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Albumin gene expression in adenocarcinomas with hepatoid differentiation

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Abstract Two cases of hepatoid adenocarcinomas were studied with an in situ hybridization technique (ISH) using a RNA probe for human albumin mRNA. In case 1 the urinary bladder of a 67-year-old woman was affected; in case 2 the tumour was located in the gastric antrum of an 80-year-old woman. In neither case had alpha fetoprotein (AFP) been determined preoperatively. Histologically these cases showed adenocarcinomatous features intermingled with hepatoid areas. These latter areas were characterized by cords of polygonal cells, each with an oval nucleus and prominent nucleoli, separated by a fine network of sinusoids. In the hepatoid areas the immunohistochemical profile was similar to that observed in hepatocellular carcinomas, in that the tumour cells were positive with AFP, alpha-1-antitrypsin (A1AAT) and albumin antisera and there was a canalicular type of reactivity with polyclonal anti-CEA (pCEA) antibody. ISH revealed albumin mRNA in virtually all hepatoid cells in case 1, and in about 50% of those in case 2. In addition, in case 2 occasional cells in the adenocarcinomatous areas showed albumin transcripts of ISH. Our findings confirm that ISH for albumin mRNA probe is a valuable method of establishing hepatocellular differentiation, and that hepatoid adenocarcinomas are tumours with true extrahepatic hepatocellular differentiation.

Key words Hepatoid adenocarcinoma · Hepatocellular carcinoma · Albumin · In situ hybridization

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Introduction

Hepatoid adenocarcinomas (HACs) are extrahepatic tumours showing areas morphologically comparable to those of hepatocellular carcinomas (HCC) [11, 12, 15, 18]. These areas are composed of cords of polygonal cells separated by sinusoids, occasionally featuring bile production and/or bile canaliculi formation [28]. In addition to this histological picture, HACs share with HCC immunohistochemical reactivity with anti-albumin, alpha fetoprotein (AFP), and alpha-1 antitrypsin (A1AAT) antibodies [1, 15, 18, 28]. Canalicular immunoreaction with polyclonal anti-CEA (pCEA) antibody [28] is also seen, together with elevated serum levels of AFP [1, 18].

Albumin is a protein produced exclusively by hepatocytes. Detection of albumin mRNA by in situ hybridization (ISH) is therefore considered a specific marker of hepatocellular differentiation [4, 9, 17, 21, 31]. Albumin mRNA has been found only in primary HCCs [4], the single exception being a case of pineal malignant germ cell tumour with hepatoid features reported by Krishna et al. [9]. Thus, albumin mRNA is a very helpful diagnostic tool to distinguish primary from metastatic liver carcinomas. To the best of our knowledge only one case of HAC has been studied by ISH to detect albumin mRNA [25]. We therefore decided to perform this test in two examples of this entity, using a cDNA for human albumin mRNA.

Materials and methods

In case 1 the urinary bladder of a 67-year-old woman was affected. This case has already been reported in detail elsewhere [28]. In case 2 a 10-cm polypoid mass, was located in the gastric antrum of an 80-year-old woman. AFP had not been determined preoperatively in either case.

Blocks showing the most representative areas of hepatoid differentiation were selected from both cases. Blocks were serially cut and stained with haematoxylin-eosin (H&E), PAS with diastase control, Alcian Blue, and immunohistochemically [8] using the following antisera: CEA (diluted 1:500), albumin (diluted 1:2000), alpha fetoprotein (diluted 1:500), and alpha-1 antitrypsin (diluted 1:300). All antisera were polyclonal and were purchased from DAKO (Denmark).

In situ hybridization was performed using a probe and a method already published elsewhere [4]. Briefly, human albumin cDNA (kindly provided by Professor C. Brechot, Paris, France) was subcloned into a Bluescript plasmid (Stratagene, La Jolla, Calif.), which was linearized with *Hin*dIII and utilized as a template in an in vitro transcription reaction for the synthesis of anti-sense probes. The albumin RNA probe consisted of a 510-base-pair (bp) fragment that corresponded to the coding region of the albumin gene [5]. Albumin probe was labelled by digoxigenin-labelled-UTP using a DIG-RNA labelling kit (Boerhringer, Mannheim, Germany). For the ISH technique, sections were cut from selected blocks and put on a polylysin-coated slide. Sections were then dewaxed in xylene and rehydrated through graded alcohol series. After digestion with proteinase K (250 µl; Sigma, Milan, Italy) in 50 mmol/l Tris-HCl/1 mmol per l EDTA for 15 min at 37°C, slides were postfixed in paraformaldehyde 4% in PBS, at 4°C for 5 min. Washing in PBS were performed for 3 min at 4°C and at room temperature.

