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Expression of cell adhesion molecules and common acute lymphoblastic leukaemia antigen in hepatoblastoma

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Abstract Hepatoblastoma is an embryonal tumour of the liver, which often contains tissue components with multidirectional differentiation. The occurrence of cell surface antigens in this tumour has not been studied systematically, and we therefore investigated 20 hepatoblastomas for the expression of common acute lymphoblastic leukaemia antigen (CALLA) and cell adhesion molecules (CAMs) in their different tissue components. Epithelial tumour cells of fetal differentiation contained E-cadherin. This protein did not occur in tumour areas with embryonal or mesenchymal differentiation. In contrast, immature embryonal and anaplastic cells expressed CALLA and the hyaluronate receptor (HCAM, CD44). Both fetal and embryonal areas stained irregularly positive for ICAM-1, which, in contrast, was not present on anaplastic cells. Immature fibrous tissue did not contain any of these molecules except for ICAM-1. However, some cells adjacent to, or enclosed in, osteoid were positive for HCAM and NCAM. Like small undifferentiated hepatoblastoma cells, primitive mesenchymal spindle-shaped cells also expressed CALLA, HCAM, and the polysialylated embryonic form of NCAM strongly. This last is not present on other epithelial or mesenchymal tumour cells. Hepatoblastoma cells of varying differentiation express distinct patterns of CAMs and CALLA. Our results give further insight into their histogenesis and cellular interactions and may explain their variable ability for invasive growth and formation of metastases.

Key words Hepatoblastoma · Adhesion molecules · CALLA · Immunohistochemistry

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Introduction

Although hepatoblastoma is a rare neoplasm, it accounts for the majority of primary liver tumours of childhood. Histologically, hepatoblastomas imitate the spectrum of different developmental stages of the liver, since their epithelial components consist of fetal, embryonal or undifferentiated small (anaplastic) tumour cells. Many of these tumours contain mesenchymal tissue with varying differentiation. In 1967 Ishak and Glunz established the most widely used histopathological classification [15], and in 1970 Kasai and Watanabe additionally described more undifferentiated histological patterns [17] of this tumour. Since then, the hepatoblastoma has been well characterized in its light microscopic and ultrastructural appearance [1, 32]. Some investigations have shown that the occurrence of immature embryonal and undifferentiated small cell tissue in hepatoblastoma is associated with aggressive growth and development of metastases, which results in a less favourable clinical course [12, 42]. Immunohistochemical investigations have been undertaken on the expression of cytoskeletal proteins [1], especially cytokeratins [32, 38], hormonal substances [28], and extracellular matrix proteins [27]. The latter study was of particular interest to us as it demonstrated a differential expression of these molecules in hepatoblastoma, which may influence the growth and adhesion properties of different hepatoblastoma cells [27]. Membrane-bound adhesion molecules have been found to have an important role in tissue embryogenesis [5] and also in the coherence, motility and metastatic potential of malignant cells [7].

We studied whether the expression of cell adhesion molecules (CAMs) provides an explanation for the varying biological behaviour of hepatoblastomas displaying distinct patterns of histological differentiation, using immunohistochemical methods. We sought the hyaluronate receptor (HCAM, CD44 antigen) [2], since this molecule facilitates extravasation of tumour cells through binding to extracellular hyaluronic acid and has been detected on a variety of carcinoma cells [13, 36].

The intercellular adhesion molecule-1 (ICAM-1, CD54 antigen) has been found to occur in melanoma [16] and several different carcinomas [20, 31] and may increase tumour aggressiveness. Basically, this adhesion molecule is constitutively expressed on only a few cell types, such as monocytes and endothelial cells. However, it can be induced on a wide variety of cells, including hepatocytes [22], the hepatoblastoma cell line HepG2 [39] and carcinoma cells [20].

E-cadherin (cell-CAM 120/80, uvomorulin) [3] can be found on virtually all normal epithelial cells and a large number of carcinomas [6]. This molecule induces homophilic adhesion of epithelial cells and is able to prevent invasive growth and metastasis [10]. E-cadherin also has an important function in fetal liver development [9] and was therefore expected to be expressed on epithelial hepatoblastoma cells. Another mediator of cell–cell interactions is the neural CAM (NCAM, CD56 antigen) [30], which is present in developing mesenchymal tissues, on many mesenchymal tumours and on neoplasms of neuroendocrine origin [8, 11, 21]. Therefore, NCAM was assumed to be detectable in immature mesenchymal tumour components of mixed hepatoblastomas.

