidence show that DTCs are in either non-proliferative or slow cycling state which indicate that mitotic arrest is an essential biological event of DTCs which supports in the acquisition of dormancy.⁸⁴ These findings collectively suggest that DTCs metastasize into different regions by homing and transform into residual CSCs and go into a dormant state which later might be reactivated to initiate tumor formation and recurrence. There are other mechanisms by which residual cancer cells acquire dormancy such as drug induced dormancy. Studies have shown that MYC oncogene inactivation induces dormancy in cancer cells. The consensus of MYC oncogene reveals that tumor cells remain dormant as long as MYC remains inactivated.⁸⁵ It is a well-known fact that sorafenib inhibits proliferation by inhibition of MEK/ERK pathway, but the consequence of MEK/ERK on MYC oncogene remained unknown for a long period. Recent studies revealed that sorafenib as well as foretinib inactivate MYC via inhibition of the MEK/ERK pathway.⁸⁶ To verify that MYC inactivation could help in effective treatment of liver cancer, MYC transgenic mice with liver cancer were treated with doxycycline. Mice treated with doxycycline did not show any expression of MYC oncogene after treatment and became free from disease. Inactivation of MYC blocked the proliferative ability of cancer cells.⁸⁷ Previous studies show that sorafenib induces tumor regression in HCC patients but not complete eradication of residual cancer cells and a significant number of HCCs are refractory to sorafenib treatment. This suggests that HCC cells acquire drug induced dormancy which may be a consequence of anti-proliferative treatment.⁸⁸ These observations provide a possible explanation for the link between anti-proliferative therapy and drug induced dormancy. Drugs that inhibit proliferation via MEK/ERK inhibition cause MYC inactivation which induce dormancy in residual cancer cells which survived the drug effect.

According to a previous study which investigated the effect of metronomic therapy on tumor recurrence in orthotopic HCC mouse model, the residual dormant CSCs formed new tumor outgrowth after withdrawal of treatment. Initially they implanted hAFP producing human HCC cells (TIL) into immunodeficient mice to form new HCC tumors. Serum hAFP showed significant correlation with tumor size. Cyclophosphamide administration induced regression of tumor mass completely but a residual hAFP were detected in serum, suggesting that there could be some residual hAFP producing cancer cells remaining in liver. This was verified using immunohistochemical analysis which showed that those cells were positive for hAFP and CD13 but negative for PCNA. Surprisingly, tumors were regrown after discontinuation of treatment following complete tumor regression. This observation confirms that hAFP+/CD13+/PCNA- cells residing after cyclophosphamide treatment are dormant CSCs.⁴⁶ Moreover, these findings suggest that drug induced tumor regression causes dormancy in residual CSCs but do not affect the tumorigenic potential of these cells which is the basis of new tumor formation after treatment withdrawal.

Revoking the dormancy to normalcy and resurrection of CSCs in HCC

CSCs remain dormant until the microenvironment becomes favorable for proliferation, and it can be reactivated to active state by any growth stimulus leading to new tumor formation commonly known as tumor recurrence. These dormant cells are the residual cancer cells which failed to respond to conventional anti-proliferative and antiangiogenic therapies.^{78,85,89} Recently it was found that, quiescent CSCs undergoes epithelial to mesenchymal transition (EMT) under the influence of interleukin-17 (IL-17) which transforms the quiescent CSCs into invasive phenotypes leading to acquisition of metastatic characters in gastric cancer.⁹⁰ But the central player in bringing back the dormant CSCs to normalcy is MYC oncogene. Dormancy and normalcy of CSCs primarily depend on inactivation and activation of MYC respectively which switch the cells between these two states.⁸⁵ To analyze the effect of doxycycline on MYC inactivation and activation, two groups of MYC transgenic mice with liver cancer were treated with doxycycline. Complete tumor regression and MYC inactivation were observed during doxycycline treatment. Then, one group was discontinued for doxycycline treatment and another group continued the treatment. Doxycycline continuing group became free of disease but doxycycline discontinuation induced reactivation and high expression of MYC in the other group which led to high expression of AFP and CSC markers. This study concluded that MYC inactivation by doxycycline induces dormancy in HCSCs and reactivation of MYC upon cessation of doxycycline treatment induced tumorigenic properties in these residual dormant CSCs.⁸⁷

The fact that MYC plays significant roles in organ, as well as embryonic development, has been well established and cannot be ignored and the involvement of MYC in the tumor biology was found to be significant. Accordingly, it is undeniable that programs found in stem cells and MYC regulated cellular programs in cancer cells are common.⁸⁵ As defined earlier, quiescent cancer cells remain in dormant state with their preserved proliferative potential. Proliferative stimulus such as growth factors and cessation of anti-proliferative therapy play crucial roles in provoking the residual dormant CSCs to normalcy. Recovery from dormancy restores the inherent proliferative programs of CSCs forming new tumors which in turn cause recurrence.

