REVIEWS

Transplantation of Embryonic Hepatocytes. Experimental Substantiation of a New Approach to the Therapy of Liver Failure

G. T. Sukhikh and A. A. Shtil'

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 134, No. 12, pp. 604-610, December, 2002
Original article submitted July 23, 2002

The use of embryonic cells for the therapy of insufficiency in vitally important organs is a promising approach of transplantation medicine. Transplantation of fetal cells can restore liver functions during severe failure of various geneses. We review modern notions about histogenesis, structure, and mechanisms of functioning of low differentiated hepatocytes and extrahepatic cells that can be used for transplantation. Methods for *ex vivo* isolation of fetal cells for transplantation are described.

Key Words: *embryonic stem cells; hepatocyte; liver; transplantation; experimental models*

Much progress in culturing of somatic cells, genetic engineering, and molecular biology was achieved in the last 25-30 years, which offers new scope for directed modulation of physiological functions. Intra- and interspecies transfer of cells is the most promising field of biology and medicine. Transplantation of cells opens up new approaches to studies of embryogenesis and organogenesis. Transplantation of specialized somatic cells is method for replacement cellular therapy of immune deficiency, hereditary abnormalities in cell metabolism, and acute functional insufficiency of organs. This procedure is widely used for intrauterine correction of hereditary defects [1].

The therapeutic effects of transplantation are mediated by embryo-specific growth factors, cytokines, or signal molecules capable of stimulating regeneration and survival of liver cells in the organism of recipients. The mechanisms underlying therapeutic activity of transplanted cells are extensively studied. The effect of fetal cells is probably associated with the activation of specialized and precursor cells. Donor cells and recipient regenerating cells partially or completely normalize molecular and cellular homeostasis.

Transplantation of Liver Cells: General Concept and Substantiation of Approaches

As noted by the World Health Organization, chronic liver failure ranks 5th among mortality factors in the Earth. In 80% patients acute liver failure (ALF) leads to a lethal outcome. Plasmapheresis, hemodialysis, ultrafiltration, and hemosorption used over the past 25 years did not produce desirable results in the therapy of patients with ALF. Plasmapheresis and hemoperfusion in the absence of biologically active cells did not decrease the mortality rate in patients with this disease. Radical therapy of these patients suggests transplantation of histocompatible liver.

Laboratory of Clinical Immunology, Research Center for Obstetrics, Gynecology, and Perinatology, Russian Academy of Medical Sciences, Institute of Biological Medicine; Institute of Carcinogenesis, N. N. Blokhin Oncology Research Center, Moscow. ***Address for correspondence:*** immunolab@pregnancy.ru. Sukhikh G. T.

0007-4888/02/1346-0519$27.00

The transplantation register of the United Nations Organization shows that the incidence of liver transplantations to adult patients and children performed in 1988-1991 increased by 85 and 30%, respectively. Then the number of surgeries decreased slightly due to inadequate supply of donor organs. More than 25,000 transplantations were performed in the United States in 1967-1997. Some patients with donor livers live 10-20 years and retain the ability to work [1].

Transplantation of somatic cells in clinical practice is limited due to insufficient abilities of organ transplantation, low quality and deficiency of donor tissues. In the United States and Europe only 15-25% patients receive adequate therapy (organ transplantation). Ten donor organs per 1 million patients are required annually to satisfy the current demand for livers. Not more than 20% patients survive up to surgery. The number of patients requiring organ transplantation by vital prescriptions increases with demographic aging and distribution of chronic viral diseases. Transplantation of somatic cells is a promising alternative to organ transplantation [1].

Various hereditary metabolic defects of the liver can be compensated by transplantation of 3-5% hepatocytes. Despite high rejection rate for donor hepatocytes in the liver of recipients, implanted donor cells undergo division, form hepatocyte steems, and stimulate regeneration of the remaining liver parenchyma. The advantage of cell transplantation is that it allows repeated treatment with cryopreserved cells (*e.g.*, immortalized human and porcine hepatoblasts) standardized for medical purposes. Organ transplantation has considerable technical difficulties: parenchymal organs may not be cryopreserved, the vascular endothelium is irreversibly damaged during heat and cold ischemia, and parenchymal cells rapidly die *in situ*.

