

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Estrogen Signaling and Thyrocyte Proliferation

Valeria Gabriela Antico Arciuch and Antonio Di Cristofano
*Department of Developmental and Molecular Biology
Albert Einstein College of Medicine, Bronx
USA*

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 (17 β -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 (Cavaliere 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
<i>RAS</i> : 20-50%	<i>BRAF</i> : 40-45%	<i>TP53</i> : 50-80%
<i>PAX8-PPARγ</i> : 20-35%	<i>RAS</i> : 10-20%	<i>BRAF</i> : 20-40%
PI3K pathway: 20%	<i>RET-PTC</i> : 10-30%	<i>RAS</i> : 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-PPAR γ* rearrangements (20–35%), which lead to a mutant protein incapable of transactivating a *PPAR γ* 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, 17 β -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 (ER α , ER β and GPR30). ER α was initially described in 1973 (Jensen and De Sombre, 1973) while ER β was identified much later (Kuiper et al., 1996). ER α and ER β 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 ER α and ER β might have different physiological functions. It has also been demonstrated that in many systems the activity of ER β is opposed to that of ER α . For example, in breast cancer cells, ER α is the receptor responsible for E2-induced proliferation, whereas activation of ER β inhibits this effect (Strom et al., 2004). In the uterus, E2 induces proliferation of both epithelial and stromal cells through ER α , which is the predominant ER in the mature organ, while in the immature uterus, ER α and ER β are found at similar expression levels in both epithelium and stroma, and ER β mediates the action of E2 as a suppressor of cell proliferation against activation of ER α 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 ER α 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). ER α interacts with a number of coactivators and corepressors in a ligand-dependent manner (Klinge, 2000). ER α 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 ER α or ER β 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 Ca²⁺ 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 ER α 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 ER α and ER β 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 ER β possesses a putative internal mitochondrial targeting peptide signal to the organelle (Chen et al., 2004). These authors observed that around 12% of total cellular ER α and 18% of ER β is present in the mitochondrial fraction in E2-treated MCF7 cells. Furthermore, the localization of both ER α and ER β 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 ER α (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 cytochrome *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 ER α agonist PPT, but down-regulated by the ER β agonist DPN in thyroid cancer cells, suggesting that ER α induces Bcl-2 expression whereas ER β reduces it (Zeng et al., 2007). In addition, it has been shown that ER β but not ER α 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 ER α and ER β (Kumar et al., 2010). Cathepsin D is a classical E2 target gene regulated by Sp1-ER α 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 siER α and siER β shows roles for ER α and ER β in regulating cyclin D1 transcription. E2 regulation of cyclin D1 transcription involves ER α -Sp1 (Castro-Rivera et al., 2001) and AP-1-ER α (Liu et al., 2002) interactions.

In Nthy-ori3-1 and BCPAP cells (derived from thyroid carcinoma), ER α was found to be complexed with Hsp90 and AKT (Rajoria et al., 2010). The complex of Hsp90 and AKT with ER α has major implications for its non-genomic signaling. In the presence of E2, Hsp90 dissociates, allowing ER α 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; Coffey and Woodgett, 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/PKB α , AKT2/PKB β and AKT3/PKB γ . 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 (Ser⁴⁷³) and the other in the activation loop (Thr³⁰⁸). Phosphorylation at Ser⁴⁷³ appears to precede and facilitate phosphorylation at Thr³⁰⁸ (Sarbasov et al., 2005). AKT is phosphorylated in Ser⁴⁷³ by mTORC2 (Ikenoue et al., 2008), while PI-3K-dependent kinase 1 (PDK1) accounts for the phosphorylation in Thr³⁰⁸ (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 (Muisse-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 I κ B 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.

