Annals of Surgical Oncology **15**(12):3601–3608
DOI: 10.1245/s10434-008-0170-1

Expression and Secretion of Endostatin in Thyroid Cancer

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Background: In thyroid cancer (TC) endostatin was identified as a powerful negative regulator of tumor angiogenesis in vitro. It is currently being evaluated in phase I trials for antiangiogenic therapy in various solid tumors. The aim of this study was to evaluate endostatin expression in archival TC specimens and its secretion following stimulation with thyrotropin (TSH) and epidermal growth factor (EGF) in TC cell lines.

Methods: Tissue microarrays of 44 differentiated and 7 anaplastic TC and their metastasis were immunostained for endostatin protein expression and compared with corresponding non-neoplastic thyroid tissue (NT). In vitro, six differentiated (FTC133, FTC236, HTC, HTC-TSHr, XTC, and TPC1) and three anaplastic (C643, Hth74, Kat4.0) TC cell lines were evaluated for basal as well as TSH (1–100 mU/ml) and EGF stimulated (1–100 ng/ml) endostatin.

Results: Endostatin was detected in all TC and more than half of the NT. Endostatin expression was more frequent and intense in differentiated as compared to anaplastic TC. In vitro, basal endostatin secretion varied between 33 ± 5 pg/ml (FTC236) and 549 ± 65 pg/ml (TPC1) and was doubled in FTC, when the "primary" (FTC133) was compared with the metastasis (FTC236). Some cell lines showed TSH-induced (e.g., 60% in XTC) or EGF-induced (e.g., 120% in TPC1) upregulation of endostatin secretion, while others did not, despite documented receptor expression.

Conclusion: This study demonstrates endostatin expression in TC, metastasis and—less frequently and intensely—in NT, suggesting a possible association to tumor progression. In vitro, endostatin secretion of some cell lines is regulated by TSH and EGF, however the individual differences deserve further functional studies. These results support rather tumor-specific than histotype-specific expression and regulation of endostatin in TC.

Tumor growth and progression basically require a sufficient blood supply and blood vessel formation connecting the primary tumor with the pre-existent host vasculature. This process, known as angiogenesis, is introduced and maintained by the tumor itself by means of secretion of proangiogenic factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF-2), and angiopoietins.[1,2] It is balanced by a large number of antiangiogenic agents, derived by the tumor itself and the tumor surrounding, especially the extracellular matrix, e.g., angiostatin, endostatin, thrombospondin-1, tumstatin, canstatin, restin, arrestin, or endorepellin.[3] This balance may be shifted towards a dominance of pro-angiogenic factors (the so-called angiogenic

Published online September 26, 2008.
This work was supported by a grant from the B. Braun Foundation to Sebastian Hoffmann.
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switch), resulting in tumor progression and metastasis.[4]

Among the large number of antiangiogenic agents identified until today, endostatin is commonly seen as one of the most important endogenous angiogenesis inhibitors. Endostatin is the carboxy-terminal segment of collagen XVIII, originally derived from hemangioendothelioma (EOMA) cells.[5] The cleavage of endostatin from collagen XVIII involves a number of proteinases, including cathepsin L, matrix metalloproteinases (MMPs), and elastases.[6] It is the only antiangiogenic factor being evaluated in phase I and II trials for clinical application.[7,8] Endostatin has been demonstrated to downregulate pathological angiogenesis of 65 different tumor types without apparent side-effects.[9] Although the exact mechanism of how endostatin inhibits angiogenesis still remains unclear, there is evidence for a tumor-specific suppression of angiogenesis by virtue of endostatin-induced apoptosis and endostatin-derived inhibition of endothelial cell migration.[10] Endostatin-related suppression of tumor growth and progression has been demonstrated for various solid tumors, including small Lewis lung cell carcinoma,[11] human breast cancer,[12] bladder tumor growth,[13] ovarian carcinomas,[14] malignant melanoma,[15] and hepatocellular carcinoma.[16] Recent studies have highlighted the importance of dosage and application to obtain optimum antitumor activity.[17,18]

Although thyroid cancer in general has a favorable prognosis when treated according to well-established clinical protocols, metastatic or undifferentiated disease is still associated with poor prognosis. Chemotherapy or radiotherapy so far serve for mere palliation[19,20] and anaplastic (ATC) or poorly differentiated carcinomas exhibit an extremely aggressive behavior, resulting in a median survival of only months.[21–23] So far, no effective treatment is available, thus endostatin might be an attractive therapeutic option in poorly differentiated and anaplastic thyroid cancer.