The origin of AFP: hepatic progenitors *versus* HCSCs

AFP synthesis and secretion is one of the important features of HCC over other cancers, though few reported that some HCC patients had low AFP levels in serum. However, high AFP in HCC was significantly associated with post-operative complications and recurrence.⁹¹⁻⁹³ Previous reports showed that 5-year survival of patients who underwent liver transplantation for HCC were 52.7% and 80.3% for patients with AFP levels >1000 ng/mL and ≤1000 ng/mL respectively.⁹⁴ An elevated AFP level predicts poor prognosis of HCC after liver resection and transplantation. A serum AFP level more than 200 ng/mL has 3.32 fold more risk for recurrence than in patients with AFP levels less than 200 ng/mL.⁹² AFP levels were often correlated with the size of the tumors. A majority of small HCC less than 2 cm do not raise AFP levels. Studies also found a significant correlation between CSC marker CD133 and AFP in HCC as well as HCV infection. On the other hand, the levels of AFP were also found to be increased in chronic liver disease with hepatocellular damage associated with HCV infection.⁹⁵ These observations demonstrate that AFP is not only involved in

HCC genesis, but primarily associated with regenerating hepatocytes. AFP is an oncofetal protein secreted by fetal liver and yolk sac. AFP synthesis declines after fetal development and birth. AFP is secreted in liver cancers due to the presence of undifferentiated hepatocytes.^{96,97} The well-known property of liver tissue to regenerate after partial hepatectomy is due to its ability to restore the loss of hepatocytes by rapid proliferation. This led to the notion that liver consists of undetermined stem cells which are responsible for rapid proliferation.⁹⁷ Hepatocyte proliferation starts immediately within 48 hours of hepatectomy with simultaneous rise in AFP for about five days, which is often detected in liver cells undergoing mitosis during this regeneration process.^{98,99} Moreover, AFP is considered as a liver progenitor cell marker in fetal liver and it is also evident that proliferating liver cells re-express stem cell markers.⁹⁷ Thus AFP secreting cells are undifferentiated hepatocytes which tend to exhibit a progenitor state mimicking the functional characters of embryonic stem cells as a consequence of dedifferentiation of mature hepatocytes. Previous studies reported that HCCs express markers of dedifferentiation including CD133 and AFP which correspond to poor prognosis with clinical implications. These are not specific HCC markers since they are expressed by hepatic progenitor cells as well as HCSCs.¹⁰⁰ The proportionate increase in AFP corresponds to the regenerating hepatocytes either in an undifferentiated state or merely a consequence of ongoing dedifferentiation of hepatocytes back to its embryonic stem cell state.^{97,101} It is conceivable that initial liver response to damage by rapid proliferation leads to reappearance of undifferentiated hepatocytes which are the sources of AFP. On the other hand, the acquisition of stemness in differentiated hepatocytes plays a crucial role in the genesis of HCSCs, which in turn become the source of AFP.^{97,100}

AFP and immune deception

The function of AFP is largely unknown, although it is hypothesized that it plays a transport role in serum, similar to albumin.¹⁰² Recent studies came up with evidences supporting the novel immunomodulatory functions of AFP which influence the proliferation and growth of tumor cells in HCC.¹⁰³ Previously it was assumed that fetal AFP circulated in maternal circulation protects the fetus from rejection from the maternal immune system during embryo development.^{104,105} To support this notion, various studies emerged with evidences culminating that AFP acts as a detrimental molecule for immune cells. Tumor derived AFP is shown to induce apoptotic cell death in antigen presenting cells (APC) which leads to ineffective immune surveillance. In addition to impairment of APCs, it was also shown to suppress the proliferation of natural killer (NK) cells and T cells.¹⁰⁶ Further studies showed that, tumor derived AFP is involved in impairment of dendritic cells (DCs) function thereby inhibits antigen presentation process to immune system. It was also shown to inhibit the differentiation of DCs.¹⁰² *In vitro* studies demonstrated that incubation of human peripheral blood DCs with AFP resulted in apoptosis via expression of p38-MAPK and caspase3 in DCs which further resulted in immune evasion of tumor cells.¹⁰⁷ HCC immune surveillance system is mainly constituted by DCs, NK cells and T cells. It is confirmed that immune escape of HCC cells is merely a consequence of the effect on these three cells by AFP.^{108,109} Thus, AFP plays critical roles in decreasing immunity to overcome the immune responses mounted against the HCC cells. Similar to fetal AFP mechanism, hepatic progenitor cell or CSC derived AFP, tunes the immune system in favor of HCC development by modulating the biology of key immune cells such as DCs, NK cells and T cells.