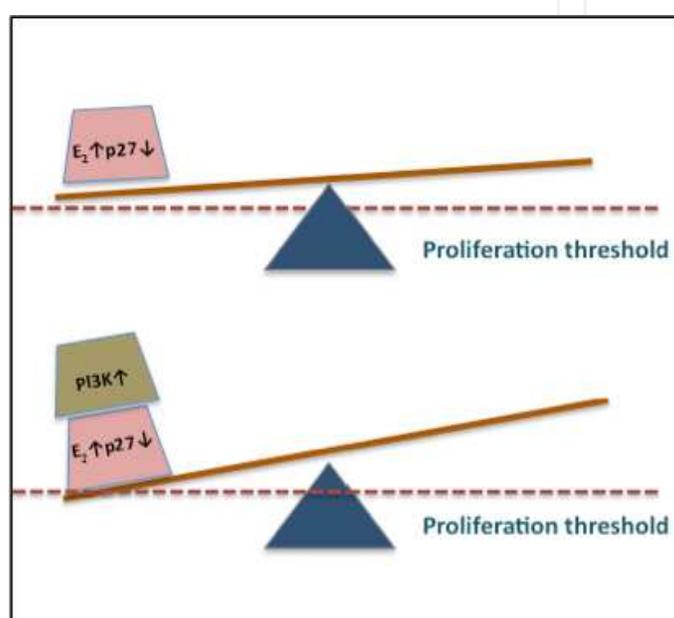


Fig. 1. Schematic model of the cooperation between estrogen signaling and PI3K activation.

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- κ B, 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.

7. References

- Antico Arciuch VG, Dima M, Liao XH, Refetoff S and Di Cristofano A. (2010) Cross-talk between PI3K and estrogen in the mouse thyroid predisposes to the development of follicular carcinomas with a higher incidence in females. *Oncogene*. 29:5678-86.
- Antico Arciuch VG, Galli S, Franco MC, Lam PY, Cadenas E, Carreras MC, and Poderoso JJ. (2009) AKT1 intramitochondrial cycling is a crucial step in the redox modulation of cell cycle progression. *PLoS One* 4: e7523.
- Antonsson B and Martinou JC. (2000) The Bcl-2 protein family. *Exp Cell Res*. 256:50-7.
- Are C and Shaha AR. (2006) Anaplastic thyroid carcinoma: biology, pathogenesis, prognostic factors, and treatment approaches. *Ann Surg Oncol*. 13:453-64.
- Arnold JT, Liu X, Allen JD, Le H, McFann KK and Blackman MR. (2007) Androgen receptor or estrogen receptor-beta blockade alters DHEA-, DHT-, and E(2)-induced proliferation and PSA production in human prostate cancer cells. *Prostate*. 67:1152-62.
- Ascenzi P, Bocedi A, Marino M. (2006) Structure-function relationship of estrogen receptor alpha and beta: impact on human health. *Mol Aspects Med*. 27:299-402.

- Bellacosa A, Testa JR, Staal SP and Tschlis PN. (1991) A retroviral oncogene, akt, encoding a serine-threonine kinase containing an SH2-like region. *Science*. 254:274-277.
- Bijur GN, Jope RS. Rapid accumulation of AKT in mitochondria following phosphatidylinositol 3-kinase activation. (2003) *J Neurochem* 87:1427-35.
- Carmeci C, Thompson DA, Ring HZ, Francke U and Weigel RJ. (1997) Identification of a gene (GPR30) with homology to the G-protein-coupled receptor superfamily associated with estrogen receptor expression in breast cancer. *Genomics*. 45:607-617.
- Casas F, Rochard P, Rodier A, Cassar-Malek I, Marchal-Victorion S, Wiesner RJ, Cabello G, and Wrutniak C. (1999) A variant form of the nuclear triiodothyronine receptor c-ErbAalpha1 plays a direct role in regulation of mitochondrial RNA synthesis. *Mol Cell Biol*. 19:7913-7924.
- Castro-Rivera E, Samudio I and Safe S. (2001) Estrogen regulation of cyclin D1 gene expression in ZR-75 breast cancer cells involves multiple enhancer elements. *J Biol Chem*. 276:30853-30861.
- Cavalieri EL and Rogan EG. (2001) Evidence that a burst of DNA depurination in SENCAR mouse skin induces error-prone repair and forms mutations in the H-ras gene. *Oncogene* 20:7945-7953.
- Cavalieri EL, Stack DE, Devanesan PD, Todorovic R, Dwivedy I, Higginbotham S, Johansson SL, Patil KD, Gross ML, Gooden JK, Ramanathan R, Cerny RL and Rogan EG. (1997) Molecular origin of cancer: catechol estrogen-3,4-quinones as endogenous tumor initiators. *Proc Natl Acad Sci U S A*. 94:10937-42.
- Chan TO, Rittenhouse SE and Tschlis PN. AKT/PKB and other D3 phosphoinositideregulated kinases: kinase activation by phosphoinositide-dependent phosphorylation. (1999) *Annu Rev Biochem* 68: 965-1014.
- Chen GG, Liu ZM, Vlantis AC, Tse GM, Leung BC and van Hasselt CA. (2004) Heme oxygenase-1 protects against apoptosis induced by tumor necrosis factor-alpha and cycloheximide in papillary thyroid carcinoma cells. *J Cell Biochem*. 92:1246-56.
- Chen GG, Vlantis AC, Zeng Q and van Hasselt CA. (2008) Regulation of cell growth by estrogen signaling and potential targets in thyroid cancer. *Curr Cancer Drug Targets*. 8:367-377.
- Chen J, Gokhale M, Li Y, Trush MA, and Yager JD. (1998) Enhanced levels of several mitochondrial mRNA transcripts and mitochondrial superoxide production during ethinyl estradiol-induced hepatocarcinogenesis and after estrogen treatment of HepG2 cells. *Carcinogenesis*. 19:2187-2193.
- Chen J, Schwartz DA, Young TA, Norris JS, and Yager JD. (1996) Identification of genes whose expression is altered during mitosuppression in livers of ethinyl estradiol-treated female rats. *Carcinogenesis*. 17:2783-2786.
- Chen JQ, Delannoy M, Cooke C and Yager JD. (2004) Mitochondrial localization of ERalpha and ERbeta in human MCF7 cells. *Am J Physiol*. 286:E1011-E1022.
- Chen WS, Xu PZ, Gottlob K, Chen ML, Sokol K, Shiyanova T, Roninson I, Weng W, Suzuki R, Tobe K, Kadowaki T and Hay N. Growth retardation and increased apoptosis in mice with homozygous disruption of the AKT1 gene. (2001) *Genes Dev* 15:2203-8.
- Cho H, Mu J, Kim JK, Thorvaldsen JL, Chu Q, Crenshaw EB 3rd, Kaestner KH, Bartolomei MS, Shulman GI and Birnbaum MJ. Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase AKT2 (PKB beta). (2001) *Science* 292:1728-1731.