However, the evidence of an endostatin-derived anti-angiogenic effect in thyroid carcinoma is rare. The only endostatin-related antitumor effect demonstrated so far concerned a transfected follicular thyroid carcinoma cell line with forced secretion of endostatin, which grew smaller experimental tumors with a significantly reduced microvessel density in nude mice.[24] In a previous paper, we found first evidence for intrinsic endostatin secretion in thyroid cancer cell lines in vitro.[25]

Yet, so far, there has not been a systematic review of clinical thyroid cancer specimens nor an attempt to confirm the secretion or regulation of endostatin in thyroid cancer in vitro. We therefore aimed to systematically analyze endostatin protein expression in archival thyroid cancer specimens of different histotypes of differentiated and anaplastic thyroid cancer as well as their metastases. Furthermore, we examined an array of well-differentiated and anaplastic thyroid carcinoma cell lines and the effect of epidermal growth factor (EGF) and thyrotropin (TSH) on the secretion of endostatin.

MATERIALS AND METHODS

Cell Lines and Culture Conditions

The following thyroid-cancer-derived cell lines were screened for basal endostatin secretion: TPC 1[26] – papillary; FTC 133, 236,[27] HTC (HTC-), HTC-TSHr (HTC+) – follicular;[28] XTC – Hürthle cell;[29] C 643, Hth 74, Kat 4.0 – anaplastic[30] thyroid cancer. Of these cell lines, TPC 1, FTC 133, HTC+, XTC, C 643, and Hth 74 were chosen for further evaluation of TSH/EGF-stimulated endostatin secretion.

Culture flasks and dishes were obtained from Corning (Corning, NY), and cell culture medium was purchased from Biochrom (Berlin, Germany). Bovine TSH and human recombinant EGF employed were from Sigma-Aldrich (Munich, Germany). Early passages of the cell lines were maintained in full growth medium (FGM = DMEM-h21/Ham's F12 1:1 (v/v), containing 25 mM Hepes, 0.055 g/l sodium pyruvate, 0.365 g/l glutamine, 10% fetal bovine serum, 10,000 U/l penicillin, and 100 mg/l streptomycin) at 37°C in a 100% humidified, 5% CO_2 atmosphere as described previously.[29]

For experiments, thyroid cancer cells in exponential growth were harvested by brief incubation with cold trypsin-EDTA (0.025%/M, Sigma) and vitality was assessed by trypan blue exclusion. Depending on the experiment, serum-free H5 medium (H5 = DMEM-h21/Ham's F12 1:1 (v/v) with glutamin, supplemented with 10 µg/ml bovine insulin, 5 µg/ml human transferrin, 10 ng/ml somatostatin, 2 ng/ml glycyl-L-histidyl-L-lysine acetate, and 10^{-8} M hydrocortisone) was used instead of FGM during the time of the experiment.

Tumor Specimens

In a series of 51 archival thyroid cancer specimens (differentiated: papillary-PTC, $n = 27$; follicular: FTC: $n = 17$; and anaplastic: $n = 7$), their lymph node metastasis and corresponding normal thyroid

tissue, expression of endostatin protein was determined by means of immunohistochemistry. Two multispecimen tissue microarrays (TMA) composed of punch biopsies of the 51 paraffin donor tissues were constructed for the present study.[31] In brief, on hematoxylin and eosin (H&E) sections corresponding to standard paraffin blocks, a representative tumor or tissue area was selected and circled with a water-resistant marker. These slides were put onto the cutting side of the complementary standard paraffin block and the same area was marked here. Then, using a punching device, tissue cores of about 1.4 mm in diameter were punched out of the marked areas and assembled together to construct a new paraffin block. For immunohistochemistry, slides from both TMAs were processed on the same day using the same incubation fluids, reaction times, and substrate to exclude day-to-day variations.

Endostatin Immunohistochemistry

Immunohistochemical staining was performed on 3-μm TMA sections. In brief, following melting and incubation in citric acid buffer for 18 h at 60°C for antigen retrieval, slides were deparaffinized and rehydrated in graded alcohol. After blocking endogenous peroxidases and unspecific binding, incubations were carried out at 4°C using anti-endostatin primary antibody (mouse monoclonal, DPC Biermann, Bad Nauheim, Germany), and further enhanced by secondary antibodies (biotinylated anti-rabbit IgG and biotinylated anti-goat IgG, Santa-Cruz, Heidelberg, Germany) and streptavidin–horseradish peroxidase conjugate (Pharmingen, SanDiego, CA, USA). Slides were stained with DAB-chromogen (DakoCytomation, Hamburg, Germany), counterstained with hematoxylin according to Mayer, and mounted. Positive immunoreactivity was scored by one observer using an approach previously described.[32] In brief cytoplasmic staining was considered positive and the total percentage of tumor cells stained was categorized as either "focal" (+; <10% of tumor cells positive), "moderate" (++; 10–50% positive), or "strong" (+++; > 50% positive). All tumors displaying a moderate or strong endostatin expression were considered as positive.

