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MORPHOLOGICAL, STRUCTURAL AND FUNCTIONAL ASPECTS OF LIVER PARENCHYMAL CELLS

ASPECTE MORFOLOGICE, STRUCTURALE ȘI FUNCȚIONALE ALE CELULELOR PARENCHIMATOASE DIN FICAT

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ABSTRACT | REZUMAT

The functional hepatic complex, regardless of condition (physiological or pathological) is ensured through networking parenchymal cells with non-parenchymal cells through a series of molecular multidirectional signals. Studying the functions and interrelations of liver cell is priority for researchers in the field, due to newly discovered and unelucidated uncertainties related to cell cooperation and possible ways of inhibiting or stimulating certain cells in various pathological conditions, in order to block disease progression and regeneration of the affected territories. This paper presents the morphological, structural and functional characteristics of liver parenchymal cells represented by hepatocytes, cholangiocytes and oval cells.

Keywords: hepatocytes, cholangiocytes, oval cells, monoclonal antibodies

Complexul funcțional hepatic, indiferent de stare (fiziologică sau patologică), este asigurat prin interrelaționarea celulelor parenchimatoase cu cele non-parenchimatoase printr-o serie de semnale moleculare multidirecționale. Studierea funcțiilor și interrelațiilor celulare hepatice constituie preocuparea prioritară a cercetătorilor din domeniu, datorită aspectelor neclare, recent descoperite și încă neelucidate legate de conlucrarea celulară și eventual de modalitățile de inhibare sau stimulare a anumitor celule în diferite stări patologice, în scopul blocării evolutive a bolii, dar și pentru regenerarea teritoriilor afectate. În această lucrare sunt prezentate caracteristicile morfologice, structurale și funcționale ale celulelor parenchimatoase din ficat reprezentate de hepatocite, colangiocite și celulele ovale.

Cuvinte cheie: hepatocite, colangiocite, celule ovale, anticorpi monoclonali

The liver makes part of vital organs without which man or animals cannot survive. As part of the animal's body, the liver plays a major role in metabolic processes with multifunctional involvement in carrying out vital functions. The importance of the liver in maintaining homeostasis of the body lies in the multitude of functions it performs.

Communication through molecular signals of liver and biliary cells is crucial both for the optimal functioning of liver and post-lesion regeneration. Molecular interrelations targeting parenchymal and non-parenchymal cells act according to restore the integrity of the lobular and functional organization (13).

Fulfilling numerous functions (e.g., synthesis and excretion of bile, storage and synthesis of compounds, detoxification, role in hematopoiesis, reservoir of blood and immune function) is achieved through multidirectional cell networking involving all the constituent cells. In recent years, several researchers (11, 51) emphasize the immunological importance of liver, some even call it "immune organ". The role of the liver in immunity is achieved by hepatocytes and the non-parenchymal cells involved in defense response of the liver with the task of neutralizing toxic substances and infectious agents, but at the same time to prevent an exaggerated immune response that could destroy the body (11).

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HEPATOCYTES – MORPHOLOGY AND FUNCTIONAL IMPLICATIONS

Hepatocytes are the functional cells with the highest share in the liver cell population. They represent about 80% of the liver's volume and 60% of total liver cells. Hepatocytes are responsible for the most specialized functions of the liver (22; Malarkey *et al.*, 2005; 41; 17).

Among domestic animal species, the hepatocytes are similar in shape and size, have a diameter of 18-20 µ, are polyhedral cells and present two poles. Intercellular contacts with neighboring hepatocytes are achieved by cell margins, and the poles are represented by the biliary pole towards the bile canaliculus and by

the vascular one towards the sinusoid capillary (9, 22, 41). The two opposite sides of the hepatocyte, which are bounded by sinusoidal capillaries, form a vascular pole, which is separated from sinusoids by perisinusoidal space or Disse space. Towards the vascular pole, the hepatocyte membrane is fitted with microvilli that increase cell surface and facilitate the exchange between hepatocyte and capillary blood (5, 17).