- Cho H, Thorvaldsen JL, Chu Q, Feng F and Birnbaum MJ. (2001) AKT1/pkbalph is required for normal growth but dispensable for maintenance of glucose homeostasis in mice. *J Biol Chem* 276:38349-52.
- Coffer PJ and Woodgett JR. (1991) Molecular cloning and characterisation of a novel putative protein-serine kinase related to the cAMP-dependent and protein kinase C families. *Eur J Biochem.* 201:475-481.
- Dalla Valle L, Ramina A, Vianello S, Fassina A, Belvedere P and Colombo L. (1998) Potential for estrogen synthesis and action in human normal and neoplastic thyroid tissues. *J. Clin. Endocrinol. Metab.* 83:3702-3709.
- Datta SR, Brunet A and Greenberg ME. Cellular survival: a play in three AKTs. (1999) *Genes Dev.* 13:2905-27.
- Dechering K, Boersma and Mosselman S. (2000) Estrogen receptors alpha and beta: two receptors of a kind? *Curr. Med. Chem.* 7:561-576.
- Demonacos CV, Karayanni N, Hatzoglou E, Tsiriyiotis C, Spandidos DA and Sekeris CE. (1996) Mitochondrial genes as sites of primary action of steroid hormones. *Steroids.* 61:226-232.
- Deroo BJ, Korach KS. (2006) Estrogen receptors and human disease. *J Clin Invest.* 116:561-70.
- Dick GM, Hunter AC and Sanders KM. (2002) Ethylbromide tamoxifen, a membrane-impermeant antiestrogen, activates smooth muscle calcium-activated large-conductance potassium channels from the extracellular side. *Mol Pharmacol.* 61(5):1105-13.
- Diehl JA, Cheng M, Roussel MF and Sherr CJ. (1998) Glycogen synthase kinase-3beta regulates cyclin D1 proteolysis and subcellular localization. *Genes Dev.* 12:3499-3511.
- Droge W. (2002) Free radicals in the physiological control of cell function. *Physiol Rev* 82, 47-95.
- Dunn KL, Espino PS, Drobic B, He S and Davie JR. (2005) The Ras-MAPK signal transduction pathway, cancer and chromatin remodeling. *Biochem Cell Biol.* 83:1-14.
- Enriquez JA, Fernandez-Silva P, Garrido-Perez N, Lopez-Perez MJ, Perez-Martos A, and Montoya J. (1999) Direct regulation of mitochondrial RNA synthesis by thyroid hormone. *Mol Cell Biol.* 19:657-670.
- Fagin JA. (2004) How thyroid tumors start and why it matters: kinase mutants as targets for solid cancer pharmacotherapy. *J Endocrinol.* 183:249-56.
- Felty Q. (2006) Estrogen-induced DNA synthesis in vascular endothelial cells is mediated by ROS signaling. *BMC Cardiovasc Disord* 6, 16-22.
- Felty Q., Singh K.P. and Roy, D. (2005) Estrogen-induced G1/S transition of G0-arrested estrogen-dependent breast cancer cells is regulated by mitochondrial oxidant signaling. *Oncogene* 24, 4883-93.
- Filardo EJ, Quinn JA, Bland KI, Frackelton AR Jr. (2000) Estrogen-induced activation of Erk-1 and Erk-2 requires the G protein-coupled receptor homolog, GPR30, and occurs via trans-activation of the epidermal growth factor receptor through release of HB-EGF. *Mol. Endocrinol.* 14:1649-1660.
- Filardo EJ, Graeber CT, Quinn JA, Resnick MB, Giri D, DeLellis RA, Steinhoff MM and Sabo E. (2006) Distribution of GPR30, a seven membrane-spanning estrogen receptor, in primary breast cancer and its association with clinicopathologic determinants of tumor progression. *Clin. Cancer Res.* 12:6359-6366.