Development of the Method for Hepatocyte Transplantation

Hepatocyte transplantation is an alternative to transplantation of the liver. This method originates from operations when surgeons decreased the volume of grafted tissues to attenuate rejection. Initially, the therapeutic effect was achieved after transplantation of the liver lobe. Then transplantation of 10-15% liver produced the desired effect. Transplantation of small segments is used for supportive therapy of patients, when appropriate donor liver is not availible [32].

It is necessary to obtain maximum number of hepatocytes per unit of volume during the development of artificial liver. This is achieved by culturing of hepatocyte monolayers on each side of an artificial filter. The relationship between monolayers of parenchymal cells forming bile canaliculi in the hepatic trabecula was studied. A three-dimensional structure of artificial livers approaches the structure of hepatic lobules. Hepatocyte monolayers are separated from the blood flow with a semipermeable membrane allowing active and passive transport of substances between the external and internal space. In the culture with high density of cells, hepatocytes and endothelium survive and retain specific functions for 25 days [79].

Apart from studies of extracorporal bioartificial liver, a method of microtransplantation of liver tissues for the correction of ALF in animals was elaborated. Preference was given to fetal hepatocytes. These cells were characterized by high survival and proliferation rates. Rejection of fetal hepatocytes was insignificant. For example, the survival rate in animals with lethal ALF increased on day 7 after intraomental transplantation of fetal hepatocytes from rats or pigs. It should be emphasized that 0.5-cm^3 fragments of the fetal liver were most effective [32]. Administration of donor hepatocytes into the omentum improved survival in rats with liver failure. The omentum contains considerable amounts of loose connective tissue with numerous capillaries and stem compartments. To increase the survival rate of non-rejected cells, hepatocytes were implanted into the omentum using polymeric carriers [41]. Inoculation of donor hepatocytes into the abdominal cavity in rats with lethal ALF also prevented death of animals. Administration of syngeneic hepatocytes into the abdominal cavity improved survival of rats with ALF produced by D-galactosamine. Biochemical parameters of the blood improved in 25% survived animals. The suspension of freshly isolated hepatocytes was most effective, while cells embedded into gel produced a less pronounced effect [38].

These data indicate that transplantation of hepatocytes holds much promise for the correction of functional insufficiency of the liver. The dynamics of protooncogene expression showed that the transplanted liver is characterized by active regeneration and contains proliferating hepatocytes. It is important that regeneration of hepatocytes in transplanted livers can compensate post-operative rejection of the transplant due to immunological incompatibility.

Therefore, successive transplantation requires the selection of suitable donor hepatocytes. The criteria for selecting these cells are described below.

Stem Cells as the Material for Transplantation

The use of isolated hepatocytes is associated with 2 difficulties. First, the therapy of liver failure requires a considerable number of mature hepatocytes (25 billion cells) [6]. And second, these hepatocytes are characterized by very low division rate. Normal adult hepato-

cytes do not proliferate in the culture without epidermal growth factor or hepatocyte growth factor [23,48]. Proliferation and differentiation are interrelated processes. The higher is the degree of hepatocyte differentiation (*i.e.*, their ability to perform specialized functions), the lower is the rate of their proliferation [40]. Transplantation should be performed with a considerable number of differentiated hepatocytes capable of proliferating (mutually exclusive conditions). For the present, only one approach can solve the problem. This method suggests the use of stem liver cells (SLC) that retain the ability to undergo proliferation and differentiation into specialized cells. Three type of SLC can be used:

— embryonic SLC, or embryonic bipotent stem precursor cells of the liver (BSPCL), differentiate into hepatocytes or bile duct cells depending on culturing conditions;
— mature hepatocytes that sometimes regain the ability to proliferate;
— oval cells are nonparenchymal liver cells with pronounced proliferative activity [26].

BSPCL originate from endodermal cells forming the hepatic diverticulum, which appears on the peritoneal side of the anterior endoderm in human embryos on day 22 of intrauterine development [17,34]. BSPCL form hepatic trabeculae by spreading in the septal mesenchyma [17,34]. BSPCL can be detected in the liver of human and rat embryos from the end of the 4th week and days 10-12, respectively [25]. These cells designated as hepatoblasts proliferate and differentiate into hepatocytes or bile duct cells. This process proceeds over several months in humans and by the end of the 2nd week in rats. At this stage differentiation of hepatocytes and bile duct cells is reversible. Hepatoblasts can differentiate into ductal cells [77]. Ductal cells are transformed into oval cells, non-differentiated elements capable of developing into hepatocytes, bile duct cells, and cells of other organs (enterocytes and pancreatic cells) [77]. Bipotency of SLC was thoroughly studied in culture, since they can be isolated from rat and mouse embryonic liver by the end of 2 weeks gestation [28]. Under various conditions of culturing SLC differentiate into hepatocytes or bile ductal cells. These cells may be easily distinguished by ultrastructural and specific markers (albumin, α-fetoprotein, and cytokeratins 1 and 18 in hepatocytes; cytokeratins 7 and 18 and γ-glutamyltransferase in duct cells) [28,66]. There are a variety of markers for identification of SLC. The major pathways for evolution of these cells were characterized [77].

