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Aberrations of growth factor control in metastatic follicular thyroid cancer *in vitro*

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The aggressiveness of follicular thyroid cancer (FTC) varies widely, and metastasis is the primary cause of death. Uncontrolled proliferation of cancer cells may be associated with loss of growth factor control. We investigated the effects of stimulating (epidermal growth factor [EGF]; thyreotropin [TSH] in low concentrations) and inhibiting growth factors (transforming growth factor beta 1 [TGF beta 1]; TSH in high concentrations) on invasion and growth of FTC cell lines from the thyroid tumor (FTC133) and from the lymph node (FTC236) and lung (FTC238) metastases of the same patient. Invasion—penetration through an 8 μm pore membrane, covered by Matrigel (basement membrane)—and growth were measured using the MTT-method. EGF (10 ng/ml) and TSH in low concentrations (1 mU/ml) stimulated invasion and growth of all FTC cell lines, but the amplitude of stimulation differed significantly. The parental cell line FTC133 was considerably more responsive to growth factor stimulation than the metastatic clones. Invasion of FTC133 was enhanced by 42% (EGF; $p < 0.02$) and 21% (TSH; $p < 0.01$), invasion of FTC236 by 8% (EGF; $p < 0.02$) and 8% (TSH; $p < 0.01$), and invasion of FTC238 by 9% (EGF; $p < 0.02$) and 8% (TSH; $p < 0.01$). Conversely, invasion and growth of FTC133 were significantly more inhibited by TGF beta 1 (10 ng/ml) and supraphysiologic concentrations of TSH (100 mU/ml) than the cell lines from the lymph node and lung metastases. At day 7, invasion of FTC133 was inhibited by 32% (TGF beta 1; $p < 0.02$) and 21% (TSH; $p < 0.01$), invasion of FTC236 by 18% (TGF beta 1; $p < 0.02$) and 11% (TSH; $p < 0.01$), and invasion of FTC238 by 16% (TGF beta 1; $p < 0.02$) and 12% (TSH; $p < 0.01$). Moreover, we analyzed growth factor independence in minimally supplemented or unsupplemented medium. Growth, but no invasion was evident when cells were cultured completely unsupplemented over 7 days. These results suggest that metastatic FTCs may have developed by escaping from the normal control of TSH and other growth factors.

Keywords: growth factors, invasion, metastatic thyroid cancer

Introduction

Malignant tumor cells are characterized by high mitogenic activity, disorganized growth, and the ability to invade adjacent tissues and form metastatic foci at distant locations. Specific growth factors and cytokines, which regulate the homeostasis of proliferation and differentiation of normal cells, also play an important role in the development and progression of malignancies. Loss of one or more of these control mechanisms is an essential characteristic of tumor cells. Uncontrolled proliferation of cancer cells may occur when they become resistant to growth inhibitors, such as transforming growth factor beta (TGF beta) or interleukin [1–3]. Some reports suggest, that the

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phenomenon of a 'multi-growth-factor-resistant phenotype' is crucial for the development of cells capable of metastasis [4, 5].

Follicular carcinomas comprise about 20% of all thyroid malignancies. They vary widely in aggressiveness. Some tumors may disseminate very early, while others with similar histology may grow to a large size and fail to metastasize [6]. Chronic thyreotropin (TSH) stimulation was associated with enhanced tumor proliferation and aggressiveness [7, 8]. Proto-oncogenes and oncogenes play a major role in invasion and proliferation of thyroid follicular cells. Unregulated cell growth was correlated with a high frequency of mutational activation of *ras* and *gsp* oncogenes in differentiated thyroid cancer [9–11]. Also, the *TRK* and *RET* proto-oncogenes, which code for membrane receptor tyrosine kinases have been found to be frequently activated in papillary thyroid cancers [12, 13]. In other human cancers, the serine/threonine kinase genes v-*mos* and v-*raf* as well as other tyrosine kinase genes v-*fms*, v-*fes* and v-*src* have been associated with metastatic phenotypes [14].