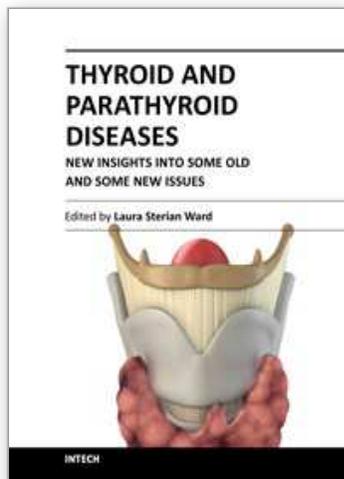
- Franke TF, Yang SI, Chan TO, Datta K, Kazlauskas A, Morrison DK, Kaplan DR and Tschlis PN. The protein kinase encoded by the AKT proto-oncogene is a target of the PDGF-activated phosphatidylinositol 3-kinase. (1995) *Cell* 2:727-36.
- Foster JS, Fernando RI, Ishida N, Nakayama KI and Wimalasena J. (2003) Estrogens down-regulate p27Kip1 in breast cancer cells through Skp2 and through nuclear export mediated by the ERK pathway. *J Biol Chem.* 278:41355-41366.
- Garcia-Rostan G, Costa AM, Pereira-Castro I, Salvatore G, Hernandez R, Hermsem MJ et al. (2005) Mutation of the PIK3CA gene in anaplastic thyroid cancer. *Cancer Res.* 65:10199-10207.
- George S, Rochford JJ, Wolfrum C, Gray SL, Schinner S, Wilson JC, Soos MA, Murgatroyd PR, Williams RM, Acerini CL, Dunger DB, Barford D, Umpleby AM, Wareham NJ, Davies HA, Schafer AJ, Stoffel M, O'Rahilly S and Barroso I. A family with severe insulin resistance and diabetes due to a mutation in AKT2. (2004) *Science* 304:1325-1328.
- Gilfillan, CP. (2010) Review of the genetics of thyroid tumours: diagnostic and prognostic implications. *ANZ Journal of Surgery.* 80:33-40.-
- Grossman A, Oppenheim J, Grondin G, St. Jean P and Beaudoin AR. (1989) Immunocytochemical localization of the [3H]estradiol-binding protein in rat pancreatic acinar cells. *Endocrinology.* 124:2857-2866.
- Gutierrez-Hartmann A, Duval DL and Bradford AP. (2007) ETS transcription factors in endocrine systems. *Trends Endocrinol Metab.* 18(4):150-8.
- Haas E, Meyer MR, Schurr U, Bhattacharya I, Minotti R, Nguyen HH, Heigl A, Lachat M, Genoni M and Barton M. (2007) Differential effects of 17beta-estradiol on function and expression of estrogen receptor alpha, estrogen receptor beta, and GPR30 in arteries and veins of patients with atherosclerosis. *Hypertension.* 49:1358-1363.
- Hall JM, Couse JF and Korach KS. (2001) The multifaceted mechanisms of estradiol and estrogen receptor signaling. *J. Biol. Chem.* 276:36869-36872.
- Handkiewicz-Junak D, Czarniecka A and Jarzab B. (2010) Molecular prognostic markers in papillary and follicular thyroid cancer: Current status and future directions. *Mol Cell Endocrinol.* 322:8-28.
- He YY, Cai B, Yang YX, Liu XL and Wan XP. (2009) Estrogenic G protein-coupled receptor 30 signaling is involved in regulation of endometrial carcinoma by promoting proliferation, invasion potential, and interleukin-6 secretion via the MEK/ERK mitogen-activated protein kinase pathway. *Cancer Sci.* 100:1051-1061.
- Hermenegildo C and Cano A. (2000) Pure anti-oestrogens. *Hum Reprod Update.* 6(3):237-43.
- Herrmann BL, Saller B, Janssen OE, Gocke P, Bockisch A, Sperling H, Mann K and Broecker M. (2002) Impact of estrogen replacement therapy in a male with congenital aromatase deficiency caused by a novel mutation in the CYP19 gene. *J. Clin. Endocrinol. Metab.* 8:5476-5484.
- Hodgson NC, Button J and Solorzano CC. (2004) Thyroid cancer: is the incidence still increasing? *Ann Surg Oncol.* 11:1093-7.
- Hou P, Ji M and Xing M. (2008) Association of PTEN gene methylation with genetic alterations in the phosphatidylinositol 3-kinase/AKT signaling pathway in thyroid tumors. *Cancer.* 113:2440-2447.

