Eur J Cancer, Vol. 27, No. 6, pp. 699–703, 1991.
Printed in Great Britain

0277–5379/91 $3.00 + 0.00

Insulin-like Growth Factor-II (IGF-II)/Mannose-6-phosphate Receptors are Increased in Primary Human Thyroid Neoplasms

Tohru Yashiro, Toshio Tsushima, Hitomi Murakami, Takao Obara, Yoshihide Fujimoto, Kazuo Shizume and Kunihiko Ito

Insulin-like growth factor (IGF)-II receptors were demonstrated in normal and neoplastic tissues of human thyroid. Specific binding of [^{125}I]IGF-II to thyroid membranes was dependent on the time and temperature of incubation, and a steady state was achieved after 22 h of incubation at 4°C. The binding of [^{125}I]IGF-II was dose-dependently displaced by unlabelled IGF-II with 50% inhibition at an IGF-II concentration of 6 ng/ml. IGF-I had a relative potency of 1% compared to IGF-II, and insulin showed no inhibition at concentrations as high as 2000 ng/ml. Scatchard analysis of the binding data revealed a single class of IGF-II receptor with high affinity. Affinity crosslinking and autoradiography demonstrated the type II IGF receptors. Specific binding of [^{125}I]IGF-II to thyroid papillary cancer tissues (mean [S.D.] 13.2 [1.3]% per 200 μg protein, $n = 8$) was significantly ($P < 0.01$) higher than that to the surrounding normal tissues (4.8 [0.5]%). The binding in follicular cancer and follicular adenoma was also significantly higher than that in the corresponding normal tissues. The higher IGF-II binding to neoplastic tissues was due to an increase in the number of binding sites without any change of affinity. These results suggest that the increased IGF-II receptors may be involved in growth or functions of thyroid neoplasms.
Eur J Cancer, Vol. 27, No. 6, pp. 699–703, 1991

INTRODUCTION

THE INSULIN-LIKE growth factors (IGF)-I and II are growth hormone-dependent growth factors that are mitogenic to many types of normal and neoplastic cells [1–4]. Many studies have shown that these peptides are also involved in the growth of thyroid cells, including those of humans [5–10]. Furthermore, recent studies have suggested that IGF-I functions as an autocrine factor in human thyroid cells [11]. These peptides exert their physiological actions through binding to specific receptors on the cell surface. There are two subtypes of IGF receptor; type I receptors, which interact with IGF-I with a high affinity and weakly with insulin; and type II receptors interact with IGF-II rather than IGF-I and do not interact with insulin [12]. We have already reported that type I IGF receptors exist on human thyroid tissues [13]. Although type II IGF receptors have been identified in rat thyroid cells [5, 14], there is no available data on type II IGF receptors on human thyroid tissues. In this study, we identified and characterised the IGF-II receptors in normal and neoplastic human thyroid tissues. We show here that type II IGF receptors are increased in neoplastic tissues in comparison with the surrounding normal tissues.

MATERIALS AND METHODS

Hormones and reagents

IGF-II and monocomponent porcine insulin were products of Eli Lilly (Indianapolis, Indiana). Recombinant IGF-I was a gift from Fujisawa Pharmaceutical Institute (Osaka). IGF-II was radioiodinated to a specific activity of 5.55 TBq (150 Ci)/g by the chloramine-T method. The radioiodinated IGF-II was purified by gel filtration over a Sephadex G-50 column.

Human thyroid tissue

Thyroid tissue was obtained from 18 patients who had undergone thyroidectomy because of papillary carcinoma (8 patients), follicular carcinoma (3) or follicular adenoma (7). A fragment of normal thyroid tissue was obtained from the excised portion surrounding the neoplasm in all patients. The tissues were immediately weighed, frozen on dry ice and stored at −70°C until use. The diagnosis for each tumour was established by histological examination.