Endodermal cells not differentiated into BSPCL express hepatocyte nuclear factor 3 (hepatocyte growth factor 3). This is an early transcriptional factor detected in the hepatic diverticulum [81]. This factor is expressed earlier than other proteins modulating transcription [18,70,81] and, probably, regulates the formation of the endoderm and differentiation of liver cells [70].

SLC used for the therapy of liver failure can be isolated after interruption of pregnancy by medicinal prescriptions. The objections against treatment with embryonic hepatocytes are associated with ethical principles. Moreover, embryonic hepatocytes differ from mature hepatocytes. Gluconeogenesis in human liver starts only on the 4th month of embryogenesis [34]. Polarity of rat hepatocytes disappears several weeks after birth. Moreover, the embryonic liver expresses α-fetoprotein, but not albumin [62]. Therefore, embryonic hepatocytes can proliferate, but do not compensate functional activity of the organ. An attempt was made to use embryonic hepatocytes for the therapy of patients with fulminant liver failure [31]. Seven patients intraperitoneally received human embryonic hepatocytes. However, this attempt was unsuccessful.

Recent experiments were performed embryonic cells isolated from human liver. Their differentiation into hepatocytes or bile duct cells was studied [47]. Precursor cells of rat embryonic liver isolated on day 14 were distributed in the liver of adult rats and formed hepatic trabeculae and bile ducts [10]. The population of precursor cells spreading in the liver and differentiating into mouse liver parenchyma can be obtained by flow cytofluorometry [71]. This finding provides a way for elaborating an alternative approach to liver transplantation. Despite great interest to these results [65], the use of human liver precursor cells is still a difficult problem.

Mature hepatocytes are slowly dividing cells. Normal liver contains 0.10-0.01% mitotic cells [16,23]. The ratio of mitoses sharply increases during liver regeneration after partial hepatectomy in rats [23,48]. However, in this experimental model the population of liver cells is replenished with proliferating (differentiated) hepatocytes [2,48,77]. In other words, mature hepatocytes are unipotent stem cells. As differentiated from other stem cells [59], they differentiate only into one type of cells.

The question arises: whether differentiated adult hepatocytes can populate the affected liver? Studies on transgenic mice with severe disorders of the liver stimulating its regeneration for a long time showed that transplantation of normal adult hepatocytes into mouse liver provides its complete repopulation [55, 60]. Under these conditions adult hepatocytes are capable of proliferating. Mature cells undergo up to 77 divisions (which is comparable with division potential of hemopoietic stem cells) [56]. This approach was used on the model of acquired liver diseases. Complete

repopulation of the liver with adult hepatocytes proceeded in rats, when the cell cycle was suppressed with alkaloid retrosine after partial hepatectomy [42]. Experiments on analbuminemic Nagase rats revealed albumin in the liver of animals after liver transplantation. In these rats plasma albumin concentration returned to normal 4 months after hepatocyte transplantation [53]. Transplanted mature hepatocytes contact with hepatocytes in hepatic lobules and bile duct cells [42,56]. Moreover, transplanted hepatocytes retain morphological and functional characteristics of adult hepatocytes and do not undergo transformation into ductal or tumor cells [56].

These interesting results raise a number of questions. It remains unclear whether all adult hepatocytes or only individual cells can proliferate. Fractionation of cells showed that medium and large hepatocytes (but not small cells) display the ability to proliferate [56]. Medium and large hepatocytes are localized in the medial and central zones of hepatic lobules, while small cells are present around the periportal space. Because of periportal localization, some authors hypothesized that small hepatocytes are adult stem cells of the liver [64,69]. However, experiments with liver regeneration after partial hepatectomy showed that all hepatocytes can proliferate independently on their localization in the lobule [4,23].