The hybridization mix contained 50% deionized formamide, 0.3 mol/l NaCl, 20 mmol/l Tris-HCl (pH 7.4), 5 mol/l EDTA, 10 mmol/l NaH_2PO_4 (pH 8), 10% dextran sulphate, 1× Denhart's, 50 mg/ml yeast *t*-RNA. Each section was covered with 25 ml of the hybridization solution containing 50 ng of digoxigenin-labelled cRNA probe. The sections were each covered with a siliconized coverslip. Hybridization was performed at 50°C for 16 h. Coverslips were removed and slides subjected to a stringent wash in 5× sodium chloride sodium citrate (SSC) for 10 min at 50°C and in 4×SSC for 20 min at 37°C. RNAse treatment was then performed at 37°C for 20 min (RNAse Boehringer, Mannheim, Germany), followed by a stringent wash in 2×SSC, 0,5×SSC and 0.1×SSC at 42°C for 20 min each. Slides were than rinsed in buffer 1 (100 mmol/l Tris-HCl, 150 mmol/l NaCl, pH 7.5) at room temperature before incubation with normal sheep serum diluted 1:50 for 30 min at room temperature and with polyclonal anti-digoxigenin antibody (Boehringer, Mannheim, Germany) diluted 1:500 for 2 h at room temperature. After 3 washes in buffer 1, sections were equilibrated in buffer 2 (100 mmol/l Tris-HCl, 100 mmol/l NaCl, 50 mmol/l MgCl2, pH 9.5) for 2 min and incubated in buffer 2 with a freshly prepared colour-substrate solution containing nitro-blue tetrazolium salt (340 mg/ml), 5-bromo-4-chloro-3-indolyl phosphate toludium salt (170 mg/ml) and levamizole (1 mmol/l). Slides were placed in a humid chamber and developed in darkness for 1–3 h at room temperature. After development, sections were washed in deionized water, counterstained with neutral green, quickly dehydrated, and mounted in Eukitt. Negative controls added to each case consisted of pretreated slides with ribonuclease A (250 mg/ml) before hybridization and omitting the probe cocktail during the ISH procedure. Sections of normal liver, added to each batch of slides, served as positive controls.

Results

Both cases showed a prominent hepatoid component associated with an adenocarcinomatous component. The hepatoid areas were composed by solid cords of round to polygonal cells with granular, eosinophilic cytoplasm (Fig. 1). The cords were separated by a fine capillary network. In case 1, areas of haemorrage and necrosis were present. A central, round to oval nucleus with a prominent nucleolus was present. In case 2, large areas predominantly composed of clear cells were also present. In both cases, rare intracytoplasmic hyaline PAS-positive globules were seen. Features suggestive of bile production were focally observed in case 1, but were absent in case 2. In both cases the second neoplastic component exhibited glandular structures lined by atypical cells.

Immunohistochemical (IHC) staining in the hepatoid areas showed intracytoplasmic positivity with albumin and A1AAT antisera. AFP stained the intracytoplasmic globules detected with PAS stain. Polyclonal anti-CEA antibody (pCEA) evidenced a diffuse canalicular type of positivity in case 1 (Fig. 2), while in case 2 canalicular structures were very rare. ISH for human albumin mRNA showed intense cytoplasmic positivity in virtually all the hepatoid neoplastic cells in case 1, and in about 50% of those in case 2 (Fig. 3).

Alcianophilic mucosubstances were histochemically demonstrated in the adenocarcinomatous areas, but not in the hepatoid areas. The tumour cells of the adenocarcinomatous component showed some intracytoplasmic staining for pCEA, but were negative for all other antisera used. Interestingly, in the adenocarcinomatous areas, there was staining of occasional neoplastic cells lining the glandular structures with ISH for albumin transcripts (Fig. 4).

