

Progress in stem cell-derived technologies for hepatocellular carcinoma

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Abstract: Primary hepatocellular carcinoma (HCC) is a common malignancy that has a poor prognosis because it is often diagnosed at an advanced stage. HCC normally develops as a consequence of underlying liver disease and is most often associated with cirrhosis. Surgical resection and liver transplantation are the current best options to treat liver cancer. However, problems associated with liver transplantation, such as shortage of donors, risk of immune rejection, and tissue damage following surgery provided the impetus for development of alternative therapies. The emerging field of stem cell therapy has raised hopes for finding curative options for liver cancer. Stem cells have the ability not only to proliferate after transplantation but also to differentiate into most mammalian cell types *in vivo*. In this review, progress on stem cell-derived technologies for the treatment of liver cancer is discussed.

Keywords: liver, cancer, stem cell, therapy, hepatocellular carcinoma

Introduction

Primary liver cancer can be classified into one of several histologically different hepatic malignancies, including hepatocellular carcinoma (HCC), cholangiocarcinoma, hepatoblastoma, and hemangiosarcoma.¹ Of these, HCC is the most common liver cancer, accounting for 70%–85% of cases, with nearly 700,000 deaths occurring worldwide each year.^{2,3} Recurrence or metastasis is quite common in patients who have had a resection, and survival rate is only 30%–40% at five years after surgery.⁴ A variety of risk factors may cause HCC which vary according to geographical region,⁵ and susceptibility to disease depends upon the host's genetic factors and pre-existing health conditions.⁶

During the past decade, advances in imaging techniques such as magnetic resonance imaging and contrast-enhanced ultrasonography with real-time low mechanical index harmonic imaging ultrasound equipment have allowed us to perform noninvasive diagnosis of HCC and replaced the need for invasive procedures like ultrasound-guided biopsy or angiography.⁷ Currently, in addition to standard chemotherapy, a number of treatment options are being used in the clinical setting.⁸ They include surgical resections such as enucleation, segmentectomy and lobectomy, percutaneous interventions such as hyperthermia and cryotherapy, transarterial interventions, and various embolization methods (chemical, thermochemical, and others). The treatment regimen that is currently being administered is shown in Figure 1. In addition to these treatment methods, efforts are being made to target cellular signaling pathways involved in HCC to suppress the tumors. Putative drug candidates for these studies include small-molecule protein kinase inhibitors, monoclonal antibodies, and antibiotics, either alone or in combinations.^{8,9} The most

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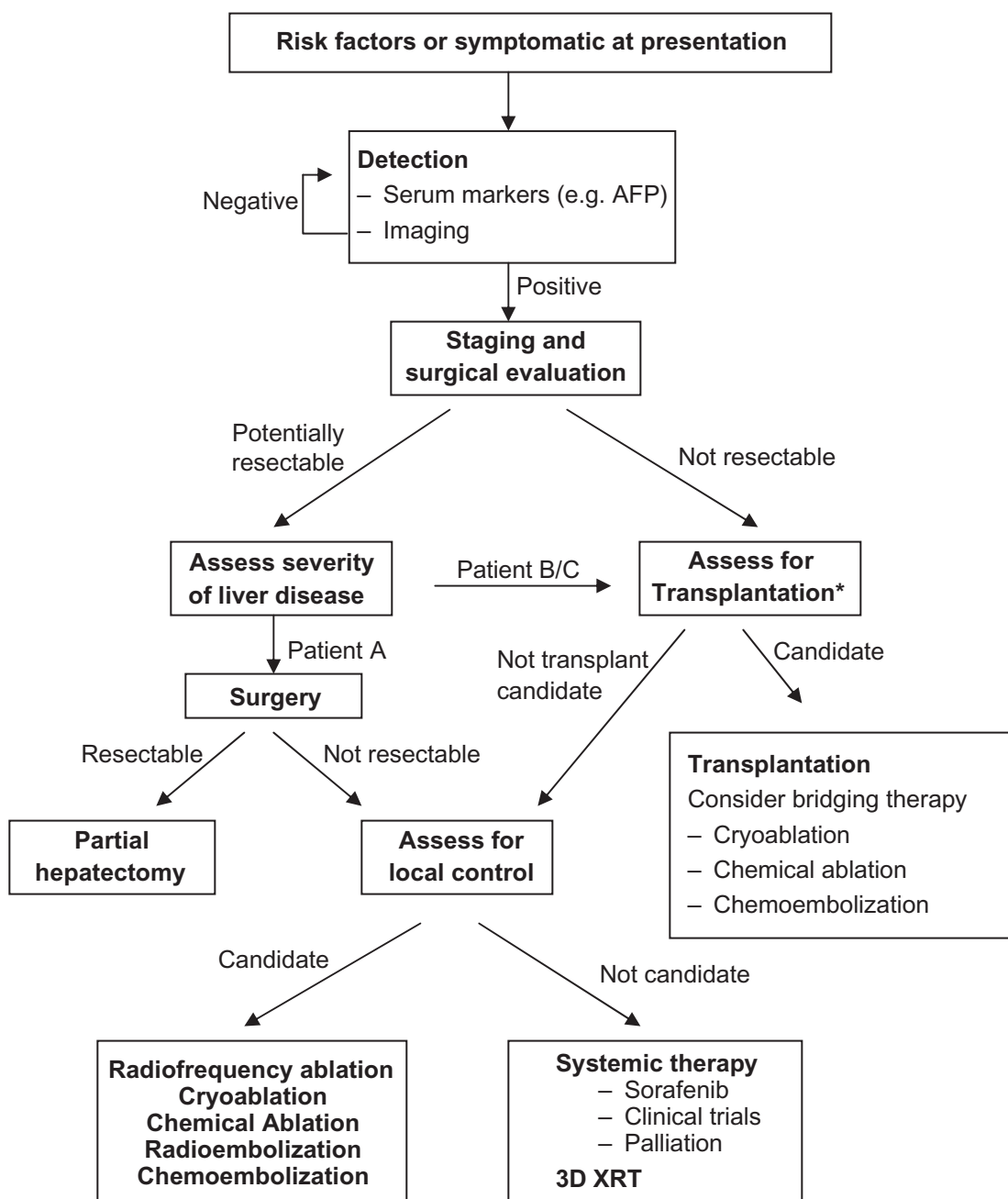


