# 10 Androgen Receptor Expression in Human Thyroid Cancer Tissues: A Potential Mechanism Underlying the Gender Bias in the Incidence of Thyroid Cancers

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**Abstract**

Sex difference in the incidence of thyroid cancer with predominance of the disease in women is well known, whereas the underlying mechanism remains obscure. Research performed during the last four decades points out that sex steroids may underlie this bias in thyroid cancer incidence. This review attempts to compile the available information in the area. The authors have taken care to include all relevant publications. If any of the important reports is not included, it is inadvertent and not intentional. A series of reports from our laboratory have established that testosterone stimulates the proliferation and growth of normal thyroid gland in rats of either sex, whereas estradiol has a specific stimulatory effect in females and an inhibitory effect in males. Early experimental studies in rats revealed that sex steroids may promote thyroid tumorigenesis;

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P.R. Sudhakaran (ed.), *Perspectives in Cancer Prevention – Translational Cancer Research*, DOI 10.1007/978-81-322-1533-2_10, © Springer India 2014

we have shown that testosterone may specifically promote malignancy. We have also shown the stimulatory effect of testosterone and estradiol in human thyroid cancer cell lines NPA-87-1 and WRO-82-1. In a recent paper we reported a positive correlation between AR ligand-binding activity and its protein expression level; AR mRNA expression had a positive correlation with its transcription factors Sp1 and a negative correlation with p53, its repressor in papillary thyroid carcinoma (PTC) or follicular adenoma (FTA) tissues of women. There was inconsistency between expression levels of AR mRNA and its protein, which was influenced by the expression level of the microRNA (miR)-124a. From our in vitro experiments using a human PTC cell line (NPA-87-1) transfected with either *miR-124a* or anti-*miR-124a* in the light of our findings from human thyroid tumor tissues, we have shown for the first time that *miR-124a* is a potent inhibitor of AR expression, and its expression pattern may determine the mitogenic effect of testosterone on thyroid cancer.

**Keywords**
Thyroid cancer • Estradiol • Tumorigenesis • Papillary thyroid carcinoma • Mitogenic effect • Testosterone

## 10.1 Introduction

Thyroid cancer has emerged as the most common endocrine malignancy. Broadly, thyroid tumors are classified into tumors of follicular and C cells origin. Benign follicular adenoma, well-differentiated papillary thyroid carcinoma (PTC), follicular thyroid carcinoma (FTC), and poorly differentiated anaplastic thyroid carcinoma (ATC) are tumors of follicular cells; medullary thyroid carcinoma (MTC) is the tumor of C cells (Hedinger et al. 1989). PTC is the major thyroid neoplasia, constituting more than 80 % of all thyroid cancers, followed by FTC, which constitutes 2–5 % thyroid cancers. Though carcinoma of the thyroid constitutes less than 2 % of total cancer cases (Landis et al. 1998; Colonna et al. 2010; Ward et al. 2010), it accounts for 90 % of all endocrine tumors and 63 % of all deaths due to endocrine malignancy (Franker 1995). The incidence of thyroid cancer has increased significantly in the past several decades (Chen et al. 2009). It is estimated that thyroid cancer accounts for approximately 140,000 of the 11 million annual global cancer cases (Nandakumar et al. 2005) and about 37,400 of the 1.5 million cases of cancers that are diagnosed annually in the USA (ACS 2012). It is the 6th most commonly diagnosed cancer among American women and the most common cancer of the endocrine system (ACS 2012).

The disease is relatively more prevalent in younger age group than adults, with almost two-thirds of the diagnosed cases being persons aged 20–55 years and the mortality rate increasing gradually by 40–45years of age (Dean and Hay 2000). Thyroid cancer had the fastest reported increase in the age-adjusted annual incidence rate, compared to other cancers recorded in the USA between 1980 and 2005 (Chen et al. 2009). In the last decade, the incidence of thyroid cancer in the USA had increased by 82 %, whereas the USA population grew at a rate of 12 % (ACS 2012). The pattern of thyroid cancer in India is different from that seen in Western countries. The Indian National Cancer Registry (INCR) reported 3,617 female and 2,007 male thyroid cancer cases between 1984 and 1993 (Rao 1999). The nationwide relative frequency of thyroid cancer among all the cancer cases was 0.1–0.2 % (Unnikrishnan and Menon 2011). In Mumbai, western India, the thyroid cancer incidence was found to be at the lowest level in both sexes but was about

three times more frequent among women than in men (Rao 1999). The age-adjusted incidence rate of thyroid cancer per 100,000 is about 1 for males and 1.8 for females as per the Mumbai Cancer Registry, which covered a population of 9.81 million subjects (Unnikrishnan and Menon 2011). One report from Chennai, South India, showed that thyroid carcinoma constitutes about 1–2 % of all cancers (Dorairajan et al. 2002). Our study conducted during the period 2005–2007 recorded 125 cases of thyroid cancer, among 381 patients who were admitted for surgical treatment of different thyroid disorders at the Department of Endocrine Surgery, Rajiv Gandhi Government General Hospital, Madras Medical College, Chennai (one of the major referral center for endocrine surgeries in South India), with the female:male ratio of 5.2:1 (unpublished data). In Goa, the incidence of PTC was reported to be higher than other thyroid cancers with a low ratio of PTC to FTC in iodine-deficient areas, where iodized salts are not in use (Arora and Dias 2006).