Conclusions

CSC theory has gained significant attention since the discovery of induced pluripotency and CSCs. The HCSCs found in liver cancer possess stem cell features which makes them relatively imperishable. The high recurrence rates after treatment phase characterizes the HCC as an aggressive cancer type. These unsatisfactory outcomes of anticancer and antiproliferative therapies intensified the scrutiny on the field of HCC recurrence and HCSCs. Recent researches had evidenced that, stem cell nature protects and transforms the HCSCs to a quiescent state, making it dormant during therapeutic interventions. These mitotically quiescent cells remain in a biologically silent state of existence which makes them durable. Dormant HCSCs surviving the cancer therapies revokes back to its normal cellular state that brings up active proliferation and self-renewal which leads to cancer recurrence. Hence, we propose that, the functionally muted state of mitotic quiescence is the basis of therapeutic resistance, tumor dormancy and cancer recurrence after therapy.

References

1. Ananthakrishnan A, Gogineni V, Saeian K. Epidemiology of primary and secondary liver cancers. *Semin Intervent Radiol* 2006;23:047-63.
2. Forman D, Mathers C, Soerjomataram I, et al. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2014;136:E359-86.
3. Liu CY, Chen KF, Chen PJ. Treatment of liver cancer. *Cold Spring Harbor Perspect Med* 2015;5:1-16.
4. Crissien AM, Frenette C. Current management of hepatocellular carcinoma. *Gastroenterol Hepatol* 2014;10:153-61.
5. Au JS, Frenette CT. Management of hepatocellular carcinoma: Current status and future directions. *Gut Liver* 2015;9:437-48.
6. Abbott A. Cancer: the root of the problem. *Nature* 2006;442:742-3.
7. Wang K, Wu X, Wang J, Huang J. Cancer stem cell theory: therapeutic implications for nanomedicine. *Int J Nanomed* 2013;8:899-908.
8. Matthai SM, Ramakrishna B. Cancer stem cells in hepatocellular carcinoma--an immunohistochemical study with histopathological association. *Indian J Med Res* 2015;142:391-8.
9. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature* 2001;414:105-11.
10. Fialkow PJ. Stem cell origin of human myeloid blood cell neoplasms. *Verh Dtsch Ges Pathol* 1990;74:43-7.
11. Lapidot T, Sirard C, Vormoor J, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature* 1994;367:645-8.
12. Lobo NA, Shimono Y, Qian D, Clarke MF. The biology of cancer stem cells. *Annu Rev Cell Dev Biol* 2007;23:675-99.
13. Pierce GB, Wallace C. Differentiation of malignant to benign cells. *Cancer Res* 1971;31:127-34.
14. Aponte PM, Caicedo A. Stemness in cancer: stem cells, cancer stem cells, and their microenvironment. *Stem Cells Int* 2017;2017:5619472.
15. Cabrera MC, Hollingsworth RE, Hurt EM. Cancer stem cell plasticity and tumor hierarchy. *World J Stem Cells* 2015;7:27-36.