Basal and TSH/EGF-Stimulated Endostatin Secretion of Thyroid Cancer Cells (ELISA)

Thyroid cancer cells were seeded at a density of 2×10^5 cells into six-well plates and allowed to resume growth for 24 h in FGM. Then cells were switched to serum-free H5 medium for 24 h and incubated under stimulated (TSH 1-100 mU/ml, EGF 1-100 ng/ml) or unstimulated conditions for another 48 h. Conditioned media (CM) was harvested and centrifuged (15,000*g*, 20 min, 4°C) to remove debris. Aliquots were stored at −80°C until analyzed. Basal (H5) and stimulated (H5 + TSH/EGF) concentrations were quantified by a commercially available endostatin enzyme-linked immunosorbent assay (ELISA) (endostatin, R&D Systems, Minneapolis, MN) using an ELISA plate reader (Emax, Molecular Devices, Sunnyvale, CA 450-570 nm). Experiments were repeated three times with similar results. The pancreatic cancer cell line BxPC-3, known to exhibit high intrinsic endostatin levels and to be endostatin-reactive, was employed as a positive control.[33]

Statistical Analysis

Unless stated otherwise, values are expressed as the mean ± standard deviation. Student's paired *t*-test was used to evaluate differences of continuous variables from samples of interest and the respective controls during TSH-/EGF-stimulation. All tests were two-tailed. A *P*-value of <0.05 was considered to indicate significance.

RESULTS

Expression of Endostatin in Thyroid Cancer Cell Lines

In vitro, endostatin was found to be secreted by all thyroid cancer cell lines examined (Fig. 1). Differences in basal endostatin secretion ranged from 33 ± 5 pg/ml for FTC 236 to 549 ± 65 pg/ml for TPC 1 cells. The largest quantities of endostatin were detected in the conditioned medium of the two most differentiated cell lines, i.e., in the TPC 1 papillary cancer and the XTC-Hürthle cell carcinoma cell lines. This observation concerned both serum-free (H5) and serum-containing conditions (FGM).

The less differentiated follicular cancer cell lines, i.e., HTC, and the FTC cell lines displayed a distinctly lower basal endostatin secretion. Among the FTC cell lines, basal endostatin secretion of the primary tumor (FTC 133) was more than twice as high as that of the metastatic (FTC 236) clone. Ambiguous findings could be detected when anaplastic tumor cell lines were analyzed. Here a more intense endostatin secretion and a strong reactivity to FGM could be demonstrated for C 643 compared with Hth 74 and Kat 4.0.

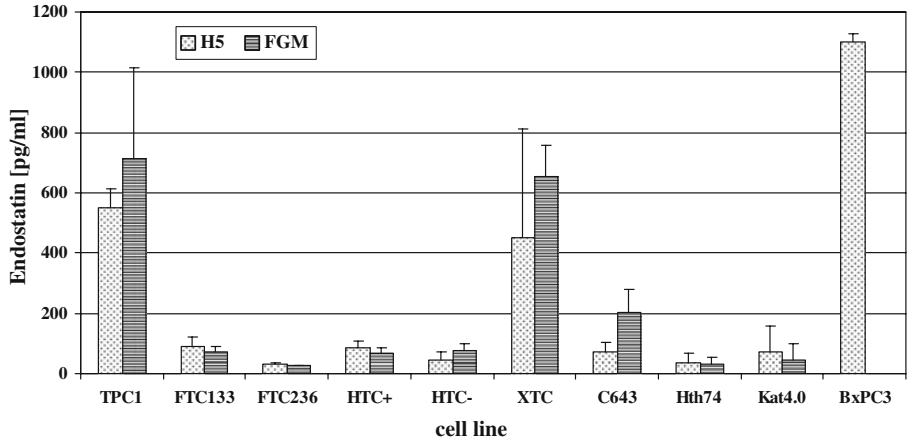

FIG. 1. Accumulation of endostatin (pg/ml) adjusted for cell number in the conditioned medium of thyroid cancer cell lines (follicular: FTC 133, FTC 236, HTC (HTC-), HTC-TSHr (HTC+); Hürthle cell: XTC; papillary: TPC 1; anaplastic: C 643, Hth 74, Kat 4.0) in serum-free (H5) and 10% calf serum containing conditions (FGM). The pancreatic cancer cell line BxPC3—known to secrete high amounts of endostatin—was employed as a positive control.