## 10.2 Etiology of Thyroid Cancer

The thyroid gland is one of the most radiosensitive organs in the human body (Ron et al. 1998; Schonfeld et al. 2011). Exposure to radiation is by far the most well-established risk factor for thyroid cancer (Reiners 2009; Lukas et al. 2012; Mazonakis et al. 2012; Tronko et al. 2012; Veiga et al. 2012). The increased incidence of thyroid cancer from 1935 to 1975 observed in the USA was attributed to therapeutic radiation administered to the head and neck area of children for the treatment of benign abnormalities (Pottern et al. 1980). The Chernobyl nuclear reactor accident resulted in a marked increase (nearly 2,400 %) in the incidence of thyroid cancer in Belarus, affecting predominantly females (Demidchik et al. 2007; Tronko et al. 2012). Even in the adjoining Ukraine, there was marked increase in the incidence of thyroid cancer among men and women (Tronko et al. 2012; Demidchik et al. 2007). This dramatically increased the number of thyroid cancer in children living in Belarus, Ukraine, and Russia was related to the radioactive iodine contamination (Pacini et al. 1997; Stiller 2005; Demidchik et al. 2007; Kesminiene et al. 2012; Tronko et al. 2012). Memon et al. (2004) suggested that family history of benign thyroid diseases may be associated with an increased risk of thyroid cancer, particularly among first-degree female relatives. Epidemiological data have revealed a four- to ten fold increase in the incidence of PTC among the first-generation relatives of PTC patients (Pal et al. 2001), suggesting the possibility of familial predisposition to PTC. Several oncogenes and a series of genetic alterations are associated with thyroid cancer. Multiple lines of evidence suggest that *RET*/*PTC* rearrangements may be an early event in thyroid cancer development (Mishra et al. 2009). Involvement of the rat sarcoma (*RAS*) gene has been reported as an early and important event in thyroid tumorigenesis (Volante et al. 2009). Activation of *MYC* oncogene has been detected in human thyroid cancers. Living in volcanic areas (Duntas and Doumas 2009), iodine intake (Franceschi 1998), obesity (Schonfeld et al. 2011), and importantly the female sex (Rahbari et al. 2010) have also been associated with higher incidence of thyroid cancer.

## 10.3 Thyroid Cancer and Gender

Thyroid cancer is one of the cancers in which the incidence rate in women far exceeds that of men (Reynolds et al. 2005). Data from cancer registries of different countries show that more women than men are diagnosed with thyroid cancer (3–5.5:1) (Mack et al. 1999; Negri et al. 1999; Cook et al. 2009; Howlader et al. 2011), a patented phenomenon observed across geographic locations and ethnicity. Our earlier report also confirmed the gender disparity in thyroid cancer incidence as indicated by a sex ratio of 3:1 for PTC, 5:1 for FTC, and 5.4:1 for FTA between women and men (Stanley et al. 2012). The incidence of thyroid cancer in women increases with age (Reynolds et al. 2005), reaches the peak during pubertal age, and decreases after menopause (Preston-Martin et al. 1987; Farahati et al. 1998). Though females have the high incidence of thyroid tumors, men have a high

rate of malignancy, usually with poor prognosis (LiVolsi 1996; Holzer et al. 2000; Wartofsky 2010). In 2012, among 56,000 adults diagnosed with thyroid cancer in the United States, 43,000 were women (ACS 2012). The difference in the incidence of thyroid cancer between men and women suggests a possible role of sex steroids in the incidence of thyroid cancer (Howlader et al. 2011). However, the exact relationship between thyroid cancer incidence and women's reproductive life remains unclear due to the great variability observed among different ethnic groups (Mack et al. 1999; Negri et al. 1999).

## 10.4 Specific Effects of Sex Steroids on Normal and Cancer Thyroid

A sex-specific difference in the ligand-binding activity (LBA) of AR in human thyroid with higher activity in males than in females was reported (Marugo et al. 1991). A series of reports from our laboratory on postnatal development/growth of thyroid gland in rats ascertained the sex-specific effects of testosterone and estradiol on thyrocytes proliferation. Banu et al. (2002) demonstrated that the peak in thyrocytes proliferation in normal male and female rats during the second postnatal week is associated with elevated levels of testosterone and estradiol and their receptors in the thyroid. Another in vitro study on thyrocytes of immature and adult rats revealed a direct mitogenic effect of sex steroids; while testosterone promoted the proliferation of thyrocytes isolated from either sex, estradiol specifically promoted the same in females, whereas it inhibited in males. The above study entrenched the role of sex steroids on thyrocytes proliferation by demonstrating the inhibitory effect of the antiestrogen tamoxifen and the antiandrogen flutamide on estradiol and testosterone-induced thyrocytes proliferation, respectively (Banu et al. 2002). Recently, we reported that the mitogenic effects of testosterone and estradiol on rat thyrocytes are associated with increased expression of IGF-1 (Stanley et al. 2010). Earlier, we reported the differential effect of testosterone and estradiol on the proliferation of human thyroid cancer cell lines; while testosterone promoted the proliferation of PTC (NPA-87-1) and FTC (WRO-82-1) cell lines, estradiol promoted the proliferation of NPA-87-1 alone, whereas it inhibited that of WRO-82-1 cell line (Banu et al. 2001b). The differential effects of estradiol and testosterone on the proliferation of thyroid cancer cell lines underscored the importance of these sex steroids in the sex-specific difference in thyroid cancer incidence. Thiruvengadam et al. (2003) from our laboratory provided evidence for the specific effects of estradiol and testosterone in the promotion of nonmalignant and malignant thyroid tumorigenesis in rat. The above study demonstrated that estradiol promotes the DHPN-induced development of thyroid adenoma in either sex, whereas excess testosterone favored the development of malignant thyroid tumors alone. The effects of testosterone in promoting the malignant thyroid tumors were obvious in gonad intact males and ovariectomized rats, suggesting a modulatory effect of endogenous estradiol on the effects of testosterone (Aruldhas et al. 1995; Thiruvengadam et al. 2003). In vitro studies from our laboratory also revealed the stimulatory effects of testosterone and estradiol on ($^3$H)-thymidine uptake by normal (Banu et al. 2001a, b; Stanley et al. 2010) and cancerous rat thyrocytes (Aruldhas et al. 1995) and human PTC and FTC cell lines (Banu et al. 2001a, b; Stanley et al. 2012), suggesting the direct action of sex steroids on the thyroid. Rossi et al. (1996) reported that 5α-dihydrotestosterone inhibited the proliferation of primary culture of goitrous thyrocytes and the PTC cell line TPC, which was associated with a reduction in the expression of *c-myc* gene, whereas thyroperoxidase and thyroglobulin genes remained unaltered. The exact reason for the inconsistency between our study and that of Rossi et al. is not clear. Probably, the difference in the cell lines used and the type of the androgen and doses employed in the two studies are the reasons for the inconsistency. Bahrami et al. (2009) reported that testosterone decreased thyroid enlargement and prevented the fall in serum free $T_4$ levels in castrated iodine-deficient rats. Testosterone decreased thyroxine

