Receptors for epidermal growth factor and thyrotropin in thyroid carcinoma

Tuulikki Mäkinen1,2, Fredrika Pekonen1,2, Kaarle Franssila3 and Bror-Axel Lamberg1,2

Minerva Foundation Institute for Medical Research1, Endocrine Research Laboratory2, and Pathology Laboratory of the Department of Radiotherapy and Oncology3, University of Helsinki, Finland

Abstract. The EGF and TSH receptor properties in malignant thyroid tumours and adjacent normal thyroid tissues were characterized using radioreceptor assays. Ten patients with papillary, 4 with medullary, 1 with Hürthle cell type follicular carcinoma, and 2 with anaplastic thyroid carcinoma were studied. In 10 out of 12 patients with papillary and anaplastic thyroid carcinomas, more EGF receptors were found in the neoplastic tissue than in the adjacent normal tissue (P < 0.01). The affinity of the EGF receptors varied between patients (from 0.5 × 10⁹ l/mol to 1.9 × 10⁹ l/mol), but was in each patient the same in the neoplastic and in the normal tissue. In medullary carcinomas and a follicular Hürthle thyroid carcinoma, the EGF receptor content was very low. The receptor number was unaltered or decreased in papillary carcinomas when compared with adjacent normal tissue. In anaplastic medullary and follicular (Hürthle cell) carcinomas, the neoplastic tissue had very few high affinity TSH receptor sites. The alterations in TSH receptor characteristics when thyroid neoplastic tissue was compared with adjacent normal tissue did not correlate to changes in EGF receptor characteristics. Our results demonstrate that the amount of EGF receptors in papillary and anaplastic thyroid carcinomas differ significantly from that in follicular and medullary carcinomas and that alterations in EGF receptor content in malignant thyroid tissues are independent of TSH receptor content.

Although thyrotropin is considered to be the main regulator in the thyroid cell, it has become increasingly evident that other factors as well are involved in the regulation of thyroid function (Westermark et al. 1983). One of these factors is epidermal growth factor (EGF), which stimulates growth and proliferation in many tissues including the thyroid (Carpenter & Cohen 1979; Roger & Dumont 1982). It has been shown that thyroid growth is regulated by an interplay between EGF and TSH (Westermark et al. 1986), but the physiological role of EGF in thyroid growth remains to be elucidated. The presence of specific high affinity EGF receptors in many tissues and their involvement in neoplastic transformation have recently been demonstrated (Editorial 1986). EGF receptors have been found in about 35% of human breast carcinomas and a negative association between the number of EGF receptors and oestrogen receptors has been shown to exist (Sainsbury et al. 1985). EGF receptors have also been found in bladder tumours (Neal et al. 1985) and thyroid neoplasms (Duh et al. 1985). Papillary thyroid carcinoma, as well as medullary, anaplastic and poorly differentiated follicular thyroid carcinomas have decreased amounts of TSH receptors and respond poorly to TSH (Carayon et al. 1980). So far very little is known about EGF receptors in thyroid neoplasms and there are no reports concerning the potential relationship between the EGF receptors and the TSH receptors. We have therefore studied the EGF and TSH receptors in thyroid neoplastic and adjacent normal tissues. The aim of our study was to clarify the role of TSH and EGF in the regulation of malignant thyroid cell growth.
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(Pharmacia
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further
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phate
months.
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stored
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14
BSA,
427
was
reaction
BSA,
27
Membrane
7.5,
containing
0.4
mCi of carrier-free
Na-125I
(IMS
30, Amersham International plc, Amersham, England). Chloramine T, 20 µg, was then added.
The reaction was stopped after 40 sec by addition of 40 µg of sodium metabisulphite. The labelled EGF was separated from labelled albumin and from unreacted Na-125I by gel filtration through Sephadex G-50 (Pharmacia Fine Chemicals, Uppsala, Sweden) with a buffer containing 10 mmol/l Tris, 50 mmol/l NaCl, and 0.1% BSA, pH 7.5 (Tris-NaCl-BSA). The labelled EGF was stored at −80°C. The specific activity was about 50 µCi/µg and the preparation remained stable for at least 2 months.