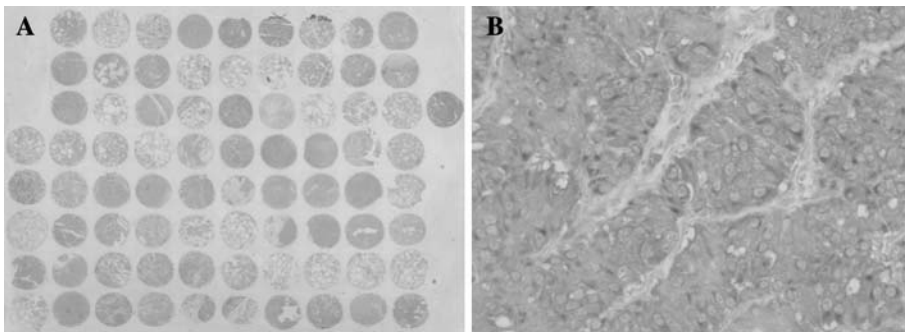

FIG. 2. Immunohistochemical staining of endostatin expression in thyroid cancer, corresponding normal tissue, and metastasis (if present). An example of a preparation of a multispecimen tissue microarray (TMA) is shown at 10× magnification (**A**) and an example of a positive-stained specimen locus is shown at 400× magnification to illustrate immunoreactivity for endostatin (**B**).

Analysis of Tissue-Type-Dependent Endostatin Expression in Tumor Specimens

Endostatin immunoreactivity was detected in the vast majority of thyroid tumor specimens. An example of a thyroid cancer multispecimen tissue microarray (TMA) with positive immunostaining for endostatin is shown in Fig. 2.

When tumor specimens were separately analyzed for the expression of endostatin, 93% of the thyroid cancer probes ($n = 51$) and 90% of the corresponding lymph node metastases ($n = 21$) stained positive for endostatin. Two lymph node metastasis of endostatin-positive ATCs showed no endostatin expression.

In contrast, only 56% of the corresponding non-neoplastic thyroid tissues ($n = 48$) had expression of endostatin with comparable intensity ($P < 0.05$). Little differences were seen when the histotype-specific expression of endostatin in the thyroid cancer specimens was analyzed. For instance, endostatin expression of anaplastic thyroid tumors was 100% ($n = 7$), whereas 94% of the follicular ($n = 17$) and 90% of the papillary thyroid cancers ($n = 27$) displayed expression of endostatin (Fig. 3).

Regulation of Endostatin Secretion by TSH/EGF

Because FGM altered endostatin accumulation into conditioned medium, thyroid cancer cell lines were then evaluated for TSH- and EGF-mediated regulation of endostatin cleavage, respectively. The results were quite different from cell line to cell line. XTC cells showed significant ($P < 0.05$ at TSH 100 mU/ml) dose-dependent upregulation of endostatin secretion induced by TSH (Fig. 4), whereas other cell lines showed no increase of endostatin by TSH despite the documented expression of a functional TSH receptor (HTC-TSHr). On the contrary, addition of TSH apparently inhibited endostatin accumulation in a number of differentiated cell lines (TPC1, HTC-TSHr, and FTC133).

Likewise, four cell lines showed increase of endostatin secretion by EGF of up to 120%, as

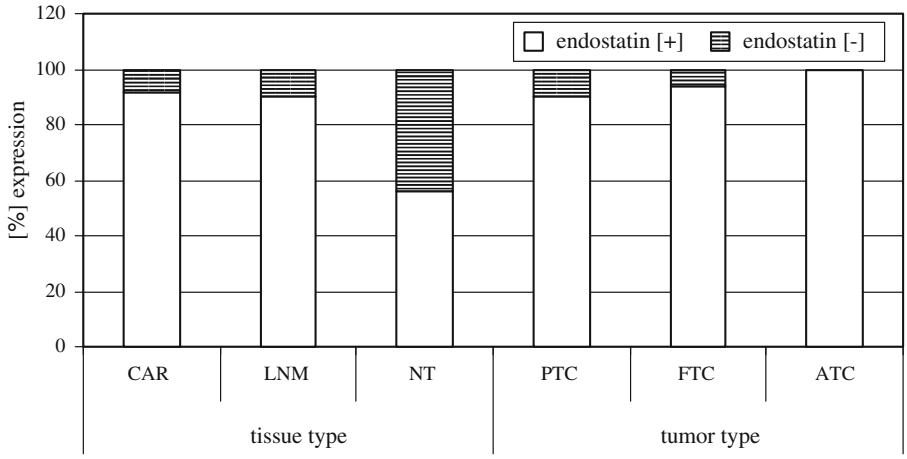

FIG. 3. Tissue-type-associated (CAR: carcinoma [$n = 51$], LNM: lymph node metastasis [$n = 21$], NT: normal thyroid tissue [$n = 48$]) and tumor-type associated (PTC: papillary [$n = 27$], FTC: follicular [$n = 17$] and ATC: anaplastic thyroid cancer [$n = 7$]) immunohistochemical detection of endostatin in thyroid cancer specimens on the TMA.