Madhan and Raju (2014) after conducting a comparative study on human and animal (cow, sheep and goat) livers found that in humans the hepatocytes are hexagonal and are larger than in the investigated animals. In sheep, goat and cow the hepatocytes are polygonal (31).

The hepatocyte can be mononucleate, binucleate (Fig. 1) and trinucleate, and the nucleus is vesicular, spherical, containing one or two nucleoli. Within the mononuclear cells the size of the nucleus are variable. Generally, the nuclei are large and euchromatic. These cells with large nuclei are considered polyploid, and the ones with small nuclei are diploid (41).

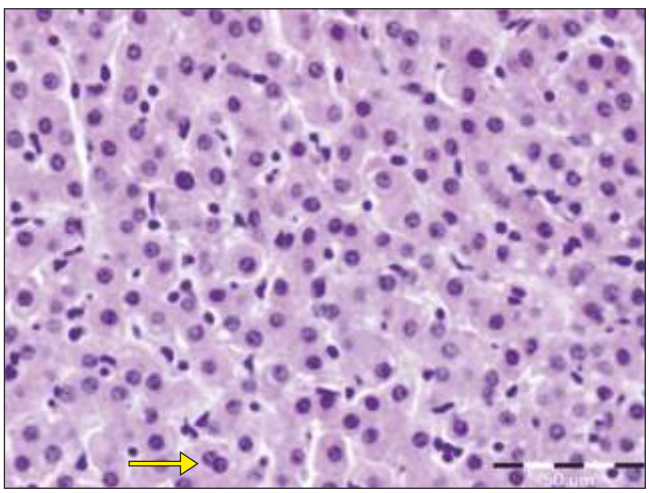

Fig. 1. Hepatocytes arranged in chords, mono and binucleate (arrow) with different sized nuclei and prominent nucleoli. Lamb liver, H&E, x400.

The hepatocytes are metabolically active cells and are characterized by a distinctive shape and a high degree of internal organisation, containing a large number of cytoplasmic organelles (e.g., rough and smooth endoplasmic reticulum, ribosomes, mitochondria, Golgi apparatus, and peroxisomes) (9, 17, 41).

Samuelson (2007) says that the most frequent organelles are the peroxisomes, the smooth endoplasmic reticulum and the lysosomes (41).

Hepatocyte shape and organisation depend on a complex of filament networks in the cytoplasm (cytoskeleton). The hepatocyte cytoskeleton consists of microtubules, microfilaments and intermediate filaments. The microtubules have a diameter of 24 nm and are composed of polymerized tubulin, the microfilaments have a diameter of 6 nm and are composed of polymerised actin, and the intermediate filaments have a diameter of 10 nm and are composed of cytokeratin subunits (41). Near the biliary pole, the hepatocyte cytoskeleton contains more microfilaments mostly made of actin and myosin, which are designed to regulate the passage of bile through the canaliculi (4). Hesketh and Prime (1996) suggest that the hepatocytes have a three-dimenstional cytomatrix, similar to a microtrabecular network. Using immunofluorescence with antitubulin antibodies the distribution of the microtubules was highlighted, and it was observed that they are distributed as a reticular network throughout the cytoplasm, but the larger amount is found near the canaliculi and around the Golgi apparatus, radiating to the periphery. These microtubules are involved in cellular mitosis, in secretory processes of plasmatic proteins and lipoproteins, and also in the intracellular translocation of the secretory vesicles and other organelles (22).

Also within hepatocytes there are a number of inclusions which contain glycogen, lipids and pigments (bilirubin, hepatosiderin). Glycogen is stored perinuclear, in a granular form, and is stained intensely using the PAS method, while lipids are in a state of small droplets and usually accumulate within pericentrolobular area hepatocytes (9, 17).