The purpose of this investigation was to determine whether the potential of follicular thyroid cancer (FTC) to metastasize is due to an altered growth factor sensitivity. Three follicular thyroid carcinoma (FTC) cell lines established from the primary tumor (FTC133) and a lymph node (FTC236) and lung (FTC238) metastases of one patient were analyzed. These cell lines provide the unique opportunity to investigate the effects of different growth factors on primary and metastatic thyroid cancer cells.

Materials and methods

Tumor cells and cell culture conditions

All three FTC cell lines were obtained from a single patient. FTC133 were established from the primary thyroid tumor in June 1987. In an ultimately fatal course of disease, the patient rapidly developed lymph node (FTC236) and lung (FTC238) metastases [15]. Under standard tissue culture incubating conditions, cells were maintained in Dulbecco's modified Eagle's media (DMEM-H21), 4.5 g/l glucose (Irvine Scientific, Santa Ana, CA, USA), supplemented with 10% fetal calf serum (Hyclone, Logan, UT, USA). In order to perform experiments under serum-free conditions and thus exclude the undesirable effects of fetal calf serum, cells were maintained for 72 h prior to any experiment in Dulbecco's MEM and modified HAM's F12 medium 1:1 (UCSF Cell Culture Facility) supplemented with a serum-free, chemically defined five hormone mixture (H5), using slight modifications of the Ambesi–Impiombato method [16].

H5 contains bovine insulin (10 μg/ml), human transferrin (5 μg/ml), somatostatin (10 ng/ml), glycyl-L-histidyl-L-lysine acetate (2 ng/ml; all from Sigma, St Louis, MO, USA) and hydrocortisone (0.36 ng/ml; Calbiochem, San Diego, CA, USA). Bovine thyrotropin, epidermal growth factor and TGF beta 1 were purchased from Sigma.

Proliferation assays

Experiments were performed in 96-well plates in triplicate. Each well contained 10000 cells in 100 μl H5. After 24 h, cells were incubated with TSH (0.1, 1, 10 or 100 mU/ml), epidermal growth factor (EGF) or TGF beta 1 (0.1, 1, 10 or 100 ng/ml). Also, we tested proliferation of all FTC cell lines in growth factor-deprived media. In these experiments, tumor cells were plated in 100 μl of media H5, H4 (H5 – somatostatin); H3 (H3 – glycyl-L-histidyl-L-acetate) H2 (H3 – transferrin), H1 (H2-hydrocortisone) and H0 (H5 without any of the hormones). The medium was changed every other day and growth was determined at days 0, 1, 3, 5 and 7 using the MTT-method [17, 18]. After removing medium from the wells, 100 μl dimethylthiazol-diphenyltetrazolium bromide (MTT, Sigma; reconstituted in PBS: 1 mg/ml) was added to each well and the cells were incubated for 4 h.

MTT is taken up by the cells and metabolized to formazan. Formazan production is proportional to the number of mitrochondria and thus reflects the number of cells. After incubation, 100 μl of Hansen's solution [19] was added to each well and plates were incubated for 1 h. Hansen's solution contains isopropanol which lyses the cells, stops any further metabolism and solubilizes formazan. Following incubation, the lysate was sonicated for 2 min and carefully triturated until completely dissolved. Cell number was then determined by optical density (OD) of 570 over 630 nm using an automatic Elisa microplate reader.

Invasion assays

Invasion was evaluated by documenting the ability of the cells to penetrate an 8 μm pore size Nucleopore polycarbonate membrane insert of a 6.5-mm Transwell (Costar, Cambridge, MA, USA), coated with an extract of basement membrane components (1:10 preparation of Matrigel, Collaborative