16. Lavau C, Szilvassy SJ, Slany R, Cleary ML. Immortalization and leukemic transformation of a myelomonocytic precursor by retrovirally transduced HRX-ENL. *EMBO J* 1997;16:4226-37.
17. Krivtsov AV, Twomey D, Feng Z, et al. Transformation from committed progenitor to leukaemia stem cell initiated by MLL-AF9. *Nature* 2006;442:818-22.
18. So CW, Karsunky H, Wong P, et al. Leukemic transformation of hematopoietic progenitors by MLL-GAS7 in the absence of Hoxa7 or Hoxa9. *Blood* 2004;103:3192-9.
19. Friedmann-Morvinski D, Verma IM. Dedifferentiation and reprogramming: origins of cancer stem cells. *EMBO Rep* 2014;15:244-53.
20. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006;126:663-76.
21. Liu X, Huang J, Chen T, et al. Yamanaka factors critically regulate the developmental signaling network in mouse embryonic stem cells. *Cell Res* 2008;18:1177-89.
22. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007;131:861-72.
23. Malik N, Rao MS. A review of the methods for human iPSC derivation. *Methods Mol Biol* 2013;997:23-33.
24. Tajima H, Ohta T, Okamoto K, et al. Radiofrequency ablation induces dedifferentiation of hepatocellular carcinoma. *Oncol Lett* 2010;1:91-4.
25. Yang SD, Lee SC, Chang HC. Heat stress induces tyrosine phosphorylation/activation of kinase FA/GSK-3 alpha (a human carcinoma dedifferentiation modulator) in A431 cells. *J Cell Biochem* 1997;66:16-26.
26. Hamburger AW, Salmon SE. Primary bioassay of human tumor stem cells. *Science* 1977;197:461-3.
27. Bonnet D, Dick JE. Human acute myeloid leukaemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med* 1997;3:730-7.
28. Magee JA, Piskounova E, Morrison SJ. Cancer stem cells: impact, heterogeneity, and uncertainty. *Cancer Cell* 2012;21:283-96.
29. Rountree CB, Barsky L, Ge S, et al. A CD133-expressing murine liver oval cell population with bilineage potential. *Stem Cells* 2007;25:2419-29.
30. Ma S, Chan KW, Hu L, et al. Identification and characterization of tumorigenic liver cancer stem/progenitor cells. *Gastroenterology* 2007;132:2542-56.
31. Cai Z, Xu K, Li Y, et al. Long noncoding RNA in liver cancer stem cells. *Discov Med* 2017;24:87-93.
32. Xiang Y, Yang T, Pang BY, et al. The progress and prospects of putative biomarkers for liver cancer stem cells in hepatocellular carcinoma. *Stem Cells Int* 2016;2016:7614971.
33. Qadir AS, Ceppi P, Brockway S, et al. CD95/Fas Increases Stemness in Cancer Cells by Inducing a STAT1-Dependent Type I Interferon Response. *Cell Rep* 2017;18:2373-86.
34. Fang T, Lv H, Wu F, et al. Musashi 2 contributes to the stemness and chemoresistance of liver cancer stem cells via LIN28A activation. *Cancer Lett* 2017;384:50-9.
35. Li N, Zhu Y. Targeting liver cancer stem cells for the treatment of hepatocellular carcinoma. *Ther Adv Gastroenterol* 2019;12:1756284818821560.
36. Zhai B, Zhang X, Sun B, et al. MK2206 overcomes the resistance of human liver cancer stem cells to sorafenib by inhibition of pAkt and upregulation of pERK. *Tumour Biol* 2016;37:8047-55.
37. Wang N, Wang S, Li MY, et al. Cancer stem cells in hepatocellular carcinoma: an overview and promising therapeutic strategies. *Ther Adv Med Oncol* 2018;10:1758835918816287.
38. Vu NB, Nguyen TT, Tran LC, et al. Doxorubicin and 5-fluorouracil resistant hepatic cancer cells demonstrate stem-like properties. *Cytotechnology* 2013;65:491-503.
39. Mancuso A. Management of hepatocellular carcinoma: Enlightening the gray zones. *World J Hepatol* 2013;5:302-10.
40. Wong R, Frenette C. Updates in the management of hepatocellular carcinoma. *Gastroenterol Hepatol* 2011;7:16-24.
41. Dong S, Wang Z, Wu L, Qu Z. Effect of surgical margin in R0 hepatectomy on recurrence-free survival of patients with solitary hepatocellular carcinomas without macroscopic vascular invasion. *Medicine* 2016;95:e5251.
42. Lee W, Han HS, Ahn S, et al. Correlation between Resection Margin and Disease Recurrence with a Restricted Cubic Spline Model in Patients with Resected Hepatocellular Carcinoma. *Dig Surg* 2018;35:520-31.
43. Guo Z, Li LQ, Jiang JH, et al. Cancer stem cell markers correlate with early recurrence and survival in hepatocellular carcinoma. *World J Gastroenterol* 2014;20:2098-106.
44. Ho DW, Lo RC, Chan LK, Ng IO. Molecular Pathogenesis of Hepatocellular Carcinoma. *Liver Cancer* 2016;5:290-302.
45. Muramatsu S, Tanaka S, Mogushi K, et al. Visualization of stem cell features in human hepatocellular carcinoma reveals in vivo significance of tumor-host interaction and clinical course. *Hepatology* 2013;58:218-28.
46. Martin-Padura I, Marighetti P, Agliano A, et al. Residual dormant cancer stem-cell foci are responsible for tumor relapse after antiangiogenic metronomic therapy in hepatocellular carcinoma xenografts. *Lab Invest* 2012;92:952-66.
47. Hadfield G. The dormant cancer cell. *Br Med J* 1954;2:607-10.
48. Gao XL, Zhang M, Tang YL, Liang XH. Cancer cell dormancy: mechanisms and implications of cancer recurrence and metastasis. *Oncotargets Ther* 2017;10:5219-28.
49. Terzi MY, Izmirlı M, Gogebakan B. The cell fate: senescence or quiescence. *Mol Biol Rep* 2016;43:1213-20.
50. Demidenko ZN, Zubova SG, Bukreeva EI, et al. Rapamycin decelerates cellular senescence. *Cell Cycle* 2009;8:1888-95.
51. Cooper S. On the proposal of a G0 phase and the restriction point. *FASEB J* 1998;12:367-73.
52. Zhu X, Raina AK, Smith MA. Cell cycle events in neurons. Proliferation or death?. *Am J Pathol* 1999;155:327-9.
53. Zetterberg A, Larsson O. Kinetic analysis of regulatory events in G1 leading to proliferation or quiescence of Swiss 3T3 cells. *Proc Natl Acad Sci U S A* 1985;82:5365-69.
54. Coller HA, Sang L, Roberts JM. A new description of cellular quiescence. *PLoS Biol* 2006;4:e83.
55. Cheung TH, Rando TA. Molecular regulation of stem cell quiescence. *Nat Rev Mol Cell Biol* 2013;14:329-40.
56. Moore N, Lyle S. Quiescent, slow-cycling stem cell populations in cancer: a review of the evidence and discussion of significance. *J Oncol* 2010;2011:396076.
57. De Francesco EM, Sotgia F, Lisanti MP. Cancer stem cells (CSCs): metabolic strategies for their identification and eradication. *Biochem J* 2018;475:1611-34.
58. Wickström M, Larsson R, Nygren P, Gullbo J. Aminopeptidase N (CD13) as a target for cancer chemotherapy. *Cancer Sci* 2011;102:501-8.
59. Wang R, Sun Q, Wang P, et al. Notch and Wnt/ β -catenin signaling pathway play important roles in activating liver cancer stem cells. *Oncotarget* 2016;7:5754-68.
60. Ikeda N, Nakajima Y, Tokuhara T, et al. Clinical significance of aminopeptidase N/CD13 expression in human pancreatic

