Estrogen and its role in thyroid cancer

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Abstract

Proliferative thyroid diseases are more prevalent in females than in males. Upon the onset of puberty, the incidence of thyroid cancer increases in females only and declines again after menopause. Estrogen is a potent growth factor both for benign and malignant thyroid cells that may explain the sex difference in the prevalence of thyroid nodules and thyroid cancer. It exerts its growth-promoting effect through a classical genomic and a non-genomic pathway, mediated via a membrane-bound estrogen receptor. This receptor is linked to the tyrosine kinase signaling pathways MAPK and PI3K. In papillary thyroid carcinomas, these pathways may be activated either by a chromosomal rearrangement of the tyrosine receptor kinase TRKA, by RET/PTC genes, or by a BRAF mutation and, in addition, in females they may be stimulated by high levels of estrogen. Furthermore, estrogen is involved in the regulation of angiogenesis and metastasis that are critical for the outcome of thyroid cancer. In contrast to other carcinomas, however, detailed knowledge on this regulation is still missing for thyroid cancer.

Key Words

- thyroid
- estrogen
- cell signaling
- growth factor receptor
- neoplasia

Introduction

Both benign and malignant thyroid tumors are 3–4 times more prevalent in females than in males (Dean & Gharib 2008, Li et al. 2013). Whereas in prepubertal girls and boys (rare), thyroid cancer is roughly equally represented, with the onset of puberty the incidence increases in females by up to 14 times (Farahati et al. 1997). After menopause, the incidence decreases again (Li et al. 2013).

In several studies, pregnancy was associated with a higher risk of thyroid cancer (Galanti et al. 1995, Rossing et al. 2000, Sakoda & Horn-Ross 2002, Horn-Ross et al. 2011). In contrast, low dose of estrogen in contraceptives and in replacement therapy in postmenopausal women does not contribute to a higher risk of thyroid cancer (Kabat et al. 2012). Some studies even found an inverse association between the use of contraceptives and the risk of thyroid cancer, which may be explained by the low concentrations of 17β-estradiol (E₂) in currently used contraceptives (Rossing et al. 2000, Sakoda & Horn-Ross 2002, Cornet 2013). However, in a recent retrospective cohort study of 145 007 postmenopausal women, no association between the risk of thyroid cancer and hormonal or reproductive factors such as age at menarche or menopause, parity, and bilateral oophorectomy was observed (Kabat et al. 2012). Only women who had a first live birth between the age of 20 and 24 years had an increased risk of papillary thyroid cancer (PTC; Kabat et al. 2012).

Genetic factors do not seem to confer a higher risk of thyroid cancer to females. In a case–control study of 344 PTCs and 452 controls, the association between PTC and 1151 tag single-nucleotide polymorphisms (SNPs) in 58 candidate gene regions involved in sex hormone synthesis and metabolism did not show a significant correlation between the investigated SNPs and the risk of PTC (Schonfeld et al. 2012).
The strongest support for estrogen as a risk factor of differentiated thyroid cancer comes from experimental studies that have clearly demonstrated that E₂, the main female sex hormone, is a potent stimulator of both human benign and malignant thyroid cells (Manole et al. 2001, Zeng et al. 2007, Chen et al. 2008, Kumar et al. 2010, Rajoria et al. 2010). Interestingly, E₂ was equally potent in its growth-promoting effect on human thyroid cells derived from male and female thyroid glands, which underlines the relevance of the hormone rather than of other sex-specific factors (Manole et al. 2001).

In summary, epidemiological, clinical, and experimental data are indicative of a role of estrogen in the pathogenesis of proliferative thyroid diseases.

Estrogen and its receptors

Estrogens are steroid hormones that play a key role in the regulation of not only growth, differentiation, and function of the reproductive organs but also bone, and the cardiovascular and immune systems in males and females (Heldring et al. 2007). Estrogens that are effective in humans include estrone, estriol, and E₂, of which E₂ has the highest affinity to the estrogen receptors (ERs) and the highest potency (Kuiper et al. 1997). The action of estrogen occurs via a classical genomic and a non-genomic pathway. The genomic mode of action is mediated through ERα and ERβ, which are members of a large family of nuclear transcription factors (Mangelsdorf et al. 1995). For activation, estrogen has to enter the cell where it binds to its receptors ERα and ERβ, which is followed by a nuclear translocation, homo- or heterodimerization of the estrogen–ER complex, and its binding to an estrogen-responsive element (ERE) in the promoter region of different target genes (Nilsson et al. 2001). For the transactivation of genes, a process of recruitment and binding of different coactivators is mandatory.

