Development and molecular composition of the hepatic progenitor cell niche

Peter Siig Vestentoft

This review has been accepted as a thesis together with three original papers by University of Copenhagen 9th of February 2012 and defended on 27th of June 2012.

Tutors: Hanne Cathrine Bisgaard and Kjeld Møllgård.

Official opponents: Jens Høiriis Nielsen, Peter Nagy and Henrik Daa Schrøder.

Correspondence: Department of Cellular and Molecular Medicine, University of Copenhagen, Blegdamsvej 3b, 2200 Copenhagen. Denmark.

E-mail: sqp608@ku.dk

Dan Med J 2013;60 (5): B4640

THE THREE ORIGINAL PAPERS ARE

1. A two- and three-dimensional approach for visualizing human embryonic stem cell differentiation. Brøchner CB, Vestentoft PS, Lynnerup N, Andersen CY, Møllgård K. Methods Mol Biol. 2010;584:179-93.
2. Three-dimensional reconstructions of intrahepatic bile duct tubulogenesis in human liver. Vestentoft PS, Jelnes P, Hopkinson BM, Vainer B, Møllgård K, Quistorff B, Bisgaard HC. BMC Dev. Biol. 2011 Sep 26;11:56.
3. Molecular composition of the remodeling extracellular matrix in hepatocellular regeneration from progenitor cells. Vestentoft PC, Bisgaard HC. Additional authors to be included. To be submitted.

ABBREVIATIONS

2-AAF/PHx: 2-acetylaminoflourene combined with 70 % partial hepatectomy
AFP: Alpha-fetoprotein
AQP1: Aquaporin 1
Dlk1: Delta-like 1 homolog
HESC: Human embryonic stem cells
ECM: Extracellular matrix
EpCAM: Epithelial cell adhesion molecule
HAI-1: Hepatocyte growth activator inhibitor type 1
HAI-2: Hepatocyte growth activator inhibitor type 2
HepPar1: Hepatocyte Paraffin 1
K7: Keratin 7
K19: Keratin 19
Prss8: Protease, serine, 8
SOX9: SRY (sex determining region Y)-box 9
Spint1: Serine protease inhibitor, Kunitz type 1
Spint2: Serine protease inhibitor, Kunitz type 2
St14: Suppresion of tumerogenicity 14
ZO-1: Tight junction protein 1

INTRODUCTION AND AIMS

The liver is a both an exocrine and endocrine gland responsible for a variety of important metabolic processes and complex functions such as plasma protein synthesis, detoxification and glycogen storage. In addition, mammalian liver possesses a regenerative capacity so enormous that its ability to regrow was renowned even in ancient times. In Greek mythology, Zeus punished the titan Prometheus for stealing fire to Man by eternally chaining him to a rock, where his liver was eaten daily by the Caucasian eagle, only to regenerate at night (1). This ability to regenerate is primarily ascribed to the immense proliferative capacity of the hepatocytes, the chief parenchymal cell type of the liver (2). However, in acute liver failure, as a result of sudden introduced hepatic injuries, or in chronic liver diseases, caused by prolonged insults, regeneration fails. Acute liver failure is relatively rare and has an incidence between 1 and 6 cases per million people each year in the developed world, accounting for 5-6% of liver transplants in the United States (3;4). In Denmark, the United Kingdom, and the United States acute liver failure is most commonly caused by excessive intake of the common over-the-counter analgesic acetaminophen (paracetamol) (5-7) . Chronic liver diseases are estimated to affect 170 million patients worldwide and eventually progresses to fibrosis and in 25-30 % of these cases ultimately cirrhosis (8). When hepatic regeneration is hindered, orthotopic liver transplantation is the only treatment that radically improves the outcome of hepatic failure (8;9). However, given the worldwide shortage of hepatic donor livers alternative strategies are needed for therapeutic treatment. Stem/progenitor cell based therapy could be employed as a novel intervention strategy for treating liver patients.

Stem cells are cells that are characterized by their ability to not only self-renew but also to develop into more differentiated progeny, thereby maintaining tissue homeostasis and repair (10). Adult stem cells are hypothesized to reside in specialized microenvironments known as “niches” (11). These niches are anatomical entities typically composed of a microenvironment containing support cells and an extracellular matrix scaffold that collectively influence stem cell activity (11-14).

The possibility of isolating and culturing hepatic stem cells raise the promise to treat liver patients even with autologous stem cells (15-19). This procedure would thereby prevent the administration of lifelong immunosuppressive medication employed for preventing allograft rejection. The lack of suitable markers for their identification and isolation is, however, a major obstacle for hepatic progenitor cell research (20-24). Nevertheless, observations suggest that activated adult hepatic progenitor cells reca-

pitulate the phenotype of primitive hepatocytes during liver development (25-29).

Identifying the components expressed in the hepatic progenitor cell niche is important for understanding the factors that controls stem cell differentiation if novel progenitor cell based strategies are to be developed (30). The overall focus of the PhD thesis was therefore to gain an increased knowledge of hepatic progenitor/oval cell nature and the spatial composition of their niche. This was achieved through studies conducted in both prenatal and adult diseased human hepatic tissues and in rodent models of liver regeneration. These studies resulted in the three papers discussed in this PhD. In brief, the aims and hypothesis of the three studies were the following:

1. To understand the spatial relationship between structures and protein-expression in biological samples we developed protocols for three dimensional visualizations. The protocols resulted in a methods chapter published in the protocol handbook "Methods in Molecular Biology" (31).
2. Hepatic tissue architecture has been much disputed and several attempts of visualizing vessel branching have been demonstrated. Furthermore, proteins expressed in the developing liver are often displayed by activated adult hepatic progenitor cells. In this study we wished to improve our previous protocols for 3D visualization and to characterize cholangiocytic tubulogenesis, thereby describing the hepatic progenitor cell niche in prenatal and adult human liver (32).
3. The extracellular matrix components expressed in regenerating liver likely influences progenitor/oval cell activation and behavior. The third study presented herein, therefore aimed to characterize molecular components specific for the progenitor mediated response and compare these in various models of liver regeneration.

STEM CELL BIOLOGY

ADULT STEM CELLS

With an estimated loss of 20 billion cells per day, the human body needs constant replenishment from renewing stem cells (33). Although there is no exact definition of what characterizes a stem cell, general consensus is that stem cells are cells that not only possess a capacity for unlimited or prolonged self-renewal, but can also give rise to at least one type of differentiated descendent (34). In the course going from stem cell to terminally differentiated progeny, there is usually an intermediate population of committed progenitor cells, with less proliferative capacity, known as transit amplifying cells (10;34). A function of the transit amplifying cells is to increase the number of differentiated cells, thereby reducing the need for division of the stem cell itself (34).

STEM CELL NICHES

Stem cells have been located in several tissues, including the hair follicle (35-37), the hematopoietic system (38;39) and the intestinal crypts (40;41) and are envisaged to reside in anatomical entities known as niches (11;34). The niche is defined as the microenvironment that harbors and maintains the stem cell population (14). This typically constitutes the stem cells themselves, surrounding support cells and the extracellular matrix scaffold, though in some cases, a basal lamina and stem cells are the only components (12;42). Cell-cell and cell-matrix interactions integrate signals that are important regulators for stem cell behavior (43). Within niches, the stem cell can undergo symmetric division to amplify the stem cell population, or asymmetric division, in which one daughter cell progresses to become a transit amplifying cell which leaves the niche and differentiates (12;33;34;44). However, leaving the niche does not necessarily induce differentiation, as hematopoietic stem cells traffic between extramedullary tissues and the niche in the bone marrow (45). Additionally, the stem cells may influence the niche environment themselves (46).Therefore, defining and understanding how the niche influences stem cell behavior is likely to be of scientific and clinical value, if therapeutic stem cell strategies are to be developed and employed (13;30).

LIVER BIOLOGY

ANATOMY

The liver is a vital organ that not only functions as an exo- and endocrine gland but also possesses remarkable regenerative capacity (1;8;15;47). Though many cell types are present, the chief parenchymal cells are the hepatocytes and cholangiocytes. Additional cell types include endothelial cells, myofibroblast, stellate cells and Kupffer cells (48-53).

In the face of discussions regarding how to define the livers structural and functional entities, the histological unit "lobule" is frequently used. The stylized lobule is a hexagonal structure delineated by portal triads, each containing branches of the portal vein, hepatic artery and bile ducts (27). One-cell thick plates of hepatocytes extend from the portal triads towards a central vein. The hepatocytes are directly linked to the portal bile ducts through a connecting structure lined by hepatocytes and cholangiocytes known as the canal of Hering (16;27;54). Blood from the portal veins and hepatic arteries converge in the fenestrated sinusoids and flows past the hepatocytes towards the central vein. Bile, on the other hand, transported from the hepatocytic network of canaliculi empties directly into the bile ducts.

NORMAL HEPATIC TISSUE HOMEOSTASIS.

Almost all blood exiting the intestines, the stomach, the spleen and the pancreas is transported to the liver through the portal vein (15). Given the plethora of important metabolic functions performed by the liver and its exposure to ingested environmental toxins, evolutionary events are hypothesized to have imparted the livers tremendous capacity for adaptation and regeneration (15;55). As a result, at least two levels of regeneration are known to exist (8;47). In normal liver, parenchymal cell turnover is slow with an average hepatocyte lifespan of 200-300 days (56;57). This turnover was once thought to follow the streaming liver hypothesis, in which hepatocytes originating periportally to the portal triads, gradually progress towards the central veins replacing aged hepatocytes (57). Although this hypothesis has some scientific support (58) it is debated (59). Despite the general quiescence of hepatocytes and cholangiocytes, experiments have revealed a highly proliferative capacity. Serial transplantations have shown that one hepatocyte can give rise to at least 50 mouse livers, while maintaining a fully differentiated state, thus ensuring liver function (2;60;61). In rodents, resecting two-thirds of the liver in accordance with the partial hepatectomy protocol leads to complete compensatory regrowth in approximately 10 days (1;15;27;62). This surgical intervention can be repeated at least 12 times without regenerative failure (61). Furthermore, under circumstances in which the biliary epithelium is chronically injured, as seen in instances of primary biliary cirrhosis and primary sclerosing cholangitis (63), periportally located hepatocytes can transform into biliary cells (64). Therefore, during normal

tissue homeostasis and in biliary diseases the hepatocytes can be regarded as the livers functional “stem cells”.

LIVER REGENERATION IN FULMINANT HEPATIC FAILURE.

In spite of the livers formidable ability to regenerate, this capacity can be compromised in several instances. Acute liver failure is the manifestation of sudden severe liver injury that can have several etiologies (3;65). Common causes are viral hepatitis or excessive drug intake, frequently acetaminophen, leading to hepatic encephalopathy, coagulopathy and often progressive multiorgan failure (3;66). Chronic liver diseases are commonly caused by prolonged hepatic insults such as alcohol consumption, non-alcoholic fatty liver disease, hepatitis B or C infection (8). Regardless, these insults can lead to fibrosis followed by the end stage cirrhosis in which hepatic architecture is greatly disturbed (65;67). This form for wound healing response is characterized by the presence of scar tissue encircled nodules of hepatocytes or the formation of collagen-rich septae linking portal areas (65;67;68). Whereas acute liver failure causes sudden massive cell death (69;70), chronic liver disease is characterized by continuous cell death (71). In both syndromes, a so-called “ductular reaction” is noted at the portal triad interface (65;72-74). This reaction is thought to represent the second level of hepatic regeneration, a form for stem cell mediated response (30;47;75;76).

HEPATIC PROGENITOR CELLS AND THEIR NICHE.

Stem cells have been located in several tissues, including the hair follicle (35-37), the hematopoietic system (38;39) and the intestinal crypts (40;41). However, opposed to the liver, these organs are generally under constant renewal, and require frequent stem cell division for tissue replenishment. Stem cells in these organs are therefore fulltime committed to stem cell function, whereas the hepatic stem or progenitor cells are seldom activated (15). As a result specific markers for hepatic stem cells have not been identified (77). In response to insults impairing hepatocyte replication as is the case in fulminant hepatic failure, cells sprout from the periportal zone and form an intertwined network of ductular structures radiating into the liver lobule (27;28;48;54;78). The result is an arborizing network of ductular structures classified as an atypical ductular reaction due to their poorly defined lumen. Early histological examinations by Farber revealed these pseudoductular structures to consist of small cells with a scant cytoplasm and oval-shaped nucleus (17;79). They are therefore termed oval cells in rodents (79) but progenitor cells in humans as rodent models and human diseases may not be exactly comparable (16). However, we will refer to them collectively as progenitor cells. Hepatic progenitor cells behave like bipotential cells capable of biliary and hepatocytic differentiation and they are therefore thought to represent transit amplifying cells (17;22;25-28;77;80-85). Although the origin of the progenitor cells has not been conclusively established, evidence points to the canal of Hering, the terminal branches of the intrahepatic biliary system adjoining the hepatocytes, as a source (16;19;48;81;86). Experiments have revealed that ligation of the common bile duct induces proliferation of the larger bile ducts. The proliferating biliary cells do however not show any signs of hepatocytic differentiation (47;87-91). Administration of the carcinogen 2-acetylaminofluorene (2-AAF) to rodents followed by two-thirds partial hepatectomy (PHx), on the other hand, blocks hepatocyte proliferation and initiates the progenitor cell response or “ductular reaction” in which biliary cells express hepatocytic markers (22;80;92-94). However, destruction of the entire biliary tree through 4,4'-methylene dianiline administration inhibits progenitor proliferation, suggesting the progenitor cells are descendants of the cholangiocytic lineage (86). Furthermore, administration of dexamethasone similarly blocks progenitor cell proliferation, but has no consequences for large bile duct proliferation (91). The location of the Canal of Hering therefore makes the prime candidate for the adult hepatic progenitor cell compartment or “niche“(16;48;86). However, the putative stem cells located in this structure, may not necessarily be dedicated stem cells, but rather subpopulations of hepatocytes or biliary cells with increased stemness relative to other cells of their lineage (15).