Tissue processing

The crude thyroid membrane fractions were prepared by a modification of the method of Atkinson *et al.* [15]. Briefly, the tissues were minced with scissors on ice and then homogenised with a Polytron PT-10 (Brinkmann Instruments, Westbury, New York) in chilled 10 mmol/l Tris-HCl buffer, pH 7.4, for 30 s at a dial setting of 6. The homogenate was further homogenised with a Dounce homogeniser and the suspension was centrifuged at 800 *g* for 10 min at 4°C. The supernatant was then centrifuged at 10000 *g* for 15 min at 4°C. The resultant supernatant was discarded and the pellet was resuspended in 10 mmol/l Tris-HCl buffer, pH 7.4, containing 50 mmol/l NaCl and 0.1% bovine serum albumin (BSA). The protein concentration of the crude thyroid membrane preparation was measured by the method of Lowry *et al.* [16] using BSA as a standard.

Binding studies

Crude membrane fractions (200 μg protein/tube) were incubated with approximately 30000 cpm of [^{125}I]IGF-II at 4°C for

Correspondence to T. Tsushima.
T. Yashiro is at the Department of Surgery, Institute of Clinical Medicine, Tsukuba University, Tsukuba City; T. Tsushima and H. Murakami are at the Department of Medicine and T. Obara and Y. Fujimoto are at the Department of Endocrine Surgery, Institute of Clinical Endocrinology, Tokyo Women's Medical College, 8-1 Kawadacho, Shinjukuku, Tokyo 162; K. Shizume is at the Institute for Growth Science, Tokyo; and K. Ito is at the Ito Hospital, Tokyo, Japan.
Revised 5 Feb. 1991; accepted 22 Feb. 1991.

22 h in the presence or absence of various concentrations of unlabelled IGF-II in a total volume of 0.5 ml Tris-HCl buffer (50 mmol/l, pH 7.4, containing 10 mmol/l $MgCl_2$ and 0.1% BSA). The reaction was terminated by adding 2 ml of ice-cold Tris-HCl buffer. The tubes were then centrifuged at 2000 *g* for 30 min at 4°C to separate membrane-bound from unbound [^{125}I]IGF-II. The supernatant was aspirated and the radioactivity of the pellet was counted with an automated gamma-counter. Non-specific binding was defined as the binding obtained in the presence of an excess amount of unlabelled IGF-II (1 μg/ml), and specific binding was calculated by subtracting non-specific binding from total binding. Percentage specific binding was defined as the percentage of specific binding against the total count added. All binding studies were carried out in duplicate.

Sodium dodecyl sulphate gel electrophoresis (SDS–PAGE) and autoradiography

Crude membrane fractions (1 mg protein) were incubated with approximately 8 × 10^5 cpm of [^{125}I]IGF-II at 4°C for 18 h in the presence or absence of excess unlabelled IGF-II. The tubes were then centrifuged at 2000 *g* for 30 min at 4°C. The pellet was treated with 0.5 mmol/l disuccinimidyl suberate for 20 min at 20°C to crosslink [^{125}I]IGF-II to the receptors according to the method described previously [17]. The reaction was terminated by addition of 10 mmol/l Tris-HCl buffer, pH 7.4, containing 1 mmol/l EDTA. The tubes were again centrifuged as described, and the pellet was solubilised with 50 mmol/l Tris-HCl buffer, pH 6.8, containing 10% glycerol, 1% SDS and 100 mmol/l dithiothreitol. After centrifugation at 10000 *g* for 20 min, 50 μl supernatant was applied to a 7.5% polyacrylamide slab gel with a 3% stacking gel and electrophoresed using a discontinuous buffer system, as described by Laemmli [18]. After completion of electrophoresis, the gels were dried and subjected to autoradiography using Kodak X-Omat film with an intensified screen.

Statistical analysis

Results are expressed as mean (S.E.) throughout. Statistical analysis of the data was performed by two-tailed Student's *t* test when the variation of the data was uniform. If the variation was not uniform, then Duncan's new multiple range test was employed. Differences between groups were considered significant if the *P* value was less than 0.05.