In contrast to the slow process of transactivation of target genes, the non-genomic action of estrogen, which is mediated by a membrane-associated estrogen receptor (mER), is very fast (Moriarty et al. 2006). Both the genomic and the non-genomic actions of estrogen are operative in benign and malignant thyroid cells (Manole et al. 2001).

Alternatively, rapid signal transduction of estrogen may be mediated by a novel G protein-coupled receptor, designated GPR30 (Revankar et al. 2005). This receptor has been detected in some thyroid carcinoma cell lines that lack classical ERs and stimulate growth via GPR30 but not in benign and malignant thyroid tissues (Vivacqua et al. 2006).

In addition, a novel membrane-bound ER, referred to as ERx, was detected in the brain and the uterus (Toran-Allerand et al. 2002). The existence of this receptor in the thyroid is still unknown.

ER expression in benign and malignant thyroid tissues

First indirect evidence for the presence of ERs in benign and malignant thyroid tissues (Table 1) came from ligand studies (Molteni et al. 1981). Later ER immunoreactivity was detected in normal thyroid tissue, thyroid adenomas, goiters, and differentiated and undifferentiated thyroid carcinomas (Diaz et al. 1991, Mizukami et al. 1991, Hiasa et al. 1993). However, widely varying and, in part, contradictory results were reported. Some authors did not find ERs by immunohistochemical staining in normal thyroid tissue and adenomas or goiters (Jaklic et al. 1995, Arain et al. 2003), while others detected ER staining in approximately 10% to more than 50% of benign thyroid lesions (Diaz et al. 1991, Mizukami et al. 1991, Hiasa et al. 1993, Tavangar et al. 2007).

A similar picture emerged when comparing immunostaining of thyroid cancer samples. Although ER immunostaining was described in less than 10% to over 60% of the samples in the majority of investigations (Haruta et al. 1990, Diaz et al. 1991, Mizukami et al. 1991, Hiasa et al. 1993, Lewy-Trenda 1998, Tavangar et al. 2007), no ER immunoreactivity was observed in other studies (Jaklic et al. 1995, Arain et al. 2003). These widely varying results were described for both papillary carcinoma and follicular thyroid carcinoma (FTC) samples. The discrepancies may be explained by technical factors such as the use of different monoclonal or polyclonal antibodies or various staining procedures.

However, using considerably more sensitive reverse transcription PCR, detection of ER mRNAs in malignant thyroid tissues also varied between less than 20% and up to 100% of samples (Yane et al. 1994, Dalla Valle et al. 1998). Therefore, it is likely that these discrepancies may be also due to epigenetic modifications of ER expression rather than only the result of technical factors. Epigenetic regulation of ER expression was demonstrated in different tumors including breast cancer (Hervouet et al. 2013).

Moreover, both isoforms, ERα and ERβ, were detected in goiter tissues, thyroid carcinomas, and different thyroid carcinoma cells (Manole et al. 2001, Santin & Furlanetto 2011). Most of the thyroid cancer cell lines that are widely used in thyroid research express both ER isoforms (Manole et al. 2001, Santin & Furlanetto 2011). However, similarly,
the patterns of ERα and ERβ expression in thyroid carcinoma tissues are widely varying. Some authors did not observe ERα immunostaining in thyroid adenomas, goiters, and papillary, follicular, and anaplastic thyroid carcinomas, but ERβ immunoreactivity in all benign and malignant thyroid samples (Ceresini et al. 2006, Vaiman et al. 2010a,b). In contrast, others reported an increased expression of ERα in PTC tissues in comparison to low or absent expression in normal thyroid tissues (Chen et al. 2008, Kansakar et al. 2009). Again, other immunohistochemical studies showed similar ERα staining in normal thyroid and tumor tissues (Hampl et al. 1985, Vaiman et al. 2010a,b). Recently, in papillary thyroid carcinomas, overexpression of ERα but lack of ERβ expression in the surrounding tissue and absence of ERβ expression in the tumor samples have been described (Di Vito et al. 2011). These authors used the sensitive technique of laser capture microdissection to isolate homogeneous cell population from PTC surgical samples to analyze ERα and ERβ expression. In this context, it is of special interest that undifferentiated thyroid stem and progenitor cells that are widely believed to be the origin of thyroid tumors predominantly express ERα with eightfold higher expression levels than differentiated thyroid cells (Xu et al. 2013).