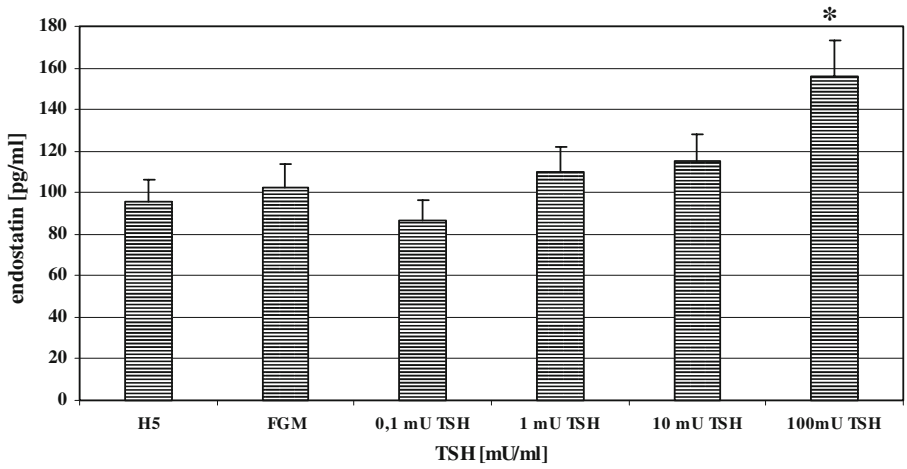

FIG. 4. Dose-dependent increase of endostatin secretion of a Hürthle cell carcinoma cell line (XTC) by TSH (0.1–100 mU/ml) in vitro. Control cells were grown in either serum-free (H5) or serum-containing (FGM) conditions. Bars indicate standard deviation; $*P < 0.05$.

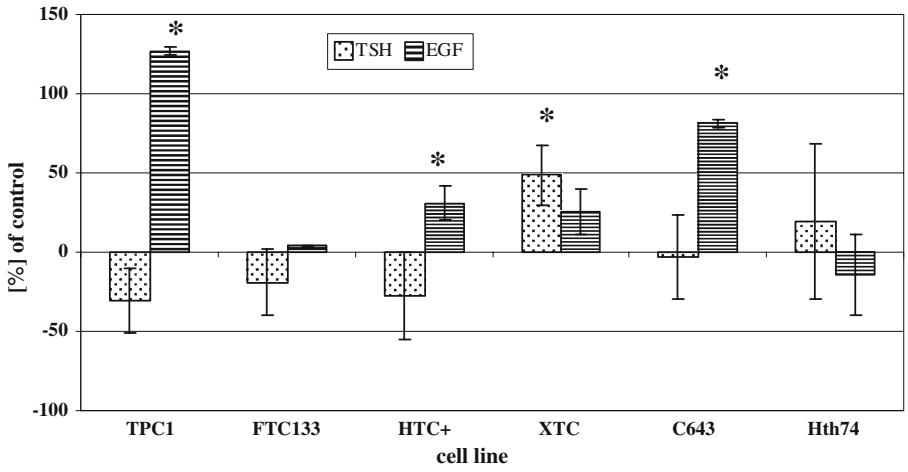

FIG. 5. TSH (100 mU/ml) and EGF (100 ng/ml) stimulated secretion of endostatin in thyroid cancer cell lines in percentage difference to unstimulated controls (follicular: FTC 133, HTC-TSHr[HTC+]; Hürthle cell: XTC; papillary: TPC 1; anaplastic: C 643, Hth 74). Bars indicate standard deviation and significance compared with the untreated control; $*P < 0.05$.

demonstrated for TPC1 and to a lesser extent for the differentiated cell lines HTC-TSHr and XTC as well as the anaplastic cell line C643 ($P < 0.05$). In FTC133 and the anaplastic Hth74 cells, endostatin was practically unchanged in response to EGF (Fig. 5).

DISCUSSION

Endostatin is currently believed to be one of the most important endogenous inhibitors of angiogenesis and has received considerable attention for possible "biological" antitumor treatment. Because an antiproliferative and anti-angiogenic effect of forced endogenous endostatin expression achieved by retroviral gene transfection was recently demonstrated in experimental follicular thyroid cancers,[24] hopes have been raised that endostatin may be a therapeutic option for undifferentiated thyroid cancers. However, to date, there has been no information on the expression of endostatin in normal thyroid tissue, nor in thyroid cancers or metastases from thyroid tumors. Moreover, it would be of considerable interest to know whether or not endostatin secretion is regulated by TSH or EGF, both of which are important cytokines in thyroid tumorigenesis.