Research, Bedford, MA, USA). Cells (200 000) were seeded in 100 μl H5 with and without TSH, EGF and TGF beta 1. The lower chambers contained 600 μl H5-media without any growth factor. As controls, 200 000 cells of each cell line were seeded with and without the growth factors in a 6.5-mm Transwell chamber without membrane insert. Again, we tested invasion in growth factor-deprived media after plating the tumor cells in the respective media (H5–H0). After 24, 48 and 72 h, the cell numbers in both chambers were separately assessed by MTT-assay. The MTT-assay (3 mg/ml) was performed as described above, adding 40 μl MTT to the upper and 90 μl to the lower chamber. However, in this case the medium was not removed and after 4 h of incubation formazan was collected separately from both chambers using cotton swabs. Formazan was then dissolved in separate glass tubes filled with 500 μl of solubilization solution, 150 μl of which were then transferred to 96-well plates (triplicates) and read with an Elisa-reader at OD 570 to 630 nm. Invasion of cells was calculated as:

$$\% \text{ invasion} = (OD_{\text{lower well}}/[OD_{\text{upper well}} + OD_{\text{lower well}}]) \times 100$$

Statistics

All experiments were performed in triplicate. For descriptive statistics we used mean ± SD. For evaluations of differences we performed Wilcoxon's rank test and one-way analysis of variance.

Results

Stimulation of invasion

Unstimulated, the lung metastatic subclone FTC238 was most invasive. After 72 h, 24.9% of FTC238 tumor cells had penetrated the reconstituted basement membrane-coated filters. This is 14% more than the lymph node metastasis FTC236 (21.8% penetrated cells) and 42% more than the parental cell line FTC133 (17.5%; $p < 0.02$—see Figure 1).

EGF enhanced invasion in all FTC cell lines. Invasion of FTC133 was increased by 42% over basal (from 17.5 to 24.9%, $p < 0.02$), invasion of FTC236 was increased by 8% (from 21.8 to 23.5%, $p < 0.02$) and the invasion of FTC238 by 9% (from 24.9 to 27.2%; $p < 0.02$; Figure 1).

The response to TSH was biphasic: TSH stimulated invasion of FTC at low concentrations (1 mU/ml) and inhibited it at high concentrations

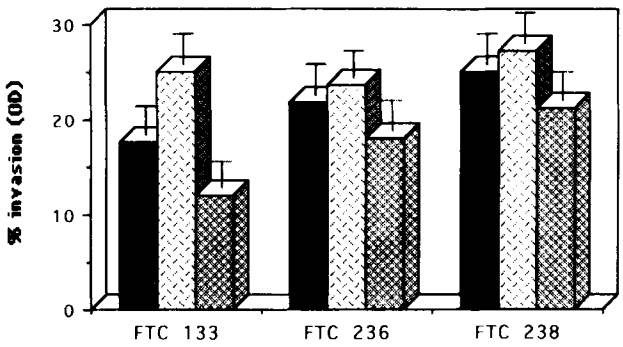

Figure 1. Effects of EGF (□; 10 ng/ml) or TGF beta 1 (▨; 10 ng/ml) on invasion of FTC133, FTC236 and FTC238 compared with basal invasion in H5 (■). Formazan production was measured as optical density (OD). Results are expressed as percentage of OD of cells which invaded through the polycarbonate membrane covered with reconstituted basement membrane (Matrigel). Individual data points represent the mean ± S.E.M. of triplicates; $p < 0.02$ (for each cell line as well as for EGF and TGF beta 1) compared with unstimulated controls.

(100 mU/ml). Again, TSH revealed the highest stimulating effect in the parental cell line FTC133, enhancing invasion by 21% of basal, whereas FTC236 and FTC238 were only stimulated by 8% each ($p < 0.01$; Figure 2).

Stimulation of growth

EGF also stimulated cell growth of all FTC cell lines and again revealed significantly different

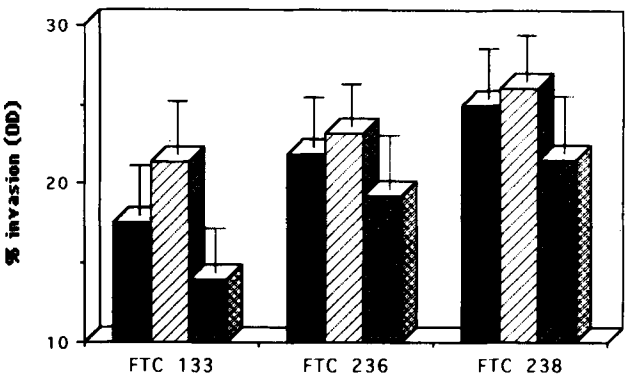

Figure 2. Biphasic effects of TSH (□; 1 or ■; 100 mU/ml) on invasion of FTC133, FTC236 and FTC238 compared with basal invasion in H5 (■). Formazan production was measured as optical density (OD). Results are expressed as percentage of OD of cells which invaded through the polycarbonate membrane covered with reconstituted basement membrane (Matrigel). Individual data points represent the mean ± S.E.M. of triplicates; $p < 0.01$ (for each cell line) compared with unstimulated controls.