Further investigations should be performed to evaluate factors initiating hepatocyte proliferation. Growth factors play an important role in this process. Continuous perfusion of hepatocyte growth factor in mice was followed by liver weight gain, stimulation of hepatocyte proliferation, and increase in the lifespan of cells carrying exogenous structures [57]. Proliferation of hepatocytes can be stimulated without partial hepatectomy. One year after administration of carnosine to rats, nearly all hepatocytes in recipients were replaced with donor cells without surgeries [43].

Oval cells (OC) are small nonparenchymal cells with the poor cytoplasm and large nucleus and nucleolus. These cells are detected after chronic treatment of animals with hepatic carcinogens or toxins [24,52,78]. OC appear in the periportal space and then occupy the hepatic lobule. It should be emphasized that the rate of invasion depends on hepatotoxicity [24]. OC are good candidates for the role of SLC: they are characterized by high proliferation capacity and can differentiate into hepatocytes and ductal cells under *in vivo* conditions and during culturing [22,29,51,73,80]. OC express proteins of hepatocytes and bile duct cells, including albumin, α-fetoprotein, cytokeratins 8 and 18 (for hepatocytes), and cytokeratins 7 and 19 (for bile duct cells) [67]. Abundance of OC simplifies their isolation and culturing [67]. Some authors believe that OC originate from cells of the Herring channel connecting hepatocytes with bile duct cells [2]. Others authors reported that these cells develop from periductal cells or unknown precursors (probably, from small hepatocytes) [64].

Due to their ability to proliferate and undergo transformation into the epithelium OC would be a good source for transplantation into affected liver. However, these attempts led to the development of cholangiocarcinoma and hepatocarcinoma [30,68]. Therefore, OC should be premodified. Transfectants of OC with the phenotype of normal hepatocytes were obtained [80].

Specific markers confirmed the presence of OC in patients with various liver diseases [8,9,61,64]. The more severe was the disease, the higher was the number of these cells [46]. Probably, affected hepatocytes are substituted with facultative stem cells, but not with unipotent committed cells (mature hepatocytes).

Stem cells of non-liver origin. Embryonic pluripotent stem cells are a promising source of proliferating stem cells. Depending on culturing conditions, these cells are transformed into muscle, nerve, or epithelial cells [21]. Neurons developing from embryonic stem cells are capable of invading affected mouse brain [11]. Transfection of hepatocyte growth factor-3 into mouse embryonic stem cells is followed by the appearance of endodermal cells expressing albumin (similarly to hepatocytes) [45] and transcriptional factors that regulate transformation of these cells into hepatoblasts. Human embryonic stem cells differentiating into intestinal cells during culturing were isolated [76].

Published data show that hemopoietic cells can differentiate into hepatocyte precursors [58,74,75]. Liver samples from female mice and rats were examined after transplantation of the bone marrow from males. Moreover, liver samples of men after transplantation of the liver from women were studied [75]. *In situ* fluorescence hybridization assay was performed to analyze chromosomes in interphase nuclei [35]. A considerable number of OC, hepatocytes, and bile duct cells contained the Y chromosome [58,74,75]. These data indicate that hemopoietic stem cells are present in the liver and develop into SLC and mature cells. Previous studies showed that hemopoietic stem cells can populate the liver and recover its biochemical functions in mice with type I hereditary tyrosinemia [44,55].

Isolation of Donor Hepatocytes

All methods for isolation of hepatocytes with collagenase impose mutually exclusive demands. The isolation medium should contain Ca^{2+} in minimum concentration to destroy desmosomes. However, Ca^{2+} ions are required for functional activity of collagenase [54]. This difficulty can be obviated by performing the pro-

cess in 2 stages. Initially the liver is washed with Ca^{2+}-free medium, which provides destruction of desmosomes. Then collagenase and Ca^{2+} are added [63]. Ca^{2+} ions in a concentration of 1 μM provide the highest rate of digestion [3].

Some researchers added 0.1-1.0 μM sodium salt of ethylenediaminetetraacetic acid or ethylene glycol-bis(β-aminoethyl ether) N,N,N,N'-tetraacetic acid (EGTA) to remove Ca^{2+}, but high yield of cells is possible without chelation of Ca^{2+}.

The method of L. C. Devirgiliis *et al*. [13] suggests that the liver is removed from embryos, dissected, placed in Ca^{2+}- and Mg^{2+}-free Hanks solution containing 0.015% collagenase, 0.005% DNase, and 2% albumin, incubated 3 times in a rotating flask with fresh enzymes and hepatocyte medium at 39°C for 15 min, and centrifuged at 40*g*. A more advanced method is described below [3].

