American Journal of Pathology, Vol. 127, No. 3, June 1987

Keratin Polypeptides in Malignant Epithelial Liver Tumors

Differential Diagnostic and Histogenetic Aspects

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Five monoclonal antibodies recognizing different keratin polypeptides in immunoblotting or different epithelial cell types in complex tissues were studied for their suitability as reagents for the differential diagnosis of primary and secondary malignant epithelial liver tumors. The broad specifity keratin antibodies lu-5 and KL-1 stained all epithelial liver neoplasms. In contrast the antibodies CK-7 (Ker-7-specific), CK-2 (Ker-18-specific) und KA-4 (Ker-19-specific in liver) allow these neoplasms to be divided into three groups: 1) Hepatocellular carcinomas were CK-2-positive and CK-7-negative. 2) Cholangiocellular carcinomas, liver metastases of extrahepatic bile duct carcinomas, liver metastases of a ductal carcinoma of breast, and a follicular thyroid carcinoma were stained positively by CK-2, CK-7, and KA-4. In 1 of 6 hepatocellular carcinomas neoplastic hepatocytes were focally labeled by KA-4. In a focal nodular hyperplasia of the liver modified hepatocytes were decorated not only by CK-2 but also by CK-7 and KA-4. 3) Liver metastases of colorectal adenocarcinomas and of a carcinoid tumor were stained positively by CK-2 and KA-4 but not by CK-7. (Am J Pathol 1987, 127:530–537)

MALIGNANT epithelial tumors of the liver can be subdivided into liver carcinomas and metastases. The distinction between the two groups is often difficult by means of conventional histologic staining. In addition, when a metastasis is confirmed, the location of the primary tumor has to be determined. Detection of the primary tumor usually involves the use of costly and invasive methods; however, in many cases, even then, the primary tumor cannot be found.

Intermediate filament (IF) typing yields information about the histogenetic origin of tumor,[1–3] because tumors continue to express the major IF type characteristic of the cell of origin. Epithelial tissues as well as carcinomas express the keratin IF type, and in man 19 individual keratins can be characterized by their differing molecular weights and isoelectric points on two-dimensional gels. Normal epithelia from various tissues contain characteristically different, sometimes overlapping keratin subsets. Adenocarcinomas and their metastases in general conserve the keratin subset present in the epithelial cell type from which they originate.[4,5] In contrast, the polypeptide expression of squamous cell carcinomas may differ from that seen in normal keratinocytes.[6] The development of a set of keratin antibodies each member of which recognizes only one (or a few) of the 19 keratin polypeptides allows the spectrum of keratin polypeptides present in normal epithelia and in carcinomas to be investigated and catalogued by immunohistologic techniques, rather than by two-dimensional gel electrophoresis.[7–11] Recently, using such a set of monoclonal antibodies specific for either keratin 7, or 8, or 18, or 19 to characterize gastrointestinal tumors, we showed that keratin 7 and keratin 19 antibodies appeared to be useful in differential diagnosis.[12] Here we document with a similar set of keratin monoclonal antibodies the usefulness of this approach in the differential diagnosis of primary and metastatic carcinomas in the liver.

Accepted for publication January 29, 1987.

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Materials and Methods

Biopsies from 19 tumors were obtained during surgery, and 6 other tumors were obtained from autopsy material. One part of each tumor was fixed in formaldehyde, embedded in paraffin, and stained with hematoxylin and eosin (H&E) for histologic classification. The other part was frozen in liquid nitrogen and stored at −70 C.

Immunohistochemical Techniques

Immunologic reactions were detected either with the alkaline phosphatase–anti-alkaline phosphatase technique (APAAP) or with the avidin–biotin method (ABC). Cryostat sections approximately 4–7 μ thick were prepared, dried for 15 minutes at 37 C, and fixed in acetone for 15 minutes. Specimens were further fixed at −10 C in chloroform for 15 minutes and then washed with Tris-buffered saline (TBS, 0.05 M Tris, 0.10 M NaCl, pH 7.6). Sections were incubated with the first antibody for 30 minutes at 20 C. After washing with TBS the appropriate second antibody was added. For the APAAP technique rabbit anti-mouse IgG followed by the APAAP complex (Dako, Klostrup, Denmark) was used. The APAAP complex was visualized with fast red. For the ABC method biotinylated rabbit-anti-mouse IgG was added, followed by the avidin–biotinlylated peroxidase complex (Vector laboratories, Burlingame, Calif). Sections were counterstained with Meyer's acid hemalum.

