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Can malignancy in insulinoma be predicted by the expression patterns of beta 1,6 branching of asparagine-linked oligosaccharides and polysialic acid of the neural cell adhesion molecule?

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Abstract We analysed the value of the expression of beta 1,6 branching of asparagine-linked oligosaccharide chains and polysialic acid of the neural cell adhesion molecule (NCAM) in predicting malignant behaviour in human insulinomas, as these glycoconjugates have been associated with invasive growth and metastatic potential. Fifty-three insulinomas from patients with well-documented clinical and follow-up data were investigated. Lectin histochemical staining for beta 1,6 branches revealed that 11 (74%) of the 15 malignant insulinomas stained more strongly than normal beta cells. However, in as many as 23 (63.1%) of the 38 benign insulinomas with a disease-free follow up for 4–18 years (average 8 years), a staining intensity equivalent to that of malignant tumours was found. Two (13%) of the malignant insulinomas and 1 of the 4 liver metastases studied were unstained. None of the 53 insulinomas (and the rat RIN insulinoma) re-expressed polysialic acid as demonstrated by immunohistochemistry and Western blotting with the monoclonal antibody 735. Therefore, histochemical staining for beta 1,6 branches and immunohistochemistry for polysialic acid are unlikely to be of value as prognostic indicators for patients with insulinomas.

Key words Insulinoma · Liver metastasis · Beta 1,6 branching · Neural cell adhesion molecule

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Introduction

Insulinomas constitute 70–75% of human pancreatic neuroendocrine tumours. Their biological behaviour cannot be predicted by conventional histopathological criteria [17, 45] with the exception of angioinvasive, poorly differentiated and fast-growing neoplasms. Since the initial reports of Braunstein et al. [3] and Kahn et al. [23], the alpha chains of human chorionic gonadotropin (HCG-alpha), and less frequently, HCG-beta have been found to be expressed by a high percentage of malignant pancreatic neuroendocrine tumours [2, 18–20, 36, 50]. However, recent studies have shown the limited value of immunohistochemical staining for HCG subunits in discriminating malignant from benign neuroendocrine tumours [14, 38]. Further studies on the ploidy status [1, 10, 13], expression of various proto-oncogenes [21], immunohistochemical detection of PCNA [37] and AgNOR labelling [46] have been inconclusive.

The malignant transformation of fibroblasts and epithelial cells is accompanied by increased β-1,6-*N*-acetylglucosamine branching of N-linked oligosaccharides. A direct correlation between this posttranslational modification and tumour stage and also progression and metastasis formation has been demonstrated in tumour cells in vitro and in human tumours [6, 11, 31, 51]. Another important oncodevelopmental antigen is the polysialic acid (poly α2,8 Neu5Ac) of the neural cell adhesion molecule, NCAM, which modulates the adhesive properties of this molecule [43, 44]. A variety of neuroendocrine tumours have been shown to express the poly α2,8 Neu5Ac of NCAM [24–27, 30, 33, 34, 44], and the immunohistochemical staining for poly α2,8 Neu5Ac has become important for diagnosis since it distinguishes small cell lung carcinoma from the more differentiated pulmonary neuroendocrine tumours [25] formerly called carcinoids [4], medullary thyroid carcinoma from other types of thyroid cancers [26], and phaeochromocytoma from adrenocortical carcinoma [27]. Recently, a direct correlation was demonstrated between poly α2,8 Neu5Ac expression and both invasive and metastatic growth of human small cell

lung carcinoma [48]. These observations provided the rationale for the present study on 53 insulinomas in which the possible predictive value of histochemical staining for beta 1,6 branching and of immunohistochemically detectable poly α2,8 Neu5Ac was evaluated.

Materials and methods

Fifty-three insulinomas from patients with hyperinsulinaemic hypoglycaemia, treated at various institutions in Switzerland, Belgium and Germany were studied. Insulinomas were defined as malignant if they showed gross infiltration of adjacent tissues, or if metastases were detected in lymph nodes and/or liver at surgical intervention. Detailed clinical data are listed in Table 1. They include long-time follow up (particularly of patients with benign insulinomas) and immunohistochemical results for insulin, glucagon, somatostatin, pancreatic polypeptide and glycoprotein hormone α-chain.