binding globulin (TBG) and total iodothyronines ($T_4$ and $T_3$), indicating the direct effect of testosterone on the thyroid gland (Tahboub and Arafah 2009).

## 10.5 Sex Steroid Receptors and Thyroid Cancer

Androgen receptor (AR), estrogen receptor (ER), progesterone receptor (PR), glucocorticoid receptor, mineralocorticoid receptor, and thyroid hormone receptor (TR) are members of the nuclear receptor super family, a diverse group of transcription factors activated by the binding of a hormone ligand (Flamant et al. 2006). In general, these intracellular receptors undergo a conformational change upon ligand binding, which allows for separation of the receptor from its cytoplasmic chaperone proteins (exception, ERs and TRs). There is subsequent dimerization and binding of the receptor to a specific steroid response element located on the promoter region of target genes, recruiting and interacting with co-activators, co-repressors, or other *cis*-acting transcription factors to regulate gene expression and activate various intracellular pathways (Thakur and Paramanik 2009).

There are two distinct ER subtypes, ERα and ERß, which are coded by separate genes. ERα is expressed most abundantly in the female reproductive tract, in particular the uterus, vagina, and ovaries, while ERß has its highest expression in the prostate, ovaries, and lungs (Saunders and Critchley 2002). Endogenous ligands for AR are testosterone and its potent metabolite 5-α-dihydrotestosterone (DHT), as well as the adrenal pre-hormones dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS); the male reproductive organs exhibit the highest level of AR expression (Cooke et al. 1991).

Thyroid tissue is responsive to sex steroids as AR and ERs are expressed in normal and cancer thyroid tissues (Marugo et al. 1991; Banu et al. 2001a, b, 2002; Banu and Aruldhas 2002; Thiruvengadam et al. 2003; Stanley et al. 2010, 2012). Though radiation exposure, low iodine diet, family history and previous history of thyroid goiter and sex chromosomes have been attributed for thyroid cancer incidence, AR and ER genes have emerged as the most significant candidates responsible for the sex-specific differences in thyroid cancer incidence (Rossi et al. 1996; Banu et al. 2001a, b; Thiruvengadam et al. 2003). Both testosterone and estradiol regulate the proliferation of thyrocytes by upregulating their respective receptors (Banu et al. 2001a, b; Stanley et al. 2010). The presence of AR, ERα, and ERß, and their regulation by respective ligands, suggests that these nuclear receptors might play an important role in the pathogenesis of thyroid tumors. We have shown that the stimulatory effect of testosterone on proliferation of human PTC (NPA-87-1) and FTC (WRO-82-1) cell lines is associated with homologous upregulation of AR, independent of thyroid-stimulating hormone (Banu et al. 2001a, b). The above study also reported homologous regulation of ER by estradiol in those cell lines. Recently, our laboratory investigated (Annapoorna et al. 2011; Annapoorna 2012) the expression pattern of ERα and ERß in 68 human PTC tissue samples (17 males, 51 females); the results from these studies revealed a varied expression pattern of ER subtypes with overexpression of ERα in 51 % of the female and the remaining subjects showing either normal (33 %) or decreased (16 %) expression; among the males 59 % showed overexpression, 24 % had decreased expression, and the remaining recorded normal expression level of ERα. The same study revealed an interesting picture of a general trend of decreased expression of ERß in most of the females (73 %) and increased expression in most of the PTC samples from males (88 %), suggesting a clear sex difference in the expression pattern. Using NPA-87-1 cell lines, Annapoorna (2012) has demonstrated that estradiol prefers ERα over ERß for its mitogenic activity, and the estradiol-induced cell proliferation is associated with activation of the MAP kinase pathway. Recently, we reported sex difference in AR status in human thyroid cancer tissues, i.e. increased expression level of AR mRNA in majority of PTC men and decreased expression in many of the women (Stanley et al. 2012). Magri et al. (2012) reported

that increased expression of AR, ERα and ERß negativity may be associated with aggressive thyroid cancer phenotypes and may represent an additional criterion in deciding whether to perform radioiodine ablation in these tumors. In a recent review Yao et al. (2011) had given an elaborative account of these aspects, and interested readers may refer to the article for additional information.

## 10.6 Androgen Receptor in Thyroid Cancer

*AR* is expressed in many tissues, including normal and malignant thyroid tumors with the highest level observed in male reproductive organs (Magri et al. 2012; Stanley et al. 2012). *AR* gene is located on the X chromosome (q11-12) and the receptor protein is well known as a ligand-inducible transcription factor that regulates target gene expression (Heinlein and Chang 2002; Lee et al. 2003; Brinkmann 2011). As a member of the nuclear receptor superfamily, the *AR* gene consists of eight exons that encode four structurally and functionally distinct domains: the $NH_2$-terminal transactivation domain, the DNA-binding domain, a hinge region, and the COOH-terminal ligand-binding domain (Matsumoto et al. 2013). Upon ligand binding, the receptor undergoes transformation, homodimerization, and nuclear translocation and binds to specific AR-responsive elements located in the promoter region of its target genes; this is followed by recruitment of specific coregulators and basal transcriptional machinery including RNA polymerase II, leading to the transcription of target genes (Heinlein and Chang 2002; Brinkmann 2011).

