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Estrogen Signaling and Thyrocyte Proliferation

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1. Introduction

The development of thyroid cancer is a multifactorial and multistep process. Several factors are thought to predispose people to thyroid cancer, including genetics, environment, and sex hormones. The incidence of thyroid cancer is three to four times higher in women than in men (Libutti, 2005; Machens et al., 2006). This difference in incidence between genders suggests that the growth and outcome of thyroid tumors may be influenced by female sex hormones, particularly E2, which has been widely implicated in the development and progression of several cancers, such as breast, ovarian and prostate cancer (Arnold et al., 2007; Stender et al., 2007). Animal studies support these epidemiological data, and suggest that exogenous estrogen (17β-estradiol, E2) can promote thyroid tumors (Mori et al., 1990; Thiruvengadam et al., 2003).

Several studies have been carried out to address the role of estrogens in the pathogenesis of proliferative and neoplastic disorders. Although the precise mechanism still remains ill-defined, a range of plausible mechanisms explaining their carcinogenic effects has been proposed. On one hand, estrogens may promote cellular proliferation through their receptor-mediated activity (Arnold et al., 2007; Lee et al., 2005). In addition, the natural estrogen E2 or its metabolites 2- hydroxy, 4-hydroxy, and 16-α-hydroxy-estradiol (2-OH-E2, 4-OH-E2, and 16-α-OH E2) can cause neoplastic transformation through a direct genotoxic effect, increasing the spontaneous mutation rate of normal cells (Cavalieri et al., 1997).

In this review, we will analyze the role of estrogen signaling in the proliferation and transformation of the thyroid gland, with a special emphasis on the cross-talk between estrogen signaling and the PI3K pathway.

2. Thyroid cancer

Thyroid carcinoma is the most common and prevalent of all endocrine malignancies, accounting for more than 95% of all endocrine-related cancers (Hodgson et al., 2004; Jemal et al., 2009). Papillary and follicular carcinomas (PTC and FTC respectively) are differentiated tumors arising from thyroid epithelial cells (thyrocytes), while medullary carcinoma originates from parafollicular cells. PTC is by far the most common type of thyroid cancer, representing up to 80% of all thyroid malignancies. Anaplastic carcinomas are undifferentiated tumors deriving from thyroid epithelial cells. They are usually lethal with no effective system therapy. The factors leading to thyroid carcinoma development are not fully understood despite some well-established associations, such as between ionizing radiation and papillary carcinoma, and between iodine deficiency and follicular carcinoma.

From the molecular point of view, papillary and follicular thyroid cancers are completely different diseases. This notion is supported by dissimilar molecular initiating events leading to neoplastic transformation and by differences in DNA ploidy level (PTCs are generally diploid, FTCs aneuploid) (Handkiewicz-Junak et al., 2010).

Follicular Carcinoma	Papillary Carcinoma	Anaplastic Carcinoma
RAS: 20-50%	*BRAF*: 40-45%	*TP53*: 50-80%
PAX8-PPARγ: 20-35%	*RAS*: 10-20%	*BRAF*: 20-40%
PI3K pathway: 20%	*RET-PTC*: 10-30%	*RAS*: 20-40%
		PI3K pathway: 20-50%

Table 1. Most frequent genetic alterations in thyroid cancer

The genetic alterations found in PTC primarily affect two central signalling pathways in thyroid cells: TSH receptor (TSHR)-mediated signalling and mitogen-activated protein kinase (MAPK) pathways (Kim and Zhu, 2009; Lemoine et al., 1998; Nikiforov, 2008). Three important initiating events, *RET/PTC* (rearranged during transfection/ papillary thyroid cancer), *RAS* (resistance to audiogenic seizures) and *BRAF* mutations, are considered mutually exclusive (Fagin, 2004). BRAF mutation and RET/PTC rearrangements differ to some extent in their effects on the shared oncogenic pathway, resulting more frequently in the classic or the solid variant of PTC, respectively, while RAS mutations are more likely to induce the follicular variant of PTC (Xing, 2005).

Follicular carcinomas are often characterized by *RAS* mutations (up to 50%) and *PAX8-PPARγ* rearrangements (20–35%), which lead to a mutant protein incapable of trans-activating a PPARγ signal (Gilfillan, 2010). Phosphatidylinositol 3-kinase (PI3K)/AKT alterations are frequently found in FTC and, even more distinctly, in ATC. In FTC, phosphorylation of AKT, the key player in this pathway, is by far more frequent than that of ERK (Liu et al., 2008).