Digoxigenin-conjugated PHA-L (leukoagglutinating form of *Phaseolus vulgaris* agglutinin), a specific reagent for the histochemical detection of beta 1,6 branches in asparagine-linked oligosaccharides (Fig. 1) [32], polyclonal sheep anti-digoxigenin antibodies, recombinant *N*-glycosidase F, and *Vibrio cholerae* neuraminidase were all purchased from Boehringer Mannheim Biochemica (Mannheim, Germany). The mouse monoclonal antibody mab 735 (protein A-purified ascites), which specifically recognizes poly α2,8 Neu 5Ac composed of at least nine α2,8-ketosidically linked sialic acid residues [12, 16, 22], was kindly provided by Dr. D. Bitter-Suermann (Hannover, Germany). Monodisperse colloidal gold with a diameter of 8 nm was prepared according to Slot and Geuze [49]. Sheep anti-dig antibodies and mab 735 were complexed to 8-nm gold particles as described by Sata et al. [47] and Lackie et al. [28], respectively. Protein A-gold (8-nm gold particles) was prepared according to standard protocol [40, 41]. Bacteriophage K1F endosialidase N (endo N) which specifically hydrolyses α2,8-linked poly α2,8 Neu5Ac, was isolated and purified according to Hallenbeck et al. [15] with modifications [39]. Both the bacteriophage and the *E. coli* strain EVE [52] were gifts from Dr. Eric Vimr (University of Illinois, Urbana, Ill.). A rabbit anti-rat NCAM antibody reactive with all major NCAM isoforms [53] was obtained from Dr. E. Bock (The Panum Institute, University of Copenhagen, Denmark). Bovine thyroglobulin, bovine serum albumin and poly-L-lysine (mol. wt. 150,000) were obtained from Sigma (St. Louis, Mo.). Silver acetate, hydroquinone and glutaraldehyde (25%, vacuum distilled) were from Fluka (Buchs, Switzerland). All other reagents were of the highest available purity.

Table 1 Results of PHA-L staining (– no staining, + fine punctate cytoplasmic labelling, ++ intense punctate cytoplasmic staining, +++ punctate cytoplasmic and intense cell surface labelling)

Staining intensity	Benign insulinomas (*n*=38)	Malignant insulinomas (*n*=15)	Metastasis (*n*=4)
–	1 (2.5%)	2 (13%)	1
+	14 (36.8%)	2 (13%)	
++	18 (47.3%)	4 (27%)	2
+++	5 (13.2%)	7 (47%)	1

The insulinomas with adjacent non-tumour pancreatic parenchyma and metastatic lesions (regional lymph nodes, liver) were obtained at surgical intervention, fixed in 4% phosphate-buffered formaldehyde or Bouin's fluid and routinely embedded in paraffin. Paraffin blocks from formaldehyde-fixed pancreas of 19-day rat embryos were obtained from our archival files. The transplantable rat insulinoma RIN, grown either subcutaneously or in monolayer culture, and isolated rat pancreatic islets were kindly provided by Dr. C. Wollheim (University of Geneva, Switzerland). They were formaldehyde-glutaraldehyde double-fixed as described above and embedded in paraffin.

Histochemical detection of beta 1,6 branches was performed using the lectin PHA-L in a two-step gold-labelling technique as previously described [31, 32].

Paraffin sections were dewaxed in two changes of xylene (10 min each), rehydrated through a series of graded ethanol, placed in PBS and processed for PHA-L lectin labelling. The sections (paraffin and Lowicryl K4M) were conditioned with PBS (pH 7.4) containing 1% bovine serum albumin, 0.05% Tween 20 and 0.05% Triton X-100 (buffer 1) for 10 min at room temperature, then incubated with dig-PHA-L (10 µg/ml in buffer 1) for 1 h at room temperature, followed by two rinses in PBS (5 min each). The sections were then covered with gold-labelled anti-dig antibodies (diluted with buffer 1 to give an absorbance at 525 nm of 0.05) for 1 h at room temperature. Following this incubation step, sections were rinsed in PBS twice for 5 min each, fixed with 1% glutaraldehyde in PBS for 20 min, briefly rinsed in PBS and several changes of double-distilled water (5–10 min) and air dried. Signal amplification by a photochemical silver reaction was carried out as previously described [31, 32]. Paraffin sections were counterstained with nuclear fast red.

Controls to demonstrate specificity of staining included omission of the lectin, preabsorption of the lectin with various glycoproteins, and pretreatment of the sections with *N*-glycanase or *Vibrio cholerae* sialidase and were performed as previously detailed [31, 32, 42]. The staining results were evaluated independently by two pathologists. The following semiquantitative evaluation scheme was followed: 0=no staining; +=fine punctate cytoplasmic labelling; ++=intense punctate cytoplasmic labelling; +++=punctate cytoplasmic and intense cell surface labelling.

For the immunohistochemical detection of poly α2,8 Neu5Ac, paraffin sections were incubated with directly gold-labeled mab 735 followed by silver intensification using silver acetate.

