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Review Article

A new understanding of goitrogenesis: role of cytokines in the regulation of normal and aberrant thyroid growth

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Summary

Several cytokines and growth factors together with their binding proteins and/or receptors are being increasingly detected in mammalian thyroid tissue. These include epidermal growth factor, insulin-like growth factors, transforming growth factors, fibroblast growth factor, interleukins, interferons, and tumour necrosis factor-alpha. Their exact role in relation to thyroid regulation has not been fully elucidated but it is clear that many of these peptide regulatory factors are mitogenic for cultured thyrocytes. Recent evidence suggests that some thyroid cancers and benign goiters over-express receptors for a number of these growth factors. Transforming growth factor-beta is unique for its dominant anti-proliferative effect on thyrocytes concurrently exposed to potent thyroid mitogens. The mammalian thyroid cell is clearly a source as well as target of myriad polypeptide factors that probably co-regulate its normal growth and differentiation. Aberrant expression of these growth factors or their receptors might be a factor in the development of goiter.

Résumé

Plusieurs facteurs cytokines et des facteurs de croissance aussi bien que leurs protéines d'agglutinage sont détectés de plus en plus dans le tissu thyroïdien mammalien. Ceux-ci comprennent les facteurs de croissance épidermale, les facteurs de croissance pareille à l'insulin; des facteurs de croissance transformant; des facteurs de croissance fibroblastes, les interleukins, les interférons et la tumeur nécrose facteur- alpha.

Leur rôle spécifique par rapport à la régulation de la thyroïde n'est pas encore élucidé mais il est clair que plusieurs de ces facteurs de régulation peptide sont mitogéniques pour les thyrocytes de culture. Des évidences récentes suggèrent que quelques cancers thyroïdes et des goitres bénins manifestent excessivement les récepteurs de certains de ces facteurs de croissance. Le facteur-beta transformant est unique pour son effet anti-proliférant dominant sur les thyrocytes exposés concurrement avec les mitogènes thyroïdes actifs.

La cellule thyroïde mammalienne est clairement la source aussi bien que la cible de plusieurs facteurs polypeptides qui probablement régularise sa croissance normale et sa différentiation à la fois. L'expression anormale de ces facteurs de croissance ou de leurs récepteurs est probablement significative dans le développement du goitre.

Introduction

Regulation of thyroid cell growth

Diffuse or focal enlargements of the thyroid gland may occur in the setting of chemical and clinical euthyroidism, iodine deficiency, autoimmune thyroid disease, primary thyroid neoplasia, secondary metastases, or may be idiopathic (Studer and Ramelli 1982). Goiter may also result from the ingestion of certain drugs (e.g. lithium), diets, (e.g. cassava), or toxins (e.g. some halogenated compounds). In the case of Graves' disease, goiter is believed to be caused by thyroid stimulating immunoglobulins (Weetman and McGregor 1984). The cause of goiter in Hashimoto's disease is not clear since both patients in the euthyroid as well as hypothyroid phases may manifest thyroid enlargement. Immunoglobulins with growth stimulating activity on thyroid cells in vitro have been reported in serum samples from several patients with both autoimmune and apparently non-immunologically mediated goiters (Schatz *et al.* 1987). The in vitro effect of these "growth stimulating immunoglobulins" has been attributed at least in part to contamination by growth factors (Gartner *et al.*, 1987; Zakariya and McKenzie 1990).

Although TSH is commonly believed to be an important regulator of thyroid growth, such an effect is not always demonstrable in thyroid cell cultures

(Eggo *et al.*, 1989). In fact, TSH is not present in fetal serum during the ontogenesis of the fetal thyroid, and goiter can develop in hypophysectomized animals (Fisher *et al.*, 1977). Thus TSH is not an obligatory factor for thyroid embryogenesis or continued postnatal growth. However, the fact that euthyroid individuals become hypothyroid following hypophysectomy indicates that TSH is required for thyroid hormone synthesis and secretion.

