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Vimentin expression of newly formed rat bile duct epithelial cells in secondary biliary fibrosis

Stefano Milani[1], Hermann Herbst[1], Detlef Schuppan[2], Gerald Niedobitek[1], Ki Young Kim[2], and Harald Stein[1]

[1] Institute of Pathology and [2]Department of Gastroenterology, Klinikum Steglitz, Free University Berlin, Hindenburgdamm 30, D-1000 Berlin 45

Summary. The intermediate filament profile and the growth fraction of hepatocytes and bile duct epithelial cells were studied in a rat model of biliary fibrosis secondary to common bile duct ligation and scission. Strong vimentin expression was observed in epithelial cells of newly formed bile ductules, while normal liver contained only few weakly positive bile duct epithelial cells. All epithelial cells reacted with a pan-cytokeratin antibody. A monoclonal antibody specific for human cytokeratin 7 selectively reacted with both normal and newly formed bile duct epithelial cells. The intermediate filament profile of hepatocytes was constant, showing no changes during proliferation or in periportal areas adjacent to excessive bile duct formations. The proliferation-associated antigen detected by the antibody Ki-67 was present in many hepatocytes, homogeneously distributed in the lobules, but was seen only in a small proportion of the epithelial cells of the newly formed bile ducts. We conclude that vimentin may serve as an indicator for cellular reorganization in the bile duct system, and that the epithelial cells of newly formed bile ductules in this particular model of secondary biliary fibrosis were most likely to be derived from an outgrowth of the biliary duct system and recruitment of preductular epithelial cells. No morphological or immunohistological evidence suggesting a derivation from hepatocytes by ductular metaplasia or from oval cells was obtained.

Key words: Immunohistology – Monoclonal antibodies – Vimentin – Cytokeratin – Intermediate filaments – bile ducts – Ki-67 – Proliferation

Offprint requests to: H. Herbst

Introduction

In routine histopathological practice, vimentin has been considered to be a specific marker for mesenchymal cells (Osborn and Weber 1983). However, recent reports indicate that this intermediate filament protein may also be found in epithelial cells of adult and embryonic tissues (Van Muijen et al. 1987; Kasper and Karsten 1988), in various epithelial neoplasms (McNutt et al. 1985), in epithelial cell cultures, and in regenerating renal tubular epithelia (Gröne et al. 1987). Gröne et al. (1987) therefore proposed that vimentin may be an indicator of proliferative and regenerative activity in some epithelial cells.

We investigated the intermediate filament profile of proliferating and non-proliferating bile duct epithelial cells in comparison with hepatocytes in an experimental model of secondary biliary fibrosis in the rat. Within few days after ligation and scission of the common bile duct, the animals show excessive bile ductule formation in portal fields and periportal marginal areas. The cellular derivation of these bile duct epithelial cells is unknown; metaplastic transformation of hepatocytes (Desmet 1985; Schulz et al. 1987; Van Eyken et al. 1988a) and derivations from pre-existing ductular structures such as the canals of Hering (Schaffner and Popper 1961; Carruthers et al. 1962) or oval cells (hepatic stem cells) (Farber 1956; Sell et al. 1981) have been postulated. The objective of this study was to establish whether, by analogy with regenerating renal tubular epithelia (Gröne et al. 1987), vimentin may be expressed in such newly formed bile duct epithelial cells. In addition, to estimate the growth fraction of the ductular epithelial cells

we applied double-immunostaining with a cytokeratin 7-specific monoclonal antibody (Tölle et al. 1985) and the monoclonal antibody Ki-67, which detects a proliferation-associated nuclear antigen (Gerdes et al. 1983).

Materials and methods

The common bile duct was interrupted by ligation and subsequent scission (Kountouras et al. 1984) in two groups of eight female Wistar rats (200–250 g, Zentralinstitut für Versuchstiere, Hannover, FRG). Animals were killed after 2, 3, 4, and 6 weeks of surgery. Untreated rats served as controls. Animal experiments were conducted in accordance with the Berlin state laws governing the use of experimental animals.