The liver is placed in a cold Petri dish with (Ca^{2+}- and Mg^{2+}-free Hanks solution with 0.05 M EGTA (pH 7.2), minced, washed 2 times, and incubated in the same solution at 37°C for 10 min under mixing to remove the blood. The floating fraction is taken, and incubation is performed under similar conditions. To remove EGTA traces the suspension is incubated with minimum Eagle medium (pH 7.2) under similar conditions.

For enzymatic digestion the suspension is incubated (at 37°C for 15 min) with modified Eagle medium containing Ca^{2+} in a physiological concentration, type I collagenase (0.5 mg per liver), and 100 μl DNase (1 mg/ml) under mixing. The mixture is passed through a filter (90 μ). The filtrate is centrifuged at 1000 rpm for 1 min. The pellet is suspended in Eagle's modified medium containing 0.3% fetal bovine serum (FBS) and placed on ice. This enzymatic treatment is performed 3 times with 2-fold lower DNase concentration. Hepatocytes are filtered (60 μ) and suspended in Eagle medium with 0.3% FBS. The number and viability of cells is estimated in the trypan blue test. This method allows obtaining 10^8 cells per 1 g liver from the embryo taken on day 21 of pregnancy. Viability of these cells reaches 90%.

From the Experiment to Clinical Practice

Only normal adult hepatocytes were used for transplantation to animals. The first results indicate that transplantation improved functional activity of the liver [33,50]. In clinical practice isolated hepatocytes were tested by 2 methods. Hepatocytes in bioreactors were placed in the artificial vascular bed in patients or grafted into various areas of the body [7,15]. Bioreactors contained normal adult allogeneic hepatocytes, xenogenetic hepatocytes, human hepatoma cells, and human immortalized hepatocytes [7]. A bioartificial liver was tested in some patients with liver failure [20,37]. The results of observations indicate that transplantation of fetal cells produces a positive effect in 22 patients with severe liver dysfunction. Transplantation of SLC as monotherapy or in combination with other treatments holds much promise for medical practice. Further studies of the mechanisms underlying differentiation and proliferation of embryonic liver cells will form the basis for clinical trials improving the state of patients with one of the most severe diseases.

We are grateful to V. V. Belov for his help in preparing this manuscript.

REFERENCES

1. V. S. Repin and G. T. Sukhikh, *Medical Cell Biology* [in Russian], Moscow (1998).
2. M. R. Alison, M. Golding, and C. E. Sarraf, *Int. J. Exp. Pathol.*, **78**, 365-381 (1997).
3. R. M. Arahuetes, J. Codesal, M. S. Garcia Barrutia, *et al.*, *Life Sci*., **68**, 763-772 (2001).
4. D. Bernuau, A. Poliard, and G. Feldmann, *Hepatology*, **8**, 977-1005 (1988).
5. M. N. Berry and D. S. Friend, *J. Cell Biol*., **43**, 506-520 (1969).
6. K. Boudjema, *Ilots de Langerhans et hepatocytes. Vers une utilizayion therapetique*, Eds. S. Franco *et al.* (1988), pp. 137-146.
7. Y. Calmus, L. Wen, R. Sarkis, *et al*., *Ibid*. (1998), pp. 29-42.
8. H. A. Crosby, S. G. Hubscher, and L. Fabris, *Am. J. Pathol.*, **152**, 771-779 (1998).
9. H. A. Crosby, S. G. Hubscher, R. E. Joplin, *et al.*, *Hepatology*, **28**, 980-985 (1998).
10. M. D. Dabeva, P. M. Petkov, and J. Sandhu, *Am. J. Pathol.*, **156**, 2017-2031 (2000).
11. T. Deacon, J. Dinsmore, L. C. Costantini, *et al*., *Am. J. Exp. Neurol*., Vol. **149**, 28-41 (1998).
12. A. A. Demetriou, F. Watanabe, and J. Rozga, *Progress in liver diseases*, Eds. J. L. Boyer, R. C. Ockner, Philadelphia (1995), **13**, pp. 331-348.
13. L. C. Devirgiliis, I. L. Dini, A. D. Pierro, *et al*., *Cell. Mol. Biol.*, **27**, 687-694 (1981).
14. J. Dinsmore, J. Ratliff, and Deacon T., *Cell Transplant*, **5**, 131-143 (1996).
15. V. Dixit, *Scand. J. Gastroenterol*, **208**, Suppl., 101-110 (1995).
16. F. Doljanski, *Int. Rev. Cytol*., **10**, 217-241 (1960).
17. A. M. Du Bois, *The liver, morphology, biochemistry, physiology*, Ed. C. Rouiller, New York (1963), **1**, pp. 1-39.
18. D. Dufort, L. Schwartz, K. Harpal, and J. Rossant, *Development*, **125**, 3015-3025 (1998).
19. B. E. Edwards, J. D. Gearhart, and E. E. Wallach, *Fertil. Steril*., **74**, 1-7 (2000).
20. A. J. Ellis, R. D. Hughes, and R. Williams, *Ilots de Langerhans et hepatocytes. Vers une utilizayion therapetique*, Eds. S. Franco *et al.*, Paris (1988), pp. 93-104.
21. *Evans M. J., Kaufman M. H.*, *Nature*, **292**, 154-156 (1981).
22. R. P. Evarts, P. Nagy, E. Marsden, and S. S. Thorgeirsson, *Carcinogenesis*, **8**, 1737-1740 (1987).
23. J. I. Fabrikant, *J. Cell Biol.*, **36**, 551-565 (1968).