Cytoplasm of hepatocytes around the centrilobular vein may contain lipofuscin granules, having uniform appearance and a yellowish-brown colour (17, 44). This pigment becomes more pronounced with age and progressively accumulates in the medium and periportal area hepatocytes (44). In general, most researchers state that hepatocytes are "parenchymal cells" and the other cells of the tissue matrix are "non-parenchymal cells". Grisham (2009) states that the name is artificial because hepatocytes alone are not capable of carrying out the essential functions of the liver, but many cell types of the tissue matrix, which act as an integrated community, can perform the multitude of liver function (21). More communication mechanisms, including signalling network involving numerous cytokines and chemokines and direct transfer of small molecules through gap junctions contribute to the functional integrity of this cellular community (3).

Hepatocytes can be easily recognized by their shape and arrangement, but in case of serious structu-

Table 1

Monoclonal antibodies used for hepatocyte labelling (6, 7, 27)

Cell type	Cell localisation	Monoclonal antibodies	Cells that are marked with the same antibody
Hepatoc yte	Withi n the structure of hepatocyte cords (Remak trabecule) in the liver parenchyma	**α-fetoprotein** **Albumin** **π-GST**	Oval cells
		CK8 **CK18**	

ral alterations or to differentiate them from other cells immunostaining can be used (Table 1).

Constantinescu *et al* (2016) quotes Ding *et al* (2010) which states that in case of partial hepatectomy, tissue recovery within three days occurs by hepatocyte proliferation, being followed by sinusoidal endothelial cell proliferation (8, 12). The information was highlighted through immunoblotting hepatocyte mitosis, positioned near unproliferated sinusoidal endothelial cells.

In humans and mice, it was proven that after performing a partial hepatectomy, the liver regenerates rather by active proliferation of highly differentiated hepatocytes than by progenitor cells expansion. Usually, hepatocytes are found in a latent state and have a limited number of divisions. Even so, they own proliferation-specific signals and a specific ability to adjust the cellular cycle (29).

Many scientists, quoted by Van Eyken and Desmet (1993), have highlighted the presence of cells with similar phenotypic characteristics to hepatocytes but also similar to bile ducts, claiming that hepatocytes can be transdifferentiated, thus inducing a "ductal metaplasia" of the cells. They believe that the most convincing argument is the hepatocytes immunoreactivity from the first acinar area (which, apparently, is found in continuity of the bile duct cells), to the cytokeratin 7 and/or 19. In support of the above statements, they proved that in the case of a long term biliary obstruction the hepatocytes from first acinar area, gradually became positive for cytokeratin 7 suggesting the apparition of an incipient "ductal metaplasia" (50).

Later, more scientists have demonstrated through independent studies the hepatocytes' capacity of "transdifferentiating" within bile duct epithelial cells in the case of chronic lesions. Hepatocytes' conversion to primitive ductal cells is gradual and it implies inducing biliary markers, decreasing cell sizes, forming a polarized epithelial layer and the appearance of a new organelle with functional potential (43, 52).

Zeisberg *et al.* (2007), through their research, have tested the hypothesis in which hepatocytes would have a direct contribution to accumulation of activated fibroblasts during hepatic fibrosis. The experiment results have shown that more than just α-SMA positive myofibroblasts are positive to fibroblast specific protein-1 (FSP1) which contributes to the evolution of hepatic fibrosis. They demonstrated for the first time that up to 45% of the fibroblasts positive to FSP1 originate from hepatocytes through epithelial - mesenchymal transition (53).

Tarlow *et al.* (2014) affirms that mature hepatocytes may suffer from reversible ductal metaplasia as a reaction to lesions and contributes to hepatocyte mass. They suggest that hepatocytes metaplasia can happen in hepatic cirrhosis in humans and that hepatocytes can be a source of the ductal reaction in cases of lengthy injuries (49).

Hepatocytes transition from latent to active form, with proliferative capacities, is done through a series of reactions in which different type of hepatic cells are implicated. The regulation of these chain reactions is done through paracrine factors such as cytokine and growth factors (36).

Polyploidy is characteristic to adult hepatocyte in mammals and contributes to specific molecular mechanisms which sustain cellular cycle (29).

