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Thyroid Cancers: Molecular Concepts

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Thyroid cancer is the most common malignancy among the endocrine carcinomas. More recently several studies are representative of a significant increase in thyroid cancer incidence which has been attributed to the different risk factors such as iodine deficiency, radiation exposure and changes in lifestyles. Histopathologically, four main subtypes of the thyroid carcinomas have been demonstrated. These subtypes origin from **(of)** different thyroid cells and include papillary thyroid cancer **(PTC)**, follicular thyroid cancer **(FTC)**, anaplastic thyroid cancer **(ATC)** and medullary thyroid cancer **(MTC)**. PTC, FTC (also called as differentiated thyroid cancers **[DTCs]**) and ATC (also known as undifferentiated thyroid cancer) derive from follicular thyroid cells and MTC subtype originates from para-follicular thyroid cells **(C-cells)**. Nowadays, progressive and astonishing understanding of the molecular mechanisms and genetic pathogenesis of thyroid carcinomas and identification of the different important cell signaling pathways has propelled the endocrine specialist to offer effective treatment strategies for thyroid cancer therapy. On the other hand, targeting of these molecular pathways has drawn the scientific particular attention

to develop different adjutant and combination therapy for patients with advanced subtypes of thyroid carcinomas. In this chapter we have focused on the molecular basis of the different subtypes of thyroid malignancies.

Keywords: Thyroid Carcinoma; Papillary Thyroid Cancer; Follicular Thyroid Cancer; Anaplastic Thyroid Cancer; Medullary Thyroid Cancer

INTRODUCTION

Thyroid cancer is the most common malignancy among the endocrine carcinomas and head and neck neoplasms. It accounts more than 90% of endocrine cancers and 1% of all cancers [1]. A large body of studies [2-6] has demonstrated a significant increase in thyroid cancer incidence which has been attributed to the different risk factors such as iodine deficiency, radiation exposure and changes in lifestyles [7]. Anatomically, the thyroid gland is a butterfly-shaped organ with 15-25 g weight, located on the anterior surface of the trachea at the base of the neck and comprised of colloid-filled lobules of spherical follicles. The lobules are surrounded by follicular epithelial cells synthesizing and secreting thyroid hormones (L-triiodothyronine **(T3)** and L-thyroxine **(T4)**). Thyroid para-follicular cells which also called C cells, are located at the junction of the upper and middle third of both thyroid lobes and produce calcitonin [8]. Generally, thyroid cancers are classified into two main groups: 1) follicular thyroid cells-derived cancers (more than 95% of all thyroid cancers) including papillary thyroid cancer **(PTC)**, follicular thyroid cancer **(FTC)**, anaplastic thyroid cancer **(ATC)** [9] and 2) para-follicular-originated cancer (roughly 3% of all thyroid cancers) including medullary thyroid cancer **(MTC)** [10]. In spite of the same cellular origin, PTC, FTC and ATC have different biological, behavioral and histological characteristics due to the various molecular and genetic alterations [11]. PTC and FTC also called differentiated thyroid cancers **(DTCs)** are most common thyroid malignancies with good prognosis whereas ATC also known as undifferentiated thyroid cancer is the most aggressive solid tumor with fatal entity [12]. In spite of the high prevalence of thyroid nodules in adults, hardly ever they convert into thyroid carcinomas. The gold standard for evaluation of thyroid nodules is fine needle aspiration biopsy **(FNAB)** followed by cytological examination [13-15]. Although, in the most cases, definite discrimination between benign and malignant nodules is possible by FNAB [15] but, roughly 10 to 30% of nodules remain indeterminate leading to inappropriate management and unnecessary surgery [16,17]. Nowadays, increasing understanding of the molecular pathogenesis of thyroid cancer has led to the discovery of different genetic alterations in cell signaling pathways [18]. Identification of many of these molecular alterations has caused to: 1) developing new diagnostic and prognostic molecular markers and therapeutic targets for thyroid carcinomas [19], 2) suggesting effective methods to risk-stratify patients before undergoing to surgery, 3) reducing the number unnecessary thyroidectomies in patients with benign nodules, and 4) identifying the patients' recurrence risk, or the risk of dedifferentiation into a more aggressive subtype. Like the other cancers, progression of thyroid malignancies has been attributed to the accumulation of genetic and epigenetic lesions which cause to disturbances in signaling pathways playing critical