Since the discovery of epidermal growth factor (EGF) and of its regulation by thyroid hormone (Fisher and Lakshmanan, 1990), other peptide regulatory factors (PRFs) have been linked to the thyroid. The messenger RNA for several of these PRFs as well as the translated products are being increasingly reported in thyroid tissue. The mammalian thyroid expresses specific receptors for many PRFs and synthesizes binding proteins for some of them. Furthermore, cultured thyroid cells respond to some PRFs more strongly than they do to TSH.

Intrathyroidal growth factors

Epidermal growth factor: Significant concentrations of immunoreactive and bioactive EGF have been demonstrated in both murine and human thyroid tissues (Hirata and Orth 1979; Dagogo-Jack *et al.*, 1986; Mizukami *et al.*, 1991). The presence of mRNA for prepro-EGF in thyroid tissue (Ozawa *et al.*, 1991) indicates that thyroidal EGF is synthesized locally rather than taken up from the circulation. The level of EGF in the mouse thyroid is unrelated independent of the submandibular gland (SMG) EGF content; in fact, sialoadenectomy augments thyroidal EGF (Dagogo-Jack, 1992). Also, the thyroid gland of neonatal mice has been found to contain near-adult levels of EGF by seven days of age when their SMG has little detectable EGF (Dagogo-Jack, 1991). The low EGF in the SMG of neonatal mice cannot be increased by thyroid hormone treatment, as obtained in adult mice (Salido *et al.*, 1990). The level of EGF in the mouse thyroid is hormone-responsive (Ozawa *et al.*, 1991).

EGF modulates the growth and differentiation of thyroid cells in vitro (Roger and Dumont, 1982) as well as in vivo (Ozawa and Spaulding, 1990), and may thus play a physiological role in thyroid regulation. However, the EGF content of goiters has not been systematically studied.

Insulin-like growth factors: Cultured human and mammalian thyroid cells have been reported to secrete immunoreactive insulin-like growth factors (IGFs) (Maciel *et al.*, 1988; Ollis *et al.*, 1989; Tode *et al.*, 1989). The secretion of IGF-1 by cultured human thyroid follicular cells is stimulated by growth hormone (20-200ug/ml) and TSH (0.1nmol/1-0.1nmol/1) but inhibited by supraphysiological doses of TSH (Ollis *et al.*, 1989). Thus, within the physiological range, TSH could stimulate thyroid growth by augmenting intrathyroidal IGF-1 concentration but at pharmacologic doses, growth hormone, but not TSH, would be growth promoting via the same mechanism. These reports might be relevant to the pathogenesis of goiter in patients with acromegaly (Miyakawa *et al.*, 1988) as well as in pregnancy, where both IGF-1 (Wilson *et al.*, 1982) and placental lactogen (a growth hormone analogue) circulate in high concentrations. Insulin and IGFs stimulate the growth of cultured thyroid cells, and TSH potentiates this mitogenic effect (Saji *et al.*, 1987; Eggo *et al.*, 1990). Several IGF binding proteins (IGFBP) and their mRNAs have been identified in sheep thyroid cells by radioligand binding and in situ hybridization techniques (Bachrach *et al.*, 1989). Known thyroid mitogens such as EGF and phorbol esters stimulate the synthesis of IGFBP-3 mRNA while differentiation stimulants such as TSH, transferrin, hydrocortisone, somatostatin, insulin, and the tripeptide glycyl-histidyl-lysine, are associated with decreased transcription of the genes for IGFBP-2 and IGFBP-3 (Bachrach *et al.*, 1991). Both insulin and the IGFs stimulate the thyroid cell growth in vitro, an effect that is potentiated by pre-incubation with TSH (Saji *et al.*, 1987; Eggo *et al.*, 1990).

