Oestrogen action on thyroid progenitor cells: relevant for the pathogenesis of thyroid nodules?

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Abstract

Benign and malignant thyroid nodules are more prevalent in females than in males. Experimental data suggest that the proliferative effect of oestrogen rather than polymorphisms is responsible for this gender difference. This study analysed whether both differentiated thyroid cells and thyroid stem and progenitor cells are targets of oestrogen action. In thyroid stem/progenitor cells derived from nodular goitres, the ability of 17β-oestradiol (E_2) to induce the formation of thyrospheres and the expression of oestrogen receptors (ERs) and the effect of E_2 on the growth and expression of markers of stem cells and thyroid differentiation (TSH receptor, thyroperoxidase, thyroglobulin and sodium iodide symporter (NIS)) were analysed. E_2 induced thyrosphere formation, albeit to a lower extent than other growth factors. Thyroid stem and progenitor cells expressed ERα (ESR1) and ERβ (ESR2) with eight times higher expression levels of *ERα* mRNA compared with the differentiated thyrocytes. E_2 was a potent stimulator of the growth of thyroid stem/progenitor cells. In contrast, TSH-induced differentiation of progenitor cells, in particular, the expression of NIS, was significantly inhibited by E_2. In conclusion, oestrogen stimulated the growth and simultaneously inhibited the differentiation of thyroid nodule-derived stem/progenitor cells. From these data and based on the concept of cellular heterogeneity, we hypothesize a supportive role of oestrogen in the propagation of thyroid stem/progenitor cells leading to the selection of a progeny of growth-prone cells with a decreased differentiation. These cells may be the origin of hypofunctioning or non-functioning thyroid nodules in females.

Key Words

- oestrogen
- thyroid stem cells
- progenitor cells
- thyroid nodules

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Introduction

Thyroid nodules are the most frequent endocrine neoplasms with three to four times higher prevalence in females than in males (Derwahl & Studer 2002, Libutti 2005, Gharib *et al*. 2008). Their prevalence ranges from 4–7% palpable nodules to up to 60% when ultrasound is applied (Dean & Gharib 2008).

The majority of these nodules are non-functioning, but only 5% of these scintigraphically 'cold' lesions are malignant tumours (Belfiore *et al*. 1992, Knudsen *et al*. 2000).

Understanding the pathogenesis of the different nodules may facilitate their diagnosis and enable a clear distinction between benign and malignant lesions.

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However, although many pathogenetic factors such as iodine deficiency, mutagenesis, overexpression of growth factors and their related receptors, altered signalling, data on gene expression profiles and genetic predisposition are known, a comprehensive concept for the pathogenesis of thyroid nodules and nodular goitres is still missing (Studer & Derwahl 1995, Krohn *et al*. 2005). At first glance, the high frequency of nodule formation in the thyroid gland is surprising because, compared with that of the highly proliferating tissues such as the colon and the breast, the growth rate of human thyroid cells is considerably lower with an estimated frequency of only about five cell divisions during adulthood (Coclet *et al*. 1989). However, tissues with a high cell turnover are more sensitive to mutagenesis and other molecular mechanisms that initiate tumour formation, whereas in resting tissues such as the thyroid gland, these mechanisms are less operative. To explain this discrepancy, it has been suggested that free radicals resulting from reactive oxygen species in the thyroid gland generate mutations more frequently (Maier *et al*. 2006).

Adult stem cells have been suggested as an alternative source of benign and malignant tumour formation (Reya *et al*. 2001, Fierabracci 2012). These cells reside in all tissues and organs and are capable of proliferating during the lifetime of the organism (Levi & Morrison 2008). Stem cells have been identified in the human thyroid gland (Thomas *et al*. 2006, Lan *et al*. 2007, Fierabracci *et al*. 2008) and cancer stem cells in thyroid cancer cells and tissues (Mitsutake *et al*. 2007, Todaro *et al*. 2010, Zheng *et al*. 2010, Malaguarnera *et al*. 2011). Orthotopic transplantation of stem cells derived from undifferentiated thyroid cancer tissues into a mouse thyroid gland recapitulated the nature and behaviour of the original tumour, which supported the concept of stem cells being the source of tumour formation (Todaro *et al*. 2010, Derwahl 2011).