E-cadherin and NCAM are expressed during the regulation of mesenchymal to epithelial transition of embryonal tissues [5, 9]. Identifying these two molecules on cells of the different tumour components of hepatoblastoma promised further insight into their histogenesis [1, 29]. This is particularly true for the polysialylated embryonic form of the NCAM molecule, which was found to be re-expressed in Wilms tumour [26], neuroectodermal tumours [8, 11], and small cell lung carcinoma [18]. Additionally, we examined the hepatoblastomas for the common acute lymphoblastic leukaemia antigen (CALLA, CD10 antigen). This neutral endopeptidase [35] occurs in a variety of tissues, especially during fetal development [24], and influences cellular adhesion [35]. In the liver it was localized to the biliary canaliculi [19]. In this region the so-called small epithelial cells [29] or oval cells [33], which have properties of liver stem cells, are located. Although expression of CALLA on these cells has not yet been investigated, it seemed interesting to evaluate the distribution of this molecule in hepatoblastomas with different characteristics of differentiation.

Materials and methods

Twenty children with hepatoblastomas underwent surgery in accordance with the protocol of the German Paediatric Liver Tumour Study HB89 [40]. There were 9 female and 11 male patients. Their age ranged from 6 months to 6.5 years (median 14 months). In 1 case, the hepatoblastoma was a recurrent tumour diagnosed 2 years after prior resection and chemotherapy. Representative tumour material was taken immediately after resection (12 patients) or biopsy (8 patients). In paraffin histology, 10 of the tumours were purely epithelial and 10 mixed epithelial and mesenchymal hepatoblastomas according to the classification of Ishak and Glunz [15]. The distribution of different tissue components in the tumours is shown in Table 1. One mixed hepatoblastoma was mainly composed of mesenchymal sarcoma-like tissue, but also contained typically fetal tumour areas. Primitive spindle cells occurred in large cord-like structures in 1 tumour and in multiple small foci in 4 others. There were 6 hepatoblastomas from which additional material was obtained at second-look surgery after treatment with two or three courses of chemotherapy [40]. These specimens showed regression with necrosis and scars, but the histological type had not changed. However, the proportion of fetal and embryonal tissue shifted toward fetal differentiation in 5 tumours, while 1 former purely embryonal hepatoblastoma still contained only embryonal cells. The mesenchymal components, in particular areas with osteoid, were more prominent in the post-treatment specimens.

Table 1 Distribution of tissue components in the 20 hepatoblastomas investigated

Tissue component	Epithelial type ($n = 10$)	Mixed type ($n = 10$)
Epithelial component:		
Pure fetal	4	1
Fetal + embryonal	5	8
Pure embryonal	1	–
Small cell undifferentiated	–	1
Mesenchymal component:		
Immature fibrous tissue	–	10[a]
Osteoid	–	10
Primitive spindle cells	–	5[b]

[a] In 1 tumour sarcoma-like
[b] In 1 tumour large areas, in 4 tumours small foci

After removal of representative pieces for conventional histological examination, tumour material was snap-frozen in liquid nitrogen and stored at –80°C. Normal liver tissue from the patients was obtained separately as control tissue for immunostaining. In addition, cytospins were prepared from 4 of the hepatoblastomas as previously described [41]. Briefly, fresh tumour material was finely minced and suspended in cold Hanks' balanced salt solution (Gibco, Berlin, Germany). Separation from debris was achieved by density gradient centrifugation on Ficoll-Paque (Pharmacia, Freiburg, Germany), and the cells were washed in phosphate-buffered saline (PBS; pH 7.4). Cytospins were prepared by centrifugation at 130 *g* on a cytospin 2 centrifuge (Shandon, Astmore, UK) and air dried. They contained fetal and embryonal tumour cells in 3 cases and embryonal cells only in 1 case.

The murine monoclonal antibodies used for immunostaining are listed in Table 2. Antibodies against cytokeratins, cytokeratin 19 and vimentin were used for better identification of tumour components. Immunostaining was performed on 6-µm cryostat sections using the APAAP technique essentially as described by Cordell et al. [4]. Briefly, the slides were fixed in ice-cold acetone for 10 min and air dried. After preincubation with normal rabbit serum (1:20), the primary MAb was added for 1 h at room temperature. Thereafter, incubations followed with rabbit anti-mouse immunoglobin (Dako, Glostrup, Denmark; 1:500) and the APAAP complex (Dako; 1:100), each for 1 h. Subsequently the last two incubation steps were repeated twice, for 30 min each. Colour development was performed with naphthol-AS-biphosphate and new fuchsin (Sigma, Deisenhofen, Germany). After washing with deionized water and counterstaining with haemalaun (Merck, Darmstadt, Germany), the slides were mounted with glycerol-gelatin (Merck). Positive reaction resulted in bright red staining.

