

STEM CELLS AND HEPATOCYTES IN THERAPY OF LIVER DISEASES

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Rezumat

Celulele stem și hepatocitele în terapia bolilor hepatice

În prezent, există un interes crescut a utilizării celulelor stem în terapia diferitor boli. Celulele stem au capacitatea de a se divide pentru o perioadă indefinită de timp, de a se autoreînnoi și de a da naștere mai multor tipuri de celule. Terapia celulară poate fi definită ca „utilizarea celulelor în restabilirea, menținerea și îmbunătățirea funcțiilor țesuturilor și organelor”. Acest articol de sinteză este adresat hepatocitelor, celulelor stem hepatice, inclusiv originii lor, rolului acestora în regenerarea ficatului și dezvoltarea fibrozei, și folosirea lor posibilă în tratarea bolii de ficat. Cele mai multe tulburări hepatice rezultă din disfuncția hepatocitelor, aici acordându-se un mare interes transplantului de hepatocite izolate. În încheiere, hepatocitele izolate și derivate trebuie să demonstreze activitatea normală fiziologică, anume în funcția de detoxificare și metabolismul specific, ce se determină prin expresia genelor a proteinelor prezente în hepatocitele adulte.

Summary

At present, there is growing interest in the therapeutic use of stem cells. A stem cell has the ability to divide for indefinite periods of time, to self-renew and to give rise to many different cell types. Cell therapy can be defined as «the use of living cells to restore, maintain or enhance the function of tissues and organs». This review will address on hepatocytes, hepatic stem cells, including their origin, their role in liver regeneration and fibrosis, and their possible use in the treatment of liver disease. As most liver disorders result from hepatocyte dysfunction, there has been great interest in transplantation of isolated hepatocytes. In summary, isolated and derived hepatocytes should demonstrate drug metabolism and detoxification activity by both gene expression and function, and they should express the hepatic transport proteins and transcription factors present in mature hepatocytes.

Diseases of the liver impose a heavy burden on society and affect approximately 17% of the world population [4]. Viral hepatitis, acute and chronic, cirrhosis and hepatic cancer present a very important medical and socio-economical problem in Republic of Moldova. More than 10000 people are infected with viral hepatitis every year [27, 28]. Cirrhosis is a progressive liver disease and is marked by the gradual destruction of liver tissue over time. Globally the main causes of cirrhosis are hepatitis B, C and alcohol abuse. There is 79,2% of patients deaths with digestive system diseases is cause of cirrhosis. Cirrhosis is one of the important and common causes of death and mortality from cirrhosis have increased last 20 years in Moldova [27].

Despite the high incidence of liver diseases that result in liver dysfunction and failure, current medical therapies are limited.

At the cirrhotic stage, liver disease is considered irreversible and the only solution is orthotopic liver transplantation (OLT). Liver transplantation is considered to be the standard treatment. Unfortunately, its extensive application is restricted by the limited availability of donor organs. In addition, liver transplantation is associated with significant morbidity and mortality. However, the increasing shortage of donor organs restricts liver transplantation, life-long dependence on immunosuppression and the poor outcome in patients not supported by liver transplantation, there is obviously a demand for new strategies to treat liver disorders [28].

Cell therapy can be defined as «the use of living cells to restore, maintain or enhance the function of tissues and organs» [1]. The use of isolated, viable cells has emerged as an experimental therapeutic tool in the past decade, due to progress in cell biology and particularly in techniques for the isolation and culture of cells derived from several organs and tissues. However, experimental cell therapy has a longer tradition in hepatology, since it has been known

for more than 30 years that isolated hepatocytes infused into the portal vein engraft into the liver cords and express normal cell function. Such a therapeutic strategy was put forward as an alternative to OLT, which requires major surgery and is limited by the availability of donors. Indeed, it was shown that significant clinical results can be obtained with the transplantation of isolated hepatocytes corresponding to as little as 1-5% of the total hepatocyte mass [2,3].

This review will address on hepatocytes, hepatic stem cells, including their origin, their role in liver regeneration and fibrosis, and their possible use in the treatment of liver disease.