Besides epidemiological data on the higher prevalence of proliferative thyroid diseases in females, several experimental studies have provided evidence that oestrogen is involved in the pathogenesis of thyroid nodules and tumours. Benign and malignant thyroid cells and tissues express functional oestrogen receptors (ERs) and their growth is stimulated by oestrogen (Furlanetto *et al*. 1999, Manole *et al*. 2001, Ceresini *et al*. 2006, Kumar *et al*. 2010, Rajoria *et al*. 2010, Di Vito *et al*. 2011). In addition, the presence of these receptors has also been demonstrated in thyroid vessels that may be relevant for the vascularization of neoplastic tissues (Ceresini *et al*. 2006).

Herein, we report for the first time that thyroid stem and progenitor cells are also targets of oestrogen action. Compared with primary human thyroid cells, stem/progenitor cells expressed more than eight times higher *ERα* mRNA levels. 17β-Oestradiol (E_2) promoted the growth of thyroid stem and progenitor cells and further up-regulated the expression of *ERα*. Accordingly, the expression of cyclin D1 cells was significantly enhanced. E_2 also promoted the formation of single stem cell-derived thyrospheres. On the other side, in response to E_2 stimulation, the levels of thyroid differentiation markers such as *TSHR*, thyroid symporter peroxidase (*TPO*) and sodium iodide (*NIS* (*SLC5A5*)) were markedly decreased with a maximal inhibition of TSH-induced *NIS* expression.

In conclusion, oestrogen stimulated the growth and simultaneously inhibited the differentiation of thyroid nodule-derived stem/progenitor cells. From these data and based on the concept of cellular heterogeneity, we hypothesize a supportive role of oestrogen in the propagation of thyroid stem/progenitor cells that may lead to the selection of a progeny of growth-prone cells with a decreased differentiation. These cells may be the origin of hypofunctioning or non-functioning thyroid nodules in females.

Materials and methods

Cell cultures and formation of thyrospheres

Human thyrocytes and primary thyrospheres isolated from nodular goitres of 58 patients after thyroidectomy were cultured as described by our group previously (Lan *et al*. 2007). The mean age of the patients (40 females and 18 males) was 53.1±14.0 years. Informed consent was obtained from all patients. The study was approved by the Ethics Committee of Charite, University Medicine Berlin.

For establishing the secondary generations of thyroid stem/progenitor cells (secondary thyrospheres), primary thyrospheres were dissociated mechanically and enzymatically into single cells and then cultured on poly-L-ornithine/fibronectin-coated (Sigma) dishes (cell density 10^5 cells/ml) with DMEM/F12 (1:1, v/v; Invitrogen) containing B-27 (1:50; Invitrogen), epidermal growth factor (EGF, 20 ng/ml; Invitrogen) and basic fibroblast growth factor (bFGF, 20 ng/ml; Invitrogen).

Conventional RT-PCR and real-time quantitative RT-PCR

Total RNA was extracted using the RNeasy Micro Kit (Qiagen) according to the manufacturer's specifications. RT-PCR was performed as described previously (Broecker *et al*. 1998). Real-time quantitative RT-PCR was performed

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with the iCycler iQ_Real-time PCR detector system (Bio-Rad), using the ABsolute QPCR SYBR Green Fluorescein Mix (Applied Thermo Fisher Scientific, Inc Schwerte, Germany.) according to the manufacturer's instructions. Cycling conditions were as follows: initial enzyme activation at 95 °C for 15 min, followed by 50 cycles at 95 °C for 15 s, 58 °C for 30 s and 72 °C for 30 s. The relative expression levels of each gene in real-time PCR were analysed using the $(2\times\text{efficiency})^{-\Delta\Delta Ct}$ method and normalized to the expression of the housekeeping gene *GAPDH*. Each sample was duplicated from independent sets of RNA preparations. The results are given as mean $\pm$ S.E.M. of three independent experiments.

For all PCR analyses, *GAPDH* was used as an internal control. In addition, *18S* was used as a second housekeeping gene. Primer sequences, product sizes, cycle numbers and annealing temperatures are listed out in Table 1.