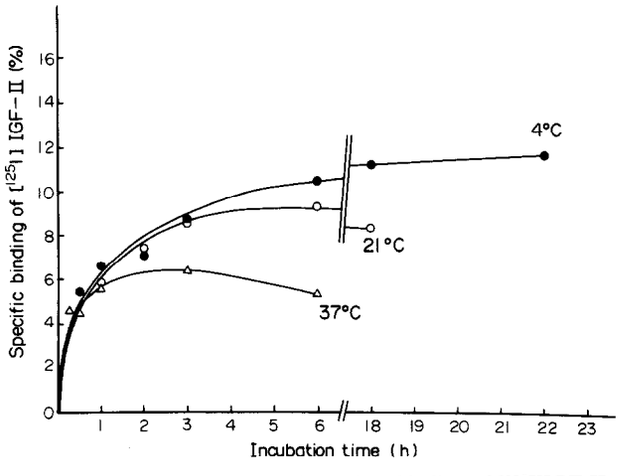

Fig. 1. Time and temperature-dependent binding of [^{125}I]IGF-II to papillary carcinoma membranes. Thyroid membranes from a patient with papillary carcinoma were incubated with [^{125}I]IGF-II at the indicated temperatures. Each value represents the mean of duplicate determinations.

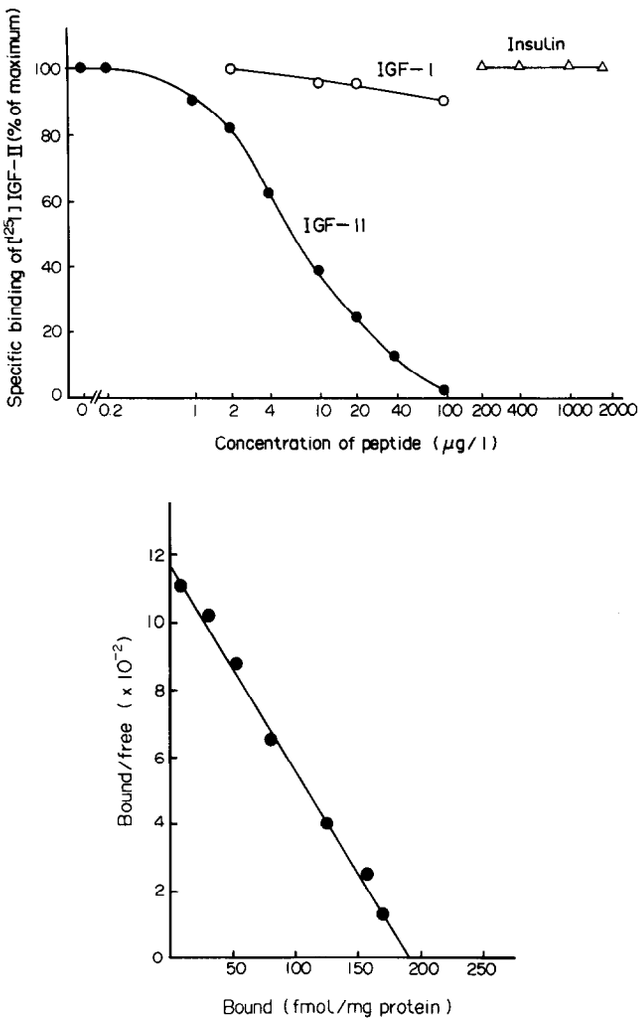

Fig. 2. (a) Specificity of [^{125}I]IGF-II binding to thyroid membranes. Crude thyroid membrane fractions obtained from a patient with papillary carcinoma were incubated with [^{125}I]IGF-II in the presence of the indicated concentrations of unlabelled IGF-II (•), IGF-I (○), or insulin (△). Each value represents the mean of duplicate determinations. (b) Scatchard plot of the data in Fig. 2a. Scatchard analysis revealed a single, high affinity binding site with a K_a of 1.9 × 10^9 mol/l.

RESULTS

Binding of [^{125}I]IGF-II to human thyroid membrane fractions

[^{125}I]IGF-II specifically bound to crude membrane fractions from cancer tissues as a function of the amount of protein (data not shown). The binding was also dependent on the time and temperature of incubation, showing maximal binding at 4°C and 22 h (Fig. 1). Subsequent studies, therefore, were conducted at pH 7.4 and 4°C for 22 h using 200 μg protein. Non-specific binding was less than 5% in all the samples and there was no difference among normal, adenoma and cancer tissues.

