Growth factors and goitrogenesis

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Abstract

By combining data from studies of multinodular non-toxic goitre (MNTG) with data from rat models of goitre induction and in vitro models, a map of the growth factors involved in goitrogenesis has been constructed. We have addressed the roles of the insulin-like growth factors, transforming growth factors, fibroblast growth factors, endothelins, etc. We hypothesise that an imbalance in the interactions between the various growth factor axes exists in MNTG which favours cell replication. Thyrotrophin, although not significantly elevated in MNTG, exerts critical effects through interactions with autocrine and paracrine factors and their receptors. Expansion of the thyroidal vascular bed through angiogenesis is closely co-ordinated with follicular cell expansion and folliculo-neogenesis, and while the integrated paracrine actions of fibroblast growth factors, vascular endothelial growth factor and endothelin probably play central roles, additional, as yet elusive, factors are probably involved. The combination of in vitro and in vivo approaches, designed to address specific questions, will undoubtedly continue to prove invaluable in dissecting further the complex interactions that exist between these growth factors, their binding proteins and receptors in goitrogenesis.

Introduction

The homoeostasis of growth in differentiated epithelia reflects a critical balance between the promotion and suppression of cell division. In accord with this generalised model, the follicular epithelium of the normal adult thyroid gland is essentially quiescent, and the loss of thyroid follicles and cell death is closely balanced against follicular neogenesis (Coclet et al. 1989). However, the thyroid gland is among the most common sites of epithelial hyperplasia, affecting up to 15% of the adult population, and frequently requiring surgical intervention to relieve compression of the trachea and laryngeal nerves. Typically presenting as 'sporadic' or multinodular non-toxic goitre (MNTG), the hyperplastic gland usually contains welldefined nodules of varying size, surrounded by a normal epithelium. Patients with MNTG are invariably euthyroid, with thyrotrophin (TSH) levels within the normal physiological range (Gutekunst & Scriba 1989), and may be contrasted with those with endemic (iodide-deficiency) goitre, in whom thyroidal enlargement reflects an increase in cell proliferation as a consequence of dietary iodine deprivation. Within the thyroid gland affected by MNTG, individual nodules typically display different rates of expansion, and several studies have shown that polyclonal and monoclonal nodules coexist within goitrous thyroid tissue (Thomas et al. 1988, Kopp et al. 1994). Pioneering studies by Studer and colleagues have provided evidence that the onset and progression of MNTG reflect the expansion of follicular cells and follicles that have acquired different patterns of growth factor responsiveness, with individual nodules typically displaying different rates of cell division (Studer et al. 1992). An explanation for the high incidence of nodules (>50%) and possibly micropapillary carcinoma (6–28.4%) found at autopsy (Wheeler 1994) is that the stem cell population of the thyroid gland has innate growth potential. Accelerated by fluxes of growth stimulation, the localised expansion of the follicular cell population will thus, given adequate time, give rise to focal hyperplasia (Studer et al. 1995). While our understanding of the cellular and humoral mechanisms underlying MNTG remains incomplete, dysregulation in the bioavailability of key growth regulators has become an intensive area of research. Progress in such studies has, to date, revealed evidence for an involvement of several autocrine growth stimulators and their receptors in the progression of MNTG. Prominent among these are the insulin-like growth factors (IGFs; Minuto et al. 1989),

fibroblast growth factors (FGFs; Thompson et al. 1998), transforming growth factor β1 (TGFβ1; Grübeck-Loebenstein et al. 1989, Perlino et al. 1996), hepatocyte growth factor (HGF; Eccles et al. 1996), and the paracrine angiogenic factors, vascular endothelial growth factor (VEGF), placental-derived growth factor (PIGF) (Viglietto et al. 1997) and endothelin (Sellitti & Hughes 1990).