Antibodies

The mouse monoclonal antibodies used were as follows: *Kl-1*, a broad-specifity monoclonal keratin antibody raised against keratin polypeptides of 55–57,000 daltons[13] (Dianova, Hamburg, FRG); lu-5, a broad-specificity monoclonal keratin antibody,[16] obtained from Dr. C. Staehli, Central Research Division, Hoffmann La Roche, Basle, Switzerland; CK-7, a monoclonal antibody specific for keratin polypeptide 7[10] (Amersham International; Boehringer GmbH, Mannheim, FRG); CK-2, a monoclonal antibody specific for keratin polypeptide 18[7,8] (CK-2 or CK-4, which has the same specificity, can be purchased from a number of different commercial sources); KA-4, a monoclonal keratin antibody with high specificity for keratin polypeptide 19[14] (a recent study has shown that KA-4 reacts not only with keratin 19 but also less strongly with keratins 14, 15, and 16[15]; this antibody was a gift from Dr. R. Nagle, of the University of Arizona). Monoclonal antibodies were used as hybridoma supernatants, with the exception of KA-4, which was used as a 1:300 dilution of ascites fluid.

Results

The reactivities of the monoclonal antibodies used in the study on human liver and on human liver tumors are summarized in Table 1. Hepatocytes, bile duct epithelial cells, and all the tumors investigated were positively stained by the pan-epithelial keratin antibodies lu-5 and KL-1. However, different epithe-

Table 1—Use of Monoclonal Antibodies Specific for Individual Keratin Polypeptides to Characterize Normal Liver and Liver Tumors

		Keratin monoclonal antibodies					
		KL-1	lu-5				
	No. of cases (biopsy/autopsy)	Most keratin polypeptides		CK-7 Ker7	CK-2 Ker18	KA-4 Ker19	Keratin polypeptides from gels[5]
Normal liver tissue							
Hepatocytes	10 (8/2)	+	+	−	+	−	8,18
Bile duct epithelia	12 (10/2)	+	+	+	+	+	7,8,18,19
Primary liver tumors							
Nodular hyperplasia of liver	1 (1/0)	+	+	*	+	*	
Hepatocellular carcinoma	6 (4/2)	+	+	−	+	−/‡	8,18
Cholangiocarcinoma	4 (3/1)	+	+	+	+	+	7,8,18,19
Liver metastases							
Bile duct carcinoma	2 (2/0)	+	+	+	+	+	(7),8,18,19
Ductal breast carcinoma	1 (1/0)	+	+	+	+	+	
Follicular thyroid carcinoma	1 (0/1)	+	+	+	+	+	
Carcinoid of large bowel	1 (1/0)	+	+	−	+	+	
Colorectal adenocarcinoma†	10 (8/2)	+	+	−	+	+	8,18,19

+, positive staining; −, no stain.
*Bile ducts 100% positive, hepatocytes mostly negative, but some focally positive areas for CK-7 and KA-4 were detected.
†Including two tumors transplanted to nude mice.
‡In 1 biopsy case 5% of tumor cells stained positively with KA-4.

lial cell types in normal liver, as well as different tumor types, could be distinguished when monoclonal antibodies specific for particular keratin polypeptides were used. In normal liver, hepatocytes were decorated by antibody CK-2 specific for keratin 18, while bile duct epithelia stained positively with the CK-2 antibody, with the CK-7 antibody specific for keratin 7, and with the KA-4 antibody, which recognizes keratin 19 with high specificity. When primary liver tumors were studied, 5 of 6 hepatocellular carcinomas (HCCs) were stained only by the CK-2 antibody specific for keratin 18 and not by the CK-7 antibody specific for keratin 7 or the KA-4 antibody, specific for keratin 19 (Figure 1A). In one of the 6 HCC specimens the antibody KA-4 yielded a positive focal staining in some tumor areas. Four specimens of cholangiocellular carcinoma of the liver were strongly positively stained by the antibodies CK-7 (Figure 1B), CK-2 (Figure 1C), and KA-4 (Figure 1D). Thus, the CK-7 antibody appears able to distinguish hepatocellular carcinomas which are not stained from cholangiocellular carcinomas which are stained.