Specificity of IGF-II binding

The specificity of [^{125}I]IGF-II binding to human thyroid membranes is shown in Fig. 2a. The data are representative of 11 experiments using thyroid cancer tissues. The binding was dose-dependently displaced by unlabelled IGF-II, half maximal inhibition occurring at a concentration of 6 ng/ml IGF-II. We

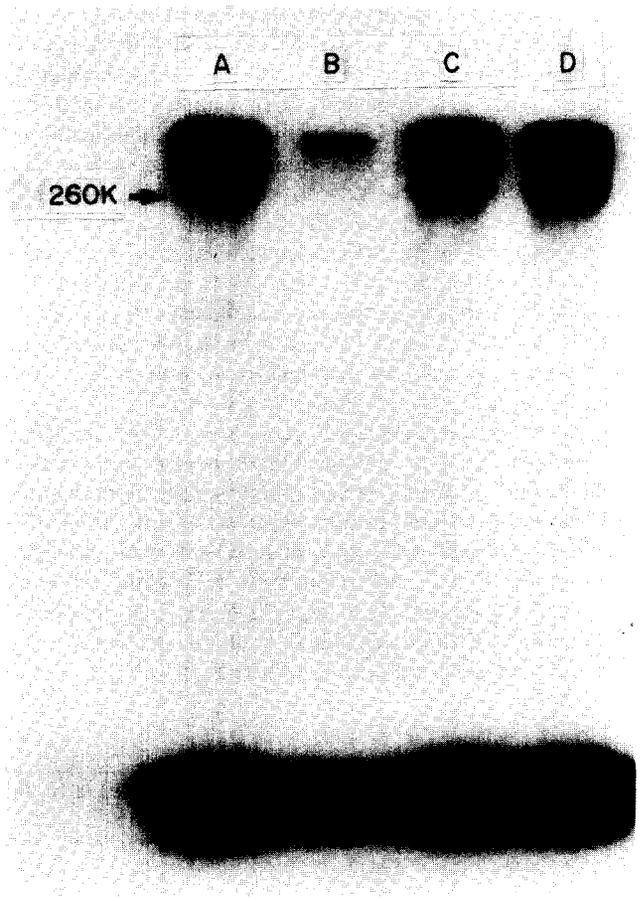

Fig. 3. Autoradiogram of [^{125}I]IGF-II crosslinked to membrane fractions of papillary carcinoma. Thyroid membranes (the same preparations used in Fig. 2a) were incubated with [^{125}I]IGF-II in the absence of unlabelled peptide (lane A) and in the presence of excess unlabelled IGF-II (lane B), IGF-I (lane C) or insulin (lane D).

have previously reported that IGF-II crossreacts with IGF-I receptors with an affinity lower than that of IGF-I [13]. Figure 2a also shows that [^{125}I]IGF-II binding was partially displaced by unlabelled IGF-I. However, the inhibition by IGF-I was only 8% or less at a concentration of 100 ng/ml, which was enough to saturate IGF-I binding sites on human thyroid membranes [13]. Furthermore, an insulin concentration as high as 2000 ng/ml did not displace [^{125}I]IGF-II binding at all to the membranes. Thus, [^{125}I]IGF-II binding was largely to type II IGF receptors. Scatchard analysis of the binding data revealed a single class of IGF-II binding sites with a K_a of 1.9×10^9 mol/l^{-1} and a binding capacity of 192.5 fmol/mg protein (Fig. 2b).

Affinity crosslinking and autoradiography

To further characterise the IGF-II binding sites on human thyroid membranes, [^{125}I]IGF-II crosslinked thyroid membranes were analysed by SDS–PAGE under reducing conditions. As seen in the gel autoradiogram, one band with an apparent molecular weight of 260 000 kD (Fig. 3, lane A), characteristic of type II IGF receptor complex [12, 17], was present. This band was abolished when thyroid membranes were incubated with [^{125}I]IGF-II in the presence of excess unlabelled IGF-II (lane B), but not by IGF-I or insulin (lanes C and D). No labelled band was detected in the position corresponding to 135 000 kD, characteristic of the type I IGF receptor complex [12, 17]. This may be due to low affinity of [^{125}I]IGF-II to type I IGF receptors.