**Experimental systems**

Much of our understanding of the growth factor-dependent regulation of thyroid cell proliferation and differentiation is derived from studies with thyroid follicular cells in primary culture which, while retaining a high level of differentiation, express and respond to numerous growth factors and cytokines (Eggo et al. 1989, Bidey 1990, Dumont et al. 1991, Eggo & Sheppard 1994). Such models, which can be maintained and manipulated under closely controlled conditions, have facilitated detailed studies of the interactions of TSH with growth factors and their respective receptors with regard to follicular cell growth and function, and have furnished clues to the roles of these factors in the goitrogenic process. It is important to recognise at the outset, however, the fundamental differences between in vitro and in vivo models, which should be borne in mind when extrapolating effects observed in cell cultures to the intact thyroid gland. The first drawback is that cell cultures condition their medium with autocrine growth-regulating factors, which may attain far greater concentrations than those found within the intact thyroid gland in vivo. The levels of thyroid hormones released from endogenous stores of iodinated thyroglobulin can also be very high and can persist for a considerable time in culture (Becks et al. 1987). Accordingly, the possibility has to be considered that regulation of cell growth and function in vitro may be deranged by the local concentrations of cell-derived factors in the medium. Furthermore, since primary cultures may be ‘contaminated’ to variable degrees with endothelial cells, C cells and fibroblasts, determination of which cell type in the thyroid produces a given growth factor is difficult (Eggo 1998). In relation to the above, however, a further critical difference deserving special attention is that, unlike follicular cells in culture, the intact thyroid follicle is closely associated with a fibroblast-containing mesenchyme. In addition to synthesising a variety of matrix molecules which may moderate follicular cell function and growth, this cell layer will also contribute to the pool of growth factors to which follicular cells are exposed, which may also contribute to the adjustment of growth control in follicular hyperplasia. The matrix itself may also, as detailed below, serve to sequester specific cell-derived growth factors and regulate their subsequent availability and cellular action. Rat models of goitrogenesis obviate the first of these problems, and these may be induced rapidly following goitrogen treatment in combination with a low iodine diet. Thyroid hypertrophy is evident after 1 week and hyperplasia at 2 weeks. While growth continues to 12 weeks, growth velocity declines. However, despite the usefulness of this model, the danger of extrapolating data from rats to humans should not be underestimated. Furthermore MNTG is not synonymous with iodide-deficiency goitre and the evolution of these two diseases may differ. Notwithstanding the inherent limitations of both in vivo and in vitro models, it is clear that this experimental approach has afforded considerable insight into the roles that autocrine, paracrine and endocrine factors play in goitrogenesis of the human thyroid gland, and in this review we shall attempt to integrate data from clinical studies, thyroid cell culture models and rat models of goitre induction to delineate possible underlying mechanisms.

**IGFs, their binding proteins and their receptors**

Acromegaly, characterised by elevated levels of serum IGF-I, has long been associated with goitre (>70% incidence; Wuster et al. 1991). Minuto et al. (1989) described a 2-fold increase in immunoreactive IGF-I in patients with MNTG compared with controls, but IGF-I levels were not elevated in goitres associated with Graves’ disease. Whether the incidence of goitres is increased in hyperinsulinaemic states such as polycystic ovary syndrome has not yet been established, although one study showed that 28 children with MNTG all had grossly elevated insulin levels (Abdullaeva et al. 1987). A more recent study showed that the elevated levels of IGF-I in MNTG were located in the follicular cells (Maiorano et al. 1994). Sheep thyroid follicles in primary culture provided the first in vitro model of the thyroid shown to express IGF-I and IGF-II (Mak et al. 1985, Bachrach et al. 1988), a finding later confirmed in follicular cell cultures from other species, including humans (Ollis et al. 1989, Tode et al. 1989) and pigs (Beere et al. 1991). Furthermore, proliferation of follicular cells from a solitary nodule was found to be independent of exogenous IGF-I, confirming the potential autocrine role of IGF-I in proliferative thyroid disease (Williams et al. 1988). Cell culture studies of follicular cells from several species have shown that IGFs synergise with, and are obligatory for, the stimulatory effects of TSH on both thyroid growth and function (Eggo et al. 1985, 1990, Ollis et al. 1989, Williams et al. 1988), whereas TSH alone is ineffective. The requirement for both TSH and IGF-I for follicular cell growth in vitro has subsequently been confirmed in vivo. Thus, in adults with hypopituitarism, increases in serum IGF-I effected by growth hormone replacement are not associated with an increased thyroid mass in the absence of TSH over a 6 month period (Cheung et al. 1996). The mechanism of interaction between TSH and IGF-I has been
extensively investigated and remains a major focus for study (Santisteban et al. 1987, 1992, Brenner-Gati et al. 1988, Saji & Kohn 1991, Liu et al. 1996, Yamamoto et al. 1996, Ortiz et al. 1997). In cultures of human thyroid follicular cells, Tode et al. (1989) found evidence for an upregulation by TSH of IGF-I expression, and a study of IGF-I mRNA levels in pig follicles maintained in culture (Hofbauer et al. 1995) supports this finding. Thyroid follicular cells express the type I IGF receptor, but, although many of the data on IGF receptor regulation have been obtained using the rat thyroid cell strain FRTL-5 (Tramontano et al. 1987, Perrotti et al. 1989, Takahashi et al. 1991, Saji et al. 1992), caution should be exercised in extrapolating such findings to thyroid follicular cells generally. In dog thyroid cells for example, TSH does not regulate IGF receptor expression, but does regulate the functionally similar insulin receptor (Burikhanov et al. 1996). Studies with follicular cells from several species have found insulin and IGF-I receptors to coexist, and to date their actions have been found to be identical. There are no data at present on the regulation of IGF or insulin receptor affinity or number in human thyroid cells, although this may prove to be an important area for future investigation.