Metastases to the liver from gastrointestinal carcinomas of known origin, as well as metastases to the liver of single examples of a carcinoid of the large bowel and of ductal breast carcinoma were studied with the keratin monoclonal antibody panel. All tumors were positive with the KA-4 and CK-2 antibodies. KA-4- and CK-2-positive staining of tumor cells in a liver metastasis from a bile duct carcinoma is shown in Figure 2A and B, respectively, and CK-2-positive staining of a metastasis from a colorectal adenocarcinoma is shown in Figure 2C. KA-4 and CK-2 positivity of the tumor cells of a liver metastasis from a follicular thyroid carcinomas is shown in Figure 2D and E, respectively. The CK-7 antibody stained tumor cells in the liver metastases from the bile duct carcinoma, from the ductal breast carcinoma, and from the follicular thyroid carcinoma. However, CK-7 antibody did not stain tumor cells in the liver metastases of the carcinoid from large bowel, or in liver metastases from eight colorectal adenocarcinomas. Two colorectal adenocarcinomas were also tested after they had been transplanted onto nude mice for 15 weeks, and the tumor cells were still negative when tested with the CK-7 antibody. The keratin content of the metastatic tumors is summarized in Table 1. Note that the liver metastasis of a carcinoid of the large bowel showed a keratin spectrum similar to that seen with the colorectal adenocarcinomas, as judged by our immunohistochemical assays.

We also examined one focal nodular hyperplasia of the liver. Nearly all hepatocytes of the liver nodules were positively stained by the CK-2 antibody (Figure 3A) and not by the KA-4 (Figure 3B) and CK-7 antibodies. Unexpectedly, however, a few hepatocytes in this material were, in addition, positively stained by the antibodies CK-7 (Figure 3C), and KA-4, antibodies which in normal liver never stain hepatocytes (Figure 2D and Table 1). Hepatocytes positive with the CK-7 antibody were mostly found in small groups located at the periphery of the nodules. Ductular proliferations, mostly separate from larger bile ducts, could by detected in the center of the liver nodules, by hematoxylin-eosin staining (Figure 3D). Single KA-4- and CK-7-positive cells or small ductular proliferations were often found in the center of the nodules (Figure 3C). The CK-7 and KA-4 antibodies decorated these apparently abnormal hepatocytes less strongly than bile duct epithelia.

Table 1 summarizes the results obtained by immunohistochemical methods on epithelial cells in normal liver, on primary liver tumors, and on tumors of known origin metastatic to the liver. Note the agreement of the results obtained by immunohistochemical techniques with the keratin polypeptide content of tumors of the same type determined by Moll and Franke by two-dimensional gel electrophoresis.[5]

Discussion

When malignant epithelial liver tumors are classified, three differential diagnostic questions have to be answered: 1) the discrimination between primary and secondary liver tumors; 2) the differential diagnosis of primary liver carcinomas; 3) the determination of the primary tumor location when liver metastases are found. In this study we have shown that the immunohistologic detection of single keratin polypeptides by means of monoclonal antibodies can help solve these diagnostic problems.

The antibodies KL-1 and lu-5 are broad-specificity keratin antibodies recognizing the different keratin polypeptides present in a wide variety of normal epithelia and carcinomas.[13,16] Both antibodies positively stained all the tumors listed in Table 1 as well as hepatocytes and bile duct epithelia in normal liver. Thus, although both antibodies are ery useful reagents with which to confirm the epithelial nature of the neoplasms, and although positive staining with such antibodies excludes metastases of sarcoma or melanoma, they have no diagnostic relevance when it comes to the subdivision of malignant epithelial liver tumors. In contrast, the CK-7, CK-2, and KA-4 antibodies allowed primary and metastatic liver neoplasms to be divided into three groups. In the first group hepatocytes and hepatocellular carcinomas

