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Review Article

Role of molecular genetics in the preoperative diagnosis of thyroid tumors

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Abstract

Incidence of thyroid tumors has been triplicated in United States and other countries during the last three decades. There is a 2.1 fold increase of thyroid carcinoma from 2001 to 2010 in Sri Lanka. Though there is a higher incidence of thyroid tumors, their malignancy rate is low. Ultra Sound Scanning (USS) and fine needle aspiration cytology (FNAC) are used as the main pre-operative diagnostic methods. However, histopathological examination of surgical sections is used as the gold standard in determining the malignancy of the thyroid nodules. In order to prevent unnecessary surgeries and the re-operations, effective pre-operative diagnosis is important.

As most of other tumours, thyroid tumours too originated as a result of single or multiple genetic alterations or mutations of molecular markers like BRAF, RAS, RET/ PTC and TERT. These markers can be effectively used to determine the disease prognosis. The presence of any of these markers in the thyroid nodule represent a 100% positive predictive value and therefore it can be used for the clinical management of the patients. The use of molecular markers can significantly increase the diagnostic accuracy of thyroid malignancies. The combined use of molecular markers with the clinical findings and other pre surgical procedures including USS and FNAC can increase the diagnostic capability of the thyroid tumors. This can also be used for the individualized surgical approaches and post-surgical management of the patients.

Keywords: Thyroid Cancer, Genetic Alterations, Molecular Markers

Introduction

Over the past 30 years thyroid carcinoma incidence has triplicated in United States and other countries, and continue to increase globally. In Sri Lanka, there is a 2.1-fold increase of thyroid carcinoma from 2001 to 2010 (Jayarajah et al., 2018). Further, in Sri Lanka thyroid carcinoma has become the second commonest type of carcinoma affecting females more than males according to the latest cancer incidence data (Ceyran et al., 2015).

Though there is a high incidence (7% of the population) of thyroid tumors, the malignancy rate is about 5% of all the thyroid tumors (Mazeh, Mizrahi, Halle, & Ilyayev, 2011). Most of the tumours in thyroid gland are benign. Benign thyroid tumours include follicular adenoma which is the commonest type. Malignant neoplasms in thyroid gland include follicular thyroid carcinoma, papillary thyroid carcinoma (PTC), medullary carcinoma (C-cell carcinoma) and undifferentiated carcinoma. Apart from these epithelial tumours there are non-epithelial tumours such as malignant lymphomas, secondary tumours and tumour like lesions (DeLellis & Williams, 2004). Papillary thyroid carcinoma is the most common type of thyroid carcinoma accounting for 85-95% of all thyroid carcinomas (Ceyran et al., 2015, Demellawy et al., 2008, Pelizzo et al., 2006, Xing, 2013, X. Liu et al., 2014).

Several factors have affected the increased incidence of thyroid cancers. Increased use of pre surgical diagnostic methods like ultra sound scan to detect small thyroid tumours is one of the key factors affected for the rise in thyroid cancer incidence. Only 40% of the nodules which are below 1.5cm diameter can be detected by the physical examination (Davies & Welch, 2006, Kitahara & Sosa, 2016, Pellegriti et al.,2013). Exposure to radiation mainly during various medical diagnostic procedures is another factor associated with high incidence of thyroid tumors. Medical and dental diagnostic procedures like X-ray and CT scan has significantly affected the thyroid gland and thyroid gland is more prone to irradiation due to it's position in the body and it's ability of concentrating Iodine (States et al., 2008). High level of TSH and autoimmune thyroiditis conditions too have affected for the high incidence of thyroid cancers due to the production of pro inflammatory cytokines and the oxidative stress. However, successful treatment to these conditions can neutralize the effect from the TSH level and autoimmune thyroiditis conditions. Other than above facts obesity and insulin resistance has also affected for the increased incidence of thyroid tumors as insulin regulates the thyroid gene expression and stimulate the thyrocyte proliferation, differentiation and transformation. Diet, life style and exposing to other detected or undetected carcinogens have also affected for this (Kitahara & Sosa, 2016, Pellegriti et al., 2013).