Figure 1 A general strategy for management of hepatocellular carcinoma based on tumor burden and the degree of liver disease. This algorithm depicts various treatment options that are used in the clinic depending upon the disease severity and the patient's previous medical history.

Note: *Molecular and genetic prognostic markers from biopsy specimens may potentially alter existing selection criteria

Abbreviations: AFP, alfa-fetoprotein; 3D XRT, three-dimensional conformal radiotherapy.

effective candidate among them is sorafenib, which showed a modest, three-month survival benefit in some patients.⁹ At present, sorafenib is the only drug approved by the Food and Drug Administration in the US for liver cancer. Prior to this, over 100 clinical trials of intravenous (IV) chemotherapy failed to show any statistically significant survival benefit in patients with HCC.¹⁰ For those patients fortunate enough to be identified, surgery is potentially curative. The shortage

of donor livers for transplantation, however, has led to the development of techniques such as split liver transplantation and living donor transplantation.¹¹ Currently, efforts are geared towards developing cell-based therapies where hepatocytes and stem/progenitor cells will be used for transplantation into damaged and diseased livers. Such transplanted cells could proliferate and repopulate the liver and eventually restore its functions.

Liver stem cells

The liver is the only organ in the human body that is capable of renewing itself following the loss of the natural tissue. Even after 70% hepatectomy, this remarkable regenerative capacity is achieved due to the proliferation of hepatocytes and cholangiocytes, and other hepatic cells such as stellate cells, macrophages, and endothelial cells.¹² Under special circumstances, stem/progenitor cells and bone marrow (BM) cells also contribute to this regeneration process.¹³ Stem/progenitor cells are critical to the tissue restoration process because they are bipotent and can differentiate into the two primary cell types of the liver, ie, hepatocytes and biliary ductal cells (cholangiocytes). In addition to their role in liver regeneration, stem/progenitor cells are important for studies of organogenesis and liver development.^{5,14,15}

To date, a number of different types of stem/progenitor cells have been successfully isolated from healthy and injured livers (adult and fetal) as well as from liver tumors.^{16–32} These include cells of human, rodent, canine, swine, and simian origins (Table 1). One such population of human liver stem cells was recently shown to contribute to the generation of liver parenchyma in severe combined immunodeficient mice.³³ Among liver stem cells, the human hepatic progenitor cells (HPCs) are the best studied. Apart from adult and fetal livers,^{15,34–36} they have been isolated and characterized from liver specimens with severe hepatocellular necrosis, chronic viral hepatitis, and chronic alcoholic liver disease.³⁷ In normal adult liver, these cells are localized in biliary ductules (canals of Hering).

