Eur J Cancer, Vol. 29A, No. 14, pp. 2070–2071, 1993.
Printed in Great Britain
0959–8049/93 $6.00 + 0.00

Letters

Immunohistochemical Expression of Oncofetal Fibronectin in Benign and Malignant Lesions of the Stomach

Leonor David, Ulla Mandel, Henrik Clausen and Manuel Sobrinho-Simões

FIBRONECTIN (FN), AN extracellular matrix adhesion molecule, is widely distributed in normal tissues. The isoform of this glycoprotein designated as oncofetal FN (onf-FN) was originally identified by immunisation with tumour FN and selection for antibodies reactive only with tumour FN and not with plasma FN [1]. Subsequent studies of the specificity of the antibodies revealed that the epitope is a specific peptide sequence in the C-terminal region of FN with O-linked glycosylation of a single threonine residue [2, 3]. Previous immunohistochemical studies demonstrated the expression of onf-FN in the stroma of human carcinomas of the breast [4] and of the oral mucosa [5] thus suggesting the possibility of using the detection of onf-FN for diagnosing the initial steps of malignant transformation. A relationship has also been found between onf-FN expression and advanced histological grading of breast cancer [4]. Our aim was to evaluate if there is, in the stomach, a tumour-associated expression of onf-FN.

We studied the expression of normal FN and onf-FN in 17 gastric carcinomas, 15 non-neoplastic mucosas (12 cases with a normal appearance, 2 cases with intestinal metaplasia and 1 case with dysplasia) and three peptic ulcers. Immunohistochemistry was performed by the indirect immunoperoxidase method on frozen sections, using monoclonal antibody FDZ directed to normal FN [1] and monoclonal antibody 5C10 directed to onf-FN [4]. The staining was semi-quantitatively scored as −, +, ++ and +++, by two independent observers.

The antibody FDZ defined the basement membranes of gastric epithelium, vessels and muscle cells in non-neoplastic mucosa. Peptic ulcers and gastric carcinomas showed, in every case, very strong staining of the connective tissue at the ulcer base and tumour stroma, respectively (Table 1).

Table 1. FDZ (anti-FN) and 5C10 (anti-onf-FN) staining in non-neoplastic mucosa, peptic ulcers and gastric carcinomas

	FDZ	5C10
Non-neoplastic mucosa	15/15	2/15
Normal appearing mucosa	12/12	1/12
Intestinal metaplasia	2/2	0/2
Dysplasia	1/1	1/1
Peptic ulcers	3/3	3/3
Gastric carcinomas	17/17	16/17

The antibody 5C10 did not stain the large majority of cases of non-neoplastic mucosa, including the hyperplastic mucosa surrounding the peptic ulcers. However, 1 case with an otherwise normal looking mucosa and a single case with dysplasia displayed linear staining along the basement membranes of gastric glands (Table 1). In the three peptic ulcers the antibody against onf-FN stained coarse fibres at the ulcer base. Sixteen of the 17 gastric carcinomas displayed onf-FN immunoreactivity along basement membranes of tumour cell nests and vessels, as well as in fibres of the tumour stroma (Table 1). A significant relationship ($P < 0.02$) was found between the staining intensity (scores ++/+++) and the presence of vascular invasion. No significant relationship was found between the staining intensity and wall penetration and lymph node metastases. In the tumours with intense desmoplasia 5C10 also depicted a coarse fibrillar pattern of the connective tissue similar to that observed at the base of the ulcers. The single negative tumour did not display any distinctive morphological features when compared with the other carcinomas of our series.

The presence of connective tissue staining in all the three peptic ulcers and the immunoreactivity in the basement membrane of a normal looking mucosa demonstrates that the expression of onf-FN is not strictly associated with malignancy. Despite this, our results show that onf-FN is extremely frequent in gastric carcinomas and rarely observed in non-neoplastic mucosas. Our results show, furthermore, that there is an association between the intensity of onf-FN expression and the vascular invasiveness of the carcinomas. The study would have to be extended to clarify whether the onf-FN immunoreactivity in our single case of dysplasia is an isolated finding or whether it may reflect a dysplasia-related intrinsic alteration of the expression of onf-FN. This hypothesis would lead to the possibility of using the detection of onf-FN as a screening method for incipient malignant transformation of the gastric mucosa. Altogether, our findings suggest that the production of this particular form of the glycoprotein is dependent on a variety of conditions having in common the capacity to induce deposition and/or remodelling of extracellular matrix.