Table 2 Murine monoclonal antibodies applied in the study

Specificity	Clone	Dilution – concentration	Source
HCAM (CD44)	J.173	1:20–10 μ g/ml	Immunotech[b]
ICAM-1 (CD54)	15.2	1:100–2 μ g/ml	Boehringer
E-cadherin	6F6	1:15–3 μ g/ml	Boehringer[c]
NCAM (CD56)	UJ13A	1:20–3 μ g/ml	Dako[a]
NCAM (CD56)	T199	1:100–5 μ g/ml	T. Pietsch[d]
Polysialylated NCAM	735	1:200–5 μ g/ml	M. Husmann[e]
CALLA (CD10)	SS2/36	1:100–3 μ g/ml	Dako
Pan-cytokeratin (CK1)	LP 34	1:100–4 μ g/ml	Dako
Cytokeratin 19	BA 17	1:100–1 μ g/ml	Dako
Vimentin	V9	1: 50–4 μ g/ml	Dako

[a] Dako A/S, Glostrup, Denmark
[b] Immunotech S.A., Marseille, France
[c] Boehringer Mannheim GmbH, Mannheim, Germany
[d] Dr. T. Pietsch, University of Bonn, Germany [23]
[e] Dr. M. Husmann, University of Mainz, Germany [25]

Results

A summary of the reactivity of hepatoblastoma tissue components for the investigated cell membrane proteins is given in Table 3. HCAM expression was moderate on embryonal and strong on undifferentiated small cells (Fig. 1), but negative on fetal tumour cells. Thus, embryonal tumour cells were easily identifiable in otherwise fetal areas. In mesenchymal areas primitive spindle cells and vimentin-positive cells enclosed in osteoid reacted strongly for HCAM.

The distribution of ICAM-1 was different; fetal as well as embryonal cells contained this molecule with a tendency to more intensive staining in fetal cells. In 8 of the tumours, the distribution was heterogeneous with areas appearing negative alternating with those of intensive staining, but there were no clear boundaries of negative and positive areas. Areas with undifferentiated small cells did not contain ICAM-1. Immature fibrous tissue of mesenchymal tumour areas stained intensively with anti-ICAM-1 Mab, while primitive spindle cells and cells enclosed in osteoid were negative.

E-cadherin was detectable only on fetal hepatoblastoma cells, which displayed a membrane-bound expression of this molecule (Fig. 2). In some tumour areas the staining was concentrated in only one membrane of the cells. Less mature embryonal or undifferentiated small epithelial cells and mesenchymal tissue were negative. NCAM stained with the antibodies T199 and UJ13A was not present on fetal and embryonal tumour cells, but was moderately expressed on undifferentiated small cells. These cells also stained positive with MAb 735, which identifies the polysialylated embryonic form of NCAM. In mixed hepatoblastomas immunoreaction appeared in some cells adjacent to osteoid and on primitive spindle cells, whereas immature fibrous tissue and the osteoid itself were negative. Interestingly, the primitive spindle cells in cord-like formations (Fig. 3) of 1 hepatoblastoma and those of the small foci surrounded by embryonal tissue in 4 other tumours were also strongly reactive for the polysialylated embryonal NCAM.

CALLA was found to be strongly expressed on embryonal and undifferentiated small epithelial hepatoblastoma cells (Fig. 4). Primitive spindle cells also stained intensively for this protein. In contrast, there was no reactivity for CALLA in more mature epithelial (fetal) and mesenchymal (fibrous septa and osteoid) tissue components. In fetal areas, irregularly arranged bile canaliculi were clearly marked between cords of epithelial tumour cells. The expression of adhesion molecules and CALLA in the sarcoma-like mesenchymal tissue of 1 tumour was identical to that delineated for the immature fibrous tissue of the other mixed hepatoblastomas.

Endothelial cells and fibroblasts of the stroma in epithelial tumour areas stained positive for HCAM, and endothelial cells also reacted strongly for ICAM-1. In samples taken from hepatoblastomas after chemotherapy, the reactivity for ICAM-1 appeared to be decreased in surviving fetal and embryonal tumour cells, and the embryonal cells did not express HCAM. Otherwise, the expression of the cell surface antigens was the same as in the specimens taken prior to treatment.

Table 3 Reactivity of epithelial and mesenchymal hepatoblastoma tissue components for cell surface proteins (– negative, + moderate, ++ strong, +++ very strong)

Antigen	Tissue					
	Epithelial			Mesenchymal		
	Fetal	Embryonal	Small cell undifferentiated	Fibrous	Osteoid	Primitive spindle cells
HCAM	–	+	++	–	++	+++
ICAM-1	–/+	–/+	–	++	–	–
E-cadherin	++	–	–	–	–	–
NCAM	–	–	+	–	–	++
Embryonal NCAM	–	–	+	–	–	++
CALLA	–	++	++	–	–	+++