The rodent counterparts of HPCs are termed “oval cells” because of their characteristic ovoid nucleus.^{38–41} They are bipotential,⁴² and express cell surface markers such as Thy1.1, CD34, Flt3-receptor, and c-kit.⁴³ They

also produce alfa-fetoprotein (AFP), cytokeratin 19, and γ -glutamyl-transferase, and stain positive for one-cut 2 transcription factor (OC2), ovalbumin (OV-6), and BD1 when treated with monoclonal antibodies. Oval cells are normally isolated from the liver following treatment with agents such as 3,5-diethoxycarbonyl-1,4-dihydrocolidine (DDC), or from animals fed on choline-deficient diets and treated with 2-acetylaminofluorene (2-AAF).^{44,45} They proliferate *in vivo* following liver damage when the hepatocytes can no longer divide.⁴⁶ In diseases such as alcoholic liver disease and during hepatitis C virus (HCV) infection, their numbers increase and correlate with the severity of the disease.⁴⁷

Recently, Liu et al isolated epithelial progenitors from fetal rat livers that were able to divide in cell culture and express liver epithelial and biliary-specific markers.⁴⁸ Upon differentiation *in vitro*, they also expressed albumin, CK-18 and AFP, and two weeks after transplantation into animals they were shown to display hepatocyte-like features *in vivo*.⁴⁸ It has been proposed that certain epithelial cells in fetal and injured adult livers undergo epithelial-mesenchymal transition and home to liver parenchyma, and some of these cells may be capable of reversing this transition and become hepatocytes or cholangiocytes.⁴⁹ This phenomenon of transition has been reported in a number of human diseases.⁵⁰

Extrahepatic sources of liver stem cells

In addition to these liver-derived stem/progenitor cells, many studies have been published demonstrating that cells of nonliver origin could also differentiate into “hepatocyte-like” cells (Figure 2). These cells could be a valuable source of hepatocytes and cholangiocytes. It is beyond the scope of this review to discuss every stem cell type that has been described, but those that have been well studied and successfully differentiated into hepatic cell types will be highlighted.

Bone-marrow derived cells

Mesenchymal stem cells (MSC) are multipotent stem cells derived from BM aspirates. They can be expanded readily in cell culture and can be induced to differentiate into many different cell types, including hepatic cells.⁵¹ These *in vitro* differentiated cells can express hepatocyte markers and possess hepatocyte-specific biochemical activities such as albumin secretion, urea production, and glycogen storage.^{52,53} It has been suggested that these BM-derived cells fuse with damaged hepatocytes after transplantation and change their

Table 1 Liver stem/progenitor cells

Cell type/name	Species	Reference(s)
Human progenitor cells	Human	18,23,26,33,34
Oval cells	Rat	31
	Mouse	25
	Hamster	17
Stem/progenitor cells	Rat	21
	Mouse	15,25,27,28
	Pig	31
	Monkey	16
	Dog	20
	Human	29,30,33–36
Liver-derived progenitor cells	Rat	19