Although the morphological appearance of progenitor cells resembles biliary cells, ductular reactions are phenotypically heterogeneous bipolar compartments (82). Whereas the end connected to the biliary tree display cholangiocytic markers such as cytokeratin 19, hepatocytic markers and transcription factors, including Hepatocyte Paraffin 1 (HepPar1) and HNF4, are expressed in the parenchymal facing end (82;93). Between these extremes, intermediate hepatobiliary cells displaying cholangiocytic and hepatocytic markers to various degrees are found (82;95). Although many hepatic progenitor cell markers have been reported, none are specific for a pure population of hepatic stem cells (20-23;82;96) and only delta-like protein 1/preadipocyte factor 1 (Dlk1) is regarded as a progenitor cell surface marker (24;97;98). In addition to Dlk1 (24;98), hepatic progenitor cells express α-fetoprotein (Afp) (84;98;99), both proteins not normally expressed in normal liver, but observed during hepatic development (100;101). Hepatic progenitor cells therefore seemingly recapitulate a prenatal phenotype similar to that of the so-called hepatoblasts or “primitive hepatocytes” (100;102), the embryonic precursors of the hepatocytic and cholangiocytic lineages during development (25-29;80;81).

LIVER DEVELOPMENT

In the human embryo, the earliest sign of prospective hepatic development is observed at the 17 somite stage, corresponding to 3 weeks +5 days post conception (103). At this stage, a thickening of endodermal cells in the embryonic foregut is discernible that matures into the hepatic diverticulum, or “liver bud” (103). Under influence of mesodermal signaling, particularly fibroblast growth factors and bone morphogenetic proteins, cords of endodermal cells positive for cytokeratin 19 and HepPar1, expand from the liver bud into the adjacent septum transversum (104-107). The endodermal cords anastomose around preexisting endothelial-lined spaces, increase in mass and become more organized, while displacing the septum transversum that form the liver capsule (107-109).

The hepatocytic and cholangiocytic lineages are hypothesized to descend from this early mass of hepatoblasts or “primitive hepatocytes” (18). Consensus it that primitive hepatocytes in contact with the mesenchyme surrounding the developing hepatic veins change phenotype and display increased expression of cytokeratins 8, 18 and 19 relative the remaining parenchymal cells (110;111). This structure known as a “ductal plate” can be imagined as a biliary sleeve surrounding the developing veins that eventually forms the portal triad. The ductal plate becomes bilayered, with lumina forming between the mesenchymal and parenchymal facing layers. Certain areas of the ductal plate then migrate into the portal mesenchyme and mature to form the intrahepatic bile ducts, while the remaining ductal plate regresses (112-114). The biliary development and maturation process is therefore said to go through a series of remodeling stages, categorized as the “ductal plate”, “remodeling” and “remodeled bile duct” stages (104;114;115).

The ductal plate is suggested not only to constitute the pre- and perinatal hepatic progenitor niche, but also to be directly antecedent to the canal of Hering, the proposed adult progenitor cell niche (19;116-118). However, during hepatic development, Dlk1 (24;97;100) and AFP (117) are expressed by the hepatoblast, but both proteins are down regulated when hepatoblasts mature into hepatocytes or ductal plate cells. The down regulation of Dlk1 and AFP in cholangiocytic structures and the reemergence in hepatic progenitor cells have therefore led authors to speculate, that expression of Dlk1 and AFP is a feature of transit-amplifying cells primed for the hepatocytic lineage and does not mark the actual putative hepatic stem cells (98;117). Given that elevated AFP levels are associated with a favorable prognosis for patients with fulminant hepatic failure these observations support the notion that AFP marks cells capable of at least hepatocytic differentiation (119;120).

THE HEPATIC PROGENITOR CELL MICROENVIRONMENT.

EXTRACELLULAR MATRIX COMPOSITION.

A key to understanding the development and response of the hepatic progenitor cell niches lies within the composition of the extracellular matrix and the microenvironment, or niche, containing stem and progenitor cells (43). The extracellular matrix is defined as the complex multi-molecular material that surrounds cells (121). A major component are the collagens, but also a wide range of other protein families, including laminins, proteoglycans, glycosaminoglycans and elastins are represented (121-124). The extracellular matrix not only functions as a physical scaffold, but also influences cellular adhesion, migration, patterning and phenotype (122-125). Many of these actions are due both to the presence of growth factors embedded in the matrix and direct interaction with cellular surface receptors, of which integrins are of particular importance. Integrins comprise a family of dimeric transmembrane receptors linked to the cytoskeleton. Through direct interactions with extracellular matrix molecules, such as collagens, laminins and fibronectin, integrins transmit signals to the cytoskeleton, consequently influencing cellular behavior (126-131). Moreover, when transplanting stem cells from old animals where self-renewal and differentiation capacity has declined, to young animals or their extracellular matrix, stem cell function is rejuvenated to a level comparable to that observed in young animals (132;133). These recent experiments suggest that aging has a negative and detrimental effect on niche composition with direct negative influence on stem cell function (132;133). Physical features such as matrix rigidity and geometry also affect cellular phenotype and behavior and can even direct stem cell lineage specification (121;125;134;135). Many of these features have directly been shown to affect how stem cells contribute to tissue homeostasis and repair.

EXTRACELLULAR MATRIX IN THE HEPATIC PROGENITOR CELL NICHE.

Whereas the hepatic progenitor cells are well studied, knowledge of their microenvironment is limited (30). Based on the blueprint from other stem cell niches it should include a basal lamina, support cells and the stem or progenitor cells themselves (12). Previous studies have established that a laminin and type IV collagen rich basement membrane are contributors to ductal plate formation in prenatal life and associate with mature bile ducts (112;136). However, when hepatic progenitor cells are activated, laminin and type I collagen intimately accompany the ductular reactions (30;48;52;137;138). In addition to certain extracellular matrix components some cellular participants have also been recognized in the hepatic progenitor microenvironment. Myofibroblasts, stellate cells and Kupffer cells, are not just recognized as intimate companions with the ductular reactions, but the latter two are directly needed for the invasion of hepatic progenitor cells into the hepatic parenchyma (48-53). Direct cellular processes through the basement membrane are observed between stellate cells and hepatic progenitor cells suggesting some sort of intercellular communication and coordination to occur (48). Kupffer cells, on the other hand, possibly aid invasiveness through their capacity to remodel the extracellular matrix and produce chemoattractants (49). What is more, apart from physically interacting with progenitor cells, myofibroblasts and stellate cells are regarded as the main synthesizers of extracellular matrix components in the liver thereby directly influencing the composition of the niche microenvironment (52;139-142). Surprisingly, the immune system, represented by T-lymphocytes and natural killer cells, is also needed for hepatic progenitor cell proliferation partially through their local production of the cytokines tumor necrosis factor-α and interferon-γ (46;143).

THREE-DIMENSIONAL RECONSTRUCTIONS OF BIOLOGICAL DATA.

Although some markers expressed by hepatic progenitor cells have been reported and certain contributors to the hepatic progenitor cell niche are documented much work is still needed to identify pure progenitor cell markers to facilitate their isolation, and to unravel what local conditions control their differentiation. These pieces of information are absolutely essential to elucidate before novel strategies for therapeutic stem cell treatment are to be employed.

Immunohistochemistry represents a technique employed for visualizing protein expression in tissue sections through the use of labeled antibodies directed against certain antigens. When analyzing histological liver sections, a somewhat unvarying composition of hepatocytes, cholangiocytes and vessels is demonstrated. This dull appearance nonetheless conceals a highly complex tissue with an architecture that is poorly understood and frequently debated (16;144;145). Visualization of tissue in three dimensions yield useful information that allows better understanding of the shapes and spatial relationships between structures and gene expression patterns (146;147). However, three dimensional (3D) shapes cannot be inferred from standard two dimensional sections. Despite the peculiar fascination 3D imaging has always held for scientist, creating 3D reconstructions is often still a laborious task. Basically, visualization of objects can be achieved through two distinct approaches: volume rendering or image segmentation (148). Volume rendering is one of the most widely applied methods to visualize tomographic data three-dimensionally (149). Tomographic data are typically consecutive grayscale images obtained from X-ray computed tomography (CT) or magnetic resonance imaging (MRI) scanners (149). Volume rendering creates a 2D projection of the consecutive 3D image stack from any desired viewpoint (148;149). Image segmentation is the process of outlining objects of interest thereby assigning them with X, Y and Z coordinates, that can be visualized as surfaces and measured upon (148). While volume rendering is a direct and fast way of presenting data, image segmentation is a tedious and laborious process, due to the frequent complexity of biological structures that have to be manually outlined

Several technologies for 3D visualization are available, each with certain benefits and drawbacks. Confocal laser scanning microscopy is a popular method in which 3D images are generated by

creating optical sections through a tissue. Confocal laser scanning microscopy suffers from a limited tissue penetration depth of about 100 µm, though technical setups can increase both penetration depth and the area of interest considerably (150;151). Optical Projection Tomography is a relative newcomer to this field that allows 3D visualization of cubic tissue blocks up to 15 millimeters thereby making reconstructions of even entire smaller animals possible. (152-154). Though capable of generating impressive images, none of these techniques incorporate the benefits of light microscopy with respect to properties such as contrast options, resolution and color presentation.

For 3D rendering many software packages are available. Nonetheless, they are often expensive, lack functionality, have a very long learning curve and few of them are designed for light microscopy (148). Techniques for 3D reconstruction date back centuries and many different recipes for 3D visualization has been published (155-161). In relation to our project these procedures suffer from several shortcomings. The protocols generally rely on either volume rendering or image segmentation, but not the two combined. Moreover, the available protocols are seldom suitable for high resolution reconstructions, presentation of immunohistochemical staining or serial tissue sections, despite the latter founding the basis for ancient visualization techniques (162-165). The use of serial histological sections, however, has certain practical advantages, given that standard immunohistochemical staining techniques can be applied to classical archived paraffin-embedded material (146). Using serial sections, we were therefore forced to evaluate and develop protocols for an extensive range of 3D imaging programs through trial and error, finally resulting in manuscript I (31). Our two initial protocols presented in manuscript I were developed to depict protein expression in serial sections of human embryonic cell cultures and prenatal liver through the process of image segmentation alone.

A TWO- AND THREE-DIMENSIONAL APPROACH FOR VISUALIZING HUMAN EMBRYONIC STEM CELL DIFFERENTIATION

When Thomson et al. first derived human embryonic stem cells (hESC) from blastocysts in 1998, it was expected that cell cultures were homogenously undifferentiated (166). Contrary to this assumption, later studies suggested micro heterogeneity in expression of hESC markers, and thereby differentiation, in these cell cultures (167;168). To study this phenomenon we wished to stain the same hESC culture for a number of hESC markers and simultaneously present their localization. For this process serial sections of hESC cultures were dehydrated, embedded into paraffin and serially sectioned.

Alternating sections were immunohistochemically stained for single proteins, covering either transcription factors and surface markers associated with undifferentiated hESCs (OCT4, NANOG, TRA-1-60), or markers of the more differentiated germ layers, endoderm (HNF-3β), mesoderm (CD34) or ectoderm (p63) (166;169-175). The sectioning and mounting of tissue sections on glass slides prior to the staining procedure results in sections that are not perfectly aligned with respect to their original position in the tissue block. Our earliest attempt to circumvent this problem relied on the cumbersome process of importing each microphotograph into the imaging software Adobe Photoshop, making 2 consecutive sections at a time semitransparent, rotating them, so they visually fitted with respect to each other, and then saving them. This process known as neighborhood alignment, or non-rigid registration, is not perfectly correct as it relies on aligning neighboring sections to fit visually with respect to each other, without taking into consideration the some structures may be more dynamic than others or that stretching of individual sections often occur (146;176). Therefore errors in alignment can be introduced, when rigid markers are not incorporated into the tissue block or section stretching is not adjusted for. Alignment can be improved by embedding socalled fiducial markers for reference with the tissue of interest (147;177-179), or constructing a Case Cryo-imaging system for episcopic image acquisition (159;160). Tissue stretching can be mathematically corrected through the use of statistical image filters (149). However, fiducial markers are generally only applicable for imaging of larger sections and not for high-power microscopy, while Case Cryo-imaging systems are large and expensive technical setups, not readily applicable for standard laboratories. Therefore we settled with the neighborhood alignment procedure, regardless of its inherent inaccuracies.