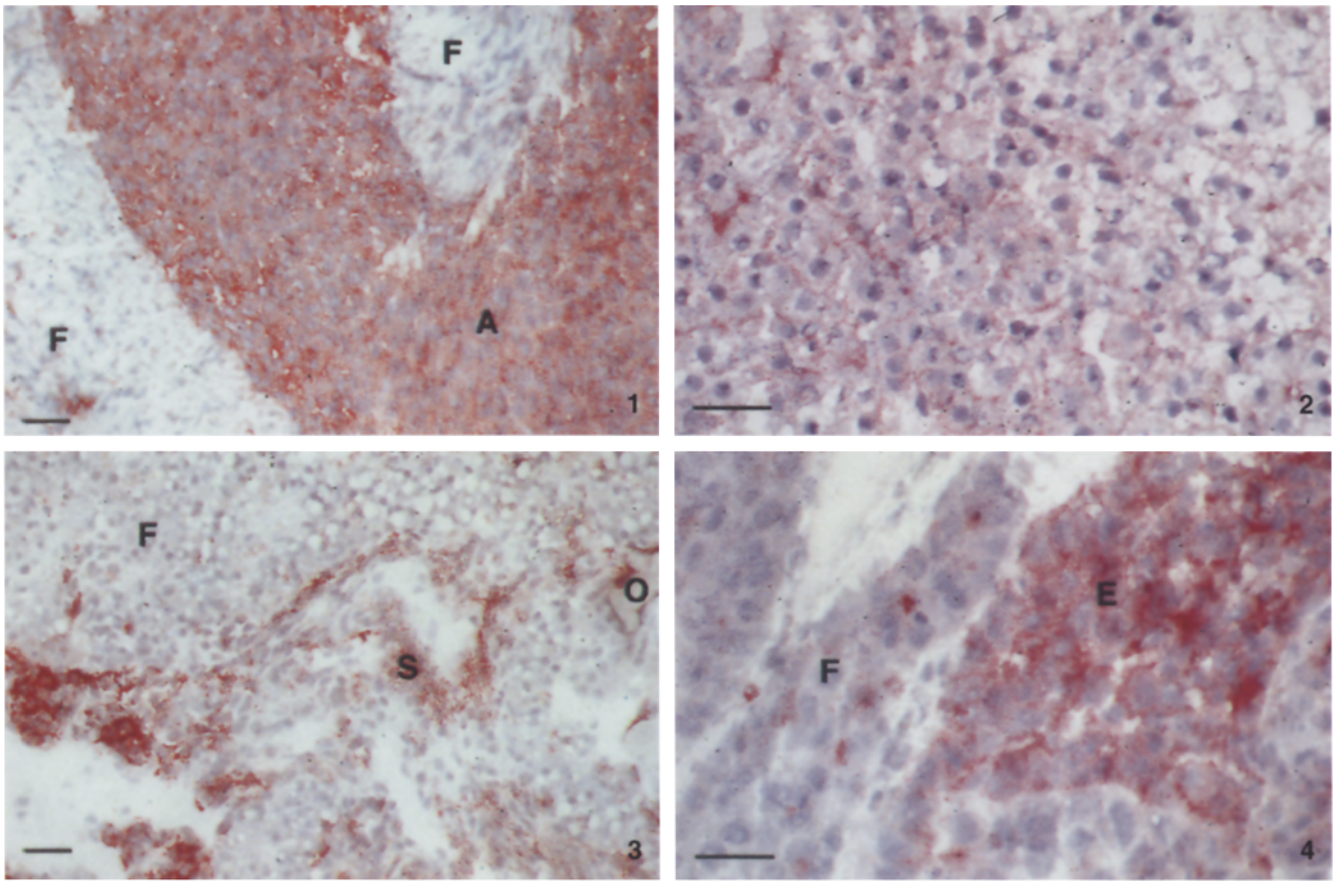

Fig. 1 Mixed hepatoblastoma with undifferentiated small cell tissue (*A*) strongly expressing HCAM besides negative immature fibrous tissue (*F*). *Bar* 50 µm; APAAP, ×200

Fig. 2 Epithelial hepatoblastoma with membrane-bound staining of fetal cells for E-cadherin. *Bar* 50 µm; APAAP, ×310

Fig. 3 Mixed hepatoblastoma showing expression of the polysialylated embryonic form of NCAM in primitive spindle-shaped mesenchymal cells (*S*) adjacent to osteoid (*O*) and fetal tumour cells (*F*), which are negative. *Bar* 50 µm; APAAP, ×200

Fig. 4 Fetal and embryonal differentiated hepatoblastoma with strong expression of CALLA in embryonal cell areas (*E*) and negative fetal cells (*F*). *Bar* 50 µm APAAP, ×310

All epithelial cells stained positive with anti-cytokeratin MAb CK1. This reactivity was stronger in fetal than in embryonal or anaplastic areas. Embryonal tumour cells also reacted with the anti-CK-19 MAb, while fetal and undifferentiated small cells were negative. In contrast to fetal and embryonal cells, undifferentiated small cells also expressed vimentin. All mesenchymal components of mixed hepatoblastomas, including primitive spindle cells, were positive for this intermediate filament protein, but negative for cytokeratins.