Dewaxed and rehydrated sections were blocked with PBS containing 2% w/v fat-free dried milk powder for 5–10 min. Sections were then incubated for 1 h with gold-labelled monoclonal antibody mAb 735 diluted to an absorbance at 525 nm of 0.04 (8-nm gold particles). The sections were then washed (2×5 min in PBS). Afterwards the sections were postfixed in 1% glutaraldehyde for 20 min and well washed in water followed by silver intensification and counterstaining with nuclear fast red. Details of the use of endo

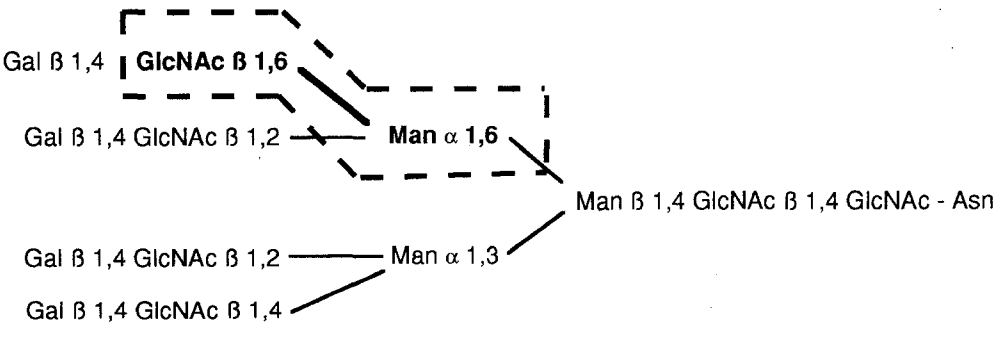

Fig. 1 Schematic presentation of an asparagine-linked oligosaccharide chain. The beta 1,6 branch is in *bold print* and outlined by the *dashed box*

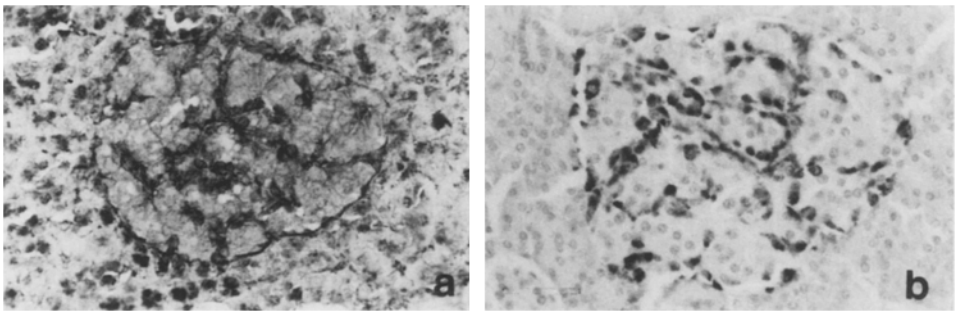

Fig. 2 Normal human pancreas, serial paraffin sections stained with **a** PHA-L and **b** glucagon antibodies, silver-intensified lectin-gold and immunogold-labelling, respectively. Strong PHA-L staining in an islet (**a**) corresponds to the distribution of the glucagon-immunoreactive cells (**b**). The majority of the endocrine cells that are beta cells show faint cytoplasmic staining. In addition, the exocrine acinar cells exhibit strong PHA-L staining over the apical portion of the cytoplasm containing the zymogen granules

N in control incubations to demonstrate specificity of immunolabelling for poly α2,8 Neu 5Ac have been described elsewhere [53].

NCAM immunoreactive sites were detected either in paraffin sections or in monolayer cultures of RIN insulinoma using rabbit polyclonal anti-rat NCAM antibodies and the protein A-gold technique [41, 53]. Sections were blocked with PBS containing 2% w/v fat-free dried milk powder for 5–10 min followed by a 1 h incubation with the primary antibody (500-fold dilution in PBS containing 1% w/v fat-free dried milk powder). Washing (2×5 min in PBS) was followed by incubation with either goat anti-rabbit IgG-gold (absorbance at 525 nm=0.05, 8-nm gold particles, diluted in PBS containing 1% bovine serum albumin and 0.05% of both Triton X100 and Tween 20) or protein A-gold (absorbance at 525 nm=0.06, 8-nm gold particles, diluted in PBS containing 1% bovine serum albumin and 0.05% of both Triton X100 and Tween 20).