As suggested for breast cancer, an imbalance between ERα and ERβ expression with overexpression of ERα was also postulated by Chen and coworkers for thyroid cancer (Chen et al. 2008, Sotoca et al. 2008). This hypothesis states that higher expression of ERα enhances cell proliferation, whereas higher expression of ERβ promotes differentiation and induces cell apoptosis. Evidence for this concept came from studies with specific agonists, which showed that selective stimulation of ERα increased thyroid carcinoma growth and expression of anti-apoptotic BCL2 protein, while selective stimulation of ERβ decreased proliferation rate and enhanced expression of apoptotic BAX protein (Zeng et al. 2007). Silencing with selective siRNAs of either ERα or ERβ showed contrary effects and thus confirmed the stimulation experiments. Very recently, a study of 89 females with PTC, which correlated ER expression with tumor size and proliferation marker Ki-67, reaffirmed the role of ERα in growth stimulation of these tumors and, on the other hand, an inhibitory effect of ERβ (Huang et al. 2014).

### Effect of estrogen on benign and malignant thyroid cells

**Estrogen and growth of benign and malignant thyroid cells**

A direct growth-stimulatory effect of estrogen was first demonstrated in the differentiated FRTL-5 rat thyroid cell line (Furlanetto et al. 1999). These cells express functional

### Table 1  Detection of estrogen receptors (ERs) α and β in benign and malignant thyroid tissues (ER-positive samples/total number of samples)

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IHC, immunohistochemical assay; RBA, radioligand-binding assay; SDG, sucrose density gradient assay.

*Tissues from female patients of reproductive age.*
ERs and enhance DNA synthesis and proliferation in response to E2 stimulation. Similar results were obtained in thyroid cells derived from goiter nodules and in human thyroid carcinoma cells (Manole et al. 2001, Zeng et al. 2007, Kumar et al. 2010, Rajoria et al. 2010). E2-stimulated cell growth was associated with increased expression of cyclin D1 protein, which plays a key role in the regulation of the G1/S transition in the cell cycle (Motokura & Arnold 1993, Manole et al. 2001). CCND1, whose overexpression has been repeatedly reported in thyroid cancer, has an ERE in the promoter region of its gene (Altucci et al. 1996, Farid 1996).

In benign and malignant thyroid cells, E2 via its membrane-bound receptor mER also stimulated activation of the MAP kinase signaling pathway through phosphorylation of extracellular receptor kinases 1 and 2, whose activity in thyroid carcinoma cells is mainly controlled by growth factors (Manole et al. 2001, Kumar et al. 2010, Rajoria et al. 2010). In addition, through mER, E2 activated the phosphatidylinositol 3-kinase (PI3K) signaling pathway (Kumar et al. 2010, Saji & Ringel 2010). Both the MAPK and the PI3K pathways are critical for proliferation and propagation of thyroid cancer. Inhibition of both signaling pathways prevents estrogen-induced mitogenesis.

**Estrogen upregulates ERα expression**

For the promotion of thyroid adenomas, goiter, and thyroid cancer in females, the effect of estrogen on its receptors is of clinical relevance. Indeed, it is well documented in benign and thyroid carcinoma cells that E2 stimulates growth and amplifies its growth-promoting effect by upregulation of ERα (Manole et al. 2001, Vivaqua et al. 2006). By contrast, ERβ expression is not affected by E2 stimulation.

**Estrogen and other mitogenic pathways of the thyroid**

In the thyroid gland, there are 3 distinct mitogenic pathways operative: i) the hormone receptor–Gs–adenyl cyclase–cAMP-dependent protein kinase system, ii) the hormone receptor–tyrosine protein kinase pathways, and iii) the hormone receptor–Gq–phospholipase C cascade (Dumont et al. 1992). In thyroid papillary cancer, the tyrosine kinase signaling pathways, Ras/Raf/MAPK and PI3K–Akt, which are also stimulated by estrogen via mER, are activated by chromosomal rearrangements of the tyrosine kinase receptor TRKA, by RET/PTC genes, and by a BRAFV600E mutation (Ciampi & Nikiforov 2007).