Following alignment in Adobe Photoshop, we imported the microphotographs images into Mimics (Materialise, Leuven, Belgium), a software package allowing 3D reconstructions of biomedical data. In order to segment or label the images, it was necessary to digitally separate the cell cultures from background. Our initial process simply involved adjusting the contrast on each image and defining a threshold interval for what part of the images should be considered cell culture. This semi-automated segmentation process is somewhat crude and we therefore manually curated the segmentation on each image. Subsequently Mimics converted the segmented sections to an actual 3D structure, onto which we could manually draw in the areas that stained for the proteins of interest. Using this procedure we visually illustrated that micro heterogeneity in multiple marker expression occurs within a culture of otherwise supposedly undifferentiated hESCs.

The experience obtained from this initial approach to 3D reconstruction was much elaborated to incorporate segmentation of immunohistochemical staining on consecutive tissue sections. To circumvent the cumbersome process of pre-aligning images in Adobe Photoshop, we turned to 3D-Doctor (Able Software Corp, MA, USA) as this 3D imaging software has built-in algorithms for alignment of tomographic material. Certain software packages, such as TissuemorphDPtm (Visiopharm, Hoersholm, Denmark), incorporate color recognition software. However, a major nuisance with 3D reconstruction software is that they only accept grayscale image files. This setup renders segmentation of immunohistochemical staining problematic, since dark staining is difficult to distinguish from tissue in nuances of gray. We dealt with this problem through simple contrast and exposure enhancements. Overexposure of immunohistochemically stained tissue sections under the microscope leads to a reduction of visible tissue, leaving darker material, such as immunostaining, behind. However, we had to make a balance between overexposure and visibility of tissue for proper alignment. 3D-doctors built-in alignment algorithm for automated alignment relies on identifying edges or similarities in intensity between consecutive sections (176). However the overexposure decreased visible tissue, thereby rendering this approach impossible. We were therefore forced to use hepatic vessels present on all consecutive sections as reference points for matching between sections. The reduction in background, however, enabled us to segment the immunostains semiautomatically by entering threshold values followed by manual corrections on each section. The approach described herein allowed us to convert classical immunostained liver sections into a 3D structure by means of segmentation.

THREE-DIMENSIONAL RECONSTRUCTIONS OF INTRAHEPATIC BILE DUCT TUBULOGENESIS IN HUMAN LIVER.

Analysis of consecutive sections immunostained for potential stem cell markers previously established the extent and composition of the human hair follicle stem cell niche (180). With a similar strategy in mind we wished to define the hepatic progenitor cell niche, describe cholangiocyte tubulogenesis and to improve our protocols for digital three-dimensional visualization in paper II (32). For this purpose we sought for hepatic progenitor cell niche candidate proteins through two distinct approaches. One approach involved identifying markers for the hepatic and cholangiocytic lineages through literature studies. The other approach was bioinformatical and relied on identifying mainly surface proteins highly upregulated in an Illumina GeneArray conducted on livers from rats subjected to the AAF/PHx protocol. By means of these two approaches we selected the following proteins for exploration: keratin 7 (K7), K19, epithelial cell adhesion molecule (EpCAM), HepPar1, sex-determining region Y (SRY)-box 9 (SOX9), aquaporin 1 (AQP1), and zone occludens 1 (ZO-1). Laminin and nestin were chosen as markers of extracellular matrix deposition.

Our two initial approaches for converting biological material into 3D structures were somewhat crude and unsophisticated. Two events changed this completely. First, attention was drawn to the software platform Amira® (Visage Imaging, Berlin, Germany). Amira is a highly adaptable software platform that allows data manipulation through a plethora of built-in modules applicable for many scientific disciplines, several of these directly suited for light microscopy and section alignment. Amira also allows execution of own developed computer libraries and algorithms permitting downstream 3D integration into other file formats for interactive presentation (181). Second, a method developed by Stephan Handschuh dealt with volume rendering microphotographs of serial cut sections in Amira (149). Thus we could preserve the advantages of light microscopy with respect to resolution and presentation of histologic sections in original colors (149).

The flexibility of Amira permitted us to modify the aligned microphotographs with professional imaging tools in Adobe Photoshop® and thereby modify copies of the same image stack for different visualization purposes. That way one image stack was adjusted, so hepatic vessels could better be discriminated from tissue, while color recognition tools permitted the detection and altering of immunostains in another copy of the same stack. These preprocessing modifications greatly facilitated vessel and immunostain segmentation in later grayscale images in Amira. Successively, contrast and brightness were modified in a third copy of the same image stack so the immunostains prominently stood out from the hepatic parenchyma in the final volume rendering. In the following volume rendering in Amira, the immunostains were therefore highly visible throughout the image stack, while the background from the hepatic parenchyma was diminished. Our developed protocol therefore managed to combine the two fundamental approaches of 3D visualization of the same material and can even display them simultaneously. Given that segmentation is a subjective process a major strength of this protocol is that the volume rendering is a direct visualization of original data and therefore verifies the manual segmentation conducted by the operator.

K19 is a marker of human cholangiocytes (19;105). Analyses of adult hepatic sections stained for K19 demonstrate not only bile ducts, but also scattered epithelial cells around the portal area (105). The nature and origin of these elusive cells was uncertain and even suggested to be remnants of the ductal plate or a sort of progenitor cell (105). Through manual tracing and coloring in of K19 on consecutive sections Theise revealed that the seemingly isolated cells represented the canals of Hering and were connected to the bile ducts (19). Using our developed protocols for 3D reconstruction we recapitulated this study in both adult human liver and from a case of paracetamol poisoning resulting in fulminant hepatic failure (32). The volume rendering and segmentations directly illustrated that in normal liver bile ducts were slender structures that had direct connections to the canals of Hering. The reconstruction of the paracetamol intoxicated liver illustrated the high proliferative capacity inherent to the canals of Hering through the depiction of numerous ductular reactions directly connected to the biliary tree. For both reconstructions it holds that had we not applied a 3D approach, K19 cells in the parenchyma could easily have been regarded as isolated cells thereby supporting previous misinterpretations (105).

We next wished to study the formation of the biliary tree, and thereby the developing human progenitor cell niche using both classical immunohistochemistry and our 3D protocol. For this we therefore identified the three major developmental stages of biliary development, i.e. the ductal plate stage (the most primitive biliary stage), the remodeling bile duct stage and the remodeled bile duct stage (104;114;115). Based on staining for K7, K19, EpCAM and SOX9 3D reconstructions of the biliary/progenitor lineages illustrated that in embryonic liver K19 and EpCAM marked both ductal plate cells and the primitive hepatocytes throughout the entire image stack (110;182-184). In the remodeling (fetal) and remodeled (adult) liver, however, these proteins only marked biliary cells, thereby corroborating the notion that K19 expression is a characteristic of not only primitive hepatocytes but less differentiated cells in general (104-106). K19 has for the same reason been called a neutral cytokeratin (185). The opposite is true for K7, as expression of this filament is regarded as a marker of final commitment into the cholangiocytic lineage (110;186). In agreement with this notion we did not detect K7 in the ductal plates and remodeling bile ducts until 13½ weeks post conception. At approximately the same time the primitive hepatocytes lost K19 and EpCAM staining, marking their commitment to the hepatocytic lineage. The relative late expression of K7 was never the less earlier than previously published (110;116-118).

SOX9 is a transcription factor involved in chondrogenesis and male gonad development (187-190). More importantly, it is also expressed in the intestinal stem cell niche (191), and is necessary for the maintenance of transit amplifying cells in the hair follicle (192;193). Recently it was shown to be the earliest maker of biliary commitment identified yet (184). This observation was supported by our 3D-models, which depicted strong SOX9 expression in the ductal plate, whereas the hepatoblast had only weak staining. Our thorough analyses of SOX9, EpCAM and HepPar1 on histological sections from approximately 6 weeks post conception furthermore revealed a remarkable aspect of cholangiocytic tubulogenesis. Several organs, such as the kidneys, lungs and pancreas, contain tubular structures facilitating transport of gas and liquids. Multiple different events can to the organization of cells into tubular organs. These events are presently characterized as wrapping, budding, cavitation, cord and cell hollowing tubulogenesis (194-197). In parallel with our investigations, studies in mouse liver revealed intrahepatic development to occur through a special mode of asymmetric cholangiocyte tubulogenesis. In this process, the mesenchymal facing ductal plate cells instruct neighboring primitive hepatocytes to form the parenchymal ductal plate layer and thereby differentiating into cholangiocytes. In support of these observations our immunohistochemical analy-

ses of the bilayered ductal plate depicted a peculiar expression pattern. While the mesenchymal facing layer only expressed the cholangiocytic markers SOX9 and EpCAM, the parenchymal layer only stained for the hepatocytic marker HepPar1. The observed reciprocal expression pattern indicate that a similar form of lateral recruitment of primitive hepatocytes also occurs in humans (198). However, how the intrahepatic bile ducts expand along the longitudinal axes was not dealt with in that or in our study and is still unresolved.

MOLECULAR COMPOSITION OF THE REMODELING EXTRACELLULAR MATRIX COMPONENTS IN HEPATOCELLULAR REGENERATION FROM PROGENITOR CELLS.

The aforementioned study of human liver directly visualized bile duct branching and suggested an asymmetric form of tubulogenesis to govern intrahepatic bile duct formation and thereby niche development. However, biliary proliferation and morphogenesis is a complex interplay between cholangiocytes, support cells and extracellular matrix components and the molecular composition in the niche is thought to change in favor of progenitor cell proliferation upon massive injury to hepatocytes.

Indeed, extracellular matrix deposition and activation of matrix-producing cells have been observed to appear prior to hepatic progenitor cell expansion and lay down a matrix for progenitor cells to invade (49;52). Subsequent studies in which isolated hepatic progenitor cells were seeded on different matrices provided evidence that laminin, in particular, was important for sustaining the biliary/hepatic progenitor cell phenotype (30). However, the exact molecular composition of the niche is unknown. Central questions to ask are therefore: Assuming that the canal of Hering truly constitutes the hepatic progenitor cell niche, which proteins are then expressed in the niche and which specific extracellular matrix molecules regulate the hepatic progenitor cell response? Of particular interest is also to clarify whether there are selective differences in extracellular matrix-composition in different models of hepatic regeneration with proliferating biliary cells. This could provide clues to the identification of factors specifically necessary for hepatic progenitor cell activation and proliferation. At present, few studies have investigated extracellular matrix (ECM)-remodeling in the activated hepatic progenitor cell niche.

To address the proposed questions we first constructed a hypothetical extracellular matrix structure based on findings in the literature. We then reasoned that some of the transcripts upregulated on global gene expression arrays from rats with activated progenitor cells would locate to the niche. We therefore employed Illumina GeneArray techonology to identify cell surface proteins and extracellular matrix molecules differentially expressed when using the 2-AAF/PHx protocol. With emphasis on the extracellular matrix components the identified transcripts were categorized into four major groups important for defining the microenvironment: Hepatic progenitor cell markers, mesenchymal cell markers, extracellular matrix proteins and a network of proteases comprising matriptase, its target prostasin, and its cognate inhibitors HAI-1 and -2, encoded by the St14, Prss8, Spint1 and Spint2 genes, respectively. Matriptase is a broad-spectrum serine protease capable of degrading extracellular matrix components and is implicated in cancer invasion and metastasis (199-205). Previous studies have identified components of this network in the hepatic progenitor cell response and we suspected it to be involved in hepatic progenitor cell proliferation and invasion (96). Additionally, given that matriptase and its inhibitors are transmembrane surface proteins, they possessed the potential to be useful for cell isolation studies. Transcriptional profiling of whole liver is unspecific for the hepatic progenitor cell compartment and transcripts upregulated in silico may not necessarily give rise to increased expression of protein. However, we realized that screening transcripts by correlating their signal intensities against K19 (Krt19) or desmin (Des) provided us with several interesting ECM components that strongly correlated with either the hepatic progenitor cell response or mesenchymal support cells. To verify microarray data we chose to investigate the predicted up-regulation of the matriptase network and Krt19 through real-time RT-PCR in unharmed rat liver, in the bile duct ligation model and in two models of hepatic progenitor cell proliferation. As predicted, transcripts for the matriptase network components and Krt19 were particularly upregulated in hepatic progenitor cell response.

With the purpose of identifying partakers in the hepatic progenitor cell response and compare expression pattern with that of bile duct ligation or unharmed liver, we applied immunohistochemistry for protein localization. This approach allowed us to make direct comparisons between the atypical ductular response, the ductular response and unharmed liver. Immunohistochemical and immunoflourescent stainings revealed matriptase and HAI-1 to decorate both the intrahepatic biliary tree and the entire proliferating hepatic progenitor cell compartment. Unfortunately, co-localization of matriptase with HAI-1 was not possible due to cross-reaction between antibodies. However, other studies have shown that matriptase and HAI-1 not only co-localize, but also that HAI-1 remarkably functions as a chaperone necessary for matriptase folding, function and inactivation (200;204;206-209). Having established matriptase and HAI-1 as markers of biliary cells, we related expression of ECM components selected from the array analysis to matriptase, HAI-1 and the two known markers of biliary/progenitor cells, Dlk1 and OV6. OV6 is an antibody that recognizes epitopes shared between keratin 14 and 19 and therefore decorates the entire intrahepatic tree (210).