5-Bromo-2′-deoxy-uridine incorporation

The proliferative potential of secondary passaged human thyroid stem/progenitor cells was evaluated by 5-bromo-2′-deoxy-uridine (BrdU) incorporation using the BrdU Labelling and Detection Kit I (Roche). Human thyroid stem/progenitor cells, 8000–10 000 cells/well, were plated into 96-well microtitre plates pre-coated with poly-L-ornithine/fibronectin in phenol red-free DMEM/F12 medium. The cells underwent starvation and adhesion for 24 h, and they were later incubated with E_2 (0.1–100 nM, dissolved in a basal medium containing up to 0.13% ethanol; Sigma), B27, EGF and bFGF for 24 h. For E_2 stimulation experiments, the controls consisted of the basal medium with only the solvent. BrdU incorporation was performed according to the manufacturer's instructions.

Immunofluorescence staining

Coverslips were coated with 0.1 mg/ml poly-L-lysine (Sigma) before introducing the cells and the medium. Human secondary passaged stem/progenitor cells were plated on the coverslips and cultured in a medium containing B27, bFGF and EGF. After 24 h, adhesive cells were rinsed with PBS, followed by fixation in pure

Table 1 Primer sequences, annealing temperatures, cycle numbers and product sizes for PCR

Target gene	Primer sequences	Annealing temperature	Cycles	Expected size (bp)
GAPDH	S: 5′-GAAGGTGAAGGTCGGAGTC-3′ AS: 5′-GAAGATGGTGATGGGATTTC-3′	58	26	226
ERα	S: 5′-CCACTCAACAGCGTGTCTC-3′ AS: 5′-GGCAGATTCCATAGCCATAC-3′	58	36	243
ERβ	S: 5′-CGCCAGTTATCACATCTGTATG-3′ AS: 5′-CCACTAACCTTCCTTTTCAGTG-3′	58	36	112
NIS	S: 5′-TCTCTCAGTCAACGCCTCT-3′ AS: 5′-ATCCAGGATGGCCACTTCTT-3′	58	36	298
TG	S: 5′-GAGCCCTACCTCTTCTGGCA-3′ AS: 5′-ATCCAGGATGGCCACTTCTT-3′	58	36	324
TSHR	S: 5′-AGCCACTGCTGTGCTTTTAAG-3′ AS: 5′-CCAAAACCAATGATCTCATCC-3′	58	36	131
TPO	S: 5′-GTCTGTCAGGCTGGTTATGG-3′ AS: 5′-CAATCACTCCGCTTGTTGGC-3′	58	36	242
PAX8	S: 5′-TTTGCTTGGCTCTTTCTACACCTC-3′ AS: 5′-GAATGTCTGTTTTAAGCTCCCTGG-3′	58	36	205
GATA4[a]	S: 5′-ACAAGATGAACGGCATCAAC-3′ AS: 5′-CGTGGAGCTTCATGTAGAGG-3′	58		174
GATA4[b]	S: 5′-CTCCTTCAGGCAGTGAGAGC-3′ AS: 5′-GAGATGCAGTGTGCTCGTGC-3′	58	36	575
OCT4	S: 5′-GACAACAATGAGAACCTTCAGGAG-3′ AS: 5′-CTGGCGCCGGTTACAGAACCA-3′	55	30	216
Cyclin D1[a]	S: 5′-ACAAACAGATCATCCGCAAACAC-3′ AS: 5′-TGTTGGGGCTCCTCAGGTTC-3′	58	30	144
18S[a]	S: 5′-TTGACGGAAGGGCACCACCAG-3′ AS: 5′-GCACCACCACCCACGGAATCG-3′	58		130

S, sense primer; AS, antisense primer.
[a]Only used for quantitative PCR.
[b]Only used for conventional RT-PCR.

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methanol at −30 °C for 5 min, permeabilized by 0.2% Triton X-100 for 10 min and then blocked by normal goat serum (10%; Sigma) for 90 min at room temperature. The coverslips were incubated with the monoclonal anti-ERα (1:50) and polyclonal anti-ERβ (1:100) antibodies (both from Santa Cruz Biotechnology) at room temperature for 1 h and then at 4 °C overnight. Unbound antibodies were removed by rinsing with a washing buffer (PBS containing 0.1% Tween-20 and 1% BSA), followed by incubation with FITC-conjugated secondary antibodies (1:400; Santa Cruz Biotechnology). FITC-labelled cells were analysed with a fluorescence Zeiss microscope using standard fluorescent filters (excitation 488 nm).

Statistical analysis

Statistical analysis was performed using the SPSS Software version 13.0. Numerical data are expressed as mean±s.e.m. Statistical differences were considered significant at $P<0.05$.