Anaplastic thyroid carcinomas (ATCs) comprise 2% of thyroid malignancies, and are usually lethal, with no effective therapy (Are and Shaha, 2006). Dedifferentiation, a common hallmark of ATC, is manifested by a loss of specific thyroid cell characteristics and functions, including expression of thyroglobulin, thyroid peroxidase, thyroid stimulating hormone receptor and the Na/I symporter (Neff et al., 2008; Smallridge et al., 2009). Molecular signature events that characterize ATC involve either *BRAF* activation or sustained hyperactivation of the PI3K/AKT cascade, together with *TP53* loss or inactivation (Kouniavsky and Zeiger, 2010).

3. Physiological functions of estrogen and estrogen receptors

3.1 Estrogen production

Estrogens are a group of steroid compounds acting as the primary female sex hormones. Estrogens regulate several physiological processes, including cell growth and development, not only in the reproductive tract but also in other tissues such as bone, brain, liver, cardiovascular system, and endocrine glands.

Although estrogens are present in both men and women, their levels are significantly higher in women of reproductive age. They are mainly produced by the adrenal cortex and ovary. The three major naturally occurring estrogens in women are: estrone, estradiol and estriol (Speroff et al., 1999). In premenopausal women, 17β-estradiol (E2), produced by the ovary, is the estrogen formed in the largest quantity and is the most potent since it has the highest affinity for estrogen receptors. In premenopausal women, the level of circulating E2 varies from 40 to 400 pg/mL during the menstrual cycle (Ruggiero et al., 2002). After menopause, the level of E2 drops to less than 20 pg/mL (Jones, 1992). The second endogenous estrogen is estrone (E1), a less potent metabolite of E2. Estrone is produced from androstenedione in adipose tissue. In postmenopausal women, the ovary ceases to produce E2 while the adrenal gland continues to produce androstenedione, with the result that the level of estrone remains unchanged while the level of E2 falls significantly. The third endogenous estrogen is estriol (E3), also a metabolite of E2. E3 is the main estrogen produced by the placenta during pregnancy, and is found in smaller quantities than E2 and E1 in nonpregnant women (Jones, 1992; Ruggiero et al., 2002).

3.2 Estrogen receptors and their ligands

The actions of estrogens occur through activation of estrogen receptors (ERα, ERβ and GPR30). ERα was initially described in 1973 (Jensen and De Sombre, 1973) while ERβ was identified much later (Kuiper et al., 1996). ERα and ERβ are encoded by separate genes, *ESR1* and *ESR2*, respectively, which share similarities in the DNA-binding domain (97% amino acid similarity) and ligand-binding domain (60% amino acid similarity) (Hall et al., 2001). These two ERs differ in their tissue distributions (Kuiper et al., 1997; Dechering et al., 2000), suggesting that ERα and ERβ might have different physiological functions. It has also been demonstrated that in many systems the activity of ERβ is opposed to that of ERα. For example, in breast cancer cells, ERα is the receptor responsible for E2-induced proliferation, whereas activation of ERβ inhibits this effect (Strom et al., 2004). In the uterus, E2 induces proliferation of both epithelial and stromal cells through ERα, which is the predominant ER in the mature organ, while in the immature uterus, ERα and ERβ are found at similar expression levels in both epithelium and stroma, and ERβ mediates the action of E2 as a suppressor of cell proliferation against activation of ERα by E2 (Weihua et al., 2000).