Mammalian liver development and hepatic induction

Embryonic stem cell are derived from the undifferentiated cell of the epiblast, which give rise to the three principal germ layers and their differentiated progeny through a process called gastrulation [25, 29]. Of the three germ cell layers the endoderm gives rise to hepatic, pancreatic, lung, and intestinal tissues in a process that is not well understood.

Using a variety of techniques, it has now been shown that fibroblast growth factors (FGFs) can substitute for cardiac mesoderm and bone morphogenic proteins (BMPs) can substitute for the septum transversum mesenchyme to work in concert to induce the ventral endoderm to adopt a hepatic fate [26]. Factors identified as proposed targets of FGF and BMP signaling include the Foxa and Gata genes, which regulate the competence of foregut endoderm to respond to hepatic inductive signals [29, 30]. In addition, the transcription factor hepatocyte nuclear factor-6 (HNF6) has been shown to play a critical role in the proper morphogenesis of both the intra- and extra-hepatic biliary tree. The mechanism by which HNF6 regulates biliary tree development also appears to involve the related transcription factor, hepatocyte nuclear factor-1 (HNF1) [20]. Hepatocytes and bile duct cells originate from a common precursor, the hepatoblast [24]. Notch signaling promotes hepatoblast differentiation toward the biliary epithelial lineage, while HGF promotes differentiation toward the hepatocyte lineage [22].

Physiological homeostasis of the liver

The normal liver contains hepatocytes, endotheliocytes, Kupffer cells, and hepatic stellate cells. Hepatic stellate cells are the key fibrogenic cells. During hepatic fibrogenesis, hepatic stellate cells undergo a response known as “activation,” which is the transition of quiescent cells into myofibroblasts. Activated hepatic stellate cells are the critical source of extracellular matrix in hepatic fibrosis [30].

Under physiological conditions, as few as one out of 2000–3000 hepatocytes divide to maintain the physiological liver mass. Liver damage or loss of liver mass can however extensively stimulate the regenerative capacity until the tissue mass has been restored by the proliferation of mature parenchymal liver cells [8]. Up to 75% of surgically removed liver mass can be regenerated within 1 week in rodents [5]. The newborn liver contains only diploid hepatocytes but polyploidisation and binuclearity occurs rapidly after birth.

Fractionation of isolated adult rat hepatocytes based on cell density has yielded subpopulations with “small” mononucleated hepatocytes and “large” hepatocytes with higher ploidy. Hepatocytes with higher ploidy have been shown to reside predominantly in the perivenous areas and to contain more DNA and to exhibit greater maturity. The “smaller” mononucleated hepatocytes are located in the periportal areas, contain less DNA and exhibit greater growth factor responsiveness [9, 28].

The “streaming liver hypothesis”, which suggests that the liver lobule is organised in a similar way to the intestinal crypt by containing a stem cell pool arising from the periportal area has, however, been disproved by the observation that nearly all hepatocytes proliferate as a response to injury, regardless of location and ploidy [24].

Although mature hepatocytes and cholangiocytes represent the first and most important resource for tissue repair, experimental data support the hypothesis that the liver also contains or activates a stem cell compartment [17]. Today the origin of hepatic stem or precursor cells is still a matter of debate.

Interestingly, oval cells express markers of Hematopoietic Stem Cells (HSCs), such as Thy-1, CD34, CD45, Sca-1, c-Kit and flt-3 [12-16]. In particular, Thy-1 is a highly conserved protein.

It has been found in the brain and in the hematopoietic system of rat, mouse and humans [16, 30]. It is also expressed on stem cells of fetal liver and in bone marrow (BM)-derived cells. In addition, the normal adult liver contains hematopoietic cells that are phenotypically similar to cells present in the BM [30]. These observations originated the hypothesis that liver stem cells may arise from a population resident in the BM. Also, experimental evidence suggests that liver parenchymal cells can originate from a specific precursor cell compartment in the liver, from pluripotent stem cells, from transdifferentiation of HSCs or cell fusion.