effects in the parental and metastatic clones. Baseline proliferation of all three FTC cell lines is shown in Figure 3. Of the tested concentrations (0.1, 1, 10 and 100 ng/ml), EGF had its optimal stimulatory effect at 10 ng/ml, with no further increase at higher concentrations. Growth of FTC133 was enhanced by 64% over basal during the exponential growth phase (at 3 days) and by 25% at confluency (at 10 days; $p < 0.01$; Figure 4). The stimulatory effect on growth of FTC236 was 26% (exponential phase) and 14% (confluency; $p < 0.01$) and 24% and 8% in FTC238 ($p < 0.01$), respectively (Figure 4).

At 1 mU/ml, TSH stimulated growth of FTC133 by 20% over basal (exponential phase; $p < 0.01$), whereas stimulation was only 9% in FTC236 and 10% in FTC238 ($p < 0.01$; Figure 4).

Inhibition of invasion

Treatment with TGF beta 1 caused inhibition of invasion of all follicular cell lines (Figure 1). TGF beta 1 had again its greatest effect in the parental cell line FTC133, inhibiting invasion by 32% (from 17.5 to 11.9%; $p < 0.02$) and was less effective in the metastatic subclones FTC236 (18%; $p < 0.02$) and FTC238 (16%; $p < 0.02$; Figure 1).

At a supraphysiologic concentration of 100 mU/ml, TSH inhibited invasion of FTC133 by 21% ($p < 0.01$; Figure 2). The inhibitive effects were smaller in both metastases (11% in FTC236 and 12% in FTC238; $p < 0.01$; Figure 2).

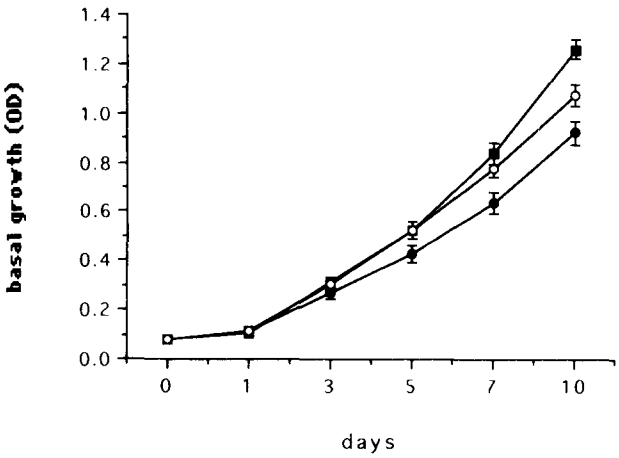

Figure 3. Baseline proliferation of FTC cell lines, FTC133 (■), FTC236 (●) and FTC238 (○), in H5-media. Formazan production was measured as optical density (OD). Individual data points represent the mean ± S.E.M. of experiments performed in triplicate.