All fetal and approximately 70% of the embryonal hepatoblastoma cells in cytospins from 4 of the tumours stained positive with MAb CK1, and the embryonal cells also for cytokeratin 19. Vimentin was positive in few mesenchymal cells of all 4 preparations. The immunoreactivity of the cells for adhesion molecules resembled that found on cryostat sections: E-cadherin was detected on fetal cells, whereas HCAM and CALLA were present on embryonal cells. The majority of tumour cells stained positive for ICAM-1 in cytospin preparations of 2 hepatoblastomas, while almost all cells in cytospins from 2 other tumours were negative.

On cryostat sections from normal liver most hepatocytes displayed positive staining for E-cadherin on the membranes directed to the sinusoids. Endothelial cells of sinusoids and larger vessels stained positive for ICAM-1, while fibrous tissue of periportal fields reacted for anti-HCAM. NCAM was found on single leucocytes occurring in periportal fields, which were negative with MAb 735. Reaction with the MAb against CALLA resulted in strong staining of bile canaliculi. Staining of normal liver confirmed specificity of the control antibodies, as hepatocytes and bile duct epithelial cells stained strongly with MAb CK1 and the bile duct epithelium also for cytokeratin-19, whilst all connective tissue displayed strong reactivity for vimentin.

After follow-up of 2–8 years (median 5.2 years), 18 of the 20 patients are alive and well: 1 patient with a multifocally disseminated tumour in the liver and 1 suffering from a recurrent tumour died. These 2 epithelial hepatoblastomas displayed a pure or predominant embryonal differentiation, respectively. However, the number of cases in this series is too small to perform a statistical analysis on the relationship between the pattern of antigen expression and the patients' survival.

Discussion

Investigation of the expression of cell adhesion molecules in embryonal tumours is most interesting, since it can reveal information on functional properties of the malignant cells, such as the capacity for invasive growth and metastasis [7]. Furthermore, such studies may give insight into tumour histogenesis, a matter of considerable interest in hepatoblastoma because of its multidirectional spectrum of differentiation [1, 29]. Hepatoblastoma can resemble all stages of liver development in its various epithelial tissue components [1, 27], and Abenoza et al. [1] proposed a scheme of hepatoblastoma differentiation on the basis of immunohistological results, suggesting that immature small tumour cells most frequently differentiate toward embryonal hepatocytes. These, in turn, would have the potential to undergo further differentiation, including the development of a mesenchymal phenotype. However, these authors also discuss the alternative possibility that very primitive precursor cells may exist, which can differentiate in various directions. The results of our study support this second hypothesis, as they show the close relationship of undifferentiated small epithelial (cytokeratin-positive) hepatoblastoma cells to the primitive mesenchymal spindle cells, both of which express CALLA, HCAM and the embryonal form of NCAM (Table 3). We assume that these cell types are directly derived from multipotential precursor cells, which may also carry the three surface proteins. The relationship of these primitive, multipotential tumour cells to the so-called oval cells of the normal liver, which have properties of liver stem cells [33], is not known, although mixed epithelial and mesenchymal hepatoblastomas can arise from these cells in rodents [37]. In a recent immunohistochemical study for cytokeratins, Ruck et al. found further evidence that hepatoblastoma cells are derived from pluripotent stem cells [29]. In the normal liver, these stem cells have been localized to the site of the transitional duct cells, which connect the terminal bile ductules (canals of Hering) with the bile canaliculi [33].

Interestingly, CALLA was found to be localized in these regions of the liver [19], as was confirmed in our control stains. However, on the cryostat sections we were not able to localize the positive staining for CALLA to such oval cells. In hepatoblastoma, we found embryonal and anaplastic but not fetal tumour cells to express this antigen. This finding corresponds with reports on the appearance of bile-duct associated cytokeratins in embryonal hepatoblastoma cells [38]. It also supports the hypothesis that embryonal cells preserve features of multipotential precursor cells of the developing liver [1, 28, 37]. CALLA acts as a neutral endopeptidase [35] in normal tissue, although its function in hepatoblastoma has yet to be elucidated. The distribution of the hyaluronate receptor HCAM on hepatoblastoma cells was similar to that of CALLA; it too was found on embryonal and anaplastic but not on fetal cells. HCAM is widely distributed in human tissue and seems to have a general role in cell–cell and cell–matrix adhesion [2]. Our findings are consistent with the fact that rapidly dividing epithelial cells, including carcinoma cells, display increased expression of hyaluronate-binding activity [13]. The antibody used in this study does not discriminate standard CD44 and the recently identified splice variants of the HCAM molecule, some of which increase the metastatic potential in carcinoma cells [14]. Further studies are needed to evaluate whether these variants are also expressed in hepatoblastoma.