Liver samples (5–7 mm^3) were immediately frozen in liquid nitrogen and stored up to one month. Cryostat sections (4 μm) were collected onto gelatin/chrome-alum coated slides, dried overnight, and fixed in acetone and chloroform for 15 min each.

Monoclonal antibodies against vimentin (V9, Osborn et al. 1984, Dakopatts, Glostrup, Denmark), human cytokeratin 7 (CK7, Tölle et al. 1985, Boehringer Mannheim, Mannheim, FRG), a common antigenic determinant on cytokeratins (lu-5, von Overbeck et al. 1985), and a proliferation-associated nuclear antigen (Ki-67, Gerdes et al. 1983) were used diluted 1:50 in 50 mM Tris/150 mM NaCl, pH 7.4 (TBS), containing 10% RPMI 1640 (Gibco, Karlsruhe, FRG), and 10% inactivated normal bovine serum. Immobilized primary antibodies were detected by the immunoalkaline phosphatase anti-alkaline phosphatase (APAAP) method (Cordell et al. 1984) with affinity-purified rabbit antimouse immunoglobulin (Ig) antibodies (Dakopatts), diluted 1:10 in a buffer containing 10% normal rat serum, and APAAP complex (Dakopatts), diluted 1:20. Alkaline phosphatase was developed with new fuchsin (Merck, Darmstadt, FRG) (Stein et al. 1985). For double staining with Ki-67 and CK7 by the immunoperoxidase and APAAP methods, slides were incubated with Ki-67 for 30 min at room temperature, washed four times in TBS, incubated with peroxidase-conjugated rabbit anti-mouse IgG serum (Dianova, Hamburg, FRG) for 30 min and, after a further washing step, with peroxidase-conjugated anti-rabbit IgG serum (Dianova). The peroxidase-reaction was carried out according to Graham and Karnovsky (1966) and the sections were then labelled with the monoclonal antibody CK7 by the APAAP procedure as described above.

Results

In normal rat liver, vimentin was found widely distributed in the cytoplasm of different cell types. In the lobule, sinusoidal and perisinusoidal cells showed a strong staining, similar in intensity to cells of the portal tract stroma. Vimentin was also observed in cells of both portal and central vein walls, including endothelial, perivascular smooth muscle, and other mesenchymal cells. Additionally, vimentin was found in mesenchymal periductular cells, but was also observed, weakly expressed, in a few scattered epithelial cells of large and small bile ducts, located predominantly in the basal cytoplasm (Fig. 1). Hepatocytes never showed any vimentin-specific staining, but were labelled by the pan-cytokeratin-specific antibody, lu-5, in addition to all bile duct epithelial cells. As previously described for human liver by Ramaekers et al. (1987), the antibody CK7 selectively stained all bile duct epithelial cells, but no hepatocytes. Very few parenchymal cells (1–2%) distributed throughout the lobule, and none of the bile duct epithelial cells showed nuclear staining with the antibody Ki-67.

