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MINIREVIEW

The Facultative Stem Cell: A New Star in Liver Pathology

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Although the unlimited capacity of hepatocytes to divide has been recently proven, more and more evidences support the existence of a primitive stem cell compartment in the liver. These cells probably do not participate in the usual maintenance of the liver mass, but they are activated in case of extensive hepatocyte injury. In vivo the oval cells show deep similarity to the primitive cells of the embryonic liver and seem to be the amplification compartment of the hepatic stem cells. A primitive epithelial cell population can be isolated from the normal liver and maintained in vitro. Studies of these two experimental systems provide most of the data about liver stem cells, which may become important for the clinical practice if we understand how their growth is regulated. (Pathology Oncology Research Vol 1, No1, 23–26, 1995)

Key words: liver, stem cell, oval cell carcinogenesis

Hepatocytes are highly differentiated cells, yet they are able to divide and maintain the liver volume within physiological limits. Their unlimited capacity to proliferate was proved recently in a very elegant experiment using transgenic mice.[26] Since hepatocytes are very vulnerable to virus infections and to several chemicals due to their high metabolic activity, the existence of a second back up system, which could regenerate the liver in case of extended parenchymal damage was hypothesized.

The first indications about the presence of a primitive epithelial cell population in the liver came from carcinogenesis studies. Farber[10] described in 1956 the proliferation of oval nucleated epithelial cells in rat liver following treatment with different carcinogens. The oval cells were later observed in most of the chemically[11] and even virally induced hepatocarcinogenesis models.[28] Their origin and importance in hepatocarcinogenesis has been the subject of an intensive discussion which has not reached a final conclusion even to day.[2]

The oval cells have been regarded by some authors as proliferating bile duct cells, based mostly on phenotypic criteria such as morphological appearance, GGT expression and similar isozyme profiles.[15] Further studies described that the oval cells produced alpha fetoprotein *(Fig.1)* and albumin,[8] proteins not expressed in normal or even in the proliferating bile duct cells. Sirica et al[33] could clearly distinguish oval cells, intrahepatic biliary cells and hyperplastic bile ductular cells from each other based on phenotypic markers. Factor et al[9] compared thoroughly the ultrastructural morphology of oval cells in a Dipin induced hepatocarcinogenesis experiment with that of proliferating bile duct cells following bile duct ligation and found well defined differences between them.

The expression pattern similarity between the oval cells and hepatoblasts - the cells which build up the embryonic liver, however is striking. Both cell types express GGT, similar isozymes, albumin and alpha fetoprotein. Keratin 14 is usually found in squamous epithelium but not in any cell population of the normal adult liver, yet, it was demonstrated being present in 12 day old rat embryo livers[4] and being transiently expressed in a subpopulation of the oval cells as well.[3] Considering this phenotypic similarity, it is not surprising that both cell types express the same battery of liver enriched transcription factors[23] *(Fig.2)*. Hepatoblasts are at least bipotential cells. During the embryonic development of the liver those cells which surround the branches of the portal veins will form the ductal plate, and eventually the bile ducts, while the rest

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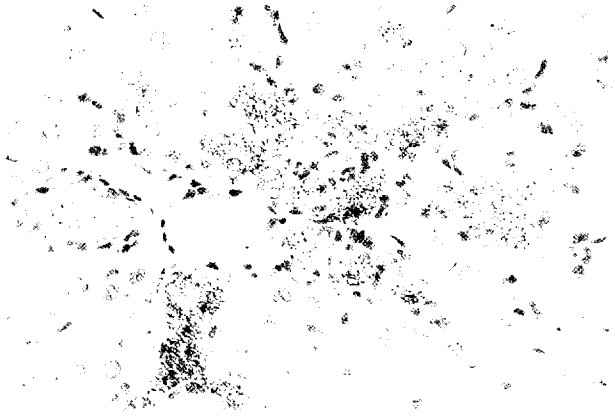

Figure 1. *Alpha fetoprotein expression in oval cells, induced by the AAF/PH protocol.[34] (In situ hybridization 1000x magnification)*

will differentiate into hepatocytes.[39] Germaine et al[13] postulated, based on results obtained using a battery of polyvalent and monoclonal antibodies, that upto embryonic day 12 near 90% of the cells in the rat liver diverticulum were bipotential progenitor cells. Although, the number of these cells gradually decreased with age, a small population was still present in the normal adult rat liver. The above described similarities between hepatoblasts and oval cells lead to the suggestion that the oval cells may be the progeny and the so called "amplification compartment"[25] of the progenitor cells which survive in the adult liver. These may function as facultative stem cells. They are called facultative, because they participate in liver regeneration only if the hepatocytes are compromised due to some cellular injury.[14]

There are controversies about the fate of the oval cells. Inaoka[17] observed the morphological transformation of oval cells into hepatocytes during butter yellow induced hepatocarcinogenesis. Tatematsu et al[34] doubted these results which conflicted with his own observations in an AAF induced oval cell proliferation model. However, when earlier timepoints were studied by Evarts et al[7] in the same experimental setup, the transfer of radioactive thymidine from the labeled oval cells to the hepatocytes was observed proving a "precursor/product" relationship between them. Lemire et al[18] demonstrated the continuity between oval cells and hepatocytes after galactosamine induced liver damage. Although, the differentiation of oval cells into bile duct cells has not been proven formally, it has been assumed to be the case. Tatematsu et al[35] described the transformation of oval cells into metaplastic intestinal glands in the liver confirming their multipotential capacity. Morphologically and functionally complete hepatocytes are formed in the pancreas after copper deficiency[26] (in this case the appearance of the hepatocytes is preceded by oval cell proliferation in the pancreas) and pancreatic metaplasia can occur in the liver.[41] These phenomenons can be explained by the presence of a multipotential stem cell compartment in these organs. These stem-cells, like the primitive endodermal cells of the midgut during ontogenesis, are able to differentiate into several different cell types.