Transforming growth factors: The mRNA transcripts for transforming growth factor (TGF) — beta (Grubeck-Loebenstein *et al.*, 1989) have been detected in human and mammalian thyroid tissues. The exposure of cultured human, rat, or porcine thyroid cells to TGF-beta results in an inhibition of DNA synthesis and resistance to the mitogenic effects of EGF, IGF-1, TSH, and TGF-alpha (Morris *et al.*, 1988: Grubeck- Loebenstein *et al.*, 1989). In addition, iodide uptake and organification by cultured thyroid cells are also affected by TGF-beta (Tsushima *et al.*, 1988). The concentration of TGF-beta mRNA was found to be reduced in thyroid tissue from patients with iodine-deficient goiters; addition of sodium iodide to follicular cells cultured

from such glands apparently increased the amount of TGF-beta released into the medium (Grubeck-Loebenstein *et al.*, 1989). TGF-beta also inhibits IGF-1 secretion as well as the mitogenic response to exogenous IGF-1 in pig thyroid cell cultures (Beere *et al.*, 1991). Intrathyroidal TGF-beta is initially secreted in a biologically inactive form, which can be activated by the enzyme, plasmin (Bechtner *et al.*, 1991). Interestingly, tissue plasminogen activator (t-PA) is secreted by cultured thyroid cells in a manner that responds to EGF stimulation (Bechtner *et al.*, 1991). It is probable that TGF-beta, a known potent inhibitor of epithelial cell growth, might be physiologically active as an autocrine inhibitor of thyroid cell growth. Exactly how TGF-beta produces its effects on thyroid cells is not known, but the inhibitory action may be mediated by regulation of proto-oncogene expression. In cultured keratinocytes, TGF-beta reduces the level of c-myc mRNA, and anti-sense c-myc oligonucleotides that inhibit synthesis of c-myc protein also inhibit cell proliferation (Coffey *et al.*, 1988; Pietenpol *et al.*, 1990).

Other growth factors: The presence of mRNA transcripts for prepro-nerve growth factor (prepro-NGF) and basic fibroblast growth factor (bFGF) has been demonstrated in thyroid tissue ((Dicou *et al.*, 1986; Emoto *et al.*, 1991). In the case of NGF, the translated peptide has not yet been demonstrated in thyroid tissue but bFGF is secreted as a protein by cultured thyroid cells (Emoto *et al.*, 1991). In vitro, bFGF stimulates thyroid cell growth and inhibits TSH-induced iodide uptake by thyrocytes (Eggo and Logan, 1991). Incubation of FRTL5 cells with TSH results in the release of an FGF-like factor that probably mediates the enhanced mitogenic response of these cells to other growth factors (Takahashi *et al.*, 1990). Further, since bFGF and the other FGFs potently stimulate angiogenesis, the vascular requirements for cellular hypertrophy and tumor formation can be met locally within the thyroid.

Other cytokines such as tumour necrosis factor (TNF)-alpha and interferon (IFN)-gamma and interleukin (IL)-1, though not yet shown to be produced in the thyroid, nevertheless, exert important effects on thyroid growth and function. TNF-alpha, produced by macrophages in response to sepsis, appears to block iodide uptake and hormone release by the thyroid (Ozawa *et al.*, 1988), and acts synergistically with IFN-gamma to inhibit thyroid cell proliferation in vitro (Nagayama *et al.*, 1987; Weetman and Rees 1988). IL-1 inhibits differentiated thyrocyte function (Sato *et al.*, 1990), inhibits the growth of human thyroid papillary and follicular carcinoma cells (Kimura *et al.*, 1991) and stimulates the proliferation of cultured Graves' thyroid cells (Mine *et al.*, 1987). Both the mitogenic and anti-mitogenic effects of IL-1 are apparently mediated through the regulation of c-myc oncogene levels (Mine *et al.*, 1987; Kimura *et al.*, 1991). Table 1 lists the various cytokines according to their in vitro effects on thyroid cell growth.

Table 1: Regulation of thyroid cell growth in vitro

Pro-mitogenic	Anti-mitogenic
Epidermal factor	Interferon-α
Fibroblast growth factor	Interleukin-1*
Insulin-like growth factor I	Transforming growth factor-β
Interleukin-1*	
Thyrotropin	
Transforming growth factor-α	

* stimulates Graves' thyroid cells but inhibits thyroid carcinoma cells.