24. E. Farber, *Cancer Res.*, **16**, 142-149 (1956).
25. N. Fausto, *The liver, biology and pathobiology*, Eds. I. M. Arias *et al.*, 3rd ed., New York (1994), pp. 1501-1518.
26. G. Feldmann, *Cell Biol. Toxicol*., **17**, 77-85 (2001).
27. J. Figueras, E. Jaurrietta, C. Valls, *et al.*, *Hepatology*, **25**, 1485-1489 (1997).
28. L. Germain, M. J. Blouin, and N. Marceau, *Cancer Res.*, **48**, 4909-4818 (1988).
29. M. Golding, C. E. Sarraf, E. N. Lalani, *Hepatology*, **22**, 1243-1253 (1995).
30. M. Goyette, R. Faris, L. Braun, *et al.*, *Cancer Res.*, **50**, 4809-4817 (1990).
31. C. M. Habibullah, I. H. Syed, A. Qamar, and Z. Taher-Uz, *Transplantation*, **58**, 951-952 (1994).
32. M. Hagihara, T. Shimura, K. Takebe, *et al.*, *Cell Transplant*, **3**, 283-290 (1994).
33. H. Hamaguchi, Y. Yamaguchi, and M. Goto, *Hepatology*, **20**, 220-224 (1994).
34. W. J. Hamilton, J. D. Boyd, H.W. Mossman, *Human embryology,* Cambridge (1964), 240-244.
35. C. Hamon-Benais, O. Ingster, B. Terris, *et al.*, *Hepatology*, **23**, 426-435 (1996).
36. A. W. Hemming, B. Langer, P. Greig, *et al*., *Am. J. Surg*., **171**, 176-180 (1996).
37. W. R. Hewitt, J. Rozga, C. J. P. Mullon, and A. A. Demetriou, *Ilots de Langerhans et hepatocytes. Vers une utilizayion therapetique*, Ed. S. Franco, Paris (1988), pp. 105-118.
38. S. Hirai, S. Kasai, and M. Mito, *Eur. Surg. Res.*, **25**, 193-202 (1993).
39. D. Houssin, *Clin. Biol*., **22**, 567-570 (1998).
40. G. Ilyin, P. Loyer, A. Corlu, *et al.*, *Ilots de Langerhans et hepatocytes. Vers une utilizayion therapetique*, Ed. S. Franco, Paris (1988), pp. 161-169.
41. L. B. Johnson, J. Aiken, D. Mooney, *et al.*, *Cell Transplant*., **3**, 273-281 (1994).
42. E. Laconi, R. Oren, and D. K. Mukhopadhyay, *Am. J. Pathol*., **153**, 319-329 (1998).
43. S. Laconi, S. Pillai, P. P. Porcu, *et al.*, *Ibid*., **158**, 771-777 (2001).
44. E. Lagasse, H. Connors, and M. Al-Dhalimy, *Nat. Med.*, **6**, 1229-1234 (2000).
45. M. Levinson-Dushnik, and N. Benvenisty, *Mol. Cell. Biol*., **17**, 3817-3822 (1997).
46. K. N. Lowes, B. A. Brennan, G. C. Yeoh, and J. K. Olynyk, *Am. J. Pathol*., **154**, 537-541 (1999).
47. H. Malhi, A. N. Irani, and S. Gupta, *Hepatology*, **30**, 317A (1999).
48. G. K. Michalopoulos and M. C. De Frances, *Science*, **276**, 60-66 (1997).
49. A. Moreau, M. Maurice and G. Feldmann, *J. Histochem. Cytochem.*, **36**, 87-94 (1988).
50. A. D. Moscioni, J. Rozga, S. Chen, *et al*., *Cell Transplant*., **5**, 499-503 (1996).