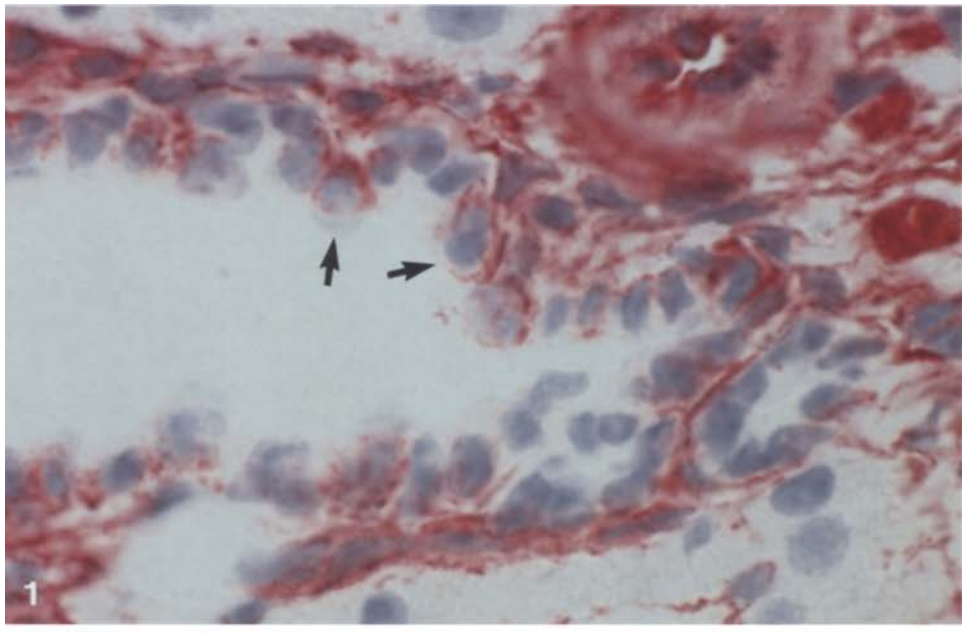
2–6 weeks after obstruction of the common bile duct, a progressive formation of small bile ductules was found in the portal fields and the periportal marginal areas. Groups of newly formed bile ducts surrounded by connective tissue infiltrated the periportal parenchymal areas displacing the liver cell plates. However, these changes were not associated with a significant degree of inflammation. Also, there was no evidence for any arrangement of hepatocytes resembling cholestatic rosettes (Butron-Vila et al. 1984). A prominent layer of vimentin-positive cells showing fine cytoplasmic extensions was observed persistently around the proliferating bile ducts. Most epithelial cells of newly formed bile ductules as well as some large bile duct epithelial cells were also intensely labelled by the antibody V9, both in the portal fields and in periportal areas of the lobule, and displayed increased staining at the basal side (Figs. 2 and 3). The portal tract stroma and the walls of blood vessels contained vimentin-positive cells in abundance. Conversely, vimentin was not observed in hepatocytes. Again, the expression of CK7 immunoreactivity

Fig. 1. Immunohistological detection of vimentin in normal rat liver (APAAP, ×400): Intense staining in sinusoidal cells of the lobule and in many stromal, perivascular, and periductular cells of portal fields, weak staining in some bile duct epithelial cells (*arrows*)

Fig. 2. Immunohistology of vimentin 4 weeks after common bile duct ligation and scission (APAAP, ×250): Multiple bile duct formations at the periphery of a lobule, variable vimentin expression in bile duct epithelial cells, sometimes with an inhomogeneous intracellular distribution (*arrows*), and strong reactivity within some perisinusoidal cells

Fig. 3. Immunohistology of vimentin 4 weeks after common bile duct ligation and scission, detail (APAAP, ×400)