Monolayer cultures were fixed in 2% formaldehyde (freshly prepared from paraformaldehyde) and 0.01% glutaraldehyde for 30 min, after which free aldehyde groups were quenched by immersion in PBS containing 50 mM NH_4Cl for 30 min at ambient temperature followed by incubation steps for the detection of

Table 2 Clinical data and results in patients with benign insulinomas (*Tu Diam* tumour diameter in cm, *Hist* histology, *sol* solid pattern, *trab* trabecular pattern, *gland* glandular pattern, *β1,6* staining intensity for beta 1,6 branches (see also Table 1), *HCG* immunostaining for glycoprotein-hormone alpha chain, *Ins* immunostaining for insulin, *gluc* immunostaining for glucagon, *Som* immunostaining for somatostatin, *PP* immunostaining for pancreatic polypeptide, *DD* died of disease, *DOC* died of other causes, *WA* well and alive, *NA* not available)

Age	Sex	Tu Diam	Hist	β1,6	HCG	Ins	Gluc	Som	PP	Follow up[a]
48	M	1.5	sol	+	–	+	–	–	–	15/WA
32	M	1.5	sol	++	(+)	+	–	–	(+)	13/WA
56	F	1.5	trab	+/++	–	+	(+)	–	–	13/WA
37	F	2	sol	++	–	+	–	–	–	12/WA
24	F	1.5	sol	++	+	+	(+)	–	(+)	12/WA
59	M	1.5	sol	+++	–	+	+	(+)	(+)	11/WA
40	M	2	sol	++	–	+	(+)	–	–	10/WA
57	F	2	sol	++	–	+	+	–	–	NA
56	F	1.5	sol	++	–	+	–	–	–	7/WA
39	M	1.5	sol	+	–	+	–	–	–	8/WA
54	F	1.5	sol	+	–	+	–	–	–	7/WA
45	F	2	trab	++	–	+	–	–	–	8/WA
41	M	0.8	trab	+	–	+	–	–	–	7/WA
66	F	2	sol	++	–	+	–	–	–	10/WA
77	M	2	trab	+	–	+	+	(+)	(+)	6/WA
51	F	1	sol	++	–	+	–	–	–	9/WA
62	F	0.8	trab	+	–	+	–	–	–	9/WA
43	M	1	sol	++/+++	–	+	–	–	–	8/WA
21	M	1	trab	+/++	–	(+)	–	–	–	9/WA
40	F	1.5	trab	++	+	+	–	+	–	1/WA
63	M	1	sol	++	–	+	–	(+)	–	3/WA
69	F	1.2	sol	+	–	+	–	+	(+)	7/WA
29	F	1.2	sol	++	–	+	–	–	–	7/WA
21	F	1.2	sol	++	–	+	–	–	(+)	6/WA
72	M	0.8	sol	+++	–	+	–	+	–	6/WA
46	F	2	trab	++	–	+	–	–	–	6/WA
27	F	2.5	sol	+++	–	+	–	(+)	–	6/WA
27	F	0.8	sol	+	–	+	–	+	–	5/WA
30	M	2	trab	+++	–	(+)	–	–	–	5/WA
57	M	2.5	trab	++	–	+	–	–	–	0.1/DOC
20	M	0.8	trab	+	–	(+)	–	–	–	4/WA
50	M	2	sol	++/+++	–	(+)	–	–	–	4/WA
65	F	1.5	trab	+++	–	+	–	–	–	4/WA
88	F	1	trab	++	–	+	–	–	–	18/DOC
63	M	0.9	gland/sol	+	–	+	–	–	–	NA
74	F	1.2	gland	–	–	+	–	–	+	NA
22	F	1.5	gland	+	–	+	–	–	–	NA
48	M	1.5	sol/trab	+	–	+	+	+	+	NA

[a] Follow up in years/clinical state

Table 3 Clinical data and results of the patients with malignant insulinomas

Age	Sex	Tu Diam	Hist	β1,6	HCG	Ins	Gluc	Som	PP	Follow up[a]
76	F	3	sol	+++	(+)	(+)	+	(+)	+	0.5/DOC
62	F	8	tab	++	−	+	+	−	+	10/WA
57	M	8	tab	+++	−	(+)	+	(+)	(+)	NA
56	M	10	sol	++/+++	−	+	−	(+)	−	4/WA
66	F	4	sol	+++	(+)	+	−	−	+	10.1/DOC
71	F	6	sol	+++	−	+	−	−	+	0.1/DOC
55	M	2	tab	++	−	+	−	+	−	9/WA
54	M	5	sol	+++	+	(+)	(+)	−	+	5/WA
73	F	3	sol	+++	(+)	+	(+)	−	+	7/DD
60	M	2	sol	+++	+	+	+	−	+	1/DD
69	M	12	sol/trab	+	+	+	−	−	−	0.1/DOC
68	M	5	sol/gland	+	+	+	+	+	+	NA
65	F	2.4	trab	−	+	+	+	+	+	6/WA
73	F	1	trab	−	+	+	+	+	+	1/WA
56	M	3.5	trab	++/+++	+	+	+	+	+	NA

[a] Follow up in years/clinical state

NCAM immunoreactive cell surface sites as described above without silver intensification. Labelled cell cultures were postfixed in 1% osmium tetroxide for 30 min followed by embedding in Epon 812. Thin sections were prepared, counterstained with lead and uranyl acetate and examined under the electron microscope.