In the present study, endostatin was shown to be secreted by all of the thyroid cancer cell lines examined and to be expressed by the vast majority of the thyroid cancer specimens as well as more than half of the non-neoplastic thyroid tissue (NT) samples. This may be an indication that endostatin is a part of the physiologic angiogenesis repertoire of the thyroid cell. The higher frequency of endostatin expression in undifferentiated thyroid carcinoma specimens and metastasis compared with in NT and differentiated thyroid cancers suggests endostatin to be associated with tumor progression. Incongruously, undifferentiated thyroid cancer cell lines displayed lower rates of endostatin secretion into conditioned medium, suggesting a different or changed mechanism of progression in this thyroid cancer histotype.

In vitro, basal secretion of endostatin revealed considerable individual differences among the thyroid cancer cell lines. Less differentiated tumors, e.g., the anaplastic cell lines Hth74 and Kat 4.0 as well as the metastatic clone FTC 236, tended to express lower amounts of endostatin, while the "differentiated" papillary thyroid cancer cell line TPC1 and the XTC-Hürthle cell line displayed the highest levels of unstimulated endostatin. Previous investigations of cancer cell lines displayed comparable levels of basic endostatin secretion, as for MCF-7 (HTB-22) human breast adenocarcinoma cells.[34] Two clones (HS-W and HS-R) of the same pancreatic cancer cell line (Hs-776T), isolated based on different growth kinetics, displayed strongly distinct levels of endostatin secretion as one possible explanation for different tumor growth in vivo.[35] However, these findings must be interpreted with caution, because it is quite likely that they represent individual in vitro characteristics of a particular cell line and do not necessarily allow the interpretation that determined amounts of endostatin secreted in vitro are linked to a particular degree of malignancy.

In fact, previous studies in animal models of retroviral endostatin gene transfer have outlined that serum levels of endostatin do not necessarily correlate with endostatin expression of the tumor tissue nor antitumor effect.[24,36] Elevated serum levels of endostatin in patients suffering from various solid tumors have been found to be associated with poor prognosis and aggressive tumor behavior, irrespective of the local expression of endostatin within the tumor.[37–40] Therefore, it is currently unclear whether or not an increase of systemic levels of endostatin or an increased level of local, i.e., tumoral, endostatin[6,41] results in reduced tumor vascularity and tumor development. Finally, patients with Down's syndrome, who commonly have elevated serum levels of endostatin, have a lower incidence of solid tumors, which argues against the hypothesis of elevated levels of serum endostatin being an indicator of malignant disease.[42]

In the present study, a similar observation was made when all of the anaplastic tumors were found to be positive for endostatin expression, in contrast to only some 50% of NT and 90% of the highly differentiated papillary thyroid tumors. This may suggest that enhanced endostatin expression by undifferentiated thyroid cancers should be read as a sign of enhanced expression of pro- and anti-angiogenic factors in general. Our findings may represent additional evidence in support of the hypothesis that a single factor influencing angiogenesis may not allow to predict the versatility of tumor-induced neo-angiogenesis. We have recently confirmed that tumor angiogenesis of thyroid cancer cells is a multifaceted process involving a large number of pro- and anti-angiogenic factors and thus that thyroid carcinomas behave quite similar to what has already been demonstrated for other solid tumors.[25]

Another novel finding in the present study is that endostatin is regulated by trophic hormones (TSH) and cytokines (EGF). So far, little is known about the regulation of endostatin and, in particular, about thyroid-specific regulation of endostatin. Since endostatin is cleaved by activity of various proteinases, such as MMPs, cathepsin L, and serine proteinase elastase, one could speculate this effect to be the result of growth-factor-induced increase of proteinase activity.[10] In fact, a previous study demon-

strated EGF-mediated increase of MMP-1 mRNA expression in thyroid cancer cell lines in vitro,[43] and we have previously shown that both EGF and TSH induce gelatinases of the metalloproteinase type in the XTC cell line.[29] Thus, the individual differences of endostatin accumulation by thyroid cancer cell lines following stimulation with EGF as observed in the current study may be explained by individual differences of EGF receptor expression and subsequent activation of proteinases.