CHOLANGIOCYTES – MORPHOLOGY AND FUNCTIONAL IMPLICATIONS

The liver contains two types of differentiated epithelial cells: hepatocytes, which predominate, and cholangiocytes, which are part of the biliary ducts structure.

Cholangiocytes form an epithelium bordering a vast network of interconnected canals, intrahepatic and extrahepatic, which have different sizes and varied functions, starting from the immediate vicinity of hepatocytes and extending towards Vater ampoule (23; 32). Tanimizu *et al.* (2009) states that the epithelial cells of

the bile ducts (cholangiocytes) differ from hepatic progenitor cells called hepatoblasts (48).

Bile ducts system is heterogeneous regarding their diameter, the small sized being joined progressively and resulting bile ducts with an increasingly larger diameter, thus forming a structure with a branched aspect, called the biliary tree. It represents a network of ducts and channels through which bile, secreted and subsequently released hepatocyte fluid, reaches the intestine where digestion occurs. In humans, it is considered that the biliary tree trunk is the common hepatic duct, large branches are the intrahepatic bile ducts and the smaller branches correspond to small bile ducts (10).

Nathanson and Boyer (1991) say that the origin of the biliary tree is the hepatocyte bile pole, responsible for producing bile (39). However, Crawford (2004) considers that the link between hepatocytes and biliary tree is via Herring channels, which are small tubular structures. In histological sections they appear as hemichannels bounded by hepatocytes semicirculary, while the other half is bounded by cuboidal epithelial cells (10, 42). Hering channels become bile ducts when cholangiocytes are arranged on a circumferential line and rest on a complete basement membrane (10).

Franchitto *et al.*, (2013) states that there is a junction point between the duct and the channel represented by the area where the bile canaliculus continues with Hering channel; at this level the channel is partially delineated by hepatocytes and partly by cholangiocytes. In addition, this is the area where undifferentiated cells have been identified, which represents the compartment of liver progenitor cells, consisting of a population of stem cells that can differentiate to the same extent into cholangiocytes and hepatocytes (16).

From the study of the biliary tree and cholangiocytes on different experimental models it was found that in both human and rat, the cholangiocytes are heterogeneous in size, their area ranging between 3 and 80 μm^2 (16), up to 100 μm^2 (4).

Depending on the diameter of the intrahepatic bile ducts in humans, their following classification was made: bile canaliculi have a diameter less than 15 μm; interlobular ducts have a diameter ranging between 15 μm and 100 μm; septal bile ducts having a diameter ranging between 100 μm and 200 μm; regional ducts diameter ranges between 300 and 400 μm; segmental ducts have values of diameter between 400 μm and 800 μm, and hepatic ducts which are larger and have values above 800 μm in diameter (10, 19, 24). In rats, unlike humans, bile duct classification comprises only of two categories: small bile ducts with a diameter below 15 m, and large bile ducts with values of more than 15 μm (19). The number of cells that line the biliary channels are believed to be 4-5 cells for small bile ducts and 10 to 100 cholangiocytes for the large bile ducts (10, 19).

Nourishment of the bile ducts epithelial cells is made through a complex vascular system called peribiliar plexus, only with oxygenated blood coming from branches of the hepatic artery, and drain within the portal venous system or within periportal sinusoids (16, 37, 38). Peribiliar plexus is seen around the large bile ducts and is not perceptible around the small bile ducts. This delivery system of bile ducts has two potential physiological implications: on the one hand substances that are absorbed by cholangiocytes from the bile ducts are subsequently transferred to hepatocytes through sinusoids then allegedly again secreted into bile. These two-way exchanges between hepatocytes and cholangiocytes are called colehepatic shunts. Secondly, the blood flow direction is counter current to the direction of flow of bile, this being favourable to bile formation (16, 19).

Cholangiocyte morphology is different depending on the size of the bile ducts. Thus, within the small bile ducts the epithelial cells are cuboidal and as these channels increase their diameter (Fig. 2), the shape gradually changes to become columnar cells; both cell types rely on a circular basement membrane (25, 34). In rats, it is considered that large cholangiocyte diameter is larger than 15 μm and the smaller below 8 μm (10).