From the array analyses we decided to dissect the expression pattern of laminin, a recognized marker of the basement membrane (211) the glycoprotein nidogen 1 (212), proteoglycan agrin (213;214) and type I collagen. Type 1 collagen, represented by its subunit collagen 1a1, is a heterotrimeric fibrillar collagen, which is primarily associated as being the main component of scarring tissue in cirrhotic liver (68;90;215). Regardless of the animal model investigated, we established that the same extracellular matrix components enclosed both the intrahepatic biliary tree, the typical and atypical ductular reactions. For laminin, this was unsurprising, as previous studies have shown similar patterns of expression and demonstrated laminins importance for maintaining the cholangiocytic phenotype (30). Nidogen 1 and agrin have both been recognized for their abilities to cross-link the extracellular matrix with the cytoskeleton and, important in our study, to bind laminin. Their pattern of expression was thus similar to that observed for laminin. More unexpectedly, was the observation, that collagen 1a1, had a comparable expression pattern, as other studies have found no relationship with hepatic progenitor cells (30) or suggested that type I collagen does not support the biliary phenotype in vitro (216;217). However, our results suggest that type I collagen is not merely a component of scar tissue, but in fact a participant in the progenitor cell niche. Interestingly, in the progenitor cell response, some of the progenitor cells adjoining the hepatocytes were not enclosed by ECM-molecules, yet still expressing the biliary markers, HAI-1 and OV-6. This led us to suspect that the extracellular matrix form a sharply limited niche

in which cells can amplify while retaining the biliary phenotype, and upon exit from the niche, differentiates towards the hepatocytic lineage. In this way, the hepatic progenitor cell niche much resembles the classical niches observed in Drosophila species (13). However, as we are unaware of the nature of the putative stem cells, it is also conceivable that certain hepatocytes or other cell types, connected to the canal of Hering transform into biliary cells.

When we analyzed tissue sections, Dlk1 was, as previously reported, only observed in the hepatic progenitor cell response. Co-staining Dlk1 with extracellular matrix components marked a subset of hepatic progenitor cells positive for Dlk1, in slender tube-like ECM structures. These observations encouraged us to triple stain sections from the hepatic progenitor cell response for Dlk1 in combination with biliary markers and extracellular matrix components, as this would provide us with more information of the spatial relationship between the proteins. This approach directly provided evidence that, while HAI-1 and OV6 marks the hepatic progenitor cells, Dlk1 expression is restricted to a subpopulation within the extracellular matrix. In order to establish if these descriptions were applicable to larger portions of the liver and not just chance findings, we reapplied our developed 3D-reconstruction protocols to investigate the spatial relationship of Dlk1, HAI-1 and agrin. Reconstructions of 208 µm control and AAF/PHx hepatic tissue, clearly highlighted, that throughout the image stacks, HAI-1 marked both portal bile ducts and hepatic progenitor cells, which were enclosed by agrin, whereas Dlk1 only stained a subset of hepatic progenitor cells.

The implications of this study with respect to the extracellular matrix molecules are therefore that, the exact same components participate in biliary proliferation, regardless of initiating insult and are no different from components found in unharmed liver. We therefore speculate that a function of these proteins is to maintain the biliary phenotype, rather than actually govern differentiation. This niche therefore resembles a transit amplifying compartment as observed in other animals and organs, in which hepatic progenitor cells can proliferate before leaving the niche (13). It seems that upon leaving this sharply restricted microenvironment the biliary phenotype is no longer supported and thus the hepatic progenitor cells differentiate towards the hepatocytic lineage. However, it is not known what governs the presumed differentiation from a cholangiocyte lineage towards the hepatocytic. However, Lorenzini et al. examined the functional roles for several extracellular matrix components and established that laminin was important for maintaining the biliary/hepatic progenitor cell phenotype (30). Type I and IV collagen was, however, incapable of preserving the biliary phenotype. Paku et al. demonstrated direct connections between activated stellate cells and the hepatic progenitor cell niche suggesting an important communication function for the mesenchymal cell compartment in the hepatic progenitor cell response (48). Moreover, both cell types are laminin producers, implying that not only the support but also the progenitor cells influence the composition of the progenitor cell niche (30). Non-parenchymal cells in the liver, including stellate cells and myofibroblast, are the main synthesizers of collagen, and are found scattered throughout the lobule (218-220). As type I collagen has a similar expression pattern, and laminin alone sustained the biliary phenotype in vitro, these finding imply that both type I collagen and desmin positive stellate cells are not essential components of the hepatic progenitor niche. However, it is difficult to conclude on cells from the mesenchymal compartment in general, as several cell types are present in the liver, and their relationships are uncertain and has previously led to misconclusions (220). Deszö et al. for instance demonstrated that Thy-1, a protein once thought to be a progenitor cell marker, actually was expressed by the hepatic myofibroblast (219). For both laminin and collagen, multiple isoforms exist. However, in both Lorenzinis paper and our study, relatively few components were immunohistochemically examined (30). We nevertheless made attempts to dissect the molecular composition of the niche by staining for several of these isoforms, but only antibodies for the mentioned components functioned. We were therefore forced to describe only total laminin, collagen 1a1, agrin, nidogen-1 and desmin. With this limited battery of extracellular markers, we nevertheless observed that, with the exception of Dlk1, the exact same components were expressed similarly in both unharmed liver, the bile duct ligation model, and in the progenitor mediated form of hepatic regeneration. These findings support our notion, that the investigated proteins were important for the biliary phenotype, rather than activating the progenitor mediated response.

The role and expression pattern of Dlk1 is somewhat enigmatic. Dlk1 a transmembrane protein with EGF-like repeats, that is a member of the EGF-like homeotic family (221-223). Dlk1 is often described as an inhibitor of differentiation and expressed in less differentiated cells (222;224). Forced expression has for instance been found to inhibit adipogenesis, whereas suppression promotes this process (222). Importantly Tanimizu et al. found that this protein was expressed in primitive hepatocytes capable of differentiating into both the hepatocytic and cholangiocytic lineages (100). It is therefore possible that Dlk1 has a negative impact on transit amplifying cell differentiation, thereby allowing these cells to multiply without differentiating, as seen in other niches (13). In this way, a large number of transit amplifying cells can be produced, before leaving the niche. We often found Dlk1 centered in the ductular reactions not directly adjoined to the hepatocytes. Our study therefore suggest the presence of a cellular hierarchy within the ductular reactions in which centrally located Dlk1 positive progenitor cells gives rise to HAI-1 positive progeny that then differentiates into hepatocytes.

CONCLUSIONS AND PERSPECTIVES

The overall aim of this project was to gain knowledge of the hepatic progenitor cell niche. We achieved this goal by first developing protocols for visualizing hepatic structures and protein expression. We thereafter dissected protein-expression in both developing and diseased human liver, and across independent models of hepatic regeneration.

Using one particular form of digital 3D reconstruction, our initial study illustrated that human embryonic stem cell cultures thought to be homogenous are actually heterogeneous and display markers for differentiation into the three germ layers. By means of a different approach for 3 modeling we were able to create a reconstruction of the ductal plate, or hepatic progenitor cell niche, during human development. These two early protocols for 3D modeling were crude and unsophisticated, but taught us valuable lessons about 3D-modelling and provided us with ideas on how to improve our protocols. Discovering the software platform Amira was in this respect the game changer that released us from the modeling strains of previous restrictive platforms and allowed us to employ our ideas. We therefore managed to build a strong protocol that combines two benefits of the two basic methods for 3D modeling; volumetric rendering and segmentation based reconstruction. This protocol was furthermore well-

suited for sections from paraffin embedded tissues, a common storage solution for preserving specimens.
With this protocol in hand we not only depicted the developing hepatic progenitor cell niche but directly visualized ductular reactions in a case of ALF. This connection had previously been established by tedious manual drawings, but our protocol not only directly visualized this connection in 3D, but can be applied to unraveling similar questions in other organs. However, there are a few shortcomings with this protocol. Even though we managed to employ color recognition techniques, the segmentation process is still painstaking work. This is not just a shortcoming of our protocol, but a general problem within the field of 3D-modelling and more refined approaches for automated cell and color recognition needs to be developed. We have however recently expanded our protocol, so we are capable of exporting the segmentation based structures and both incorporate them into the Portable Document Format (PDF) and present them in augmented reality scenes. The latter is a technique in which 3D structures are presented in real environment through the use of digital cameras. As volumetric renderings do not contain structures assigned with x, y and z coordinates their presentation with these techniques is more complicated. We are nevertheless attempting to accomplish this through the use of certain computer libraries originally created for Astronomy.
Other contributions from our studies to the field of hepatic research involved identifying the transcription factor SOX9 as an early marker of the cholangiocytic lineage in humans. Most importantly, this study also revealed that formation of the human intrahepatic biliary tree occurs through a newly identified form of asymmetrical tubulogenesis. Unraveling exactly how the mesenchymal ductal plate layer apparently instructs primitive hepatocytes to develop into cholangiocytes could provide useful information for replicating this process in vitro for future therapeutic treatment. Moreover, the question of how the biliary tree branches into the developing liver is also unresolved.
Even though the impact that extracellular matrix components can have on cell phenotype and behavior is well known, presently few studies have dissected the microenvironment in the activated progenitor cell response. The array approach used in our study identified several candidate genes for both hepatic progenitor cell surface markers and their niche. Through immunohistochemistry and immunofluorescence we located the protein product of several of these genes. We found that the niche forms a restricted zone in which hepatic progenitor cells proliferate before leaving the microenvironment to differentiate into hepatocytes. However, this niche was no different from that observed in unharmed liver or in other models of hepatic injury. In order to evaluate if the microenvironment truly is different in progenitor mediated hepatic regeneration, it will therefore be necessary to perform immunohistochemical staining's for additional candidate components in future studies. We did however identify matriptase and HAI1 as surface markers for both cholangiocytes and progenitor cells. The implication of this is that in future studies these two proteins can be used for cell isolation experiments in conjunction with Dlk1 to shed light on differences in "stemcellness" of biliary subpopulations. Interestingly we observed that expression of several transcripts strongly correlated with markers of hepatic progenitor cells or the mesenchymal compartment. It would be of interest to evaluate trough in situ hybridizations, if this simple calculation can be used as a predictor of what cell types express which extracellular matrix components.
Taken together the three studies have provided protocols for digitally reconstructing tissue and provided us with knowledge of the development and tubulogenesis of the intrahepatic biliary system. Important, we gained deeper insights into the molecular composition of the hepatic progenitor cell niche.

SUMMARY

End-stage liver diseases represent major health problems that are currently treated by liver transplantation. However, given the world-wide shortage of donor livers novel strategies are needed for therapeutic treatment. Adult stem cells have the ability to self-renew and differentiate into the more specialized cell types of a given organ and are found in tissues throughout the body. These cells, whose progeny are termed progenitor cells in human liver and oval cells in rodents, have the potential to treat patients through the generation of hepatic parenchymal cells, even from the patient's own tissue. Little is known regarding the nature of the hepatic progenitor cells. Though they are suggested to reside in the most distal part of the biliary tree, the canal of Hering, the lack of unique surface markers for these cells has hindered their isolation and characterization. Upon activation, they proliferate and form ductular structures, termed "ductular reactions", which radiate into the hepatic parenchyma. The ductular reactions contain activated progenitor cells that not only acquire a phenotype resembling that observed in developing liver but also display markers of differentiation shared with the cholangiocytic or hepatocytic lineages, the two parenchymal hepatic cell types. Interactions between the putative progenitor cells, the surrounding support cells and the extracellular matrix scaffold, all constituting the progenitor cell niche, are likely to be important for regulating progenitor cell activity and differentiation. Therefore, identifying novel progenitor cell markers and deciphering their microenvironment could facilitate clinical use.
The aims of the present PhD thesis were to expand knowledge of the hepatic progenitor cell niche and characterize it both during development and in disease. Several animal models of hepatic injury are known to induce activation of the progenitor cells. In order to identify possible progenitor cell markers and niche components, we examined several genes upregulated in a global gene expression array conducted on one of these models, in which progenitor cells are activated. The protein expression patterns were evaluated in our collections of human embryonic and fetal livers, human liver diseases, and rodent hepatic injury models. When analyzing standard histological liver sections underlying connections and tissue architecture are not immediately evident. We therefore developed models for digitally reconstructing not only protein expression in serially cut tissue sections, but also vessels of the portal area.
Article I constituted our earliest attempts to create three-dimensional reconstructions of biological material. Human embryonic stem cell cultures were previously thought to consist of homogenously undifferentiated cells. The protocols for three dimensional reconstructions developed in this study demonstrated micro heterogeneity in expression of differentiation markers and provided the basis for later reconstructions of hepatic tissues.
In article II we examined the expression patterns of chosen proteins seen upregulated in the gene array as well as classical hepatocytic and cholangiocytic markers in human liver disease and during prenatal development. Previous studies had indicated direct connections between activated progenitor cells apparently isolated in the parenchyma and the intrahepatic biliary tree. Our developed protocols for 3D reconstructions visually demonstrated direct connections between these entities. Analysis of