role in cell proliferation and survival. The discovery of the molecular alterations has led to the identification of several molecular biomarkers that may cause to development of early diagnosis and good prognosis of the disease [18]. In this chapter, the molecular bases of different subtypes of thyroid cancers and the biomarkers related to each subtype have been discussed.

PAPILLARY THYROID CARCINOMA

Papillary thyroid carcinoma **(PTC)** is the most frequent thyroid cancer and roughly accounts 80-90% of thyroid cancers [20]. Nowadays, there is a good prognosis for PTC but identification of the patients with metastasis or recurrence risk has remained as a major clinical challenge. Before surgery, the molecular prediction of PTC can provide insight about the aggressiveness of the disease and contribute to select an appropriate treatment [21]. Generally, prognostic factors for PTC include tumor size, lymph node metastasis and extra-thyroidal invasion [22].

Immunohistochemistry is a method investigating protein expression and their location within the cells. By this method several thyroid specific antigens have been identified which are used for PTC diagnosis including thyroglobulin **(Tg)**, thyroid peroxidase **(TPO)**, thyroid transcription factor-1 **(TTF-1)**, sodium iodide symporter **(NIS)** and solute carrier family 26 **(SLC26A4)**. Tg and TPO present in all thyroid tumors derived from follicular cells including metastatic thyroid tumors. The presence of TTf-1 in PTC is associated with the tumor aggressiveness and overexpression of NIS has been demonstrated for this subtype [23]. In a study carried out by Fan three proteins including haptoglobin alpha-1 chain, C-I and C-III apolipoproteins were introduced as molecular biomarkers for PTC diagnosis. The results demonstrated that haptoglobin alpha-1 chain significantly was overexpressed in patients with PTC and it may play a crucial role in PTC development. On the contrary, a significant decrease has been observed in the expression of C-I and C-III apolipoproteins in PTCs [20]. Haptoglobin has an important role in iron binding to hemoglobin, therefore its overexpression leads to increase of hemoglobin/iron and consequently to raise the possibility of a causative involvement in iron-derived oxidative stress in the tumour development. Recently, several studies have demonstrated overexpression of haptoglobin in ovarian, prostatic, pancreatic [24-26] and breast cancers [27]. Midkine is a multifunctional cytokine which has been considered as a biomarker in different cancers and nuclear factor-κB **(NF-κB)** is an important transcription factor with determining role in tumorigenesis. In a study, Zhang et al found that these proteins could be utilized potentially in differential diagnosis between PTC and multi nodular goiter. In this study a significant increase in the level of these proteins in PTC tissue samples has been reported [28].

Mitogen-activated protein kinase **(MAPK)** and phosphatidylinositol-4,5-bisphosphate 3-kinase/protein kinase B **(PI3K-AKT)** pathways are the most important signal transduction pathways in thyroid cancers pathogenesis. Both of the pathways are triggered by coupling to the cell membrane tyrosine kinase receptors **(TRKs)**, have fundamental role in the regulation of critical cell processes such as cell proliferation, differentiation, apoptosis, and survival. When

an extracellular signal stimulates TKRs, a downstream cascade of molecular events is triggered. Activation of TKRs is followed by the activation of Rat Sarcoma **(RAS)**, Rapidly Accelerated Fibrosarcoma **(B-RAF)**, mitogen-activated protein kinase kinase **(MEK)**, and extracellular signal-regulated kinases **(ERK)**. ERK is a nuclear transcription factor inhibiting tumor suppressor genes and thyroid iodide-handling genes. Various mechanisms have been identified in this pathway leading to thyroid tumorigenesis, including aberrant hypermethylation, hypomethylation, upregulation of oncogenic proteins such as chemokines, matrix metalloproteinases, nuclear factor-κB, and vascular endothelial growth factor A [18]. The most common genetic alteration in PTC is BRAF mutations which are found in 50% of the cases [18]. The presence of BRAF mutations in serum samples of PTCs may be representative of the necessity of treatment by surgery followed by radiotherapy owing to the aggressive entity of the tumor in these cases [21, 29].