G protein-coupled receptor 30 (GPR30), a novel transmembrane ER, was identified in different cells by four laboratories between 1996 and 1998 (Takada et al., 1997; Owman et al., 1996; Carmeci et al., 1997; O'Dowd et al., 1998). Since its ligand was unknown at that time, it was named based on its homology to the G protein-coupled receptor (GPCR) super-family. In addition, this receptor was found to be associated with ER expression in breast cancer cell lines (Carmeci et al., 1997). Later in 2000, Filardo et al. demonstrated that estrogen promptly activated ERK1/2 in two breast cancer cell lines, MCF-7 and SKBR3, with the cell line SKBR3 non-expressing ERs. These results demonstrated that estrogen might be a potential ligand for GPR30 (Filardo et al., 2000). This fact was further confirmed by the observation that estrogen did not activate ERK1/2 in the breast cancer cell line MDA-MB-231 without GPR30 expression, whereas ERK1/2 was activated by estrogen after GPR30 transfection into the cells (Filardo et al., 2000). Therefore, GPR30 is necessary for the activation of ERK1/2 by estrogen. So far, GPR30 has been detected in numerous human tissues such as heart, liver, lung, intestine, ovary, brain, breast, uterus, placenta and prostate (He et al., 2009; Filardo et al., 2006; Zhang et al., 2008; Haas et al., 2007; Hugo et al., 2008).

3.3 Genomic and non-genomic actions of estrogen receptors

In the classical, genomic estrogen-signaling pathway, estradiol (E2)-activated ERα translocates to the nucleus, dimerizes, and binds to the 15-bp palindromic estrogen response element (ERE) or interacts with other transcription factors on target genes, recruits coactivators, and stimulates gene transcription thereby promoting cell proliferation (Klinge, 2000). ERα interacts with a number of coactivators and corepressors in a ligand-dependent manner (Klinge, 2000). ERα may also function in a non-traditional manner, interacting with other DNA-binding transcription factors such as activator protein 1 (AP-1) or Sp-1, that in turn bind their cognate DNA elements, leading to remodeling of chromatin, and interactions with components of the basal transcription machinery complex (Ascenzi et al., 2006; Deroo and Korach, 2006).

Another more rapid mechanism of estrogen action is termed 'non-genomic' or 'membrane-initiated' because it involves E2 activation of plasma membrane-associated ERα or ERβ and leads to rapid activation of intracellular signaling pathways, e.g., ERK1/2 and PI3K/AKT (Wong et al., 2002; Watson et al., 2007; He et al., 2009). It can also result in an increase of Ca^{2+} or nitric oxide and the promotion of cell cycle progression. The ERs may be targeted to the plasma membrane by adaptor proteins such as caveolin-1 or Shc (Kim et al., 2008). GPR30 also activates ERK1/2 and PI3K/AKT signaling, although its exact role in estrogen action remains controversial (Pedram et al., 2006). GPR30 ligands, for example, estrogen (Muller et al., 1979), tamoxifen (Dick et al., 2002) and ICI 182780 (Hermenegildo and Cano, 2000) bind to GPR30, and activate heterotrimeric G proteins, which then activate Src and adenylyl cyclase (AC) resulting in intracellular cAMP production. Src is involved in matrix metalloproteinases (MMP) activation, which cleave pro-heparan-bound epidermal growth factor (pro-HB-EGF) and release free HB-EGF. The latter activates EGF receptor (EGFR), leading to multiple downstream events such as activation of phospholipase C (PLC), PI3K, and MAPK. Activated PLC produces inositol triphosphate (IP3), which further binds to IP3 receptor and leads to intracellular calcium mobilization. The activation of MAPK and PI3K results in activation of numerous cytosolic pathways and nuclear proteins, which further regulate transcription factors such as serum response factor and members of the E26 transformation specific (ETS) family by direct phosphorylation (Posern and Treisman, 2006; Gutierrez-Hartmann et al., 2007).

The non-genomic pathway may cross-talk with the genomic pathway, since ERα can be translocated from the membrane into the nucleus both in a E2-dependent or independent manner (Lu et al., 2002). It has also been demonstrated that E2-induced ERK activation stimulates the expression of AP-1-mediated genes via both serum response factor ELK-1 (ER activated in the membrane) and the recruitment of coactivators to AP-1 sites on gene promoters by the nuclear ER (Ascenzi et al., 2006). The intricate relationship between membrane and nuclear effects induced by estrogens has also been observed in the regulation of many other genes including PI3K (Ascenzi et al., 2006).

Therefore, integrative signaling by E2 from several places in the cell can lead to both rapid and sustained actions, which synergize to provide plasticity for cell response.

3.4 Estrogen receptors in the mitochondria

Glucocorticoid and thyroid hormones have been shown to modify the levels of mtDNA-encoded gene transcripts. These effects are mediated through direct interactions of their receptors with mtDNA. It has also been established that thyroid hormone can cause the direct stimulation of mitochondrial RNA synthesis (Casas et al., 1999; Enriquez et al., 1999) and that a variant form of the thyroid hormone receptor is imported in and localized within liver mitochondria (Casas et al., 1999; Wrutniak et al., 1995).