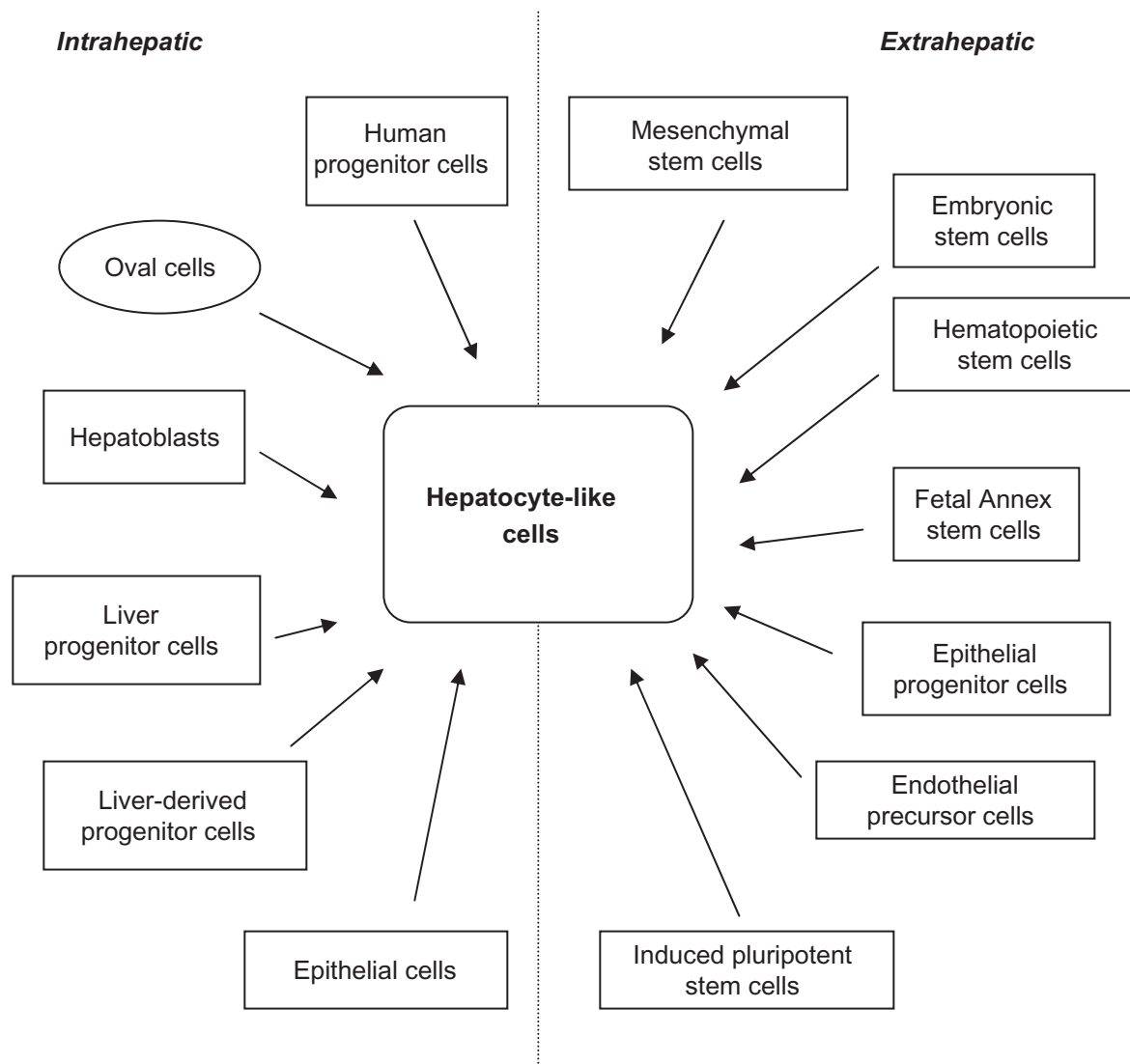


Figure 2 Intra- and extrahepatic sources of stem cells from different origins that have been demonstrated to differentiate into hepatocyte-like cells.

gene expression patterns to that of mature hepatocytes.^{41,54} Furthermore, implanted MSCs protect the liver by secreting soluble factors that possess antiapoptotic and promitotic properties, as shown in experiments with rats where hepatic failure was caused by D-galactosamine.⁵⁵ In support of these studies, Lagasse et al showed that transplanted MSCs could differentiate into mature hepatocytes *in vivo* and rescue fumarylacetoacetate hydrolase-deficient mice.⁵⁶ Transplanted MSCs were also demonstrated to restore liver function in albumin urokinase transgenic mice and in hepatitis B virus (HBV) transgenic mice.

Hematopoietic stem cells (HSC) are another type of stem cell present in the BM. They have the potential to differentiate into liver cells, both *in vitro* and *in vivo*, without fusion and could contribute to liver regeneration.⁵⁷ A comparative study

of the hepatocyte differentiation capacity of HSCs and MSCs in a rat model has shown that MSCs are more potent than HSCs in differentiating into hepatocytes.⁵⁸

Embryonic stem cells

Embryonic stem cells (ESCs) are derived from the inner mass of an early-stage embryo known as the blastocyst.⁵⁹ They are pluripotent and can be maintained in cell culture for prolonged periods of time without disturbing their developmental potential.⁶⁰ In the early studies, embryonic stem cells from murine embryos were shown to differentiate into functional hepatocytes *in vitro*.^{61–63} Later it was shown that both murine- and human BM-derived MSCs could differentiate into hepatocytes both *in vitro* and *in vivo*.^{52,64} Several studies have shown that ESCs could be induced to differentiate into liver cells.

For example, Hay et al differentiated human ESCs into hepatocyte-like cells by treating them with hepatocyte growth factor,⁶⁵ and Ishizaka et al managed to differentiate mouse ESCs into hepatocytes by treating them with hepatocyte nuclear factor-3 β .⁶⁶ Studies with BM transplant recipients have shown that these cells could home to liver and differentiate into normal hepatocytes.^{67,68} Because of major ethical concerns with the use and handling of ESCs, and due to difficulties in controlling their robust proliferative and differentiation potential, their use is currently limited to *in vitro* studies and to animal models.