[^{125}I]IGF-II binding to neoplastic and normal thyroid tissues

The results of [^{125}I]IGF-II binding to membrane fractions derived from thyroid neoplastic and normal tissues are summarised in Table 1. Specific binding of [^{125}I]IGF-II to thyroid membranes from 8 patients with papillary carcinoma was (mean [S.E.]) 13.2 [1.3]% per 200 μg protein (n = 8) and was significantly ($P < 0.01$) higher than the value in adjacent normal tissues (4.8 [0.5]%) or in adenoma tissues (6.9 [0.4]%, n = 7). The value in follicular carcinoma was higher than that in surrounding normal tissues, although the number of samples was limited. [^{125}I]IGF-II binding to adenoma tissues was also significantly different from that in the corresponding normal tissues. It can be seen that the IGF-II binding to neoplastic tissues was higher than that to the surrounding normal tissues in all patients except 1 (Fig. 4). There was no difference in the values for normal tissues surrounding cancer and adenoma. Scatchard analysis of the binding data was performed for all the tissues. The affinity constant of IGF-II receptors in neoplastic tissues, either papillary carcinoma, follicular carcinoma or adenoma, was not significantly different from that in the corresponding normal tissue. In contrast, binding capacity of neoplastic tissues was significantly higher than that in surrounding normal tissues. The value in papillary carcinoma (184.9 [23.9] fmol/mg protein) was approximately 3-fold higher compared to that in adjacent normal tissues (65.9 [9.5] fmol/mg protein). There was no relationship between IGF-II receptor levels and tumour size.

DISCUSSION

In the present study, we have demonstrated that human thyroid tissues, both normal and neoplastic, have IGF-II receptors. The

Table 1. Binding of [^{125}I]IGF-II to neoplastic and normal thyroid tissues from 18 patients

Diagnosis	Specific binding (%/200 μg protein)	Affinity (10^9 mol/l)	Capacity (fmol/mg protein)
Neoplastic tissue			
Papillary carcinoma (n = 8)	13.2(1.3)*†	1.8(0.2)	184.9(23.9)*†
Follicular carcinoma (n = 3)	8.7(0.4)*†	2.3(0.1)	92.8 (8.2)‡
Follicular adenoma (n = 7)	6.9(0.4)*	2.1(0.2)	77.9 (6.0)‡
Normal tissue			
Papillary carcinoma (n = 8)	4.8(0.5)	1.8(0.1)	65.9 (9.5)
Follicular carcinoma (n = 3)	5.2(0.5)	2.3(0.1)	55.3 (2.5)
Follicular adenoma (n = 7)	4.5(0.6)	1.8(0.2)	53.3 (8.1)

Mean (S.E.).
*Significantly ($P<0.01$) higher than that for adjacent normal tissue; †significantly ($P<0.01$) higher than that for adenoma tissue; ‡significantly ($P<0.01$) higher than that for adjacent normal tissue.

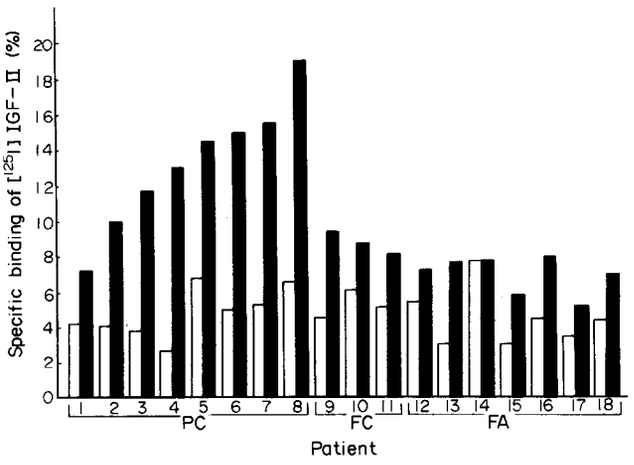

Fig. 4. Specific binding of [^{125}I]IGF-II to human thyroid membranes. [^{125}I]IGF-II binding to neoplastic tissues (closed column) was compared to the binding of adjacent normal tissues (open column) in 18 patients. PC = papillary carcinoma, FC = follicular carcinoma, FA = follicular adenoma.