These findings suggest that mitochondria could also be a target site for the action of estrogens. Monje and colleagues (Monje and Boland 2001; Monje et al., 2001) demonstrated the presence of both ERα and ERβ in mitochondria of rabbit uterine and ovarian tissue, and ER translocation into mitochondria suggests the presence of E2 effects on mitochondrial function and protein expression (Chen et al., 2004). The mitochondrial genome contains estrogen response elements (ERE)-like sequences (Demonacos et al., 1996; Sekeris et al., 1990). Furthermore, several studies have detected the presence of estrogen-binding proteins (EBPs) in the organelle (Grossman et al., 1989; Moats and Ramirez 2000). Estrogen treatment increases the transcript levels of several mitochondrial DNA (mtDNA)-encoded genes in rat hepatocytes and human Hep G2 cells (Chen et al., 1996; Chen et al., 1998).

Estrogen response elements have been found in the D-loop, in the master regulatory region, and within the structural genes of the mtDNA (Demonacos et al., 1996). As a consequence, E2 may exert coordinated effects on both nuclear and mitochondrial gene expression. E2 can increase mtDNA transcripts for cytochrome oxidase IV subunits I and II in cultured cancer cells (Chen et al., 2004). E2 profoundly affects mitochondrial function in cerebral blood vessels, enhancing efficiency of energy production and suppressing mitochondrial oxidative stress by increasing protein levels of Mn-SOD and aconitase, and stabilizing mitochondrial membrane (Stirone et al., 2005).

The mechanisms of ER translocation into mitochondria are still quite elusive but recent data in MCF7 cells demonstrated that human ERβ posses a putative internal mitochondrial targeting peptide signal to the organelle (Chen et al., 2004). These authors observed that around 12% of total cellular ERα and 18% of ERβ is present in the mitochondrial fraction in E2-treated MCF7 cells. Furthermore, the localization of both ERα and ERβ to mitochondria in response to E2-treatment is accompanied by a concomitant time- and concentration-dependent increase in the transcript levels of the mtDNA-encoded genes (Chen et al., 2004).

3.5 Target molecules of estrogen receptors in the thyroid gland

Besides the adrenal cortex and ovary, also the human thyroid gland has the ability to synthesize estrogens and such ability seems to be higher in women than men (Dalla Valle et al., 1998). In the thyroid gland, E2 provokes a considerable increase in the thyroid weight, stimulates thyroid iodide uptake, enhances thyroperoxidase activity, and increases the level of T3 (Lima et al., 2006).

ERK1/2 regulate various cellular activities, such as gene expression, mitosis, differentiation, proliferation, and cell survival/apoptosis (Roberts and Der, 2007; Dunn et al., 2005). Zeng and colleagues have demonstrated that E2 can activate ERK1/2 in the thyroid by inducing its phosphorylation (Zeng et al., 2007). ERK1/2 activation by E2 depends on the interaction between estradiol and ERα (Zeng et al., 2007).

Bcl-2 family proteins play a central role in controlling mitochondrial-mediated apoptosis. They include proteins that suppress apoptosis such as Bcl-2 and Bcl-XL, and proteins that promote apoptosis such as Bax, Bad and Bcl-X_S (Antonsson and Martinou, 2000). Bcl-2 proteins localize or translocate to the mitochondrial membrane and modulate apoptosis by permeabilization of the inner and/or outer membrane, leading to the release of citochrome *c* or stabilization of the barrier function. Bcl-2 family members are altered in thyroid cancer (Kossmehl et al., 2003) and their levels are regulated by estrogen in some cell systems (Song and Santen, 2003). The antiapoptotic member Bcl-2 is up-regulated by E2 and by the ERα agonist PPT, but down-regulated by the ERβ agonist DPN in thyroid cancer cells, suggesting that ERα induces Bcl-2 expression whereas ERβ reduces it (Zeng et al., 2007). In addition, it has been shown that ERβ but not ERα promotes the expression of Bax (Lee et al., 2005; Zeng et al., 2007).