Micro ribonucleotide acids **(miRNAs)** are the other predictive biomarkers in thyroid cancers which have drawn the researchers' attention recently. The mature miRNA is a small single strand non-coding molecule with 19-22 nucleotides regulating gene expression in post-transcription step. These molecules pair with 3′UTR of target mRNA and causes its upregulation or downregulation [30]. The circulating miRNAs have been considered as appropriate biomarkers owing to the high stability and lack of the post-translational changes [31]. In a study carried out by Lee et al. miRNA-222 and miRNA146b present in thyroid tissue and blood samples were introduced as predictor biomarkers for PTC recurrence. According to these findings the plasma levels of miRNA-222, miRNA-146b and miRNA-221 significantly were higher in PTC patients in comparison to the patients affected by multi nodular goiter. Moreover, the expression of these molecules significantly was high in recurrent tumors [32]. Recent studies have been demonstrated an association between invasion of tumor cells and BRAF mutations with the overexpression of miRNA-146b-5 [22,33]. In the other study, Yu et.al provided a serum miRNA profile for patients with PTC and benign thyroid tumors. The results showed a significant increase in the levels of let-7e, miRNA-222 and miRNA-151-5p in PTCs [34]. According to the study perfprmed by Nikiforova et al., miRNA-181b, miRNA-187 and miRNA-224 were overexpressed in PTC patients [35]. In overall, researchers gathered that the serum profiles of miRNAs may be used as the novel diagnostic biomarkers for PTC [34].

FOLLICULAR THYROID CARCINOMA

Follicular thyroid carcinoma **(FTC)** is the second common thyroid cancer [36]. Differential diagnosis between FTC and follicular thyroid adenoma **(FTA)** is the most difficult aspect of thyroid pathology. The control algorithms of FTC and FTA are different and the patients affected by FTA require total thyroidectomy, radioactive iodine therapy and long-term follow-up. Capsular invasion and angiogenesis are diagnostic criteria for FTC having a wide diversity, therefore molecular markers potentially may provide differential diagnosis between FTC and FTA in the samples after surgery [37]. Various single immunohistochemical biomarkers have been suggested for FTC diagnosis. Among these molecules galectin-3 is the most widely accepted single protein

that improves diagnostic accuracy, even in the case of minimally invasive FTC [37]. Nowadays, commercial kits are available for the assessment of this protein. The other histochemical biomarkers such as HBME-1, Ki67 and QPRT are also proposed for differential diagnosis of FTC and FTA but their specificity and sensitivity are not appropriate for clinical applications. The assessment of serum Tg is used for the diagnosis of thyroid cancer recurrence or the residual thyroid tissue after surgery but its sensitivity is not sufficiently high and reliable in the presence of thyroglobulin antibody [38].

Several mutations involved in pathogenesis of follicular tumors that the most important of these include PAX8/ peroxisome proliferator-activated receptor*γ* gene (PPAR) translocation and point mutations in the RAS gene. The first mutation occurs in 35-47% of FTCs and more than 13% of FTAs [37]. In the PAX8/PPAR translocation, PAX8 gene from chromosome 2 is translocated on chromosome 3 and causes to create a fusion gene. The protein which is produced from this gene has a different function in comparison to the wild type gene production [35]. RAS mutations in FTC can provide useful information about tumor behavior, but sometimes these mutations are found in both FTC and FTA cases, therefore it seems the these mutations have not high diagnostic value in differentiation of FTC from FTA. In a study performed by Nikiforov et al. a molecular panel including RAS mutations had been proposed and finally 85% positive cases of these mutations were identified as FTC [38]. Several studies have determined miRNA profiles for differential diagnosis between FTC and FTA and some of the miRNAs have been proposed as sensitive biomarkers [38]. Weber et al. demonstrated a significant increase in the expression of miRNA197 and miRNA346 in cancerous cells of FTC [35]. It seems that a treatment algorithm comprising FNA and miRNA panels will be used in the future to provide optimum analysis parameters [37].