While the relative expression levels of IGF peptide and receptors may become deranged in the goitrogenic process, the availability of IGF-I to component cells of the thyroid follicle may be more generally influenced and more tightly controlled by the IGF-binding proteins (IGFBPs) (Minuto et al. 1989, Tode et al. 1989, Eggo et al. 1996). IGFBPs are synthesised and secreted by thyroid cells in culture, and their production is determined by several regulators of thyroid growth and function. Although the types of IGFBPs produced by thyroid cells differ between species, inhibition of synthesis by TSH is common. This could increase the availability of IGF-I and contribute to the TSH-mediated increase in growth and function of the follicular epithelium (Bachrach et al. 1988, 1989, 1991, Eggo et al. 1991, Wang et al. 1991). Conversely, inhibitors of thyroid function production increases in thyroidal IGFBP synthesis, particularly that of IGFBP-3 (Bachrach et al. 1991, Eggo et al. 1991, 1996). IGFBPs are not, however, always inhibitory to IGF bioactivity and some of the forms produced, for example those bound to the extracellular matrix, may facilitate IGF-I interaction with its receptor. IGFBPs may also prolong the half-life of IGF internalised in cells by allowing nuclear uptake and localisation (Michell et al. 1997) or they may have effects independent of their interaction with IGFs. A final mechanism controlling the equilibrium of IGF–IGF receptor–IGFBP interaction may reside in the cell-mediated processing of IGF-I. Proteolytic removal of three amino acids from the N-terminal region produces a product that is able to interact with the IGF receptor but not the binding protein. The processing of IGF-I to a truncated form in this way, as first detected in porcine uterus (Ogasawara et al. 1989), may effectively circumvent the IGF-I–sequestering actions of the IGFBPs. Whether a protease capable of effecting such a cleavage reaction exists within the thyroid gland has yet to be determined.

Control of IGF-I bioavailability within the follicular microenvironment may also be effected by iodide supply. In cultured porcine thyroid cells, IGF-I gene transcription and peptide release are potentiated by intracellular organified iodide (Beere et al. 1995, Hofbauer et al. 1995), in parallel with a progressive reduction in the rate of cell proliferation in FRTL-5 cells (Becks et al. 1988, Tramontano et al. 1989). The reactive species of iodide is postulated to be an iodolactone derivative of arachidonic acid (Dugrillon et al. 1994, Pisarev et al. 1994), although further studies of the role of iodide per se, as well as thyroid hormones and other organified intermediates of iodide, are needed. Although not yet conclusively proven, derepression of IGF-I synthesis and release after transfer of follicular cells to iodide-deficient conditions may be the in vitro counterpart of a mechanism that contributes, at least partially, to the pathogenesis of iodide-deficiency goitre. Even under iodide-replete conditions, loss or impairment of a component critical to the correct intracellular processing of iodide may similarly derepress the synthesis and autocrine bioavailability of thyroidal IGF-I.

Figure 1 summarises the current knowledge of the production and effects of IGF-I and IGFBPs by the cells comprising the human thyroid, i.e follicular, endothelial,
fibroblast and C cells. The contribution of these cells to the pool of IGFs and IGFBPs within the gland is shown in solid arrows and, where known, the biological effects of IGFs and IGFBPs on the growth of the cells are shown. Little is known of the paracrine role of the C cells, from which medullary thyroid carcinomas arise. One of their products, somatostatin, has been shown to have inhibitory effects on both FRTL-5 cell growth and function, but their contribution to the IGF/IGFBP pool is not known. The figure also shows the effect of TSH, which is probably due to its interaction with thyroid follicular cells. However, TSH binding to its receptors will result in increased cAMP which freely diffuses from the cells. Similarly prostaglandin synthesis and release will be altered by TSH, which undoubtedly can have paracrine effects. Another effect of TSH will be to increase thyroid hormone (tri-iodothyronine (T3) and thyroxine (T4)) synthesis and release. All the cells in the thyroid will be responsive to T3 and T4, although their effects on IGF/IGFBP synthesis and release are not known. Iodide effects may be mediated through their ability to modulate T3 and T4 synthesis which will be stimulatory at low concentrations and inhibitory at high concentrations. The direct effects of iodide per se in the thyroid follicular cells, which can maintain a high intracellular iodide concentration, and the effects of the organified intermediate are also possible mediators of follicular production of growth factors. Also included in the figure are the effects of epidermal growth factor (EGF) and protein kinase C (PKC) activation. Thyroid cells may be exposed to EGF in the bloodstream or through TGFβ, secreted by endothelial cells, which interacts with the EGF receptor. Activation of the EGF receptor or PKC stimulates IGFBP secretion (Eggo et al. 1996) from the thyroid follicular cells.