The presence of AR in normal and cancer thyroid tissues of rodent, primate, and humans favors its role in the pathogenesis of thyroid cancer. A series of reports ascertained the presence of AR in human thyroid tissues (Table 10.1). The rate of thyroid malignancy is high in men with poor prognosis, compared to women. Marugo et al. (1991) showed the presence of higher nuclear AR concentration in the male rather than in the female thyroid cancer tissues. We have reported a varied pattern of AR expression in human thyroid tumor tissue, which could be categorized into subjects with increased, decreased, and unaltered expression (Stanley et al. 2012). These findings suggest that one cannot generalize about the expression pattern of AR in thyroid cancer, and it needs an individualized or categorized approach based on the tumor type, grade, and sex. Recently, based on their immunohistochemical observations of AR in 122 of thyroid cancer tissues, Magri et al. (2012) reported that AR(+) tumors are more aggressive than the AR(−) tumors due to a significantly higher prevalence of capsular invasion. The capsular invasion is a major factor increasing the risk of nodal metastasis in PTC (Pisanu et al. 2009).

All these studies pointed out that androgens have a regulatory role on thyroid tumorigenesis and sex disparity in the incidence and aggressiveness of thyroid cancer. However, the exact mechanism underlying the effect of AR on thyroid cancer and the factors responsible for its varied expression among subjects afflicted with the disease remain obscure. With this background in mind, we investigated 125 thyroid tumor tissue samples excised during surgery for PTC ($n = 68$; 17 males and 51 females), FTC ($n = 6$; 1 male and 5 females), and FTA ($n = 51$; 8 males and 43 females) at the Department of Endocrine Surgery, Madras Medical College, Chennai, India; the parameters studied include testosterone concentration, AR ligand-binding activity, *AR* expression using real-time PCR and western blot techniques, and expression pattern of a few specific regulators of AR mRNA expression (Stanley et al. 2012). The following were the salient features of our report: (1) Peripheral and thyroid tissue concentration increased in women with thyroid tumors, whereas the same decreased in male subjects with the disease. (2) AR ligand-binding activity increased in a majority of male subjects (41 % PTC, 100 % FTC, 88 % FTA) and in carcinoma tissues of females (51 % PTC and 80 % FTC), whereas in 75 % of FTA tissues from females, it decreased. Interestingly, it remained unaltered in 35 % and decreased in 24 % of PTC tissues from males and decreased in 35 %

**Table 10.1** List of reports on androgen receptor expression in human thyroid tissues

| | Author | Tissue type | Notes |
|---|---|---|---|
| 1 | Prinz et al. (1984) | PTC, FTA, NTG | AR was detected in 4/5 malignant and 9/20 nonmalignant human thyroid tissues.<br>No significant difference between sex. |
| 2 | Miki et al. (1990) | PTC, ATC, FTA, NTG | AR was detected in 8/29 nonneoplastic and 4/26 neoplastic tissues.<br>No data on sex or sex disparity. |
| 3 | Ruizeveld de Winter et al. (1991) | No detail provided | Immunostaining of AR in thyroid tissues ($n = 2$).<br>No data on sex or sex disparity. |
| 4 | Marugo et al. (1991) | NTG | *AR* expression detected in 12 female and 6 male thyroid tissues.<br>*AR* expression was higher in men than women. |
| 5 | Kimura et al. (1993) | No detail provided | AR immunostaining detected in human thyroid follicular cells.<br>No data on sex or sex disparity. |
| 6 | Rossi et al. (1996) | | AR was detected in all the noncancerous ($n = 7$) and few of the cancerous samples ($n = 3$) studied.<br>No data on sex or sex disparity. |
| 7 | Zhai et al. (2003) | MTC | AR was detected in 12/16 male and 7/9 female thyroid tissues. |
| 8 | Bléchet et al. (2007) | MTC, CCH | *AR* expression was detected mainly in men and lower expression in women.<br>No sex disparity observed. |
| 9 | Magri et al. (2012) | PTC | *AR* expression detected in 16/78 female, 3/13 male tissues.<br>No data on sex or sex disparity. |
| 10 | Stanley et al. (2012) | PTC, FTC, FTA | *AR* expression was detected in all the 125 samples analyzed; *AR* expression increased in men and decreased in women. Varied *AR* expression and the testosterone levels might contribute for the sex disparity. |

and remained unaltered in 14 % of PTC females. (3) Western blot analysis revealed normal pattern of AR protein expression level in a majority of cancer tissues from males. However, a majority of cancer tissues from female subjects recorded decreased expression level of AR protein; a few samples from either sex recorded increased expression of AR protein. When we subjected the data on AR ligand-binding activity and western blot to Pearson's correlation coefficient analysis, there was a positive correlation between the two parameters in PTC and FTA females and males. (4) In an attempt to comprehend the mechanism underlying the expression AR gene, we determined AR mRNA expression by real-time PCR analysis which showed either increased or normal expression (41 % in each) in males, but decreased expression was evident in a majority of thyroid tumor tissues from women. Pearson's correlation coefficient analysis revealed a positive correlation between AR mRNA and protein in malignant and nonmalignant tissues of women and only in PTC tissues of men. In general, our findings revealed the existence of a sex-specific difference in the expression and ligand-binding activity of AR in thyroid cancer. As discussed earlier, AR is a ligand-mediated transcription factor (Lee and Chang 2003; Flamant et al. 2006) and undergoes homologous upregulation in response to testosterone stimulation (Banu et al. 2001a, b). Therefore, based on the findings of correlation analysis of thyroidal concentration of testosterone and AR ligand-binding activity, and expression levels of AR mRNA and protein, we suggested that the tissue level of testosterone in the thyroid may be an important marker for the prognosis of thyroid cancer in men and women (Stanley et al. 2012).