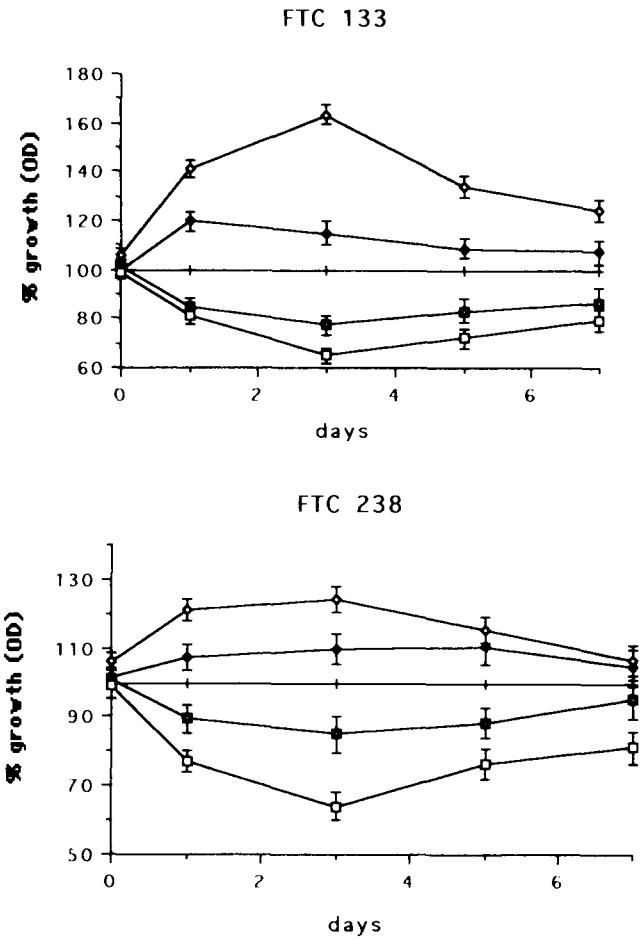

Figure 4. Dose-response studies of the effects of EGF (◇; 10 ng/ml), TGF beta 1 (□; 10 ng/ml) and TSH (◆; 1 or ◘; 100 mU/ml) on growth of FTC133 and FTC238 cells in culture. Formazan production (MTT) was measured as optical density (OD) and results are expressed as percentage of basal cell growth in H5-medium. Individual data points represent the mean ± S.E.M. of triplicate. For both cell lines p-values were: $p < 0.02$ (EGF); $p < 0.02$ (TGF beta 1) and $p < 0.01$ (TSH) compared with unstimulated controls.

Inhibition of growth

All FTC cell lines were growth inhibited by TGF beta 1 treatment with a maximal effect at 10 ng/ml. Cell growth decreased by 35% (exponential phase) and 31% (confluency) in FTC133 (Figure 4). During exponential growth, TGF beta 1 inhibited growth of both metastatic subclones also by more than a third, whereas at confluency, growth was inhibited by 15% in the lymph node metastasis FTC236, and by 17% in the lung metastasis FTC238 ($p < 0.01$; Figure 4).

At 100 mU/ml, TSH had also an inhibiting effect on tumor cell growth. It inhibited growth of

FTC133 by 23% (exponential phase; $p < 0.01$). Again, the amplitude of inhibition was markedly smaller in both metastases (16% in FTC236, 15% in FTC238; $p < 0.01$; Figure 4).

Effects of minimally supplemented medium on invasion

In another experiment, we tested the effect of a gradual withdrawal of the five components of the serum-free defined medium on invasion of all FTC cell lines. Figure 5 shows, that the absence of somatostatin did not influence invasion. Even in 'H2-medium' (insulin, hydrocortisone), 52% of basal invasiveness still occurred. However, no invasion took place after withdrawal of all five factors. The amplitude of decrease of invasion was comparable in all three FTC cell lines.

Effects of minimally supplemented medium on growth

All FTC cells grew rapidly in the serum-free defined H5 medium. FTC133 had an average population doubling time of 27 h, FTC236 of 34 h and FTC238 of 31 h. When tumor cells were cultured without any supplement (H0), growth of all FTC cell lines was considerably decreased. However, compared with growth in H5, FTC133 still had a growth rate of 21% after 7 days in completely unsupplemented culture media ($p < 0.03$; Figure 6). While the lack of somatostatin and glycyl-L-histidyl-L-lysine acetate did not appreciably affect growth of all FTC tumor cells, a withdrawal of transferrin, hydrocortisone and insulin reduced the growth rate by nearly 10%, each. In general, results in FTC236 and FTC238 were comparable. However, the overall decrease of cell growth was smaller than in the parental cell line (Figure 6).