Fetal annex stem cells

Umbilical cord blood contains multiple populations of pluripotent stem cells. Each of these populations can serve as a source of hepatocytes for liver regeneration. For instance, mesenchymal stromal cells isolated from the umbilical cord could be induced to differentiate into hepatocyte-like cells in cell culture after treating them with hepatocyte growth factor and fibroblast growth factor-4.⁶⁹ Such differentiated cells express CK-18, AFP, and albumin. Similarly, umbilical cord matrix stem cells could also differentiate into hepatocyte-like cells.⁷⁰ In addition to these cells, human mononuclear cells from umbilical cord blood could differentiate into functional hepatocytes when transplanted *in utero* into fetal rats⁷¹ and into carbon tetrachloride (CCl₄)-injured mouse liver.⁷²

Placenta-derived stem cells are another source of hepatocytes. They can be expanded in cell culture for more than 20 populations.⁷³ Recently, Chien et al cultured these cells from human placentae, differentiated them into hepatocytes, and examined their hepatocyte-specific functions.⁷⁴ When compared with ESCs, there are no ethical issues involved in using these cells because the collection of placentae does not harm either the human mother or the infant.⁷³

Induced pluripotent stem cells

The induced pluripotent stem cell (iPS) is an inducible cell type that can be generated by epigenetic reprogramming following induced expression of certain transcription factors. Takahashi and Yamanaka demonstrated this in a landmark study where they overexpressed four transcription factors Oct-3/4, Sox2, c-Myc, and Klf4 in mouse fibroblasts using a retrovirus to generate first iPS cells.⁷⁵ In subsequent studies, Yamanaka et al and Yu et al demonstrated the generation of iPS cells from murine and human somatic cells.^{76,77} Recently, Si-Tayeb et al and Song et al reported the generation of human hepatocyte-like cells from iPS cells obtained from human ESCs.^{78,79} Hepatocyte-like cells produced in this procedure

expressed various hepatic markers such as HNF-4a, FoxA2, AFP, and secreted albumin. Aoi et al isolated pluripotent stem cells from adult mouse liver and generated iPS cells by expressing these four transcription factors from a retrovirus in adult mouse hepatocytes and gastric epithelial cells.⁸⁰ This finding suggests that functional hepatocytes and other liver cells can be reprogrammed to generate stem cells.

Endothelial progenitor/precursor cells

Endothelial precursor cells (EPCs) constitute a cell type that has the potential to differentiate into mature functional endothelial cells that form a capillary or line the lumen of a blood vessel.⁸¹ They were first isolated from a population of CD34⁺ peripheral mononuclear blood cells of the BM.⁸² Subsequently, they have been isolated from sources other than BM, such as the vessel wall.⁸³ It has been reported that EPCs could differentiate into hepatocytes when cultured in hepatic differentiation media.⁸⁴

Others

The adipose tissue contains MSCs that are multipotent and can be differentiated into functional hepatocyte-like cells by treatment with a cocktail of cytokines.⁸⁵ In a recent study it was shown that, after transplantation, these human adipose tissue cells get incorporated into the liver parenchyma after transplantation into mice.⁸⁶ After differentiation, they express a number of hepatocyte markers and biologic activities of liver cells. Recently, Ikeda et al showed that tooth germ progenitor cells derived from adipose tissue could prevent liver fibrosis and improve liver function after transplantation in rodents with liver injury caused by CCl₄.⁸⁷ Other adipose tissue cells, such as salivary gland progenitor cells, differentiate into hepatocyte-like cells and express alpha-1 antitrypsin and albumin after transplantation into the murine liver via the portal vein.⁸⁸ A novel cell type, the liver-derived progenitor cell, was recently discovered and isolated from healthy, uninjured rat livers.¹⁹

Based on these numerous reports of successful *in vitro* and *in vivo* hepatocyte-like differentiation of stem/progenitor cells isolated from different organs, it is predictable that other stem cells, such as amniotic epithelial cells⁸⁹ and very small embryonic-like stem cells⁹⁰ might also be induced to produce cells of hepatocyte and biliary lineages.

Role of stem cells in liver cancer

In the normal adult liver, there is little proliferation. However, following partial hepatectomy or injury, hepatocytes proliferate rapidly and repopulate the liver to restore its

physical mass and physiologic functions. Initial experiments with rodents have demonstrated the ability of hepatocytes to proliferate and to populate the liver mass after partial hepatectomy.^{91,92} The data regarding the ability of human hepatocytes to undergo extensive cell division came from studies on chronic hepatitis with HCV and HBV. In the livers of patients with chronic viral hepatitis, there is an ongoing excessive hepatocyte death and compensatory proliferation. Based on the staining for cell proliferation markers, such as proliferating cell nuclear antigen and Ki-67, it was estimated that about 1% to 3% of hepatocytes die daily in the diseased liver, as opposed to <0.01% in normal healthy livers. This excessive hepatocyte death obviously requires extensive proliferation to maintain a stable liver mass, suggesting that an individual hepatocyte would have to divide many times during the life of a patient with chronic hepatitis. Such prolonged self-replication in an inflammatory microenvironment could result in the accumulation of genetic lesions that cause cancer formation.⁹³⁻⁹⁵