**Estrogen and in thyroid cancer**

Figure 1

Estrogen-dependent genomic and non-genomic signaling pathways. The genomic mode of action is mediated through ERα and ERβ, which are members of a large family of nuclear transcription factors. For activation, estrogen has to enter the cell where it binds to its receptors ERα and ERβ, which is followed by nuclear translocation, homo- or hetero-dimerization of the estrogen–ER complex, and its binding to an estrogen-responsive element (ERE) in the promoter region of different target genes (Nilsson et al. 2001). For the transactivation of genes, a process of recruitment and binding of different coactivators is mandatory (left). The non-genomic signaling of E2 occurs via the membrane-bound receptor mER, which stimulates activation of the MAP kinase and the PI3 kinase signaling pathways, whose activity in thyroid carcinoma cells is mainly controlled by growth factors (right). In PTC with aberrant activation of these tyrosine kinase pathways by chromosomal rearrangement of the tyrosine kinase receptor TRKA, by RET/PTC genes, or by a BRAF mutation (or a RAS mutation in FTCs), E2 synergistically stimulates these pathways (see text). In addition, in thyroid cancer, the PI3K/Akt pathway may also be activated by mutational inactivation of PTEN, a tumor-suppressor gene, or decreased/absent expression of its mRNA or protein (Bruni et al. 2000). Thus, estrogen-dependent signaling targets the mitogenic pathways that are critical for growth regulation of thyroid cancer (Fig. 1).

**Estrogen and thyroid stem cells**

There is increasing evidence that mutated thyroid stem cells or progenitor cells rather than primary thyroid cells are the origin of thyroid cancer (Derwahl 2011). Support for this concept comes from the finding that normal differentiated thyrocytes divide only five times during adult lifetime with a mean turnover rate of 8.5 years (Dumont et al. 1992). Cell division is, however, a prerequisite for the accumulation of mutations and/or other genetic and epigenetic changes and in turn the generation of a tumor. Indeed, cancer occurs more frequently in organs with a higher cellular turnover such as the colon.

As an alternative source for the generation of tumors including thyroid cancer, stem and progenitor cells have
been proposed (Davies et al. 2011, Derwahl 2011). Stem cells are undifferentiated cells that possess the capacity of self-renewal and the generation of more differentiated progenitor cells (Davies et al. 2011). These cells that reside in any organ and tissue for the lifetime of the organism have been detected in the human thyroid gland and in goiters and cancer stem cells in thyroid cancer cell lines and thyroid cancer tissues (Thomas et al. 2006, Lan et al. 2007, Mitsutake et al. 2007, Fierabracci et al. 2008, Todaro et al. 2010, Zheng et al. 2010, Malaguarnera et al. 2011).

Proliferation of stem cells is regulated by niches, signals from the surrounding microenvironment, which prevent the undifferentiated cells from undergoing uncontrolled cell division (Rezza et al. 2014). Under certain conditions, e.g., induction of apoptosis, and intense stimulation with hormones and growth factors, the strict control of niche may be overcome, resulting in proliferation of stem cells. Owing to their longevity, stem cells are prone to acquiring mutations and other molecular aberrations (Dick 2008).

In differentiated thyroid cells and thyroid cancer cells, it has been demonstrated that apoptotic stimuli can overcome the control of niches, which in vitro in response to intense stimulation with growth factors results in the outgrowth of thyroid stem and progenitor cells as spheroids, designated thyrospheres, in vitro (Lan et al. 2007, Fierabracci et al. 2008, Zheng et al. 2010).

Very recently, it has been reported that thyroid stem and progenitor cells are also a target of estrogen action (Xu et al. 2013). Thyroid stem and progenitor cells derived from thyroid nodules express ERα and ERβ with eight times higher expression levels of ERα compared with normal thyroid cells (Xu et al. 2013). This high ERα expression in undifferentiated stem/progenitor cells is reminiscent of the above-mentioned overexpression of ERα in some papillary thyroid carcinomas (Di Vito et al. 2011). High expression levels of ERα in thyroid stem/progenitor cells were further upregulated by stimulation with E2, which is in accordance with findings for human benign and malignant thyroid cells (Manole et al. 2001, Xu et al. 2013). A predominant expression of ERα was also reported in stem and progenitor cells derived from other tissues such as prostate (Hu et al. 2011).

