

Clinical Observations

## THE EXPRESSION OF CYTOKERATINS IN HUMAN HEPATOCELLULAR AND CHOLANGIOCELLULAR CARCINOMAS\*

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In order to determine the usefulness value of the antibodies to cytokeratins (CK) of "bile duct type" in the differential diagnosis between hepatocellular carcinoma (HCC) and cholangiocellular carcinoma (CC), we have made an immunocytochemical investigation, using the antibodies specifically recognizing CK19 and CK18, separately, in liver, and laminin (LN) antibody. All the CC examined (10 cases) were found CK19-positive; interestingly, CK19-positive cancer cells were also observed in 38% of HCCs (14/37). Therefore, CK19 was not a reliable marker in differentiating HCC from CC, in our consideration. The CK19 expression in HCC was showed to be irrelevant to their differentiation degree, but related to the histologic subtypes which indicated the directions of their differentiation. CK19 expression was observed in all the HCC cell nests with glandular differentiation, and an uncontinuous LN-positive basement membrane-like structure was immunolocalized around these cells, which indicated that the glandular differentiation and CK19 expression in HCC were also related to the LN deposition, as in fetal liver and some chronic liver disorders.

**Key words:** Cytokeratin, Laminin, Differentiation, Hepatocellular carcinoma, Cholangiocellular carcinoma, Human.

In normal adult liver, hepatocytes express only 2 kinds of cytokeratin (CK) polypeptides—CK18 and CK8, and bile duct (BD) epithelial cells contain CK19 and CK7 in addition to CK18 and CK8.<sup>1</sup> The difference can be clearly recognized by immunohistochemistry with CK19 monoclonal antibodies (MAb) or polyclonal antibodies (PAb) to epidermal keratin (EK),<sup>2</sup> hepatocytes are negative, but BD epithelium is positive.<sup>1-4</sup> Furthermore, this difference has been traced back to the early stage of the morphogenesis of intrahepatic BD in embryos.<sup>4,5</sup> Many authors<sup>6-8</sup> suggested that the CK patterns of hepatocytes and BD epithelium could be maintained in hepatocellular carcinoma (HCC) and cholangiocellular carcinoma (CC). However, the results of Bannasch et al.<sup>9</sup> and Eyken et al.<sup>10</sup> did not support this suggestion. In this research, we have demonstrated that CK19 could also be expressed in HCC. The significance and mechanism of "the abnormal CK expression" was discussed.

Accepted June 23, 1994.

\*This project partly supported by The National Natural Science Foundation of China (NSFC 3880376 and 39470778).

## MATERIALS AND METHODS

### Tissue Specimens

Tissue specimens were collected from the biopsy file of Xijing Hospital. All the tissues were fixed with 10% of buffered formalin, routinely embedded with paraffin and sections of 4  $\mu$ m in thickness were prepared.

### Immunohistochemistry

The first antibodies used in this research included a MAb (K174) and a PAb (RAK1) against EK which could specifically recognize CK19 in liver, a MAb (HK2) to CK18<sup>2</sup> and a PAb against laminin (LN).<sup>5,11</sup> ABC immunohistochemical staining was carried out as we described previously.<sup>2</sup>

## RESULTS

Altogether 47 cases of primary liver cancer (37 cases of HCC and 10 cases of CC) were examined. HCC tissues were graded according to the suggestions of Edmondson and typed according to the standards of WHO.

In all CCs examined, all cancer cells were showed positive for CK19 and CK18 (Figure 1, 2), even the mitotic cells were also CK19-positive (Figure 3). In 38% of HCCs (14/38), CK19-positive cancer cells were also observed. No difference of statistical significance was found among the cancer tissues of different grades ( $\chi^2=0.3894$ ,  $P>0.05$ ) (Table 1). In HCCs of Edmondson grade II and III, the cancer tissue with glandular differentiation was CK19-positive. The intensity seemed to vary with the differentiation grade, and the positivity was stronger in the cancer tissue of Edmondson grade II than in the tissue of grade III ( $P<0.01$ , Mann-Whitney test) (Figure 4, 5).