- Hou P, Liu D, Shan Y, Hu S, Studeman K, Condouris S et al. (2007) Genetic alterations and their relationship in the phosphatidylinositol 3-kinase/AKT pathway in thyroid cancer. *Clin Cancer Res.* 13:1161-1170.
- Hugo ER, Brandebourg TD, Woo JG Loftus J, Alexander JW, Ben-Jonathan N. (2008) Bisphenol A at environmentally relevant doses inhibits adiponectin release from human adipose tissue explants and adipocytes. *Environ. Health Perspect.* 116:1642-1647.
- Ikenoue T, Inoki K, Yang Q, Zhou X and Guan KL. Essential function of TORC2 in PKC and AKT turn motif phosphorylation, maturation and signaling. (2008) *EMBO J* 23:1919-31.
- Jemal A, Siegel R, Ward E, Hao Y, Xu J and Thun MJ. (2009) Cancer statistics, 2009. *CA Cancer J Clin.* 59:225-49.
- Jensen EV, DeSombre ER. (1973) Estrogen-receptor interaction. *Science.* 182:126-134.
- Jones KP (1992). Estrogens and progestins: what to use and how to use it. *Clin. Obstet. Gynecol.* 32:871-883.
- Jones PF, Jakubowicz T, Pitossi FJ, Maurer F and Hemmings BA. (1991) Molecular cloning and identification of a serine/threonine protein kinase of the second-messenger subfamily. *Proc Natl Acad Sci U S A.* 88:4171-4175.
- Kennedy SG, Kandel ES, Cross TK and Hay N. (1999) AKT/Protein kinase B inhibits cell death by preventing the release of cytochrome c from mitochondria. *Mol Cell Biol* 19:5800-5810.
- Kim CS and Zhu X. (2009) Lessons from mouse models of thyroid cancer. *Thyroid.* 19:1317-31.
- Kim KH, Moriarty K and Bender JR. (2008) Vascular cell signaling by membrane estrogen receptors. *Steroids.* 73(9-10):864-9.
- Klinge CM. (2000) Estrogen receptor interaction with co-activators and co-repressors. *Steroids* 65: 227-25.
- Kossmehl P, Shakibaei M, Cogoli A, Infanger M, Curcio F, Schönberger J, Eilles C, Bauer J, Pickenhahn H, Schulze-Tanzil G, Paul M and Grimm D. (2003) Weightlessness induced apoptosis in normal thyroid cells and papillary thyroid carcinoma cells via extrinsic and intrinsic pathways. *Endocrinology.* 144:4172-9.
- Kouniavsky G and Zeiger MA. (2010) Thyroid tumorigenesis and molecular markers in thyroid cancer. *Curr Opin Oncol.* 22:23-9.
- Kuiper GG, Enmark E, Peltö-Huikko M, Nilsson S and Gustafsson JA. (1996) Cloning of a novel receptor expressed in rat prostate and ovary. *Proc Natl Acad Sci USA.* 93:5925-5930.
- Kuiper GG, Carlsson B, Grandien K, Enmark E, Häggblad J, Nilsson S and Gustafsson JA. (1997) Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. *Endocrinology.* 138:863-870.
- Kumar A, Klinge CM and Goldstein RE. (2010) Estradiol-induced proliferation of papillary and follicular thyroid cancer cells is mediated by estrogen receptors alpha and beta. *Int J Oncol.* 36:1067-1080.
- Lee ML, Chen GG, Vlantis A C, Tse GM, Leung BC and van Hasselt CA. (2005) Induction of thyroid papillary carcinoma cell proliferation by estrogen is associated with an altered expression of Bcl-xL. *Cancer J.* 11:113-121.
- Lemoine NR, Mayall ES, Wyllie FS, Farr CJ, Hughes D, Padua RA, Thurston V, Williams ED and Wynford-Thomas D. (1988) Activated ras oncogenes in human thyroid cancers. *Cancer Res.* 48:4459-4463.