**Latent and activated forms of TGFβ and their receptors**

While an increase in IGF-I availability is a probable factor in enlargement of the thyroid mass, the actions are likely to be integrated with those of further factors of autocrine or paracrine origin within the thyroid follicle. As observed in numerous other epithelial models, TGFβ1 is a potent inhibitor of thyroid follicular cell proliferation in vitro (Morris et al. 1988, Tsushima et al. 1988, Grübeck-Loebenstein et al. 1989, Taton et al. 1993), reflecting blockade of the cell cycle through suppression of the actions of the cyclins and their regulating kinases (Ewen et al. 1993, Geng & Weinberg 1993, Koff et al. 1993). The potent suppression of cell proliferation afforded by TGFβ1 thus serves to oppose both the effect of TSH and the mitogenic action of autocrine growth factors. Interestingly, several links have been demonstrated between TGFβ1 and thyroidal IGF-I bioavailability. For example, exposure of follicular cells to TGFβ1 leads to an increase in the release of IGFBP-3 (human) (Eggo et al. 1996) and a decrease in IGF-I synthesis (porcine) (Beere et al. 1991). Both actions are consistent with a decline in follicular IGF-I bioavailability, which may serve to reinforce the direct suppression of cell proliferation activity afforded by TGFβ1. TGFβ1 synthesis at an mRNA level has been confirmed within follicular cell cultures derived from the thyroid glands of numerous species. In porcine follicular cells (Cowin et al. 1992), only TGFβ1 is present and TGFβ2 and 3 have not been detected. Analysis of TGFβ1 in thyroid cell-conditioned culture medium by bioassay and receptor assay has revealed predominantly the mature (i.e. active) form of the peptide, suggesting that normal follicular cells in culture both synthesise TGFβ1 and retain an endogenous mechanism for TGFβ1 activation (Cowin et al. 1992, Cowin & Bidey 1995). Indirect evidence for the presence of active TGFβ1 in such cultures has also been confirmed by the enhanced rate of follicular cell proliferation after immunoadsorption of autocrine TGFβ1 (Cowin & Bidey 1995). The precise mechanism of thyroidal TGFβ1 activation remains to be elucidated, but support for a contributory role of the plasminogen/plasminogen activator (PA) system comes from the observed release of tissue (t-) and urokinase (u-)–like PAs in response to TSH (Mak et al. 1984), the upregulation by TSH of endogenous cathepsins (Phillips et al. 1989), and the increased TGFβ1 bioactivity in medium conditioned by plasmin-treated follicular cells (Cowin & Bidey 1994).

Although exposure to iodide induces a potent downregulation of IGF-I synthesis in thyroid follicular cells from at least some species (Beere et al. 1995, Hofbauer et al. 1995), changes in the expression of TGFβ1 have also been observed under similar conditions. Thus the major TGFβ1 mRNA species (2.5 kB) is upregulated in porcine follicular cells after exposure to iodide (Cowin et al. 1992, Yuasa et al. 1992), and this was initially thought to explain, at least in part, the expansion of the thyroid follicular mass in endemic iodide deficiency (Grübeck-Loebenstein et al. 1989). However, in rats, thyroid hyperplasia induced by iodide deficiency and goitrogen is paradoxically accompanied by an increase in TGFβ1 expression and the arrest of goitre growth after 4 weeks. This initially surprising result is thought to reflect a critical role of TGFβ1 in stabilising goitre mass (Logan et al. 1994). Evidence that this may reflect a direct action of TSH on TGFβ1 synthesis is supported by the in vitro findings of a direct stimulatory effect of TSH on thyroidal TGFβ1 expression in FRTL-5 cells (Pekary et al. 1995) and of a greatly enhanced proliferative response to TSH after TGFβ1 immunoadsorption from porcine follicular cell cultures (Cowin et al. 1996). Presumably therefore, under conditions of iodide deficiency in vivo, the reduction of TGFβ1 expression would be masked and indeed reversed by the TGFβ1 synthesis response to TSH.

The role of TGFβ1 in MNTG, as opposed to iodine-insufficiency goitre, is less clear. Some forms of epithelial
hyperplasia are known to be associated with aberrant TGFβ1-mediated growth control (Cui et al. 1995, Roberts 1998), but clarification of the role for TGFβ1 in MNTG is clouded by conflicting reports; Perlino et al. (1996), for example, have reported a reduction in TGFβ1 in hyperplastic, compared with normal, thyroid tissue, while a contemporary report (Morosini et al. 1996) has recorded higher levels in recurrent goitre after thyroid surgery than in patients remaining in remission. Nevertheless, the evidence from the rat model implicating a critical role of TGFβ1 in the limitation of iodide-deficiency goitre suggests that dysregulation of the stimulatory growth factor profile of the hyperplasia of MNTG may include a key change in TGFβ1 bioavailability, which is clearly deserving of further study.