protein expression in prenatal liver revealed the formation of the intrahepatic tree to occur through a special form of asymmetric tubulogenesis, only recently described in mice.
In order to describe the composition of the hepatic progenitor cell niche and the localization of cell surface proteins in article III, the expression patterns of certain genes upregulated in the gene array analysis were analyzed in different models of rodent liver regeneration. We observed that the extracellular matrix molecules collagen 1a1, laminin, nidogen-1 and agrin embraced the biliary cells and sharply defined the hepatic progenitor cell niche, which was encircled by desmin positive support cells. In all injury models biliary cells expressed the cell surface proteins matriptase and HAI-1. However, in the so-called 2-AAF/PHx model of progenitor cell activation, a subpopulation of hepatic progenitor cells was positive for Dlk1. 3D reconstructions clarified that the Dlk1-subpopulation was entirely located in the portal area periphery, and connected to the bile ducts via HAI-1 positive biliary cells. The heterogeneous expression patterns of matriptase, HAI-1 and Dlk1 in this particular injury model indicate the presence of a cellular hierarchy containing possibly less differentiated Dlk1-positive hepatic progenitor cells.
In conclusion, our studies characterized the hepatic progenitor cell niche in humans and rodents. We successfully developed protocols for digitally visualizing, not only hepatic, but virtually any tissue through two fundamentally distinct approaches. The identification of an asymmetric form of tubulogenesis in humans added new knowledge to the development of the intrahepatic biliary tree, and thereby the formation of the progenitor cell niche. The identification of heterogeneously expressed cell surface proteins and extracellular matrix components provided knowledge of the constituents defining the niche. These pieces of information are important for future isolation and characterization studies of biliary subpopulations and their differentiation abilities in vitro.

REFERENCES

1. Michalopoulos GK, DeFrances MC. Liver regeneration. Science 1997 Apr 4;276(5309):60-6.
2. Overturf K, Al-Dhalimy M, Ou CN, Finegold M, Grompe M. Serial transplantation reveals the stem-cell-like regenerative potential of adult mouse hepatocytes. Am.J.Pathol. 1997 Nov;151(5):1273-80.
3. Bernal W, Auzinger G, Dhawan A, Wendon J. Acute liver failure. Lancet 2010 Jul 17;376(9736):190-201.
4. Larson AM. Diagnosis and management of acute liver failure. Curr.Opin.Gastroenterol. 2010 May;26(3):214-21.
5. Larsen FS, Kirkegaard P, Rasmussen A, Hansen BA. The Danish liver transplantation program and patients with serious acetaminophen intoxication. Transplant.Proc. 1995 Dec;27(6):3519-20.
6. Rolando N, Wade J, Davalos M, Wendon J, Philpott-Howard J, Williams R. The systemic inflammatory response syndrome in acute liver failure. Hepatology 2000 Oct;32(4 Pt 1):734-9.
7. Ostapowicz G, Fontana RJ, Schiodt FV, Larson A, Davern TJ, Han SH, McCashland TM, Shakil AO, Hay JE, Hynan L, et al. Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. Ann.Intern.Med. 2002 Dec 17;137(12):947-54.
8. Russo FP, Parola M. Stem and progenitor cells in liver regeneration and repair. Cytotherapy. 2011 Feb;13(2):135-44.
9. Riordan SM, Williams R. Use and validation of selection criteria for liver transplantation in acute liver failure. Liver Transpl. 2000 Mar;6(2):170-3.
10. Li L, Clevers H. Coexistence of quiescent and active adult stem cells in mammals. Science 2010 Jan 29;327(5965):542-5.
11. Zipori D. Stem Cell Niches. Biology of Stem Cells and the Molecular Basis of the Stem State. In Humana Press; 2009. p. 109-50.
12. Raymond K, Deugnier MA, Faraldo MM, Glukhova MA. Adhesion within the stem cell niches. Curr.Opin.Cell Biol. 2009 Oct;21(5):623-9.
13. Losick VP, Morris LX, Fox DT, Spradling A. Drosophila stem cell niches: a decade of discovery suggests a unified view of stem cell regulation. Dev.Cell 2011 Jul 19;21(1):159-71.
14. Spradling AC, Nystul T, Lighthouse D, Morris L, Fox D, Cox R, Tootle T, Frederick R, Skora A. Stem cells and their niches: integrated units that maintain Drosophila tissues. Cold Spring Harb.Symp.Quant.Biol. 2008;73:49-57.
15. Michalopoulos GK. Liver regeneration. J.Cell Physiol 2007 Nov;213(2):286-300.
16. Roskams TA, Theise ND, Balabaud C, Bhagat G, Bhathal PS, Bioulac-Sage P, Brunt EM, Crawford JM, Crosby HA, Desmet V, et al. Nomenclature of the finer branches of the biliary tree: canals, ductules, and ductular reactions in human livers. Hepatology 2004 Jun;39(6):1739-45.
17. Wilson JW, Leduc EH. Role of cholangioles in restoration of the liver of the mouse after dietary injury. J.Pathol. 1958;76(2):441-9.
18. Wilson JW, Groat CS, Leduc EH. Histogenesis of the liver. Annals of the New York Academy of Sciences 1963;111(1):8-24.
19. Theise ND, Saxena R, Portmann BC, Thung SN, Yee H, Chiriboga L, Kumar A, Crawford JM. The canals of Hering and hepatic stem cells in humans. Hepatology 1999 Dec;30(6):1425-33.
20. Hixson DC, Allison JP. Monoclonal antibodies recognizing oval cells induced in the liver of rats by N-2-fluorenylacetamide or ethionine in a choline-deficient diet. Cancer Res. 1985 Aug;45(8):3750-60.
21. Evarts RP, Nagy P, Marsden E, Thorgeirsson SS. A precursor-product relationship exists between oval cells and hepatocytes in rat liver. Carcinogenesis 1987 Nov;8(11):1737-40.
22. Evarts RP, Nagy P, Nakatsukasa H, Marsden E, Thorgeirsson SS. In Vivo Differentiation of Rat Liver Oval Cells into Hepatocytes. Cancer Res 1989 Mar 15;49(6):1541-7.
23. Van Den Heuvel MC, Slooff MJ, Visser L, Muller M, de Jong KP, Poppema S, Gouw AS. Expression of anti-OV6 antibody and anti-N-CAM antibody along the biliary line of normal

and diseased human livers. Hepatology 2001 Jun;33(6):1387-93.
24. Jensen CH, Jauho EI, Santoni-Rugiu E, Holmskov U, Teisner B, Tygstrup N, Bisgaard HC. Transit-amplifying ductular (oval) cells and their hepatocytic progeny are characterized by a novel and distinctive expression of delta-like protein/preadipocyte factor 1/fetal antigen 1. Am.J.Pathol. 2004 Apr;164(4):1347-59.
25. Alison MR, Golding MH, Sarraf CE. Pluripotential liver stem cells: facultative stem cells located in the biliary tree. Cell Prolif. 1996 Jul;29(7):373-402.
26. Shafritz DA, Dabeva MD. Liver stem cells and model systems for liver repopulation. Journal of Hepatology 2002 Apr;36(4):552-64.
27. Fausto N, Campbell JS. The role of hepatocytes and oval cells in liver regeneration and repopulation. Mech.Dev. 2003 Jan;120(1):117-30.
28. Dabeva MD, Shafritz DA. Hepatic stem cells and liver repopulation. Semin.Liver Dis. 2003 Nov;23(4):349-62.
29. Alison MR, Vig P, Russo F, Bigger BW, Amofah E, Themis M, Forbes S. Hepatic stem cells: from inside and outside the liver? Cell Prolif. 2004 Feb;37(1):1-21.
30. Lorenzini S, Bird TG, Boulter L, Bellamy C, Samuel K, Aucott R, Clayton E, Andreone P, Bernardi M, Golding M, et al. Characterisation of a stereotypical cellular and extracellular adult liver progenitor cell niche in rodents and diseased human liver. Gut 2010 May;59(5):645-54.
31. Brochner CB, Vestentoft PS, Lynnerup N, Andersen CY, Mollgard K. A two- and three-dimensional approach for visualizing human embryonic stem cell differentiation. Methods Mol.Biol. 2010;584:179-93.
32. Vestentoft PS, Jelnes P, Hopkinson BM, Vainer B, Mollgard K, Quistorff B, Bisgaard HC. Three-dimensional reconstructions of intrahepatic bile duct tubulogenesis in human liver. BMC.Dev.Biol. 2011 Sep 26;11(1):56.
33. Fuchs E. The tortoise and the hair: slow-cycling cells in the stem cell race. Cell 2009 May 29;137(5):811-9.
34. Watt FM, Hogan BL. Out of Eden: stem cells and their niches. Science 2000 Feb 25;287(5457):1427-30.
35. Tumbar T, Guasch G, Greco V, Blanpain C, Lowry WE, Rendl M, Fuchs E. Defining the epithelial stem cell niche in skin. Science 2004 Jan 16;303(5656):359-63.
36. Morris RJ, Potten CS. Highly persistent label-retaining cells in the hair follicles of mice and their fate following induction of anagen. J.Invest Dermatol. 1999 Apr;112(4):470-5.
37. Cotsarelis G, Sun TT, Lavker RM. Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. Cell 1990 Jun 29;61(7):1329-37.
38. Foudi A, Hochedlinger K, Van BD, Schindler JW, Jaenisch R, Carey V, Hock H. Analysis of histone 2B-GFP retention reveals slowly cycling hematopoietic stem cells. Nat.Biotechnol. 2009 Jan;27(1):84-90.
39. Wilson A, Laurenti E, Oser G, van der Wath RC, Blanco-Bose W, Jaworski M, Offner S, Dunant CF, Eshkind L, Bockamp E, et al. Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair. Cell 2008 Dec 12;135(6):1118-29.
40. Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M, Haegebarth A, Korving J, Begthel H, Peters PJ, et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature 2007 Oct 25;449(7165):1003-7.
41. Sato T, Vries RG, Snippert HJ, van de Wetering M, Barker N, Stange DE, van Es JH, Abo A, Kujala P, Peters PJ, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature 2009 May 14;459(7244):262-5.
42. O'Reilly AM, Lee HH, Simon MA. Integrins control the positioning and proliferation of follicle stem cells in the Drosophila ovary. J.Cell Biol. 2008 Aug 25;182(4):801-15.
43. Spradling A, Drummond-Barbosa D, Kai T. Stem cells find their niche. Nature 2001 Nov 1;414(6859):98-104.
44. Morrison SJ, Kimble J. Asymmetric and symmetric stem-cell divisions in development and cancer. Nature 2006 Jun 29;441(7097):1068-74.
45. Cao YA, Wagers AJ, Beilhack A, Dusich J, Bachmann MH, Negrin RS, Weissman IL, Contag CH. Shifting foci of hematopoiesis during reconstitution from single stem cells. Proc.Natl.Acad.Sci.U.S.A 2004 Jan 6;101(1):221-6.
46. Knight B, Akhurst B, Matthews VB, Ruddell RG, Ramm GA, Abraham LJ, Olynyk JK, Yeoh GC. Attenuated liver progenitor (oval) cell and fibrogenic responses to the choline deficient, ethionine supplemented diet in the BALB/c inbred strain of mice. J.Hepatol. 2007 Jan;46(1):134-41.
47. Riehle KJ, Dan YY, Campbell JS, Fausto N. New concepts in liver regeneration. J.Gastroenterol.Hepatol. 2011 Jan;26 Suppl 1:203-12.
48. Paku S, Schnur J, Nagy P, Thorgeirsson SS. Origin and structural evolution of the early proliferating oval cells in rat liver. Am.J.Pathol. 2001 Apr;158(4):1313-23.
49. Van HN, Lanthier N, Espanol SR, Abarca QJ, van RN, Leclercq I. Kupffer cells influence parenchymal invasion and phenotypic orientation, but not the proliferation, of liver progenitor cells in a murine model of liver injury. Am.J.Pathol. 2011 Oct;179(4):1839-50.
50. Yin L, Lynch D, Ilic Z, Sell S. Proliferation and differentiation of ductular progenitor cells and littoral cells during the regeneration of the rat liver to CCl4/2-AAF injury. Histol.Histopathol. 2002 Jan;17(1):65-81.
51. Pintilie DG, Shupe TD, Oh SH, Salganik SV, Darwiche H, Petersen BE. Hepatic stellate cells' involvement in progenitor-mediated liver regeneration. Lab Invest 2010 Aug;90(8):1199-208.
52. Van Hul NK, Abarca-Quinones J, Sempoux C, Horsmans Y, Leclercq IA. Relation between liver progenitor cell expansion and extracellular matrix deposition in a CDE-induced