- Leto G, Tumminello FM, Crescimanno M, Flandina C and Gebbia N. (2004) Cathepsin D expression levels in non-gynecological solid tumors: clinical and therapeutic implications. *Clin Exp Metastasis*. 21:91-106.
- Libutti SK. (2005) Understanding the role of gender in the incidence of thyroid cancer. *Cancer J*. 11:104-105.
- Lima LP, Barros IA, Lisbôa PC, Araújo RL, Silva AC, Rosenthal D, Ferreira AC and Carvalho DP. (2006) Estrogen effects on thyroid iodide uptake and thyroperoxidase activity in normal and ovariectomized rats. *Steroids*. 71:653-9.
- Liu MM, Albanese C, Anderson CM, Hilty K, Webb P, Uht RM, Price RH Jr, Pestell RG and Kushner PJ. (2002) Opposing action of estrogen receptors alpha and beta on cyclin D1 gene expression. *J Biol Chem*. 277:24353-24360.
- Liu Z, Hou P, Ji M, Guan H, Studeman K, Jensen K, Vasko V, El-Naggar AK and Xing M. (2008) Highly prevalent genetic alterations in receptor tyrosine kinases and phosphatidylinositol 3-kinase/akt and mitogen-activated protein kinase pathways in anaplastic and follicular thyroid cancers. *J. Clin. Endocrinol. Metab*. 93:3106-3116.
- Lu Q, Ebling H, Mittler J, Baur WE and Karas RH. (2002) MAP kinase mediates growth factor-induced nuclear translocation of estrogen receptor alpha. *FEBS Lett*. 516(1-3):1-8.
- Manning BD, and Cantley LC. (2007) AKT/PKB signaling: navigating downstream. *Cell* 129:1261-74.
- Machens A, Hauptmann S and Dralle H. (2006) Disparities between male and female patients with thyroid cancers: sex difference or gender divide? *Clin Endocrinol (Oxf)*. 65:500-5.
- Manole D, Schildknecht B, Gosnell B, Adams E and Derwahl M. (2001) Estrogen promotes growth of human thyroid tumor cells by different molecular mechanisms. *J Clin Endocrinol Metab*. 86:1072-1077.
- Mayo LD and Donner DB. (2001) A phosphatidylinositol 3-kinase/AKT pathway promotes translocation of Mdm2 from the cytoplasm to the nucleus. *Proc Natl Acad Sci U S A*. 98:11598-603.
- Miller KA, Yeager N, Baker K, Liao XH, Refetoff S and Di Cristofano A. (2009) Oncogenic Kras requires simultaneous PI3K signaling to induce ERK activation and transform thyroid epithelial cells in vivo. *Cancer Res*. 69:3689-3694.
- Moats RK II and Ramirez VD. (2000) Electron microscopic visualization of membrane-mediated uptake and translocation of estrogen-BSA:colloidal gold by hep G2 cells. *J Endocrinol*. 166:631-647.
- Monje P and Boland R. (2001) Subcellular distribution of native estrogen receptor alpha and beta isoforms in rabbit uterus and ovary. *J Cell Biochem*. 82:467-479.
- Monje P, Zanello S, Holick M and Boland R. (2001) Differential cellular localization of estrogen receptor alpha in uterine and mammary cells. *Mol Cell Endocrinol*. 181:117-129.
- Mori M, Naito M, Watanabe H, Takeichi N, Dohi K and Ito A. (1990) Effects of sex difference, gonadectomy, and estrogen on N-methyl-N-nitrosourea induced rat thyroid tumors. *Cancer Res*. 50:7662-7.
- Muise-Helmericks RC, Grimes HL, Bellacosa A, Malstrom SE, Tsichlis PN and Rosen N. (1998) Cyclin D expression is controlled post-transcriptionally via a phosphatidylinositol 3-kinase/AKT-dependent pathway. *J. Biol. Chem*. 273:29864-29872.
- Müller RE, Johnston TC and Wotiz HH. (1979) Binding of estradiol to purified uterine plasma membranes. *J Biol Chem*. 254(16):7895-900.