specificity of [^{125}I]IGF-II binding was consistent with that of type II IGF receptors, which have a preference for IGF-II over IGF-I and do not interact with insulin [12]. The presence of 260 000 species of [^{125}I]IGF-II crosslinked membranes demonstrated by SDS–PAGE is also compatible with the characteristics of the type II IGF receptors Although IGF-II crossreacts with type I IGF receptors on human thyroid membranes [13], only 8% or less of specific binding of [^{125}I]IGF-II was displaced by a high concentration of unlabelled IGF-I, indicating that the majority of [^{125}I]IGF-II binding to thyroid membranes is to type II IGF receptors. IGF-II receptors have already been identified in rat thyroid cells [5, 14]. To our knowledge, the present study is the first to demonstrate IGF-II receptors in human thyroid tissue.

We showed that IGF-II binding to thyroid neoplastic tissues was significantly higher than that to surrounding normal tissues. Furthermore, the IGF-II binding to cancer tissues was higher compared to that for adenoma tissues. Scatchard analysis of the binding data revealed that the increased IGF-II binding to neoplastic tissues was due to a higher binding capacity without any change of affinity. The difference of IGF-II binding in neoplastic and normal tissues may be due to difference in proportion of non-epithelial cells having IGF-II receptors. This is unlikely, however. Fibroblastic or lymphocytes were found in some of cancer tissues, but the proportion was less than 1%. Therefore, it is most likely that the increased IGF-II binding in neoplastic tissues is due to the higher IGF-II binding to neoplastic thyroid cells than to non-neoplastic cells. An increase in the number of IGF-II receptors has also been reported in rat thyroid tissues during compensatory growth [14].

It is well established that several types of cells synthesise IGF-binding proteins (IGF-BPs) as well as IGF. Minuto *et al.* [10] reported the presence of IGF-BP of 35–50K molecular weight in extracts of normal and nodular thyroid tissues. We have also found that the level of IGF-BPs in extracts of thyroid cancer tissues (papillary carcinoma) is much higher than that in normal tissues (T.Y. *et al.*). The IGF-BPs were largely found in 100 000 *g* supernatant of tissue extracts (data not shown). SDS–PAGE and autoradiography of [^{125}I]IGF-II crosslinked to thyroid membranes did not detect any band corresponding to IGFs (Fig. 3). Thus, contribution of IGF-BPs to [^{125}I]IGF-II binding to membrane fractions is unlikely.

The functional role of IGF-II receptors on human thyroid membranes remain to be determined. IGF-II stimulates DNA synthesis of rat or porcine thyroid cells in culture, although the potency of IGF-II in stimulating DNA synthesis is several times lower than that of IGF-I [5, 6]. Furthermore, the function of IGF-II as an autocrine growth stimulator in rat thyroid cells has been described [8]. However, recent studies using antibody to type I IGF receptors indicate that the mitogenic activity of IGF-II in a variety of cultured cells is largely mediated by type I IGF receptors [19–21]. Recently, it has been found that the IGF-II receptor is identical to the cation-independent mannose 6-phosphate (M6P) receptor [22, 23], which participates in transport and uptake of lysosomal enzymes [24]. Furthermore, M6P receptors appear to be involved in the secretion and uptake of other proteins containing M6P. A number of M6P-containing glycoproteins such as cathepsin D-like enzymes, transforming growth factor β1 precursor and proliferin have been identified in conditioned medium of various types of cultured cells [25–27]. It would be possible that these materials affect the behaviour of neoplastic cells by interacting with IGF-II/M6P receptors. However, further studies are required to determine whether an increase in IGF-II receptor content in thyroid neoplastic tissues is causally related to their uncontrolled growth.