For immunoblotting homogenates were prepared from deep-frozen tissues (insulinomas, normal pancreas, brain of 18-day rat embryos) and from isolated rat pancreatic islets and monolayer cultures of the rat insulinoma RIN. Samples were homogenized in PBS containing 1% Triton X-100, 0.15 mM PMSF and 1% aprotinin. SDS-PAGE using 3–10% polyacrylamide gradient gels and immunoblotting using directly gold-labelled mAb 735 and anti-NCAM antibodies was performed as previously described [28, 53]. Pretreatment of embryonic rat brain homogenates with endoneuraminidase N was performed as previously described [53].

Results

In normal human pancreata, staining with PHA-L was observed in both islets and exocrine acinar cells and fibroblasts, lymphocytes, red blood cells and vascular en-

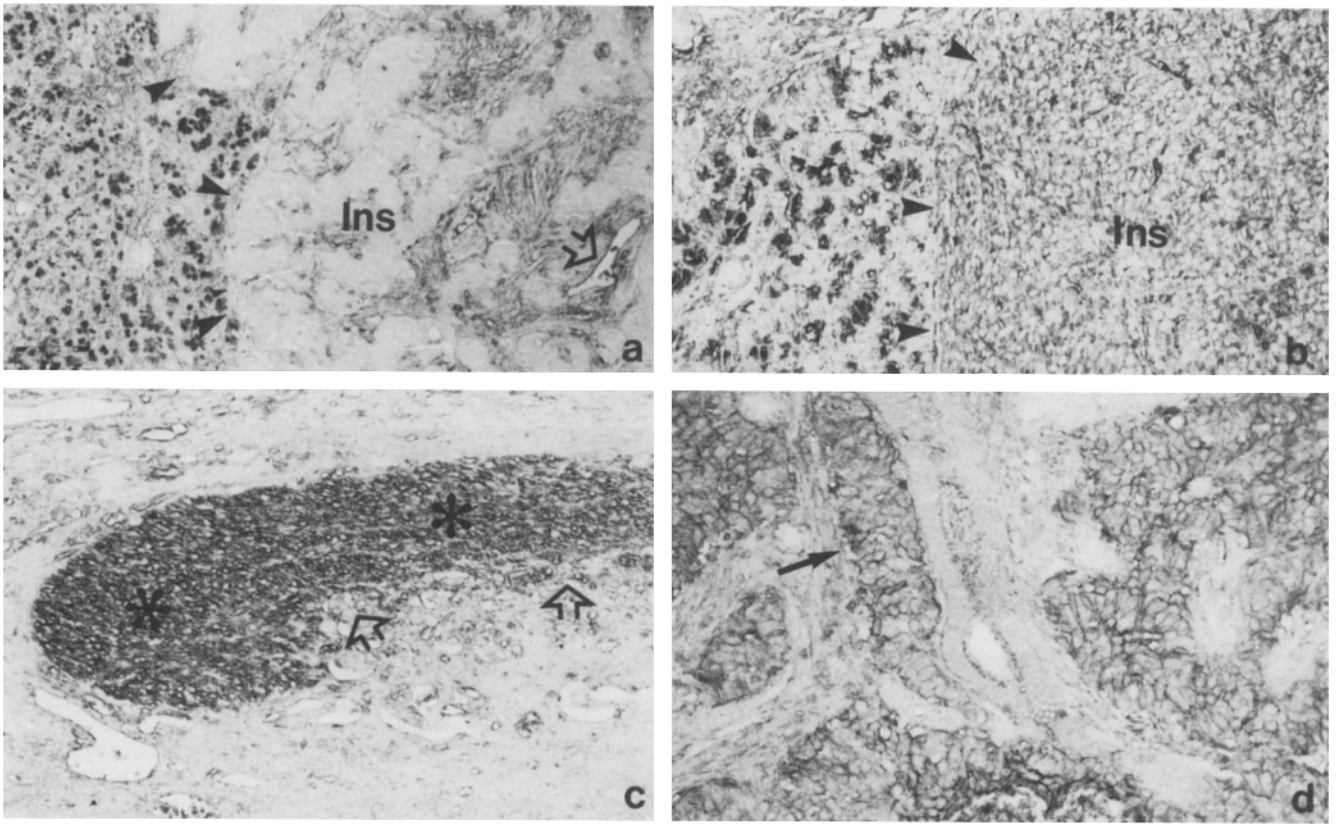

Fig. 3 Malignant insulinomas (*Ins*) may be unreactive (**a**) or intensely stained (**b**) when incubated with PHA-L. **c** A PHA-L unreactive malignant insulinoma, together with a partially invaded (*open arrows*), PHA-L-positive nerve (*asterisks*). Note the PHA-L