Hepatocyte isolation

As most liver disorders result from hepatocyte dysfunction, there has been great interest in transplantation of isolated hepatocytes. However, their clinical application is also dependent on the availability of good quality donor livers. To overcome the problem of limited donor organs and to make hepatocytes available for other applications, several approaches to isolate and propagate liver stem cells or progenitor cells have been developed.

Hepatocyte isolation from human livers is now universally performed with a “two-step” collagenase procedure developed by Berry and Friend [28]. Originally developed for the isolation of rat hepatocytes, this procedure has been modified by various laboratories for the isolation of hepatocytes from several animal species, including human [3,4].

The procedure involves the initial perfusion of the liver with a warm (37°C) divalent ion-free, EGTA-containing, isotonic buffer (Step 1) to remove blood and to loosen cell-cell junctions, followed by perfusion with a warm, isotonic, collagenase solution (Step 2) to dissociate the liver parenchyma into single cells. In general, a higher amount of collagenase is required for the isolation of hepatocytes from human livers than that required for rat livers. As collagenase is a mixture of proteases, its composition can affect its effectiveness in the dissociation of the hepatocytes as well as its cytotoxicity. It is a common practice to evaluate multiple lots of collagenase to select the one lot yielding the highest number of viable hepatocytes from a liver. After digestion, the cells are harvested by low speed centrifugation. A density gradient such as Percoll is commonly used to enrich for viable cells. The isolated cells can be used in suspension for experiments requiring a relatively short time duration (hours), plated on tissue culture surfaces pretreated with attachment substrates (e.g. collagen; Matrigel) for longer term studies, or cryopreserved for future use [10].

The method of isolation of human hepatocytes from a human liver is by no means optimized. Currently, a so called “good” yield of human hepatocytes from a human liver is approximately 10–30 billion viable cells when a whole liver is perfused. Using an approximation of 1.5 kg as an average weight of a human liver, this leads to a yield of or approximately 7–20 million hepatocytes per gram of liver (e.g. [5, 6, 29]), which is considerably less than the total number of hepatocytes (approximately 300 billion) in the human liver. It is to be noted that the yield of human hepatocytes (in terms of number of hepatocytes per gram liver) is in general higher from smaller (e.g., 10 to 300 g) liver fragments than whole livers or lobes [24, 29].

Cryopreservation

Hepatocytes, especially human hepatocytes, are now routinely used after they are cryopreserved [7, 8]. The general procedures for hepatocyte cryopreservation have not deviated extensively from the original procedures [9]. Via the use of equipments to control freezing rates (e.g. programmable control-rate freezer) and appropriate cryopreservation agents (e.g. dimethyl sulfoxide), hepatocytes now can be stored in liquid nitrogen (lower than -150 °C) for an extensively time period (years) with the retention of high viability and drug metabolizing enzyme activity [7]. The most recent advancement of human hepatocyte cryopreservation is the ability of the thawed hepatocytes to be plated as monolayer cultures (“plateable” hepatocytes) [10,11].

Stem cell differentiation into hepatocytes

Methods for differentiating stem cells into hepatocytes can be separated into those that involve spontaneous formation of liver-like cells and those involving directed differentiation [17, 22].

Spontaneous differentiation involves formation of EBs, plating of ES cells on an adherent matrix as a monolayer, or following transplantation into an hepatic environment [22,29]

Directed differentiation usually involves addition of growth factors and cytokines to cells *in vitro* on extracellular matrices. Combinations of these techniques, involving both formation of EBs with expansion in growth factors and/or co-culture with cells supplying additional factors, have also been successful [29]. In addition, culture in sodium butyrate, a histone deacetylase inhibitor, leads to an increase in the number of cells expressing mature hepatocyte-specific genes.

Selection of hepatocyte-like cells based on the use of liver-specific promoters that drive reporter gene expression has been a relatively successful strategy for selecting a homogeneous population of cells with hepatic characteristics [22,29]

In the majority of settings, the resultant cells have morphological features similar to those of primary hepatocytes and most of the cells express liver-associated proteins. Whether such cells have the functional characteristics of a mature liver cell will require a more comprehensive analysis.

To adequately assess the extent to which *in vitro* differentiation of stem cells has been effective, it will be important to clearly demonstrate cellular characteristics and activities that can only be performed by primary hepatocytes.