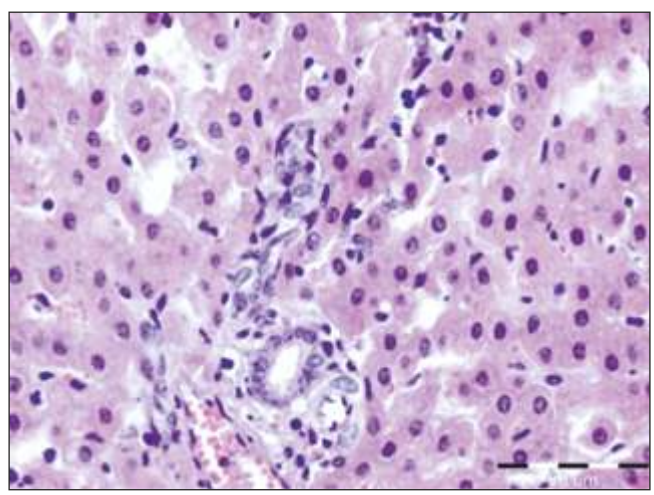

Fig. 2. Liver, small billiary duct delineated by small cholangiocytes. H&E, x400.

Cholangiocytes have a characteristic ultrastructure represented by positioning nuclei at the basal

Table 2

Types of monoclonal antibodies used for cholagiocyte labelling (6, 8, 20, 27)

Cell type	Cell localisation	Monoclonal antibodies	Cells that are marked with the same antibody
Cholangiocyte	Delineates biliary ducts	**CK-7, CK-19, OV-6**	Oval cells
		CK17 **AE1/AE3** **KRT19** **3OV-6**	
		α-tubulin (marks the cilia)	Bile duct glands

pole of the cells, the presence of a clear Golgi apparatus located between the apical pole of the cell and the nucleus. They possess lysosomes, some mitochondria and in the subapical area many vesicles. The amount of cytoplasm is also different, small cholangiocytes have less cytoplasm than large ones and hence the nucleus/cytoplasm ratio being different in the two types of cells. Epithelial cells of the small bile ducts the nucleus / cytoplasm ratio is high and it is considered that these cholangiocytes are primitive, undifferentiated cells, unlike large cholangiocytes, for which the nucleus / cytoplasm ratio is small and they are considered differentiated and specialized cells, specifically equipped to meet a secretory function. Luminal cell edge is provided with numerous short microvilli which increase the absorption surface of cells (24).

Immunostaining cholangiocytes can be done by using several monoclonal antibodies, some of them highlighting other cells also (Table 2).

Studies have increasingly revealed the importance of cholangiocytes regarding their participation to a variety of fundamental physiological processes which are essential for the normal functioning of the liver (23). Firstly, these cells form a barrier which stops the bile from passing beyond the bile ducts by delimiting the bile duct network. Through their absorption and secretion properties, they have an active contribution to bile forming, volume, pH and structure adjustment, closely correlated with the physiological needs (10, 25).

Structurally, cholangiocytes not only cover two aspects of their shape depending of the diameter of bile ducts, they also functionally present a certain functional specialization. Thus, large cholangiocytes have a physiological large secretory capacity because on their surface they possess receptors for the secretory hormone, secretin, while the smaller ones show a minimum response or, often, don't respond to the same stimuli (10).

Another ability of cholangiocytes, especially of the smaller ones, is that they manifest a large capacity of proliferation as reaction to hepatic lesions. Following the lesioning of the biliary tree, but in the presence of a proper vascularization, the biliary epithelium may recover considerably. The bipotent regenerative compartment, represented by small cholangiocytes from within the small biliary ducts assure the cellular reservoir because epithelial cells may proliferate, resulting in two cellular populations: cholangiocytes and hepatocytes (1, 10, 25).

