Review Article
Role of Estrogen in Thyroid Function and Growth Regulation

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Thyroid diseases are more prevalent in women, particularly between puberty and menopause [1], and women are more susceptible to the goitrogenic effect of iodine deficiency [2]. Carcinomas of the thyroid are three-times more frequent in women than in men, and the peak rates occur earlier in women [3]. These epidemiological data suggest a role of estrogen in the pathogenesis of thyroid diseases.

Estrogen has a well-known indirect effect on thyroid economy, increasing the thyroxine binding globulin [4], and the need for thyroid hormone in hypothyroid women [5]. Direct effects of estrogen on thyroid cells have been described more recently [6], so the aim of the present paper was to review the evidences of these effects on thyroid function and growth regulation, and its mechanisms.

1. Introduction

Thyroid diseases are more prevalent in women particularly between puberty and menopause [1], and women are more susceptible to the goitrogenic effect of iodine deficiency [2]. Carcinomas of the thyroid are three-times more frequent in women than in men, and the peak rates occur earlier in women [3]. These epidemiological data suggest a role of estrogen in the pathogenesis of thyroid diseases.

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2. Estrogen and Its Receptors

17-β-estradiol (E2) is a lipophilic hormone with low-molecular weight that occurs naturally. Cellular signaling of estrogen is mediated classically upon the binding on two soluble intracellular nuclear receptors, estrogen receptor (ER) alpha, and ER beta [7]. The isoform β is smaller than the isoform α, and the DNA-binding domains of both subtypes are highly conserved. After binding of E2, ER forms a stable dimer that interacts with specific sequences called estrogen response elements (EREs) to initiate the transcription of target genes. Ligand-bound ERs can also interact with other transcription factors complexes and influence transcription of genes that do not harbor EREs. Third and fourth mechanisms of ERs regulatory actions are, respectively, non-genomic and the ligand independent pathway. A variety of rapid signaling events such as activation of kinases and phosphatases and increases in ion fluxes across membranes has been described. These and other aspects of signaling and targets of ERs have been reviewed recently [7].

Recently, a transmembrane intracellular nonclassical ER mediating rapid cell signaling was described, a G protein-coupled receptor (GPCR), named GPR30 [8].

2.1. Expression of ERs in Human Thyroid Tissue. Classically, the presence of ER is fundamental for a direct action of estrogen in a given cell. ER has been described in both neoplastic and nonneoplastic human thyroid tissues, but the results are discordant. Immunohistochemical assays,
| Study                          | Method | Normal | All benign lesions | All neoplastic lesions | All carcinoma | Benign lesions | Carcinoma |
|-------------------------------|--------|--------|--------------------|------------------------|---------------|----------------|-----------|
|                              |        |        |                    |                        |               | Adenoma        | Goiter    | Papillary | Follicular | Medullary | Anaplastic |
| Tavangar et al. [10]; 2007    | IHC    |        | 8/37               | 31/130                 | 37/119        | 2/18           | 0/35      | 0/12      |
| Arain et al. [11]; 2003       | IHC    | 0/25   | 0/9                | 0/8                    | 0/19          | 0/10           | 0/4       |
| Lewy-Trenda et al. [12]; 1998| IHC    |        | 2/19               | 0/20                   | 4/8           | 3/5            | 0/4       |
| Valle et al. [13]; 1998       | RT-PCR | 28/33  | 12/12              | 6/7                    | 26/26         | 1/1            | 1/1       | 1/1       |
| Bonacci et al. [14]; 1996     | DCC    | 26/38  | 11/28              |                        | 7/20          |                |           |
| Jaklic et al. [15]; 1995      | IHC    | 0/1    | 0/5                | 0/4                    | 0/4           |                |           |
| Colomer et al. [16]; 1996     | IHC    |        |                    |                        |               |                |           |
| Inoue et al. [17]; 1993       | IHC    |        |                    |                        | 24/74         |                |           |
| Inoue et al. [18]; 1993       | IHC    |        |                    |                        | 18/70         |                |           |
| Yane et al. [19]; 1994        | RT-PCR |        |                    |                        | 5/27          |                |           |
| Yane et al. [20]; 1993        | IHC    | 0/10   | 2/19               | 2/12                   | 0/7           |                |           |
| Hiasa et al. [21]; 1993       | IHC    |        | 44/130             | 23/39                  | 19/115        | 7/23           | 0/6       |
| Diaz et al. [22]; 1991        | IHC    |        | 20/30              |                        | 23/30         | 11/20          |           |
| Mizukami et al. [23]; 1991    | IHC    | 8/18   | 4/8                |                        | 47/62         |                |           |
| Takeichi et al. [24]; 1991    | IHC    |        |                    |                        | 11/12         |                |           |
| Hong et al. [25]; 1991        | IHC    |        |                    |                        | 1/27          | 1/20           |           |
| Miki et al. [26]; 1990        | DCC    | 0/14   | 12/46              |                        | 7/23          | 5/11           | 2/12      | 6/20      | 0/1       | 1/1       |
| Haruta et al. [27]; 1990      | IHC    |        |                    |                        | 30/52         |                |           |
| Chaudhuri et al. [28]; 1989   | SDG    | 3/8    |                    |                        | 7/9           | 5/23           | 8/8       | 0/6       |
| Money et al. [29]; 1989       | IHC    |        | 20/22              |                        |               |                |           |
| Clark et al. [30]; 1985       | SDG    |        |                    |                        | 14/15         |                |           |
| Hampl [15]; 1985              | RBA    | 0/8    |                    |                        | 0/5           |                |           |
| Molteni et al. [37]; 1981     | SDG    | 0/2    |                    |                        | 2/4           |                |           |