The *AR* 5′ upstream promoter region lacks a typical TATA box and CAAT box but contains binding sites for key regulatory elements like specificity protein 1 (Sp1) (Mizokami et al. 1994), cAMP response element-binding protein (CBP) (Takane and McPhaul 1996), and p53 (Alimirah et al. 2007). Both Sp1 and CBP were reported to enhance *AR* expression (Faber et al. 1993; Aarnisalo et al. 1998), while p53 was reported to repress it (Alimirah et al. 2007) Increased p53 decreased the expression of AR mRNA and protein in 22Rv1 and LNCaP prostate cancer cell lines (Cronauer et al. 2004; Alimirah et al. 2007). Cyclin D1 was reported to function as a strong corepressor of *AR* in LNCaP cell line (Petre-Draviam et al. 2003).

We also detected the expression level of *AR* transcription factors CBP and SP1 by western blot, in an attempt to understand the regulation of AR transcription. There was no significant correlation between AR mRNA and CBP expression in thyroid tumor tissues from females and males. However, there was a positive correlation between AR mRNA and SP1expressions in PTC tissues of women alone. Increased level of testosterone is known to suppress AR expression via inhibition of Sp1 in rat prostate (Beklemisheva et al. 2007), suggesting that Sp1 and increased testosterone bioavailability could contribute to the downregulation of *AR* in a majority of thyroid tumors in females. Based on the above findings, we suggested that (1) SP1 may be the major positive regulator of *AR* expression in thyroid cancer, and (2) it may be one of the putative factors contributing for the specific decrease in AR expression in PTC women (Stanley et al. 2012).

As there was no consistency between AR mRNA and the positive regulators of its transcription in males, we looked for the expression of repressors of *AR* transcription, P53 and cyclin D1; there was a negative correlation between (decreased) expression of AR mRNA and P53/cyclin D1 proteins in females and an opposite trend between AR mRNA and P53 in males. We also reported a positive association between tissue testosterone level and cyclin D1 expression in PTC tissues from women and an opposite trend in males. An important finding in our study was an inconsistency between AR mRNA and protein expressions in a number of thyroid cancer tissues, particularly in males. Therefore, we looked for the existence of a specific microRNA (miRNA) that may decrease the translation of AR mRNA into its protein. miRNAs regulate translation and stability of particular target mRNAs by imperfect base pairing (Olsen and Ambros 1999). miRNAs are noncoding single-stranded RNAs of about 22 nucleotides. A large number of miRNAs are involved in almost every major cellular function like proliferation, differentiation, and apoptosis, and as a consequence, deregulation of miRNAs has also been linked to a broad variety of cancers (Ha 2011). The role of miRNAs in thyroid cancer is incompletely understood. Several studies have analyzed miRNA expression in numerous and different types of thyroid tumors, suggesting a deregulation of miRNAs in cancer tissues, compared with their normal counterparts (Ricarte-Filho et al. 2009; Abraham et al. 2011; Marini et al. 2011; Leonardi et al. 2012; Vriens et al. 2012). Using bioinformatics analysis we predicted the presence of a binding site (GUGCCUU) for miR-124a at the nucleotide position 88–94 of *AR* 3′ UTR. We found an increase in miR-124a expression in all women who had decreased expression of AR protein, suggesting a negative correlation between the two parameters in those cases that had an inconsistency between AR mRNA and protein. The finding of negative correlation between AR protein and miR-124a in PTC and FTA tissues from women alone also pointed out the sex-specific variation in the regulation of AR in thyroid cancers. We also confirmed the negative effect of miR-124a on AR protein and, thus, on testosterone-mediated proliferation of thyroid cancer cells in NPA-87-1 PTC cell line transfected with miR-124a and anti-miR-124a. *miR-124a* also decreased the AR reporter luciferase activity and the thyroid cancer cell proliferation, suggesting that *miR-124a* can be a potential candidate that determines *AR* gene expression pattern in human thyroid tumors, which could be a putative mechanism underlying the sex bias in the incidence of thyroid cancers. Thus,

for the first time, to the best of our knowledge, we provided evidence that *AR* gene is the direct target for miR-124a, and its expression level determines the expression pattern of *AR* in thyroid tumor tissues. Our study also reported for the first time the existence of a sex-specific relation between testosterone bioavailability and *AR* expression pattern in thyroid tumors (Stanley et al. 2012).

## 10.7 Conclusion

Based on our findings and existing literature, it is concluded that the varying pattern of testosterone level and AR status in thyroid tissues of men and women might contribute to the sex-specific incidence of thyroid tumors. *miR-124a* plays a significant role in determining *AR* gene expression pattern and may direct AR influence on sex-specific thyroid cancer incidence.

**Acknowledgments** This study was supported by the Government of India, Department of Biotechnology (DBT) in the form of a major research project (BT/PR4841/Med/12/187/2004) to Prof. M. Michael Aruldhas, and financial support from University Grants Commission under the Special Assistance Programme and the Department of Science and Technology under FIST Programme is gratefully acknowledged.