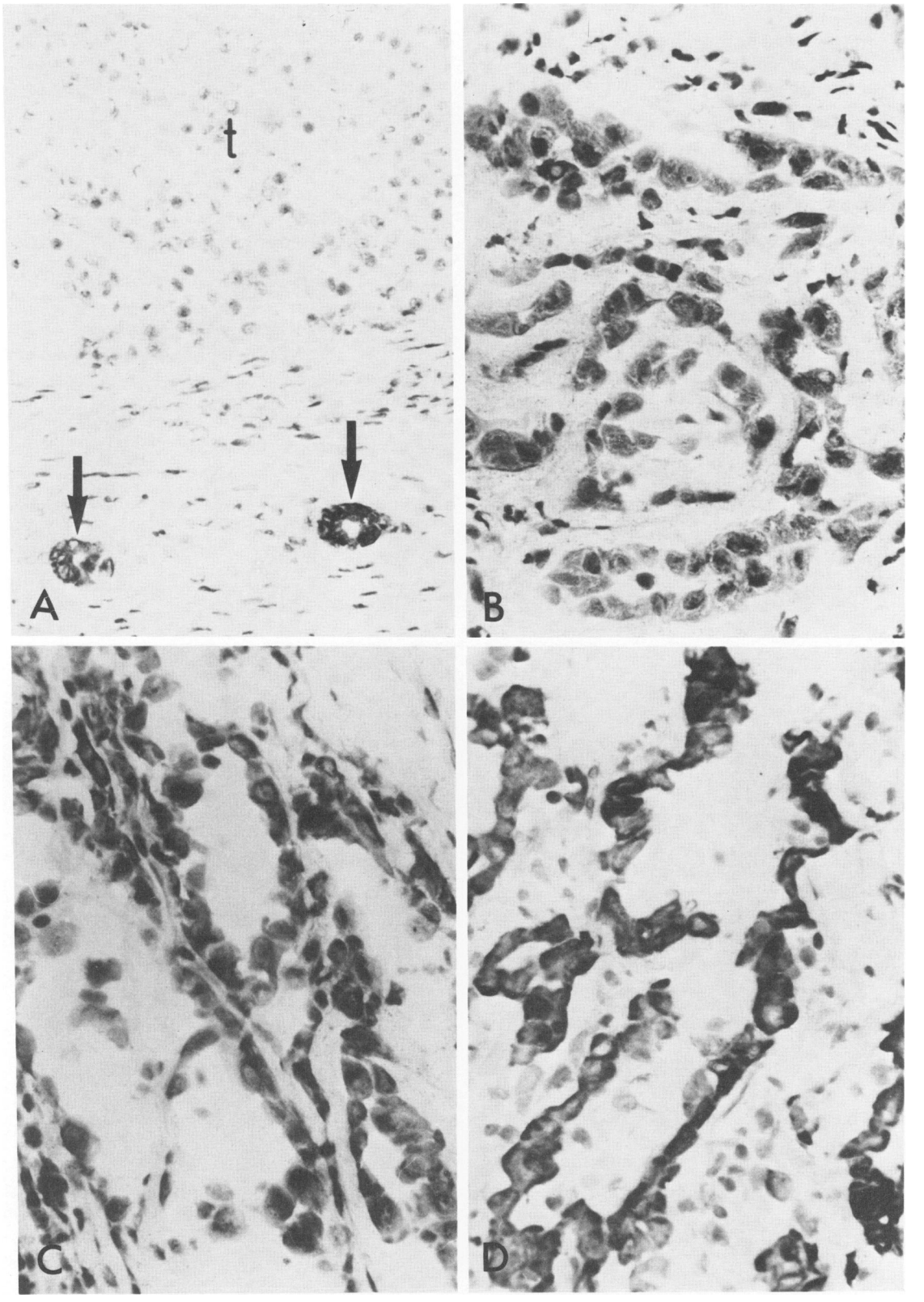

Figure 1—Primary liver carcinomas stained with monoclonal antibodies specific for keratin polypeptides. CK-7 specific for keratin 7 does not stain tumor cells in hepatocellular carcinoma (*t*) but stains bile ducts (*arrows*) (**A**). CK-7 (**B**), CK-2 specific for keratin 18 (**C**), and KA-4 (**D**) with high specificity for keratin 19 stain cholangiocarcinoma positively. (APAAP, **A**, ×200; **B–D**, ×320)