- Neff RL, Farrar WB, Kloos RT and Burman KD. (2008) Anaplastic thyroid cancer. *Endocrinol Metab Clin North Am.* 37:525-38.
- Nikiforov YE. (2008) Thyroid carcinoma: molecular pathways and therapeutic targets. *Mod Pathol.* 2:S37-S43.
- O'Dowd BF, Nguyen T, Marchese A, Cheng R, Lynch KR, Heng HH, Kolakowski LF Jr and George SR. (1998) Discovery of three novel G-protein-coupled receptor genes. *Genomics.* 47:310-313.
- Owman C, Blay P, Nilsson C and Lolait SJ. (1996) Cloning of human cDNA encoding a novel heptahelix receptor expressed in Burkitt's lymphoma and widely distributed in brain and peripheral tissues. *Biochem. Biophys. Res. Commun.* 228:285-292.
- Pedram A, Razandi M and Levin ER. (2006) Nature of functional estrogen receptors at the plasma membrane. *Mol Endocrinol* 20:1996-2009.
- Pestell RG, Albanese C, Reutens AT, Segall JE, Lee RJ and Arnold A. (1999) The cyclins and cyclin-dependent kinase inhibitors in hormonal regulation of proliferation and differentiation. *Endocr Rev.* 20:501-534.
- Posern G and Treisman R. (2006) Actin' together: serum response factor, its cofactors and the link to signal transduction. *Trends Cell Biol.* 16(11):588-96.
- Rajoria S, Suriano R, Shanmugam A, Wilson YL, Schantz SP, Geliebter J and Tiwari RK. (2010) Metastatic phenotype is regulated by estrogen in thyroid cells. *Thyroid.* 20:33-41.
- Roberts PJ and Der CJ. (2007) Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. *Oncogene.* 26:3291-310.
- Ruggiero RJ and Likis FE. (2002) Estrogen: physiology, pharmacology, and formulations for replacement therapy. *J. Midwifery Womens Health.* 47:130-138.
- Sarbassov DD, Guertin DA, Ali SM and Sabatini DM. Phosphorylation and regulation of AKT/PKB by the rictor-mTOR complex. (2005) *Science* 18:1098-1101.
- Sekeris CE. (1990) The mitochondrial genome: a possible primary site of action of steroid hormones. *In Vivo.* 4:317-320.
- Smallridge RC, Marlow LA and Copland JA. (2009) Anaplastic thyroid cancer: molecular pathogenesis and emerging therapies. *Endocr Relat Cancer.* 16:17-44.
- Song RX and Santen RJ. (2003) Apoptotic action of estrogen. *Apoptosis.* 8:55-60.
- Speroff L, Glass RH and Kase NG. (1999) *Clin. Gynecol. Endocrinol. Infert.* (6th ed). Lippincott Williams & Wilkins: Philadelphia.
- Stender JD, Frasor J, Komm B, Chang KC, Kraus WL and Katzenellenbogen BS. (2007) Estrogen-regulated gene networks in human breast cancer cells: involvement of E2F1 in the regulation of cell proliferation. *Mol Endocrinol.* 21:2112-23.
- Stirone C, Duckles SP, Krause DN and Procaccio V. (2005) Estrogen increases mitochondrial efficiency and reduces oxidative stress in cerebral blood vessels. *Mol Pharmacol.* 68:959-965.
- Strom A, Hartman J, Foster JS, Kietz S, Wimalasena J and Gustafsson JA. (2004) Estrogen receptor beta inhibits 17beta-estradiol-stimulated proliferation of the breast cancer cell line T47D. *Proc. Natl. Acad. Sci. USA.* 101:1566-1571.
- Takada Y, Kato C, Kondo S, Korenaga R and Ando J. (1997) Cloning of cDNAs encoding G protein-coupled receptor expressed in human endothelial cells exposed to fluid shear stress. *Biochem. Biophys. Res. Commun.* 240:737-741.
- Testa JR and Bellacosa A. AKT plays a central role in tumorigenesis. (2001) *Proc. Natl. Acad. Sci. USA* 98:10983-10985.