Fine Needle Aspiration Cytology (FNAC) is a well-established technique used in preoperative diagnosis of thyroid tumors. FNAC is done radiology guided or by palpations. Main objective of conducting FNAC is to stratify patients with a risk of malignancy, subjecting them to surgeries and thus preventing unnecessary surgeries for benign nodules. However, sensitivity and specificity of this technique is low as individual cell morphologies are considered and also as there is a chance of missing micro carcinomas (Wright et al., 2008). Further, the main limitation of the FNAC technique is the indeterminate cytopathology results. According to the Bethesda system for reporting thyroid cytopathology, 20-30% of thyroid cases falls into 111, 1V and V categories which are considered as indeterminate or suspicious nodules. Also according to final pathological results in these indeterminate nodules, the malignancy rates range from 6-75% (Khan & Zeiger, 2020). Therefore, to detect whether the tumor is malignant or benign, pathological identification of tumors is done by the examination of routine Hematoxylin-Eosin stained tissue sections. However, for this process lobectomy or total thyroidectomy specimens are required. Though the surgeries are done on indeterminate nodules, only 20-30% of nodules contain malignant cells. Therefore, 80% of

the patients with inderminate thyroid nodules undergo unwanted surgeries (Mazeh, Mizrahi, Halle, & Ilyayev, 2011). Pre-operative accurate diagnosis of thyroid tumors are very important to determine disease prognosis and treatment plan.

As most other carcinomas, thyroid carcinoma too results due to the accumulation of multiple genetic and epigenetic alterations in the genome (Raman & Koenig, 2014). With the objective of improving the diagnostic capacity, gene mutation markers and gene expression panels are currently used. BRAF and RAS point mutations and RET/ PTC and PAX8/ PPARγ are considered as the common genetic alterations in the thyroid carcinoma. These mutations are non-overlapping and found in 70% of papillary and follicular thyroid carcinoma FNAC specimens and the surgically resected specimens. The use of molecular markers can significantly affect on increasing the diagnostic accuracy of thyroid carcinoma as the BRAF like markers can be used for the disease prognostication and some other markers can be used for the individualized management of the patients pre and post surgically (Nikiforov, 2011).

Common Genetic Mutations in Thyroid Malignancy

Somatic mutations, alterations of the gene expression, microRNA expression and gene promoter methylation have been reported as causes for thyroid cancers in the past few decades. These can be due to point mutations or due to chromosomal rearrangements (Nikiforov & Nikiforova, 2011).

Common sites where point mutations occur are BRAF gene and RAS gene while RET/PTC and PAX8-PPARγ result due to chromosomal rearrangements. Activation of MAPK and P13K-AKT signaling pathways by their mutations leads to tumorigenesis and subsequent occurrence of carcinoma. Activation of MAPK pathway is essential for the tumor initiation. The mutations that affect the P13K-AKT path way is important for the progression and differentiation of these tumors.