ANAPLASTIC THYROID CARCINOMA

Anaplastic thyroid carcinoma **(ATC)** is uncommon (less than 5% of all thyroid cancers), most aggressive and fatal form of thyroid cancer derived from follicular cells [39]. ATC diagnosis is usually based on clinical examination and FNA results. The tumor is in single or multi nodule forms and its size is usually more than 5cm, these clinical signs are associated with congestion and hoarseness. Such diagnosis is approved using FNA and in the 90% of patients is accurate and correct [40].

Mutation in p53 gene (one the most important tumor suppressor genes) causes to PTC or FTC conversion to ATC [41]. The patients affected by ATC are older than the patients with the other thyroid cancers and 60-70% of all ATCs are women. More than 25% of ATCs coincidentally show FTC in histological tests. This can confirm the hypothesis emphasizing ATC development from FTC and PTC. On the other hand studies performed on BRAF and p53 verify this hypothesis. Development of an effective treatment strategy has been encountered with difficulties owing to low incidence of ATC, its aggressive and fatal entity [39]. Radiotherapy, chemotherapy and surgery alone is rarely effective and it seems that combination of these methods together has

a greater impact on the course of treatment. Therefore, new therapeutic strategies required and molecule-based treatments are being investigated [39,41]. Contrary to PTC, there are few proteins and genetic therapeutic targets for ATC [41]. Mutations in BRAF, RAS, p53, β-catenin, PIK3CA, Oxin, APC and PTEN have been reported in ATC. The studies carried out by comparative genome hybridization **(CGH)** have demonstrated abnormalities in some genes including EGFR, MET, BRAF, K-RAS, CCND1, FOSL1, UBE2C and CDKN2A [42]. High prevalence of BRAFV600E shows that origins from typical forms of PTC and BRAF mutations may be associated with tumor progression. The frequency of RAS mutations in ATC is between 0 and 60% and it may increase in malignant tumors [43].

As mentioned previously, there is an association between thyroid tumors and specific panels of miRNA. Four miRNAs discovered related to ATC may interfere in the function of proteins involved in thyroid cancers progression. The other targets of miRNAs include PTEN, E2F and hTERT [44]. Vinson et al. compared the miRNA profiles of ATC and normal thyroid tissue by miRNACHIP microarray. According to the results there was a significant different between miRNA profiles in ATC and normal thyroid tissue samples. Significant decrease in the expression of miRNA-30a-5pγ miRNA-30dγ miRNA-125b and miRNA-26a was demonstrated in ATC samples in comparison to normal tissue [45]. Although the histopathological features of ATC cells are determinable, but owing to very high invasion speed there is no good prognosis for ATC. FNA is the most effective approach for accurate diagnosis of ATC, therefore there is no challenging problem in ATC distinguish from the other thyroid cancer subtypes. In spite of different molecular biomarkers discovered for ATC diagnosis, they are related to the end stages of the disease and they could not to achieve FDA approval [44].