Data are shown as number of ER-positive samples/total number of samples. IHC: immunohistochemical assay; DCC: dextran-coated charcoal assay; RT-PCR: reverse transcriptase-polymerase chain reaction technique; SDG: sucrose density gradient assay; RBA: radioligand binding assay.
Table 2: Estrogen receptors (ER) α and β in human normal thyroid, and benign and malignant thyroid diseases, by immunohistochemistry (IHC).

| Study                      | Isoform | All benign | All carcinoma |
|----------------------------|---------|------------|---------------|
|                            |         | Benign lesions | Carcinoma       |
|                            |         | Adenoma | Goiter | Papillary | Follicular | Medullary | Anaplastic |
| Vaiman et al. [31]; 2010   | ERα     | 0/34     | 0/150   | 0/90      | 0/6      | 0/4       | 0/5        |
|                            | ERβ     | 30/34    | 126/150 | 60/90     | 4/6      | 3/4       | 3/5        |
| Winters et al. [32]; 2010  | ERα     | 1/1      | 10/11   | 8/11      |          |           |            |
| Vannuchi et al. [33]; 2010 | ERα     | 12/38    |          |           | 26/28    |           |            |
| Cho et al. [34]; 2007      | ERα     |          | 0/28     |           |          |           |            |
| Bléch et al. [35]; 2007    | ERα     |          | 1/1      | 17/17     | 14/17    |           |            |
| Ceresini et al. [36]; 2006 | ERα     | 0/17     | 10/11   | 8/11      |          |           |            |

Data are shown as number of ER-positive samples/total number of samples.

with monoclonal antibodies, are the most commonly used methods for establishing receptor status. As may be seen in Table 1, some studies have found ER-positivity in normal and abnormal thyroid tissue while others have not detected ER protein in any tissue studied. This discrepancy could be due to methodological issues; the development of monoclonal antibodies against ER with high sensitivity and specificity, and others factors such as tissue fixation, tissue processing, interpretation of immunohistochemistry, and cutoffs for positive results, could have contributed to the sensitivity of the techniques employed [9].

2.2. Expression of ERα and ERβ in Human Thyroid Tissue. ER expression in human thyroid was first reported in 1981 [37]. ERα was first described in 1973 [38], and ERβ was identified in 1996 [39], so only from this moment on it was possible to evaluate the relationship between isoforms of ERs in thyroid tissue. An important role of different patterns of distribution and expression of subtypes ERs in thyroid carcinoma has been proposed: estrogen binding to ERα would promote cell proliferation and growth, and, in contrast, ERβ would promote apoptotic actions and other suppressive functions in thyroid tumors, as reviewed by Chen et al. [40]. Then, ERα:ERβ ratio could have a role in the pathophysiology of thyroid cancer [40], similar to that postulated for breast cancer [41].