Subsequent experiments with mouse urokinase plasminogen activator have shown that transplanted hepatocytes could regenerate the liver in its entirety after undergoing 12 rounds of replication.⁹⁶ Serial transplantation of hepatocytes into fumarylacetoacetase hydrolase-deficient mice indicated that transplanted hepatocytes could divide at least 69 times *in vivo*, and when normal hepatocytes from wild-type mice are injected into these mutant mice, they colonized the mutant liver effectively.⁹⁷ In another study, Laconi et al transplanted dipeptidyl peptidase IV (DPPiV)-positive cells into DPPiV-deficient F344 rats, and showed that transplanted cells could repopulate 40% to 60% of female rat livers within a year and 98% to 99% of hepatic mass in male rats within nine months after transplantation.⁹⁸ Liu et al demonstrated that transplanted BM-derived EPCs could ameliorate the damage caused by CCl₄ injury in rats.⁹⁹ They transplanted EPCs into portal veins of female rats 12 weeks after treating the animals with CCl₄ and found that EPCs home to the liver and regenerate into hepatocytes. In a similar study, Nakamura et al have showed that transplanted EPCs derived from BM could improve the outcome in a cirrhotic liver rat model.⁸⁴ These findings demonstrate that the transplantation of hepatocytes and extrahepatic stem cells could be an effective treatment strategy to combat liver cancer.

Recent studies have demonstrated that the capacity to sustain tumor formation and growth resides in a small proportion of stem cells known as “cancer stem cells” (CSCs).^{100,101} They have a greater colony-forming efficiency, higher proliferation potential, and greater ability to form tumor in animal

models. The identification of CSCs in a number of tissues including brain,¹⁰²⁻¹⁰⁴ prostate,¹⁰⁵ breast,¹⁰⁶ myeloid,¹⁰⁷ gastric,¹⁰⁸ colon,^{109,110} and lung¹¹¹ reinforced the notion that stem cells might also exist in the liver. CSCs were later identified and isolated from the liver.¹¹²

The presence of CSCs and successful isolation of oval cells from cancerous tissue suggests that stem/progenitor cells play a key role in tumor formation in the liver. The clonality of HCC was later established, based on studies examining integration sites of HBV in tumor samples,¹¹³⁻¹¹⁵ as well as on the determination of restriction fragment length polymorphisms of X-linked genes such as the androgen receptor gene in tumor cells.¹¹⁶ However, the cell type that gives rise to HCC is not well understood as yet.¹¹⁷ In general, cell proliferation at the time of carcinogen exposure is a prerequisite for injury to the genome to take a heritable form. Therefore, it is logical to expect that the “target cell” has both the capacity to undergo extensive cell division and to remain viable for extended periods of time allowing for the accumulation of additional mutations needed for malignant transformation.

Additional evidence regarding the origin of HCC from hepatocytes comes from several studies done in rodents. In one such experiment, Gournay et al labeled hepatocytes with a retroviral vector expressing the β -galactosidase gene after two-thirds hepatectomy and fed the animals with 2-AAF to induce HCC.¹¹⁸ They later observed that some of the neoplastic nodules in the liver samples of these animals contained cells expressing β -galactosidase, indicating that they were directly derived from retrovirally-labeled hepatocytes. In another study, using similar retroviral marking of hepatocytes with β -galactosidase gene, Bralet et al showed that following chronic administration of diethyl-nitrosamine (DEN), some of the tumors that developed in the liver contained cells expressing β -galactosidase, confirming that mature hepatocytes can give rise to HCC in a clonal manner.¹¹⁹ While these studies demonstrated the capacity of resting hepatocytes to re-enter the cell cycle and divide in response to injury, others have showed that stem cells in the liver are also activated by injury.^{120,121} In fact, oval cells can generate hepatocytes and cholangiocytes when hepatocytes fail to respond after injury.^{42,122}

The concept that oval cells and HPCs are involved in the development of HCC is based on numerous studies performed with animal models and with human clinical liver tumor specimens. Most models of hepatocarcinogenesis are characterized by very prominent proliferation of oval cells. One well-known example is the “Solt-Farber” model.¹²³