In summary, the presented data demonstrate for the first time that endostatin is frequently expressed in a large series of thyroid cancer specimens. Endostatin is also expressed in NT and may therefore be part of the physiologic angiogenesis repertoire of the thyroid cell. The higher frequency of endostatin expression of undifferentiated thyroid carcinoma and metastasis compared with non-neoplastic thyroid tissue and, at a nonsignificant level, with differentiated thyroid carcinoma, suggests endostatin to be associated with tumor progression. This study also shows that endostatin appears to be regulated by TSH and EGF, although the individual differences of basal as well as stimulated endostatin levels were notable and deserve further functional studies. However, expression and regulation of endostatin in thyroid cancer cell lines appear to be specific to an individual tumor rather than a particular thyroid carcinoma histotype. Future investigations on drug-induced enhancement of endogenous endostatin as well as application of recombinant endostatin for antitumor action are strongly required.

ACKNOWLEDGEMENTS

We are grateful to Markus Jäckel and Dr. Magali Marwedel, Pathology, Hannover University Medical School, for help in preparing the thyroid tissue microarrays.

REFERENCES

1. Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med* 1971; 285:1182–6.
2. Folkmann J. What is the evidence that tumors are angiogenesis dependent? *J Natl Cancer Inst* 1990; 82:4–6.
3. Kalluri R. Basement membranes: structure, assembly and role in tumor angiogenesis. *Nat Rev Cancer* 2003; 3:422–33.
4. Hanahan D, Folkmann J. Patterns and emerging mechanisms of the angiogenetic switch during tumorigenesis. *Cell* 1996; 86:353–64.
5. O'Reilly MS, Boehm T, Shing Y, et al. Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. *Cell* 1997; 88:277–85.
6. Ferreras M, Felbor U, Lenhard T, et al. Generation and degradation of human endostatin proteins by various proteinases. *FEBS Lett* 2000; 486:247–51.
7. Herbst RS, Hess KR, Tran HT, et al. Phase I study of recombinant human endostatin in patients with advanced solid tumors. *J Clin Oncol* 2002; 20:3792–803.
8. Eder JP Jr, Supko JG, Clark JW, et al. Phase I clinical trial of recombinant human endostatin administered as a short intravenous infusion repeated daily. *J Clin Oncol* 2002; 20:3772–84.
9. Folkman J. Antiangiogenesis in cancer therapy—endostatin and its mechanisms of action. *Exp Cell Res* 2005; 312:594–606.
10. Wickstrom SA, Alitalo K, Keski-Oja J. Endostatin signaling and regulation of endothelial cell-matrix interactions. *Adv Cancer Res* 2005; 94:197–229.
11. Yang W, Li XY. Anti- tumor effect of pEgr-interferon-gamma-endostatin gene-radiotherapy in mice bearing Lewis lung carcinoma and its mechanism. *Chin Med J* 2005; 118:296–301.
12. Liby K, Neltner B, Mohamet L, et al. Endostatin expression by MDA- MB- 435 breast cancer cells effectively inhibits tumor growth. *Cancer Biol Ther* 2003; 2:53–4.
13. Kikuchi E, Menendez S, Ohori M, et al. Inhibition of orthotopic human bladder tumor growth by lentiviral gene transfer of endostatin. *Clin Cancer Res* 2004; 10:1835–42.
14. Subramanian IV, Gherbre R, Ramakrishnan S. Adeno-associated virus-mediated delivery of mutant endostatin supresses ovarian carcinoma growth in mice. *Gene Ther* 2005; 12:30–8.
15. Kirsch M, Weigel P, Pinzer T, et al. Therapy of hematogenous melanoma brain metastasis with endostatin. *Clin Cancer Res* 2005; 11:1259–67.
16. Tsuboi S, Nouso K, Tomono Y, et al. Anti-endostatin monoclonal antibody enhances growth of human hepatocellular carcinoma cells by inhibiting activity of endostatin secreted by transplantes cells in nude mice. *Int J Oncol* 2004; 25:1267–71.
17. Celik I, Surucu O, Dietz C, et al. Therapeutic efficacy of endostatin exhibits a biphasic dose-response curve. *Cancer Res* 2005; 65:11044–50.
18. Tjin Tham Sjin RM, Naspinski J, Birsner AE, et al. Endostatin therapy reveals a U-shaped curve for antitumor activity. *Cancer Gene Ther* 2006; 13:619–27.
19. Caron NR, Clark OH. Well differentiated thyroid cancer. *Scand J Surg* 2004; 93:261–7.
20. Diehl S, Umbricht CB, Dackiw AP, et al. Modern approaches to age-old questions about thyroid tumors. *Thyroid* 2005; 15:575–82.
21. Ain KB. Anaplastic thyroid carcinoma: a therapeutic challenge. *Semin Surg Oncol* 1999; 16:64–9.
22. McIver B, Hay ID, Giuffrida DF, et al. Anaplastic thyroid carcinoma: a 50-year experience at a single institution. *Surgery* 2001; 130:1028–34.
23. Pasieka JL. Anaplastic thyroid cancer. *Curr Opin Oncol* 2003; 15:78–83.
24. Ye C, Feng C, Wang S, et al. Antiangiogenic and antitumor effects of endostatin on follicular thyroid carcinoma. *Endocrinology* 2002; 143:3522–8.
25. Hoffmann S, Wunderlich A, Celik I, et al. Paneling human thyroid cancer cell lines for candidate proteins for targeted anti-angiogenic therapy. *J Cell Biochem* 2006; 98:954–65.
26. Ishizaka Y, Itoh F, Tahira T, et al. Presence of aberrant transcripts of ret proto-oncogene in a human papillary thyroid carcinoma cell line. *Jpn J Cancer Res* 1989; 80:1149–53.
27. Hoelting T, Zielke A, Siperstein AE, et al. Aberrations of growth factor control in metastatic follicular thyroid cancer in vitro. *Clin Exp Metastasis* 1994; 12:315–21.
28. Derwahl M, Kuemmel M, Goretzki PE, et al. Expression of human TSH receptor in a human thyroid carcinoma cell line that lacks endogenous TSH receptor: growth inhibition by cAMP. *Biochem Biophys Res Commun* 1993; 191:1131–7.