Recent work on the WRO thyroid cancer cells revealed that E2 increases cathepsin D transcription and that cathepsin D expression is inhibited upon siRNA-mediated knockdown of ERα and ERβ (Kumar et al., 2010). Cathepsin D is a classical E2 target gene regulated by Sp1-ERα promoter binding (Wang et al., 1997). It is well established that cathepsin D expression is elevated in thyroid tumors and correlates with disease aggressiveness (Leto et al., 2004).

The expression of another classical E2 target gene, cyclin D1 (Pestell et al., 1999), is stimulated by E2 in thyroid cancer cell lines, and co-treatment with siERα and siERβ shows roles for ERα and ERβ in regulating cyclin D1 transcription. E2 regulation of cyclin D1 transcription involves ERα-Sp1 (Castro-Rivera et al., 2001) and AP-1-ERα (Liu et al., 2002) interactions.

In Nthy-ori3-1 and BCPAP cells (derived from thyroid carcinoma), ERα was found to be complexed with Hsp90 and AKT (Rajoria et al., 2010). The complex of Hsp90 and AKT with ERα has major implications for its non-genomic signaling. In the presence of E2, Hsp90 dissociates, allowing ERα to dimerize and induce gene expression. At the same time, AKT is also rendered free to participate in the signal transduction cascade.

Rajoria and colleagues observed that E2 dramatically increases the ability of thyroid cells to adhere (137-140%) and migrate (27-75%). They also found downregulation of β-catenin in the thyroid cells treated with E2 (Rajoria et al., 2010).

4. PI3K-AKT pathway

In 1991, three independent research groups identified the gene that encodes for the serin/threonin kinase AKT/PKB (Jones et al., 1991; Bellacosa et al., 1991; Coffer and Woodgent, 1991). AKT plays a major role in cell proliferation, survival, adhesion, migration, metabolism and tumorigenesis. The effects of AKT activation are determined by the phosphorylation of its downstream effectors located in the cytoplasm, nucleus and mitochondria (Manning and Cantley, 2007; Bijur and Jope, 2003; Antico Arciuch et al., 2009). Mammals have three closely related PKB genes, encoding the isoforms AKT1/PKBα, AKT2/PKBβ and AKT3/PKBγ. Although the AKT isoforms are ubiquitously expressed, evidence suggests that the relative isoform expression levels differ between tissues. AKT1 is the mainly expressed isoform in most tissues, while AKT2 is highly enriched in insulin target tissues. *Akt1* deficient mice show normal glucose tolerance and insulin-stimulated glucose clearance from blood, but display severe growth retardation (Cho et al., 2001). It has also been shown that cells derived from *Akt1* deficient mouse embryos are also more susceptible to pro-apoptotic stimuli (Chen et al., 2001). On the other hand, deficiency of *AKT2* alone is sufficient to cause a diabetic phenotype in mice (Withers et al., 1998; Cho et al., 2001) and a loss-of-function mutation in AKT2 is associated with diabetes in one human family (George et al., 2004).

AKT kinases are typically activated by engagement of receptor tyrosine kinases by growth factors and cytokines, as well as oxidative stress and heat shock. AKT activation relies on phosphatidylinositol 3,4,5-triphosphate (PtdIns-3,4,5-P3) which is produced from phosphatidylinositol 4,5-biphosphate (PtdIns-4,5-P2) by phosphatidylinositol 3-kinase (PI3K) (Franke et al., 1995). The interaction between the Pleckstrin homology (PH) domain of AKT with PtdIns-3,4,5-P3 favors its phosphorylation at two residues, one in the C-terminal tail (Ser^{473}) and the other in the activation loop (Thr^{308}). Phosphorylation at Ser^{473} appears to precede and facilitate phosphorylation at Thr^{308} (Sarbassov et al., 2005). AKT is phosphorylated in Ser^{473} by mTORC2 (Ikenoue et al., 2008), while PI-3K-dependent kinase 1 (PDK1) accounts for the phosphorylation in Thr^{308} (Chan et al., 1999).

The proliferative effects of AKT result from phosphorylation of several substrates. For example, GSK3*β* once phosphorylated is inactivated and this prevents degradation of cyclin D1 (Diehl et al., 1998). Furthermore, AKT activation leads to increased translation of cyclin D1 and D3 transcripts via mTOR (Muise-Helmericks et al., 1998). AKT phosphorylates the cell cycle inhibitors $p21^{WAF1}$ and $p27^{Kip1}$ inducing their cytoplasmic retention (Testa and Bellacosa, 2001).