1. Froesch ER, Schmid C, Schwander J, Zapf J. Actions of insulin-like growth factors. *Annu Rev Physiol* 1985, **47**, 443–467.
2. Baxter RC. The somatomedins: insulin-like growth factors. *Adv Clin Chem* 1986, **25**, 49–115.
3. Nakanishi Y, Mulshine JL, Kasprzyk PG, *et al.* Insulin-like growth factor-I can mediate autocrine proliferation of human small cell lung cancer cell lines *in vitro*. *J Clin Invest* 1988, **82**, 354–359.
4. Yee D, Cullen KJ, Pail S, *et al.* Insulin-like growth factor II mRNA expression in human breast cancer. *Cancer Res* 1988, **48**, 6691–6696.
5. Tramontano D, Moses AC, Picone R, Ingbar SH. Characterization and regulations of the receptor for insulin-like growth factor-I in the FRTL-5 rat thyroid follicular cell line. *Endocrinology* 1987, **120**, 785–790.
6. Saji M, Tsushima T, Isozaki O, *et al.* Interaction of insulin-like growth factor I with porcine thyroid cells cultured in monolayer. *Endocrinology* 1987, **1221**, 749–756.
7. Bachrach LK, Eggo MC, Hintz RL, Burrow GN. Insulin-like growth factors in sheep thyroid cells; action, receptors and production. *Biochem Biophys Res Commun* 1988, **154**, 861–867.
8. Maciel RM, Moses AC, Villone G, Toramontano D, Ingbar SH. Demonstration of the production and physiological role of insulin-like growth factor II in rat thyroid follicular cells in culture. *J Clin Invest* 1988, **82**, 1546–1553.
9. Williams DM, Williams ED, Wynford-Thomas D. Loss of dependence on IGF-I for proliferation of human thyroid adenoma cells. *Br J Cancer* 1988, **57**, 535–539.
10. Minuto F, Barreca A, Del Monte P, *et al.* Immunoreactive insulin-like growth factor I (IGF-I) and IGF-I binding protein content in human thyroid tissue. *J Clin Endocrinol Metab* 1989, **68**, 621–626.
11. Tode B, Serio M, Rottela CM, *et al.* Insulin-like growth factor-I: autocrine secretion by human thyroid follicular cells in primary culture. *J Clin Endocrinol Metab* 1989, **69**, 639–647.
12. Massague J, Czech MP. The subunit structures of two distinct receptors for insulin-like growth factors I and II and their relationship to the insulin receptors. *J Biol Chem* 1982, **257**, 5038–5041.
13. Yashiro T, Ohba Y, Murakami H, *et al.* Expression of insulin-like growth factor receptors in primary human thyroid neoplasms. *Acta Endocrinol* 1989, **121**, 112–120.
14. Polychronakos C, Guyda HJ, Patel B, Posner BI. Increase in the number of type II insulin-like growth factor receptors during propylthiouracil-induced hyperplasia in the rat thyroid. *Endocrinology* 1986, **119**, 1204–1209.
15. Atkinson S, Smith PA, Taylor JJ, Kendal-Taylor P. Specific epidermal growth factors on porcine thyroid membranes. *FEBS Lett* 1983, **153**, 88–92.