Discussion

Prat et al. in 1982 [24] first reported cases of ovarian yolk sac tumours with features of hepatic cell differentiation. Subsequently numerous cases of gonadal and extragonadal [10, 15, 19] germ cell tumours, epithelial and sex cord stromal tumours with hepatoid differentiation have been reported [19]. In addition, cases of extrahepatic carcinomas with features superimposable on those of HCC of the liver but not associated with germ cell tumours have also been described. In most of these cases adenocarcinomatous areas were also present, closely intermixed with the hepatoid foci. On the basis of these observations Ishikura et al. [12] introduced the term hepatoid adenocarcinomas (HACs).The most frequent sites of origin of these tumours are stomach and ovary. In addition, isolated cases have been reported in the lung [2, 13], renal pelvis [14], papilla of Vater [7], uterus [32], placenta [3], and urinary bladder [28]. HACs are aggressive tumours, which frequently metastatise to the liver [1, 15]. They share with HCC the tendency to be associated with an elevated se-

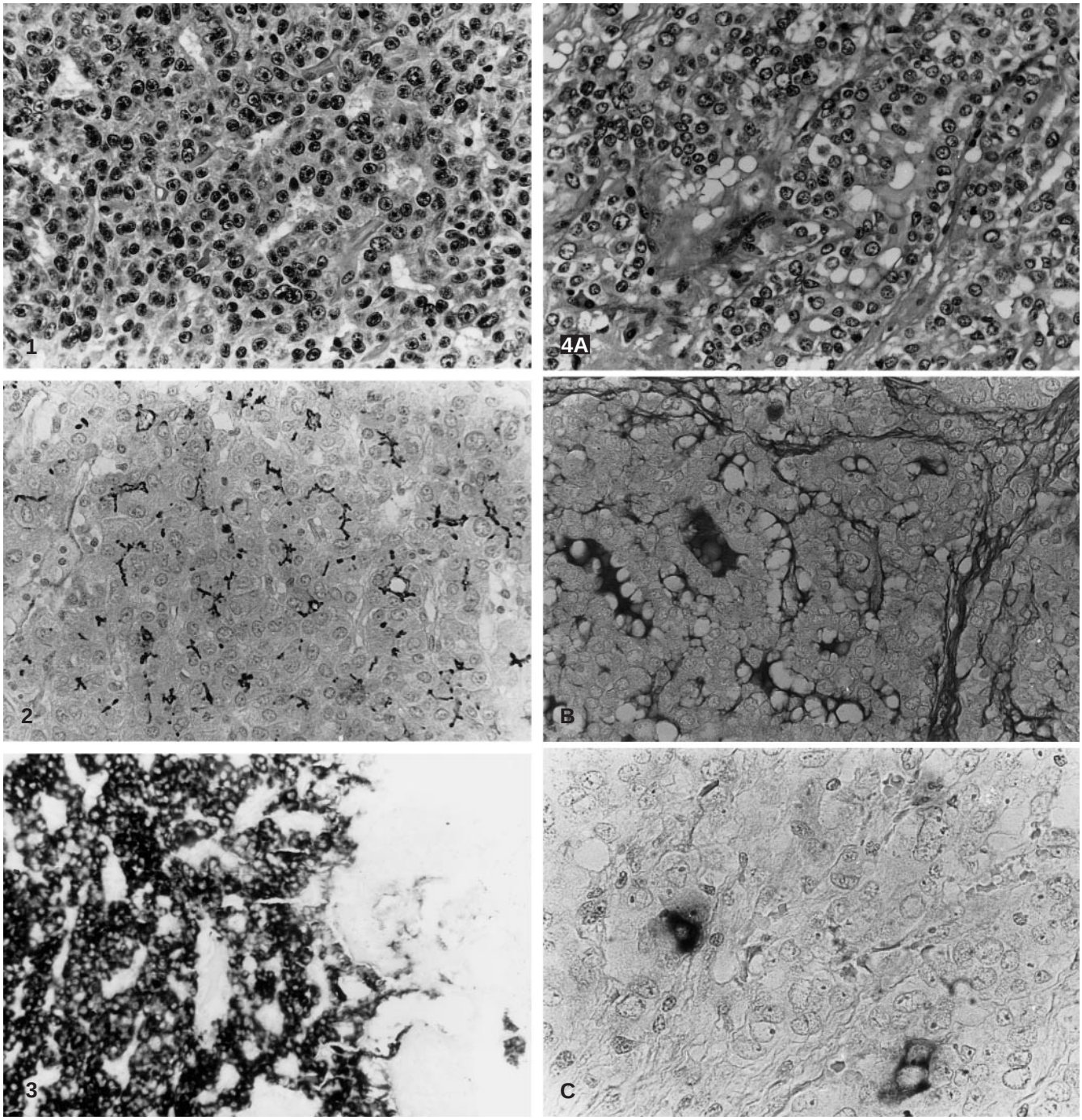

Fig. 1 At high power the hepatoid areas are composed of cords of polygonal cells, with oval nucleus and prominent nucleolus. H&E, ×250