## References

Aarnisalo P, Palvimo JJ, Janne OA (1998) CREB-binding protein in androgen receptor-mediated signaling. Proc Natl Acad Sci USA 95:2122

Abraham D, Jackson N, Gundara JS, Zhao J, Gill AJ et al (2011) MicroRNA profiling of sporadic and hereditary medullary thyroid cancer identifies predictors of nodal metastasis, prognosis, and potential therapeutic targets. Clint Cancer Res 17:4772–4781

ACS (2012) Cancer facts and figures. American Cancer Society, Atlanta, pp 1–66

Alimirah F, Panchanathany R, Cheny J, Zhang X, Ho S, Choubey D (2007) Expression of androgen receptor is negatively regulated by p53. Neoplasia 9:1152–1159

Annapoorna K (2012) Mitogenic effect of estradiol on human papillary thyroid carcinoma is mediated through MAPK signaling downstream of estrogen receptor alpha. PhD thesis, University of Madras, India

Annapoorna K, Stanley J, Neelamohan R, Aruldhas MM (2011) Estradiol-induced proliferation of human papillary thyroid cancer by activating Mitogen-activated protein kinase (MAPK) signaling is mediated through its specific receptor subtype. In: AACR International conference on New Horizons in Cancer Research: Biology to Prevention to Therapy, December 13–16, 2011. The Leela Kempinski Gurgaon, Gurgaon, Delhi (NCR), India. Poster No. B29

Arora R, Dias A (2006) Iodine and Thyroid Cancer in Goa. J Health Allied Sci 5:1–3

Aruldhas MM, Thiruvengadam A, Banu SK, Govindarajulu P (1995) Thyroidal concentration of testosterone and estradiol determines the promotion of thyroid tumours in N-nitrosodiisopropanolamine (DHPN) treated Wistar rats. In: 12th Asia Pacific Cancer Conference: towards total cancer control, Singapore, p 43

Bahrami Z, Hedayati M, Taghikhani M, Azizi F (2009) Effect of testosterone on thyroid weight and function in iodine deficient castrated rats. Horm Metab Res 41:762–766

Banu KS, Aruldhas MM (2002) Sex steroids regulate TSH-induced thyroid growth during sexual maturation in Wistar rats. Exp Clint Endocrinol Diabetes 110:37–42

Banu SK, Arosh JA, Govindarajulu P, Aruldhas MM (2001a) Testosterone and estradiol differentially regulate thyroid growth in Wistar rats from immature to adult age. Endocr Res 27:447–463

Banu SK, Govindarajulu P, Aruldhas MM (2001b) Testosterone and estradiol have specific differential modulatory effect on the proliferation of human thyroid papillary and follicular carcinoma cell lines independent of TSH action. Endocr Pathol 12:315–327

Banu SK, Govindarajulu P, Aruldhas MM (2002) Testosterone and estradiol up-regulate androgen and estrogen receptors in immature and adult rat thyroid glands in vivo. Steroids 67:1007–1014

Beklemisheva AA, Feng J, Yeh YA, Wang LG, Chiao JW (2007) Modulating testosterone stimulated prostate growth by phenethyl isothiocyanate via Sp1 and androgen receptor down-regulation. Prostate 67: 863–870

Bléchet C, Lecomte P, De Calan L, Beutter P, Guyétant S (2007) Expression of sex steroid hormone receptors in C cell hyperplasia and medullary thyroid carcinoma. Virchows Arch 450:433–439

Brinkmann AO (2011) Molecular mechanisms of androgen action – a historical perspective. Methods Mol Biol 776:3–24

Chen AY, Jemal A, Ward EM (2009) Increasing incidence of differentiated thyroid cancer in the United States, 1988–2005. Cancer 115:3801–3807

Colonna M, Bossard N, Guizard AV, Remontet L, Grosclaude P (2010) Descriptive epidemiology of thyroid cancer in France: incidence, mortality and survival. Ann Endocrinol (Paris) 71:95–101

Cook MB, Dawsey SM, Freedman ND, Inskip PD, Wichner SM et al (2009) Sex disparities in cancer incidence by period and age. Cancer Epidemiol Biomarkers Prev 18:1174–1182

Cooke PS, Young P, Cunha GR (1991) Androgen receptor expression in developing male reproductive organs. Endocrinology 128:2867–2873

Cronauer MV, Schulz WA, Burchardt T, Ackermann R, Burchardt M (2004) Inhibition of p53 function diminishes androgen receptor-mediated signaling in prostate cancer cell lines. Oncogene 23:3541–3549

Dean DS, Hay ID (2000) Prognostic indicators in differentiated thyroid carcinoma. Cancer Control 7:229–239

Demidchik YE, Saenko VA, Yamashita S (2007) Childhood thyroid cancer in Belarus, Russia, and Ukraine after Chernobyl and at present. Arq Bras Endocrinol Metabol 51:748–762

Dorairajan N, Pandiarajan R, Yuvaraja S (2002) A descriptive study of papillary thyroid carcinoma in a teaching hospital in Chennai, India. Asian J Surge 25:300–303

Duntas LH, Doumas C (2009) The "rings of fire" and thyroid cancer. Hormones 8:249–253

Farahati J, Parlowsky T, Mader U, Reiners C, Bucsky P (1998) Differentiated thyroid cancer in children and adolescents. Langenbecks Arch Surge 383:235–239

Flamant F, Baxter JD, Forrest D, Refetoff S, Samuels H et al (2006) International Union of Pharmacology. LIX. The pharmacology and classification of the nuclear receptor superfamily: thyroid hormone receptors. Pharmacol Rev 58:705–711

Franceschi S (1998) Iodine intake and thyroid carcinoma – a potential risk factor. Exp Clint Endocrinol Diabetes 106(Suppl 3):S38–S44

Franker DL (1995) Radiation exposure and other factors that predispose to human thyroid neoclassic. Surge Clint North Am 75:365–375

Ha T (2011) Micro RNAs in human diseases: from cancer to cardiovascular disease. Immune Netw 11:135–154