**Estrogen and thyroid function**

There is little known about the effect of estrogen on thyroid function. In the FRTL-5 rat thyroid cell line, E2 downregulated expression of the sodium–iodide symporter (NIS) and consecutively inhibited radioactive iodine uptake (Furlanetto et al. 1999, 2001). In contrast, expression of thyroglobulin was enhanced in response to E2 stimulation (del Senno et al. 1989).

In thyroid-progenitor-cell-derived thyroid nodules, E2 decreased the expression of TSH-induced differentiation markers such as TPO, TSHR, and NIS mRNAs (Xu et al. 2013). The most pronounced effect of E2 was observed on NIS expression. This result is in accordance with the report on primary FRTL-5 cells mentioned above. Thyroglobulin expression was not affected by E2 stimulation in thyroid progenitor cells.

**ERs and hypoxia and inflammation**

Owing to insufficient neovascularization, necrosis is frequently observed in solid tumors. The resulting hypoxia is critical for the progress of the tumor (Bertout et al. 2008). Under this condition, the hypoxia-inducible factor 1 (HIF1), a transcription factor, induces the transcription of several genes involved in angiogenesis (e.g., vascular endothelial growth factor (VEGFA)), glucose metabolism, apoptosis resistance, inflammation, invasion, and metastasis (Semenza 2012).

HIF1α is overexpressed in thyroid cancer with the highest expression in therapy-resistant, undifferentiated thyroid carcinomas (Burrows et al. 2010). Interestingly, HIF1α also regulates ERα expression (Tafani et al. 2011).

Lymphocytic infiltrations are frequently observed in thyroid cancer ( Guarino et al. 2010). Both HIT1α and nuclear factor xB (NFxB), a transcription factor that coordinates immune and inflammation processes, have very recently been demonstrated to upregulate pro-inflammatory genes that play a key role in the progression of transformed thyroid cells to a malignant phenotype (Tafani et al. 2013). Based on the links between ERα, hypoxia (HIF1α activation), and inflammation (NFxB activation), a concept has very recently been proposed that postulates a major role for the interaction between ERα and the two transcription factors in the progression of thyroid cancer ( Tafani et al. 2013).

**ER expression and outcome of thyroid cancer**

Clinical proof for the concept that ERα promotes proliferation while ERβ mediates differentiation came from studies of breast cancer, ovarian cancer, and lung cancer, which demonstrated that low ERβ expression is associated with a poor outcome of these tumors (Omoto et al. 2001, Mauro et al. 2010, Halon et al. 2011). In general, ERβ is believed to play a protective role in carcinogenesis
in that it mediates anti-proliferative and pro-apoptotic signals and thus counteracts ERα-induced cell proliferation (Gustafsson & Warner 2000, Satake et al. 2006, Rajoria et al. 2010). There is some evidence that loss of ERβ expression is a common step in estrogen-dependent tumor progression (Bardin et al. 2004).

Although for thyroid tumors, as mentioned above, data on expression of ER isoforms are, at least in part, contradictory, some recent studies have been in accordance with the findings for other carcinomas that low or even loss of ERβ expression is also a hallmark of thyroid carcinomas with a poor prognosis. In a very recent study with follicular thyroid adenomas (FTAs) and FTCs, ERβ expression was significantly lower in FTCs than in FTAs (Heikkila et al. 2013). This lower level of expression was correlated with a poor clinical outcome. Interestingly, ERβ expression was a stronger prognostic marker than expression of the Ki-67 proliferation marker routinely used in the histological diagnosis of thyroid cancer (Tallini et al. 1999). Moreover, the transition from FTAs to FTCs seemed to go along with the loss of ESR2 expression (Heikkila et al. 2013). This fits well with the lack of ERβ expression in papillary carcinomas and the finding of ERβ negativity in some small differentiated thyroid carcinomas with a more aggressive phenotype (Di Vito et al. 2011, Magri et al. 2012). Lower expression of ERβ was also described in undifferentiated thyroid stem and progenitor cells when compared with differentiated human thyrocytes (Xu et al. 2013). Thus, lower or even loss of ERβ expression that maintains cell differentiation and the epithelial phenotype may also be a hallmarks of dedifferentiation in thyroid cancer as in other carcinomas (Thomas & Gustafsson 2011).