Results

E_2 had the potential to induce thyrosphere formation

Primary human thyrospheres that were enriched with thyroid progenitor/stem cells (Lan *et al.* 2007) were dissociated and stimulated either with EGF (20 ng/ml) and bFGF (20 ng/ml) or with 1 nM E_2. After 6 days of stimulation, secondary thyrospheres were reformed in both the groups. However, the size of the spheres formed in the E_2 group was smaller than that of those formed in the group grown with growth factors (Fig. 1).

As revealed by the RT-PCR, the mRNA expression of the stem cell marker octamer transcription factor 4 (*OCT4* (*POU5F1*)) was increased in the primary and secondary thyrospheres than in the primary thyrocytes. In contrast, the expression of *NIS* mRNA, a differentiation marker of thyroid follicular cells, was not detected in the primary and secondary thyrospheres (Fig. 2).

ERα (ESR1) and *ERβ* (*ESR2*) mRNA expression and immunofluorescence staining in thyroid stem/progenitor cells derived from thyroid nodules

Using RT-PCR, the expression of *ERα* and *ERβ* mRNAs was detected in both thyrocytes and thyrospheres with the expected amplicon sizes of 243 and 112 bp respectively. MCF-7 human breast cancer cells were used as a positive control (Fig. 3A). Quantitative PCR analysis

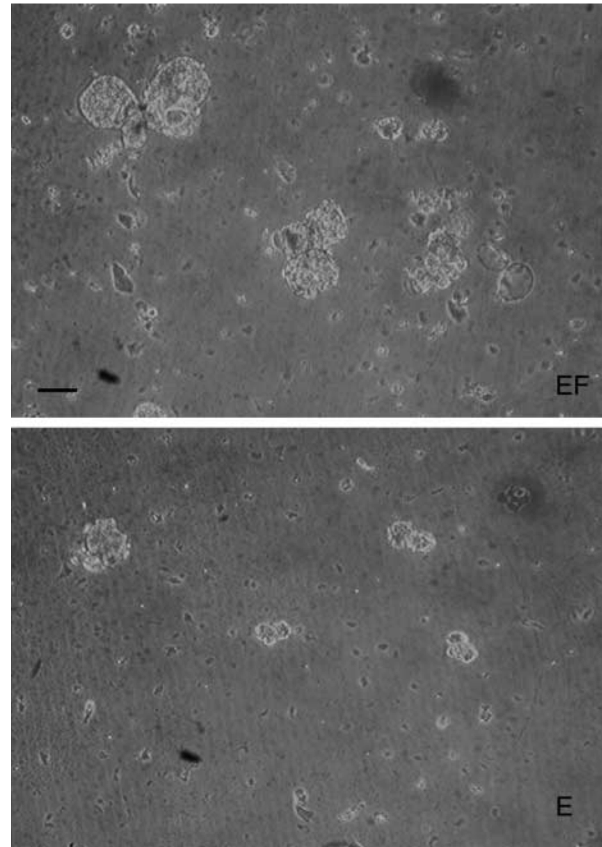

Figure 1
Thyrosphere formation was observed in both the groups with a smaller size of the spheres in the E2 group. Primary thyrospheres were dissociated and seeded in a serum-free DMEM/F12 medium with EGF (20 ng/ml) and bFGF (20 ng/ml) or with 1 nM E2. EF, EGF and bFGF; E, E2. Scale bar: 100 mm.

revealed an eightfold higher expression of *ERα* mRNA in the thyrospheres than in the thyrocytes (8.85±0.81 vs 1.10±0.35, $^{***}P<0.001$). No significant difference in the expression of *ERβ* mRNA was observed between the thyrospheres and thyrocytes (Fig. 3B).

By immunofluorescence staining, ERα and ERβ were detected in human thyroid stem/progenitor cells. Whereas ERα was enriched in the nucleus, ERβ was located in the perinuclear region of the cytoplasm (Fig. 4A, B, C, D, E and F). This distribution of ERα and ERβ immunostaining corresponds to findings in breast cancer tissues (Jarzabek *et al.* 2005).