Hedinger C, Williams ED, Sobin LH (1989) The WHO histological classification of thyroid tumors: a commentary on the second edition. Cancer 63:908–911

Heinlein CA, Chang C (2002) Androgen receptor (AR) coregulators: an overview. Endocri Rev 23:175–200

Holzer S, Reiners C, Mann K, Bamberg M, Rothmund M et al (2000) Patterns of care for patients with primary differentiated carcinoma of the thyroid gland treated in Germany during 1996. U.S. and German Thyroid Cancer Group. Cancer 89:192–201

Howlader N, Noone AM, Krapcho M, Neyman N, Aminou R, Waldron W, Altekruse SF, Kosary CL, Ruhl J, Tatalovich Z, Cho H, Mariotto A, Eisner MP, Lewis DR, Chen HS, Feuer EJ, Cronin KA, Edwards BK (2011) SEER Cancer Statistics Review, 1975–2008, National Cancer Institute. Bethesda. http://seer.cancer.gov/csr/1975_2008

Kesminiene A, Evrard AS, Ivanov VK, Malakhova IV, Kurtinaitise J et al (2012) Risk of thyroid cancer among Chernobyl liquidators. Radiat Res 178:425–436

Kimura N, Mizokami A, Oonuma T, Sasano H, Nagura H (1993) Immunocytochemical localization of androgen receptor with polyclonal antibody in paraffin-embedded human tissues. J Histochem Cytochem 41:671–678

Landis SH, Murray T, Bolden S, Wingo PA (1998) Cancer statistics. CA Cancer J Clin 48:6–29

Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J et al (2003) The nuclear RNase III Drosha initiates microRNA processing. Nature 425:415–419

Lee HJ, Chang C (2003) Recent advances in androgen receptor action. Cell Mol Life Sci 60:1613–1622

Leonardi GC, Candido S, Carbone M, Colaianni V, Garozzo SF et al (2012) MicroRNAs and thyroid cancer: biological and clinical significance (Review). Int J Mol Med 30:991–999

LiVolsi VA (1996) Well differentiated thyroid carcinoma. Clin Oncol (R Coll Radiol) 8:281–288

Lukas J, Drabek J, Lukas D, Dusek L, Gatek J (2012) The epidemiology of thyroid cancer in the Czech Republic in comparison with other countries. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2012 Nov 2. doi: 10.5507/bp.2012.086. [Epub ahead of print]

Mack WJ, Preston-Martin S, Bernstein L, Qian D, Xiang M (1999) Reproductive and hormonal risk factors for thyroid cancer in Los Angeles County females. Cancer Epidemiol Biomarkers Prev 8:991–997

Magri F, Capelli V, Rotondi M, Leporati P, La Manna L et al (2012) Expression of estrogen and androgen receptors in differentiated thyroid cancer: an additional criterion to assess the patient's risk. Endocr Relat Cancer 19:463–471

Marini F, Luzi E, Brandi ML (2011) MicroRNA role in thyroid cancer development. J Thyroid Res 2011:12 p. Article ID 407123. doi:10.4061/2011/407123

Marugo M, Torre G, Bernasconi D, Fazzuoli L, Cassulo S et al (1991) Androgen receptors in normal and pathological thyroids. J Endocrinol Invest 14:31–35

Matsumoto T, Sakari M, Okada M, Yokoyama A, Takahashi S et al (2013) The androgen receptor in health and disease. Annu Rev Physiol 75:201–224

Mazonakis M, Kourinou K, Lyraraki E, Varveris H, Damilakis J (2012) Thyroid exposure to scattered radiation and associated second cancer risk from paediatric radiotherapy for extracranial tumours. Radiat Prot Dosimetry 152:317–322

Memon A, Berrington De Gonzalez A, Luqmani Y, Suresh A (2004) Family history of benign thyroid disease and cancer and risk of thyroid cancer. Eur J Cancer 40:754–760

Miki H, Oshimo K, Inoue H, Morimoto T, Monden Y (1990) Sex hormone receptors in human thyroid tissues. Cancer 66:1759–1762

Mishra A, Agrawal V, Krishnani N, Mishra SK (2009) Prevalence of RET/PTC expression in papillary thyroid carcinoma and its correlation with prognostic factors in a north Indian population. J Postgrad Med 55:171–175

Mizokami A, Yeh SY, Chang C (1994) Identification of 3′,5′-cyclic adenosine monophosphate response element and other cis-acting elements in the human androgen receptor gene promoter. Mol Endocrinol 8:77–88

Nandakumar A, Gupta PC, Gangadharan P, Visweswara RN, Parkin DM (2005) Geographic pathology revisited: development of an atlas of cancer in India. Int J Cancer 116:740–754

Negri E, Ron E, Franceschi S, Dal Maso L, Mark SD et al (1999) A pooled analysis of case–control studies of thyroid cancer. I. Methods. Cancer Causes Control 10:131–142

Olsen PH, Ambros V (1999) The lin-4 regulatory RNA controls developmental timing in Caenorhabditis elegans by blocking LIN-14 protein synthesis after the initiation of translation. Dev Biol 216:671–680

Pacini F, Vorontsova T, Demidchik EP, Molinaro E, Agate L et al (1997) Post-Chernobyl thyroid carcinoma in Belarus children and adolescents: comparison with naturally occurring thyroid carcinoma in Italy and France. J Clint Endocrinol Metab 82:3563–3569

Pal T, Hamel N, Vesprini D, Sanders K, Mitchell M et al (2001) Double primary cancers of the breast and thyroid in women: molecular analysis and genetic implications. Fam Cancer 1:17–24

Petre-Draviam CE, Cook CL, Burd CJ, Marshall TW, Wetherill YB, Knudsen KE (2003) Specificity of cyclin D1 for androgen receptor regulation. Cancer Res 63:4903–4913

