

Research Article

Thyroid Peroxidase Identified in Human Granulosa Cells: Another Piece to the Thyroid-Ovary Puzzle?

Monteleone P^{1*}, Faviana P² and Artini PG³¹Department of Reproductive Medicine and Child Development, University of Pisa, Italy²Department of Surgical, Medical and Molecular Pathology and Critical Care, University of Pisa, Italy³Department of Experimental and Clinical Medicine, University of Pisa, Italy

***Corresponding author:** Monteleone P, Department of Reproductive Medicine and Child Development, University of Pisa, Pisa, USL Nordovest, Ospedale San Francesco, Barga (LU), Italy

Received: September 23, 2016; **Accepted:** November 09, 2016; **Published:** November 10, 2016

Abstract

Thyroid hormones seemingly influence the maturation of the human oocyte. Thyroid hormone receptors have been isolated in granulosa mural and cumulus cells and the mature oocyte of the human ovarian follicle. Thyroid hormones are present in follicular fluid in concentrations similar to those in serum. Most importantly, enzymes involved in the chain that regulate the generation of thyroid hormones have been found in granulosa cells. For the first time we have isolated thyroid peroxidase by immunocytochemistry in the granulosa cumulus cells of the human ovarian follicle, thereby supporting the hypothesis that the human ovarian follicle may be an independent thyroid-hormone producing unit.

Keywords: Thyroid peroxidase; Ovary; Granulosa cells; Follicle development

Introduction

Thyroid hormones support nearly every body system and in humans they guarantee normal development of the brain, skeleton, and organs. It seems that this important role is exerted ever since the first stages of life, even before the conceptus can produce its own thyroid hormones. Studies on humans have reported the presence of thyroid hormones, Thyroxine (T₄) and Triiodothyronine (T₃) in the fluid of ovulatory follicles, with levels within the normal range for serum [1,2]. TSH was found in human follicular fluid, in concentrations similar to, or above, that in serum, has been reported [3] and oocytes, granulosa cells, surface epithelial cells and stromal cells of healthy human ovaries have been found to express TSH receptor [4].

Luteinized granulosa cells from healthy young women have been found positive for alpha-1 and beta-1 Thyroid hormone Receptors (TR) [1]. RT-PCR amplification has shown that human mature oocytes and granulosa mural and cumulus cells express TR α 1 and TR β 1, TR β 2 [5]. More recent studies confirmed the presence of thyroid hormone receptors in human ovarian surface epithelium [6]. Aghajanova and colleagues [4] performed a more detailed study showing that TR α 1, TR α 2 and TR β 1 proteins are not expressed in granulosa cells of primordial and primary follicles, while antral follicle granulosa cells express low amounts of TR α 1 and moderate amounts of TR β 1 and granulosa cells of secondary follicles express TR β 1. This suggests a very fine regulation of thyroid hormone responsivity in various stages of follicle maturation. Positive faint-to-moderate immunostaining was found for TR α 1 and TR β 1 proteins in human oocytes [4]. These findings together allow to hypothesize that T₃ may influence oocyte maturation by acting directly upon the oocyte itself or by influencing granulosa cell activity.

Ovarian granulosa cells may not only be programmed to respond to thyroid hormones, however. Rae and colleagues found that human ovarian surface epithelium expresses enzymes that generate T₃ from T₄ and that inactivate T₃ to reverse T₃ in thyroid hormone target tissues [6]. Deiodinase type 2 and deiodinase type 3 enzymes are