- carcinoma. *Clin Cancer Res* 2003;9:1503-8.
61. Murakami H, Yokoyama A, Kondo K, et al. Circulating aminopeptidase N/CD13 is an independent prognostic factor in patients with non-small cell lung cancer. *Clin Cancer Res* 2005;11:8674-9.
 62. Yamanaka C, Wada H, Eguchi H, et al. Clinical significance of CD13 and epithelial mesenchymal transition (EMT) markers in hepatocellular carcinoma. *Jpn J Clin Oncol* 2018;48:52-60.
 63. Adachi E, Hashimoto H, Tsuneyoshi M. Proliferating cell nuclear antigen in hepatocellular carcinoma and small cell liver dysplasia. *Cancer* 1993;72:2902-9.
 64. Gade TPF, Tucker E, Nakazawa MS, et al. Ischemia Induces Quiescence and Autophagy Dependence in Hepatocellular Carcinoma. *Radiology* 2017;283:702-10.
 65. Carr BI, Cavallini A, Lippolis C, et al. Fluoro-Sorafenib (Regorafenib) effects on hepatoma cells: growth inhibition, quiescence, and recovery. *J Cell Physiol* 2013;228:292-7.
 66. Carr BI, D'Alessandro R, Refolo MG, et al. Effects of low concentrations of regorafenib and sorafenib on human HCC cell AFP, migration, invasion, and growth in vitro. *J Cell Physiol* 2013;228:1344-50.
 67. Zieglschmid V, Hollmann C, Böcher O. Detection of disseminated tumor cells in peripheral blood. *Crit Rev Clin Lab Sci* 2005;42:155-96.
 68. Chiappini F. Circulating tumor cells measurements in hepatocellular carcinoma. *Int J Hepatol* 2012;2012:684802.
 69. Luzzi KJ, MacDonald IC, Schmidt EE, et al. Multistep nature of metastatic inefficiency: dormancy of solitary cells after successful extravasation and limited survival of early micrometastases. *Am J Pathol* 1998;153:865-73.
 70. Wong IH, Lau WY, Leung T, et al. Hematogenous dissemination of hepatocytes and tumor cells after surgical resection of hepatocellular carcinoma: a quantitative analysis. *Clin Cancer Res* 1999;5:4021-27.
 71. Kienle P, Weitz J, Klaes R, et al. Detection of isolated disseminated tumor cells in bone marrow and blood samples of patients with hepatocellular carcinoma. *Arch Surg* 2000;135:213-18.
 72. Pua U. Rapid intra-hepatic dissemination of hepatocellular carcinoma with pulmonary metastases following combined loco-regional therapy. *Korean J Radiol* 2013;14:640-2.
 73. Minagawa N, Sakihama H, Kobayashi N, et al. A pilot study for cellular detection of circulating tumor cells and disseminated tumor cells of patients with hepatocellular carcinoma. *J Clin Oncol* 2014;15:11041.
 74. Li QG, Yang GS, Yang Q, et al. Disseminated tumor cells homing into rats' liver: a new possible mechanism of HCC recurrence. *World J Gastroenterol* 2004;10:903-5.
 75. Shiozawa Y, Berry JE, Eber MR, et al. The marrow niche controls the cancer stem cell phenotype of disseminated prostate cancer. *Oncotarget* 2016;7:41217-32.
 76. Weidenfeld K, Barkan D. EMT and Stemness in Tumor Dormancy and Outgrowth: Are They Intertwined Processes?. *Front Oncol* 2018;8:381.
 77. Gay LJ, Malanchi I. The sleeping ugly: Tumour microenvironment's act to make or break the spell of dormancy. *Biochim Biophys Acta Rev Cancer* 2017;1868:231-8.
 78. Giancotti FG. Mechanisms governing metastatic dormancy and reactivation. *Cell* 2013;155:750-64.
 79. Balic M, Lin H, Young L, et al. Most early disseminated cancer cells detected in bone marrow of breast cancer patients have a putative breast cancer stem cell phenotype. *Clin Cancer Res* 2006;12:5615-21.