Fig. 2 Immunohistochemical staining with pCEA showed a diffuse canalicular type of positivity in case 1. ABC, ×125

Fig. 3 ISH with albumin mRNA probe is positive in virtually all hepatoid cells of case 1. ISH, ×40

Fig. 4 In case 2, the adenocarcinomatous component shows **A** glandular formation on H&E, **B** with production of alcianophilic mucosubstances. **C** In the same area ISH revealed albumin mRNA in the cytoplasm of occasional cells. **A** H&E×125, **B** Alcian blue, pH 2.5, ×125, **C** ISH×125

rum level of AFP, and they also share a similar immunohistochemical profile. This includes positivity with AFP, A1AAT, alpha-1 antichymotrypsin, and albumin antisera, and a canalicular pattern of imunoreactivity with pCEA [1, 15, 18, 28]. Furthermore in the present case 2 AFP stained the intracytoplasmic PAS-positive granules, similarly to the staining in HCC [21].

Albumin is the most important protein produced by normal hepatocytes as it plays a fundamental part in the maintenance of the plasma oncotic pressure [23]. Since synthesis of this product is generally retained by neoplastic hepatocytes, IHC with albumin antibodies has

been used as a marker of hepatocellular differentiation. Unfortunately, since albumin is ubiquitously present in plasma and extravascular spaces [23], immunohistochemical interpretation is hampered by strong background staining. The phenomenon of protein diffusion is a well-known pitfall in IHC having been described with myoglobin [6], glial fibrillary acidic protein [27], and thyroglobulin [26]. ISH obviates the nonspecific immunocytochemical staining shown to occur when a soluble protein is used, and has therefore proved to be a superior alternative to IHC in selected situations [20]. This is the case with albumin mRNA, which when revealed with ISH has proved of great value in the diagnosis of HCC [9, 21].

The two cases presented here showed the histopathological features of HCC, and their IHC profile was similar to that of HCC [22]. The demonstration in both cases of albumin mRNA with an ISH technique further confirms differentiation toward hepatocellular elements in both cases, not only on morphological grounds but also in terms of their secretion products. This renders the differential diagnosis of HACs against metastases from HCC more difficult. In the present cases the latter possibility was excluded, as both were associated with carcinomas typical of the organs involved and did not show any liver lesion. Conversely, HAC metastases to the liver are histologically indistinguishable from primitive liver cell carcinomas [15]. Thus, in the evaluation of hepatic carcinomas the possibility of HAC metastasis should always be excluded.

The histogenesis of HACs is a matter of debate. For cases located in the digestive tract and for those associated with yolk sac or germ cell tumours, the presence of hepatoid areas has been explained on the basis of a common embryology [16]. However, cases arising in such sites as lung, ovary or urinary bladder are difficult to reconcile with such a hypothesis. In the human embryo, hepatocytes develop, during the 3d week of gestation [30], as a bud of cells from the endodermal epithelium lining the foregut in continuity with the yolk sac [29, 30]. Hepatocellular development is paralleled by sinusoidal growth and mesenchyme formation [30], which stimulate cellular differentiation toward hepatocytes [30]. It has been suggested that the hepatocellular differentiation in nongastrointestinal HACs is the result of a tentative divergent differentiation in the presence of an appropriate stromal environment [2]. This view is supported by the observation that, in our case 2, ISH for albumin mRNA was detected in neoplastic cells in the frankly adenocarcinomatous areas, while failing to show any positive cells in ordinary adenocarcinomas of the gastrointestinal tract (data not shown).

The tumour cells present in HACs are identical to those in HCCs. ISH for albumin mRNA is a valuable method for the detection of hepatocellular differentiation, whatever its mechanism of development.

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