E_2 increased BrdU incorporation into human thyroid progenitor/stem cells

In a dose-dependent manner, E_2 concentrations, ranging from 0.1 to 100 nM, increased BrdU incorporation into human thyroid progenitor/stem cells, with the maximum

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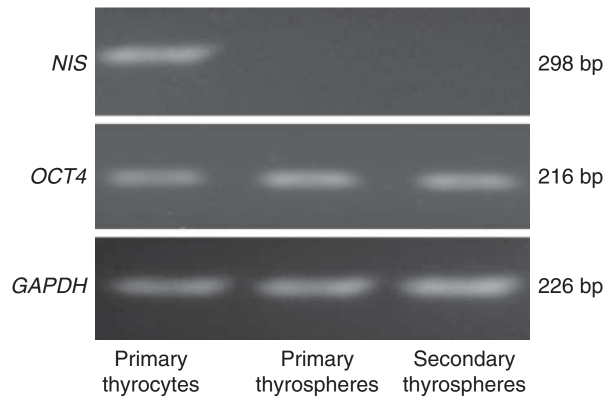

Figure 2
Expression of Na^{+}/I^{-} symporter (*NIS*) and *OCT4* mRNAs in primary thyrocytes and primary and secondary thyrospheres. The expression of the stem cell marker *OCT4* was increased in the primary and secondary passaged thyrospheres than in the thyrocytes, whereas that of the differentiation marker *NIS* was not detected in the thyrospheres. *GAPDH* was used as an internal control.

incorporation being observed at the concentration of 1 nM (167.20±4.07% vs control, $P<0.01$; Fig. 5), showing a proliferative effect of E_2 on human thyroid stem/progenitor cells.

E_2 up-regulated the expression of *ERα* mRNA and enhanced the expression of cyclin D1 in human thyroid stem/progenitor cells

After incubation with 1 nM E_2 for 24 h, the expression of *ERα* mRNA in human thyroid stem/progenitor cells was significantly increased by almost fivefold, whereas the expression of *ERβ* mRNA was not increased significantly (Fig. 6A).

Real-time PCR analysis revealed that the expression of cyclin D1 mRNA in human stem/progenitor cells was significantly increased 12 h after 1 nM E_2 stimulation (Fig. 6B), which indicates that E_2 is capable of up-regulating the expression of cyclin D1, a main regulator of the cell cycle.

E_2 decreased the expression of the differentiation markers

To explore the effects of E_2 and/or TSH on the differentiation of human thyroid progenitor cells, cell differentiation was induced using a medium with 10% serum and 5 mU/ml TSH for 3 days. The cells were further incubated with or without E_2 for an additional 3 days, before the expression of the mRNA of thyroid differentiation markers (paired box gene 8 (*PAX8*), thyroglobulin (*Tg*), *NIS*, *TSHR* and *TPO*) was analysed at day 6. A significantly decreased expression of the differentiation markers *TSHR*, *NIS* and *TPO* was observed, accompanied with an increased expression of the endodermal marker *GATA4* (Fig. 7A).

E_2 inhibited TSH-induced NIS expression

To evaluate the effects of E_2 on TSH-stimulated NIS expression, thyroid progenitor/stem cells were cultured with 10% serum and exposed to 1 nM E_2 or/and 5 mU/ml TSH for 21 days. As expected, TSH significantly increased the expression of *NIS* mRNA (1.98±0.25 vs control, $P<0.01$), whereas TSH-stimulated NIS expression was significantly suppressed by co-incubation with 1 nM E_2 (0.89±0.04 vs 1.98±0.25, $P<0.01$; Fig. 7B).