E-cadherin, which is the homologous molecule to the liver cell adhesion molecule of the chicken, is normally expressed on epithelial cells [6]. It aggregates embryonic liver cells [3] and is therefore important for the development of this organ [9]. Here it is reciprocally expressed with NCAM during the regulation of the mesenchymal–epithelial transition [9]. We were able to detect E-cadherin only on relatively mature fetal hepatoblastoma cells, where it is expressed only on the membranes directed towards the sinusoids, as in normal hepatocytes [6]. Since E-cadherin-mediated adhesion minimizes the invasiveness of carcinoma cells [10], its absence from embryonal and undifferentiated small cells of hepatoblastoma may contribute to the loss of coherence and subsequently increased potential for invasive growth and metastasis [12]. Fetal and embryonal cell areas were negative for NCAM. The occurrence of the latter adhesion molecule with vimentin in the undifferentiated small cell tumour areas confirms the multidirectional phenotype of these cells [1], which is underlined by the expression of the polysialylated embryonal form of the NCAM molecule by these cells [28]. Interestingly, Roskams et al. found NCAM to be present on reactive bile ductules [25] as one of several markers for a neuroendocrine differentiation of these cells. Like immature hepatoblastoma cells [27], these seem to be directly derived from the facultative stem cells of the liver [25]. Thus, there is a close relationship with immature hepatoblastoma cells, on which neuroendocrine markers other than NCAM can also be found [28].

The occurrence of ICAM-1 in epithelial hepatoblastoma areas was not restricted to morphological boundaries or any differentiation of the tumour cells, although anaplastic cells were negative. The expression varied from absent to very intensive. It was shown that the expression of ICAM-1 can be induced on cells of the hepatoblastoma cell-line HepG2 and on human lung adenocarcinoma cells by interleukin-1β [20, 39]. In a recent investigation, we found this cytokine to be produced by epithelial hepatoblastoma cells [41]. Their expression of ICAM-1 may be induced by the local secretion of this factor or other inflammatory cytokines also secreted in hepatoblastoma tissue [41, 43], which may explain the irregular distribution of ICAM-1-positive cells in the tumour tissue. The reduced expression of ICAM-1 in hepatoblastomas after chemotherapy might be an indicator for impaired secretory activity of tumour cells treated with cytotoxic drugs. Another possible explanation for the irregular and diffuse occurrence of ICAM-1 in epithelial he-

patoblastoma areas could be that soluble serum ICAM-1, found to be produced by hepatocellular and other carcinomas [34], was stained during immunohistochemistry. However, positive cellular staining on the cytospin preparations indicates that ICAM-1 can be produced by hepatoblastoma cells themselves. It is intriguing that this molecule was found to increase the metastatic potential of melanoma cells [16], but its role in hepatoblastoma remains unclear.

Most of the molecules investigated, except for ICAM-1, were absent from immature fibrous tissue. The strong expression of HCAM and NCAM on cells in the vicinity of osteoid is remarkable. These molecules are most likely to be present on the surface of activated lymphocytes and monocytes [2, 23], which we found concentrated around osteoid in hepatoblastoma in a recent investigation [43]. Since HCAM binds chondroitin sulfate and other extracellular matrix proteins besides hyaluronate [2], its occurrence on these cells might be associated with the formation of this cartilage-like substance. Most interesting was the pattern of surface antigen expression on primitive spindle-shaped mesenchymal cells [1, 15], which we found in larger areas of 1 and in small foci of 4 other mixed hepatoblastomas. The intensive staining of these cells for CALLA confirms that they are immature. This is underlined by the occurrence of the polysialylated embryonic form of the NCAM molecule [26] on their surface. Like the undifferentiated small hepatoblastoma cells, these primitive mesenchymal cells also preserve an extremly immature progenitor phenotype that is not yet fully committed to the embryonal mesenchymal–epithelial transformation [5]. In contrast to the undifferentiated small cells, they do not have the capability to form HLA class I proteins, which can be found on all other cell types in hepatoblastoma (unpublished observation).

Different hepatoblastoma tissue components express distinct patterns of adhesion molecules, which may be responsible for the local interactions of tumour cells and their reactions with stromal cells. The adhesion molecules found on immature epithelial and mesenchymal tumour cells enable them to invade surrounding tissue and vessels, grow multifocally in the liver, and develop distant metastases. All these features are significantly associated with worsening of the prognosis, especially in the case of undifferentiated tumours [12, 42]. Further studies are necessary to evaluate the functional properties of these adhesion molecules and CALLA in hepatoblastoma.