Role in thyroid neoplasia

Owing to their inherent mitogenic effects as well as structural homology with certain viral oncogenes (Waterfield *et al.*, 1983; Downward *et al.*, 1984), peptide growth factors and their receptors have been extensively studied with regard to their role in carcinogenesis. The EGF receptor (Klijn *et al.*, 1992) and erbB2 oncogene protein (Slamon *et al.*, 1989) are over-expressed in patients with breast cancer and other malignancies. Recently the ligand for the $p185^{erbB2}$ protein, called heregulin, has been purified from media conditioned by breast cancer cells and shown to belong to the EGF family (Holmes *et al.*, 1992).

With regard to the thyroid, there have been several reports of significant alteration in EGF receptor numbers and/or binding affinity in both benign and malignant thyroid tissues (Miyamoto *et al.*, 1988; Kanamori *et al.*, 1989; DiCarlo *et al.*, 1990). The affinity of the EGF receptor in cancerous thyroids was reported to be either lower (Miyamoto *et al.*, 1989) or greater (Kanamori *et al.*, 1989;

DiCarlo *et al.*, 1990) than that of normal or adenomatous glands but the EGF binding capacity is generally increased in nearly 50% of thyroid cancers. Makinen *et al.*, 1988 reported that papillary as well as anaplastic, but not medullary, thyroid cancers over-express EGF receptors. An inverse relationship between EGF receptor density and TSH responsiveness has been observed in anaplastic thyroid cancer (DiCarlo *et al.*, 1990). Correlations have also been found among EGF receptors, TSH receptors, and TSH-stimulated adenylate kinase activity in differentiated thyroid malignancies (Duh *et al.*, 1990). Data on the receptors for other growth factors in the thyroid are scanty. The aberrant expression of the platelet-derived growth factor (PDGF) receptor has been reported in one anaplastic thyroid carcinona cell line (Heldin *et al.*, 1988). The co-expression of IGF peptides and IGF receptors has been demonstrated in rat thyroid medullary carcinoma cells (Okimura *et al.*, 1989) and cultured human thyroid cells (Vannelli *et al.*, 1990). Human thyroid nodules appear to express greater IGF-1 receptors (Vannelli *et al.*, 1990) and tend to contain more IGF-1 peptide (Minuto *et al.*, 1989) than normal thyroid tissue. The genes encoding TGF-alpha and the EGF receptor are co-expressed in some papillary thyroid cancers, including lymph node metastases (Aasland *et al.*, 1990).

Concluding remarks

Most of the data reviewed here have been acquired from in vitro studies and would require to be confirmed using in vivo thyroid models such as that developed by Ozawa and Spaulding (1990). Prospective studies comparing receptors for EGF, IGF-1, TGF-beta, and TSH in the same tissues could be revealing. The results of such studies may possibly uncover the presence of a hierarchy among the thyroactive cytokines.

The emerging data on the anti-proliferative effects of TGF-beta and modulation of its intrathyroidal levels by iodide are of considerable theoretical interest, offering potential insights into the pathogenesis of endemic goiter and thyroid neoplasia. In the case of endemic goiter, the available bits of evidence permit a hypothetical construct of a probable mechanism of goitrogenesis (Fig. 1). In this model, TGF-beta assumes a prominent role as an autocrine inhibitor of thyroid cell proliferation pitched against an array of thyroid mitogens. Differential regulation of intrathyroidal TGF-beta levels by a variety of dietary and environmental "goitrogens" may conceivably activate or deactivate the thyrocyte response to local mitogens (Morris *et al.*, 1988; Grubeck-Loebenstein *et al.*, 1989).

