Review Article

Clear cell hepatocellular carcinoma: origin, metabolic traits and fate of glycogenotic clear and ground glass cells

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ABSTRACT: Clear cell hepatocellular carcinoma (CCHCC) has hitherto been considered an uncommon, highly differentiated variant of hepatocellular carcinoma (HCC) with a relatively favorable prognosis. CCHCC is composed of mixtures of clear and/or acidophilic ground glass hepatocytes with excessive glycogen and/or fat and shares histology, clinical features and etiology with common HCCs. Studies in animal models of chemical, hormonal and viral hepatocarcinogenesis and observations in patients with chronic liver diseases prone to develop HCC have shown that the majority of HCCs are preceded by, or associated with, focal or diffuse excessive storage of glycogen (glycogenosis) which later may be replaced by fat (lipidosis/steatosis). In ground glass cells, the glycogenosis is accompanied by proliferation of the smooth endoplasmic reticulum, which is closely related to glycogen particles and frequently harbors the hepatitis B surface antigen (HBsAg). From the findings in animal models a sequence of changes has been established, commencing with preneoplastic glycogenotic liver lesions, often containing ground glass cells, and progressing to glycogen-poor neoplasms via various intermediate stages, including glycogenotic/lipidotic clear cell foci, clear cell hepatocellular adenomas (CCHCA) rich in glycogen and/or fat, and CCHCC. A similar process seems to take place in humans, with clear cells frequently persisting in CCHCC and steatohepatitic HCC, which presumably represent intermediate stages in the development rather than particular variants of HCC. During the progression of the preneoplastic lesions, the clear and ground glass cells transform into cells characteristic of common HCC. The sequential cellular changes are associated with metabolic aberrations, which start with an activation of the insulin signaling cascade resulting in preneoplastic hepatic glycogenosis. The molecular and metabolic changes underlying the glycogenosis/lipidosis are apparently responsible for the dramatic metabolic shift from gluconeogenesis to the pentose phosphate pathway and Warburg-type glycolysis, which provide precursors and energy for an ever increasing cell proliferation during progression.

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KEY WORDS: clear cell hepatocellular carcinoma; phenotypic heterogeneity; glycogenosis; lipidosis; preneoplastic glycogenosis; progression; metabolic aberrations

Introduction

Hepatocellular carcinoma (HCC) remains the fifth most common malignancy in men and the eighth in women worldwide, with increasing incidence in Western countries.[1] The risk factors for HCC include chronic viral infection (hepatitis B or C), alcohol abuse, dietary aflatoxin contamination, hemochromatosis, oral contraceptive use and, as recognized recently, obesity and type 2 diabetes.[1, 2] In addition, a relatively rare but heuristically informative inborn error of metabolism, namely hepatic glycogen storage disease (glycogenosis), is a risk factor for the development of hepatocellular adenomas (HCA) and HCC when the patients pass through adolescence.[3] The prognosis of the majority of HCC is poor, ranking the second most common cause of cancer-related mortality.[4] However, specific forms of HCC, such as clear cell hepatocellular carcinoma (CCHCC), might have a better prognosis. A closer look at CCHCC is not only of interest from a clinical point of

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view, but also provides important new insights for our understanding of the pathogenesis of HCC. The present article was therefore to review the cellular origin and composition, the metabolic traits, and the prognosis of CCHCC.

Historical background

CCHCC is considered an uncommon histological variant of HCC as observed in conventional hematoxilin-eosin (HE) staining.[5-11] Special staining shows that the clear cells, appearing "empty", "watery", "vacuolated", "foamy" or even "ballooned" in HE-staining, accumulate glycogen[6, 7, 12] and/or fat.[7, 13] In addition to CCHCC, clear cells containing abundant glycogen are also found in focal nodular hyperplasia[11, 14] and in many clear cell hepatocellular adenomas (CCHCA).[7, 8, 11, 12, 15]