In this model, DENA is administered first as an initiator, and after two weeks the animals were fed with 2-AAF to inhibit the proliferation of non-initiated hepatocytes. Two-thirds partial hepatectomy was then performed in these animals to stimulate proliferation of cells that were initiated with DENA. The most prominent histologic feature observed in this model is proliferation of oval cells within biliary ductular structures, which peaked at 1–3 weeks, followed by the appearance of dysplastic nodules where oval cells surrounded the nodules, and migration into them. The development of HCC then occurred within 14 months in these rats. The comparison of the phenotypes between various cell populations has shown that tumor cells have a phenotype similar to that of oval cells but not hepatocytes.¹²³

Another example of the experimental models supporting the involvement of progenitor/stem cells in hepatocarcinogenesis is the choline-deficient diet model.⁴⁵ In this model, administration of the carcinogens ethionine or AAF to animals on a choline-deficient diet results in a rapid proliferation of oval cells beginning 1–2 days after the administration of the diet and results in tumor formation. Even though it is not clear whether oval cells or periductular progenitor cells are ultimately responsible for cancer formation, it is apparent that some type of hepatic progenitor cell, and not the mature hepatocyte, is the target for malignant transformation. The most direct evidence for the involvement of oval cells in hepatocarcinogenesis was provided by Dumble et al.¹²⁴ This group isolated oval cells from p53 null mice and then transplanted them into athymic nude mice where the p53-deficient oval cells produced hepatocellular carcinomas, conclusively demonstrating the ability of oval cells to serve as the target cells of malignant transformation in HCC.

In another study, Yang et al used CD90 expression as a marker to characterize CSCs in HCC cell lines, tumor specimens, and blood samples.¹²⁵ They showed that CD45⁻ and CD90⁺ cells, but not CD90⁻ cells, had tumorigenic potential in cell lines. All tumor specimens and most blood samples from HCC patients contained a population of CD90⁺ and CD44⁻ cells which were capable of generating tumor nodules when transplanted in immunodeficient mice. The CD90⁺CD44⁺ cells demonstrated a more aggressive phenotype than the CD90⁺CD44⁻ cells and formed metastatic lesions in the lungs of these mice. The gene expression profile of CD45⁻CD90⁺ cells indicated a stem cell-like phenotype, and blockage of CD44 expression prevented the formation of local and metastatic tumor nodules, suggesting that CD44 be a viable target in the treatment for HCC.

HCC originating from hematopoietic progenitor cells was inferred from the observation that many HCC specimens contain a mixture of mature cells that were phenotypically similar to HPCs expressing OV-6, CK7, CK19, and chromogranin-A. This would suggest an origin from stem cells with partially and fully differentiated malignant progeny existing within the same tumor sample.¹²⁶ Cells resembling HPCs have also been described in hepatoblastoma.^{32,127} Hepatoblastoma is generally thought to be stem cell-derived because both epithelial and mesenchymal tissue components can be found in such tumor specimens.¹²⁸ Other studies have identified cells with an HPC phenotype in a rare subset of hepatic cancers. These tumors have two major components, ie, an HCC component and a cholangiocarcinoma component, suggesting that the tumor could originate from a bipotential progenitor cell.¹²⁹ More recently, Zhang et al studied 12 cases of combined hepatocellular cholangiocarcinoma where the majority of the tumor cells expressed hepatic, biliary, as well as stem cell markers simultaneously, suggesting that these tumors were derived from HPCs.¹³⁰

Stem cell therapy for treatment of HCC

Due to the problems associated with orthotopic liver transplantation, transplantation of hepatocytes has been proposed as an alternative treatment option for liver disease. However, widespread use of this approach is severely limited due to the shortage of reproducible sources of hepatocytes.¹³¹ As a result of this, as well as the emergence of the stem cell field, stem/progenitor cells with the capacity to differentiate into hepatocyte-like cells appear to be a promising curative option in liver disease. These cells could regenerate the liver mass because they can proliferate for prolonged periods of time and differentiate into hepatic cells after transplantation.

The most important implication of liver CSCs is their potential clinical impact in developing novel therapeutic approaches for HCC. Recently, several groups have reported isolation and characterization of human HCC stem cells. For example, CD133 has been reported to be a marker of CSCs in various tissues (brain, pancreas, prostate, colon) and to identify CSCs in hepatocellular carcinoma cell lines.²⁷ They found that HCC was hierarchically organized and originated from a population of progenitor cells that expressed CD133⁺. These progenitor cells also possessed characteristics similar to that of normal stem cells and the ability to self-renew and differentiate. In a follow-up study by the same researchers, it has been shown that CD133⁺ HCC stem cells were the cell

population responsible for the chemotherapy (doxorubicin and 5-fluorouracil) resistance seen in HCC, and could be the source of tumor recurrence after chemotherapy.¹³² They also demonstrated that CD133⁺ HCC cells survived chemotherapy significantly better than most tumor cells which did not express CD133, and that the underlying mechanism was the constitutive activation of the serine/threonine protein kinase Akt and Bcl-2 cell survival pathways. An obvious clinical implication of this finding is the fact that specific inhibitors of these pathways would potentially be useful in the treatment of HCC.

