Journal of Steroid Biochemistry & Molecular Biology 130 (2012) 105–124

Contents lists available at SciVerse ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

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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ARTICLE INFO

Article history:
Received 20 May 2011
Received in revised form 6 February 2012
Accepted 11 February 2012

Keywords:
Mir-124a
Testosterone
Thyroid tumors
p53
Sp1
CBP
Cyclin D1

ABSTRACT

Gender bias in the incidence of thyroid cancer is well known, however, the underlying mechanism is largely unknown. The current study determines variations in the molecular characteristics of thyroid cancers between men and women. Normal and cancerous thyroid tissues were collected from a total of 125 men and women who underwent surgical thyroidectomy. Testosterone levels in serum and thyroid cancer tissues were elevated in women while it decreased in men compared to respective control groups; whereas, ligand binding activity increased in men and decreased in women. Androgen receptor (AR) mRNA expression increased in a majority of men while it decreased in a majority of women except those with follicular thyroid carcinoma (FTC). In thyroid cancers of women, Pearson's correlation analysis showed a positive correlation of AR mRNA with AR protein, CBP and Sp1, whereas AR mRNA showed a negative correlation with p53. In case of men, AR mRNA showed a positive correlation with AR and cyclin D1 proteins in papillary thyroid carcinoma (PTC); and CBP and Sp1 in follicular thyroid adenoma (FTA), whereas AR mRNA showed a positive correlation with p53. Our study identified for the first time that AR is posttranscriptionally regulated by *miR-124a* in thyroid cancer tissues. Further, our *in vitro* studies with a PTC cell line (NPA-87-1) showed *miR-124a* as the potent inhibitor of AR that impairs cell proliferation even in the presence of testosterone. Thus, the current study suggests that: (i) the varying pattern of testosterone level and AR status in thyroid tissues of men and women may predispose to the gender specific incidence of thyroid tumors and (ii) *miR-124a* plays a significant role in determining the AR gene expression pattern and thus, androgen mediated thyroid tumor growth.

1. Introduction

Thyroid carcinoma accounts for 90% of all endocrine tumors and 63% of all deaths due to endocrine cancers [1]; although it constitutes less than 2% of total cancer incidence in developed countries [2]. Thyroid cancer incidence in several countries has been increasing during the last 30 years [3–5]. Papillary (PTC), follicular (FTC) and anaplastic or undifferentiated thyroid carcinoma (ATC) are cancers of thyroid follicle cells [6], while medullary thyroid carcinoma (MTC) is of parafollicular or C-cell origin [7]. Thyroid cancer incidence shows an age and gender dependent variation [8], where more women than men are diagnosed with the disease at a ratio of 3–5.5:1 [9,10]. While females have an increased incidence of thyroid tumors, males have a high rate of malignancy with poor prognosis [11]. Hormonal exposure is a risk factor for thyroid cancer in women of <35 years of age [12]. The peak in thyroid cancer incidence seen in women during their reproductive years identifies hormonal changes related to irregular menstrual cycle [13], abortion [14], oral contraceptives [15], hormone replacement therapy [16] and fertility drugs [17] as relevant etiological factors.

Sex steroids exert many of their functions through intracellular receptors [18]. As they are considered to play important roles in the development of soft tissue lesions [19], sex steroid receptors are widely studied in head and neck tumors [20]. Even though the role of estradiol is known in thyroid cancers, only limited information is available on the role of androgens. Androgen receptor (AR) is expressed in both normal and malignant thyroid

Abbreviations: PTC, papillary thyroid carcinoma; FTC, follicular thyroid carcinoma; ATC, anaplastic thyroid cancer; MTC, medullary thyroid cancer; FTA, follicular thyroid adenoma; AR, androgen receptor; ARE, androgen response element; E_2, estradiol; ER, estrogen receptor; DHT, dihydrotestosterone; Sp1, specificity protein1; CBP, cAMP response element binding protein; p53, tumor protein 53; OD, optical density; miR/miRNA, micro RNA; CDK, cyclin dependent kinase; UTR, untranslated region; LBA, ligand binding activity.

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doi:10.1016/j.jsbmb.2012.02.004

Table 1
Baseline characteristics of thyroid cancer patients.

Variable	Papillary thyroid cancer		Total	Follicular thyroid cancer		Total	Follicular thyroid adenoma		Total
	Males ($n=17$)	Females ($n=51$)	$n=68$	Males ($n=1$)	Females ($n=5$)	$n=6$	Males ($n=8$)	Females ($n=43$)	$n=51$
Mean age (years)	38.8 ± 3.6	36.2 ± 2.5		60.0 ± 0	28.2 ± 4.3		28.9 ± 3.7	35.1 ± 1.7	
Mean height (cm)	164 ± 2.38	157.1 ± 1.74		172 ± 0	154.2 ± 1.6		164.1 ± 2.1	152.5 ± 1.7	
Mean Weight (kg)	57.8 ± 2.59	54.3 ± 2.42		70 ± 0	54.7 ± 7.7		64.0 ± 7.5	53.7 ± 2.6	
Pathological stage									
Grade 1	–	–		–	–		–	–	
Grade 2	14	28		–	2		3	31	
Grade 3	2	23		1	3		5	9	
Grade 4	1	–		–	–		–	3	

cm, centimeter; kg, kilogram; Grade-Tumor stage at the time of diagnosis.