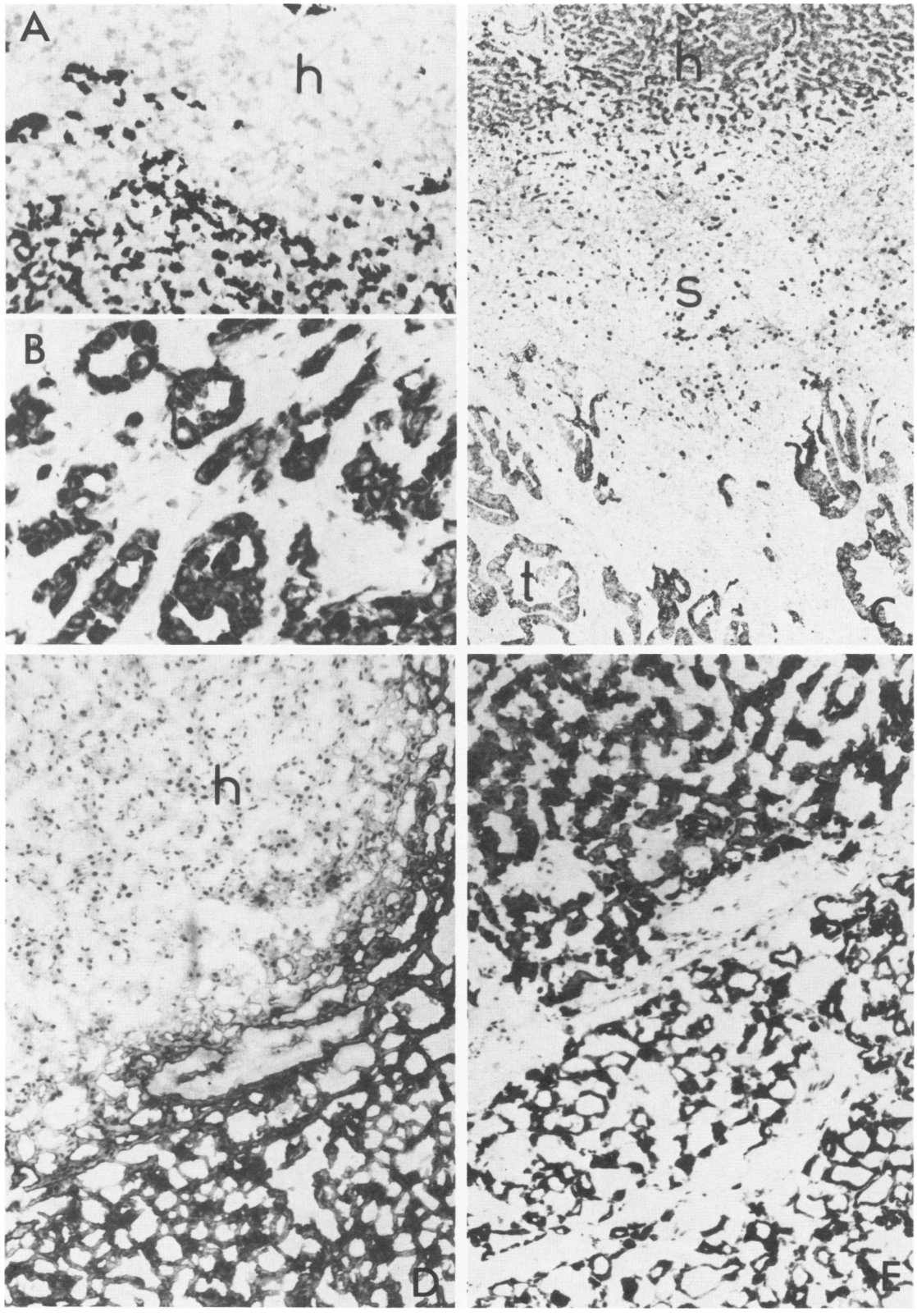

Figure 2—Carcinomas metastatic to the liver stained with monoclonal antibodies specific for individual keratin polypeptides. KA-4 decorates a metastasis of a bile duct carcinoma but does not stain hepatocytes (*h*) (**A**). CK-2 reacts with a bile duct carcinoma (**B**). CK-2 stains a colorectal adenocarcinoma (*t*) as well as hepatocytes (*h*) and bile ducts (**C**). KA-4 (**D**) and CK-2 (**E**) react with a follicular thyroid carcinoma, but KA-4 does not stain hepatocytes (*h*). (ABC, **A**, ×32; **B**, ×200; **C**, ×32; **D** and **E**, ×80)