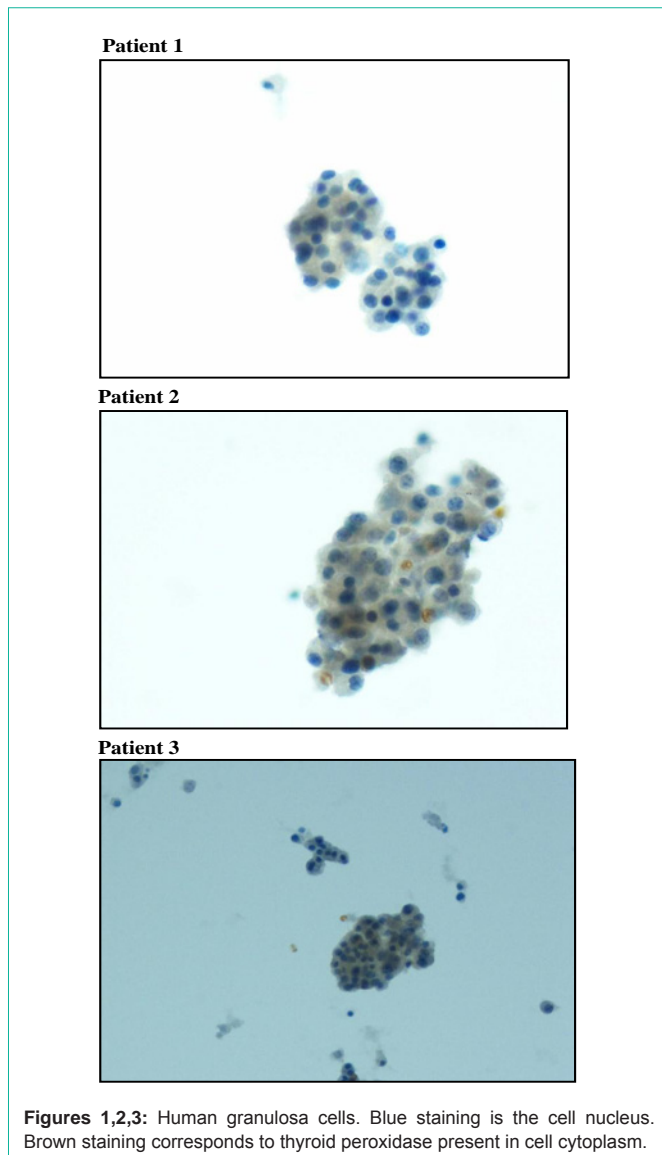
expressed in luteinized human granulosa cells [4], giving these cells the ability to regulate the conversion of T₄ to either T₃ or reverse T₃, locally controlling thyroid hormone activity [4].

Aim of the study

Thyroid peroxidase is important for the incorporation of iodine into thyroglobulin to produce thyroid hormone and its expression is upregulated by TSH, to which granulosa cells are sensitive. Because it is becoming increasingly clear that thyroid hormones not only act upon components of the ovarian follicle but may be produced inside this unit, the aim of the present study was to test whether thyroid peroxidase is present in human granulosa cells of the cumulus oophorus.

Materials and Methods

Human cumulus granulosa cells were collected after denudation of human oocytes obtained by follicular aspiration in three patients undergoing *in vitro* fertilization at the Department of Reproductive Medicine and Child Development of the University of Pisa. The cells were collected in formalin and delivered to the Department of Pathology of the University of Pisa. Cytological sections were stained with hematoxylin and eosin for cytological examination. Immunocytochemistry was performed on formalin fixed, paraffin-embedded cells, using previously validated protocols for the antibody. Cell sections were deparaffinized in xylene and rehydrated in a graded ethanol series. Slides were stained using a diaminobenzidine detection system preceded, only for anti-TPO, by heat-induced epitope retrieval involving immersion of cells in a pre-warmed buffer solution (Target Retrieval Solution, DakoCytomation, Carpinteria, CA) and maintaining heat in a steamer at 98°C for 50 min. To reduce nonspecific staining caused by endogenous biotin, the Endogenous Biotin Blocking Kit (Ventana Medical Systems, SA, Illkirch, Cedex, France) was employed, according to Manufacturer's instructions. Anti-TPO immunostaining was performed using monoclonal antibodies by Abcam (1:100 dilution). Negative controls were obtained by omission of the primary antibodies.



Figures 1,2,3: Human granulosa cells. Blue staining is the cell nucleus. Brown staining corresponds to thyroid peroxidase present in cell cytoplasm.

Results

Immunocytochemistry staining revealed the presence of thyroid peroxidase in all the examined samples of human granulosa cell. Staining scores were determined by two pathologists. All cells showed intense and ubiquitous cytoplasmic TPO-positive staining, greater than 80%, indicating the important role of this enzyme in granulosa cells of the mature ovarian follicle.

Discussion

Thyroid peroxidase assists the chemical reaction that adds iodine to thyroglobulin, a critical step in generating thyroid hormones, thyroxine and triiodothyronine. Thyroid hormones, in particular T₃, may play an important role in ovarian folliculogenesis and oocyte maturation. Indeed, earlier *in vitro* studies have shown that T₃ can synergize with chorionic gonadotrophin [7] to stimulate granulosa cell proliferation, and with follicle stimulating hormone to induce differentiation of granulosa cells from porcine follicles [8]. In particular, T₃ was demonstrated to enhance FSH action and increase

LH/hCG receptor formation and induce steroidogenic enzymes 3 beta-hydroxysteroid dehydrogenase and aromatase in granulosa cells from porcine follicles [8]. The ovary can uptake iodine from the blood circulation. In particular, small and growing follicles take up more iodine than large ones indicating that the presence of this molecule is crucial for follicular development [9]. Thyroid receptors are present in granulosa cells of primordial, primary, and secondary follicles [4]. Mature oocytes are also positive TR α 1, TR α 2, TR β 1, and TR β 2 mRNA [5]. Moreover, the presence of deiodinase types 2 and 3 in granulosa cells indicates a possibility of conversion of peripheral T₄ on ovarian tissue [4].