In differentiated thyroid follicular tumors, the expression of ERα has been associated with well-differentiated tumors and reduced incidence of disease recurrence [54]. ERα protein [55] and ERα mRNA [19, 56] are expressed in normal and neoplastic follicular cells of the thyroid. Also, the expression of ERα and ERβ was detected in human medullary thyroid cancer [34] with an increased ratio of ERα/ERβ, suggesting a possible role in tumor growth and progression. A few studies evaluated ERα and ERβ expression in normal and abnormal thyroid tissue, as shown in Table 2.

The effects of the agonists of ERα and ERβ, respectively, propyl-pyrazole-triol (PPT) and diarylpropionitrile (DPN), in the proliferation of thyroid cancer cell lines has been studied: PPT had a stimulatory effect, while inhibition of proliferation and DNA fragmentation were observed after DPN [45]. In the same study, small interference ribonucleic acid (siRNA) blocking ERα or ERβ demonstrated that knockdown of the ERα attenuated E2-mediated B-cell lymphoma 2 (Bcl-2) expression, an important antiapoptotic protein, while knockdown of the ERβ enhanced E2-induced Bcl-2 expression [45].

2.3. Expression of GPR30 in Thyroid Cells Lines. Growing evidence suggests that estrogens are also able to exert non-genomic events mediated by GPR30 [8]. Vivacqua and colleagues analyzed the effects of E2 and the phytoestrogen genistein in human follicular thyroid carcinoma cell lines, WRO and FRO, and ARO, a human anaplastic thyroid carcinoma cell line [46]. Both hormones stimulated in vitro proliferation of these cell lines through the GPR30 and mitogen-activated protein kinase signaling cascade [46]. In other human benign and malignant thyroid tissue, the expression of GPR30 has not been studied.

3. Response to E2 Stimulation In Vitro

3.1. Proliferation. Several studies described proliferation of thyroid cells induced by E2, as shown in Table 3. Some of the most commonly used assays are incorporation of bromodeoxyuridine (BrdU) [6], 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) [45, 47, 50, 57], [(3)H]-thymidine incorporation [48, 52, 53], and trypan blue solution [43]. Cotreatment with ICI182780, fulvestrant, an antagonist of E2 by inhibition and degradation of ER [58], significantly attenuated these proliferative effects.

Based in these studies, E2 increases proliferation of thyroid cells.

3.2. ER-Dependent Effects on Thyroid Differentiation Proteins. Few studies evaluated E2 effect on gene transcription of
differentiation proteins in thyroid cells. In Fischer rat derived thyroid cell line, FRTL-5, E2 treatment decreased the sodium-iodide symporter (NIS) gene expression [50], and the iodide uptake [49]. E2 increased the thyroglobulin gene expression in suspension cultures of human thyroid follicles of adenoma and carcinoma [52]. These data are shown in Table 3. The opposite effects of E2 on the NIS gene expression and iodide uptake, in FRTL-5 cells, and the thyroglobulin gene expression, in suspension culture of thyroid cells, could be due to the different systems studied; it cannot be excluded that estradiol affects these genes by different intracellular pathways. These results, together with the increase in cell growth caused by estrogen, could implicate this hormone in the pathogenesis of goiter and thyroid carcinoma; nevertheless, as just one study evaluated the effect of estrogen on thyroid differentiated proteins in human thyroid tissue, more studies should be done to better understand the role of estrogen in thyroid differentiated protein expression.