16. Lowry OH, Rosebrough NJ, Farr AL, Randal RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951, **193**, 265–275.
17. Kasuga M, Van Obberghen E, Nissley SP, Rechler MM. Demonstration of two subtypes of insulin-like growth factor receptors by affinity crosslinking. *J Biol Chem* 1981, **256**, 5305–5308.
18. Laemmli DZ. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature* 1970, **227**, 680–685.
19. Ewton DZ, Falen SL, Florini JR. The type II insulin-like growth factor (IGF) receptor has low affinity for IGF-I analogs: pleiotypic actions of IGFs on myoblasts are apparently mediated by type I receptor. *Endocrinology* 1987, **120**, 115–123.
20. Morgan DO, Roth RA. Acute insulin action requires insulin receptor kinase activity: introduction of an inhibitory monoclonal antibody into mammalian cells blocks the rapid effects of insulin. *Proc Natl Acad Sci USA* 1987, **84**, 41–45.
21. El-Badry OM, Romanus JA, Helman LJ, *et al.* Autonomous growth of a human neuroblastoma cell line is mediated by insulin-like growth factor II. *J Clin Invest* 1989, **84**, 829–839.
22. Morgan DO, Edman JC, Standring DN, *et al.* Insulin-like growth factor II receptor as a multifunctional binding protein. *Nature* 1987, **329**, 301–307.
23. Tong PY, Tollefsen SE, Kornfeld S. The cation-independent mannose 6-phosphate receptor binds insulin-like growth factor II. *J Biol Chem* 1988, **263**, 2585–2588.
24. Dahms NM, Lobel P, Kornfeld S. Mannose 6-phosphate receptors and lysosomal enzyme targetting. *J Biol Chem* 1989, **264**, 12115–12118.
25. Capony F, Morisset M, Barret AJ, *et al.* Phosphorylation glycosylation, and proteolytic activity of the 52-KD estrogen-induced protein secreted by MCF-7 cells. *J Cell Biol* 1987, **104**, 253–262.
26. Purchio AF, Cooper JA, Brunner AM, *et al.* Identification of mannose 6-phosphate in two asparagine-linked sugar chains of recombinant transforming growth factor-B1 precursor. *J Biol Chem* 1988, **263**, 14211–14215.
27. Lee S-J, Nathans D. Proliferin secreted by cultured cells binds to mannose 6-phosphate receptors. *J Biol Chem* 1988, **263**, 3521–3527.

Acknowledgements—We wish to thank Mr M. Okada and Miss M. Arai for their technical assistance. This work was supported by grants from the University of Tsukuba for Project Research, the Ministry of Education and the Ministry of Health and Welfare, Japan.

Eur J Cancer, Vol. 27, No. 6, pp. 703–710, 1991.
Printed in Great Britain

0277–5379/91 $3.00 + 0.00
Pergamon Press plc

In vitro and Clinical Characterisation of a Newcastle Disease Virus-modified Autologous Tumour Cell Vaccine for Treatment of Colorectal Cancer Patients

Winfrid Liebrich, Peter Schlag, Maria Manasterski, Burkhard Lehner, Michael Stöhr, Peter Möller and Volker Schirrmacher

A virus-modified autologous tumour cell vaccine prepared from human colorectal cancer cells is described. After dissociation an average of 5×10^7 cells/g tissue were obtained from primary tumours and 9×10^7/g tissue from metastases with an average viability of 72% and 51%, respectively. Following irradiation (200 Gy), inactivation of the proliferative activity of the cells was demonstrated by their degeneration in tissue culture and the absence of incorporation of ^{3}H-labelled thymidine. One third of the cells were still metabolically active, as shown by the incorporation of ^{3}H-uridine and a mixture of ^{3}H-aminoacids. The dissociated cells expressed MHC class I and II antigens in a qualitatively similar way to tissue sections. Epithelium-specific antigens (detected by MAb HEA125) were expressed on an average of more than 75% cells of the suspension, while leucocyte-specific antigens (detected by MAb CD53) were expressed on an average of less than 25% cells. The vaccine was prepared by admixing the nonlytic strain Ulster of Newcastle disease virus (NDV) with the tumour cell suspension. The NDV adsorption at tumour cells was shown by electron microscopy. Clinically, the treatment with the vaccine was associated with an increased sensibilisation against autologous tumour cells, measured by DTH skin reactivity. First results in 23 patients with colorectal liver metastases who underwent "curative" liver resection followed by vaccination show a clear correlation between the induced increase of DTH skin reaction against autologous tumour cells and the recurrence-free interval. No correlation was found for DTH reaction caused by standard antigens (Mérieux test), NDV alone or autologous normal liver tissue. The results demonstrate the possibility of preparing immunogenic virus-modified autologous tumour cell vaccine from colorectal cancer tissue, which could be used for cancer therapy.
Eur J Cancer, Vol. 27, No. 6, pp. 703–710, 1991

INTRODUCTION

MODIFYING TUMOUR cells by means of viruses may increase their immunogeneity [1, 2]. The commonly used procedures in clinical tumour vaccination studies is the use of oncolysates or membranes from tumour cells infected with viruses such as vaccinia virus [3] parainfluenza virus [4], or Newcastle disease