To date there are no studies verifying the presence of thyroid peroxidase in the granulosa cells of the human ovarian follicle. Although the number of cases is very small, we can affirm to have isolated thyroid peroxidase in mature human granulosa cells of the cumulus oophorus thus indicating that the human ovarian follicle may be independent in producing thyroid hormones.

This finding may also be important to explain reproductive failure often associated to thyroid peroxidase autoimmunity in women. It is increasingly evident that thyroid autoimmunity, rather than thyroid hormone concentrations, may impair IVF cycle outcomes [10,11]. We have found thyroid peroxidase antibodies in follicular fluid at levels similar to those in serum [12]. These antibodies may therefore disrupt the function of thyroid peroxidase, generate an inflammatory response, and alter the milieu of the maturing oocyte. Indeed, it was recently demonstrated that positive thyroid peroxidase antibody status negatively affects embryo quality in euthyroid women, independently of whether TSH is low-normal or high-normal [11].

In conclusion, there are multiple suggestions in the literature that thyroid hormones influence the maturation of the human oocyte. It is becoming increasingly clear that the cellular components of the human ovarian follicle can produce these substances on their own and may not need to rely on supply from blood circulation. Most studies regarding thyroid hormone production in human granulosa cells, including ours, have been carried out in cells from gonadotropin-stimulated cycles. Further research needs to be done during natural cycles, possibly sampling follicles at different stages of maturation. At the same time, follicular cells may be targeted by thyroid peroxidase antibodies and their activity upset during such an important and delicate process as oocyte maturation.

References

1. Wakim AN, Paljug WR, Jasnosz KM, Nawar Alhakim, Anne B Brown, Dennis R Burholt. Thyroid hormone receptor messenger ribonucleic acid in human granulosa and ovarian stromal cells. *Fertility and Sterility*. 1994; 62: 531–534.
2. Wakim AN, Polizotto SL, Buffo MJ, Marrero MA, Burholt DR. Thyroid hormones in human follicular fluid and thyroid hormone receptors in human granulosa cells. *Fertility and Sterility*. 1993; 59: 1187–1190.
3. De-Silva M. Detection and measurement of thyroid stimulating hormone in human follicular fluid. *J Reprod Med*. 1994; 39: 679–680.
4. Aghajanova L, Lindeberg M, Carlsson IB, Stavreus-Evers A, Zhang P, Scott JE, et al. Receptors for thyroid-stimulating hormone and thyroid hormones in human ovarian tissue. *Reprod Biomed Online*. 2009; 18: 337-347.
5. Zhang SS, Carrillo AJ, Darling DS. Expression of multiple thyroid hormone receptor mRNAs in human oocytes, cumulus cells, and granulosa cells. *Molecular Human Reproduction*. 1997; 3: 555–562.
6. Rae MT, Gubbay O, Kostogiannou A, Price D, Critchley HOD, Hillier SG.

- Thyroid hormone signaling in human ovarian surface epithelial cells. *Journal of Clinical Endocrinology and Metabolism*. 2007; 92: 322–327.
7. Goldman S, Dirnfeld M, Abramovici H, Kraiem Z. Triiodothyronine (T3) modulates hCG-regulated progesterone secretion, cAMP accumulation and DNA content in cultured human luteinized granulosa cells. *Mol Cell Endocrinol*. 1993; 96: 125-131.
 8. Maruo T, Hayashi M, Matsuo H, Yamamoto T, Okada H, Mochizuki M. The role of thyroid hormone as a biological amplifier of the actions of follicle-stimulating hormone in the functional differentiation of cultured porcine granulosa cells. *Endocrinology*. 1987; 121: 1233-1241.
 9. Slebodziński AB. Ovarian iodide uptake and triiodothyronine generation in follicular fluid. The enigma of the thyroid ovary interaction. *Domest Anim Endocrinol*. 2005; 29: 97-103.
 10. Scoccia B, Demir H, Kang Y, Fierro MA, Winston NJ. *In vitro* fertilization pregnancy rates in levothyroxine-treated women with hypothyroidism compared to women without thyroid dysfunction disorders. *Thyroid*. 2012; 22: 631–636.
 11. Weghofer A, Himaya E, Kushnir VA, Barad DH, Gleicher N. The impact of thyroid function and thyroid autoimmunity on embryo quality in women with low functional ovarian reserve: a case-control study. *Reprod Biol Endocrinol*. 2015; 13: 43.
 12. Monteleone P, Parrini D, Faviana P, Carletti E, Casarosa E, Uccelli A, et al. Female infertility related to thyroid autoimmunity: the ovarian follicle hypothesis. *Am J Reprod Immunol*. 2011; 66: 108-114.