3.3. Non-Genomic Effects of E2. Some of the actions of E2 in the proliferation of thyroid cells are mediated by the activation of signal transducing pathways, as shown in

Table 3: E2 effects on thyroid protein expression, function, and proliferation in vitro.

| Study                  | Thyroid cells | Presence of ERα/ERβ | Erα expression | Erβ expression | Proliferation | Nis expression | Iodide uptake | TG mRNA |
|------------------------|---------------|---------------------|----------------|----------------|---------------|----------------|---------------|---------|
| Kumar et al. [42]; 2010| NPA87         | ERα+/ERβ+           | §              | §              |               | §              | §             | §       |
| KAT5                   | ERα+/ERβ+     | §                   | §              | §              | §             | §              | §             | §       |
| WRO                    | ERα+/ERβ+     | §                   | §              | §              | §             | §              | §             | §       |
| Rajoria et al. [43]; 2010| BCPAP        | ERα+/ERβ+           | §              | §              | §             | §              | §             | §       |
| Nthy-3-1               | ERα+/ERβ+     | §                   | §              | §              | §             | §              | §             | §       |
| Zeng et al. [44]; 2008 | KAT5          | ERα+/ERβ+           | §              | §              | §             | §              | §             | §       |
| FRO                    | ERα+/ERβ+     | §                   | §              | §              | §             | §              | §             | §       |
| Zeng et al. [45]; 2007 | KAT5          | ERα+/ERβ+           | §              | §              | §             | §              | §             | §       |
| FRO                    | ERα+/ERβ+     | §                   | §              | §              | §             | §              | §             | §       |
| ARO                    | ERα+/ERβ+     | §                   | §              | §              | §             | §              | §             | §       |
| Vivacqua et al. [46]; 2006| WRO        | ERα+/ERβ−           | §              | §              | §             | §              | §             | §       |
| FRO                    | ERα+/ERβ−     | §                   | §              | §              | §             | §              | §             | §       |
| ARO                    | ERα−/ERβ−     | §                   | §              | §              | §             | §              | §             | §       |
| Lee et al. [47]; 2005  | KAT5          | §                   | §              | §              | §             | §              | §             | §       |
| Banu et al. [48]; 2001 | NPA87         | ER+                 | §              | §              | §             | §              | §             | §       |
| WRO                    | ER+           | §                   | §              | §              | §             | §              | §             | §       |
| Manole et al. [6]; 2001| HTC-TSHr      | ERα+/ERβ+           | §              | §              | §             | §              | §             | §       |
| Goiter                 | ERα+/ERβ+     | §                   | §              | §              | §             | §              | §             | §       |
| XTC-133                | §             | §                   | §              | §              | §             | §              | §             | §       |
| Furlanetto et al. [49]; 2001| FRTL-5    | §                   | §              | §              | §             | §              | §             | §       |
| Furlanetto et al. [50]; 1999| FRTL-5  | §                   | §              | §              | §             | §              | §             | §       |
| Nagy et al. [51]; 1999*| Mng           | §                   | §              | §              | §             | §              | §             | §       |
| Ca                     | §             | §                   | §              | §              | §             | §              | §             | §       |
| Ade                    | §             | §                   | §              | §              | §             | §              | §             | §       |
| Del Senno et al. [52]; 1989**| N        | §                   | §              | §              | §             | §              | §             | §       |
| Ade                    | §             | §                   | §              | §              | §             | §              | §             | §       |
| Ca                     | 0             | 0                   | §              | §              | §             | §              | §             | §       |
| Yang et al. [53]; 1988  | TT            | §                   | §              | §              | §             | §              | §             | §       |

Estrogen receptor (ER) +: presence of expression, –: absence of expression; NPA87, KAT5, and BCPAP: human papillary thyroid carcinoma cell lines; WRO and FRO: human follicular thyroid carcinoma cell lines; Nthy-3-1: human normal transformed thyroid cell line; ARO: human anaplastic thyroid carcinoma cell line; HTC-TSHr: human thyroid carcinoma cell line lacking endogenous TSH receptor; XTC-133: thyroid cancer cell line of Hurthle cell origin; FRTL-5: Fischer rat thyroid cell line. Mng: multinodular goiter; Ca: carcinoma; Ade: adenoma; N: normal thyroid; TT: human medullary thyroid carcinoma cell line; §: increase, §: decrease, and 0: no effect, after E2 exposure. *: thyroid tissue obtained in surgical resection, under organotypic culture conditions for 48 hours; **: suspension cultures of thyroid follicles.
Table 4: Non-genomic estrogen effects on thyroid cells.