staining of the stroma and capillaries (*open arrow* in **a**) in the unreactive malignant insulinomas (**a**,**c**). The *arrowheads* in **a** and **b** indicate the border to the exocrine pancreatic parenchyma, the latter being intensely stained. Note that there is no accentuation of PHA-L staining in the invasion front of the malignant insulinoma shown in **b** or the angioinvasive tumour cell complexes of another malignant insulinoma (*arrow* in **d**)

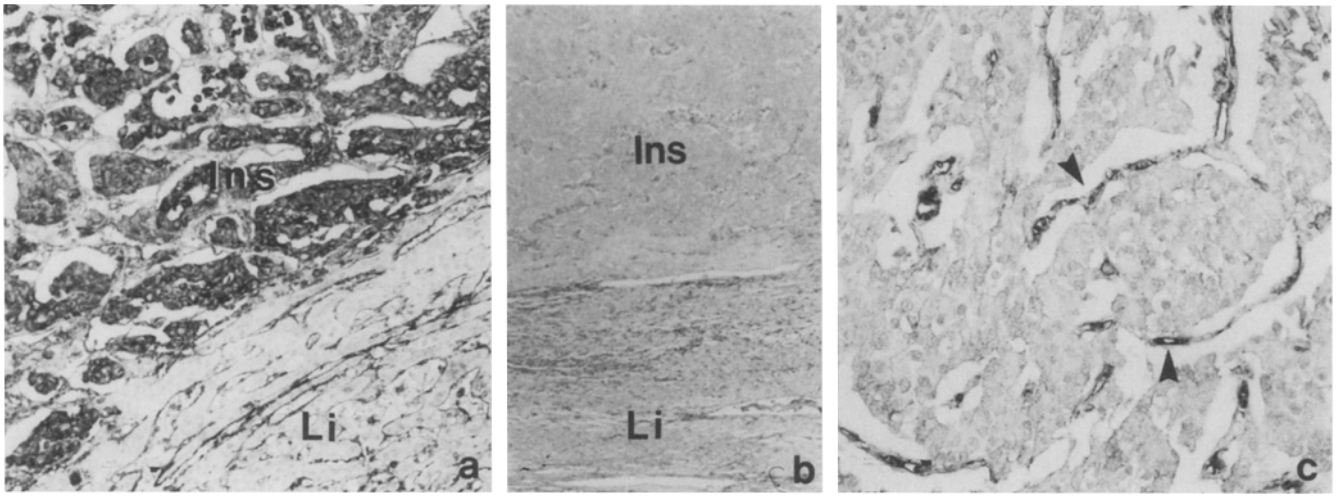

Fig. 4 Liver metastases of malignant insulinomas (*Ins*) may be **a** intensely stained by PHA-L or **b** unreactive. **c** Absence of PHA-L staining in a liver metastasis can be clearly seen at higher magnification, whereas the capillaries (*arrowheads*) are positive

dothelium (Fig. 2). Strongly PHA-L-positive cells in the islets corresponded to glucagon-producing alpha cells (cf. Fig. 2a,b). Insulin-producing beta-cells exhibited weak cytoplasmic staining (Fig. 2a) and cells with secretory granules typical of somatostatin-producing delta cells were not labelled (not shown).