The considerably differing expression patterns of ERα make it difficult to define its role in the pathogenesis of thyroid cancer. However, there is no doubt that estrogen is a potent growth factor for benign and malignant thyroid cells and that proliferation is mediated via ERα-dependent signaling (Manole et al. 2001, Lee et al. 2005, Chen et al. 2008).

This assumption is also supported by the findings that ERα positivity was associated with a more aggressive phenotype of differentiated thyroid cancer and of an eight to ten times higher expression of ERα in undifferentiated, growth-prone thyroid stem and progenitor cells than in differentiated thyrocytes (Magri et al. 2012, Xu et al. 2013).

### ER expression and outcome of thyroid cancer in pregnancy

During pregnancy, iodine deficiency, the TSH receptor-stimulating effect of hCG and high estrogen levels are believed to promote growth of benign and malignant nodules (Kimura et al. 1990). However, there are only a few studies that have assessed the outcome of thyroid cancer, the overall survival, and the DTC-related death in pregnancy (recently reviewed by Alves et al. 2011). Remarkably, no differences in survival, recurrence, or death were observed between pregnancy-associated thyroid cancer and cancer in aged-matched non-pregnant women (Herzon et al. 1994, Moosa & Mazzaferri 1997, Yasmeen et al. 2005).

By contrast, a more recent study has found a significantly higher rate of recurrent or persistent disease in pregnant women (Vannucchi et al. 2010). These authors demonstrated a higher expression of ERα immuno-reactivity in tumor samples from pregnant women than in those from controls. Two recent studies have confirmed the clinical outcome of this study but not the increased ERα expression: Shindo et al. (2014) reported on the enlargement of a papillary thyroid microcarcinoma in four out of nine pregnant patients (44%). However, in all three patients who underwent surgery ER expression was not detectable. In the other study, which included 38 pregnant patients, DTC persistence and recurrence were significantly higher in pregnant than in non-pregnant women (Messuti et al. 2014). But, no differences among the groups were observed regarding ER expression patterns, NIS expression, and BRAF mutations. The results of the two studies do not indicate a role of ER expression in thyroid cancer during pregnancy.

### ERs and epigenetic regulation in thyroid cancer

The reasons for the absence of ERα expression in some immunohistochemical studies of differentiated and undifferentiated thyroid cancers or its restriction to few samples remain unclear.

A most likely explanation is that ER and ER-targeted genes in thyroid tumors are also controlled by epigenetic pathways, as has demonstrated for other cancers including breast cancer (recently reviewed by Hervouet et al. 2013). Epigenetic pathways regulate gene transcription by DNA methylation and post-translational modification of histones. DNA methylation may silence the promoter of the ESR1 gene that encodes ERα, which occurs in the pathogenesis of different cancers (Yao et al. 2010, Wei et al. 2012). In breast cancer, DNA hypermethylation is a major reason for the loss of ERα expression and confers a poor prognosis to this tumor (Ramos et al. 2010).
Estrogen and angiogenesis in thyroid cancer

Neovascularization that increases the local blood supply is a prerequisite for any growing tumor. While estrogen and other growth factors stimulate angiogenesis in endothelial cells, inhibition of ER results in decreased angiogenesis (Losordo & Isner 2001).

VEGF has the capacity to induce angiogenesis by detachment of quiescent endothelial cells from their parental vessels followed by migration of the cells into the neighboring stroma (Krock et al. 2011). In vitro thyroid cancer cells secreted VEGF in response to estrogen treatment (Kamat et al. 2011). Incubation of human umbilical vein endothelial cells with a conditioned medium from estrogen-treated cancer cells resulted in angiogenesis-associated events such as tubulogenesis and migration.

VEGF expression or its overexpression has been reported in samples from differentiated, anaplastic, and metastatic thyroid carcinomas (Viglietto et al. 1995, Klein et al. 1999). In comparison with normal thyroid cells, higher TSH-stimulated VEGF expression and secretion were reported for thyroid carcinoma cells (Soh et al. 1996, 1997). Remarkably, in tumor tissues, the number of blood vessels and the tumor size were directly related to the VEGF concentrations (Soh et al. 1997, Fenton et al. 2000). In contrast, others described higher VEGF expression in the surrounding tissues than in cancer tissue (Kansakar et al. 2009).