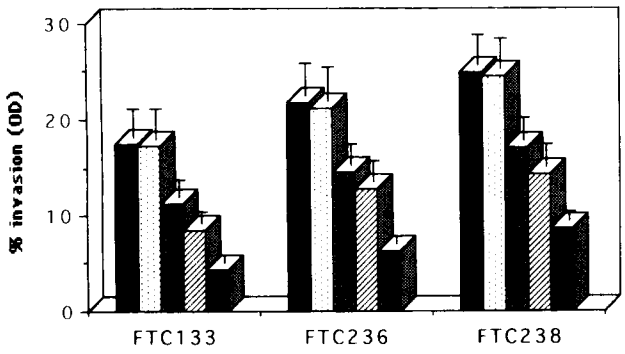

Figure 5. Effects of different media conditions on invasion of FTC133, FTC236 and FTC238 compared with basal invasion in the five-hormone medium H5 (■). Media were: H4 (▣, H5 – somatostatin); H3 (■, H4 – glycyl-L-histidyl-L-acetate); H2 (▨, H3-transferrin) and H1 (■, insulin only). Formazan production was measured as optical density (OD). Results are expressed as percentage of OD of cells which invaded through the polycarbonate membrane covered with reconstituted basement membrane (Matrigel). Individual data points represent the mean ± S.E.M. of triplicate, $p < 0.02$ for all cell lines compared with baseline proliferation.

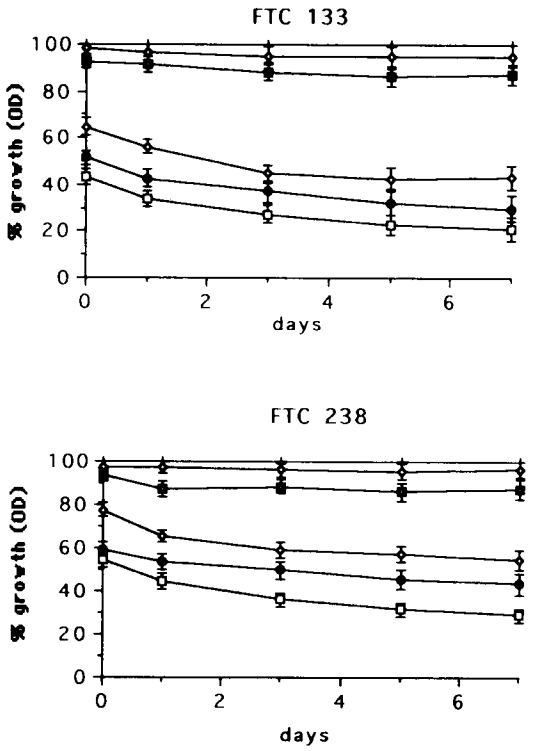

Figure 6. Dose-response studies of the effects of different media conditions on growth of FTC133 and FTC238 cells in culture. Media were: the five-hormone medium H5 (+); H4 (◇, H5 – somatostatin; H3 (◘, H4 – glycyl-L-histidyl-L-acetate); H2 (◇, H3 – transferrin), H1 (●, insulin only) and H0 (□, hormone-free medium). Formazan production (MTT) was measured as optical density (OD) and results are expressed as percentage of basal cell growth in H5 medium. Individual data points represent the mean ± S.E.M. of triplicates; $p < 0.03$ for both cell lines compared with baseline proliferation.

Discussion

Differentiated thyroid cancers are generally characterized by slow growth, an indolent clinical course and good prognosis. However, the aggressiveness of thyroid cancers is unpredictable and varies widely, and some patients develop local

invasion or metastatic disease [6, 20]. Metastatic dissemination is the primary cause of death from well-differentiated thyroid cancer [21, 22].

It is of interest, that FTCs infrequently (10%) involve the lymph nodes and are usually unifocal within the thyroid, whereas papillary thyroid carcinomas are multifocal and frequently involve the regional cervical lymph nodes. Follicular cancers frequently metastasize to distant sites via the blood stream [6, 23, 24]. The histopathology of follicular tumors is critical and cytology findings generally cannot discriminate between benign and malignant follicular neoplasms. The prognosis of patients with FTC is improved by early detection. Analyses of growth stimulating and inhibiting factors should provide more insight into the mechanisms of neoplastic transformation, cancer invasion and metastases. New information could improve diagnostic accuracy and predict the invasive and metastatic potential of the individual tumor.