 80. Shiozawa Y, Berry JE, Eber MR, et al. The marrow niche controls the cancer stem cell phenotype of disseminated prostate cancer. *Oncotarget* 2016;7:41217-32.
 81. Shiozawa Y, Pedersen EA, Taichman RS. GAS6/Mer axis regulates the homing and survival of the E2A/PBX1-positive B-cell precursor acute lymphoblastic leukemia in the bone marrow niche. *Exp Hematol* 2010;38:132-40.
 82. Liu J, Wang K, Yan Z, et al. Axl expression stratifies patients with poor prognosis after hepatectomy for hepatocellular carcinoma. *PLoS One* 2016;11:e0154767.
 83. Wu G, Ma Z, Hu W, et al. Molecular insights of Gas6/TAM in cancer development and therapy. *Cell Death Dis* 2017;8:e2700.
 84. Sosa MS, Bragado P, Aguirre-Ghiso JA. Mechanisms of disseminated cancer cell dormancy: an awakening field. *Nat Rev Cancer* 2014;14:611-22.
 85. Shachaf CM, Felsher DW. Tumor dormancy and MYC inactivation: pushing cancer to the brink of normalcy. *Cancer Res* 2005;65:4471-4.
 86. Duncan JS, Whittle MC, Nakamura K, et al. Dynamic reprogramming of the kinome in response to targeted MEK inhibition in triple-negative breast cancer. *Cell* 2012;149:307-21.
 87. Shachaf CM, Kopelman AM, Arvanitis C, et al. MYC inactivation uncovers pluripotent differentiation and tumor dormancy in hepatocellular cancer. *Nature* 2004;431:1112-7.
 88. Nishida N, Kitano M, Sakurai T, Kudo M. Molecular Mechanism and Prediction of Sorafenib Chemoresistance in Human Hepatocellular Carcinoma. *Dig Dis* 2015;33:771-9.
 89. Wheelock EF, Weinhold KJ, Levich J. The tumor dormant state. *Adv Cancer Res* 1981;34:107-40.
 90. Jiang YX, Yang SW, Li PA, et al. The promotion of the transformation of quiescent gastric cancer stem cells by IL-17 and the underlying mechanisms. *Oncogene* 2016;36:1256-64.
 91. Carr BI, Akkiz H, Üsküdar O, et al. HCC with low- and normal-serum alpha-fetoprotein levels. *Clin Pract* 2018;15:453-64.
 92. Schraiber Ldos S, de Mattos AA, Zanotelli ML, et al. Alpha-fetoprotein Level Predicts Recurrence After Transplantation in Hepatocellular Carcinoma. *Medicine* 2016;95:e2478.
 93. Ma WJ, Wang HY, Teng LS. Correlation analysis of preoperative serum alpha-fetoprotein (AFP) level and prognosis of hepatocellular carcinoma (HCC) after hepatectomy. *World J Surg Oncol* 2013;11:212.
 94. Hameed B, Mehta N, Sapisochin G, et al. Alpha-fetoprotein level >1000 ng/mL as an exclusion criterion for liver transplantation in patients with hepatocellular carcinoma meeting the Milan criteria. *Liver Transpl* 2014;20:945-51.
 95. Mansour AH, Elkhodary TR, Anwar R, et al. Regulation of Cancer Stem Cell Marker (CD133) by Transforming Growth Factor Beta in Hepatocellular Carcinoma. *Int J Cancer Res* 2014;10:65-73.
 96. Gitlin D. Sites of alpha-fetoprotein synthesis. *N Engl J Med* 1971;285:1436-7.
 97. Sell S. Alpha-fetoprotein, stem cells and cancer: how study of the production of alpha-fetoprotein during chemical hepatocarcinogenesis led to reaffirmation of the stem cell theory of cancer. *Tumour Biol* 2008;29:161-80.
 98. Sell S, Nichols M, Becker FF, Leffert JL. Hepatocyte proliferation and alpha1-fetoprotein in pregnant, neonatal and partially hepatectomized rats. *Cancer Res* 1974;34:865-71.
 99. Sell S. Heterogeneity of Alpha-fetoprotein (AFP) and albumin containing cells in normal and pathological permissive states for AFP production: AFP containing cells induced in adult rats recapitulate the appearance of AFP containing hepatocytes in