| Study                          | Cells   | GPR30 | MAPK | PI3k | Cyclin D1 | c-fos | Bcl-2 | Bax |
|-------------------------------|---------|-------|------|------|-----------|-------|-------|-----|
| Kumar et al. [42]; 2010       | NPA87   | −     | +    | −    | +         | −     | +     | +   |
|                               | KAT5    | −     | +    | −    | +         | −     | +     | +   |
|                               | WRO     | +     | +    | −    | −         | −     | +     | +   |
| Zeng et al. [45]; 2007        | KAT5    | +     | +    | −    | +         | −     | +     | +   |
|                               | FRO     | +     | +    | −    | +         | −     | +     | +   |
|                               | WRO     | +     | +    | −    | +         | −     | +     | +   |
| Vivacqua et al. [46]; 2006    | WRO     | +     | +    | −    | +         | −     | +     | +   |
|                               | FRO     | +     | +    | −    | +         | −     | +     | +   |
|                               | ARO     | +     | +    | −    | +         | −     | +     | +   |
| Manole et al. [6]; 2001       | HTC-TSHr| +     | +    | −    | +         | −     | +     | +   |
|                               | Goiter  | +     | +    | −    | +         | −     | +     | +   |
|                               | XTC-133 | +     | +    | −    | +         | −     | +     | +   |

NPA87 and KAT5: human papillary thyroid carcinoma cell lines; WRO and FRO: human follicular thyroid carcinoma cell lines; HTC-TSHr: human thyroid carcinoma cell line lacking endogenous TSH receptor; XTC-133: thyroid cancer cell line of Hurthle cell origin; Goiter: primary culture of human thyroid cells isolated from goiter nodules. (+): presence of expression; (−) absence of expression; (↑): increase, (↓): decrease, and (0): no effects, after E2 exposure.

Table 4. E2 can induce activation of phosphatidylinositol 3-kinase (PI3K) [42] and phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2) in follicular thyroid carcinoma cells, mainly due to interaction via membrane-associated ER [42, 45, 46]. PI3K and Erk1/2 signaling may play a critical role in preventing apoptosis and inducing cell cycle progression by induction of key genes expression [59].

Expression of early response genes and regulatory genes of the cell cycle are necessary for proliferation of cells. As E2 has been demonstrated to stimulate the growth of thyroid cells, it is important to study the expression of key cell-cycle genes such as cyclin D1 after stimulation with E2. Cyclin D1 regulates the cell progression cycle facilitating G1 to S phase transition and also has an estrogen-responsive regulatory region [60], that is likely different from the canonical ERs. Overexpression of cyclin D1 in thyroid malignancies has been reported [61–65], moreover, its expression has been associated with an aggressive behavior in papillary thyroid microcarcinomas, because over 90% of the metastasizing microcarcinomas expressed cyclin D1 [66].

E2 significantly increased the expression of cyclin D1 in a human thyroid carcinoma cell line lacking endogenous TSH receptor (HTC-TSHr cells), and in a thyroid cancer cell line of Hurthle cell origin (XTC-133), which was abolished by PD.098059 that blocked G0/G1 to S phases [6]. E2 upregulated cyclins A and D1, as well as the proto-oncogene c-fos, in WRO, FRO, and ARO cells [46]. Cyclin D1 was also shown to be upregulated by E2 in KAT5, a papillary thyroid cancer cell line, and WRO cells [42].

Together, these results are very compelling, pointing to an ability of E2 to regulate genes mediating cell cycle progression in thyroid cells, and potentially contributing to the pathogenesis of thyroid cancer or thyroid hyperplasia.

4. Conclusions

There are evidences that estrogen may have direct actions in human thyroid cells by ER-dependent mechanisms or not, modulating proliferation, and function. Different patterns of distribution, expression, and ratios of ERα and ERβ may have a role in thyroid cancer cells proliferation, as well as in the outcome of thyroid cancer. Studying estrogen effects on thyroid cells is a potential tool to better understand the pathogenesis of thyroid diseases, and to develop targets to its treatment. Further studies on the influence of E2 on the growth and function of the thyroid are needed, preferably in primary culture of normal and abnormal human thyroid cells.

Conflict of Interests

The authors declare that there is no conflict of interests.

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