The process of hepatocarcinogenesis including the development of CCHCC cannot be systematically studied in the human. However, various animal models of chemical, hormonal, and viral hepatocarcinogenesis have been established which allow us to follow the origin, the metabolic traits and the fate of the clear cells.[16-19] Among the different approaches to induce hepatocarcinogenesis in rodents by chemicals, investigations in rats predominated. In addition to the continuous oral exposure of chemical carcinogens to rats, single dose application, initiation-promotion protocols, the stop protocol and the complex resistant hepatocyte model have been widely used.[20, 21] The great advantage of the stop protocol is that a single carcinogen, e.g. 4-dimethylaminoazobenzene or N-nitrosomorpholine (NNM), is applied for a limited time period (3-7 weeks) and the evolution of preneoplastic and neoplastic lesions can be analyzed after withdrawal of the carcinogen. This protocol distinguishes reversible changes from persistent lesions and allows us to follow the persistent alterations without any interference with acute hepatotoxic effects or other experimental manipulations.[16, 22] It were particularly studies in the NNM-stop model of hepatocarcinogenesis which revealed that excessive storage of glycogen and/or fat reflects fundamental metabolic changes starting in a multicentric fashion in foci of altered hepatocytes (FAH) early during neoplastic transformation.[3, 16, 18, 23, 24] Hepatocellular glycogenosis is often accompanied by hypertrophy of the smooth endoplasmic reticulum which is visible as an acidophilic cytoplasmic network under the light microscope.[12, 25] In patients chronically infected with HBV, Hadziyannis et al[26] found that hypertrophy of the smooth endoplasmic reticulum is frequently associated with expression of the hepatitis B surface antigen (HBsAg) which is localized in the lumen of the smooth endoplasmic reticulum. The authors called these cells "ground glass hepatocytes", which have since become an important diagnostic marker in patients with chronic hepatitis B. In animal models of chemical hepatocarcinogenesis, cells with a proliferation of the smooth endoplasmic reticulum were called "acidophilic (eosinophilic) cells". In models of hepadnaviral hepatocarcinogenesis in mice and woodchucks, and in patients with chronic HBV and/or HCV infections, the terms "ground glass hepatocyte" and "acidophilic cell" are used interchangeably, irrespective of the presence of HBsAg. We consider the hypertrophy of the smooth endoplasmic reticulum visible as an acidophilic cytoplasmic inclusion under the light microscope as the common denominator, and call all of these types of hepatocytes "acidophilic ground glass hepatocyte" (AGGH) in this review.

Historically, human liver tumors composed of "clear cells" had been mistakenly classified as "adrenal rest tumors", "hypernephromas" or "hypernephroid tumors" until 1970. Hamperl[27] examined 26 cases and found that all were in fact HCAs or HCCs.[7] The phenotypic similarity of cells from CCHCC and renal cell carcinoma (RCC) or clear cell carcinomas of other sites complicates the differential diagnoses of CCHCC and metastases of extrahepatic clear cell carcinomas to the liver.[6, 7] In the past two decades, CCHCC has attracted great attention in clinical studies because of its controversial prognosis.[28-38]

In view of the impressive conformity of the process of hepatocarcinogenesis in humans and in various animal species,[17, 39] particularly in rodents exposed to chemicals,[18, 21] hepadnaviridae (with and without exposure to aflatoxin B1)[40, 41] or local hyperinsulinism,[42, 43] we addressed comparative aspects which might be helpful for elucidation of CCHCC pathogenesis, highlighting the roles of glycogenotic clear and AGGH in neoplastic transformation.

Cytopathology of CCHCC

Mixtures of various types of neoplastic cells

There is a general agreement that the clear cells in CCHCC accumulating glycogen and/or fat are usually mixed with various other types of cells. This holds for "conventional" or "common", granular eosinophilic or basophilic neoplastic hepatocytes,[7-9, 11, 12, 27, 30, 44, 45] glycogenotic AGGH,[10, 12, 25] and different types of "intermediate" cells. The cytoplasm of the latter is partly clear or acidophilic and rich in glycogen or fat, partly basophilic and poor in glycogen or fat.[8-12]

Two types of glycogenotic cells have to be distinguished in CCHCC: glycogenotic/lipidotic clear cells and