1. Gene expression by differentiated “hepatocyte-like” cells should be compared to the gene expression profile of human fetal and/or mature liver cells [29];
2. Evidence of basal and inducible CYP450 isoform function should be assessed [23,29];
3. Metabolism of xenobiotics or other endogenous substances (hormones and ammonia) should be determined [21,29];
4. Synthesis and/or secretion of the following should be performed: albumin, clotting factors, complement, transporter proteins, bile acids, and lipids and lipoproteins. [29];
5. Evidence of restoration of liver function in appropriate animal models, or evidence of repopulation of the liver by derived “hepatocytes” should be examined.

In summary, derived hepatocytes should demonstrate drug metabolism and detoxification activity by both gene expression and function, and they should express the hepatic transport proteins and transcription factors present in mature hepatocytes.

The procedure seems relatively safe, provided portal pressure and/or portal flow are monitored during cell infusion in order to prevent vascular thrombosis [30]. Hepatocyte transplantation has recently been used as an alternative to OLT in patients with liver-based congenital metabolic disorders, such as Crigler-Najjar disease, α -1-antitrypsin deficiency glycogen storage disease type Ia, 10 ornithine transcarbamoylase deficiency and the deficiency of factor VII [7, 8, 9] The role of hepatocyte transplantation in the treatment of acute and chronic liver disease is less clear due to difficulty in organizing large-scale clinical trials.

Indeed, the main factor limiting the practice of hepatocyte transplantation is again the availability of liver grafts for cell isolation. Moreover, the metabolic effects of cell transplantation seem to be fading with time, a problem which can partially be solved by repeated hepatocyte infusions but which probably indicates the progressive loss of the terminally-differentiated exogenous cells. In theory, both problems could be solved by replacing the hepatocyte with stem or precursor cells, provided they can be isolated from a more affordable source [11, 12].

At present, there is growing interest in the therapeutic use of stem cells [28]. A stem cell has the ability to divide for indefinite periods of time, to self-renew and to give rise to many different cell types. Embryonic stem cells originate from the inner cell mass of the mammalian blastocyst and are totipotent. Adult stem cells are more specialized, being committed to give rise to cells with a particular function within their own specific tissue or organ. Precursor/progenitor cells are defined as cells rapidly dividing and already partially determined towards specific differentiation pathways [28-30].

However, experimental evidence suggests that some adult stem cells are able to develop into different types of specialized cells (a process also known as transdifferentiation), depending on the microenvironment where they are homed, including the liver.

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CĂILE DE ÎMBUNĂTĂȚIRE A REZULTATELOR TRATAMENTULUI CHISTURILOR HIDATICE SUPURATE A FICATULUI

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Summary

Ways to improve treatment outcomes in suppurated liver echinococcus

We analyzed the treatment outcomes in 63 patients with suppurated liver echinococcus and concluded that intraparenchymal cysts suppurate more often due to compression and communication with the bile ducts. The medication adjustment must be done before surgery. The volume of surgery must be appreciated during the operation in dependence of localization of the cyst and patients condition. We recommend application of the immunotention of the liver parenchyma with the mononuclear cells to ameliorate the reparative properties.

Rezumat

Noi am analizat rezultatele tratamentului a 63 de bolnavi cu echinococoza supurată a ficatului și am ajuns la concluzie că mai des supurează chisturile intraparenchimotoase din cauza compresiei și comunicării cu ducturile biliare. Înainte de operație trebuie de efectuat corecția medicamentoasă. Volumul operație trebuie de apreciat intraoperator în dependență de localizarea chistului și stării pacientului. Noi recomandăm aplicarea imunostimulării parenchimului ficatului cu celulele mononucleare pentru ameliorarea proprietăților reparative.

Actualitate

Echinococoza, fiind o patologie regională pentru Moldova, este o problemă medicală serioasă. Deși stabilirea diagnosticului de echinococoza hepatică în prezent nu este dificilă, stadiile primare asimptomatice duc la adresarea tardivă a pacienților. Majoritatea operațiilor se efectuează când cea mai mare parte a parenchimului ficatului este înlocuită cu chist parazit și