In the insulinomas, a wide variation in the intensity of PHA-L staining was observed (Tables 1–3, Figs. 3, 4). Of the 38 clinically benign insulinomas, 23 (63.1%) exhibited lectin staining that was more intense than that of normal beta cells (Tables 1, 2). In the group with clinically benign insulinomas, patients were alive and disease free for 4–18 years of follow up (average 8 years). The 15 clinically malignant insulinomas included 11 (74%) that were histochemically strongly positive for β 1,6 branches (Fig. 4a, Tables 1, 3). Notably, 2 (13%) were histochemically unreactive for β 1,6 branches (Fig. 3a) but HCG-alpha positive, and 2 (13%) showed a weak cytoplasmic staining equal to that observed for beta cells in normal islets. It should be noted that the stroma and capillary endothelia and also the surrounding exocrine pancreas of the histochemically negative benign and malignant insulinomas were reactive for β 1,6 branches (Fig. 3a). Figure 3c shows a stained peripheral nerve that is partially invaded by unstained insulinoma cells. Of the 11 clinically malignant and intensely β 1,6 branching positive insulinomas, 5 were immunohistochemically unreactive for HCG-alpha (Table 3). Variability in the intensity of staining for beta 1,6 branching was observed but was not consistent with the tumour invasion front, vascular invasion, or particular morphological features of the insulinomas (Fig. 3d). The studied metastases did not exhibit a consistent pattern (Fig. 4). In particular, one was unstained by PHA-L although the surrounding liver parenchyma exhibited strongly stained capillaries (Fig. 4c).

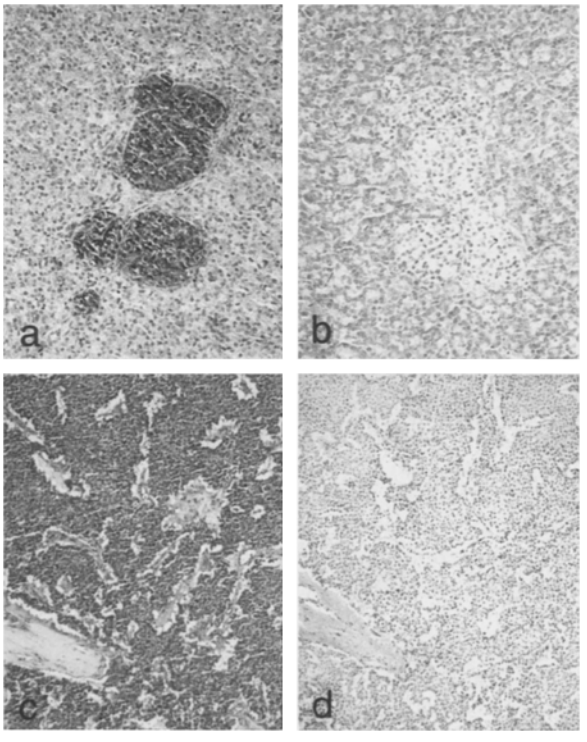

Fig. 5 Consecutive serial paraffin sections of normal human pancreas (**a**,**b**) and a malignant insulinoma (**c**,**d**); immunogold-silver staining. The islets of Langerhans (**a**) and the insulinoma (**c**) exhibit immunostaining for NCAM. The exocrine parenchyma (**a**) and the tumour stroma (**c**) are unreactive. No immunoreactivity for polysialic acid is detectable in the islets of Langerhans and the exocrine parenchyma (**b**) or in the insulinoma (**d**)

In normal adult human pancreas, immunostaining for NCAM was detectable in all cells of the islets of Langerhans and pancreatic nerve fibres, but undetecable in the exocrine acinar cells (Fig. 5a). Immunoreactivity for poly α2,8 Neu5Ac was undetectable in the pancreas except for nerve fibres (Fig. 5b). Specimens of human embryonic pancreas were not available for study, but in pancreata from 19-day embryonic rats, NCAM immunostaining was observed in the exocrine and endocrine pancreas, which is consistent with previously published data [29].

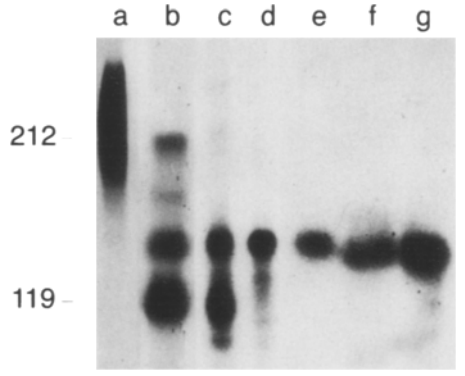

Fig. 6 Western blot analysis. *Lane a* 18-day rat embryonic brain exhibits the typical high molecular weight (≥250 kDa) broad band of polysialic acid; directly gold-labelled mab 735. *Lane b* 18-day rat embryonic brain, pretreatment with endo N to remove polysialic acid reveals the typical NCAM isoforms; rabbit anti-NCAM antibodies followed alkaline phosphatase conjugated secondary antibodies. *Lane c* adult normal human pancreas shows a 120 kDa and a 140 kDa NCAM isoform without pretreatment by endo N. This demonstrates absence of polysialic acid on NCAM, since its presence would result in a broad, high-molecular-weight band as seen in *lane a*. *Lane d* human insulinoma exhibits a 140-kDa nonpolysialylated NCAM. *Lane e* isolated rat islets with a 140-kDa nonpolysialylated NCAM. *Lanes f and g* subcutaneously grown tumour and monolayer, respectively, of rat insulinoma RINm5F. Nitrocellulose stripes shown in *lanes c–e* were incubated with anti-NCAM antibodies followed by alkaline phosphatase conjugated secondary antibody as detailed in Materials and methods; no endo N treatment