Cholangiocytes reactions to different lesions are defined by a so-called neuro-endocrine-like trans-differentiation whose functions are represented by biliary proliferation support and by the immune response together with the implications in inflammatory process and fibrosis (33).

A new concept has appeared in human pathology, related to the role manifested by cholangiocytes, which considers that bile duct epithelial cells actively participate in the inflammatory process. In different pathological states, cholangiocytes secrete cytokine with chemotaxis and proinflammatory cytokines as well as growth factors capable of activating mesenchymal cells and extracellular matrix synthesis or able of reducing matrix production and leukocyte adhesion (45).

More evidence suggest that bile duct cells have an active role in stimulating fibrogenic response, probably by activating latent portal fibroblasts or by activating hepatic stellate cells (45).

Ramadori *et al.* (2008) affirms that hepatocytes necrosis and lobular biliary stroke may happen in cases where the biliary tract is obstructed for prolonged periods (weeks or months) (40). In this situation, around the portal area a duct reaction with extensions towards neighboring porto-biliary areas and within the parenchyma together with an extended proliferation of periductal (mio)fibroblasts.

Glaser *et al.* (2009) support the previous theory, proving that there are numerous studies which insinuated that cholangiocytes proliferation does induce fibrosis. This may also be produced by two ways, directly through epithelial and mesenchymal transition or indirectly by activating other two types of cells. Epithelial and mesenchymal transition refers to the process in which mature epithelial cells loose cell-to-cell contacts and epithelial cells characteristics, and gain phenotypical characteristics of mesenchymal cells (19, 53).

Sell (2001), quoted by Crawford (2004), considers that bile ducts represent the entry site for extra-hepatic stem cells which are able to proliferate and differentiate, turning into either cholangiocytes or hepatocytes (10).

More researchers have proven that hepatic stem/progenitory cells localized around the portal vein are the first to behave, firstly as precursory biliary cells and later giving birth to cholangiocytes (26).

Interrelations between biliary epithelial cells and other liver cells, including cells that have origins within hematopoietic marrow, are done by Gap junctions. Cholangiocytes may interact with various infectious agents, they may be influenced by basal membrane components and matrix proteins and are less involved in drug metabolism (10).

OVAL CELLS – MORPHOLOGY AND FUNCTIONAL IMPLICATIONS

The oval cells represent a cell population, localized in the Hering canals' structure and present a certain heterogeneity in expressing proteins and surface markers, which may differentiate in hepatocytes, cholangiocytes, enterocytes and other cells of the exocrine pancreas (16, 27).

Conigliaro *et al.* (2010) and Burt *et al.* (2011) state that the one that described the oval cells for the first time was Farber in 1956 and on the base of the cytological characteristics they received the name of "oval cells" (5, 6). In rats and mice they use the expression "oval cells" for this type of cells and in humans they prefer the term „hepatic progenitor cells" (15).

Lowes *et al.* (2003) claim that these cells have been acknowledged by Kinosita *et al.*, in 1937 (28). Kinosita *et al.* have observed small cells, with oval nucleus within rat livers exposed to a nitrogen-based staining ("Butter Yellow") which has carcinogenic effects (28). Although they were observed many years ago, they have been taken into consideration and recognized as hepatic progenitor cells after the year 1987 (2, 6).

Oval cells usually exist in the liver. They are found at the junction between hepatocytes and cholangiocytes, at the Hering canal level. Usually they are in a latent state (46). Thorgeirsson *et al.* have demonstrated through ultrastructural and immunohistochemical studies that the oval cells come from Hering canal's undifferentiated cells (35).

In physiological conditions the number of latent oval cells is low, but in the case of quick alterations of micro medium (which leads to hepatic cell demise), the oval cells quickly proliferate, changes in morphology, cellular composition and surface markers can be seen, therefore we can observe a differentiation (6). It appears that there is a high increase in oval cells number when hepatic lesions are produced simultaneously with the impairment of hepatocytes proliferation (35).