Fig. 4. Detection of CK7 (APAAP) and Ki-67 immunoreactivity 2 weeks after common bile duct ligation and scission (×400): CK7-specific red cytoplasmic labelling in bile duct epithelial cells but not in hepatocytes, and brownish nuclear staining by the antibody Ki-67 in a small number of bile duct epithelial cells (*arrows*)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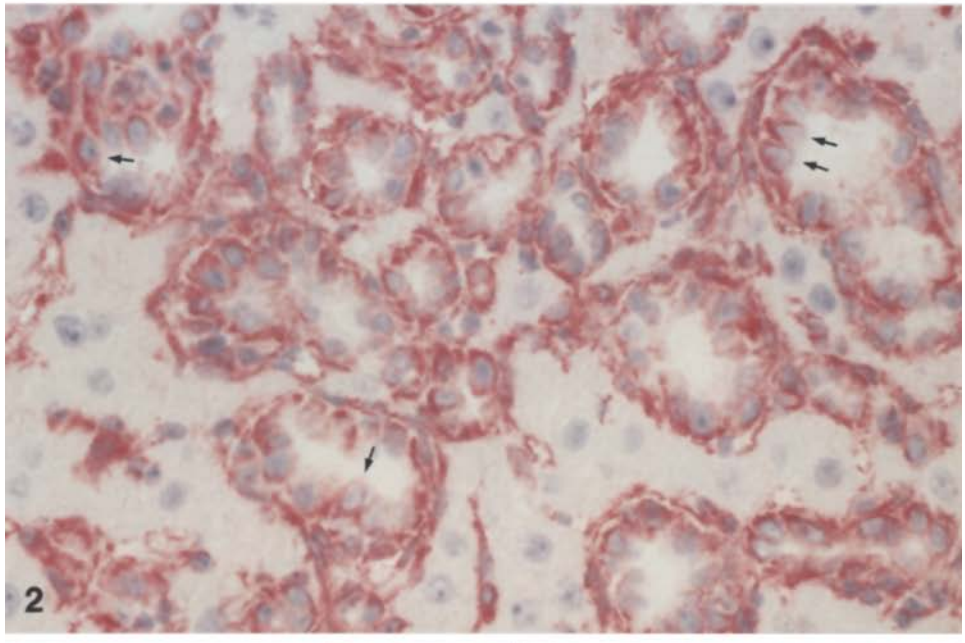
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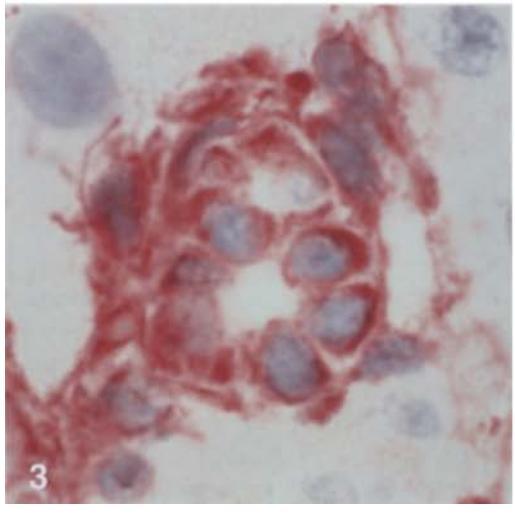
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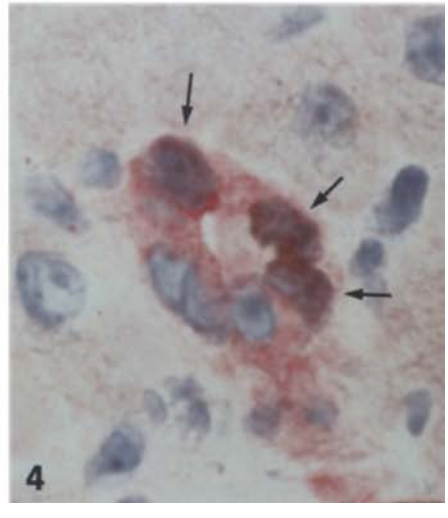
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was limited to bile duct epithelial cells; newly formed bile ducts were clearly labelled (Fig. 4). The growth fraction was evaluated on sections simultaneously labelled with antibodies Ki-67 and CK7. Approximately 8% of the hepatocytes in all locations of the lobule displayed nuclear staining with the antibody Ki-67, whereas only few epithelial cells (1–2%) of the newly formed bile ducts showed both Ki-67 and CK7 immunoreactivity.

Discussion

We have demonstrated the expression of the intermediate filaments vimentin and cytokeratin in newly formed bile ducts. Previously, a similar coexpression has been reported for certain types of tumours, such as renal cell carcinoma (Holthöfer et al. 1983), thyroid carcinoma (Henzen-Logmans et al. 1987), or pleomorphic adenoma of salivary glands (Achtstätter et al. 1986). Coexpression is also seen in certain normal cell types, like myoepithelial cells of the parotid gland (Born et al. 1987), and in proliferating and regenerating human and rat renal tubular epithelial cells (Gröne et al. 1987). Thus our findings strongly support the view that vimentin may be associated with certain differentiation and growth processes in some epithelia. However, the low percentage of Ki-67 positive bile duct epithelial cells observed in small ductules indicates a rather small growth fraction. Therefore, although proliferation may coincide with vimentin-associated differentiation, vimentin cannot be considered a proliferation marker per se, but may rather be indicative of cellular reorganization. Vimentin has been observed in various types of epithelial cells in vitro and its expression was thought to be related to intercellular and cell-matrix interactions (Ben Ze'ev et al. 1984). Since a massive production and deposition of extracellular matrix (basement membrane proteins) was observed around expanding bile ducts after common bile duct ligation and scission (Milani et al., submitted work), it is conceivable that this material may influence intermediate filament expression patterns in newly formed bile duct epithelial cells or vice versa.

