Published in final edited form as:
Exp Mol Pathol. 2010 June ; 88(3): 331–340. doi:10.1016/j.yexmp.2010.01.003.

The identification of stem cells in human liver diseases and hepatocellular carcinoma

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Abstract

Liver stem cells are thought to preside in bile ducts and the canals of Hering. They extend into the liver parenchyma at a time when normal liver cell proliferation is suppressed and liver regeneration is stimulated. In the present study 69 liver biopsies and surgically excised liver tumors were studied for the presence of liver stem cells. It was found that human cirrhotic livers and hepatocellular carcinomas (HCC) frequently exhibited isolated single scattered hepatocyte stem cells within the liver parenchyma rather than in the portal tract, bile duct or the canal of Hering. These cells expressed liver stem cell markers. HCCs also contained isolated tumor cell which expressed the same stem cell markers. The markers used were GST-P, OV-6, CK-19, Oct-3/4 and FAT10. They were identified by immunofluorescent antibody staining. HGF, EGF, CK19, AIR, H19, Nanog, Oct-3/4 and FAT10 were identified by RNA-FISH. H19 is a non-coding RNA, which is expressed in most HCCs. Results: Immunohistochemistry and RNA-FISH performed on human livers identified isolated stem cells in liver parenchyma as follows: Stem cells identified by immunohistochemical markers (OV-6 and GST-P) and RNA-FISH markers (HGF, EGF, CK19 and H19) were found scattered in the liver parenchyma of cirrhotic livers and within hepatocellular carcinomas (HCCs). Precirrhotic ASH or NASH all stained negative for these stem cells. In HCCs, 13 out of 15 had stem cells located within the tumor (78%). In cirrhotic livers, 12 out of 28 (37%) had liver parenchymal stem cells present. In one case of stage 3 precirrhosis, stem cells were also found. Double staining for the markers showed colocalization of the markers in stem cells. Stem cells were found in 33% of HBV, 47% of HCV, 25% of alcoholic steatohepatitis (ASH) and 17% of non-alcoholic steatohepatitis (NASH). The frequency of stem cells found in the different disease categories correlates with the frequency of HCC occurring in these different diseases.

Keywords

Epidermal growth factor (EGF); Hepatocyte growth factor (HGF); Glutathione S-transferase placental (GST-P); O. volvulus 6 (OV-6); AIR (antisense Igf2r)

Introduction

The liver plays a central role in metabolic homeostasis, as it is responsible for the metabolism, synthesis, storage and redistribution of nutrients, carbohydrates, fats and vitamins (Saxema et al., 2003). Importantly, it is the main detoxifying organ of the body, which removes wastes and xenobiotics by metabolic conversion and biliary excretion. The main cell type of the liver that carries out most of these functions is the parenchymal cell, or

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hepatocyte, which makes up ~80% of cells, in the liver. Although adult hepatocytes are long lived and normally have a low rate of cell division, they maintain the ability to proliferate in response to toxic injury and infection (Cantz et al., 2008). The amazing regenerative capacity of the liver is most clearly shown by the two-thirds partial-hepatectomy model in rodents, which was pioneered by Higgins and Anderson in 1931 (Higgins and Anderson, 1931). Cell division is rarely seen in hepatocytes in the normal adult liver, as these cells are in the G0 phase of the cell cycle (Michalopoulos and DeFrances, 1997; Taub et al., 1999). The degree of replication of these cells correlates with the degree of inflammation and fibrosis in diseases such as chronic hepatitis, hemochromatosis, alcoholic and non-alcoholic steatohepatitis (Libbrecht et al., 2000; Lowes et al., 1999). However, after partial hepatectomy approximately 95% of hepatic cells, which are normally quiescent, rapidly re-enter the cell cycle. The onset of DNA synthesis is well synchronized in hepatocytes, beginning in cells that surround the portal vein of the liver lobule and proceeding towards the central vein (Minuk, 2003). Many growth factors are involved in the regeneration of the liver: hepatocyte growth factor (HGF) (Nishino et al., 2008), epidermal growth factor (EGF) (Natarajan et al., 2007), transforming growth factors (TGFs) (Weymann et al., 2009), insulin (Stefano et al., 2006), glucagon (Kothary et al., 1995) and insulin like growth factor (Sanz et al., 2005).

In animal models, in which hepatocytes are directly damaged and thereby induced to undergo necrosis. His resembles simulates growth-factor- and cytokine-mediated pathway up regulation seen after partial hepatectomy (Dabeva et al., 1993; Dabeva and Shafritz, 1993). Proliferation of hepatocytes is also involved in the liver regeneration that occurs after massive hepatocytic necrosis or apoptosis that is induced by hepatic toxins such as CCl_4 (Fausto, 1999).

Human hepatic stem cells rather than quiescent hepatocytes are responsible for regeneration when cirrhosis develops, in order to compensate for the regenerative response to liver injury due to the up regulation of p21 in the cirrhotic liver (Kato et al., 2005). P21 inhibits cell cycling at the G2 stage of mitosis in the cirrhotic liver (Ridley et al., 1988). Identification of liver stem cells promises new therapeutic treatments for a wide range of liver pathological conditions (e.g. cirrhosis, hepatocarcinogenesis). It has been speculated that human stem cells could be the precursors of HCC as well as cholangiocarcinomas, which also drive HCC cellular growth (Theise et al., 1999). Indeed, several studies showed that HCC expressed markers of stem cells such as OV6, A6, OV1, AFP, CK7 and CK19 (Bottinger et al., 1997; Chiba et al., 2006; Herrera et al., 2006; Kim et al., 2004; Roskams et al., 1998; Roskams et al., 2004; Xiao et al., 2003). Other markers that can be used to identify stem cells are: CD34+, Thy-1+, c-Kit+ and Flt3+ (Burke et al., 2007). HCCs expressing these markers are likely to have significantly more negative prognosis and a higher recurrence after surgical resection and liver transplantation because of the resistance of stem cells to chemotherapy (Sell, 2008).

In this study, we used stem cell marker (OV-6) to identify these cells in the biopsies of different patients (Mishra et al., 2009). GST-P was also used to identify these cells, but GST-P is a marker that identifies oval cells also (Hepatic progenitor cells) in the damaged mammalian liver (Alison et al., 2009). Oval cells are defined as small cells with an oval nucleus and scanty cytoplasm and are considered to be progenitor cells with the ability to differentiate into hepatocytes and cholangiocytes (Farber, 1956; Rountree et al., 2007). They are said to proliferate when the cell cycle of normal hepatocytes is suppressed by up regulation of p21. They are located in the portal tract (Roskams, 2006). In addition, we used two new stem cell markers (EGF and HGF). There was a positive correlation between the causes of the chronic liver disease studied and the frequency of the stem cells found in the cases.

Materials and methods

Immunofluorescent staining

Liver sections were stained with rabbit anti-ubiquitin polyclonal antibody (DAKO, Carpinteria, CA). Antibody binding was detected with Texas-Red labeled and FITC-labeled secondary antibodies (Jackson, West Grove, PA). DAPI was used for nuclear staining. The slides were examined using a Nikon-400 fluorescent microscope with a FITC, Rhodamine and a triple color band cube to detect simultaneously FITC, Texas Red and DAPI staining. Confocal microscopy was performed using a Laica fluorescent microscope.

Amplification and cloning of human and mouse probes

The probes were amplified by using Phusion™ Hot Start High-Fidelity DNA Polymerase (Finnzymes Inc., Woburn, MA). The conditions for PCR are: 98 °C 30 s, 98 °C 30 s, 60 °C 30 s, 72 °C 30 s (40 cycles), 72 °C 5mn. The PCR product is separated in a 1% Agarose gel using the NucleoSpin Extract II (Macherey-Nagel, Bethlehem, PA). The purified PCR products are cloned in the pGMET vectors, overnight at 16 °C, following the instructions of the company (Promega, Madison, WI). JM109 bacteria are transformed with the ligation product (Zymo Research, Orange, CA). Positive clones are selected with EcoRI digestion. All the clones are sequenced. Sequence of the primers used to amplify the probe:

Hybridization in situ of RNA (RNA-FISH)

The slides were placed in Xylene 10 mn, in 1:1 Xylene/EtOH 10 mn and finally in 100% EtOH 10 mn (Sigma-Aldrich, St. Louis, MO). They were washed in PBS and placed in digestion buffer (PBS+SDS 0.05%+Proteinase K 10 μg/ml) (Roche, Indianapolis, IN), at room temperature for 10mn. They were then fixed in cold fresh-made 4% paraformaldehyde, at 4 °C, 20 mn. They were washed in PBS and placed in 0.1 M PBS/ Tween20 0.1%, for 30 mn. They were then placed in the prehybridization buffer (1:1 Formamide/5× SSC) for 2 h at 65 °C. The probe was made using a Fluorescein High-Prime, and using Tetramethyl-rhodamine-5-dUTP, following the instructions of the company (Roche, Indianapolis, IN). The probe was incubated with the slides at 65 °C, 16 h. The slides were then washed in 2× SSC, for 30 mn at RT, 1 h at 65 °C in 2× SSC, 1 h at 65 °C with 0.2× SSC, 10 mn at PBS/Tween20 65 °C and 10 mn with PBS/Tween20 at room temperature.

Results

Stains for twelve stem cell markers (Stem Cells/Progenitors cells SCPs) were performed either by RNA-FISH or immunohistochemistry. The control livers stained negative for the stem cells/progenitor cells (SCPs) except in one case in which one parenchyma liver cell stained positive for OV-6 and GST-P. Tables 2–7 give the staining results. The results are broken down as to age, sex, HBV+, HCV+, HCC+, cirrhosis and Mallory–Denk Body formation. Each stain result was broken down to indicate whether the positive cells were present in cirrhosis, HCC or others (non cirrhotic and non HCC).

Table 1 showed that of 18 HCCs, there were 14 cases in which SCPs were identified by one or more stains (78%). The high frequency of stem cells in HCC influenced the frequency of stem cells found in the various liver diseases studied. That is at caused the frequency to be higher when the HCC was also present (Tables 2–7).

The high frequency influenced the frequency in stem cells form in the other liver diseases sampled where the frequency of HCC was high (Tables 2–7).

The frequency of positive stem cell markers in the various diseases roughly correlated with the relatively frequency of HCC that develops in the clinical setting *i.e.* HCV 47%, HBV 35%, cirrhosis 33%, ASH 25%, NASH 16.5% (Table 8). The positive stem cell markers found in cirrhosis and HCCs are illustrated in Fig. 1. All of the RNA-FISH and immunostaining that were positive in cirrhosis and HCCs were double stained which when viewed by confocal microscopy showed that the same cells stained positive for both markers and when merged appeared yellow indicating that the two proteins or mRNAs colocalized (Fig. 2). The individual stem cells were single in the liver parenchyma with few exceptions and had abundant cytoplasm and were polyhedral shaped fitting into liver cell cords and tumor areas period. They resemble the normal liver cells and tumor cells. These cells were not identified as different morphogically from their neighboring cells.

Discussion

The stem cells identified by immunostaining or RNA-FISH were commonly found in HCCs. The frequency, 78% is higher than previously reported by Thorgeirsson and Grisham (Thorgeirsson and Grisham, 2006). These stem cells, which were positive for a host of different stem cell markers, were not located in the portal tracts, bile ducts or canals of Hering. They have been characterized as oval cells or hepatic progenitors cells in animal and human chronic liver diseases and cirrhosis (Theise et al., 1999; Roskams et al., 1998; Roskams et al., 2004; Chiba et al., 2006; Mishra et al., 2009). Instead, they were located and scattered as single cells in the liver parenchyma and in tumors with no relationship to the portal tracts in this present study. They had the morphology of their neighboring hepatocytes in both cirrhosis and in HCCs. They stained positive for CD133 in both HCCs and cirrhosis as described by others (Alison et al., 2009). These results were presented in part in an abstract (Oliva et al., 2009).

Acknowledgments

The study was supported by NIH/NIAAA grant 8116 and the Alcohol Center Grant on Liver and Pancreas P50-011999, including the morphology core.

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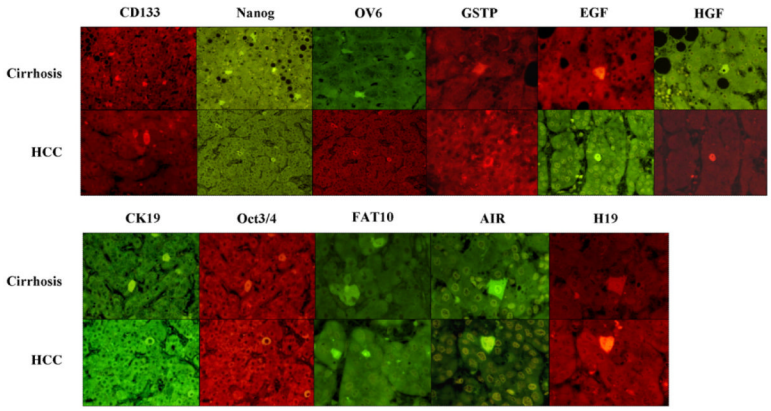

Fig. 1.
EGF, HGF, CD133, FAT10, AIR and H19 were done by RNA-FISH. Oct4, CK19, GST-P, OV6 and Nanog were done by immunohistochemistry.

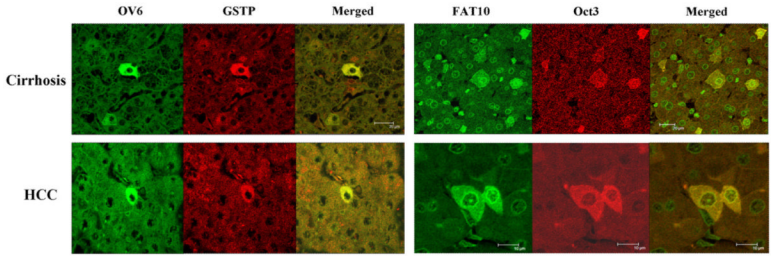

Fig. 2.
OV6 and GST-P were done by immunohistochemistry. FAT10 and Oct3 were done by RNA-FISH.

Human primers			
EGF	NM_001963	Forward	GTAGCCAGCTCTGCGTTCCT
	NM_001963	Reverse	CTTTCTCGCTGGGAACCATC
HGF	NM_001010934	Forward	AGGGATCACTGGAAGCTTGA
	NM_001010934	Reverse	TAGTCCCCCTCCCCAAATAC
CK19	NC_000017.	Forward	AAGGAGGGAGGCTTGGTAAA
	NC_000017	Reverse	GGTCTGTGGGTCTGGGTCTA
Oct3/4	NM_203289.3	Forward	GGTATTCAGCCAAACGACCA
	NM_203289.3	Reverse	CACACTCGGACCACATCCTT
Nanog	NM_024865.2	Forward	GTGATTTGTGGGCCTGAAGA
	NM_024865.2	Reverse	ACACAGCTGGGTGGAAGAGA
Sox2	NM_003106.2	Forward	GACAGTTACGCGCACATGAA
	NM_003106.2	Reverse	TAGGTCTGCGAGCTGGTCAT
FAT10	NM_006398	Forward	AATGCTTCCTGCCTCTGTGT
	NM_006398	Reverse	TTTCACTTGTGCCACTGAGC
H19	NR_002196	Forward	CCTCATCAGCCCAACATCAA
	NR_002196	Reverse	GGGGAAACAGAGTCGTGGAG
AIR	GQ166646	Forward	AAGTCAGGATCACCAGCCTTT
	GQ166646	Reverse	TACACTCACTAGACCCACCCG

Table 1

Antibodies	Company
Goat Anti CK19	Abcam, Cambridge, MA
Rabbit alpha GST-P	Lifespan Biosciences, Seattle, WA
Rabbit anti Oct3/4	Abgen, San Diego, CA
Mouse anti OV-6	R&D Systems, Minneapolis, MN

Table 2

Summary of the cases positive with hepatitis B virus (HBV).

Age	Sex	HBV	HCV	rrhos	HCC	NASH	ASH	MDB			OV6			GST-P			HGF			EGF			CK19			H19			AIR			Ubd			Nanog			Oct4			Oct3			CD133	
								C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T
44	M	Pos	Neg	Pos							Neg			Neg																															
63	M	Pos	Pos	Pos	Pos						Pos	Pos		Pos	Pos		Pos	Pos								Pos	Pos		Pos	Pos					Pos	Pos		Pos	Pos					
67	M	Pos	Pos	Pos	Pos				Pos		Pos	Pos								Neg	Neg														Neg	Neg		Neg	Neg					
40	M	Pos	Neg	Pos							Neg			Neg																													
34	M	Pos	Neg	Pos							Neg																																	
47	M	Pos	Neg	Pos	Pos									Neg	Pos		Neg																											
18	M	Pos	Neg										Neg			Neg			Neg			Neg			Neg			Neg			Neg			Neg			Neg			Neg				
67	M	Pos		Pos		Pos							Neg																						Neg									
45	M	Pos	Neg		Pos							Pos			Neg																					Neg								Neg	
63	M	Pos	Pos	Pos	Pos						Neg	Neg		Neg	Neg																												
59	M	Pos	Pos										Neg			Neg																												
40	M	Pos	Pos	Pos	Pos				Pos		Pos	Pos		Pos	Pos																				Pos	Pos					Pos				
45	F	Pos	Pos	Pos							Neg			Neg																														
51	F	Pos	Neg			Pos																																						
46	F	Pos	Neg	Pos		Pos					Neg			Neg																														
58	F	Pos	Neg	Pos							Pos			Pos																														
69	F	Pos	Pos	Pos							Neg			Neg																														

Table 3

Summary of the cases positive with hepatitis C virus (HCV).

Age	Sex	HBV	HCV	Cirrhosis	HCC	NASH	ASH	MDB			OV6			GST-P			HGF			EGF			CK19			H19			AIR			Ubd			Nanog			Oct4			Oct3			CD133		
								C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O
59	M	Neg	Pos	Pos	Pos		Pos		Pos		Pos	Pos		Pos	Pos		Pos	Pos		Pos	Pos		Neg	Neg		Neg	Neg		Neg	Neg					Pos	Neg		Pos	Pos							
51	M	Neg	Pos	Pos							Neg			Neg																														
63	M	Pos	Pos	Pos	Pos						Pos	Pos		Pos	Pos		Pos	Pos								Pos	Pos		Pos	Pos					Pos	Pos		Pos	Pos							
67	M	Pos	Pos	Pos	Pos				Pos		Pos	Pos			Pos					Neg	Neg														Neg	Neg		Neg	Neg							
67	M	Pos	Pos		Pos							Neg																								Neg										
50	M	Neg	Pos	Pos	Pos												Pos	Pos		Pos	Pos															Pos						Pos	Pos		Pos	Pos	
63	M	Pos	Pos	Pos	Pos						Neg	Neg		Neg	Neg																														
59	M	Pos	Pos										Neg			Neg																														
40	M	Pos	Pos	Pos	Pos				Pos		Pos	Pos		Pos	Pos																				Pos	Pos				Pos						
45	F	Pos	Pos	Pos							Neg			Neg																														
62	F	Neg	Pos																Pos			Pos																								
53	F	Neg	Pos	Pos	Pos									Neg	Pos		Pos	Pos																												
62	F	Neg	Pos	Pos	Pos						Neg	Pos		Neg	Pos			Pos						Neg																						
42	F	Neg	Pos										Neg			Neg																														
62	F	Neg	Pos	Pos							Neg			Neg																																
69	F	Pos	Pos	Pos							Neg			Neg																																
62	F	Neg	Pos								Neg			Neg																																

Table 4

Summary of the cases with cirrhosis.

Age	Sex	HBV	HCV	Cirrhosis	HCC	NASH	ASH	MDB			OV6			GST-P			HGF			EGF			CK19			H19			AIR			Ubd			Nanog			Oct4			Oct3			CD133		
								C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O
64	M	Neg	Neg	Pos	Pos						Neg	Pos		Neg	Pos		Neg	Neg		Neg	Neg		Neg	Neg																					
53	M	Neg	Neg	Pos		Pos																			Neg			Neg																
44	M	Pos	Neg	Pos							Neg			Neg																														
59	M	Neg	Pos	Pos	Pos		Pos		Pos		Pos	Pos		Pos	Pos		Pos	Pos		Pos	Pos		Neg	Neg		Neg	Neg		Neg	Neg					Pos	Neg		Pos	Pos						
51	M	Neg	Pos	Pos							Neg			Neg																														
75	M	Neg	Neg	Pos	Pos				Pos		Pos	Pos		Pos	Pos		Pos	Pos		Pos	Neg					Pos	Pos					Pos	Neg		Neg	Pos					Pos	Neg		Neg	Pos	
65	M	?	?	Pos	Pos				Pos		Neg	Neg																																
62	M	Neg	Neg	Pos	Pos				Pos		Pos	Pos			Pos																														
63	M	Pos	Pos	Pos	Pos						Pos	Pos		Pos	Pos		Pos	Pos								Pos	Pos		Pos	Pos					Pos	Pos		Pos	Pos						
67	M	Pos	Pos	Pos	Pos				Pos		Pos	Pos			Pos					Neg	Neg														Neg	Neg		Neg	Neg						
40	M	Pos	Neg	Pos							Neg			Neg																														
48	M	?	?	Pos				Pos			Pos			Pos																														
34	M	Pos	Neg	Pos							Neg																																		
47	M	Pos	Neg	Pos	Pos									Neg	Pos		Neg																												
50	M	Neg	Pos	Pos	Pos												Pos	Pos		Pos	Pos														Pos	Pos					Pos	Pos		Pos	Pos	
61	M	Neg	Neg	Pos			Pos				Neg			Neg																														
63	M	Pos	Pos	Pos	Pos						Neg	Neg		Neg	Neg																														
44	M	Neg	Neg	Pos			Pos				Neg			Neg																														
40	M	Pos	Pos	Pos	Pos				Pos		Pos	Pos		Pos	Pos																				Pos	Pos					Pos					
45	F	Pos	Pos	Pos							Neg			Neg																														
46	F	Pos	Neg	Pos			Pos				Neg			Neg																														
61	F	Neg	Neg	Pos				Pos						Pos																														
14	F	?	?	Pos																Pos																								
44	F	Neg	Neg	Pos			Pos	Pos			Neg			Pos																														
58	F	Pos	Neg	Pos							Pos			Pos																														
53	F	Neg	Pos	Pos	Pos									Neg	Pos		Pos	Pos																										
62	F	Neg	Pos	Pos	Pos						Neg	Pos		Neg	Pos			Pos						Neg																				
67	F	Neg	Neg	Pos	Pos						Neg	Pos		Neg	Neg			Neg					Pos	Neg			Neg																	
62	F	Neg	Neg	Pos		Pos		Pos			Neg																																		
51	F	Neg	Neg	Pos				Pos			Neg																																		
31	F	Neg	Neg	Pos	Pos						Neg	Neg		Neg	Neg																														
43	F	Neg	Neg	Pos			Pos				Neg			Neg																														
62	F	Neg	Pos	Pos							Neg			Neg																														
76	F	Neg	Neg	Pos							Neg			Neg																														

Age	Sex	HBV	HCV	Cirrhosis	HCC	NASH	ASH	MDB			OV6			GST-P			HGF			EGF			CK19			H19			AIR			Ubd			Nanog			Oct4			Oct3			CD133		
								C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O
69	F	Pos	Pos	Pos							Neg			Neg																															

Table 5

Summary of the cases positive with hepatocellular carcinoma (HCCs).

Age	Sex	HBV	HCV	Cirrhosis	HCC	NASH	ASH	MDB			OV6			GST-P			HGF			EGF			CK19			H19			AIR			Ubd			Nanog			Oct4			Oct3			CD133		
								C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O
64	M	Neg	Neg	Pos	Pos						Neg	Pos		Neg	Pos		Neg	Neg		Neg	Neg		Neg	Neg																					
59	M	Neg	Pos	Pos	Pos		Pos		Pos		Pos	Pos		Pos	Pos		Pos	Pos		Pos	Pos		Neg	Neg		Neg	Neg		Neg	Neg					Pos	Neg		Pos	Neg						
75	M	Neg	Neg	Pos	Pos				Pos		Pos	Pos		Pos	Pos		Pos	Pos		Pos	Neg					Pos	Pos					Pos	Neg		Neg	Pos					Pos	Neg		Neg	Pos	
65	M	?	?	Pos	Pos				Pos		Neg	Neg																																	
83	M	Neg	Neg		Pos							Pos												Pos			Pos																			
62	M	Neg	Neg	Pos	Pos				Pos		Pos	Pos			Pos																															
63	M	Pos	Pos	Pos	Pos						Pos	Pos		Pos	Pos		Pos	Pos								Pos	Pos		Pos	Pos					Pos	Pos		Pos	Pos						
67	M	Pos	Pos	Pos	Pos				Pos		Pos	Pos			Pos					Neg	Neg														Neg	Neg		Neg	Neg						
47	M	Pos	Neg	Pos	Pos									Neg	Pos		Neg																												
67	M	Pos			Pos							Neg																								Neg									
45	M	Pos	Neg		Pos							Pos			Neg																						Neg								Neg	
50	M	Neg	Pos	Pos	Pos												Pos	Pos		Pos	Pos														Pos	Pos		Pos	Pos		Pos	Pos		Pos	Pos	
63	M	Pos	Pos	Pos	Pos						Neg	Neg		Neg	Neg																															
40	M	Pos	Pos	Pos	Pos				Pos		Pos	Pos		Pos	Pos																				Pos	Pos					Pos					
53	F	Neg	Pos	Pos	Pos									Neg	Pos		Pos	Pos																											
62	F	Neg	Pos	Pos	Pos						Neg	Pos		Neg	Pos			Pos						Neg																					
67	F	Neg	Neg	Pos	Pos						Neg	Pos		Neg	Neg			Neg					Pos	Neg			Neg																		
31	F	Neg	Neg	Pos	Pos						Neg	Neg		Neg	Neg																															

Table 6

Summary of the cases positive with alcoholic steaotehepatitis (ASH).

Age	Sex	HBV	HCV	Cirrhosis	HCC	NASH	ASH	MDB			OV6			GST-P			HGF			EGF			CK19			H19			AIR			Ubd			Nanog			Oct4		
								C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O
58	M	Neg	Neg				Pos						Neg			Neg											Neg			Neg										
33	M	Neg	Neg				Pos																				Pos												
59	M	Neg	Pos	Pos	Pos		Pos		Pos		Pos	Pos		Pos	Pos		Pos	Pos		Pos	Pos		Neg	Neg		Neg	Neg		Neg	Neg					Pos	Neg		Pos	Pos	
61	M	Neg	Neg	Pos			Pos				Neg			Neg																								
44	M	Neg	Neg				Pos						Neg			Neg																								
44	M	Neg	Neg	Pos			Pos				Neg			Neg																								
51	F	Pos	Neg				Pos						Neg			Neg																								
46	F	Pos	Neg	Pos			Pos				Neg			Neg																								
44	F	Neg	Neg	Pos			Pos	Pos			Neg			Pos																								
47	F	Neg	Neg				Pos			Pos			Neg			Neg																								
54	F	Neg	Neg				Pos						Neg			Neg																								
43	F	Neg	Neg	Pos			Pos				Neg			Neg																								

Table 7 Summary of the cases positive with non-alcoholic steaotehepatitis (NASH).

Age	Sex	HBV	HCV	Cirrhosis	HCC	NASH	ASH	MDB			OV6			GST-P			HGF			EGF			CK19			H19			AIR		
								C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O	C	T	O
14	M	Neg	Neg			Pos							Neg			Neg															
53	M	Neg	Neg	Pos		Pos																				Neg			Neg		
22	M	Neg	Neg			Pos										Neg															
22	F	Neg	Neg			Pos				Pos			Neg			Neg					Neg						Neg			Neg	
40	F	Neg	Neg			Pos				Pos						Pos											Neg			Neg	
62	F	Neg	Neg	Pos		Pos		Pos			Neg																				

Table 8

Summary of the cases analyzed for the stem cells.

Liver disease	# of cases	# of cases with at least 1 positive stem cell marker(s)	% of cases with at least 1 positive stem cell marker(s)
HCC	18	14	78
Hepatitis C	17	8	47
Hepatitis B	17	6	35
Cirrhosis	35	13	37
ASH	12	3	25
NASH	6	1	17