MEDULLARY THYROID CARCINOMA

Medullary thyroid cancer **(MTC)** is a scarce neoplasia arising from thyroid para-follicular cells (or C-cells secreting calcitonin in thyroid gland) [46,47]. It accounts approximately 5-10% of all thyroid cancers and is in two forms including inheritance (25% of cases) and sporadic (75% of cases) [48-50]. The inheritance form is subdivided into multiple endocrine neoplasias **(MEN)** 2a, MEN2b and familial MTC **(FMTC)**. In addition of thyroid, other organs such as parathyroid and adrenal glands are involved in inheritance form of MTC [47]. The main feature of MEN2 is MTC and its inheritance pattern is autosomal dominant owing to mutations in the RET proto-oncogene in germline cells [46,51-53]. Various clinical, pathological and genetic variables have been considered as prognostic biomarkers for MTC including age at the diagnosis time, nodule size, metastasis distance, pathological stage and mutagen lesions in tumor suppressor genes [54]. Tumor cells in MTC secret many proteins which can be used as biomarkers. Clinically, calcitonin and carcinoma embryonic antigen **(CEA)** are the most important and informative molecules for MTC diagnosis. In the previous studies a correlation between calcitonin and CEA levels with lymph node metastasis and metastases distance had been demonstrated, nevertheless routine measurement of serum calcitonin has remained challenging owing to some anxieties about

methodology, sensitivity, specificity and effectiveness of the tests. Calcitonin levels are related to the disease prognosis and used to disease follow-up [42]. The physician suspects to MTC in patient by physical examination and high levels of calcitonin. Cytological and histological conformation is necessary for MTC diagnosis and ultrasound, computed tomography **(CT)** scan and magnetic resonance imaging **(MRI)** are used to determine the tumor size. Effectiveness of treatment and survival of patient depend on the disease diagnosis time [43,47]. The 10-year overall survival rate for MTC patients diagnosed in stage I and II is 90–100%, in stage III is 55-85% and for patients diagnosed in stage IV is 20-55%. Over the past 30 years, epidemiologic studies have showed that no change has occurred in diagnosis or improvement of survival rate. This issue emphasizes the importance of initial diagnosis in both heritable and sporadic forms of MTC [43].

Calcitonin is a hormone specifically produced by the thyroid C cells, therefore increase in its plasma level can be used as a specific biomarker for initial diagnosis of MTC and C-cell hyperplasia [29,55]. However, sometimes increase in calcitonin levels is not observed. Normal level of calcitonin in blood circulation is less than 20 pg/ml and this amount may vary according to the type of the assay method and some physician use 20 pg/ml as the cutoff [55]. Although the high levels of calcitonin is representative to MTC/CCH, but the increase in calcitonin amounts is not so specific and it may increase in the individuals' plasma without diseases related to C-cells [43,55]. Moreover, calcitonin level is related to body mass index (BMI, especially in men) and age, on the other hand smoking may increase the plasma concentration of calcitonin and causes to false positive results. In order to overcome this problem, stimulation of calcitonin secretion by calcium or pentagastrin is required. It should be noted that large tumors without lymph node metastasis and small tumors with metastasis to the lymph node secret the similar amounts of calcitonin therefore basal level of calcitonin cannot be reliable to discriminate these cases from each other. In 1984, Miyauchi et al. reported a strong relationship between MTC recurrence and calcitonin doubling time. Recently several studies have confirmed this finding demonstrating that nowadays calcitonin doubling time in the plasma is the most sensitive biomarker for assessment of MTC progression [43]. Although calcitonin is the gold-standard biomarker for MTC diagnosis and follow-up, but owing to its circadian variability it has a variable hlalf-life, therefore to overcome this problem a more stable hormone such as procalcitonin **(PCT)** has been suggested. PCT is the precursor molecule of calcitonin and in comparison to calcitonin it is more stable in high temperature and has less circadian variability and longer half-life. According to the results of a systematic review performed by Karagiannis et al. it seems that PCT can be used as a biomarker in conjunction whit calcitonin for the diagnosis and follow-up of MTC, particularly in a few tumors which are calcitonin-negative or secreting low amounts of calcitonin [56].

