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Ultrastructural characteristics of the respective forms of hepatic stellate cells in chronic hepatitis B as an example of high fibroblastic cell plasticity. The first assessment in children

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ABSTRACT

Purpose: Activation of hepatic stellate cells (HSCs), mainly responsible for extracellular matrix synthesis, is assumed to be central event in the process of liver fibrogenesis. The major objective of the research was to analyze the ultrastructural profile of activated HSCs in children with chronic hepatitis B (chB), with respect to fibrosis intensity.

Materials/methods: Ultrastructural investigations of HSCs were conducted on liver bioptates from 70 children with clinicopathologically diagnosed chB before antiviral treatment. Biopsy material, fixed in paraformaldehyde and glutaraldehyde solution, was routinely processed for electron-microscopic analysis.

Results: In children with intensive liver fibrosis (S-2 and S-3), the ultrastructural picture showed almost total replacement of quiescent HSCs (Q-HSCs) by activated, i.e. transitional HSCs (T-HSCs). Among T-HSCs, two types of cells were distinguished: cells exhibiting initiation of HSC activation (Ti-HSCs), never before described in chB, that were frequently accompanied by activated Kupffer cells, and cells with features of perpetuation of activation (Tp-HSCs). Tp-HSCs were elongated and characterized by substantial loss of cytoplasmic lipid material; they contained an increased number of cytoskeletal components, extremely dilated channels of granular endoplasmic reticulum and activated Golgi apparatus, which indicated their marked involvement in intensive synthesis of extracellular matrix proteins. Many collagen fibers were found to adhere directly to Tp-HSCs.

Conclusions: The current study showed T-HSCs to be an important link between Q-HSCs and myofibroblastic HSCs (Mf-HSCs). Transformation of HSCs into new morphological variations (Ti-HSCs; Tp-HSCs and Mf-HSCs), observed along with growing fibrosis, indicates their high plasticity and a key role in fibrogenesis in pediatric chB.

1. Introduction

Hepatic stellate cells (HSCs), due to their morphology, biology and numerous functions within the organ, have long been defined in literature as a most fascinating and at the same time poorly known and enigmatic human cells [1]. The existence of numerous synonyms for this cell population, e.g. fat storing cells, Ito cells, perisinusoidal stellate cells, liver interstitial cells, liver specific pericytes or vitamin A storing cells seems to reflect the case [1–4]. It is assumed that HSCs belong to the population of nonparenchymal hepatic cells (NPCs), which apart from stellate cells accounting for approximately 20% of NPCs, also includes endothelial cells (c. 40%), Kupffer cells/macrophages (KCs/MPs) (c. 20%) and a heterogeneous subpopulation of intrahepatic lymphocytes (c. 20%) [4].

HSCs are easy to identify using transmission electron microscopy (TEM) or immunohistochemical (IHC) stainings, especially for smooth muscle actin (alpha-SMA) and desmin [1–3,5,6,7], but not with routine histological staining. Ultrastructural investigations allowed researchers to distinguish three main morphological forms of HSCs, quiescent HSCs (Q-HSCs), transitional HSCs (T-HSCs) and myofibroblastic HSCs (Mf-HSCs) [1,3,8–11].

It is assumed that HSCs which undergo transformation to metabolically active T-HSCs and myofibroblast-type cells are

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mainly responsible for the synthesis of protein components of the extracellular matrix (ECM) and play a key role in the initiation and progression of liver fibrosis [5,6,10,12–15]. They are also a potential target for the treatment of liver fibrosis [12,14,15,16–19].

Up to now literature data on the sequence of morphological events observed at the ultrastructural level in the pathogenesis of liver fibrosis have been limited to adult patients and experimental studies. Similar reports on pediatric patients are lacking. Taking that into consideration, the aim of the current study was to characterize the respective forms of hepatic stellate cells in children with chronic hepatitis B (chB), in relation to the advancement of liver fibrosis, based on abundant retrospective biopsy material.

The study is a continuation of our many years' morphological and clinical research, including IHC and submicroscopic investigations, into the process of fibrogenesis and development of liver fibrosis in children with chB and other nonviral chronic diseases affecting this organ [20–25].

2. Material and methods

2.1. Study patients' profile

The retrospective morphological analysis was based on liver oligobiopsy specimens obtained blind from 70 children with chB (46 boys and 24 girls), aged 3–17 years (mean 11), who were qualified for alpha interferon or lamivudine treatment, but still prior to the initiation of scheduled antiviral therapy in the Department of Pediatrics, Gastroenterology and Allergology, Medical University of Bialystok. The patients had a documented HBV infection of >6 months' duration, with HBsAg and HBeAg-positive sera. Patients with HCV, with other liver disorders and with cirrhosis were excluded from the study.