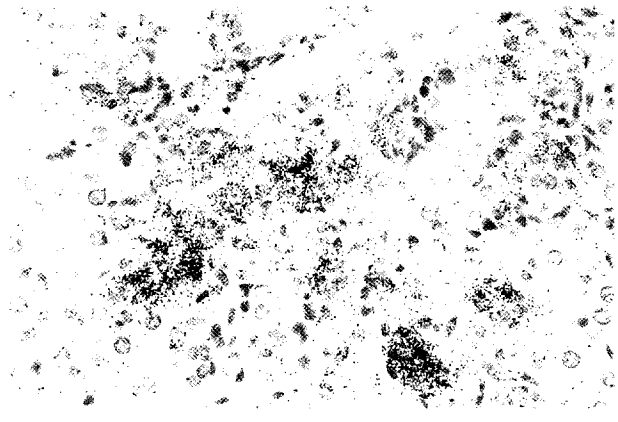

Figure 2. *C/EBP beta (member of the liver enriched transcriptional factors) expression in oval cells, induced by the AAF/PH protocol.[34] (In situ hybridization 1000x magnification)*

In vitro experimental data also support the hypothesis that a primitive multipotential cell population exists in the liver. Williams et al[40] isolated undifferentiated epithelial cells from rat liver. Several laboratories have established similar cell lines from adult or newborn rat livers, these are usually called rat liver epithelial (RLE) cells. After the transformation of the RLE cells Tsao et al[37] observed different kinds of tumors, including tumors that resembled differentiated hepatocellular carcinomas. TGF-beta treatment of RLE cells induced the appearance of hepatocytic traits (albumin, AFP).[24] Another study described similar changes after sodium butyrate treatment.[21] Fausto et al[11] using the sandwich technology, were able to influence hepatocytic or bile duct directed differentiation of their RLE cells by changing the growth factor composition of the feeding layer (personal communication). The most convincing data about the stem cell potential of these RLE cells were provided by Coleman et al[6] who injected beta-galactosidase labeled RLE cells into rat liver and in this natural environment they were incorporated into the liver plate and differentiated into morphologically mature hepatocytes.

Where do these stem cells reside in the liver? Since we do not know any specific marker of these cells it is very difficult to identify them. Indirect evidence show that they harbor somewhere in the periportal region of the liver lobule. They are either among the cells of the Hering canal or form an undescribed periductal cell population.[30] Marceau[20] using phenotypic markers, localized bipotential precursor cells in a region that corresponds to the borderline between the biliary ductule epithelial cells and the

hepatocyte plate. The oval cells, the presumed progeny of these stem cells always appear first periportally and invade in the centrilobular direction. Lemire and Fausto,[19] assuming that the 2.1 Kb alpha fetoprotein (AFP) messenger RNA can be a potential marker of the stem cells, used an AFP probe for in situ hybridization and found the signal in the periportal region in rat liver.

Arber et al[1] introduced the notion of "streaming liver". According to this idea, the liver acinus is kinetically similar to the intestinal crypt villus system, and the hepatocytes in the periportal zone correspond to the stem cells and as they move from the portal zone toward the terminal hepatic vein, they cross the three acinar zones, each of them representing a differentiation stage. Although this hypothesis is challenging, it conflicts with previous observations and was recently definitely refused based on cell lineage studies using retroviral markers.[5] On the other hand more and more data support that there exists a facultative stem cell population in the liver. Studying these cells is not only interesting for understanding basic biological processes but they may likely to be active participants in pathological processes of the liver.

Although, all the data described above derive from animal studies recent evidences indicate a similar situation in human liver. Shah et al,[31,32] during the ontogenesis of the human liver, described the differentiation of hepatoblasts to hepatocytes and bile ducts after the formation of ductal plates, and found that in humans it happens in the same ways as in rats. Similarly, oval cell proliferation has been described in human livers in more and more cases following viral or chemical injury.[12,16,36,38] This fact gives further clinical importance of the quest for the liver stem cell. These progenitor cells are probably less vulnerable than the hepatocytes to harmful agents due to their low, or not detected, phase I and high phase II activities which favour detoxification over activation of hepatotoxic chemicals.[22,29] A better understanding of the factors regulating the activation and expansion of this cell compartment may form the basis for a completely new approach to the treatment of several liver diseases. The restoration of the damaged liver parenchyme from the stem cells would be a attractive alternative to liver transplantation. Furthermore, the isolated and transfected stem cells would be an ideal vector for gene transfer into the liver to correct genetic metabolic diseases.

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