Following the observations carried out on the liver in humans, it was noticed that oval cells are small cells, with little, slightly basophilic cytoplasm, with oval, faintly blue colored nuclei and the nucleus/cytoplasm ratio has great value because they have little cytoplasm (18, 30, 47). Oval cells size in humans is approximatively 10 μm and, regarding the identification possibilities, some authors consider that they can't be observed using hematoxiline-eozine staining (18), and others say that they are easy to recognize using routine histological stainings (47).

Conigliaro *et al.* (2010) affirms that oval cells represent a bipotent precursory population which can at the same time express tipical celular markers used for cholangiocytes (CK-7, CK-19 and OV-6) but also for hepatocytes (α-fetoprotein and albumin) suggesting the existence of a tight relationship between oval cells and differentiated hepatic parenchimal cells (6) (Table 3).

In addition, they express stem cell factor, bcl-2 and cytokeratin 14 which are considered characteristic markers of stem cells. Due to immunoreactivity to the hematopoietic cell line antibodies (i.e., CD34 and c-kit), the oval cells are considered to have characteristics similar to those of hematopoietic cells and precursory cells of the hepatic parenchyma. This hypothesis is supported by recent studies which suggest that oval cells could be hepatic stem cells. The study was effected in a population of bone marrow cells from humans and rats and it was observed that they show similar functions to those of hepatocytes. Bone marrow cells from rats were administrated intra-portal in the rats' liver and they noticed that these have integrated within the hepatocyte cords and have differentiated in

Table 3

Types of monoclonal antibodies used for oval cells labelling (6, 7, 14, 27)

Cell type	Cell localisation	Monoclonal antibodies	Cells that are marked with the same antibody
Oval cells	Hering channels	**α-fetoprotein Albumin π-GST**	Hepatocytes
		CK-7, CK-19, OV-6	Cholangiocytes
		bcl-2, CK14	Stem cells; Bcl-2- Billiary epithelial cells of small channels, small lymphocytes, Schwann cells, muscle cells from blood vessel walls
		CD34, c-kit	Hematopoietic cells
		M2-PK	Some lymphocytes
		CK20	

mature hepatocytes (14).

The transformation of progenitor liver cells in hepatocytes or in mature cholangiocytes is passing through a transit stage defined by the appearance of intermediate phenotype cells which are polygonal shaped and their size varies between the hepatic progenitor cells and cholangiocytes or hepatocytes sizes (47).

The multiplication of progenitor hepatic cells is highlighted by the presence of groups of oval cells which extend within the liver parenchyma along the hepatic cords towards the centrilobular vein, differentiating themselves into hepatocytes, or appear as branched ducts which start from the Hering channels and head towards the center of the portal tract, followed by the forming of small and large bile ducts. Through this multiplication and orientation of the oval cells, these have the ability of reestablishing cell mass and also liver functions (47).

Paku *et al.*, quoted by Esrefoglu (2013), have proved that the oval cell proliferation is closely related to the stellate cells, suggesting that the non-parenchymal cells support the oval cells growth and differentiation through growth factors and cytokines but also by direct cell-cell interactions (14).

In humans, but also in rodents, it has been observed that oval cells proliferate in the immediate vicinity of the stellate cells which exhibit morphological characteristics of the myofibroblasts (15). The author affirms that in rats the oval cells are arranged in the form of "ductules" separated by a basal membrane which actually represents extensions of the Hering canals. Moreso, between the stellate cells and the oval cells of the "ductules" contact is made directly by stellate cells crossing the basal membrane (15).

Within a definitive structured liver, the regeneration is provided by hepatocytes and cholangiocytes, which can multiply and restore damaged tissue. Even so, there are situations in which the proliferation of the hepatocytes is inhibited and liver repair is taken over by the progenitor hepatic cells (oval cells) which migrate, proliferate and differentiate in two directions: hepatocytes and cholangiocytes (5, 15, 46). Inhibition of hepatocytes proliferation can be induce through toxic injuries and extended hepatic necrosis (5, 46).

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