Estrogen and metastasis of thyroid cancer

In breast cancer, it was demonstrated that the three different steps of metastasis, adhesion, invasion, and migration, are controlled by estrogen (Malek et al. 2006, Planas-Silva & Waltz 2007, Baranwal & Alahari 2009). Analogously, through downregulation of β-catenin, E2 also affected the metastatic phenotype of thyroid cancer cells by enhancing their adhesion, migration, and invasiveness (Rajoria et al. 2010). Downregulation of proteins of the cadherin–catenin complexes correlated with increased proliferation, adhesion, migration, and invasion of cancer cells in vitro and in vivo (Cheng et al. 2008, Kalluri & Weinberg 2009).

In breast cancer, estrogen expanded cancer stem cells and enhanced their potential for tumor sphere formation (Fillmore et al. 2010). Interestingly, when cancer-stem-cell-enriched thyrospheres derived from an undifferentiated, metastatic thyroid cancer were transplanted onto nude mice, a metastatic disease was generated in the mice (Todaro et al. 2010). This indicates the presence of both cancer stem cells and metastatic cancer stem cells in undifferentiated thyroid cancer (Derwahl 2011). There is some evidence that a small subpopulation of cancer stem cells is critical for metastatic colonization, the initial expansion of cancer cells at the secondary site (Malanchi et al. 2011). Whether or not estrogen is involved in the growth regulation of metastatic cancer stem cells is still unknown.

Concluding remarks

Estrogen and its receptors play a pivotal role in the pathogenesis and progression of cancers in females, particularly breast cancer. In contrast, in thyroid cancer, there is still a substantial gap in understanding the significance of estrogen and ERs in the pathogenesis and progression of this tumor.

Certainly, epidemiological and clinical studies demonstrated a three to four times higher prevalence of differentiated thyroid cancer in females than in males and an increase in the incidence of this tumor with the onset of puberty in females is well documented (Farahati et al. 1997, Dean & Gharib 2008, Li et al. 2013). Furthermore, there is some recent evidence for the progression of papillary microcarcinoma during pregnancy and a higher recurrence rate of thyroid cancer due to pregnancy (Messuti et al. 2014, Shindo et al. 2014). Results from all these studies have indicated that estrogen and its receptors are relevant to the regulation of thyroid cancer growth.

In fact, results from in vitro stimulation experiments unanimously support this view. As outlined above, however, in contrast to these data, there are numerous, in part, contradictory results showing widely varying ERα and ERβ expression patterns, loss of expression of one or the other form or overexpression of one of them in thyroid cancer tissues.

To clarify these apparent contradictions, it must be kept in mind that the current state of expression patterns of a receptor may not allow conclusions to be drawn regarding its role in the pathogenesis of a tumor. This is very well known for TSH and its receptor, whose expression is decreased or may even be absent in thyroid cancer. Nevertheless, TSH is a major regulator of thyroid growth and also involved in the regulation of thyroid cancer proliferation, which justifies a TSH-suppressive treatment in patients with differentiated thyroid cancer.

In addition, the high complexity of ER expression and variants, with multiple phosphorylation sites and different subcellular localizations, their probable interactions with...
miRNAs, their epigenetic modifications, and the putative influence of SNPs of ERs have to be taken into account. All these molecular mechanisms are operative in breast cancer and affect the pathogenesis and progression of this tumor (recently reviewed by Burns & Korach 2012). It is scarcely conceivable that such molecular mechanisms, at least in part, are not operative in the pathogenesis, recurrence, and metastasis of thyroid cancer.

In conclusion, there is strong evidence that estrogen is involved in the pathogenesis of thyroid cancer in females. However, our knowledge about the influence of this hormone on this tumor is still very limited. The comparison of ER expression patterns alone may not allow conclusions to be drawn regarding the relevance of estrogen and its receptors to the pathogenesis of thyroid cancer in females. More detailed studies are necessary to fill this gap, which may have considerable consequences for the treatment of thyroid cancer in females.

Declaration of interest
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References


Fierabracci A, Puglisi MA, Giuliani L, Mattarocci S & Gallinella-Muzi M 2008 Identification of an adult stem/progenitor cell-like population in


Thyroid 22 151–156. (doi:10.1089/thy.2011.0309)


Thyroid 22 151–156. (doi:10.1089/thy.2011.0309)


Thyroid 22 151–156. (doi:10.1089/thy.2011.0309)


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