Reexpression of Cytokeratin-19 in a Primary Human Hepatocyte Culture

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Isolated human hepatocytes cultured in liver biomatrix express cytokeratins 8 and 18. By the end of the first and during the second week in culture, hepatocytes express these cytokeratins and react with antibodies to cytokeratin-19. Cytokeratin-7 was found in individual cholangiocytes contaminating the culture. The presence of cytokeratin-19 in hepatocytes during the second week of culturing can be regarded as its reexpression.

Key Words: *hepatocytes; cytokeratins; cell culture*

Intermediate filaments of epitheliocyte cytoskeleton consist of cytokeratins (CKR) that represent a family of 20 proteins differing by molecular weight and isoelectric properties [5,6]. CKR polypeptides are the products of various genes. They are subdivided into acid (type I, CKR-9-20 according to R. Moll's catalogue) and neutral-base (type II, CKR-1-8). Generally, CKR are expressed by pairs, i. e., an epitheliocyte contains one type I and a paired type II protein. Mammalian liver contains two types of epitheliocytes: hepatocytes and cholangiocytes. Intermediate filaments of hepatocyte cytoskeleton consist of CKR-8 and 18, while cholangiocytes also contain CKR-7 and 19. CKRs-7 and 19 are regarded as specific linear cholangiocyte markers, and antibodies to them are widely used in the diagnosis of many diseases of the liver [12]. The set of expressed CKR depends on the level and direction of neoplastic cell differentiation in a mixed human tumor [2]. If tumor foci are hepatoma-like, the cells express CKR-8 and 18, while tubular structures and foci of mixed structure express CKR-7 and 19. Expression of CKR-19, which is uncommon for hepatocytes, occurs in chronic hepatitis B and cirrhosis of the liver [10], in hepatoblastoma cells [13], hepatocellular carcinoma [11], and cultured hepatic tumor cells [1]. These data allow a hypothesis that under certain conditions, hepatocytes express CKR-19 [9]. There are no published reports about the expression of cholangiolar CKR by cultured human hepatocytes. We studied the spectrum of CKR in a primary human hepatocyte culture.

MATERIALS AND METHODS

Human hepatocytes were isolated and cultured at the Laboratory of Hepatology, Catholic University of Leuven (Belgium). Hepatocytes were isolated from donor liver unfit for transplantation by double perfusion with a collagenase solution [8]. Cell suspension was twice filtered through nylon membranes and centrifuged. After second centrifugation the cells were resuspended in Williams E medium containing 10% fetal calf serum, antibiotics, and antifungal drugs. Cells were cultured in Petri dishes on slides coated with liver biomatrix [7]. Viability of isolated cells was assessed by Trypan Blue staining. Slides were fixed in absolute acetone after 1, 3, 5, 8, 13, and 17 days of culturing and stored at -20°C.

The indirect immunoperoxidase test was carried out with monoclonal antibodies to CKR-8 (clone 4.1.18, dilution 1:10, Boehringer Mannheim), CKR-7 (clone Ks 7-18, dilution 1:5, Boehringer Mannheim), CKR-19 (clone 170.2.2, dilution 1:5, Boeh-