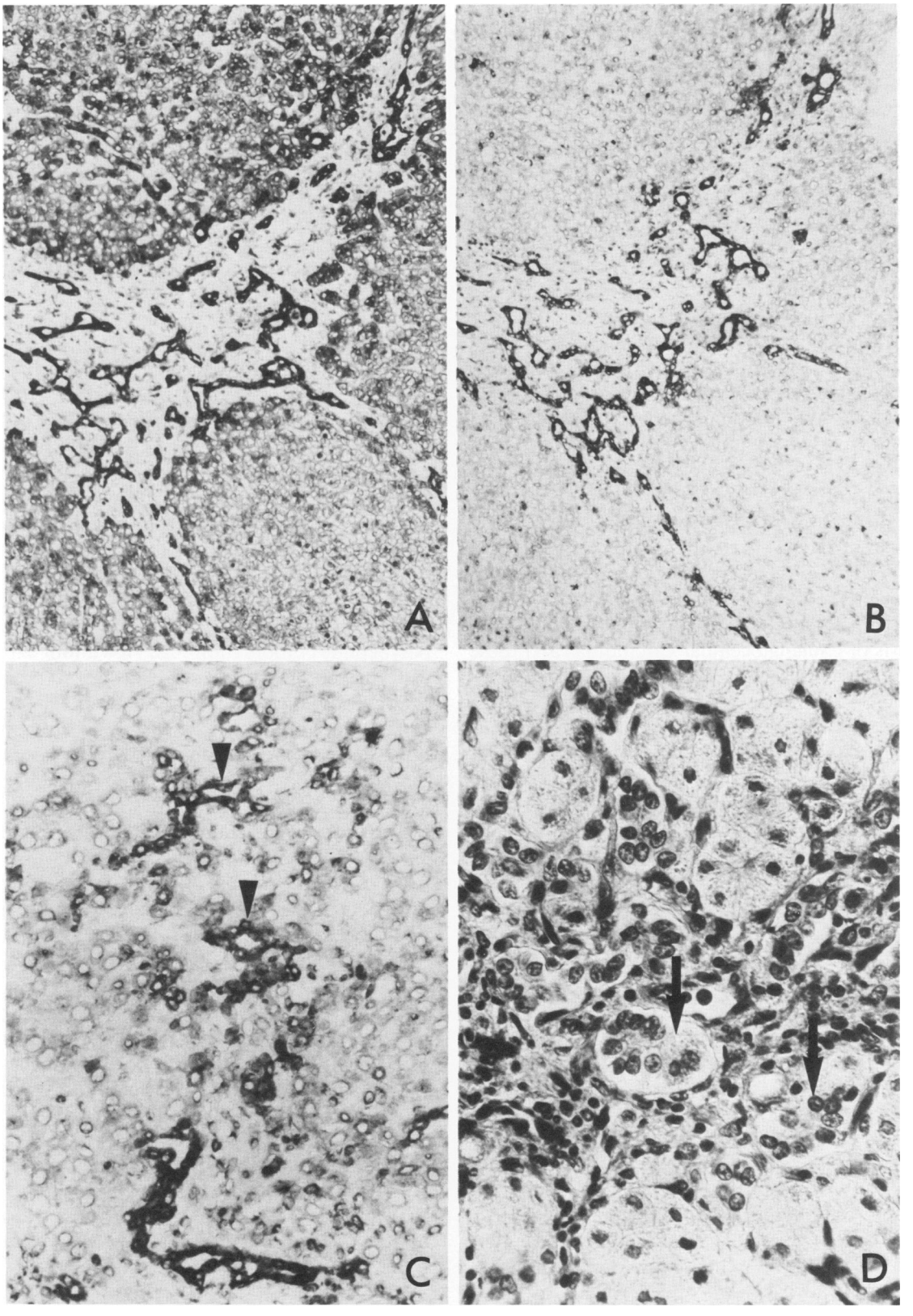

Figure 3—Focal nodular hyperplasia of liver decorated with monoclonal antibodies specific for individual keratin polypeptides. CK-2 stains hepatocytes as well as bile duct proliferation (**A**). KA-4 marks only bile ducts (**B**). CK-7 reacts with small groups of hepatocytes in the center of the liver nodules. Note early stages of ductular modification (*arrow*) (**C**). H&E staining shows ductular transformation of hepatocytes in **D**. (**A–C**, ABC, **A** and **B**, ×80; **C**, ×200; **D**, H&E, ×500)