As in cells from other forms of epithelial hyperplasia, studies of follicular cells from MNTG have revealed a high incidence of insensitivity to the actions of TGFβ1 (Asmis et al. 1996) irrespective of the total TGFβ1 content of the cell-conditioned medium (Cowin & Bidey 1996). The concept of a localised cellular resistance to TGFβ1 within the MNTG-affected thyroid gland is supported by a loss of response to TGFβ1 in follicular cells from feline goitres (Asmis et al. 1996). Although changes in TGFβ1 receptor (TβR) expression or signal transduction remain to be confirmed as factors effecting loss of TGFβ1 responsiveness in thyroid hyperplasia, mutations in the type 2 TβR isoform are commonplace in epithelial neoplasia (Kim & Kim 1996), while other epithelial tumours are deficient in the ALK-5 isoform of the type I TβR (Baldwin et al. 1996). Interestingly, and as seen in other epithelia (Kim & Kim 1996), follicular cells from some human MNTGs also demonstrate impaired TGFβ1 processing (Cowin & Bidey 1996). This suggests that a loss of a component critical to the cell membrane-mediated proteolysis of the latent TGFβ1 precursor may impair local TGFβ1 bioavailability at the follicular cell level. In some hyperplastic epithelia, including the prostate (Timme et al. 1995) and endometrium (Gold et al. 1994), TGFβ1 resistance is accompanied by high levels of endogenous TGFβ1, which may induce expansion of the underlying TGFβ1-responsive mesenchyme. Accordingly, and in contrast with the growth-suppressive effect exerted on epithelia, it is conceivable that TGFβ1 of follicular cell origin may enhance the growth of perifollicular fibroblasts within the immediate vicinity of the thyroid follicle, so that the perifollicular mesenchyme may continue to expand. This may contribute to the fibrotic lesions and excessive matrix deposition commonly found in MNTG-affected thyroid tissue. Whether as a consequence of cellular TGFβ1 resistance or diminished cellular processing therefore, the balance of current evidence suggests that, as in thyroid tumorigenesis (Blaydes et al. 1996), a localised change in TGFβ1 bioavailability within the follicular microenvironment represents a key factor in the development of thyroid hyperplasia.

Figure 2 summarises our current knowledge of TGFβ production and effects in the thyroid. Some of the regulatory data shown have not been confirmed in human thyroids. The contributions of all the cell types to the pool of this growth factor are shown and the effects of TGFβ on these cell types are shown, where known. The role of PAs as potential activators of latent TGFβ is also shown. TSH effects, which, as noted previously, may invoke the secretion of powerful paracrine factors from the follicular cells, and the effects of iodide and T3 and T4 are shown.

**FGFs and FGF receptors**

Follicular cells from MNTG tissue express both FGF-1 and FGF-2, together with FGF receptor-1 (FGFR1) (Thompson et al. 1998), and this expression is greatly increased compared with the normal thyroid. In rats, compelling evidence for the role of FGF in goitrogenesis comes from in vivo experiments in which administration of FGF-1 produced a 43% increase in thyroid weight by 6 days and a decrease in thyroid deiodinase in 2–8 h (Chanoine et al. 1992, DeVito et al. 1992). FGF has been found in pig (Emoto et al. 1991) and sheep thyroid cells in culture, and TSH has been shown to increase FGF-2 expression in the latter (Hill et al. 1994). There is indirect evidence for FGF production in FRTL-5 cells, for which these factors are potent mitogens (Logan et al. 1992). In primary cultures, the effects of FGF on thyroid cells appear inconsistent but this may be related to regulation of receptor expression. An initial report of studies with dog thyroid cells showed mitogenic (Roger & Dumont 1984) and dedifferentiating effects (Gerard et al. 1989), while studies using human thyroid cells found no effect on
growth (Taylor et al. 1993). FGFs are not normally secreted in free form, and their effectiveness as mitogens is dependent upon release from the extracellular matrix. As is the case with TGFβ therefore, the role of the FGFs in thyroid remodelling very likely depends on the presence of specific proteases to release them from sequestration within the extracellular matrix. Potential candidates for such cleavage are the PAs, which thyroid follicular cells produce in abundance (Mak et al. 1984) as well as cathepsins and matrix metalloproteinases.