Highly purified bovine TSH (30 1U/mg, a gift from Dr J. G. Pierce) was labelled and receptor purified according to the methods of Smith & Hall (1981).

Membrane preparations
Human thyroid tissue was obtained from 17 patients undergoing thyroidectomy owing to thyroid malignancies with the permission of the local ethics committee. Ten patients had papillary carcinomas, 4 medullary carcinomas, one patient had follicular carcinoma of the Hürthle cell type, and 2 patients anaplastic carcinomas. In 14 patients both normal and malignant thyroid tissues were obtained. The clinical data of the patients are given in Table 1. The prognostic indexes of the papillary carcinomas are given according to the EORTC thyroid Cancer Cooperation Group (Byar et al. 1979). The neoplastic and the adjacent, histologically normal tissues were stored at −80°C until membranes were prepared as described by Smith & Hall (1981). Briefly, the tissue was thawed (all steps during membrane preparations were performed at +4°C), cut into small pieces and homogenized with an Ultraturrax (Ika-Werk, Freiburg, FRG) homogenizer in 10 mmol/l Tris-HCl, pH 7.5. After centrifugation at 800 × g for 5 min, the pellet was discharged and the supernatant was further centrifuged at 30 000 × g for 20 min. The pellet was resuspended in Tris-NaCl-BSA buffer containing 0.02% NaN₃, and the protein content was adjusted to 6.0 g/l. Membranes were stored in −80°C until analysed. For determination of high affinity TSH receptors, the membranes were exposed to ammonium sulphate as earlier described (Pekonen & Weintraub 1980).

Radioceptor assays
The EGF receptor content in thyroid tissue was assessed by radioreceptor assay. Briefly, 100 µl of thyroid membrane (final protein concentration 2.4 g/l) in Tris-NaCl-BSA buffer, 100 µl of serially diluted unlabelled EGF (Tris-NaCl-BSA buffer) and 50 µl of [125I]EGF (100 000 cpm) were incubated at +37°C. The reaction was stopped after 60 min by adding 250 µl of ice-cold buffer. The bound and free EGF were separated by centrifugation at 30 000 × g for 20 min at +4°C. The supernatant was aspirated and the radioactivity in the pellet was counted. The binding data are expressed as saturable binding (binding inhibited by 6.6 × 10⁻⁸ mol/l EGF). The specificity of the receptor assay was measured in the presence of thyrotropin, insulin, hCG, and transferrin (Fig. 1). All determinations were performed in duplicate.

Binding of [125I]TSH to the ammonium sulphate treated membranes was measured according to Pekonen & Weintraub (1980). The membrane protein concentration was 0.8 g/l. Neoplastic and normal tissues from the same patients were measured in the same assay.

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Diagnosis</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Prognostic index</th>
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<td>M</td>
<td>51</td>
<td>60+</td>
</tr>
<tr>
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<td>Pap</td>
<td>F</td>
<td>43</td>
<td>43</td>
</tr>
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<td>M</td>
<td>28</td>
<td>30+</td>
</tr>
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<td>Pap</td>
<td>F</td>
<td>34</td>
<td>35</td>
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<tr>
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<td>53+</td>
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<td>46+</td>
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<td>F</td>
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<td>Pap</td>
<td>F</td>
<td>68</td>
<td>68</td>
</tr>
<tr>
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<td>Pap</td>
<td>F</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>10</td>
<td>Pap</td>
<td>F</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>11</td>
<td>Ana</td>
<td>F</td>
<td>63</td>
<td></td>
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<td>M</td>
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<td>Med</td>
<td>F</td>
<td>69</td>
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<td>M</td>
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<td>Med</td>
<td>F</td>
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<td></td>
</tr>
<tr>
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<td>Med</td>
<td>M</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Hür</td>
<td>F</td>
<td>53</td>
<td></td>
</tr>
</tbody>
</table>

The prognostic index is given according to the EORTC thyroid Cancer Cooperation Group (Byar et al. 1979).
Specificity and sensitivity of EGF receptor assay. The EGF receptor binding was measured by incubating 600 µg of thyroid membranes with $^{125}$IEGF (100,000 cpm) and varying amounts of unlabelled proteins in a final volume of 250 µl of Tris-NaCl-BSA buffer at 37°C for 60 min. The reaction was stopped by adding cold buffer and centrifugation at 30,000 × g for 20 min. The radioactivity bound to the pellet was counted. EGF ●, TSH ■, hCG △, insulin □, transferrin ○.