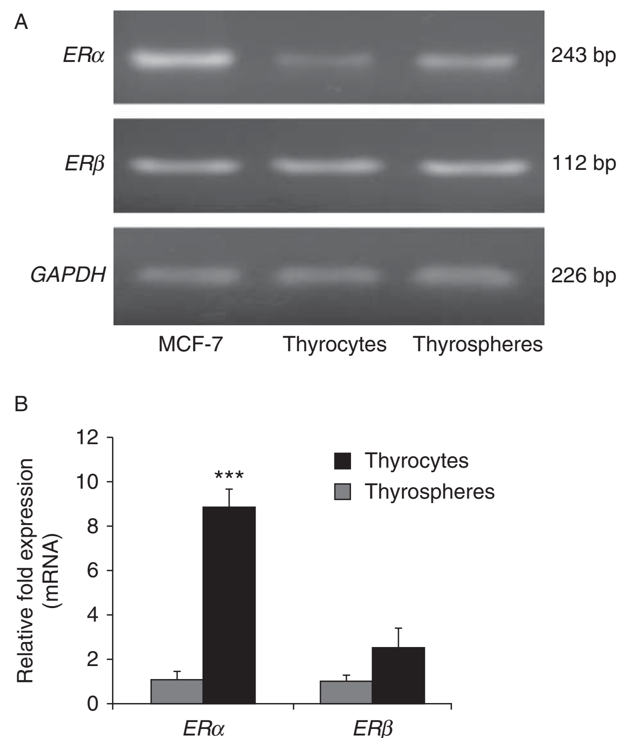

Figure 3
Expression of *ERα* and *ERβ* mRNAs in human thyrocytes and thyrospheres. (A) RT-PCR analysis of the expression of *ERα* and *ERβ* mRNAs, with the expected amplicon sizes of 243 and 112 bp respectively. MCF-7 human breast cancer cells were used as a positive control and *GAPDH* as an internal control. (B) Results of quantitative real-time PCR. A significantly higher expression of *ERα* was observed in the thyrospheres. The *C*t value of the real-time RT-PCR was calculated by the $(2\times\text{efficiency})^{-\Delta\Delta Ct}$ method and normalized by the value of the internal control *GAPDH*. Data are presented as the mean of fold change ±S.E.M. vs the control, and they were derived from three independent experiments (***$P<0.01$).

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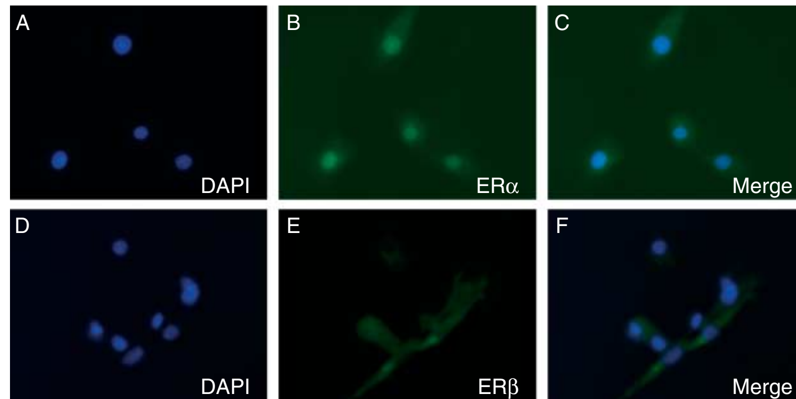

Figure 4
Localization of ERα and ERβ in human thyroid stem/progenitor cells by immunofluorescence staining. In human thyroid stem/progenitor cells, both ERα and ERβ were observed, with ERα being mainly localized in the nucleus and ERβ in the perinuclear region of the cytoplasm. (A, B and C), ERα; (D, E and F), ERβ in the stem/progenitor cells.

Discussion

There is a large body of evidence that suggests that oestrogen is involved in the pathogenesis of thyroid nodules and differentiated thyroid cancer cells (Santin & Furlanetto 2011). Oestrogen exerts its effect via genomic and non-genomic signalling (Manole *et al*. 2001). It controls the central signalling pathways of thyroid growth regulation such as MAP kinase and PI3 kinase pathways (Manole *et al*. 2001, Zeng *et al*. 2007, Antico-Arciuch *et al*. 2010). In tissues derived from human nodular goitres, it has been demonstrated that E_2 stimulated the growth of thyroid cells derived from female and male glands to the same extent (Manole *et al*. 2001). In line with this finding, no common genetic variants in sex hormone pathway genes that may explain the higher incidence rates of proliferative thyroid diseases in females have been detected yet (Schonfeld *et al*. 2012). Another argument for a pathogenetic role of oestrogen is the increasing incidence of differentiated thyroid cancer in females but not in males with the onset of puberty and the simultaneous rise in the levels of sex hormones (Farahati *et al*. 1997).

In the present work, we demonstrated that not only mature thyrocytes but thyroid-derived stem and progenitor cells also expressed ERs (Fig. 3A). The proof for the presence of ERs in stem cells comes from the finding that E_2 stimulation under starvation conditions, i.e. in a serum-free medium, results in the formation of thyrospheres, although the size of the spheres is smaller than that of those generated in the presence of EGF and bFGF (Fig. 1) or in response to insulin and IGF1 and IGF2 stimulation (Malaguarnera *et al*. 2011, Chen *et al*. 2012). Spheres including thyrospheres are derived from a single stem cell that generates a self-copy of itself and differentiates into progenitor cells (Lan *et al*. 2007, Deleyrolle & Reynolds 2009).