murine model of chronic liver injury. Hepatology 2009 May;49(5):1625-35.
53. Evarts RP, Hu Z, Fujio K, Marsden ER, Thorgeirsson SS. Activation of hepatic stem cell compartment in the rat: role of transforming growth factor alpha, hepatocyte growth factor, and acidic fibroblast growth factor in early proliferation. Cell Growth Differ. 1993 Jul;4(7):555-61.
54. Newsome PN, Hussain MA, Theise ND. Hepatic oval cells: helping redefine a paradigm in stem cell biology. Curr.Top.Dev.Biol. 2004;61:1-28.
55. Starzl TE, Fung J, Tzakis A, Todo S, Demetris AJ, Marino IR, Doyle H, Zeevi A, Warty V, Michaels M, et al. Baboon-to-human liver transplantation. Lancet 1993 Jan 9;341(8837):65-71.
56. Wood RL. Regeneration of Liver and Kidney. JAMA: The Journal of the American Medical Association 1971 Nov 22;218(8):1306-7.
57. Zajicek G, Oren R, Weinreb M, Jr. The streaming liver. Liver 1985 Dec;5(6):293-300.
58. Fellous TG, Islam S, Tadrous PJ, Elia G, Kocher HM, Bhattacharya S, Mears L, Turnbull DM, Taylor RW, Greaves LC, et al. Locating the stem cell niche and tracing hepatocyte lineages in human liver. Hepatology 2009 May;49(5):1655-63.
59. Duncan AW, Dorrell C, Grompe M. Stem cells and liver regeneration. Gastroenterology 2009 Aug;137(2):466-81.
60. Ankoma-Sey V. Hepatic Regeneration-Revisiting the Myth of Prometheus. News Physiol Sci. 1999 Aug;14:149-55.
61. Stocker E, Wullstein HK, Brau G. [Capacity of regeneration in liver epithelia of juvenile, repeated partially hepatectomized rats. Autoradiographic studies after continous infusion of 3H-thymidine (author's transl)]. Virchows Arch.B Cell Pathol. 1973 Nov 28;14(2):93-103.
62. Fausto N. Liver regeneration and repair: hepatocytes, progenitor cells, and stem cells. Hepatology 2004 Jun;39(6):1477-87.
63. Crosby HA, Hubscher S, Fabris L, Joplin R, Sell S, Kelly D, Strain AJ. Immunolocalization of putative human liver progenitor cells in livers from patients with end-stage primary biliary cirrhosis and sclerosing cholangitis using the monoclonal antibody OV-6. Am.J.Pathol. 1998 Mar;152(3):771-9.
64. Laconi E, Oren R, Mukhopadhyay DK, Hurston E, Laconi S, Pani P, Dabeva MD, Shafritz DA. Long-term, near-total liver replacement by transplantation of isolated hepatocytes in rats treated with retrorsine. Am.J.Pathol. 1998 Jul;153(1):319-29.
65. Dechene A, Sowa JP, Gieseler RK, Jochum C, Bechmann LP, El FA, Schlattjan M, Saner F, Baba HA, Paul A, et al. Acute liver failure is associated with elevated liver stiffness and hepatic stellate cell activation. Hepatology 2010 Sep;52(3):1008-16.
66. O'Grady JG. Acute liver failure. Postgraduate Medical Journal 2005 Mar 1;81(953):148-54.
67. Guo J, Friedman SL. Hepatic fibrogenesis. Semin.Liver Dis. 2007 Nov;27(4):413-26.
68. Rojkind M, Giambrone MA, Biempica L. Collagen types in normal and cirrhotic liver. Gastroenterology 1979 Apr;76(4):710-9.
69. Rutherford A, Chung RT. Acute liver failure: mechanisms of hepatocyte injury and regeneration. Semin.Liver Dis. 2008 May;28(2):167-74.
70. Riordan SM, Williams R. Mechanisms of hepatocyte injury, multiorgan failure, and prognostic criteria in acute liver failure. Semin.Liver Dis. 2003 Aug;23(3):203-15.
71. Canbay A, Friedman S, Gores GJ. Apoptosis: the nexus of liver injury and fibrosis. Hepatology 2004 Feb;39(2):273-8.
72. Xiao JC, Jin XL, Ruck P, Adam A, Kaiserling E. Hepatic progenitor cells in human liver cirrhosis: immunohistochemical, electron microscopic and immunofluorencence confocal microscopic findings. World J.Gastroenterol. 2004 Apr 15;10(8):1208-11.
73. Richardson MM, Jonsson JR, Powell EE, Brunt EM, Neuschwander-Tetri BA, Bhathal PS, Dixon JB, Weltman MD, Tilg H, Moschen AR, et al. Progressive fibrosis in nonalcoholic steatohepatitis: association with altered regeneration and a ductular reaction. Gastroenterology 2007 Jul;133(1):80-90.
74. Chen YK, Zhao XX, Li JG, Lang S, Wang YM. Ductular proliferation in liver tissues with severe chronic hepatitis B: an immunohistochemical study. World J.Gastroenterol. 2006 Mar 7;12(9):1443-6.
75. Roskams TA, Libbrecht L, Desmet VJ. Progenitor cells in diseased human liver. Semin.Liver Dis. 2003 Nov;23(4):385-96.
76. Libbrecht L, Roskams T. Hepatic progenitor cells in human liver diseases. Semin.Cell Dev.Biol. 2002 Dec;13(6):389-96.
77. Yovchev MI, Grozdanov PN, Joseph B, Gupta S, Dabeva MD. Novel hepatic progenitor cell surface markers in the adult rat liver. Hepatology 2007 Jan;45(1):139-49.
78. Santoni-Rugiu E, Jelnes P, Thorgeirsson SS, Bisgaard HC. Progenitor cells in liver regeneration: molecular responses controlling their activation and expansion. APMIS 2005 Nov;113(11-12):876-902.
79. Farber E. Similarities in the sequence of early histological changes induced in the liver of the rat by ethionine, 2-acetylamino-fluorene, and 3'-methyl-4-dimethylaminoazobenzene. Cancer Res. 1956 Feb;16(2):142-8.
80. Evarts RP, Hu Z, Omori N, Omori M, Marsden ER, Thorgeirsson SS. Precursor-product relationship between oval cells and hepatocytes: comparison between tritiated thymidine and bromodeoxyuridine as tracers. Carcinogenesis 1996 Oct;17(10):2143-51.
81. Wang X, Foster M, Al-Dhalimy M, Lagasse E, Finegold M, Grompe M. The origin and liver repopulating capacity of murine oval cells. Proc.Natl.Acad.Sci.U.S.A 2003 Sep 30;100 Suppl 1:11881-8.
82. Zhou H, Rogler LE, Teperman L, Morgan G, Rogler CE. Identification of hepatocytic and bile ductular cell lineages and

candidate stem cells in bipolar ductular reactions in cirrhotic human liver. Hepatology 2007 Mar;45(3):716-24.
83. Tatematsu M, Ho RH, Kaku T, Ekem JK, FARBER E. Studies on the proliferation and fate of oval cells in the liver of rats treated with 2-acetylaminofluorene and partial hepatectomy. Am.J.Pathol. 1984 Mar;114(3):418-30.
84. Evarts RP, Nagy P, Marsden E, Thorgeirsson SS. In situ hybridization studies on expression of albumin and alpha-fetoprotein during the early stage of neoplastic transformation in rat liver. Cancer Res. 1987 Oct 15;47(20):5469-75.
85. Grisham JW, Thorgeirsson SS. 8 - Liver stem cells. In: Potten CS, editor. Stem Cells. London: Academic Press; 1997. p. 233-VIII.
86. Petersen BE, Zajac VF, Michalopoulos GK. Bile ductular damage induced by methylene dianiline inhibits oval cell activation. Am.J.Pathol. 1997 Oct;151(4):905-9.
87. Slott PA, Liu MH, Tavoloni N. Origin, pattern, and mechanism of bile duct proliferation following biliary obstruction in the rat. Gastroenterology 1990 Aug;99(2):466-77.
88. Polimeno L, Azzarone A, Zeng QH, Panella C, Subbotin V, Carr B, Bouzahzah B, Francavilla A, Starzl TE. Cell proliferation and oncogene expression after bile duct ligation in the rat: evidence of a specific growth effect on bile duct cells. Hepatology 1995 Apr;21(4):1070-8.
89. Sugihara T, Koda M, Matono T, Maeda K, Yamamoto S, Ueki M, Murawaki Y. Extracellular matrix metabolism-related gene expression in bile duct-ligated rats. Mol.Med.Report. 2009 May;2(3):345-51.
90. Veidal SS, Vassiliadis E, Bay-Jensen AC, Tougas G, Vainer B, Karsdal MA. Procollagen type I N-terminal propeptide (PINP) is a marker for fibrogenesis in bile duct ligation-induced fibrosis in rats. Fibrogenesis.Tissue Repair 2010;3(1):5.
91. Nagy P, Kiss A, Schnur J, Thorgeirsson SS. Dexamethasone inhibits the proliferation of hepatocytes and oval cells but not bile duct cells in rat liver. Hepatology 1998 Aug;28(2):423-9.
92. Ohlson LC, Koroxenidou L, Hallstrom IP. Inhibition of in vivo rat liver regeneration by 2-acetylaminofluorene affects the regulation of cell cycle-related proteins. Hepatology 1998 Mar;27(3):691-6.
93. Nagy P, Bisgaard HC, Thorgeirsson SS. Expression of hepatic transcription factors during liver development and oval cell differentiation. J.Cell Biol. 1994 Jul 1;126(1):223-33.
94. Grozdanov PN, Yovchev MI, Dabeva MD. The oncofetal protein glypican-3 is a novel marker of hepatic progenitor/oval cells. Lab Invest 2006 Dec;86(12):1272-84.
95. Sell S. Liver stem cells. Mod.Pathol. 1994 Jan;7(1):105-12.
96. Yovchev MI, Grozdanov PN, Zhou H, Racherla H, Guha C, Dabeva MD. Identification of adult hepatic progenitor cells capable of repopulating injured rat liver. Hepatology 2008 Feb;47(2):636-47.
97. Tanimizu N, Tsujimura T, Takahide K, Kodama T, Nakamura K, Miyajima A. Expression of Dlk/Pref-1 defines a subpopulation in the oval cell compartment of rat liver. Gene Expr.Patterns. 2004 Dec;5(2):209-18.
98. Jelnes P, Santoni-Rugiu E, Rasmussen M, Friis SL, Nielsen JH, Tygstrup N, Bisgaard HC. Remarkable heterogeneity displayed by oval cells in rat and mouse models of stem cell-mediated liver regeneration. Hepatology 2007 Jun;45(6):1462-70.
99. Bisgaard HC, Nagy P, Ton PT, Hu Z, Thorgeirsson SS. Modulation of keratin 14 and alpha-fetoprotein expression during hepatic oval cell proliferation and liver regeneration. J.Cell Physiol 1994 Jun;159(3):475-84.
100. Tanimizu N, Nishikawa M, Saito H, Tsujimura T, Miyajima A. Isolation of hepatoblasts based on the expression of Dlk/Pref-1. J.Cell Sci. 2003 May 1;116(Pt 9):1775-86.
101. Floridon C, Jensen CH, Thorsen P, Nielsen O, Sunde L, Westergaard JG, Thomsen SG, Teisner B. Does fetal antigen 1 (FA1) identify cells with regenerative, endocrine and neuroendocrine potentials? A study of FA1 in embryonic, fetal, and placental tissue and in maternal circulation. Differentiation 2000 Aug;66(1):49-59.
102. Tee LB, Kirilak Y, Huang WH, Morgan RH, Yeoh GC. Differentiation of oval cells into duct-like cells in preneoplastic liver of rats placed on a choline-deficient diet supplemented with ethionine. Carcinogenesis 1994 Dec;15(12):2747-56.
103. Severn CB. A morphological study of the development of the human liver. I. Development of the hepatic diverticulum. Am.J.Anat. 1971 Jun;131(2):133-58.
104. Fabris L, Cadamuro M, Libbrecht L, Raynaud P, Spirli C, Fiorotto R, Okolicsanyi L, Lemaigre F, Strazzabosco M, Roskams T. Epithelial expression of angiogenic growth factors modulate arterial vasculogenesis in human liver development. Hepatology 2008 Feb;47(2):719-28.
105. Haruna Y, Saito K, Spaulding S, Nalesnik MA, Gerber MA. Identification of bipotential progenitor cells in human liver development. Hepatology 1996 Mar;23(3):476-81.
106. Terrace JD, Currie IS, Hay DC, Masson NM, Anderson RA, Forbes SJ, Parks RW, Ross JA. Progenitor cell characterization and location in the developing human liver. Stem Cells Dev. 2007 Oct;16(5):771-8.
107. Lemaigre FP. Mechanisms of liver development: concepts for understanding liver disorders and design of novel therapies. Gastroenterology 2009 Jul;137(1):62-79.
108. Severn CB. A morphological study of the development of the human liver. II. Establishment of liver parenchyma, extrahepatic ducts and associated venous channels. Am.J.Anat. 1972 Jan;133(1):85-107.
109. Lemaigre F, Zaret KS. Liver development update: new embryo models, cell lineage control, and morphogenesis. Curr.Opin.Genet.Dev. 2004 Oct;14(5):582-90.
110. Van EP, Sciot R, Callea F, van der Steen K, Moerman P, Desmet VJ. The development of the intrahepatic bile ducts in man: a keratin-immunohistochemical study. Hepatology 1988 Nov;8(6):1586-95.