In all study cases, the ultrastructural analysis of liver biopsies was preceded by semiquantitative numerical histologic assessment of the extent of liver fibrosis (staging – S) (using scoring system according to Batts and Ludwig – 26) as well as IHC staining for alpha SMA. The IHC and statistical results relative to the above patients were described in our earlier report [5]. All morphological investigations of the oligobiopsy material obtained from children with chB, together with TEM assessment, were performed in the Department of Medical Pathomorphology, Medical University of Bialystok.

The study was approved by the Bioethics Committee, Medical University of Bialystok.

2.2. Ultrastructural analysis

For ultrastructural investigations, small fresh liver blocks (1 mm^3 volume) were fixed in a solution containing 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 mol/L cacodylate buffer, pH 7.4. The specimens were postfixed in 2% osmium tetroxide (OsO_4) in 0.1 mol/L cacodylate buffer, pH 7.4, for 1 h. Then, the material was routinely processed for TEM analysis, i.e. dehydrated through a graded series of ethanols and propylene oxide, embedded in Epon 812 and sectioned on a Reichert ultramicrotome to obtain semithin sections (0.5–1 μm thick). These semithin sections were stained with 1% methylene blue in 1% sodium borate and examined under a light microscope. Ultrathin sections were double stained with uranyl acetate and lead citrate, and examined using an Opton 900 transmission electron microscope (Zeiss, Oberkochen, Germany). This processing procedure had been used in our earlier ultrastructural investigations of the liver in children [20–22].

HSCs were determined by a microscopist who was blinded to the clinical information.

3. Results

3.1. Baseline features

The semiquantitative numerical scoring system evaluating staging, i.e. the extent of liver fibrosis, according to Batts and Ludwig [26], preceding TEM analysis, allowed us to distinguish the following four study groups (from S-0 to S-3): group I (S-0) – consisting of 5 patients (2 boys; 3 girls); group II (S-1) – 27 patients (18 boys; 9 girls); group III (S-2) – 31 patients (21 boys; 10 girls) and group IV (S-3) – 7 patients (5 boys; 2 girls).

3.2. Group I (S-0)

EM analysis of the liver of children with chB in group I (S-0) allowed easy identification of dispersed Q-HSCs in biopsy specimens. They were observed in the perisinusoidal spaces of Disse, between sinusoidal endothelial cells and vascular pole of hepatocytes. They frequently intruded into the space in between adjacent hepatocytes. The presence of numerous lipid vacuoles exerting a pressure on cell organelles and constituting up to 80% of the cytoplasm volume was a characteristic feature of Q-HSCs (Fig. 1A,B). Among poorly developed cell organelles, narrow

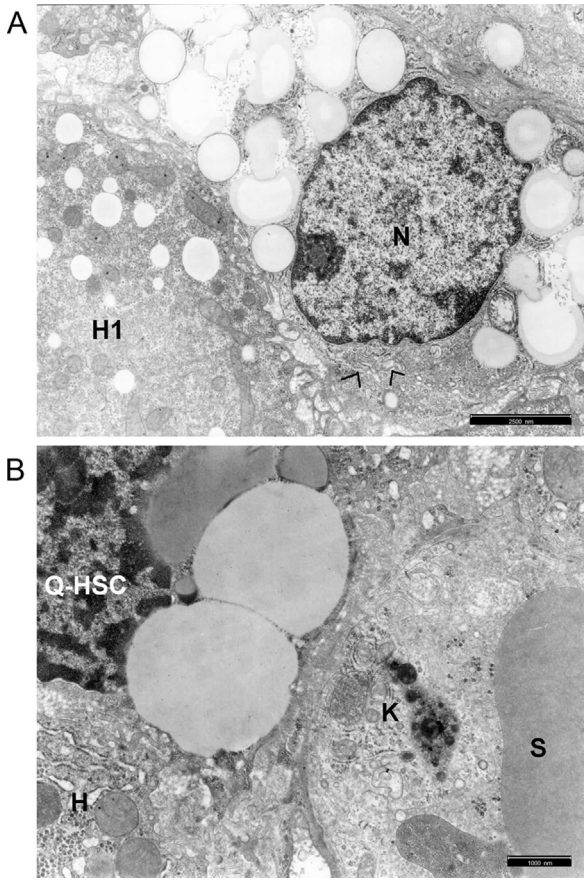

Fig. 1. 1A, 1B: The ultrastructure of quiescent HSCs (Q-HSCs) in biopsy material obtained from children with chB; group I (S-0).
A) The view of Q-HSC – located in the perisinusoidal space of Disse – filled up with lipid droplets; poorly developed cell organelles; visible Golgi apparatus in the perinuclear location (>); cell nucleus (N) contains abundance of euchromatin and heterochromatin mainly accumulating underneath the nuclear envelope and nucleolar body. The vascular pole of hepatocyte (H1) sends numerous microvilli towards Q-HSC (scale bar 2,5 μm, original magnification 4400×).
B) Fragment of Q-HSC containing large lipid droplets with an activated Kupffer cell (K) closely adhering to this cell. H – hepatocyte; S – sinusoidal lumen (scale bar 1 μm, original magnification 7000×).