29. Zielke A, Tezelman S, Jossart GH, et al. Establishment of a highly differentiated thyroid cancer cell line of Hürthle cell origin. *Thyroid* 1998; 8:475–83.
30. Heldin NE, Westermark B. The molecular biology of the human anaplastic thyroid carcinoma cell. *Thyroidology* 1991; 3:127–31.
31. Mengel M, Kreipe H, von Wasielewski R. Rapid and large-scale transition of new tumor biomarkers to clinical biopsy material by innovative tissue microarray systems. *Appl Immunohistochem Mol Morphol* 2003; 11:261–8.
32. Langner C, Ratschek M, Rehak P, et al. Are heterogenous results of EGFR immunoreactivity in renal cell carcinoma related to non-standardised criteria for staining evaluation? *J Clin Pathol* 2004; 57:773–5.
33. Kisker O, Becker CM, Prox D, et al. Continuous administration of endostatin by intraperitoneally implanted osmotic pump improves the efficacy and potency of therapy in a mouse xenograft tumor model. *Cancer Res* 2001; 61:7669–74.
34. Nilsson UW, Dabrosin C. Estradiol and tamoxifen regulate endostatin generation via matrix metalloproteinase activity in breast cancer in vivo. *Cancer Res* 2006; 66:4789–94.
35. Schuch G, Kisker O, Atala A, et al. Pancreatic tumor growth is regulated by the balance between positive and negative modulators of angiogenesis. *Angiogenesis* 2002; 5:181–90.
36. Feldman AL, Alexander HR, Hewitt SM, et al. Effect of retroviral endostatin gene transfer on subcutaneous and intraperitoneal growth of murine tumors. *J Natl Cancer Inst* 2001; 93:1014–20.
37. Shaarawy M, El-Sharkawy SA. Biomarkers of intrinsic angiogenic and anti-angiogenic activity in patients with endometrial hyperplasia and endometrial cancer. *Acta Oncol* 2001; 40:513–8.
38. Suzuki M, Iizasa T, Ko E, et al. Serum endostatin correlates with progression and prognosis of non-small cell lung cancer. *Lung Cancer* 2002; 35:29–34.
39. Feldman AL, Alexander HR Jr, Yang JC, et al. Prospective analysis of circulating endostatin levels in patients with renal cell carcinoma. *Cancer* 2002; 95:1637–43.
40. Woo IS, Kim KA, Jeon HM, et al. Pretreatment serum endostatin as a prognostic indicator in metastatic gastric carcinoma. *Int J Cancer* 2006; 119:2901–9.
41. Sasaki T, Larsson H, Kreuger J, et al. Structural basis and potential role of heparin/heparan sulfate binding to the angiogenesis inhibitor endostatin. *EMBO J* 1999; 18:6240–8.
42. Zorick TS, Mustacchi Z, Bando SY, et al. High serum endostatin levels in Down syndrome: implications for improved treatment and prevention of solid tumors. *Eur J Hum Genet* 2001; 9:811–4.
43. Korem S, Resnick MB, Kraiem Z. Similar and divergent patterns in the regulation of matrix metalloproteinase-1 (MMP-1) and tissue inhibitor of MMP-1 gene expression in benign and malignant human thyroid cells. *J Clin Endocrinol Metab* 1999; 84:3322–7.