In comparison with primary thyroid cells, thyroid stem/progenitor cells expressed about eight times higher levels of *ERα* mRNA (Fig. 3B). A predominant expression of *ERα* was also observed in other stem and progenitor cells (Hu *et al*. 2011, Matsubara & Matsubara 2012). Furthermore, overexpression of *ERα* was also reported in papillary thyroid carcinomas (Di Vito *et al*. 2011).

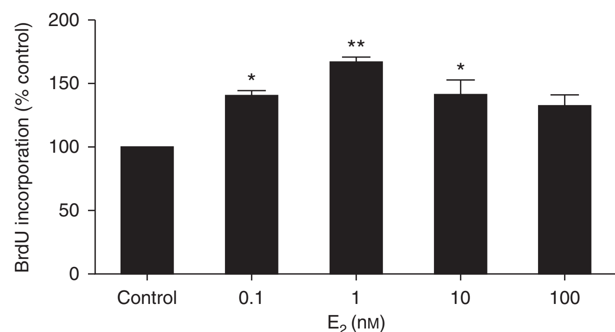

Figure 5
Effects of E_2 on BrdU incorporation in human thyroid stem/progenitor cells. Secondary passaged thyroid stem/progenitor cells were plated into 96-well microtitre plates pre-coated with poly-L-ornithine/fibronectin in phenol red-free DMEM/F12 medium. The cells underwent starvation and adhesion for 24 h, and they were later incubated with E_2 (ranging from 0.1 to 100 nM) and growth factors for 24 h. BrdU incorporation was measured by BrdU ELISA. Data were derived from three independent experiments conducted with four to six replicates. Results are plotted as the percentage of vehicle controls (mean±S.E.M., $*P<0.05$ and $**P<0.01$).

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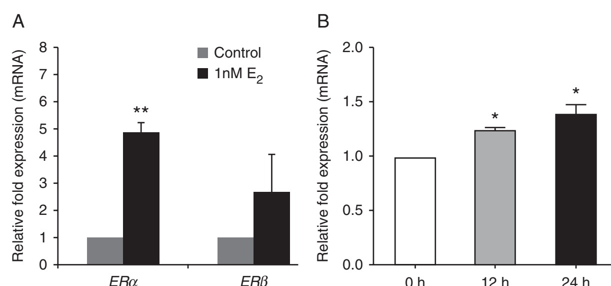

Figure 6
Expression of *ER* and cyclin D1 mRNAs in thyroid progenitor/stem cells in response to 1 nM E_2 stimulation determined by quantitative real-time PCR. (A) Expression of *ERα* and *ERβ* mRNAs in thyroid stem/progenitor cells in response to 1 nM E_2 stimulation for 24 h. As revealed by the real-time PCR, an almost fivefold increase in the expression of *ERα* mRNA was observed after incubation with E_2. No significant change in the expression of *ERβ* mRNA was observed. (B) Up-regulation of cyclin D1 mRNA levels induced by 1 nM E_2 treatment in thyroid stem/progenitor cells. The Ct values were calculated by the $(2\times\text{efficiency})^{-\Delta\Delta Ct}$ method and normalized by the value of the internal control *GAPDH*. Data are presented as the mean of fold change±S.E.M. vs the control, derived from three independent experiments (*$P<0.05$; **$P<0.01$).

An increased expression of *ERα* in highly proliferating stem/progenitor cells, which was further up-regulated by E_2 stimulation (Fig. 6), can be explained by the finding that cell growth is primarily regulated via ERα, whereas ERβ is involved in the control of apoptosis and suppressive functions (Chen *et al*. 2008). In taking this view, two recent reports that correlated ERα positivity and loss of ERβ expression in differentiated thyroid carcinomas with a more aggressive phenotype and a poor outcome are of interest (Heikkila *et al*. 2012, Magri *et al*. 2012).

E_2 stimulated the proliferation of thyroid stem/progenitor cells with maximal effect at 1 nM (Fig. 5). In contrast, in primary human thyroid cells, five times higher E_2 concentrations and twice the time of stimulation were necessary to achieve maximal growth stimulation (Manole *et al*. 2001). These differences are attributable to the higher expression levels of *ERα* in stem/progenitor cells (Fig. 3B) and to the lower proliferation rate of differentiated thyrocytes.