The notion of CSCs initiating and advancing tumors is now well accepted in the scientific community. Unlike the great majority of cells in a given organ, only a small number of CSCs possess tumorigenic potential and the necessary phenotype of “stemness” to initiate tumors.¹³³ Therefore, a critical step towards developing effective cancer treatments is to understand cellular, molecular, and biochemical differences between normal stem cells and CSCs. Knowledge of such differences would allow us to identify key targets for therapeutic applications. One common method that is being used to distinguish CSCs from other cells is identifying their expression of cell surface markers. Unfortunately, this approach has not been very useful because several of these surface markers are shared both by CSCs as well as normal stem cells of the corresponding tissue. For instance, both CSCs and normal stem cells of the liver express CD133.^{27,134–136} With the improvement in assays to determine gene expression profiles, it should be possible to identify the differences between the two cell types in the near future. Other approaches, such as studies of heritable epigenetic gene silencing, genetic alterations, and mutations in specific oncogenes of these cells, as well as the resident microenvironment of tumors, may allow us to understand the conditions that give rise to CSCs. Similarly, targeting specific cellular signaling pathways involved in HCC is another approach towards finding new treatments.^{5,8} Furthermore, removing the stemness of CSCs to eliminate their ability to proliferate and differentiate, and inhibiting their maintenance of stem cell state, is another approach that might provide us with important breakthroughs.¹³⁷ It has been shown in a number of cancers that generation of tumors can be achieved by transplanting CSCs into animals. Therefore, transplantation of CSCs and stem/progenitor cells expressing oncogenes such as *c-Myc* might allow us to develop animal models to study the disease process and to develop novel cancer therapies.

As discussed above, stem/progenitor cells have been isolated from almost every mammalian organ, and shown to differentiate into hepatocytes and other cell types. The

important question is: are all these stem cells created equal? In other words, can we use stem cells from any organ for transplantation into livers for therapeutic purposes? To address this question, we need a clear understanding of the similarities and differences between various stem cells prior to and after their differentiation into the desired cell type. A couple of recent investigations have dealt with this issue. In one study, Sharova et al looked at the similarities and differences among mouse stem cells of different origins and strains using gene expression profiling.¹³⁸ They found that ESCs and embryonic germ cells were indistinguishable in their gene expression pattern. Very few differences were observed when these cells were grown in normal growth medium and in the medium that promotes differentiation. However, using stringent statistical analysis and quantitative real-time polymerase chain reaction, the authors were able to identify a signature profile containing 20 genes that distinguish each cell type. Interestingly, the variation in gene expression was greater between ESCs and embryonic germ cells of different mouse strains than within a single strain. In another study, Noël et al evaluated global gene and protein expression profiling of human adipose tissue-derived and multipotential stromal cells using microarrays, 2D gel electrophoresis, and functional assays. They found that cell type-specific differences do exist between these cells, although they possess similar differentiation potentials.¹³⁹ Their data suggest that stem cells are different and express genes depending upon their tissue origin. Further studies are needed to understand molecular mechanisms involved in the regulation of stemness and differentiation.

Conclusions

Advances in stem cell technology provide opportunities to develop novel approaches with an ability to reduce the morbidity and mortality associated with liver cancer. Liver cancer is a multifactorial disease with many different underlying pathogenic mechanisms caused by a variety of risk factors. Despite enormous progress during the past several decades, patient survival remains very low. Prospective, randomized human clinical trials are expensive, time-consuming, and very difficult to perform. A severe shortage of livers for orthotopic transplantation has compounded this problem even further. Unfortunately, hepatocyte transplantation has achieved little success in humans so far and there is a greater need to isolate and enrich stem cells with greater clonogenic potential to use them for therapy.¹¹⁷ However, stem cell therapy for the treatment of liver cancer is a long way away from reality. Rather than using treatments such as tetrasine, which are clinically unacceptable,

enhancing the clonogenic potential of stem/progenitor cells isolated from healthy livers might prove critical for creating novel cancer therapeutics. Towards this goal, a better understanding of the molecular mechanisms involved in tumor formation and progression, development of new antifibrotic agents, experimental animal models that closely mimic the human disease, and antiviral agents are critical for the success of cell-based therapies for liver cancer.

Disclosure

The author reports no conflict of interest in this work.

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