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References

1. Abenoza P, Manivel JC, Wick MR, Hagen K, Dehner LP (1987) Hepatoblastoma: an immunohistochemical and ultrastructural study. Hum Pathol 18: 1025–1035
2. Aruffo A, Stamenkovic I, Melnick M, Underhill CB, Seed B (1990) CD44 is the principal cell surface receptor for hyaluronate. Cell 61: 1303–1313
3. Bertolotti R, Rutishauser U, Edelman GM (1980) Cell surface molecule involved in aggregation of embryonic liver cells. Proc Natl Acad Sci USA 77: 4831–4835
4. Cordell JL, Falini B, Erber WN, Ghosh AK, Abdulaziz Z, MacDonald S, Pulford KAF, Stein H, Mason DY (1974) Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase. J Histochem Cytochem 32: 219–229
5. Edelman GM (1988) Morphoregulatory molecules. Biochemistry 27: 3533–3543
6. Eidelman S, Damsky CH, Wheelock MJ, Damjanov I (1989) Expression of the cell–cell adhesion glycoprotein cell-CAM 120/80 in normal human tissues and tumors. Am J Pathol 135: 101–110
7. Fawcett J, Harris AL (1992) Cell adhesion molecules and cancer. Curr Opin Oncol 4: 142–148
8. Figarella-Branger DF, Durbec PL, Rougon GN (1990) Differential spectrum of expression of neural cell adhesion molecule isoforms and L1 adhesion molecules on human neurectodermal tumors. Cancer Res 50: 6364–6370
9. Fleming S (1991) Cell adhesion and epithelial differentiation. J Pathol (Lond) 164: 95–100
10. Frixen UH, Behrens J, Sachs M, Eberle G, Voss B, Warda A, Löchner D, Birchmeier W (1991) E-cadherin-mediated cell–cell adhesion prevents invasiveness of human carcinoma cells. J Cell Biol 113: 173–185
11. Garin-Chesa P, Fellinger EJ, Hivos AG, Beresfold HR, Melamed MR, Triche TJ, Rettig WJ (1991) Immunohistochemical analysis of neural cell adhesion molecules. Differential expression in small round cell tumors of childhood and adolescence. Am J Pathol 139: 275–286
12. Haas JE, Muczynski KA, Krailo M, Ablin A, Land V, Vietti TJ, Hammond GD (1989) Histopathology and prognosis in childhood hepatoblastoma and hepatocarcinoma. Cancer 64: 1082–1095
13. Heider K-H, Hofmann M, Hors E (1993) A human homologue of the rat metastasis- associated variant of CD44 is expressed in colorectal carcinomas and adenomatous polyps. J Cell Biol 120: 227–234
14. Hofmann M, Rudy W, Zöller M, Tölg C, Ponta H, Herrlich P, Günthert U (1991) CD44 splice variants confer metastatic behavior in rats: homologous sequences are expressed in human tumor cell lines. Cancer Res 51: 5292–5297
15. Ishak KG, Glunz PR (1967) Hepatoblastoma and hepatocarcinoma in infancy and childhood. A report of 47 cases. Cancer 20: 396–422
16. Johnson JP, Stade BG, Holzmann B, Schwäble W, Riethmüller G (1989) De novo expression of intercellular adhesion molecule-1 in melanoma correlates with increased risk of metastasis. Proc Natl Acad Sci USA 86: 641–644
17. Kasai M, Watanabe I (1970) Histologic classification of liver-cell carcinoma in infancy and childhood and its clinical evaluation. A study of 70 cases collected in Japan. Cancer 25: 551–563
18. Komminoth P, Roth J, Lackie PM, Bitter-Suermann D, Heitz PU (1991) Polysialic acid of the neural cell adhesion molecule distinguishes small cell lung carcinoma from carcinoids. Am J Pathol 139: 297–304
19. Loke SL, Chiu KY, Yau WL, Cheung LM (1990) Localization of CD10 to biliary canaliculi by immunoelectron microscopical examination. J Clin Pathol 43: 654–656
20. Mickelson JK, Kukielka G, Bravenec JS, Mainolfi E, Rothlein R, Hawkins HK (1995) Differential expression and release of CD54 induced by cytokines. Hepatology 22: 866–875
21. Miettinen M, Cupo W (1993) Neural cell adhesion molecule distribution in soft tissue tumors. Hum Pathol 24: 62–66
22. Nanji AA, Griniuviene B, Yacoub LK, Fogt F, Tahan SR (1995) Intercellular adhesion molecule-1 expression in experimental alcoholic liver disease: relationship to endotoxemia and TNF alpha messenger RNA. Exp Mol Pathol 62: 42–51
23. Pietsch T, Hadam MR (1989) Epitope analysis of the NCAM/LEU19 molecule (CD56) using monoclonal antibody T-199. In: Knapp W, Dörken B, Gilks WR, Rieber EP, Schmidt