tumors [21,22]. On activation by the binding of androgens, AR undergoes rapid homodimerization and nuclear translocation, and binds to androgen-responsive elements located in the promoter region of its target genes [23]. After binding to promoters, AR recruits the co-regulators together with the basal transcriptional machinery, and modulates the transcription of target genes leading to cell proliferation, differentiation and survival [24]. AR is present in higher concentrations in the thyroid glands of males than in females [22]. Differences in the incidence of thyroid cancers between males and females and the presence of AR and estrogen receptor (ER) α and β sub-types in normal and tumor thyroid tissues of humans and experimental animals suggest a role for these receptors in the etiology of the disease [21,25]. Experimental studies from our laboratory showed a promoting effect of testosterone on N-bis (2-hydroxypropyl) nitrosamine-induced thyroid tumors and a protective effect of estradiol (E_2) against the development of thyroid carcinoma in male rats [26]. The potent androgen 5α-dihydrotestosterone (5α-DHT) inhibited proliferation of an AR-positive PTC cell line *in vitro* [27]. However, an *in vitro* study from our laboratory revealed stimulatory effect of testosterone on proliferation of human PTC (NPA-87-1) and FTC (WRO-82-1) cell lines, through homologous up-regulation of AR, independent of thyroid stimulating hormone (TSH) [28]. We have also demonstrated that testosterone stimulated the proliferation of thyrocytes from normal immature rats of both males and females, whereas, E_2 showed a pleiotrophic effect, where it was stimulatory on thyrocyte proliferation in females, and inhibitory in males, showing a differential response based on sex [25,29]. However, the underlying mechanism behind these differential effects of testosterone and E_2 are not known.

Although thyroid cancer is heterogeneous in its etiology and progression, AR and ER genes have emerged as the most significant contributor to its proliferation and development [27,28,30–32]. The gene for the AR is located on the long arm of the X chromosome (q11-12) and consists of 8 exons spanning a region of 75 kb [33]. 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) [34], cAMP response element binding protein (CBP) [35] and p53 [36]. Both Sp1 and CBP were reported to enhance AR expression [37,38] while p53 was reported to repress it [36]. Therefore, the current study was designed to understand the interaction/association between AR and its downstream transcriptional factors CBP, Sp1 and p53 in thyroid cancers from men and women.

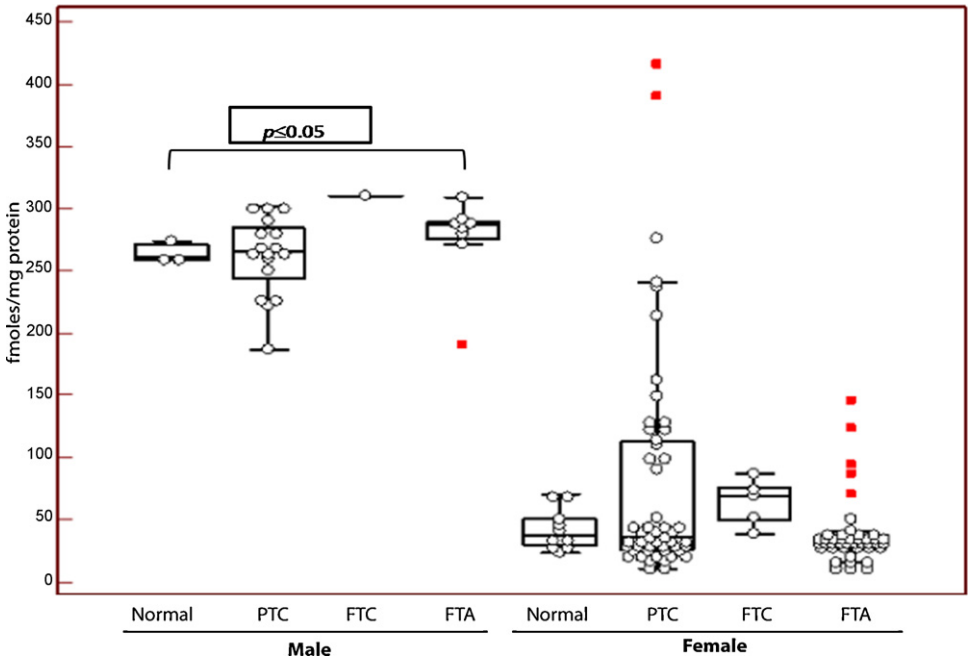

Fig. 1. AR nuclear ligand binding activity in thyroid cancer tissues from men and women. The box and whisker plot comprises 3 components: The horizontal line in the box indicate the central tendency of location (median value), a box to indicate variability (standard error) that represents values from the lower to the upper quartiles (25th to 75th percentile), the line extends from the minimum (5th percentile) to the maximum (95th percentile) values, excluding 'outside' values, that is, values smaller than the lower quartile minus 1.5-fold the inter quartile range, or larger than the upper quartile plus 1.5-fold the inter quartile range, plotted as a square marker. Please refer Table 1 for number of cases and characteristics of tumor samples.