Correspondence to L. David.
L. David and M. Sobrinho-Simões are at the Institute of Molecular Pathology and Immunology (IPATIMUP), Medical School, Hospital S. João, University of Porto, 4200 Porto, Portugal; and U. Mandel and H. Clausen are at the Department of Oral Diagnostics, Faculty of Health Sciences, University of Copenhagen, Norre Alle 20, DK-2200, Copenhagen, Denmark.
Received 4 June 1993; accepted 10 June 1993.

1. Matsuura H, Hakomori S. The oncofetal domain of fibronectin defined by monoclonal antibody FDC-6: its presence in fibroblasts from fetal and tumor tissues and its absence in those from normal adult tissues and its absence in those from normal adult tissues and plasma. *Proc Natl Acad Sci USA* 1985, **82**, 6517–6521.
2. Matsuura H, Takio K, Titani K, *et al.* The oncofetal structure of human fibronectin defined by monoclonal antibody FDC-6. Unique structural requirement for the antigenic specificity provided by a glycosylhexapeptide. *J Biol Chem* 1988, **263**, 3314–3322.
3. Matsuura H, Hakomori S. An alpha-*N*-acetylgalactosaminylation at the threonine residue of a defined peptide sequence creates the oncofetal peptide epitope in human fibronectin. *J Biol Chem* 1989, **264**, 10472–10476.

4. Loridon-Rosa B, Vielh P, Matsuura H, Clausen H, Cuadrado C, Burtin P. Distribution of oncofetal fibronectin in human mammary tumors: immunofluorescence study on histological sections. *Cancer Res* 1990, **50**, 1608–1612.
5. Mandel U, Therkildsen MH, Reibel J, *et al.* Cancer-associated changes in glycosylation of fibronectin. Immunohistological localization of oncofetal fibronectin defined by monoclonal antibodies. *Acta Path Microbiol Scand* 1992, **100**, 817–826.

Acknowledgements—The authors thank the support of JNICT and the Danish Cancer Society. The authors also thank the expert technical assistance of Dina Leitão.

Eur J Cancer, Vol. 29A, No. 14, pp. 2071–2072, 1993.
Printed in Great Britain
0959–8049/93 $6.00 + 0.00

Delayed Thyroid-Stimulating Hormone Suppression by L-Thyroxine in the Management of Differentiated Thyroid Carcinoma

Carlo L. Maini, Rosa Sciuto and Anna Tofani

THYROID STIMULATING hormone (TSH) SUPPRESSION BY L-thyroxine (THY) is a cornerstone in the management of patients with differentiated thyroid carcinoma (DTC) after thyroidectomy, as is radioiodine ablation to avoid any TSH stimulation of tumour growth [1–3]. Induction of hypothyroidism by the withdrawal of THY once or twice a year is necessary to perform diagnostic ^{131}I total body scanning (TBS) or radioiodine treatment. After the TBS and treatment, shortening of the exposure period to inappropriate elevated TSH serum levels is advisable. Prolonged exposure to TSH stimulation can greatly increase the chance of further mutation and allow tumour progression by clonal selection [4]. Accordingly, THY therapy is restarted 24 h after radioiodine administration and continued thereafter with the highest tolerated dosage. Mean recommended THY suppressive doses range from 150 to 300 μg/day [5–8]. Unfortunately, these dosages may be dangerous if administered from the beginning in highly hypothyroid patients because of cardiovascular side-effects. Administration of a low THY dose is often mandatory in elderly subjects or those with cardiac disease to avoid tachyarrhythmias or angina. This is a limiting factor in the approach to TSH suppressive treatment in patients with DTC. Most studies report on the adequacy of TSH suppression by THY therapy in terms of final TSH serum concentrations but provide limited information on the time-course necessary to obtain such results [9,10].