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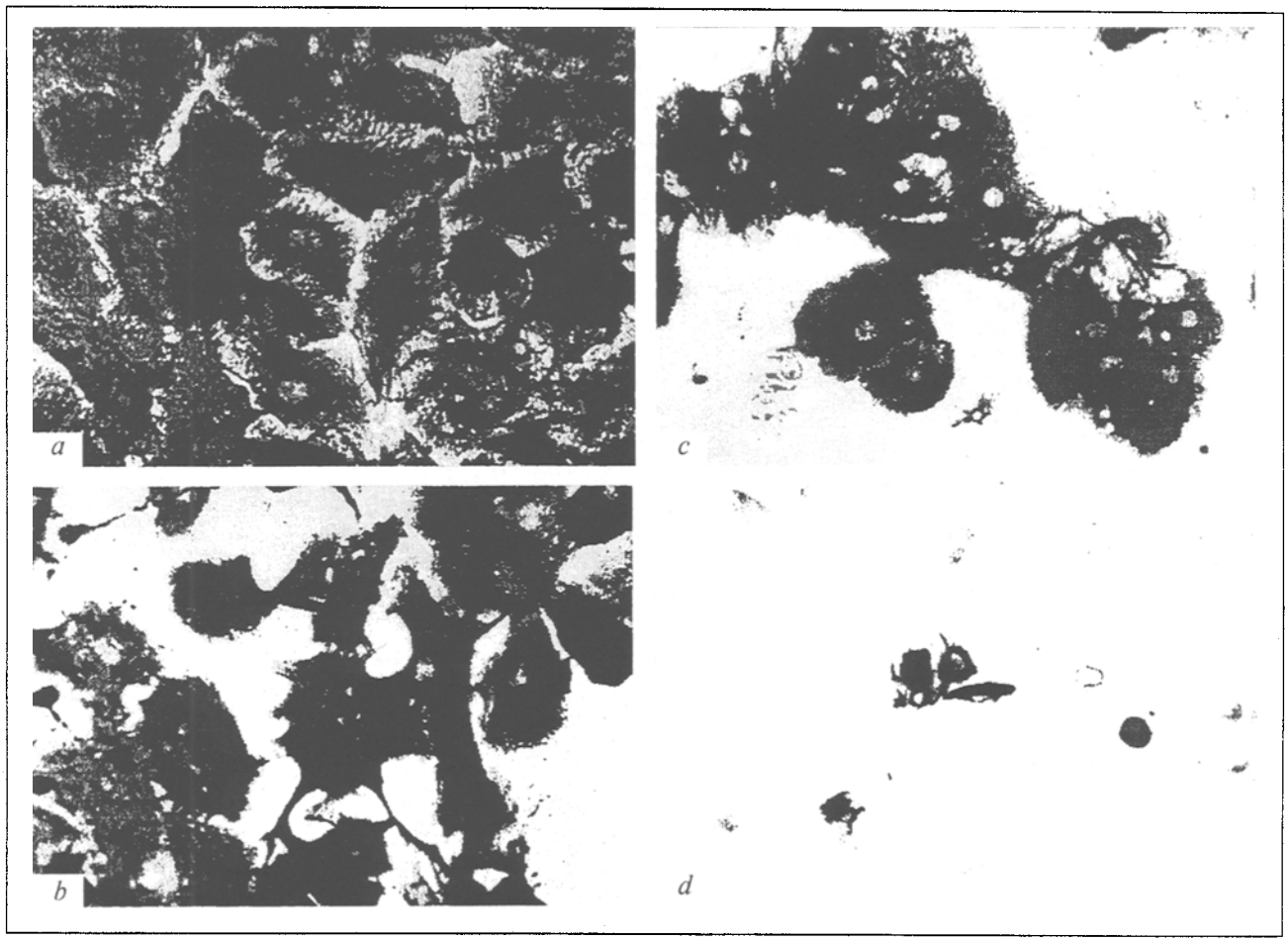

Fig. 1. Primary human hepatocyte culture stained with antibodies to cytokeratin-18 (*a*), 19 (*b*, *c*), and 7 (*d*). Days 17 (*a*), 8 (*b*), and 13 (*c*, *d*) of culturing. ×200.

ringer Mannheim), and CKR-18 (RGE-53, dilution 1:5, ICN Biomedicals). Secondary antibodies were rabbit immunoglobulins to mouse Ig conjugated with horse radish peroxidase (dilution 1:100, Dako). The specificity of immunohistochemical reaction was tested by replacing primary antibodies with nonimmune murine serum. Cryostat sections of normal human liver biopsy specimens served as a positive control. Antibodies to CKR-8 and 18 reacted with hepatocytes and cholangiocytes. The product of histochemical reaction with antibodies to CKRs-7 and 19 was detected only in epitheliocytes of intrahepatic bile ducts.

RESULTS

Immunocytochemical analysis of cultured cells showed that during the first 5 days of culturing the bulk of cells is represented by hepatocytes stained only by antibodies to CKRs-8 and 18. Reaction with anti-CKR-7 and anti-CKR-19 antibodies at this terms was observed only in some small cells. These cells are apparently cholangiocytes contaminating the suspension of isolated hepatocytes. Starting from day 8 of culturing, we observed hepatocyte staining with antibodies to CKR-19, presenting as a cytoplasmatic reticulum (Fig. 1, *b, c*), in addition to CKR-8 and 18 (Fig. 1, *a*). Anti-CKR-7 antibodies reacted only with cholangiocytes which formed clusters of small cells on the second week of culturing (Fig. 1, *d*). Hepatocyte cytoskeleton was not stained by antibodies to CKR-7 at any period of investigation.

A relationship between CKR production and degree of liver parenchyma cell differentiation has been hypothesized. At the early stages of embryogenesis human liver is represented by precursor cells whose intermediate filaments consist of CKRs-8, 18, and 19 [4,9]. The CKR-19 is not expressed during prenatal development of hepatocytes, probably due to morphogenetic rearrangement of hepatic tissue, which reflects qualitative changes in the spectrum of CKR produced in the liver. Our results indicate a reexpression of CKR-19 in a primary hepatocyte culture. This reexpression may be caused by disordered cell-to-cell interactions and alteration of normal microenvironment under conditions of cul-

turing, deeply modifying the cell phenotype and the CKR gene expression [3]. Reversion of embryonal hepatocyte phenotype *in vitro* raises doubts about probability of using antibodies to CKR-19 for detection of linear markers of cholangiocytes and confirms the hypothesis about probable reexpression of this protein by hepatocytes in some liver diseases.

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