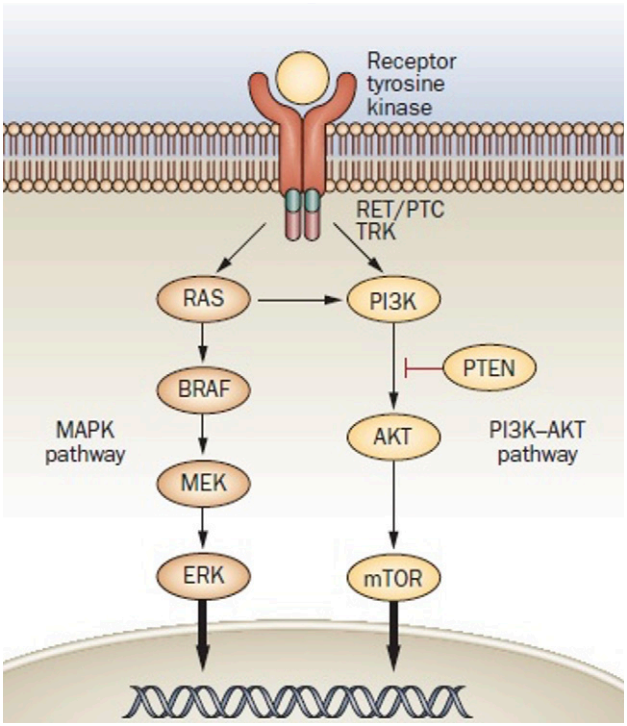

Figure1 - The main signaling pathways involved in thyroid carcinogenesis are the MAPK and PI3K–AKT pathways. These pathways are involved in propagation of signals from various cell membrane receptor tyrosine kinases into the nucleus, and they regulate multiple cell processes including proliferation, differentiation and survival. Activation of the MAPK pathway by oncogenic stimuli such as mutated BRAF, RAS or the chimeric fusion proteins RET/PTC and TRK is a common tumor initiating event in well differentiated papillary carcinoma and in some follicular carcinomas. Mutations involving the effectors of the PI3K–AKT pathway such as the PI3K subunit PIK3CA, AKT1 and PTEN are found more frequently in follicular carcinomas and in less differentiated types of thyroid cancer (Nikiforov & Nikiforova, 2011).

BRAF V600E mutation is considered as the most common type of mutation in thyroid cancers including papillary thyroid carcinoma with a rate of 99.3% (Raman & Koenig, 2014). This mutation is absent in the follicular variant of papillary thyroid carcinoma and the benign nodules (Trovisco et al., 2004). This mutation results due to the replacement of a glutamate

to valine amino acid at 600 residue (Val600Glu) as a result of the thymine to adenine substitution at 1799th position of this gene (Nikiforov & Nikiforova, 2011). This mainly happen around the nucleotide 600. BRAF gene mutation causes the production of BRAF-V600E mutant protein which activates the BRAF kinase and then MAPK signaling pathway. This point mutation gets positive in 15-40% of indeterminate thyroid cytology specimens and 40-45% of papillary thyroid carcinoma specimens. Although this marker has a high specificity, its sensitivity is low when used independently. Further, BRAF V600E gene is also used for the prognosis of the thyroid disease as this mutation is associated with the re-occurrence of the tumors and the mortality (Y. Liu et al., 2013, Soares et al., 2017,Xing, 2013). A significant association has been identified between the BRAF mutation and extra thyroidal invasion, lymph node metastasis and advanced tumor stages. This marker can also be used for the risk stratification of the thyroid tumors (Xing et al., 2005).

PAX8/ PPAR γ promotes the differentiated follicular thyroid neoplasia. This is due to a fusion between the paired domain transcription factor and the peroxisome proliferator – activated receptor genes [t (2;3)(q13;p25) chromosomal translocation] (Hsiao & Nikiforov, 2014). Thirty to thirty five percent (30-35%) of thyroid follicular carcinoma and the follicular variant papillary carcinoma carries the PAX8/ PPAR γ mutant protein. This act as a dominant negative inhibitor of PPAR γ or as a PPAR γ like transcription factor depending on the target gene and the cellular context. This mutant protein increases the growth rate of the cells and inhibit the apoptosis process (Raman et al., 2014). RAS mutation rarely get overlapped with this mutation as it has distinct pathogenetic pathways in the follicular thyroid carcinoma development. Presence of this marker in a follicular lesion doesn't confirm the malignancy. However, this can be used as an intensive tool for the vascular and capsular invasion (Bhaijee & Nikiforov, 2011).

TERT gene, the catalytic sub unit of the gene is responsible to keep the cells immotile by maintaining the telomere length at the end of chromosomes. Two mutations can occur in this TERT promoter region namely C228T and C250T leading to shortening of telomere length and thus increase the rate of cell replication and carcinogenesis. C250T gene mutation is rare. However, both C228T and C250T are mutually exclusive. This gene has been identified as the best marker of aggressiveness, poor prognosis and distance metastasis of all the types of thyroid carcinoma (Melo et al., 2014, Landa et al., 2013, X. Liu et al., 2014, Soares et al., 2017, X. Liu et al., 2013).

RET/ PTC is a chromosomal rearrangement that leads to cause papillary thyroid carcinoma at a significant rate. Currently the rate of RET/ PTC rearrangement is progressively decreasing. High frequency in this rearrangement is found in the children and the young adults who has an exposure to radiation (Hsiao & Nikiforov, 2014). Usually RET gene codes for the protein which is a signaling subunit of a receptor complex for ligands of the glial-derived neurotrophic factor (GFL) family and chimeric forms for this receptor develops due to this chromosomal rearrangement. There are about twelve (12) forms of this rearrangement. This is considered as a very early event in the development of thyroid malignancies as this mutation leads to the chronic activation of MAPK pathway. Moreover, this is present only in a small fraction of poorly differentiated thyroid cancers and also this oncoprotein may not confer a high risk of disease progression (Marrow et al., 2014). This RET gene acts on thyroid C cells and not on the follicular cells.