We have evaluated short-term TSH suppression in 30 patients with DTC after TBS and THY. Patients were divided into three groups in relation to THY dosage; they were selected according to age, body weight and clinical condition. 10 patients received 100 μg/day (group 1), 10 patients received 150 μg/day (group 2) and 10 received 200 μg/day (group 3). Serum TSH, T_3 and T_4 were measured on the day of TBS (day 0) and after 7, 14, 21, 30, 45, 60 and 90 days. Serum levels of T_3 and T_4 were assayed by specific radioimmunoassays (RIA) (Radim) and TSH by an ultrasensitive immunoradiometric assay (Byk-Mallinckrodt). A TSH level below 0.5 mU/l was taken as evidence of complete suppression. The results are reported as mean concentration values and S.E.M. in Fig. 1. Mean basal TSH serum levels do not differ significantly among the three groups, ranging from 117 to 100.3 mU/l. The pattern of TSH inhibition was, however, different. Complete TSH suppression was achieved after 3 months in group 1, between 45 and 60 days in group 2 and between 30 and 45 days in group 3. Serum T_4 and T_3 increased

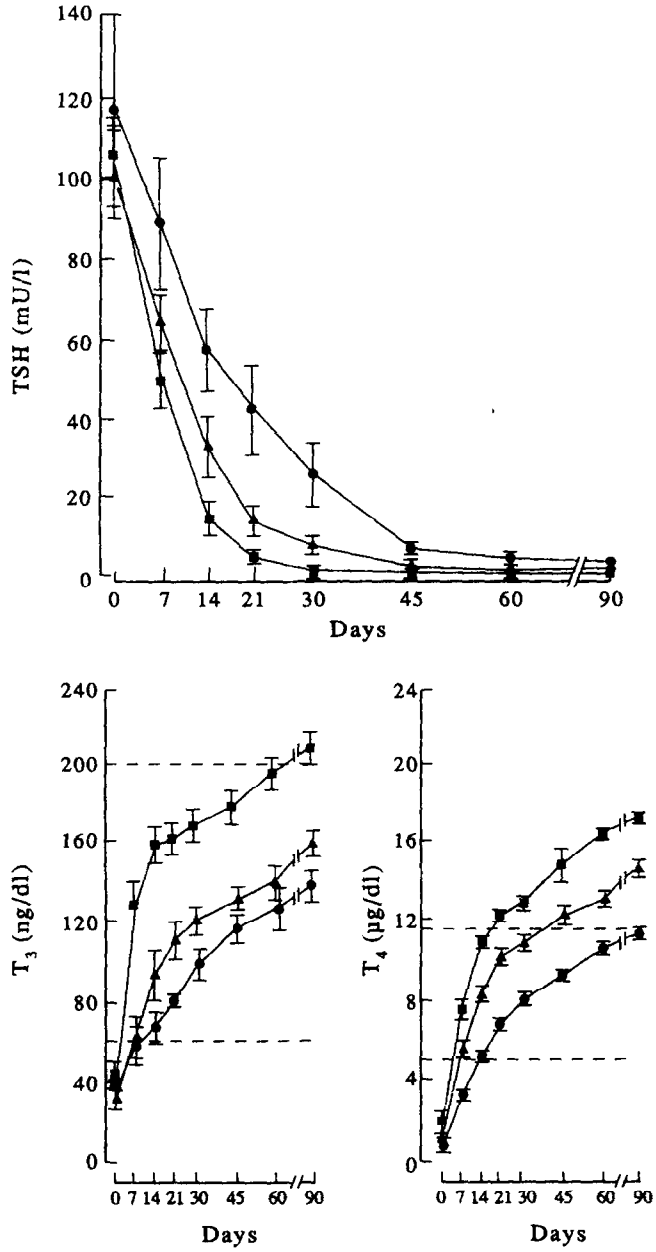

Fig. 1. Mean serum TSH (top panel) and T_3 and T_4 (bottom panel) levels and S.E.M. in three groups of patients with differentiated thyroid carcinoma after restarting of different doses of L-THY. Group 1 : 100 μg of L-THY (circles); group 2 : 150 μg of L-THY (triangles); group 3 : 200 μg of L-THY (squares). Normal range for T_3 and T_4 is shown by broken lines.

Correspondence to C.L. Maini at La Pietra Pizzuta, I-03010 Patrica (FR), Italy.
R. Sciuto and A. Tofani are at the Nuclear Medicine Department, "Regina Elena" National Cancer Institute, Viale Regina Elena 291, Rome, Italy.
Revised 9 June 1993; accepted 29 June 1993