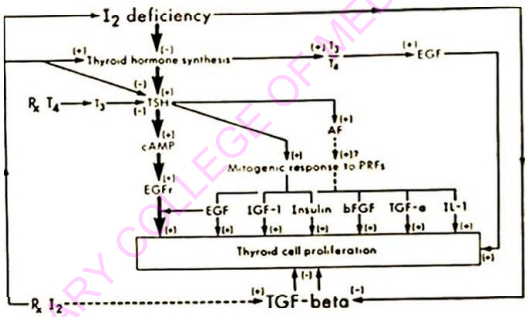

Fig. 1: A new understanding of goitrogenesis. Schematic representation of the antagonistic actions of TGF-beta vs. several growth factors, and the role of iodine in the regulation of thyroid cell growth.
AF = amplification factors, EGFr = EGF receptors, PRF = peptide regulatory factors. Symbols indicate increased (+) or decreased (—) responses to stimuli. Solid lines are pathways supported by the literature; broken lines denote hypothetical paths.

Traditional beliefs about the pathogenesis of goiter have been based on rather simplistic models that cannot account for the common occurrence of goiter in a variety of metabolically disparate conditions. Abundant evidence now indicates that several polypeptide growth factors probably act in concert to co-regulate normal thyroid growth and differentiation. The aberrant expression or imbalance of some of these regulatory peptides and/or their receptors might be the proximate event underlying the processes of goitrogenesis and neoplastic transformation.

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References

1. Aasland R, Akslen LA, Varhaug JE, Lillehaug JR. Co-expression of the genes encoding transforming growth factor-alpha and its receptor in papillary carcinomas of the thyroid. Int. J. Cancer. 1990; 46: 382-387.
2. Bachrach LK, Eggo MC, Burrow GN, Liu F, Tram T, Powell DR. Regulation of insulin-like growth factor-binding protein messenger ribonucleic acid levels in sheep thyroid cells. Endocrinology. 1967-1973, 1991; 128.
3. Bechtner G, Potscher CH, Rafferzeder M, Spannagel M. Transforming growth factor-beta release from porcine thyroid follicles and the mechanism of activation by plasmin. 19th ETA Meeting, Annales d'Endocrinologie, 1991: Abstract no. 159.
4. Beere HM, Soden J, Tomlinson S, Bidey SP. Insulin-like growth factor 1 production and action in porcine thyroid follicular cells in monolayer: regulation by transforming growth factor-beta. J. Endocrinol. 1991; 130: 3-9.
5. Byyny RL, Orth DN, Cohen S. Radio-immunoassay of epidermal growth factor. Endocrinology 1972; 90: 1261-1266.
6. Coffey RJ, Bascom CC, Sipes NJ, Graves-Deal R, Weisman BE, Moses B. Selective inhibition of growth-related gene expression in murine keratinocytes by transforming growth factor-beta. Mol. Cell Biol. 1988; 8: 3088-3093.
7. Cohen S. Isolation of a mouse submaxillary gland protein accelerating incisor eruption and eyelid opening in the newborn animal. J. Biol. Chem. 1962; 237: 1555-1562.