- Thiruvengadam A, Govindarajulu P and Aruldhas MM. (2003) Modulatory effect of estradiol and testosterone on the development of N-nitrosodiisopropanolamine induced thyroid tumors in female rats. *Endocr Res.* 29:43-51.
- Vasko VV and Saji M. (2007) Molecular mechanisms involved in differentiated thyroid cancer invasion and metastasis. *Curr Opin Oncol.* 19:11-17.
- Vivacqua A, Bonofiglio D, Albanito L, Madeo A, Rago V, Carpino A et al. (2006) 17beta-estradiol, genistein, and 4-hydroxytamoxifen induce the proliferation of thyroid cancer cells through the G protein-coupled receptor GPR30. *Mol Pharmacol.* 70:1414-1423.
- Wang F, Porter W, Xing W, Archer TK and Safe S. (1997) Identification of a functional imperfect estrogen-responsive element in the 5'- promoter region of the human cathepsin D gene. *Biochemistry.* 36:7793-7801.
- Wang Y, Hou P, Yu H, Wang W, Ji M, Zhao S et al. (2007) High prevalence and mutual exclusivity of genetic alterations in the phosphatidylinositol-3-kinase/akt pathway in thyroid tumors. *J Clin Endocrinol Metab.* 92:2387-2390.
- Watson CS, Alyea RA, Jeng YJ and Kochukov MY. (2007) Nongenomic actions of low concentration estrogens and xenoestrogens on multiple tissues. *Mol Cell Endocrinol* 274:1-7.
- Weihua Z, Saji S, Makinen S, Cheng G, Jensen EV, Warner M and Gustafsson JA. Estrogen receptor (ER) beta, a modulator of ERalpha in the uterus. *Proc. Natl. Acad. Sci. USA.* 97:5936-5941.
- Withers DJ, Gutierrez JS, Towery H, Burks DJ, Ren JM, Previs S, Zhang Y, Bernal D, Pons S, Shulman GI, Bonner-Weir S and White MF. (1998) Disruption of IRS-2 causes type 2 diabetes in mice. *Nature* 391:900-904.
- Wong CW, McNally C, Nickbarg E, Komm BS and Cheskis BJ. (2002) Estrogen receptor-interacting protein that modulates its nongenomic activity-crosstalk with Src/Erk phosphorylation cascade. *Proc Natl Acad Sci U S A.* 99:14783-14788.
- Wrutniak C, Cassar-Malek I, Marchal S, Rasclé A, Heusser S, Keller JM, Flechon J, Dauca M, Samarut J, Ghysdael J and Cabello G. (1995) A 43-kDa protein related to c-Erb A alpha 1 is located in the mitochondrial matrix of rat liver. *J Biol Chem.* 270:16347-16354.
- Xing M. (2005) BRAF mutation in thyroid cancer. *Endocr. Relat. Cancer.* 12:245-262.
- Yeager N, Brewer C, Cai KQ, Xu XX and Di Cristofano A. (2008) mTOR is the key effector of PI3K-initiated proliferative signals in the thyroid follicular epithelium. *Cancer Res.* 68:444-449.
- Yeager N, Klein-Szanto A, Kimura S and Di Cristofano A. (2007) Pten loss in the mouse thyroid causes goiter and follicular adenomas: insights into thyroid function and Cowden disease pathogenesis. *Cancer Res.* 67:959-66.
- Zeng Q, Chen GG, Vlantis AC and van Hasselt CA. (2007) Oestrogen mediates the growth of human thyroid carcinoma cells via an oestrogen receptor-ERK pathway. *Cell Prolif.* 40:921-35.
- Zhang Z, Duan L, Du X, Ma H, Park I, Lee C, Zhang J and Shi J. (2008) The proliferative effect of estradiol on human prostate stromal cells is mediated through activation of ERK. *Prostate.* 68:508-516.



Thyroid and Parathyroid Diseases - New Insights into Some Old and Some New Issues

Edited by Dr. Laura Ward

ISBN 978-953-51-0221-2

Hard cover, 318 pages

Publisher InTech

Published online 07, March, 2012

Published in print edition March, 2012

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.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Valeria Gabriela Antico Arciuch and Antonio Di Cristofano (2012). Estrogen Signaling and Thyrocyte Proliferation, Thyroid and Parathyroid Diseases - New Insights into Some Old and Some New Issues, Dr. Laura Ward (Ed.), ISBN: 978-953-51-0221-2, InTech, Available from:
<http://www.intechopen.com/books/thyroid-and-parathyroid-diseases-new-insights-into-some-old-and-some-new-issues/estrogen-signaling-and-thyrocyte-proliferation>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen