MINI REVIEW

Human cirrhosis: Monoclonal regenerative nodules derived from hepatic progenitor cells abutting ductular reaction

Cirrhose chez l’homme : nodules de régénération monoclonaux dérivés de cellules souches hépatiques en connexion avec la réaction ductulaire

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Available online 28 April 2010

Summary  Cirrhosis is a premalignant condition leading to hepatocellular carcinoma. Cirrhotic nodules are surrounded by a rim of CK 7/CK19-positive biliary cells termed ductular reaction. Half of all regenerative cirrhotic nodules are thought to be monoclonal by studying the pattern of inactivation of the X-linked human androgen receptor gene (HUMARA). Using a new technique for lineage tracing in human liver based on the identification in the mitochondrial DNA of mutations in the cytochrome c oxidase (CCO) gene, the authors discovered that 20% of regenerative nodules were monoclonal; in addition they showed that hepatic progenitor cells within abutting CCO-deficient cells of the ductular reaction had the same mutations as the adjacent regenerative nodule, indicating a common cell origin. It is the first direct evidence that regenerative nodules in cirrhosis can be derived from hepatic progenitor cells.

Cirrhosis is defined histologically as a diffuse process in which the normal anatomical lobules are replaced by architecturally abnormal nodules separated by fibrous tissue. Knowing that cirrhosis is a premalignant condition leading to hepatocellular carcinoma, a number of attempts have been made to establish the clonality of regenerative nodules (RNs), often by examination of X chromosome-linked markers in females. In liver cirrhosis, approximately half of all RNs have been found monoclonal by studying the pattern of inactivation of the X-linked human androgen receptor gene (HUMARA) [1]. However, in this type of approach the distribution of X-inactivated cells in the liver is not taken into account (the progeny of a single X-inactivated embryonic cell may be clustered together giving a false information concerning clonality).
Cirrhotic nodules are surrounded by a rim of CK 7/CK19-positive biliary cells termed ductular reaction (DR). Examination of cirrhotic explants of diverse diseases reveals that most intraseptal hepatocytes (ISH), whether as nodules or as loose clusters, are associated with DR [2]. Three-dimensional reconstruction of ISH in hepatitis C related cirrhosis demonstrates that in that setting virtually all ISH are associated with DRs, which form a structural and probably physiological link to the biliary tree. Therefore, if proliferation of hepatocytes in the early stages of chronic hepatitis C accounts for much of hepatocyte regeneration, in cirrhosis, the biliary tree becomes more proliferative as hepatocyte replication diminishes.

A reaction of ductular phenotype, possibly but not necessarily of ductular origin, in chronic liver disease may arise from:

- proliferation of preexisting cholangiocytes;
- progenitor cells (local so-called hepatic progenitor cells [HPCs]) and/or circulating cells probably bone marrow-derived;
- rarely, biliary metaplasia of hepatocytes.

The progenitor functioning of the DR attracts much attention [3]. In particular, cells of intermediate morphology and intermediate immunophenotyping are of interest. These cells are referred to as intermediate hepatobiliary cells, defined as larger than 6 microns in diameter (the approximate size of the normal canal of Hering cell, i.e., the smallest cholangiocytes), but less than 40 microns (the typical size of a hepatocyte), with other features suggesting dual characteristics of both hepatocytes and cholangiocytes. These include, but are not limited to: simultaneous expression of biliary antigens (e.g. keratins 19, 7, OV-6) and hepatocyte antigens (e.g., HepPar1, albumin, alpha-1-antitrypsin, biliary glycoprotein-1 detected by canalicular staining with polyclonal anti-CEA, and, occasionally, alpha-fetoprotein), other markers such as NCAM-1/CD56, and structural features such as basement membrane formation typical of cholangiocytes and canalicular membranes typical of hepatocytes.

Staining with NCAM, CK19, and HepPar1 has revealed a distinctly bipolar structure to DRs that are embedded in cirrhotic tissue. Spatial analysis of cells that are singly HepPar1-positive, or CK19-positive, has revealed hepatocytic and biliary poles, respectively, in the DRs. The location of singly NCAM-positive cells in DRs suggests that they may be bipotent liver stem/progenitor cells. The locations of other intermediate hepatobiliary cells, which have combinations of markers, suggest that CK19+/NCAM+ cells are transitional cells in the biliary lineage and that rare cells that are negative for all three markers are transitional cells in the hepatocytic lineage.

The working cell lineage model for DRs is presented below [4]:

**Hepatocyte** ↔ **Stem cell** → **Bile duct**

Transitional cells in the hepatic lineage are negative for the three markers.

Transitional cells for the bile duct lineage are positive for NCAM and CK19.

The liver has a multi-tiered, flexible system of regeneration rather than a single stem/progenitor cell location. Four possible hepatic stem cell niches have been identified: the canal of Hering (proximal biliary tree), intralobular bile ducts, periductal "null" mononuclear cells, and peribiliary hepatocytes [5].

A new technique for lineage tracing in human liver has been recently discovered [6]. It has been shown that human gastrointestinal stem cells and their progeny contain non-pathogenic mutations in their mitochondrial DNA, including mutations in the cytochrome c oxidase (CCO) gene, a component of complex IV of the respiratory chain, that are relatively common. The mitochondrial genome is prone to mutation. Mutations can expand stochastically within a cell and over time cells will become either homoplasmic—all the mitochondria in the cell are mutated—or heteroplasmic—the cell contains a mixture of mutated and wild-type mitochondria. This stochastic expansion is a lengthy process, often taking many years, and for a mutated cellular phenotype to be observed, homoplasmic or a high degree of heteroplasmity must be present. Thus, stem cells are the only cells that have a sufficient lifespan to accumulate these mitochondrial mutations to a level that results in a detectable biochemical deficiency. Many patches of CCO-negative hepatocytes have been identified in human liver, invariably connected to the portal areas suggesting an origin from an area close to the limiting plate, the stem cell niche. mtDNA sequencing of laser-captured individual hepatocytes from these patches has provided unequivocal proof that each patch was monoclonaclly derived.

The same authors using the same approach in human cirrhosis [7] have shown that most RNs are either uniformly CCO-positive or CCO-deficient, less than 3% are CCO-mixed nodules, suggesting that most RNs could be monoclonal (Fig. 1). mtDNA sequence analysis revealed that within a given CCO-deficient RN, all cells harbored the same mutation(s), which proves monoclonality. Furthermore, HPCs within abutting CCO-deficient DRs had the same mutations as the adjacent RN, indicating a common cell origin. It is the first direct evidence that RNs in cirrhosis can be derived from HPCs.

RNs can therefore be formed by monoclonal expansion, and not simply by fibrotic dissection of preexisting liver parenchyma and that HPCs within the abutting DRs can have the same cell of origin as the monoclonal RN. RNs examined histologically revealed no dysplastic features but their malignant potential is unknown.

This study showing that RNs in cirrhosis can be monoclonal, with the cell of origin likely to be a facultative stem cell from the biliary tree elegantly confirms the first study [2]. This obviously changes our concept of the formation of RN.

Cirrhosis formation takes years. After exhaustion of the hepatocytic proliferation capability, the potential stem cell compartment is activated. This process may perhaps start sooner than thought. Indeed, extracellular matrix deposition and activation of matrix-producing cells occurs as an early phase of chronic liver injury, and it may be that the fibrotic environment is important for establishing the niche...
for the process of activation and differentiation of HPCs. Differentiation of HPCs to hepatocytes occurs at this fibrotic interface, leading to buds of intraseptal hepatocytes, and the RNs we see may represent further evolution of this process.

This study opens new questions especially in the field of carcinogenesis. Are those monoclonal nodules the one who will transform into high grade dysplastic nodule? We should not wait very long before knowing the answer.

Conflict of interest statement

The authors have not declared any conflict of interest.

References