111. Shah KD, Gerber MA. Development of intrahepatic bile ducts in humans. Immunohistochemical study using monoclonal cytokeratin antibodies. Arch.Pathol.Lab Med. 1989 Oct;113(10):1135-8.
112. Terada T, Nakanuma Y. Expression of tenascin, type IV collagen and laminin during human intrahepatic bile duct development and in intrahepatic cholangiocarcinoma. Histopathology 1994 Aug;25(2):143-50.
113. Shah KD, Gerber MA. Development of intrahepatic bile ducts in humans. Possible role of laminin. Arch.Pathol.Lab Med. 1990 Jun;114(6):597-600.
114. Lemaigre FP. Development of the biliary tract. Mech.Dev. 2003 Jan;120(1):81-7.
115. Libbrecht L, Cassiman D, Desmet V, Roskams T. The correlation between portal myofibroblasts and development of intrahepatic bile ducts and arterial branches in human liver. Liver 2002 Jun;22(3):252-8.
116. Schmelzer E, Zhang L, Bruce A, Wauthier E, Ludlow J, Yao HL, Moss N, Melhem A, McClelland R, Turner W, et al. Human hepatic stem cells from fetal and postnatal donors. J.Exp.Med. 2007 Aug 6;204(8):1973-87.
117. Zhang L, Theise N, Chua M, Reid LM. The stem cell niche of human livers: symmetry between development and regeneration. Hepatology 2008 Nov;48(5):1598-607.
118. Schmelzer E, Wauthier E, Reid LM. The phenotypes of pluripotent human hepatic progenitors. Stem Cells 2006 Aug;24(8):1852-8.
119. Taketa K. Alpha-fetoprotein: reevaluation in hepatology. Hepatology 1990 Dec;12(6):1420-32.
120. Schmidt LE, Dalhoff K. Alpha-fetoprotein is a predictor of outcome in acetaminophen-induced liver injury. Hepatology 2005 Jan;41(1):26-31.
121. Geiger B, Spatz JP, Bershadsky AD. Environmental sensing through focal adhesions. Nat.Rev.Mol.Cell Biol. 2009 Jan;10(1):21-33.
122. Hynes RO. The extracellular matrix: not just pretty fibrils. Science 2009 Nov 27;326(5957):1216-9.
123. Badylak SF, Freytes DO, Gilbert TW. Extracellular matrix as a biological scaffold material: Structure and function. Acta Biomater. 2009 Jan;5(1):1-13.
124. Guilak F, Cohen DM, Estes BT, Gimble JM, Liedtke W, Chen CS. Control of stem cell fate by physical interactions with the extracellular matrix. Cell Stem Cell 2009 Jul 2;5(1):17-26.
125. Brown BN, Barnes CA, Kasick RT, Michel R, Gilbert TW, Beer-Stolz D, Castner DG, Ratner BD, Badylak SF. Surface characterization of extracellular matrix scaffolds. Biomaterials 2010 Jan;31(3):428-37.
126. Mould AP, Askari JA, Barton S, Kline AD, McEwan PA, Craig SE, Humphries MJ. Integrin activation involves a conformational change in the alpha 1 helix of the beta subunit A-domain. J.Biol.Chem. 2002 May 31;277(22):19800-5.
127. Rocco M, Rosano C, Weisel JW, Horita DA, Hantgan RR. Integrin conformational regulation: uncoupling extension/tail separation from changes in the head region by a multiresolution approach. Structure. 2008 Jun;16(6):954-64.
128. Ahmed N, Riley C, Rice G, Quinn M. Role of integrin receptors for fibronectin, collagen and laminin in the regulation of ovarian carcinoma functions in response to a matrix microenvironment. Clin.Exp.Metastasis 2005;22(5):391-402.
129. Yi XY, Wayner EA, Kim Y, Fish AJ. Adhesion of cultured human kidney mesangial cells to native entactin: role of integrin receptors. Cell Adhes.Commun. 1998 Mar;5(3):237-48.
130. Ruoslahti E, Pierschbacher MD. New perspectives in cell adhesion: RGD and integrins. Science 1987 Oct 23;238(4826):491-7.
131. Giancotti FG, Ruoslahti E. Integrin signaling. Science 1999 Aug 13;285(5430):1028-32.
132. Sun Y, Li W, Lu Z, Chen R, Ling J, Ran Q, Jilka RL, Chen XD. Rescuing replication and osteogenesis of aged mesenchymal stem cells by exposure to a young extracellular matrix. FASEB J. 2011 May;25(5):1474-85.
133. Ryu BY, Orwig KE, Oatley JM, Avarbock MR, Brinster RL. Effects of aging and niche microenvironment on spermatogonial stem cell self-renewal. Stem Cells 2006 Jun;24(6):1505-11.
134. Discher DE, Mooney DJ, Zandstra PW. Growth factors, matrices, and forces combine and control stem cells. Science 2009 Jun 26;324(5935):1673-7.
135. Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. Cell 2006 Aug 25;126(4):677-89.
136. Yasoshima M, Tsuneyama K, Harada K, Sasaki M, Gershwin ME, Nakanuma Y. Immunohistochemical analysis of cell-matrix adhesion molecules and their ligands in the portal tracts of primary biliary cirrhosis. J.Pathol. 2000 Jan;190(1):93-9.
137. Paku S, Dezso K, Kopper L, Nagy P. Immunohistochemical analysis of cytokeratin 7 expression in resting and proliferating biliary structures of rat liver. Hepatology 2005 Oct;42(4):863-70.
138. Paku S, Nagy P, Kopper L, Thorgeirsson SS. 2-acetylaminofluorene dose-dependent differentiation of rat oval cells into hepatocytes: confocal and electron microscopic studies. Hepatology 2004 May;39(5):1353-61.
139. Kwiecinski M, Noetel A, Elfimova N, Trebicka J, Schievenbusch S, Strack I, Molnar L, von BM, Tox U, Nischt R, et al. Hepatocyte Growth Factor (HGF) Inhibits Collagen I and IV Synthesis in Hepatic Stellate Cells by miRNA-29 Induction. PLoS.One. 2011;6(9):e24568.
140. Knittel T, Janneck T, Muller L, Fellmer P, Ramadori G. Transforming growth factor beta 1-regulated gene expression of Ito cells. Hepatology 1996 Aug;24(2):352-60.
141. Kikkawa Y, Mochizuki Y, Miner JH, Mitaka T. Transient expression of laminin alpha1 chain in regenerating murine liver: restricted localization of laminin chains and nidogen-1. Exp.Cell Res. 2005 Apr 15;305(1):99-109.

142. Knittel T, Kobold D, Piscaglia F, Saile B, Neubauer K, Mehde M, Timpl R, Ramadori G. Localization of liver myofibroblasts and hepatic stellate cells in normal and diseased rat livers: distinct roles of (myo-)fibroblast subpopulations in hepatic tissue repair. Histochem.Cell Biol. 1999 Nov;112(5):387-401.
143. Strick-Marchand H, Masse GX, Weiss MC, Di Santo JP. Lymphocytes support oval cell-dependent liver regeneration. J.Immunol. 2008 Aug 15;181(4):2764-71.
144. Saxena R, Theise ND, Crawford JM. Microanatomy of the human liver-exploring the hidden interfaces. Hepatology 1999 Dec;30(6):1339-46.
145. Saxena R, Theise N. Canals of Hering: recent insights and current knowledge. Semin.Liver Dis. 2004 Feb;24(1):43-8.
146. Gijtenbeek JM, Wesseling P, Maass C, Burgers L, van der Laak JA. Three-dimensional reconstruction of tumor microvasculature: simultaneous visualization of multiple components in paraffin-embedded tissue. Angiogenesis. 2005;8(4):297-305.
147. Bussolati G, Marchio C, Volante M. Tissue arrays as fiducial markers for section alignment in 3-D reconstruction technology. J Cell Mol Med. 2005 Apr;9(2):438-45.
148. Clendenon JL, Byars JM, Hyink DP. Image processing software for 3D light microscopy. Nephron Exp.Nephrol. 2006;103(2):e50-e54.
149. Handschuh S, Schwaha T, Metscher BD. Showing their true colors: a practical approach to volume rendering from serial sections. BMC.Dev.Biol. 2010;10:41.
150. Wanninger A. The application of confocal microscopy and 3D imaging software in Functional, Evolutionary, and Developmental Zoology: reconstructing myo- and neurogenesis in space and time. Modern Research and Educational Topics in Microscopy 2007;1:353-61.
151. Sands GB, Gerneke DA, Hooks DA, Green CR, Smaill BH, Legrice IJ. Automated imaging of extended tissue volumes using confocal microscopy. Microsc Res Tech 2005 Aug 1;67(5):227-39.
152. Kerwin J, Scott M, Sharpe J, Puelles L, Robson SC, Martinez-de-la-Torre M, Ferran JL, Feng G, Baldock R, Strachan T, et al. 3 dimensional modelling of early human brain development using optical projection tomography. BMC Neurosci 2004 Aug 6;5:27.
153. Sharpe J, Ahlgren U, Perry P, Hill B, Ross A, Hecksher-Sorensen J, Baldock R, Davidson D. Optical projection tomography as a tool for 3D microscopy and gene expression studies. Science 2002 Apr 19;296(5567):541-5.
154. Sharpe J. Optical projection tomography. Annu Rev Biomed Eng 2004;6:209-28.
155. Gerstenfeld LC, Alkhiary YM, Krall EA, Nicholls FH, Stapleton SN, Fitch JL, Bauer M, Kayal R, Graves DT, Jepsen KJ, et al. Three-dimensional reconstruction of fracture callus morphogenesis. J Histochem.Cytochem. 2006 Nov;54(11):1215-28.
156. Fu YY, Lin CW, Enikolopov G, Sibley E, Chiang AS, Tang SC. Microtome-free 3-dimensional confocal imaging method for visualization of mouse intestine with subcellular-level resolution. Gastroenterology 2009 Aug;137(2):453-65.
157. Haug JT, Haug C, Maas A, Fayers SR, Trewin NH, Waloszek D. Simple 3D images from fossil and recent micromaterial using light microscopy. J Microsc 2009 Jan;233(1):93-101.
158. Soufan AT, Ruijter JM, van den Hoff MJ, de Boer PA, Hagoort J, Moorman AF. Three-dimensional reconstruction of gene expression patterns during cardiac development. Physiol Genomics 2003 May 13;13(3):187-95.
159. Wilson D, Roy D, Steyer G, Gargesha M, Stone M, McKinley E. Whole Mouse Cryo-Imaging. Proc.Soc.Photo.Opt.Instrum.Eng 2008 Jan 1;6916:69161I-69161I9.
160. Roy D, Steyer GJ, Gargesha M, Stone ME, Wilson DL. 3D cryo-imaging: a very high-resolution view of the whole mouse. Anat Rec (Hoboken) 2009 Mar;292(3):342-51.
161. Chen G, Li XC, Wu GQ, Zhang SX, Xiong XF, Tan LW, Yang RG, Li K, Yang SZ, Dong JH. Three-dimensional reconstruction of digitized human liver: based on Chinese Visible Human. Chin Med.J (Engl.) 2010 Jan 20;123(2):146-50.
162. Strasser H. Ueber das Studium der Schnittserien und uber die Hulfsmittel, welche die Reconstruction der zerlegten Form erleichtern. Z Wiss Mikrosk 1886;3:179-95.
163. Strasser H. Ueber die Methoden der plastischen Rekonstruktion. Z Wiss Mikrosk 1887;4:168-208.
164. Lebedkin S. Zur Technik der graphischen Rekonstruktionen: "Projektionsrekonstruktionen" und "Stereoskopische Rekonstruktionen". Z Wiss Mikrosk 1926;43:1-86.
165. Born G. Die Plattenmodelliermethode. Archiv f mikroskop Anatomie 1883;22:584-99.
166. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. Science 1998 Nov 6;282(5391):1145-7.
167. Laursen SB, Mollgard K, Olesen C, Oliveri RS, Brochner CB, Byskov AG, Andersen AN, Hoyer PE, Tommerup N, Yding AC. Regional differences in expression of specific markers for human embryonic stem cells. Reprod.Biomed Online. 2007 Jul;15(1):89-98.
168. Stewart MH, Bosse M, Chadwick K, Menendez P, Bendall SC, Bhatia M. Clonal isolation of hESCs reveals heterogeneity within the pluripotent stem cell compartment. Nat Methods 2006 Oct;3(10):807-15.
169. Boiani M, Scholer HR. Regulatory networks in embryo-derived pluripotent stem cells. Nat Rev Mol Cell Biol 2005 Nov;6(11):872-84.
170. Heins N, Englund MC, Sjoblom C, Dahl U, Tonning A, Bergh C, Lindahl A, Hanson C, Semb H. Derivation, characterization, and differentiation of human embryonic stem cells. Stem Cells 2004;22(3):367-76.
171. Trounson A. The production and directed differentiation of human embryonic stem cells. Endocr.Rev 2006 Apr;27(2):208-19.