Expression of growth factors and their receptors in thyroids of goitrogen-treated rats

In the goitrogen-treated rat, thyroïdal FGF-2 expression is maximally induced after 1 week of treatment, precedes DNA synthesis and subsequently declines in parallel with the growth velocity of the gland. An increase in FGF-2 immunoreactivity in the goitrous thyroid glands of these animals has been histologically localised to the thyrocytes (Logan et al. 1994, Patel et al. 1996). Large reserves of FGF-2 are sequestered within the perifollicular stroma of the rat thyroid gland, unlike that in human thyroid, and an earlier stage in the goitrogenic response may involve proteolysis of FGF-2 and release from the stromal store. FGF-2 mRNA is also expressed in the thyrocytes and increases approximately 1 week after that of FGF-2 mRNA. This observation provides evidence that FGF-2 may drive the expression of its own receptor (Becks et al. 1994). In the same model, thyrocyte IGF-I mRNA and peptide are induced within 1 week of goitrogen treatment, and are increased further at 2 weeks. Like FGF-2, IGF-I levels thereafter decline with growth velocity and are reduced further during goitre regression. IGFBP-2 and IGFBP-3 mRNAs and protein levels increase progressively with goitre regression and are not influenced by T3 in the rat thyroid gland (Humphries & Phillips 1994). In these studies, the negative regulation of the mitogenic action of IGF as goitre homoeostasis is established. These data contrast with those from in vitro cell cultures where TSH inhibits follicular cell IGFBP synthesis. Whether the differences are due to the species, to the contributions from the other cells in the thyroid (either the follicular cells or the pool of IGFBPs during the goitrogenic response) or to differences in the aetiology of iodide-deficiency goitre and MNTG remains to be clarified. IGFBP-5 is expressed only in the C cells and its expression declines during goitre regression but is more abundant during goitre regression as C cell hyperplasia occurs. TGFβ1 is present in C cells even in normal animals and is abundant during the C cell hyperplasia that accompanies goitre regression (Logan et al. 1994).

Hypophysectomised rats have been used to determine the intermediary role of TSH in triggering the changes in growth factor expression in experimentally induced goitre. Hypophysectomy prevents the rapid rise in FGF-2 and FGF-R1 expression and that of IGF-I and IGFBP-2 and -3, and under such conditions no goitre or histological changes are evident, despite the fall in T3 to almost undetectable levels as a consequence of iodide deficiency. Subsequent treatment of goitrogen-exposed hypophysectomised rats with TSH gives rise to a hyperplastic tissue response and increased abundance of FGF-2 and FGF-R1, IGF-I, IGFBP-2 and IGFBP-3. Hypophysectomy also prevents an increase in TGFβ expression in response to goitrogen that is not reversed by treatment with T3. These results suggest that in iodine-deficiency goitre in the rat, TSH is a direct mediator of the FGF and IGF expression which drives the goitre, while TGFβ expression may also be linked to prevailing T3 levels.

Figure 3 summarises current knowledge of FGF production and actions in the thyroid and is limited to FGF-1 and FGF-2. Some of the data have not been confirmed in human thyroid cells. The role(s) of other members of the FGF family and the expression of FGF-R isoforms in MNTG compared with normal tissue await to be determined. The role of known modulators of thyroid function/growth, i.e. TSH and iodide, are shown (dotted lines) at the bottom. The putative role of the PAs, which are positively regulated by TSH, in releasing FGF from the matrix is shown. The effects of T3 and T4 on FGF production and FGF receptor expression in any of the different cell types are not known.

**Angiogenic factors**

Expansion of the follicular epithelium during thyroid hyperplasia is dependent upon a co-ordinated expansion of the vascular bed supplying the thyroid gland. As a
consequence of their established angiogenic actions, the FGFs may participate in paracrine actions between follicular and endothelial cells within the expanding tissue mass, in addition to autocrine actions between adjacent follicular cells. Goodman & Rone (1987) showed that rat thyroid cells in culture (both primary and FRTL cells) produced endotheliotropic chemoattractant activity. They did not identify the factor, but their data convincingly supported the hypothesis of Wollman et al. (1978) that the growth of perifollicular blood vessels was stimulated by angiogenic factors secreted by follicular cells. Similarly, Greil et al. (1989) showed the release of an endothelial cell growth factor from pig thyroid cells. Recent data have shown that VEGF, an angiogenic growth factor, is synthesised by thyroid follicular cells and is increased in goitres in the rat, and in human thyroid cells in vitro, following TSH stimulation (Sato et al. 1995). Although this growth factor increases the permeability of the endothelium and is mitogenic for endothelial cells in the thyroid as elsewhere (Sato et al. 1997), its effects on human thyroid follicular cell growth and function are not known. KDR and Flt, the receptors for VEGF, were until recently thought to be exclusive to cells of the vasculature. However, they have now been located in diverse tissues, including trophoblast cells (Ahmed et al. 1997) and smooth muscle cells from the uterus (Brown et al. 1997). Significantly, recent data obtained using FRTL-5 cells indicate that this clonal thyroid cell line produces not only VEGF but also the Flt-1 receptor, as demonstrated by reverse transcriptase PCR (Wang et al. 1998). Addition of VEGF to FRTL-5 cultures blocks TSH-stimulated growth and function, presumably through the Flt-1 receptor because the KDR receptor was not found (Wang et al. 1998). PlGF has also been identified as a product of rat thyroid follicular cells. Within the thyroid gland of goitrogen-treated rats, PlGF binds only to the Flt-1 receptor and this is increased by chronic activation of the thyroid by TSH (Vigilietto et al. 1997). Presumably therefore PlGF may exert the same effect as VEGF, at least in FRTL-5 cells. These novel findings may implicate VEGF in the growth attenuation found during goitrogenesis in the rat. Induction of VEGF synthesis by TSH, while initially promoting angiogenesis, may also act as a brake on the mitogenic stimulation of the follicular cells by TSH, in an analogous manner to TGFβ. The study of Vigilietto et al. (1997) did not, however, reveal consistent staining of VEGF receptors on the follicular cells of goitrogen-treated rats, although endothelial cell VEGF receptors were increased. Whether human thyroid follicular cells possess VEGF receptors and can respond to VEGF remains to be determined.