The three different FTC cell lines from one patient offer a unique opportunity to study different effects of specific growth factors on the primary and the metastatic clones from the same original tumor. A frequent criticism of studies investigating growth mechanisms in metastatic competent cells concerns the establishment of different clones from the same parental cell line. Those techniques cannot exclude the possibility that results may be in part due to some clonal artifacts. For example, Kath *et al.* [25] subsequently established several cell lines with different metastatic properties *in vivo*. Using one parental human melanoma cell line, they selected those tumor cells that were most invasive *in vitro*. Interestingly, their results were in part consistent with our findings. They found a strong correlation between *in vitro* invasiveness, *in vivo* metastatic capacities and a lack of dependence on serum for growth of these cells in culture. In this regard, we cannot, theoretically, exclude the possibility that in our tumor model the lung metastasis is descended from the lymph node metastasis. However, regarding the natural history of metastatic FTC, it would be more likely that the lung metastasis developed via blood stream. Therefore, we and others consider the two cell lines independent metastases [26].

In this study we investigated whether the potential of follicular thyroid cancer to metastasize is due to an altered growth factor sensitivity. Our results document that the metastatic FTC cell lines are more invasive than the primary tumor. The basal invasive capacity of the lymph node metastatic cell line FTC236 was 25% and that of the lung metastatic cell line FTC238 was 42%, higher than that of the primary tumor FTC133. These findings are consistent with other reports which demonstrated increased invasiveness in metastatic subclones, such as in T-cell lymphoma [27], melanoma [28] and cancers of the colon [29] and breast [30]. Our results show, that stimulating and inhibiting effects of the various growth factors tested appeared to at least partly depend on cell culture kinetics. When cell cultures became confluent, the amplitudes of stimulation or inhibition of invasion or growth by TSH, EGF and TGF beta 1 were significantly smaller in both metastatic cell lines. Unstimulated FTC238 cells had the highest invasive potential, but it was only minimally affected by the stimulation of EGF (9% of basal) and TSH (8% of basal). In contrast, the parental cell line FTC133 had the lowest basal invasiveness, but was considerably stimulated by EGF (42%) and TSH (21%). TGF beta 1 inhibited invasion of FTC133 by 32% of basal, but was only half as effective (16%) in inhibition of FTC238 invasion.

These findings highlight two aberrations of growth regulation which may favor progression of malignant disease and acquisition of metastatic competence: (i) resistence to growth factor inhibitors and (ii) growth autonomy of metastatic FTC cells. A growing literature has established the close association between malignant tumor progression and growth regulatory aberrations in cancer cells. Most of these studies have focused on the phenomenon that, in contrast to their parental cell lines, many advanced and more aggressive tumors or metastases lose the sensitivity to growth inhibitors, such as transforming growth factor beta 1. Some metastatic cancer cell lines are even stimulated rather than inhibited by TGF beta 1 [1, 2, 31, 32]. This 'inhibitor-to-stimulator switch' phenomenon has lead to the use of a variety of illustrative terms, such as 'clonal dominance' of primary tumors by metastatically competent tumor cell variants [3], 'progressive emancipation' from external negative growth control [33], or 'multicytokine-resistant phenotype' [34]. Growth and invasion of FTC236 and FTC238 were less inhibited by TGF beta 1 than was the primary tumor. Several mechanisms have been speculated for the altered response to TGF beta, including alterations in the ratio of the different TGF beta subtypes [35], altered or reduced TGF beta receptors [36] or altered growth factor responsiveness by oncogene-induced changes, e.g. enhanced levels of the c-*myc* gene [37]. Nicolson has proposed [38] that the *nm23* gene could code for regulator pro-

teins which act at the level of gene expression or signal transduction. This tumor suppressor gene was associated with low metastatic tumor potential [39].