AKT activity prevents apoptosis through the phosphorylation and inhibition of pro-apoptotic mediators such as Bad, FOXO family members, and IκB kinase-β (IKK-β) (Datta et al., 1999). AKT activity also attenuates the response of cells to the release of cytochrome *c* into the cytoplasm (Kennedy et al., 1999).

AKT can also antagonize p53-mediated cell cycle checkpoints by modulating the subcellular localization of Mdm2. Phosphorylation of Mdm2 by AKT triggers its localization to the nucleus, where Mdm2 can complex with p53 to promote its ubiquitin/proteasome-mediated degradation (Mayo and Donner, 2001).

The crucial role of the PI3K signaling cascade in the pathogenesis of thyroid neoplastic disorders has been recently confirmed by the development and study of a relevant mouse model (Yeager et al., 2007, 2008; Miller et al., 2009), as well as by solid clinicopathological data (Garcia-Rostan et al., 2005; Hou et al., 2007, 2008; Vasko and Saji, 2007; Wang et al., 2007). Thyrocyte-specific deletion of the *Pten* tumor suppressor constitutively activates the PI3K signaling cascade, leading to hyperplastic thyroid glands at birth, and to the development of thyroid nodules and follicular adenomas by 6-8 months of age (Yeager et al., 2007) and thyroid carcinomas by one year of age (Antico Arciuch et al., 2010).

5. PI3K-estrogen cooperation during proliferation

The *Pten* mouse model of thyroid disease displays a unique and remarkable characteristic: the higher proliferative index of female mutant thyrocytes, compared with males. This difference leads to increased cellularity in the thyroids of female mutants at a young age, to an increased incidence of thyroid adenomas in mutant females at 8 months of age (Yeager et al., 2007), and to an increased incidence of thyroid carcinomas in mutant females at one year of age (Antico Arciuch et al., 2010). The direct role of estrogen signaling in determining this difference in proliferative response to PI3K activation is underlined by the fact that these effects could be completely reversed by estrogen depletion in the females, and by slow-release estrogen pellet implantation in the males.

Several groups had anticipated a role for estrogen in thyroid proliferation, based on the effects of estradiol on thyroid carcinoma cells in culture (Manole et al., 2001; Vivacqua et al., 2006; Chen et al., 2008; Kumar et al., 2010; Rajoria et al., 2010). The *Pten* mouse model represents the first *in vivo* validation of the direct role played by estrogen in establishing the increased prevalence of thyroid disorders in the female.

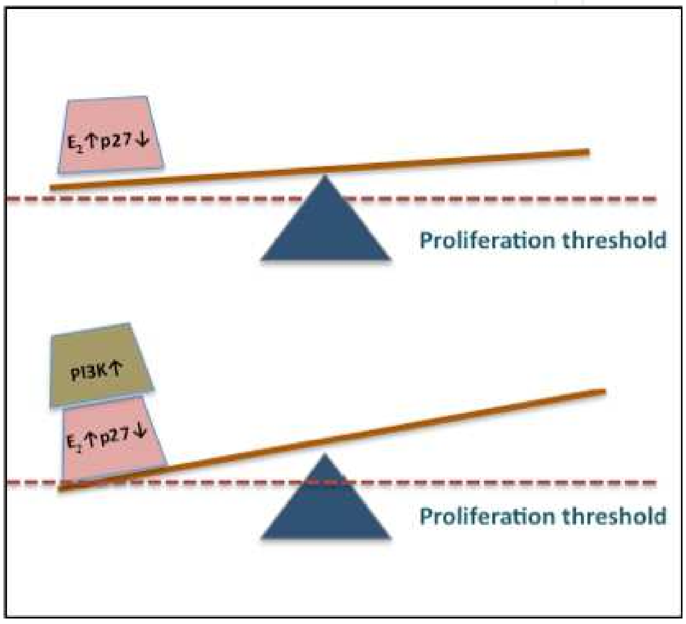

Fig. 1. Schematic model of the cooperation between estrogen signaling and PI3K activation.