51. P. Nagy, H. C. Bisgaard, and S. S. Thorgeirsson, *J. Cell Biol.*, **126**, 223-233 (1994).
52. E. L. Opie, *J. Exp. Med.*, **80**, 231-246 (1944).
53. R. Oren, M. D. Dabeva, P. M. Petkov, *et al.*, *Hepatology*, **29**, 75-81 (1999).
54. J. Overton, *J. Exp. Zool*., **168**, 203-214 (1968).
55. K. Overturf, M. Al-Dhalimy, and R. Tanguay, *Nat. Genet*., **12**, 266-273 (1996).
56. K. Overturf, M. Al-Dhalimy, C. N. Ou, *et al*., *Am. J. Pathol*., **155**, 2135-2143 (1999).
57. G. A. Patijn, A. Lieber, D. B. Schowalter, *et al*., *Hepatology*, **28**, 707-716 (1998).
58. B. E. Petersen, W. C. Bowen, and K. D. Patrene, *Science*, **284**, 1168-1170 (1999).
59. C. S. Potten and M. Loeffler, *Development*, **110**, 1001-1020 (1990).
60. J. A. Rhim, E. P. Sandgren, J. L. Degen, *et al*., *Science*, **263**, 1149-1152 (1994).
61. T. Roskams, R. De Vos, P. Van Eyken, *et al*., *J. Hepatol*., **29**, 455-463 (1998).
62. J. M. Sala-Trepat, J. Dever, T. D. Sargent, *et al*., *Biochemistry*, **18**, 2167-2178 (1979).
63. P. O. Seglen, *Methods Cell Biol*., **13**, 29-83 (1976).
64. S. Sell, *Hepatology*, **27**, 317-331 (1998).
65. D. A. Shafritz, *Ibid*., **32**, 1399-1400 (2000).
66. N. Shiojiri, and T. Mizuno, *Anat. Embryol*., **187**, 221-229 (1993).
67. A. E. Sirica, G. A. Mathis, N. Sano, and L. W. Elmore, *Pathobiology*, **58**, 44-64 (1990).
68. P. Steinberg, R. Steinbrecher, and S. Radaeva, *Lab. Invest*., **71**, 700-709 (1994).
69. A. J. Strain, *Hepatology*, **29**, 288-290 (1999).
70. N. J. Sund, S. L. Ang, and S. D. Sackett, *Mol. Cell Biol*., **20**, 5175-5183 (2000).
71. A. Suzuki, Y. W. Zheng, and R. Kondo, *Hepatology*, **32**, 1230-1239 (2000).
72. H. Taniguchi, T. Toyoshima, K. Fukao, and H. Nakauchi, *Nat. Med.*, **2**, 198-203 (1996).
73. L. B. G. Tee, Y. Kirilak, W. H. Huang, *et al*., *Carcinogenesis*, **15**, 2747-2756 (1994).
74. N. D. Theise, S. Badve, and R. Saxena, *Hepatology*, **31**, 235-240 (2000).
75. N. D. Theise, M. Nimmakayalu, R. Gardner, *et al*., *Ibid*, **32**, 11-16 (2000).
76. J. A. Thomson, J. Itskovitz-Eldor, and S. S. Shapiro, *Science*, **282**, 1145-1147 (1998).
77. S. S. Thorgeirsson, *FASEB J*., **10**, 1249-1256 (1996).
78. I. Tournier, L. Legres, D. Schoevarert, *et al.*, *Lab. Invest*., **59**, 657-665 (1988).
79. M. C. Wake, A. G. Mikos, G. Sarakinos, *et al*., *Cell Transplant*., **4**, 275-279 (1995).
80. O. Yasui, N. Miura, K. Terada, *et al*., *Hepatology*, **25**, 329-334 (1997).
81. K. S. Zaret, *Annu. Rev. Physiol*., **58**, 23-251 (1996).