CEA is the other molecular biomarker for MTC follow-up. This protein is a cytosolic enzyme secreted by several tumors related to endocrine system and other tissues; therefore it is not a specific biomarker for MTC. Compared to calcitonin CEA has low diagnostic accuracy [43,55]. Nevertheless, there is an association between high levels of CEA and tumor size, lymph node

metastasis, MTC recurrence and its prognosis. Moreover, there is a strong relationship between CEA doubling time and MC progression. In some patients with metastatic disease, post surgery levels of CEA is in the normal range, therefore the assessment of CEA before surgery has more diagnostic value and shows the disease progression. Plasma levels of CEA may be used in calcitonin-negative MTCs [43]. Whenever the physician encountered with high levels of CEA, the assessment of calcitonin levels and thyroid ultrasound will be required [42].

About 20 to 25 percent of the MTC may be related to MEN2 syndrome. This syndrome has an autosomal dominant inheritance pattern due to the several mutations in the RET proto-oncogene. About 75 to 80 percent of MTC cases are sporadic form. Based on the syndrome form (hereditary or sporadic forms), clinical characteristics, treatment and prognosis of MTC will be different [55]. RET proto-oncogene was discovered during human DNA transferring into the NIH3TT cells and therefore nominated as Rearranged during Transfection. The protein product of this gene is a membrane tyrosine kinase receptor [49] and usually expressed in the central, peripheral and enteric nervous systems, adrenal medulla chromaffin cells and thyroid C-cells. Normal function of RET proto-oncogene is essential for development of mammalian embryo [57]. Genetic studies have demonstrated the role of RET gene in inheritance form and lesser in sporadic form. In spite of strong phenotype-genotype correlation, clinical heterogeneous in the families with the same RET mutation even among the carriers in the same family has been observed. Additionally, single nucleotide polymorphism in RET gene in general population and among the patients affected by MTC have been reported. Some studies have reported the association between polymorphisms and MTC progression and several clinical studies show the effective and important role of RET polymorphisms in development of inheritance form of MTC. On the other hand, there are some studies failed to demonstrate an association between RET variants and MTC progression. Such differences in the results may be due to the differences among ethnic groups or because of defects in methodologies [55]. RET mutations have been demonstrated in 98% of families affected by MEN2. The presence of these mutations in germline cells shows the inheritance form of MTC and the risk of death in the mutations carriers is approximately 100%, therefore genetic screening is recommended in all affected families by MEN2. Identification of the carriers of germline RET mutations provides prophylactic surgery and biochemical follow-up for MTC metastasis and recurrence. RET mutations in somatic cells in tumor tissue have been identified in 23-69% of MTC patients. There is an association between M918T mutation and disease stage in sporadic form. After total thyroidectomy the risk of MTC recurrence and metastasis is very high and the patient survival rate decreases. This shows that the presence of RET mutations in somatic cells can be used as prognostic biomarkers [43]. Nowadays, early diagnosis of the carriers of RET mutations is possible and genetic screening of first degree relatives is necessary [58]. In the absence of RET proto-oncogene mutations in family members, their risk of MTC will be similar to the general population and the need for preventive measures such as total thyroidectomy will be remedied. On the other hand identification of mutant alleles in RET gene in the patient's family members is representative of hereditary form of the disease and it will be the basis for prophylactic surgery [47].

In the recent years, the role of miRNAs in pathogenesis and prognosis of MTC has been demonstrated. In a comprehensive study carried out by Pennelli et al. a large group of MTC patients (hereditary and sporadic forms) was investigated. In this study the role of miRNA-21 in MTC pathogenesis was demonstrated. miRNA-21 is an important carcinogen molecule showing overexpression in different human tumors particularly thyroid tumors. Recent studies show that miRNA-21 convert normal cells into tumor cells by suppression of tumor suppressor genes such as PTRN, PDCD4, RECK and TPM-1. The protein product of PDCD4 gene plays important roles in apoptosis, cell transformation, and tumor invasion and progression. This protein activates via interaction with eukaryotic transcription initiation factor 4A **(eIF4A)** and its expression decreases in human cancers. miRNA-21 overexpression causes PDCD4 dysregulation, therefore there is a strong correlation between expression of miRNA-21/PDCD4 and pathogenesis and prognostic findings in MTC but miRNA-21 could not achieve FDA approval for clinical application so far [31].

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