**Results**

Papillary thyroid carcinomas had higher EGF tracer binding than adjacent normal thyroid tissue in 9 of the 10 patients, whereas the tumour tissue bound slightly less EGF than adjacent normal tissue in one patient (Fig. 2). In papillary carcinomas, the difference between EGF binding to neoplastic and adjacent normal tissues was highly significant ($P < 0.01$, Wilcoxon matched-pairs signed ranks test). There was, however, a clear correlation between EGF receptor binding in neoplastic and adjacent normal tissue ($r = 0.86$, $P < 0.001$, Fig. 3). Thus, the higher the EGF binding to neoplastic tissue the higher was also the binding to adjacent normal tissue. In both anaplastic carcinomas, high EGF tracer binding was observed. This binding was higher than the binding to any of the normal tissues tested and higher than the binding to adjacent normal tissue (Patient No. 11). In the two medullary carcinomas where adjacent normal tissues also were available, a significantly lower maximal binding was observed in the neoplastic tissue than in the normal tissue. In the other two medullary carcinomas, where no normal tissue was available, the neoplastic tissue showed low EGF binding. In the follicular Hürthle cell carcinoma, EGF binding was lower than in the adjacent normal tissue (Fig. 2).

Owing to the limited amount of available tissue, Scatchard analysis of EGF receptor binding could be performed only in 7 pairs of tissues. The affinities and capacities of the receptors are given in Table 2. The affinity of the EGF receptors varied between patients from $0.5 \times 10^9$ l/mol to $1.9 \times 10^9$ l/mol, but was virtually the same in each pair of neoplastic and adjacent normal tissues.

The TSH receptors were measured in 8 pairs of normal and neoplastic thyroid tissues. In papillary carcinoma, the TSH receptor content, contrary to the EGF receptor content, was either unaltered (Patients Nos. 4, 6, 7 and 8) or decreased (Patient No. 10) as compared with adjacent normal tissue (Table 2, Figs. 2 and 4). In anaplastic carcinoma, the TSH receptor content was decreased, whereas the EGF receptor binding activity in this tissue was the highest seen in any of the tissues tested (Patient No. 11, Table 2, Fig. 2). In follicular carcinoma of Hürthle cell type and in medullary carci-
Displaceable EGF binding to normal (□) and neoplastic (■) tissues of 17 patients with various thyroid neoplasms. Pap: papillary carcinoma, Ana: anaplastic carcinoma, Med: medullary carcinoma, Hur: Hürthle cell follicular carcinoma. For clinical data see Table 1.

Correlation between EGF binding to normal thyroid and adjacent neoplastic papillary tissue. EGF binding was measured as described in legend to Fig. 1. Non-displaceable binding in the presence of $6.6 \times 10^{-8}$ mol/l EGF was subtracted from all values.