8. Dagogo-Jack S, Atkinson S, Kendall-Taylor P. Demonstration of significant epidermal growth factor activity in murine thyroid tissue. J. Endocrinol. 1986; 110: 67-72.
9. Dagogo-Jack S, Kendall-Taylor P. Iodine deficiency depletes EGF in mouse thyroid and submaxillary glands. Hellenic Endocrine Society International Symposium on "Iodine and the Thyroid", Athens, Greece, Abstract no. 47, 1990.
10. Dagogo-Jack S. Age related changes in immuno-reactive epidermal growth factor concentrations in murine thyroid and submaxillary glands. Endocrine Regulations. 1991; 25: 221-224.
11. Dagogo-Jack S. Sialoadenectomy increases the concentration of epidermal growth factor in the mouse thyroid gland. Thyroid 1992a; 2: 137-140.
12. Dagogo-Jack S. Testosterone regulates epidermal growth factor levels in the thyroid gland of hypothyroid mice. Endocr. Res. 1992b; 18: 201-212.
13. DiCarlo A, Mariano A, Pisano G, Parmeggiani U, Beguinot L, Macchia V. Epidermal growth factor receptor and thyrotropin response in human thyroid tissues. J. Endocrinol. Invest. 1990; 13: 293-299.
14. Dicou E, Lee J, Brachet P. Synthesis of nerve growth factor mRNA and precursor protein in the thyroid and parathyroid glands of the rat. Proc. Natl. Acad. Sci. USA 1986; 83: 7084-7088.
15. Downward J, Yarden Y, Mayes E, *et al.* Close similarity of epidermal growth factor receptor and verbB oncogene protein sequences. Nature. 1984; 307: 521-527.
16. Duh Q-Y, Gum ET, Gerend PL, Raper SE, Clark OH. Epidermal growth factor receptors in normal and neoplastic thyroid tissue. Surgery 1985; 98: 1000-1007.
17. Duh Q-Y, Siperstein AE, Miller RA, Sancho JJ, DeMeure MJ, Clark OH. Epidermal growth factor receptors and adenylate cyclase activity in human thyroid tissues. World J. Surg. 1990; 14: 410-417.
18. Eggo MC, Pratt MAC, Becks GP, Burrow GN. Regulation of growth and differentiation in follicle cells. Adv. Exp. Med. Biol. 1989; 261: 341-356.
19. Eggo MC, Bachrach LK, Burrow GN. Interaction of TSH, insulin and insulin-like growth factors in regulating thyroid growth and function. Growth factors. 1990; 2: 99-104.
20. Eggo MC, Logan A. Basic fibroblast growth factor in sheep thyroid cells in culture: synthesis and biological effects. Endocrine Society, 73rd Meeting, Washington D.C., 1991: Abstract no. 1266.
21. Emoto N, Isozaki O, Arai M, *et al.* Identification and characterization of basic fibroblast growth factor in porcine thyroids. Endocrinology 1991; 128: 58-64.
22. Fisher DA, Dussault JH, Sack J, Chopra IJ. Ontogenesis of hypothalamic-pituitary-thyroid