No data are as yet available on the goitrogenic role of the newest member of the angiogenic growth factor family, angiopoietin (ANG) and its receptor Tie-1, although it is reasonable to predict its involvement in goitrogenesis. Whether its actions are restricted to the endothelium will also need to be determined. Other paracrine factors may also be involved in controlling the angiogenic response within the hyperplastic thyroid gland. One of the products of plasminogen cleavage by plasmin, and hence angiostatin (Stathakis et al. 1997). Since the synthesis of both uPA and tPA are increased by TSH (Mak et al. 1984), angiotatin levels may also be raised. Conceivably, this factor may act to inhibit the angiogenic response and limit goitre size. As in the control of thyroid follicular cell proliferation itself, angiogenesis within the hyperplastic thyroid gland is dependent upon specific factors being available in the correct location at a given time. In order for angiotatin to be effective therefore, plasminogen and glutathione, or another reducing agent, must both be available. Figure 4 illustrates our current knowledge of the secretion and effects of VEGF, PlGF and ANG in the thyroid. The effects of known modulators of thyroid function, i.e. TSH and iodide, are shown at the bottom of the figure. As noted above, TSH stimulates follicular cell secretion of paracrine factors. Thrombospondin, which is secreted by thyroid follicular cells (Prabakaran et al. 1993), is generally thought to have a negative effect on the angiogenic process. In the rat model of goitre, thrombospondin is reduced to negligible levels within 2 weeks of goitrogen treatment (Patel et al. 1996) consistent with inhibition by TSH of its secretion, shown in vitro (Bellon et al. 1994). The effects of T3 and T4 on the production of the angiogenic factors and their receptors are not known.

**Endothelins, atrial natriuretic peptide (ANP) and their receptors**

Endothelin-1 (ET-1) is secreted by rat and pig thyroid follicular cells (Colin et al. 1992) and human (Tseng et al. 1999).
1993) and FRTL-5 cells (Vainio et al. 1996). ET-1 is a 21-amino acid polypeptide formed by proteolytic cleavage from a large precursor molecule by the action of endothelin converting enzyme (ECE), a membrane-bound neutral metalloprotease. ET-1 is widely distributed throughout the body and is synthesised primarily, but not exclusively, by the vascular endothelium. ET-2 and ET-3 are synthesised by vascular smooth muscle cells. Receptors for ETs are classified as ETA, which, when expressed on smooth muscle cells, are responsible for vasoconstriction, and ETB, which are found in the endothelial cells and are responsible for the release of nitric oxide, prostacyclin and ANP. The role of ET-1 in the thyroid itself has been examined using ET-1 knock-out mice. Homozygous mice were found to have smaller thyroid glands than normal, which showed no midline fusion (Kurihara et al. 1995). ET-2 and -3 have been found to bind to porcine thyroid cells, implying redundancy in the ligands capable of interacting with the receptors. ET-1 inhibits iodide uptake in pig thyroid cells but not apparently by inhibiting cAMP formation (Tsuchima et al. 1994). In contrast, in FRTL-5 cells, ET-1 inhibits TSH-stimulated DNA synthesis by interfering with cAMP production (Miyakawa et al. 1992).

In human thyroid cells in culture, Eguchi et al. (1993) found that nanomolar concentrations induced growth, and Tseng et al. (1993) found that TGFβ1 stimulated ET secretion and increased ET receptor number. This is in contrast with the effects of TSH which had no effect on either ET synthesis or receptor number. In pig thyroid cells, treatment with TSH reduced the mRNA for ET-1 whereas supraphysiological levels of iodide (0·1 mM) increased both mRNA and protein levels (Isozaki et al. 1993). In studies of goitre induction and involution in rats, Colin et al. (1994, 1995) showed that mature ET-1 within the thyroid gland was increased 5-fold during the hyperplastic stage, and remained high in the subsequent rapid phase of involution. Both ETA and ETB receptors were detectable, as was ECE. These workers also examined the nitric oxide synthase genes (NOS I, II and II) and found that NOS I and III were increased in the expansive stage of goitrogenesis, whereas during involution their levels rapidly returned to normal. Concurrent treatment with both a goitrogen and a NOS inhibitor reduced the vascular expansion by more than 30%. Because of the highly vascularised nature of thyroid tissue, it is not surprising that factors capable of controlling vascular contractility are available locally. That these factors have effects on follicular cells is more surprising, but illustrates again the diversity of paracrine communications between the component cell types within the thyroid gland, and the delicate balance that exists between expression of growth factors, their receptors and other mediators such as binding proteins and proteases.