Discussion

Different histological types of thyroid carcinoma differ in their EGF and TSH receptor characteristics. In papillary thyroid carcinoma, the neoplastic tissue had higher EGF tracer binding than the adjacent normal tissue, whereas the TSH receptor content was normal in 4 out of 5 papillary carcinoma tissues and decreased in one. Our results are supported by a recent report by Duh et al. (1985) who showed that there are more EGF receptors in neoplastic thyroid tissue than in normal. The TSH receptor results are, however, conflicting. Initially, Ichikawa et al. (1976) reported normal amount of TSH receptors in differentiated thyroid carcinoma, in agreement with our results. They did not, however, specify the type of carcinoma and did not compare malignant and adjacent normal thyroid tissue. Carayon et al. (1980) have shown that the TSH binding site capacity is lower in papillary thyroid carcinoma than in normal thyroid tissue. The different results may be due to the fact that we, in the present work, predominantly measured high affinity TSH receptors. The results of Carayon et al. (1980), on the other hand, are based on a TSH receptor assay which predominantly measures low affinity binding as is evident from the TSH displacement curves depicting their receptor assay (Carayon et al. 1978). In support of lowered TSH receptor activity in papillary carcinoma are results by Field et al. (1978) who reported decreased cyclic AMP response to TSH in papillary carcinoma. The TSH receptor concentration is, however, not directly related to cAMP stimulation (Carayon et al. 1980).
In medullary carcinoma, of parafollicular origin, the amounts of both EGF and TSH receptors were low. To our knowledge there are no other reports concerning EGF receptors in medullary carcinoma. The low EGF receptor concentration in a patient with follicular Hürthle cell carcinoma agrees with the results of Duh et al. (1985) who also reported low amounts of EGF receptors in one patient with Hürthle cell carcinoma.

The highest EGF tracer binding was observed in a patient with anaplastic carcinoma, suggesting that high EGF receptor content could be a marker of the malignant character of anaplastic thyroid carcinoma. The TSH binding in the anaplastic carcinoma was low, supporting results by Carayon et al. (1980). In breast carcinoma, high EGF receptor content has been correlated to poor prognosis as well as to low oestrogen receptor content (Sainsbury et al. 1985). Neal et al. (1985) have shown, by indirect immunoperoxidase technique, that the presence of a high intensity staining for EGF receptors in human urinary bladder tumours is associated with poor differentiation and with invasion.

Table 2.

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Diagnosis</th>
<th>Tissue type</th>
<th>EGF receptors</th>
<th>TSH receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Affinity (10^9 l/mol)</td>
<td>Capacity (fmol/mg protein)</td>
</tr>
<tr>
<td>4</td>
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<td>normal</td>
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</tr>
<tr>
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<td>carcinoma</td>
<td>n. d.</td>
<td>n. d.</td>
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<td>normal</td>
<td>1.9</td>
<td>10</td>
</tr>
<tr>
<td></td>
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<td>carcinoma</td>
<td>1.3</td>
<td>39</td>
</tr>
<tr>
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<td>normal</td>
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<td>normal</td>
<td>1.1</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>Pap</td>
<td>carcinoma</td>
<td>1.3</td>
<td>37</td>
</tr>
<tr>
<td>11</td>
<td>Pap</td>
<td>normal</td>
<td>n. d.</td>
<td>n. d.</td>
</tr>
<tr>
<td></td>
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<td>carcinoma</td>
<td>n. d.</td>
<td>n. d.</td>
</tr>
<tr>
<td>14</td>
<td>Med</td>
<td>normal</td>
<td>0.6</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Med</td>
<td>carcinoma</td>
<td>0.6</td>
<td>14</td>
</tr>
<tr>
<td>17</td>
<td>Hür</td>
<td>normal</td>
<td>1.0</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Hür</td>
<td>carcinoma</td>
<td>1.4</td>
<td>7</td>
</tr>
</tbody>
</table>

In papillary thyroid carcinoma, the prognosis of the disease shows a negative correlation to the age of the patient. We did not, however, find any correlation between the EGF receptor amount and the patient's age nor between EGF receptor content and the prognostic index of EORTC (Byar et al. 1979), suggesting that EGF receptor content is not a determinant for the degree of malignancy. Thus, our observations, except those in anaplastic carcinomas, do not support the conclusion by Duh et al. (1985) that thyroid tumours with a poorer prognosis appear to have higher EGF binding than tumours with a better prognosis. Further studies including more patients are, however, needed to firmly establish the relation between EGF receptors and the prognosis of thyroid carcinoma.

Acknowledgments

This work was supported by Finska Läkaresällskapet, Nordiska Insulinfonden and Liv och Hälsa.

References


Received April 2nd, 1987.
Accepted September 11th, 1987.

Tuuilikki Mäkinen, MA,
Minerva Foundation,
PO Box 819,
SF-00101 Helsinki 10,
Finland.