The analysis of *Pten* mutant mice also shed some light on the molecular basis of the differential thyrocyte proliferative index and risk of adenoma and carcinoma development between male and female mutant mice. Genetic approaches, by crossing *Pten* mutant mice and *p27* mutant mice, and cell culture-based experiments have provided evidence that these gender-based differences in this mouse model are due, at least in part, to the ability of estrogens to down-regulate p27 levels through mechanisms that include transcriptional regulation, in addition to the known effects on p27 protein degradation through regulation of Skp2 (Antico Arciuch et al., 2010; Foster et al., 2003).

Thus it is conceivable that, in thyroids harboring mutations that confer elevated proliferative signals and thus a low cell cycle progression threshold, E2-mediated p27 depletion further increases the thyrocyte proliferative index (Figure 1).

Additional mechanisms, including E2-mediated mitochondrial effects, are also likely to contribute to this phenotype. Maintenance of a normal intracellular redox status plays an important role in such processes as DNA synthesis, gene expression, enzymatic activity, and others. Signaling cascades involving protein tyrosine kinases can be enhanced by oxidative inhibition of protein tyrosine phosphatases, and pathways involving NF-kB, JNK, p38 MAPK, and AP-1 are strongly responsive to redox regulation (Droge, 2002). Recent data have suggested that physiological concentrations of E2 trigger a rapid production of intracellular reactive oxygen species (ROS) in endothelial and epithelial cells, and that E2-induced DNA synthesis is at least in part mediated by ROS signaling in these cells (Felty et al., 2005; Felty, 2006). This notion is particularly intriguing, since E2-mediated ROS production in thyroid follicular cells would have two effects: an immediate stimulation of cell proliferation, and a long-term accumulation of oxidative DNA damage. Furthermore, these effects would be further enhanced if PI3K activation resulted in an alteration of the thyrocyte antioxidant and detoxification system. Strikingly, in an ongoing proteomic effort (manuscript in preparation), we have recently identified Glutathione S-transferase Mu 1 (GSTM1), an enzyme important for the reduction (detoxification) of hydrogen peroxide, as one of the most significantly down-regulated proteins in mutant thyroids, suggesting that, indeed, PI3K-mediated GSTM1 reduction might indeed further amplify the effects of ROS in the thyroid.

Finally, the increased expression level of *Tpo*, *Duox1* and *Slc5a5* genes in female mice, irrespective of their genotype, strongly suggests that estrogen has a significant role in their transcriptional regulation, providing additional targets for future studies on the role of estrogen in the pathophysiology of the thyroid gland.

6. Conclusion

A role for estrogen in thyroid proliferation has been proposed for several years, based on the analysis of the effects of estrogen on thyroid cells in culture. Now, for the first time, our hormone manipulation experiments in a relevant mouse model of thyroid proliferative disorders and neoplastic transformation have provided *in vivo* evidence that circulating estrogens increase thyroid follicular cells proliferation. It is tempting to suggest that the relatively mild effect of estrogens on thyroid cells is uncovered and amplified by oncogenic events lowering the thyrocyte proliferation threshold. Further studies will validate this hypothesis in the context of different oncogenic mutations.

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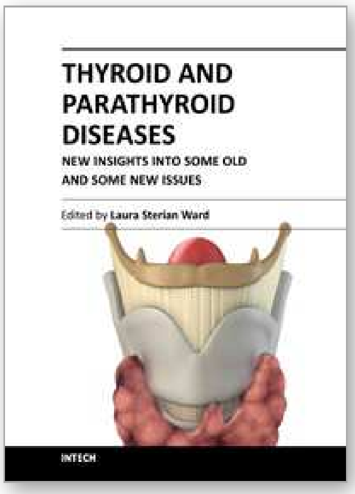

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This book was designed to meet the requirements of all who wish to acquire profound knowledge of basic, clinical, psychiatric and laboratory concepts as well as surgical techniques regarding thyroid and parathyroid glands. It was divided into three main sections: 1. Evaluating the Thyroid Gland and its Diseases includes basic and clinical information on the most novel and quivering issues in the area. 2. Psychiatric Disturbances Associated to Thyroid Diseases addresses common psychiatric disturbances commonly encountered in the clinical practice. 3. Treatment of Thyroid and Parathyroid Diseases discusses the management of thyroid and parathyroid diseases including new technologies.

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