In accordance with the growth-stimulatory effect of E_2 on thyroid stem/progenitor cells, the expression of cyclin D1, an important regulator of the cell cycle, whose gene harbours an oestrogen-responsive regulatory region, was up-regulated (Fig. 6B). Similar results were observed before in differentiated human thyroid cells (Manole *et al*. 2001). In addition, overexpression of cyclin D1 was detected in several papillary thyroid carcinomas (Lazzereschi *et al*. 1998, Shi *et al*. 2001, Khoo *et al*. 2002, Kumar *et al*. 2010).

Although a large body of reports analysed the effects of oestrogen on benign and malignant thyroid growth, the knowledge on the influence of oestrogen on thyroid differentiation is still very limited. In this work, it has been shown that TSH-induced differentiation was inhibited by E_2.

When thyroid progenitor cells were pre-treated with TSH, E_2 resulted in a 60–70% decrease in NIS expression. The inhibitory effect of E_2 on the expression of *TSHR* and *TPO* mRNAs was significant but substantially lower (Fig. 7). Radio-iodide uptake was not measured, since iodide uptake is absent in a monolayer of progenitor cells (Lan *et al*. 2007). An inhibitory effect of E_2 on NIS expression and on radio-iodide uptake was first demonstrated in differentiated FRTL5 rat thyroid cells (Furlanetto *et al*. 1999, 2001).

Oestrogen action on thyroid stem/progenitor cells, i.e. stimulation of growth and inhibition of differentiation, in particular, of NIS expression, generates a progeny of cells with a higher proliferation rate and a reduced differentiation. Since E_2 influences all cells to the same extent, the question arises as to whether all progenitor cells have the same intrinsic growth potential. In the pathogenesis of thyroid nodules and nodular goitres as in many other endocrine and non-endocrine organs, the concept of the heterogeneity of growth and function is generally accepted (Derwahl & Studer 2002). Each thyroid cell has

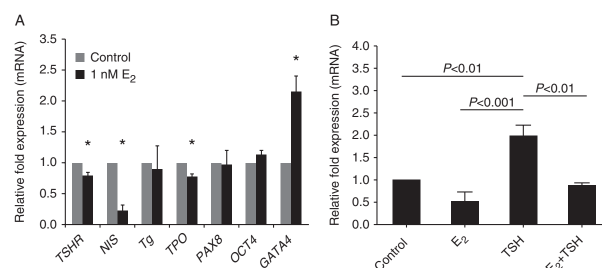

Figure 7
Effect of E_2 on the expression of the differentiation markers. (A) Effect of E_2 on TSH-induced differentiation of human thyroid stem/progenitor cells after 3 days of E_2 stimulation. As revealed by the real-time PCR, the expression of the differentiation markers *TSHR*, *NIS* and *TPO* was significantly decreased, whereas the expression of the endodermal marker *GATA4* was increased. Data were derived from three independent experiments. (B) Real-time PCR analysis of the suppressive effect of E_2 on the expression of *NIS* mRNA. Human thyroid progenitor/stem cells were cultured with 10% serum and exposed to 1 nM E_2 or/and 5 mU/ml TSH. *NIS* expression was significantly increased in the TSH-stimulated group. When co-incubated with TSH and E_2, the up-regulated *NIS* expression induced by TSH was significantly suppressed by E_2. Data were derived from four independent experiments. The Ct values were calculated by the $(2\times\text{efficiency})^{-\Delta\Delta Ct}$ method and normalized by the value of the internal control *GAPDH*. Data are presented as the mean of fold change±S.E.M. vs the control (*$P<0.05$ and **$P<0.01$).

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its individual growth potential and individual function. There is some evidence that heterogeneity may be operative in stem cells also (Collins *et al*. 2005, Graf & Stadtfeld 2008). Thus, oestrogen may contribute to the propagation of thyroid progenitor cells with a higher-than-average growth potential and simultaneously decrease their function.

In conclusion, we hypothesize a supportive role of oestrogen in the propagation of thyroid stem/progenitor cells that may lead to the selection of a progeny of growth-prone cells with a decreased function. These cells may be the origin of hypofunctioning or non-functioning thyroid nodules in females.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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