function and metabolism in man, sheep, and rat. Recent Prog. Horm. Res. 1977; 33: 59-

23. Fisher DA, Lakshmanan J. Metabolism and effects of epidermal growth and related growth factors in mammals. Endocr. Rev. 1990; 11: 418-442.
24. Gartner R, Tsavella C, Bechtner G, Greil W. Evidence that thyroid growth promoting activity of immunoglobulin preparations is due to contamination with EGF. Acta. Endocrinol. (Copenh), suppl. 1987; 281: 256-259.
25. Grubeck-Loebenstein B, Buchan G, Sadeghi R, *et al.* Transforming growth factor beta regulates thyroid growth. Role in the pathogenesis of nontoxic goiter. J. Clin. Invest. 1989; 83: 764-770.
26. Heldin NE, Gustavsson B, Claesson-Welsh L, *et al.* Aberrant expression of receptors for platelet-derived growth factor in an anaplastic thyroid carcinoma cell line. Proc. Natl. Acad. Sci. USA. 1988; 85: 9302-9306.
27. Hirata Y, Orth DN. Epidermal growth factor (urogastrone) in human tissues. J. Clin. Endocrinol. Metab. 1979; 48: 667-672.
28. Holmes WE, Sliwkowski MX, Akita RW, *et al.* Identification of heregulin, a specific activator of $p185^{erbB2}$. Science 1992; 256: 1205-1210.
29. Kanamori A, Abe Y, Yajima Y, Manabe Y, Ito K. Epidermal growth factor receptors in plasma membranes of normal and diseased human thyroid glands. J. Clin. Endocrinol. Metab. 1989; 68: 899-903.
30. Kimura H, Tominaga T, Tsuruta M, *et al.* Interleukin-1 inhibits human thyroid carcinoma cell growth. 19th Eur. Thyr. Assoc. Meeting, Annales d'Endocrinologie, 1991: Abstract no. 101.
31. Maciel RM, Moses AC, Villone G, Tramontano 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.
32. Makinen T, Pekonen F, Franssila K, Lamberg BA. Receptors for epidermal growth factor and thyrotropin in thyroid carcinoma. Acta Endocrinol. (Copenh) 1988; 177: 45-50.
33. Mine M, Tramontano D, Chin WW, Ingbar SH. Interleukin-1 stimulates thyroid cell growth and increases the concentration of c-myc proto-oncogene mRNA in thyroid follicular cells in culture. Endocrinology. 1987; 120: 1212-1214.
34. Minuto F, Barreca A, Del Monte P, Cariola G, Torre GC, Giordano G. Immunoreactive insulin-like growth factor 1 (IGF-1) and IGF-1 binding protein content in human thyroid tissue. J. Clin. Endocrinol. Metab. 1989; 68: 621-626.
35. Miyakawa M, Saji M, Tsushima T, Wakai K, Shizume K. Thyroid volume and serum thyroglobulin levels in patients with acromegaly: correlation with plasma insulin-like growth factor 1 levels. J. Clin. Endocrinol. Metab. 1988; 67: 973-978.
36. Miyamoto M, Sugawa H, Mori T, Hase K, Kuma K, Imura H. Epidermal growth factors on cultured neoplastic human thyroid cells and effects of epidermal growth factor and thyroid - stimulating hormone on their growth. Cancer Res. 1988; 48: 3652- 3656.
37. Miyamoto M, Sugawa H, Kuma K, Mori T, Imura H. Analysis of epidermal growth factor (EGF) receptor and effect of EGF on the growth of cultured Graves' and nonneoplastic human thyroid cells. J. Endocrinol. Invest. 1989; 12: 111-117.
38. Mizukami Y, Nonomura A, Hashimoto T, *et al.* Immunohistochemical demonstration of epidermal growth factor and c-myc oncogene product in normal, benign and malignant thyroid tissues. Histopathology. 1991; 18: 11-18.
39. Morris JC, Ranganathan G, Hay ID, Nelson RE, Jiang NS. The effects of transforming growth factor-beta on growth and differentiation of the continuous rat thyroid follicular cell line, FRTL-5. Endocrinology. 1988; 123: 1385-1344.
40. Nagayama Y, Izumi M, Ashizawa K, *et al.* Inhibitory effects of inteferon-gamma on the response of human thyrocytes to thyrotropin stimulation: relationship between the response to TSH and the expression of DR antigen. J. Clin. Endocrinol. Metab. 1987; 64: 949-956.
41. Okimura Y, Kitajima N, Uchiyama T, *et al.* Insulin- like growth factor 1(IGF-1) production and the presence of IGF-1 receptors in rat medullary thyroid carcinoma cell line 6-23 (clone 6). Biochem. Biophys. Res. Commun. 1989; 161: 589-595.
42. Ollis CA, Hill DJ, Munro DS. A role for insulin-like growth factor-1 in the regulation of human thyroid cell growth by thyrotrophin. J. Endocrinol. 1989; 123: 495-500.
43. Ozawa M, Sato K, Han DC, Kawakami M, Tsuchima T, Shizume K. Effects of tumor necrosis factor-alpha cachectin on thyroid

hormone metabolism in mice. Endocrinology. 1988; 123: 1461-1467.
44. Ozawa S, Spaulding SW. Epidermal growth factor inhibits radioiodine uptake but stimulates deoxyribonucleic acid synthesis in newborn rat thyroids grown in nude mice. Endocrinology 1990; 127: 604-612.
45. Ozawa S, Sheflin LG, Spaulding SW. Thyroxine increases epidermal growth factor levels in the mouse thyroid in vivo. Endocrinology. 1991; 128: 396-1403.
46. Pientopol JA, Holt JT, Stein RW, Moses HL. Transforming growth factor-beta 1 suppression of c-myc gene transcription: role in inhibition of keratinocyte proliferation. Proc. Natl. Acad. Sci. USA. 1990; 87: 3758-3762.
47. Roger PP, Dumont JE. Epidermal growth factor controls the proliferation and the expression of differentiation in canine thyroid cells in primary culture. FEBS Letts. 1982; 144: 209-212.
48. Salido EC, Lakshmanan J, Koy S, Rarajas L, Fisher DA. Effect of thyroxine administration on the expression of epidermal growth factor in the kidney and submandibular gland of neonatal mice. An immunocytochemical and in situ hybridization study. Endocrinology. 1990; 127: 2263-2269.
49. Sainsbury JRC, Farndon JR, Needham GK, Malcolm AJ, Harris AL. Epidermal growth factor receptor status as predictor of early recurrence of, and death from, breast cancer. Lancet 1987; 1: 1398-1402.
50. Saji M, Tsushima T, Isozaki O, *et al.* Interaction of insulin-like growth factor 1 with porcine thyroid cells cultured in monolayer. Endocrinology 1987; 121: 749-756.
51. Sato K, Satoh T, Shizume K, *et al.* Inhibition of ^{125}I organification and thyroid hormone release by interleukin-1, tumor necrosis factor-alpha, and interferon-gamma in human thyrocytes in suspension culture. J. Clin. Endocrinol. Metab. 1990; 70: 1735-1743.
52. Schatz H, Pschierer-Berg K, Nickel JA, *et al.* Assay for thyroid growth stimulating immunoglobulins: stimulation of [3H] thymidine incorporation into isolated thyroid follicles by TSH, EGF, and immunoglobulins from goitrous patients in an iodine-deficient region. Acta Endocrinol. (Copenh) 1986; 112: 523-530.
53. Slamon DJ, Godolphin W, Jones LA, *et al.* Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science. 1989; 244: 707-712.
54. Takahashi S, Conti M, Van Wyk JJ. Thyrotropin potentiation of insulin-like growth factor 1 dependent deoxyribonucleic acid synthesis in FRTL-5 cells: mediation by an autocrine amplification factor(s). Endocrinology. 1990; 126: 736- 745.
55. Tode B, Serio M, Rotella CM, Galli G, Franceschelli F, Tanini A, Tocafondi R. Insulin-like growth factor 1: autocrine secretion by human thyroid follicular cells in primary culture. J. Clin. Endocrinol. Metab. 1989; 69: 639-647.
56. Tsushima T, Arai M, Saji M. *et al.* Effects of transforming growth factor-beta on deoxyribonucleic acid synthesis and iodine metabolism in porcine thyroid cells in culture. Endocrinology. 1988; 123: 1187-1194.
57. Vannelli GB, Barni T, Modigliani U, *et al.* Insulin-like growth factor-1 receptors in nonfunctioning thyroid nodules. J. Clin. Endocrinol. Metab. 1990; 71: 1175-1182,
58. Weetman AP, McGregor AM. Autoimmune thyroid disease: developments in our understanding. Endocr. Rev. 1984; 5: 309-355.
59. Weetman AP, Rees AJ. Synergistic effects of recombinant tumour necrosis factor and interferon-gamma on rat thyroid cell growth and Ia antigen expression. Immunol. 1988; 63: 285-289.
60. Waterfield MD, Scrace GT, Whittle N, *et al.* Platelet-derived growth factor is structurally related to the putative transforming protein p.28^{sis} of simian sacroma virus. Nature 1983; 304: 35-39.
61. Wilson DM, Bennett A, Adamson GD, *et al.* Somatomedins in pregnancy: a cross-sectional study of insulin-like growth factors I and II and somatomedin peptide content in normal human pregnancies. J. Clin. Endocrinol. Metab. 1982; 55: 858-861.
62. Zakarija M, McKenzie JM. Do thyroid growth-promoting immunoglobulins exist? J. Clin. Endocrinol. Metab. 1990; 70: 308- 310.