- fetal rats. *Oncodevelopmental Biol Med* 1980;1:93-105.
100. Ker C, Kuo K, Chang W, et al. Clinical significance of hepatic cancer stem cells. *Formosan J Surg* 2011;44:205-10.
101. Abelev GI, Erasler TL. Cellular aspects of alpha-fetoprotein reexpression in tumors. *Semin Cancer Biol* 1999;9:95-107.
102. Pardee AD, Shi J, Butterfield LH. Tumor-derived α -fetoprotein impairs the differentiation and T cell stimulatory activity of human dendritic cells. *J Immunol* 2014;193:5723-32.
103. Tomasi TB Jr. Structure and function of alpha-fetoprotein. *Annu Rev Med* 1977;28:453-65.
104. Crainie M, Semeluk A, Lee KC, Wegmann T. Regulation of constitutive and lymphokine-induced Ia expression by murine alpha-fetoprotein. *Cell Immunol* 1989;118:41-52.
105. Nicholas NS, Panayi GS. Immunosuppressive properties of pregnancy serum on the mixed lymphocyte reaction. *Br J Obstet Gynaecol* 1986;93:1251-5.
106. Meng W, Bai B, Bai Z, et al. The immunosuppression role of alpha-fetoprotein in human hepatocellular carcinoma. *Discov Med* 2016;21:489-94.
107. Trompezinski S, Migdal C, Tailhardat M. Characterization of early events involved in human dendritic cell maturation induced by sensitizers: Cross talk between MAPK signalling pathways. *Toxicol Appl Pharmacol* 2008;230:397-406.
108. Schmidt N, Neumann-Haefelin C, Thimme R. Cellular immune responses to hepatocellular carcinoma: lessons for immunotherapy. *Digestive Dis* 2012;30:483-91.
109. Wang X, Wang Q. Alpha-Fetoprotein and Hepatocellular Carcinoma Immunity. *Can J Gastroenterol Hepatol* 2018; 2018:9049252.