Rodeck *et al.* [40] and Mancianti *et al.* [41] postulated the loss of sensitivity to growth inhibitory factors as another contributor of growth factor independence in metastatic tumor cells. They found a stage dependent subsequent loss of exogenous growth factor requirements in human melanoma cell lines. The majority of metastatic cells grew in peptide growth factor-free media, whereas melanoma cells derived from the preceding tumor stage often required at least one exogenous growth factor. Chadwick and Lagarde [42] reported a coincidental acquisition of growth autonomy and metastatic potential during the malignant transformation of factor-dependent CCL39 lung fibroblasts. In contrast, others showed metastatic cell lines which remained serum-dependent, suggesting that growth autonomy is not an obligatory component of the metastatic phenotype [5, 43].

We cultured the FTC cell lines three days prior to the experiments in the serum-free, chemically defined media H5, which was modified from the H6 media used to culture the rat thyroid cell line FRTL-5 [16]. Growth and invasion of the FTC were similar in H5 or in media containing FCS. The doubling time of FTC133 cells was 27 h in H5, compared with 24 h in 10% FCS. Similar differences were obtained in FTC238 (doubling times 31 and 29 h, respectively). In contrast, PTC-UC3, a papillary thyroid cancer cell line recently established in our laboratory, is quiescent in H5 and grows in 10% FCS (data not shown). By deleting the individual components of H5, we found that all FTC cell lines grew without any supplement, indicating a complete growth factor independence for tumor cell growth. However, they all failed to invade after complete withdrawal of all growth factors.

Thus, the enhanced invasive capacities of the metastatic FTC cell lines are unlikely to be only due to a loss of sensitivity to growth inhibitors. Classically, TSH is considered the most important trophic factor for the thyroid gland [6, 7, 44, 45]. However, the exact role of TSH is not clear and results are controversial, because the effect of TSH on growth of follicular thyroid cells differs in different species. TSH effects vary from stimulation [46], to a dose-dependent biphasic response [47], to inhibition [48]. Normal thyroid cells grow minimally in the absence of serum or growth factors, such as TSH or insulin [49, 50].

Interestingly, the FTC cell lines in our tumor model have been demonstrated to lack TSH receptors and not to depend on TSH for growth. However, they contain thyroglobulin, have intact thyroid functions and response to TSH [51, 52]. These cells accumulate cyclic AMP when stimulated by TSH, indicating an intact TSH–AC axis [51]. Interestingly, when Derwahl *et al.* [52] stably transfected FTC133 with the human TSH receptor cDNA, the transfected cells grew more slowly and bound more ^{125}I-TSH, suggesting a differentiating effect of TSH in these cells. We found that TSH has a biphasic effect in follicular and papillary thyroid carcinomas, stimulating growth and invasion at physiologic and inhibiting them at supraphysiologic concentrations. Again, the amplitudes of both, stimulating and inhibiting effects were smaller in the FTC metastases.

Epidermal growth factor also stimulates the thyroid gland. EGF and TSH have a complex relationship in thyroid growth and differentiation. TSH stimulates both thyroid cell growth and differentiation. EGF, in contrast, stimulates growth but inhibits differentiation [53]. Membranes from thyroid neoplasms bind more EGF compared to histologically normal thyroid tissue removed from the same patients [54]. EGF and its receptor have been demonstrated to be involved in the metastatic cascade, favoring proliferation [51], proteolysis [55], migration and invasion in nonthyroid cancer cells [55, 56]. In our studies, EGF caused an increase of growth and invasion in all FTCs and again, stimulation by EGF was lower in FTC236 and FTC238. It is possible that our results may in part be due to some clonal artifact of one or more of these cell lines, and this aberration in growth factor control in the metastatic cell lines may not be generalized to all tumor metastases. There are no other thyroid cell lines available that offer this unique situation of having the primary thyroid cancer and the two metastatic clones from the same patient for comparison.

In conclusion, the altered sensitivity to and decreased requirement for exogenous growth factors and the coincidentally enhanced basal invasive capacities indicate that metastatic follicular thyroid cancer may have developed by escaping from the normal control of TSH and other growth factors.

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