RE, Stein H, von dem Borne AEG (eds) Leucocyte typing, IV. Oxford University Press, Oxford, p 704
24. Platt JL, Le Bien TW, Micheal AF (1983) Stages of renal ontogenesis identified by monoclonal antibodies reactive with lymphohemopoietic differentiation antigens. J Exp Med 157: 155–172
25. Roskams T, Campos RV, Drucker DJ, Desmet VJ (1993) Reactive human bile ductules express parathyroid hormone-related peptide. Histopathology 23: 11–19
26. Roth J, Zuber C, Wagner P, Taatjes DJ, Weisgerber C, Heitz PU, Goridis C, Bitter- Suermann D (1988) Reexpression of poly(sialic acid) units of the neural cell adhesion molecule in Wilms tumor. Proc Natl Acad Sci USA 85: 2999–3003
27. Ruck P, Kaiserling E (1992) Extracellular matrix in hepatoblastoma: an immunohistochemical investigation. Histopathology 21: 115–126
28. Ruck P, Harms D, Kaiserling E (1990) Neuroendocrine differentiation in hepatoblastoma. An immunohistochemical investigation. Am J Surg Pathol 14: 847–855
29. Ruck P, Xiao J-C, Kaiserling E (1996) Small epithelial cells and the histogenesis of hepatoblastoma. Electron microscopic, immunoelectron microscopic, and immunohistochemical findings. Am J Pathol 148: 321–329
30. Rutishauser U, Acheson A, Hall AK, Mann DM, Sunshine J (1988) The neural cell adhesion molecule (NCAM) as a regulator of cell–cell interactions. Science 240: 53–57
31. Santarosa M, Favaro D, Quaia M, Spada A, Sacco C, Talamini R, Galligioni E (1995) Expression and release of intercellular adhesion molecule-1 in renal cancer patients. Int J Cancer 62: 271–275
32. Schmidt D, Harms D, Lang W (1985) Primary malignant hepatic tumours in childhood. Virchows Arch [A] 407: 387–405
33. Sell S (1990) Is there a liver stem cell? Cancer Res 50: 3811–3815
34. Shimizu Y, Minemura M, Tsukishiro T, Kashii Y, Miyamoto M, Nishimori H, Higuchi K, Watanabe A (1995) Serum concentration of intercellular adhesion molecule-1 in patients with hepatocellular carcinoma is a marker of the disease progression and prognosis. Hepatology 22: 525–531
35. Shipp MA, Stefano GB, Switzer SN, Griffin JD, Reinherz EL (1991) CD10 (CALLA)/neutral endopeptidase 24.11 modulates inflammatory peptide-induced changes in neutrophil morphology, migration, and adhesion proteins and is itself regulated by neutrophil activation. Blood 78: 1834–1841
36. Terpe H-J, Tajrobehkar K, Günthert U, Altmannsberger M (1993) Expression of cell adhesion molecules alpha-2, alpha-5 and alpha-6 integrin, E-cadherin, N-CAM and CD44 in renal cell carcinomas. Virchows Arch [A] 422: 219–224
37. Tsao M-S, Grisham JW (1987) Hepatocarcinomas, cholangiocarcinomas, and hepatoblastomas produced by chemically transformed cultured rat liver epithelial cells. Am J Pathol 127: 168–181
38. Van Eyken P, Sciot R, Callea F, Rameakers F, Schaart G, Desmet VJ (1990) A cytokeratin-immunohistochemical study of hepatoblastoma. Hum Pathol 21: 302–308
39. Volpes R, Van der Oord JJ, Desmet VJ, Yap S-H (1992) Induction of intercellular adhesion molecule-1 (CD54) on human hepatoma cell line HepG2: influence of cytokines and hepatitis B virus transfection. Clin Exp Immunol 87: 71–75
40. Von Schweinitz D, Bürger D, Weinel P, Mildenberger H (1992) The treatment of malignant liver tumours in childhood. An interim report of the multicentric study HB89 of the GPOH. Klin Pädiatr 204: 214–220
41. Von Schweinitz D, Hadam MR, Welte K, Mildenberger H, Pietsch T (1993) Production of interleukin-1β and interleukin-6 in hepatoblastoma. Int J Cancer 53: 728–734
42. Von Schweinitz D, Wischmeyer P, Leuschner I, Schmidt D, Wittekind C, Harms D, Mildenberger H (1994) Clinico-pathological criteria with prognostic relevance in hepatoblastoma. Eur J Cancer [A] 30: 1052–1058
43. Von Schweinitz D, Schmidt D, Fuchs J, Welte K, Pietsch T (1995) Extramedullary hematopoiesis and intratumoral production of cytokines in childhood hepatoblastoma. Pediatr Res 38: 555–563