The exclusive reactivity of the antibody CK7 with rat bile duct epithelial cells points to homologous cytokeratin expression patterns in rat and human liver. Human hepatocytes contain cytokeratin types 8 and 18, and human bile duct epithelial cells additionally express cytokeratin types 7 and 19 (Van Eyken et al. 1987). Recent studies suggest that the development of bile ducts in embryonal liver (Van Eyken et al. 1988a) and in chronic liver disease (Desmet 1985) is associated with an increasing expression of bile duct-specific cytokeratins in some hepatocytes along a metaplastic differentiation pathway towards bile duct epithelial cells.

Such gradual changes, however, were not visible in our model of secondary biliary fibrosis in adult rats. At all stages, distinct intermediate filament expression patterns were found in hepatocytes and bile duct epithelial cells. Vimentin was expressed in epithelial cells of newly formed bile ductules, but not in hepatocytes. CK7 immunoreactivity was detected exclusively in cells with the histomorphological characteristics of bile duct epithelial cells. Also, there was no morphological evidence for cholestatic rosette formation by hepatocytes, or for any colocalization of hepatocytes and bile duct epithelial cells in the same ductular formation. Therefore, our findings are well in line with the hypothesis that the proliferation of biliary ductules secondary to complete bile duct obstruction may be due to the transformation of pre-existing structures, such as canals of Hering (preductules) into bile ductules (Schaffner and Popper 1961; Carruthers et al. 1962). Alternatively, metaplasia of hepatocytes into bile duct epithelial cells may explain the increased numbers of bile duct cells, but then the expression of CK7 immunoreactivity, vimentin, and basement membrane proteins (Milani et al., submitted work) must constitute a very late event secondary to the acquisition of the histomorphological character of bile duct epithelial cells. Furthermore, derivation of the proliferating bile duct epithelial cells from oval cells can be considered. After carcinogen administration, these cells appear to arise from undifferentiated hepatic stem cells (Farber 1956; Sell et al. 1981) and to differentiate into hepatocytes. It has been suggested that duct-like structures observed in carcinogen treated rat livers may also be derived from oval cells. As shown by Dunsford et al. (1985), however, the duct-like structures produced during chemical hepatocarcinogenesis are most likely derived from pre-existing bile ductules.

Although the morphology of the model described here does not parallel human secondary biliary fibroses in all details, it may be particularly useful for studying the formation of new bile ducts in the absence of almost any inflammatory infiltrate. In clinical situations of chronic major bile duct obstruction, formations of bile ductules have been observed at the margins of portal tracts, termed "marginal duct proliferation", and considered to be due to an outgrowth of pre-existing ductules (Christoffersen and Poulsen 1970; Desmet 1985). In contrast, bile duct generation by ductular

metaplasia has been suggested to be restricted to certain types of chronic cholestasis accompanied by inflammation, such as incomplete bile duct obstruction, primary biliary cirrhosis, primary sclerosing cholangitis, and chronic inflammatory liver diseases (Desmet 1985; Van Eyken et al. 1988b). The liver appears to have the potential to generate new bile ducts by different mechanisms. It is therefore conceivable that our observations concerning the pattern of vimentin and cytokeratin 7 expression, if confirmed in human liver, may prove useful in distinguishing bile duct formations secondary to complete and abrupt obstruction of large bile ducts from those associated with chronic inflammatory liver disease.

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