The immunostaining in the neighbouring sections with CK19 antibody and LN antibody seperately demonstrated that the CK19-positive tissue of HCC with glandular differentiation were enclosed by LN-positive basement membrane-like structure and fibrotic tissue,

some of the cancer cells were also LN-positive (Figure 6, 7). CK19-positive cancer cells were also observed in 7 of the 17 cases of trabecular type of HCC, which included 2 cases of wide-trabecular type and 5 cases of fine-trabecular type. But the CK19-positivity was much weaker and the positive cells was much less than in the glandular type of HCC. The positive cells were found scattered near the fine septa of LN-positive fibrotic tissue (Figure 8–10). The positive products was observed at or beneath the cell membrane. No CK19-positive cells were observed in 2 cases of clear-cell type of HCC. In 2 of 4 cases of HCC of Edmondson grade IV, some spindle cancer cells were found positive for CK19.

All of 37 cases of HCC were found CK18-positive.

Table 1. CK19 expression in HCC of 37 cases

Edmondson grade	Cases examined	Cases positive for CK19 (%)
II	15	5 (33)
III	18	7 (39)
IV	4	2 (50)
Total	37	14 (38)

## DISCUSSION

It has been demonstrated that CC has same CK composition with normal BD epithelium and is positive for EK antibody.<sup>6, 7</sup> Similarly, many authors reported that HCC was EK-negative, and suggested that HCC might have the same CK pattern with the hepatocytes in normal condition.<sup>6-8</sup> This hypothesis was supported by the results of biochemical analysis by Denk et al.<sup>6</sup> However, Bannasch et al. (1981) reported that some HCCs of trabecular type were EK-positive.<sup>9</sup> Similarly, the immunostaining with CK MAbs by Eyken et al. (1988) showed some well-differentiated HCCs was positive for CK19 and/or CK7, and all of 7 cases of poorly-differentiated HCCs were CK19-positive.<sup>10</sup> In this research, we have demonstrated that CK19 was