172. Brunner HG, Hamel BC, Bokhoven HH. P63 gene mutations and human developmental syndromes. Am.J Med.Genet 2002 Oct 15;112(3):284-90.
173. Mandal A, Tipnis S, Pal R, Ravindran G, Bose B, Patki A, Rao MS, Khanna A. Characterization and in vitro differentiation potential of a new human embryonic stem cell line, ReliCellhES1. Differentiation 2006 Mar;74(2-3):81-90.
174. Mikkola M, Olsson C, Palgi J, Ustinov J, Palomaki T, Horelli-Kuitunen N, Knuutila S, Lundin K, Otonkoski T, Tuuri T. Distinct differentiation characteristics of individual human embryonic stem cell lines. BMC Dev Biol 2006;6:40.
175. Yang A, McKeon F. P63 and P73: P53 mimics, menaces and more. Nat Rev Mol Cell Biol 2000 Dec;1(3):199-207.
176. Ourselin S, Roche A, Subsol G, Pennec X, Ayache N. Reconstructing a 3D structure from serial histological sections. Image and Vision Computing 19[1], 25-31. 2001.
1. Ref Type: Abstract
177. Hansen JM, Qvortrup K, Friis M. Vestibular tributaries to the vein of the vestibular aqueduct. Acta Otolaryngol. 2011 Jan;131(1):9-13.
178. Cassoni P, Gaetano L, Senetta R, Bussolati B, Molinaro L, Bussolati G. Histology far away from Flatland: 3D roller-coasting into grade-dependent angiogenetic patterns in oligodendrogliomas. J Cell Mol Med. 2008 Apr;12(2):564-8.
179. Vuillemin M, Pexieder T, Wong YM, Thompson RP. A two-step alignment method for 3D computer-aided reconstruction based on fiducial markers and applied to mouse embryonic hearts. Eur.J Morphol 1992;30(3):181-93.
180. Ohyama M, Terunuma A, Tock CL, Radonovich MF, Pise-Masison CA, Hopping SB, Brady JN, Udey MC, Vogel JC. Characterization and isolation of stem cell-enriched human hair follicle bulge cells. J.Clin.Invest 2006 Jan;116(1):249-60.
181. Ruthensteiner B, Baeumler N, Barnes DG. Interactive 3D volume rendering in biomedical publications. Micron. 2010 Oct;41(7):886.
182. Litvinov SV, Velders MP, Bakker HA, Fleuren GJ, Warnaar SO. Ep-CAM: a human epithelial antigen is a homophilic cell-cell adhesion molecule. J.Cell Biol. 1994 Apr;125(2):437-46.
183. de Boer CJ, Van Krieken JH, Janssen-van Rhijn CM, Litvinov SV. Expression of Ep-CAM in normal, regenerating, metaplastic, and neoplastic liver. J.Pathol. 1999 Jun;188(2):201-6.
184. Antoniou A, Raynaud P, Cordi S, Zong Y, Tronche F, Stanger BZ, Jacquemin P, Pierreux CE, Clotman F, Lemaigre FP. Intrahepatic bile ducts develop according to a new mode of tubulogenesis regulated by the transcription factor SOX9. Gastroenterology 2009 Jun;136(7):2325-33.
185. Stasiak PC, Purkis PE, Leigh IM, Lane EB. Keratin 19: predicted amino acid sequence and broad tissue distribution suggest it evolved from keratinocyte keratins. J.Invest Dermatol. 1989 May;92(5):707-16.
186. Shiojiri N, Lemire JM, Fausto N. Cell lineages and oval cell progenitors in rat liver development. Cancer Res. 1991 May 15;51(10):2611-20.
187. Bell DM, Leung KK, Wheatley SC, Ng LJ, Zhou S, Ling KW, Sham MH, Koopman P, Tam PP, Cheah KS. SOX9 directly regulates the type-II collagen gene. Nat.Genet. 1997 Jun;16(2):174-8.
188. Wright E, Hargrave MR, Christiansen J, Cooper L, Kun J, Evans T, Gangadharan U, Greenfield A, Koopman P. The Sry-related gene Sox9 is expressed during chondrogenesis in mouse embryos. Nat.Genet. 1995 Jan;9(1):15-20.
189. Bi W, Huang W, Whitworth DJ, Deng JM, Zhang Z, Behringer RR, de CB. Haploinsufficiency of Sox9 results in defective cartilage primordia and premature skeletal mineralization. Proc.Natl.Acad.Sci.U.S.A 2001 Jun 5;98(12):6698-703.
190. Bi W, Deng JM, Zhang Z, Behringer RR, de CB. Sox9 is required for cartilage formation. Nat.Genet. 1999 May;22(1):85-9.
191. van der Flier LG, van Gijn ME, Hatzis P, Kujala P, Haegebarth A, Stange DE, Begthel H, van den Born M, Guryev V, Oving I, et al. Transcription factor achaete scute-like 2 controls intestinal stem cell fate. Cell 2009 Mar 6;136(5):903-12.
192. Nowak JA, Polak L, Pasolli HA, Fuchs E. Hair follicle stem cells are specified and function in early skin morphogenesis. Cell Stem Cell 2008 Jul 3;3(1):33-43.
193. Vidal VP, Chaboissier MC, Lutzkendorf S, Cotsarelis G, Mill P, Hui CC, Ortonne N, Ortonne JP, Schedl A. Sox9 is essential for outer root sheath differentiation and the formation of the hair stem cell compartment. Curr.Biol. 2005 Aug 9;15(15):1340-51.
194. Lubarsky B, Krasnow MA. Tube morphogenesis: making and shaping biological tubes. Cell 2003 Jan 10;112(1):19-28.
195. Hogan BL, Kolodziej PA. Organogenesis: molecular mechanisms of tubulogenesis. Nat Rev Genet 2002 Jul;3(7):513-23.
196. Chung S, Andrew DJ. The formation of epithelial tubes. J Cell Sci 2008 Nov 1;121(Pt 21):3501-4.
197. Martin-Belmonte F, Mostov K. Regulation of cell polarity during epithelial morphogenesis. Curr.Opin.Cell Biol 2008 Apr;20(2):227-34.
198. Raynaud P, Tate J, Callens C, Cordi S, Vandersmissen P, Carpentier R, Sempoux C, Devuyst O, Pierreux CE, Courtoy P, et al. A classification of ductal plate malformations based on distinct pathogenic mechanisms of biliary dysmorphogenesis. Hepatology 2011 Jun;53(6):1959-66.
199. Satomi S, Yamasaki Y, Tsuzuki S, Hitomi Y, Iwanaga T, Fushiki T. A role for membrane-type serine protease (MT-SP1) in intestinal epithelial turnover. Biochem.Biophys.Res.Commun. 2001 Oct 5;287(4):995-1002.
200. List K, Bugge TH, Szabo R. Matriptase: potent proteolysis on the cell surface. Mol.Med. 2006 Jan;12(1-3):1-7.
201. Uhland K. Matriptase and its putative role in cancer. Cell Mol.Life Sci. 2006 Dec;63(24):2968-78.
202. Tripathi M, Potdar AA, Yamashita H, Weidow B, Cummings PT, Kirchhofer D, Quaranta V. Laminin-332 cleavage by matriptase alters motility parameters of prostate cancer cells. Prostate 2010 Jul 29.

203. Shi YE, Torri J, Yieh L, Wellstein A, Lippman ME, Dickson RB. Identification and characterization of a novel matrix-degrading protease from hormone-dependent human breast cancer cells. Cancer Res. 1993 Mar 15;53(6):1409-15.
204. Benaud CM, Oberst M, Dickson RB, Lin CY. Deregulated activation of matriptase in breast cancer cells. Clin.Exp.Metastasis 2002;19(7):639-49.
205. List K, Szabo R, Molinolo A, Sriuranpong V, Redeye V, Murdock T, Burke B, Nielsen BS, Gutkind JS, Bugge TH. Deregulated matriptase causes ras-independent multistage carcinogenesis and promotes ras-mediated malignant transformation. Genes Dev 2005 Aug 15;19(16):1934-50.
206. Szabo R, Kosa P, List K, Bugge TH. Loss of matriptase suppression underlies spint1 mutation-associated ichthyosis and postnatal lethality. Am.J.Pathol. 2009 Jun;174(6):2015-22.
207. Oberst MD, Williams CA, Dickson RB, Johnson MD, Lin CY. The activation of matriptase requires its noncatalytic domains, serine protease domain, and its cognate inhibitor. J.Biol.Chem. 2003 Jul 18;278(29):26773-9.
208. Oberst MD, Chen LY, Kiyomiya K, Williams CA, Lee MS, Johnson MD, Dickson RB, Lin CY. HAI-1 regulates activation and expression of matriptase, a membrane-bound serine protease. Am.J.Physiol Cell Physiol 2005 Aug;289(2):C462-C470.
209. Chen YW, Wang JK, Chou FP, Chen CY, Rorke EA, Chen LM, Chai KX, Eckert RL, Johnson MD, Lin CY. Regulation of the matriptase-prostasin cell surface proteolytic cascade by hepatocyte growth factor activator inhibitor-1 during epidermal differentiation. J.Biol.Chem. 2010 Oct 8;285(41):31755-62.
210. Bisgaard HC, Parmelee DC, Dunsford HA, Sechi S, Thorgeirsson SS. Keratin 14 protein in cultured nonparenchymal rat hepatic epithelial cells: characterization of keratin 14 and keratin 19 as antigens for the commonly used mouse monoclonal antibody OV-6. Mol.Carcinog. 1993;7(1):60-6.
211. Ogawa K, Suzuki J, Mukai H, Mori M. Sequential changes of extracellular matrix and proliferation of Ito cells with enhanced expression of desmin and actin in focal hepatic injury. Am.J.Pathol. 1986 Dec;125(3):611-9.
212. Ho MS, Bose K, Mokkapati S, Nischt R, Smyth N. Nidogens-Extracellular matrix linker molecules. Microsc.Res.Tech. 2008 May;71(5):387-95.
213. Tatrai P, Dudas J, Batmunkh E, Mathe M, Zalatnai A, Schaff Z, Ramadori G, Kovalszky I. Agrin, a novel basement membrane component in human and rat liver, accumulates in cirrhosis and hepatocellular carcinoma. Lab Invest 2006 Nov;86(11):1149-60.
214. Bezakova G, Ruegg MA. New insights into the roles of agrin. Nat.Rev.Mol.Cell Biol. 2003 Apr;4(4):295-308.
215. Bornstein P, Sage H. Regulation of collagen gene expression. Prog.Nucleic Acid Res.Mol.Biol. 1989;37:67-106.
216. Clayton E, Forbes SJ. The isolation and in vitro expansion of hepatic Sca-1 progenitor cells. Biochem.Biophys.Res.Commun. 2009 Apr 17;381(4):549-53.
217. McClelland R, Wauthier E, Uronis J, Reid L. Gradients in the liver's extracellular matrix chemistry from periportal to pericentral zones: influence on human hepatic progenitors. Tissue Eng Part A 2008 Jan;14(1):59-70.
218. Duffield JS, Forbes SJ, Constandinou CM, Clay S, Partolina M, Vuthoori S, Wu S, Lang R, Iredale JP. Selective depletion of macrophages reveals distinct, opposing roles during liver injury and repair. J.Clin.Invest 2005 Jan;115(1):56-65.
219. Dezso K, Jelnes P, Laszlo V, Baghy K, Bodor C, Paku S, Tygstrup N, Bisgaard HC, Nagy P. Thy-1 is expressed in hepatic myofibroblasts and not oval cells in stem cell-mediated liver regeneration. Am.J.Pathol. 2007 Nov;171(5):1529-37.
220. Ramadori G, Saile B. Mesenchymal cells in the liver--one cell type or two? Liver 2002 Aug;22(4):283-94.
221. Laborda J, Sausville EA, Hoffman T, Notario V. dlk, a putative mammalian homeotic gene differentially expressed in small cell lung carcinoma and neuroendocrine tumor cell line. J.Biol.Chem. 1993 Feb 25;268(6):3817-20.
222. Smas CM, Sul HS. Pref-1, a protein containing EGF-like repeats, inhibits adipocyte differentiation. Cell 1993 May 21;73(4):725-34.
223. Jensen CH, Krogh TN, Hojrup P, Clausen PP, Skjodt K, Larsson LI, Enghild JJ, Teisner B. Protein structure of fetal antigen 1 (FA1). A novel circulating human epidermal-growth-factor-like protein expressed in neuroendocrine tumors and its relation to the gene products of dlk and pG2. Eur.J.Biochem. 1994 Oct 1;225(1):83-92.
224. Moon YS, Smas CM, Lee K, Villena JA, Kim KH, Yun EJ, Sul HS. Mice lacking paternally expressed Pref-1/Dlk1 display growth retardation and accelerated adiposity. Mol.Cell Biol. 2002 Aug;22(15):5585-92.