Pisanu A, Reccia I, Nardello O, Uccheddu A (2009) Risk factors for nodal metastasis and recurrence among patients with papillary thyroid microcarcinoma: differences in clinical relevance between nonincidental and incidental tumors. World J Surg 33:460–468

Pottern LM, Stone BJ, Day NE, Pickle LW, Fraumeni JF Jr (1980) Thyroid cancer in Connecticut, 1935–1975: an analysis by cell type. Am J Epidemiol 112: 764–774

Preston-Martin S, Bernstein L, Pike MC, Maldonado AA, Henderson BE (1987) Thyroid cancer among young women related to prior thyroid disease and pregnancy history. Br J Cancer 55:191–195

Prinz RA, Sandberg L, Chaudhari PK (1984) Androgen receptors in human thyroid tissue. Surgery 96:996–1000

Rahbari R, Zhang L, Kebebew E (2010) Thyroid cancer gender disparity. Future Once 11:1771–1779

Rao DN (1999) Epidemiological observations of thyroid cancer. In: Shah AH, Samuel AM, Rao RS (eds) Thyroid cancer – an Indian perspective. Quest Publications, Mumbai, pp 3–16

Reiners C (2009) Radioactivity and thyroid cancer. Hormones (Athens) 8:185–191

Reynolds RM, Weir J, Stockton DL, Brewster DH, Sandeep TC et al (2005) Changing trends in incidence and mortality of thyroid cancer in Scotland. Clint Endocrinol (Oxf) 62:156–162

Ricarte-Filho JC, Fuziwara CS, Yamashita AS, Rezende E, da-Silva MJ et al (2009) Effects of let-7 microRNA on cell growth and differentiation of papillary thyroid cancer. Transl Once 2:236–241

Ron E, Doody MM, Becker DV, Brill AB, Curtis RE et al (1998) Cancer mortality following treatment for adult hyperthyroidism. Cooperative Thyrotoxicosis Therapy Follow-up Study Group. JAMA 280:347–355

Rossi R, Zatelli MC, Franceschetti P, Maestri I, Magri E et al (1996) Inhibitory effect of dihydrotestosterone on human thyroid cell growth. J Endocrinol 151:185–194

Ruizeveld de Winter JA, Trapman J, Vermey M, Mulder E, Zegers ND, van der Kwast TH (1991) Androgen receptor expression in human tissues: an immunohistochemical study. J Histochem Cytochem 39:927–936

Saunders PTK, Critchley HOD (2002) Estrogen receptor subtypes in the female reproductive tract. Reprod Med 10:149–164

Schonfeld SJ, Ron E, Kitahara CM, Brenner A, Park Y, et al (2011) Hormonal and reproductive factors and risk of postmenopausal thyroid cancer in the NIH-AARP Diet and Health Study. Cancer Epidemiol 35:e85–e90

Stanley JA, Aruldhas MM, Yuvaraju PB, Banu SK, Anbalagan J et al (2010) Is gender difference in postnatal thyroid growth associated with specific expression patterns of androgen and estrogen receptors? Steroids 75:1058–1066

Stanley JA, Aruldhas MM, Chandrasekaran M, Neelamohan R, Suthagar E et al (2012) Androgen receptor expression in human thyroid cancer tissues: a potential mechanism underlying the gender bias in the incidence of thyroid cancers. J Steroid Biochem Mol Biol 130:105–124

Stiller CA (2005) Thyroid cancer in Belarus. Int J Epidemiol 34:714

Tahboub R, Arafah BM (2009) Sex steroids and the thyroid. Best Pract Res Clin Endocrinol Metab 23:769–780

Takane KK, McPhaul MJ (1996) Functional analysis of the human androgen receptor promoter. Mol Cell Endocrinol 119:83–93

Thakur MK, Paramanik V (2009) Role of steroid hormone coregulators in health and disease. Hor Res 71:194–200

Thiruvengadam A, Govindarajulu P, 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

Tronko M, Mabuchi K, Bogdanova T, Hatch M, Likhtarev I et al (2012) Thyroid cancer in Ukraine after the Chernobyl accident (in the framework of the Ukraine-US Thyroid Project). J Radiol Prot 32:N65–N69

Unnikrishnan A, Menon U (2011) Thyroid disorders in India: an epidemiological perspective. Indian J Endocr Metab 15:S78–S81

Veiga LH, Lubin JH, Anderson H, de Vathaire F, Tucker M et al (2012) A pooled analysis of thyroid cancer incidence following radiotherapy for childhood cancer. Radiat Res 178:365–376

Volante M, Rapa I, Gandhi M, Bussolati G, Giachino D, Papotti M, Nikiforov YE (2009) RAS mutations are the predominant molecular alteration in poorly differentiated thyroid carcinomas and bear prognostic impact. J Clint Endocrinol Metab 94:4735–4741

Vriens MR, Weng J, Suh I, Huynh N, Guerrero MA et al (2012) MicroRNA expression profiling is a

potential diagnostic tool for thyroid cancer. Cancer 118:3426–3432

Ward EM, Jemal A, Chen A (2010) Increasing incidence of thyroid cancer: is diagnostic scrutiny the sole explanation? Future Once 6:185–188

Wartofsky L (2010) Increasing world incidence of thyroid cancer: Increased detection or higher radiation exposure? Hormones 9:103–108

Yao R, Chiu G, Strugnell SS, Gill S, Wiseman SM (2011) Gender differences in thyroid cancer. Expert Rev Endocrinol Metab 6:215–243

Zhai QH, Ruebel K, Thompson GB, Lloyd RV (2003) Androgen receptor expression in C-cells and in medullary thyroid carcinoma. Endocr Pathol 14(Summer):159–165, PubMed PMID: 12858007