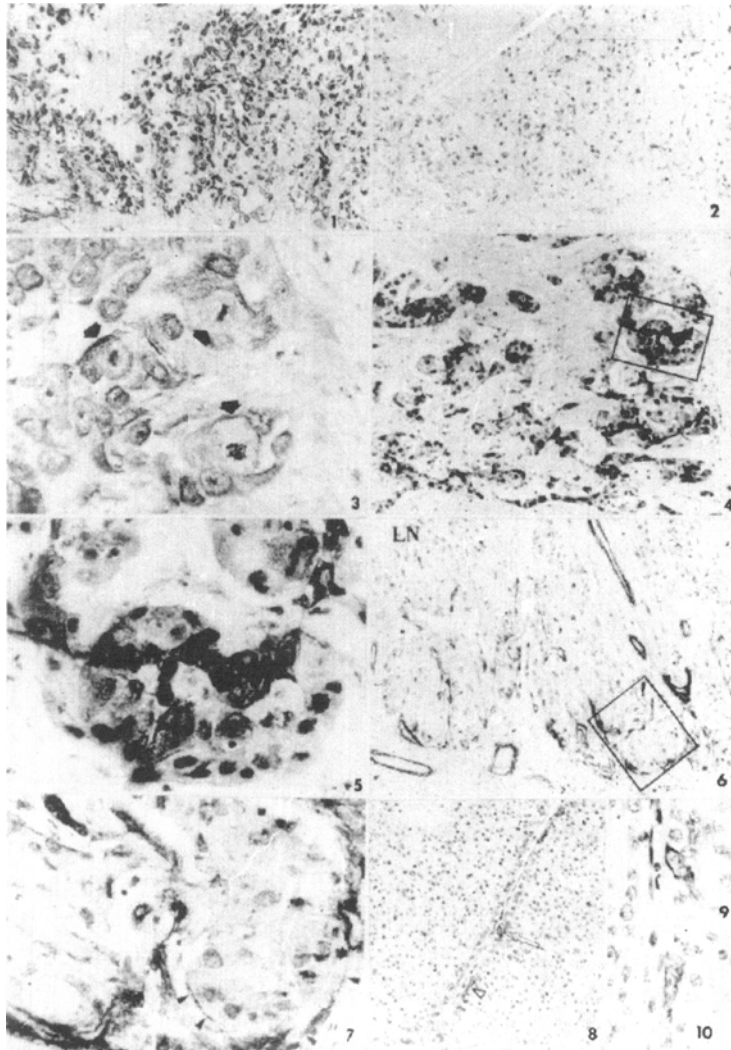


Fig. 1–3. Moderately differentiated CC. Fig. 1, 2. The same area of the neighbouring sections incubated with RAK1 and with the preimmune serum in same dilution, respectively. ( $\times 10$ ) Fig. 3. Mitotic cancer cells (dark arrow) are also CK19-positive. RAK1 ( $\times 40$ )

Fig. 4, 5. HCC of grade II, labelled with K174. Fig. 4. The glandular area are CK19-positive. ( $\times 10$ ) Fig. 5. Detail of the square-marked area in Figure 4. ( $\times 40$ )

Fig. 6, 7. Similar area in the adjacent section as Figure 4, labelled with LN antibody. Fig. 6. ( $\times 10$ ). Fig. 7. Detail of the square-marked area in Figure 6. The dark arrowheads denote LN-positive basement membrane-like structure. ( $\times 4$ )

Fig. 8–10. HCC of grade II, wide-trabecular type, labelled with RAK1. Fig. 9 ( $\times 10$ ) and Fig. 10 ( $\times 40$ ) are the local magnification of two paraseptal CK19-positive cells marked by an empty arrow and an empty arrowhead, respectively, in Figure 8 ( $\times 10$ ). All the sections above are stained with ABC method and counterstained with hematoxylin.

expressed in 38% of HCCs (14/37), in accordance with the results of Bannasch et al.<sup>9</sup> and Eyken et al.<sup>10</sup> We suggested, therefore, that CK19 expression could not be used as a reliable marker in the differential diagnosis between HCC and CC. The contradiction in the literature listed above might be caused, in our consideration, by the fact that both the two-dimensional electrophoresis analysis by Denk et al.<sup>6</sup> and the immunostaining by most investigators in the past were carried out with only several cases of specimens.

CK19 expression was observed in this research in HCCs of various grades. No definite relationship was found between it and the differentiation degree. In HCCs of Edmondson grade II and III, however, CK19 expression was found to be related to the histologic patterns which indicated the directions of differentiation of the cancer cells. The fact that HCC cells with glandular differentiation can express CK19, a "BD type" CK, imply that some cancer cells in HCC could indeed differentiate towards BD, as the ductular metaplasia of hepatocytes in some benign liver disorders<sup>11, 12</sup> and the morphogenesis of intrahepatic BD in embryonic livers.<sup>4, 5</sup>

It was suggested by Goodman et al. that HCCs of glandular type might belong to hepatocellular cholangiocellular combined carcinoma.<sup>13</sup> But the clinical manifestations and the prognosis of this type of "combined carcinoma" is different from the classical combined carcinoma. So we thought that this suggestion might be a little farfetched and of no remarkable significance for the practice of surgical pathology.

The growth and differentiation of cancer cells is determined mainly by their genetic characteristics, but more or less they are still responsible o some changes of the environments around them. The CK19 expression in some HCCs might be an indication of this ability of the cancer cells. The immunostaining in the neighbouring sections demonstrated that most of the CK19-positive cell nests with glandular differentiation could express LN and were enclosed by LN-positive basement membrane-like structure. This indicated that HCC cells with glandular differentiation might be able to express LN receptors and response to LN. Their differentiation might be modulated by LN produced in as the develop-

ment of intrahepatic BD in embryos.<sup>5</sup>

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