ANP has been identified in human and pig thyroid follicular cells and is secreted basolaterally by pig thyroid cells in culture (Sellitti & Hughes 1990). High-affinity ANP receptors are found on human thyroid cells which (at least at high ANP concentrations) are coupled to the release of cGMP (Tseng et al. 1990). ANP treatment inhibits thyroglobulin release from human thyroid cell cultures, secondary to a reduction in intracellular cAMP (Sellitti et al. 1989). In FRTL-5 cells, cGMP accumulation is functionally coupled to the ANP receptor. TSH down-regulates ANP receptor number (Tseng et al. 1991) in human thyroid cells but there are no data on the regulation of ANP expression. In endothelial cells, both ANP and ET-1 regulate VEGF synthesis, ANP being inhibitory whereas ET-1 is stimulatory (Pedram et al. 1997). In this way, these vasoactive peptides can effect a close regulation of endothelial cell proliferation and invasion. Since VEGF, ANP and ET-1 are all products of thyroid follicular cells, derangements in their mutual regulation and interaction are likely to be important in determining the balance of cell growth promotion and limitation in the angiogenesis associated with goitrous expansion of the thyroid gland.

Other growth factors and their receptors

HGF is secreted from normal human thyroid cells and is a potent mitogen for both these cells (Eccles et al. 1996) and those from dog thyroids (Dremier et al. 1994). In the latter study, HGF, like FGF, was only found to have growth-promoting and dedifferentiating effects if added shortly after the cells were plated, i.e. at the rapid growth stage. This would indicate a cell density-dependence of HGF receptor expression. An early study of mRNA encoding the HGF receptor, known as met, in normal human thyroid tissue demonstrated abundant mRNA expression but an absence of protein (Di Renzo et al. 1991). However, in thyroid carcinoma, c-met protein levels were elevated over 100-fold. Expression of met and HGF has recently been examined in non-neoplastic nodules using immunostaining (Trovato et al. 1998). In 40% of the samples analysed, 1–3% of the cells were positive for HGF and its receptor. By comparison with normal (0%) and non-papillary carcinoma (0%), this is elevated but does not approach the positivity found in papillary carcinoma (15–46% cells positive for HGF and c-met in 90% of the samples). The potency of HGF as a mitogen is significantly greater than that of other growth factors, therefore this factor clearly has the potential to influence thyroid growth and function. In considering the actions of factors such as HGF, FGF and VEGF in the goitrogenic process, the possible existence of binding proteins that may determine or modify the nature of the cellular responses should be considered. These may be truncated forms of the receptors as found for FGF (Hanneken et al. 1994) or, like the IGFBPs, they may have a completely different structure. In any event, their contribution to the regulation of the biological activity of the corresponding growth factor, which is at present unknown, may be critical.
With the exception of IGFs, all of the autocrine/paracrine factors in the thyroid have dedifferentiating effects on thyroid follicular cells and, except for TGFβ and possibly VEGF, are mitogenic. Also, consistent with the dedifferentiating actions of the mitogenic growth factors described in this review, it can be concluded that, in contrast with iodine-deficiency goitre, a low intracellular iodide content in MNTG is a consequence, rather than a cause, of thyroid hyperplasia (Aeschimann et al. 1994).

Conclusions

The in vitro approach, combined with data from rat models of goitrogenesis, has already allowed us to construct an accurate map of the growth factors involved in goitrogenesis due to iodide deficiency, while also yielding valuable data on some of the likely mechanisms underlying MNTG. The overall pattern that is emerging is of an imbalance in the interactions between the various growth factor axes, which alters the replicative and differentiation stimuli in the direction of cell replication. Although TSH is not elevated in MNTG, its interactions with autocrine and paracrine factors and their receptors appear to be critical. Expansion of the thyroidal vascular bed through angiogenesis is closely co-ordinated with follicular cell expansion and folliculoneogenesis, and while the integrated paracrine actions of FGF-2, VEGF, PIGF and endothelin probably play central roles, additional as yet elusive factors are probably involved. The combination of in vitro and in vivo approaches, each designed to address specific questions, will undoubtedly continue to prove invaluable in dissecting further the complex interactions that exist between these growth factors, their binding proteins and receptors in the development and progression of thyroid hyperplasia.

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