All benign and malignant insulinomas showed immunostaining for NCAM (Fig. 5c) but none for poly α2,8 Neu5Ac (Fig. 5d), which was confirmed by Western blot analysis (Fig. 6). The 120- and 140-kDa NCAM protein isoforms were detectable in normal pancreas (Fig. 6, lane c) and a 140-kDa NCAM isoform, in the insulinomas (Fig. 6, lane d). Similar results were obtained with isolated rat islets and the transplantable rat insulinoma RIN (Fig. 6, lanes e–g). For comparison, embryonic rat brain was studied, which exhibited a broad band of high molecular mass typical of polysialylated NCAM (Fig. 6, lane a) [43, 44, 53] which could be resolved in distinct NCAM protein isoform bands by enzymatic removal of poly α2,8 Neu5Ac with endo N (Fig. 6, lane b) [35, 53].

Discussion

In the present study we investigated two carbohydrate antigens, beta 1,6 branches and poly α2,8 Neu5Ac, to assess their value in predicting malignancy in 53 human insulinomas. The rationale for this investigation was based on observations that increased cellular expression of beta 1,6 branching was directly associated with the acquisition of metastatic potential of certain tumour cell lines [7–9]. The poly α2,8 Neu5Ac of NCAM has also been shown to be useful in the immunohistochemical classification of various neuroendocrine tumours and tumours of other origins [44, 45] and to be associated with invasive and metastatic growth [48].

Of the 53 insulinomas in our study, 72% appeared to be benign on the basis of morphological criteria at surgical intervention and showed a clinical course consistent with the initial diagnosis. In these tumours, the intensity of histochemical staining for beta 1,6 branches was highly variable. Most notably, 23 (63.1%) of the benign insulinomas presented more intense staining than normal pancreatic beta cells and their staining intensity equalled that of some malignant insulinomas. Only 14 of the benign insulinomas (34%) showed the same staining intensity as normal pancreatic beta cells. All 15 malignant insulinomas displayed unequivocal criteria of malignancy at surgery. As in the benign insulinomas, however, a high staining variability was observed. Two of the malignant insulinomas (13%) were unreactive and the staining of 2 others (13%) was equal in intensity to that of normal pancreatic beta cells. These findings are at variance with previous work on colon and breast carcinomas [8, 11], in which the carcinoma cells were consistently more intensely stained with PHA-L than the cells of the corresponding normal tissue and the benign (preneoplastic) lesions. A study on oesophageal carcinomas showed most intense PHA-L staining at the invasion front [51]. No such staining pattern has been identified for insulinomas, however. This also holds for the liver metastases from malignant insulinomas we studied, which did not show a consistent PHA-L staining profile. We were therefore unable to relate the histochemical staining for beta 1,6 branches in insulinomas to their biology. This agrees with a recent study on breast carcinomas, which indicates that PHA-L histochemical staining may not be predictive of either the disease-free interval or the overall survival of the patients [5].

None of the insulinomas stained for poly α2,8 Neu5Ac of NCAM, although NCAM protein was detectable. Hence, poly α2,8 Neu5Ac is expressed in the developing pancreas in the fetus, but not in insulinomas. This is in agreement with results of previous studies [24–27, 30, 33, 44, 48] demonstrating consistent NCAM protein expression in neuroendocrine tumour types but highly variable poly α2,8 Neu5Ac immunolabelling patterns. For example, medullary thyroid carcinoma, small cell lung carcinoma and phaeochromocytoma were positive for both poly α2,8 Neu5Ac and NCAM protein, whereas neuroendocrine tumours (carcinoids) of the lung and gastrointestinal tract were unreactive for poly α2,8 Neu5Ac. Such differential poly α2,8 Neu5Ac expression is helpful in discriminating between neuroendocrine tumours. However, the reason for the differences in poly α2,8 Neu5Ac expression among the neuroendocrine tumour is not known.

We conclude from our study that histochemical staining for beta 1,6 branches and immunohistochemistry for poly α2,8 Neu5Ac are of no value in predicting the biological behaviour of insulinomas.

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