Second commonest type of mutation found in thyroid malignancies is the RAS mutation. There are three isoforms of RAS mutations namely NRAS, KRAS and HRAS. Among these three NRAS is the predominant one in thyroid tumors, specially in the follicular thyroid carcinoma. This mutation occurs in the codon 61 and 12. This RAS mutation activate both MAPK signaling pathway and the PI3K–AKT pathway and P13-AKT is involved in the tumorigenesis. RAS mutation is

seen in 40-50% follicular thyroid carcinoma and 10-20% of papillary thyroid carcinoma. Most of the RAS positive papillary thyroid carcinoma are follicular variant. Further, it is also positive in 20-40% of follicular thyroid adenoma. Basically these three markers present in follicular cell derived thyroid tumors and HRAS and NRAS can also be found in the medullary tumors (Hsiao & Nikiforov, 2014). The PPV of this mutation is comparatively low for cancer when considering other common mutations and translocations like BRAF, PAX8/ PPAR γ and RET/ PTC. Although this RAS mutation is positive in the Follicular adenomas there are reported evidences that these are present in precancerous stages which can transform into malignancies (Zhu et al., 2003).

PI3K/ AKT plays a major role in the thyroid tumour generation. It can be mainly affected by activating mutations in PIK3CA and AKT1 and inactivation of PTEN mutation. Somatic mutations in PTEN can be seen in follicular thyroid tumours and anaplastic thyroid carcinomas. Germline mutations in the PTEN gene can be seen in the thyroid follicular tumours in patients with Cowden syndrome(Hsiao & Nikiforov, 2014). From the two activating mutations, PIK3CA mutation occur in the 9 and 20 exons and it is found in follicular thyroid carcinomas, poorly differentiated thyroid carcinomas, and anaplastic thyroid carcinomas while AKT1 is seen in metastatic thyroid tumors (Hsiao & Nikiforov, 2014).

Diagnosis of common genetic mutations in thyroid tumours

Detection of genetic markers in the cytological indeterminate nodules play an important role in the accurate diagnosis of the thyroid tumors. As the pre-operative accurate diagnosis is crucial in the thyroid diseases, several panels of genetic mutations and the genetic expressions can be used. This can be accomplished with the use of next generation sequencing in which more than one mutation or a genetic expression can be diagnosed simultaneously. These expressions can be used for the diagnosis, prognosis and the therapeutic management of the patients. Panel of gene mutations and gene expressions are currently used as the sensitivity is low when the markers used independently.

BRAF and RAS mutations and the RET/ PTC and PAX8/ PPARγ rearrangements are widely used for the diagnosis of thyroid malignancies. A high specificity and high positive predictive value was observed in any of the mutations which include BRAF, KRAS, HRAS, NRAS and RET/PTC1. RET/PTC3 and PAX8/ PPARγ. It has been found that the presence of any of the above mutations except the RAS mutation can be used as an indication for thyroidectomy (Buryk et at, 2013).

In the mutation negative thyroid nodules with atypia of Undetermined Significance/Follicular Lesion of Undetermined Significance (AUS/ FLUS), there is a malignancy risk of 6% and invasive carcinoma risk of 2.3% from the 14% prevalence of thyroid diseases. The negative predictive value of these AUS/ FLUS nodules is 94% which is a very high rate. Further, as there is a low chance of missing the nodules with a risk of malignancy and also as majority of missed nodules are intra-thyroidal, these molecular markers can be used to eliminate surgery of AUS/FLUS nodules which is a burden to the health care system (Nikiforov et al., 2011). Other than nodules with undetermined significance, panel of molecular markers aids in preventing 60% of the thyroidectomies or lobectomies in the children. This is due to the high specificity of the molecular markers in the diagnosis of thyroid nodules (Buryk et al. 2013).

Conclusion

The use of molecular markers can significantly increase the diagnostic accuracy of thyroid malignancies. The combined use of molecular markers with the clinical findings and other pre surgical procedures including ultrasound scan and fine needle aspiration cytology could increase the diagnostic capability of the thyroid tumors. This can also use for the individualized surgical approaches and post-surgical management of the patients.

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Conflict of interests:
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