were positive with the CK-2 antibody, negative with the CK-7 antibody, and, with the exception of one hepatocellular carcinoma where focal staining with the KA-4 antibody was seen, negative with KA-4. The keratin complement found by immunohistologic methods is consistent with the keratin 8 and 18 positively determined by two-dimensional gels.[4,5,17] In the second group, bile duct epithelial cells and cholangiocellular carcinomas stained positively with the CK-7, CK-2, and KA-4 antibodies, ie, were positive for keratin 7, 18, and 19. Again, for cholangiocellular carcinomas, the keratin complement determined by immunohistochemical methods is consistent with the keratin 7, 8, 18, and 19 positively determined by gel electrophoresis. Liver metastases from extrahepatic bile duct carcinomas were also positive with the CK-2, CK-7, and KA-4 antibodies, as were ductal carcinomas of pancreas.[12] In addition, the majority of adenocarcinomas of endometrium and ovary as well as adenocarcinomas of the lung have a similar keratin complement from gel electrophoresis.[5] The liver metastasis of a ductal breast carcinoma contained the same spectrum of keratin subtypes corresponding to the keratin polypeptides found in most ductal and lobular breast carcinomas.[5,18] Thus, a differential diagnosis of cholangiocellular carcinomas and these metastatic liver tumors is not possible by keratin typing, because all these tumor types appear to express the same keratin polypeptide spectrum. However, tumors in the third group, ie, the liver metastases of all 10 colorectal adenocarcinomas we examined and of the carcinoid tumor of large bowel reacted with the CK-2 and KA-4 antibodies but were not decorated by the CK-7 antibody. The same keratin polypeptides are found in normal epithelial of small and large bowel.[5] The negative reaction with the CK-7 antibody and the positive staining with the KA-4 antibody allows colorectal carcinomas to be distinguished from all other tumors in our study. Here, also, however, it has to be noted that keratin polypeptide 7 is expressed heterogeneously in a few ductular breast carcinomas and is absent in some adenocarcinomas of ovary and endometrium.[5]

Differences in keratin polypeptide content between hepatocellular carcinoma and cholangiocarcinoma have been noted not only in this but also in other studies.[5,12,19] Although the results have usually been interpreted as implying an origin of cholangiocellular carcinoma from bile duct epithelial cells, the situation may be more complex. One of our HCCs contained small areas focally labeled by the KA-4 antibody. The nodular hyperplasia of liver likewise showed some CK-7- and KA-4-positive hepatocytes, and in some instances ductular transformation was also noted. In this situation it seems that hepatocytes can express additional keratin polypeptides normally seen only in bile duct epithelia. Possibly the focal expression of the keratin polypeptide reacting with the KA-4 antibody in HCC is a first step of differentiation or metaplastic transformation which later can result in cholangiocarcinoma. Ductular differentiation of hepatocytes has been observed in other instances. In fetal liver, for example small bile ducts develop from primitive hepatocytes.[20,21] In liver dystrophia, as well as in focal hyperplasia, ductular structures can be observed in connection with hepatocytes which are far from preformed bile ducts.[22,23]

Our results show that the transition from hepatocytes to bile duct epithelia is accompanied by a distinct program of keratin polypeptide expression. Thus, the relatively simple combination of keratin polypeptides characteristic of hepatocytes and most hepatocellular carcinomas, ie, 8 and 18, appears to be followed by the additional expression of keratin 19, which we observed focally in one hepatocellular carcinoma. In nodular hyperplasia some modified hepatocytes begin to synthesize polypeptide 7, and this seems to be the last step before duct formation characterized by expression of 7, 8, 18, and 19. Thus far, the polypeptide combination 7, 8, 18 without expression of 19 has not been observed.

Some investigators assume that cholangiocellular carcinomas as well as some cholangiocarcinomas are more or less metaplastic HCCs.[22-26] The 1 HCC that showed focal reactivity with KA-4 might support this hypothesis. Then, if this point of view is further extended, cholangiocellular carcinoma could originate also from hepatocytes.

Although the number of tumors investigated is still rather small, there appears to be a high diagnostic value to immunohistochemical staining of liver tumors with antibodies specific for keratins 7, 8, and 19.

Our conclusions are as follows: 1) These antibodies allow the differential diagnosis of cholangiocarcinomas and most hepatocellular carcinomas. 2) Discrimination between extrahepatic and intrahepatic bile duct carcinomas as well as of the majority of adenocarcinoma metastases, eg, of breast, pancreas, or lung carcinomas, is not possible. 3) The keratin pattern characteristic of primary liver tumors and of liver metastases of breast, pancreas, or lung carcinomas differs from that of colorectal adenocarcinomas.

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