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Molecular Diagnosis in Clinical Management and Diagnosis of Thyroid Cancer

Abbas Hussein Saleh Hussein[1,*]

[1]General Surgeon, Ahalia Hospital, Mussaffah, Abu Dhabi

Abstract

The prevalence of thyroid cancer is rapidly increasing worldwide, majorly due to overdiagnosis and overtreatment methods of differentiated thyroid cancer. The emergent and potent preclinical models, high-throughput molecular techniques, and genetic expression microarrays have delivered deeper insights into understanding the molecular features in oncogenesis. Thus, molecular markers have become a promising tool in managing thyroid cancer for differentiating benign and malignant tumors, prognosis, recurrence, and determination of novel therapeutic targets. In differentiated thyroid cancer, molecular markers are majorly utilized for guiding the development of indeterminate thyroid nodules on fine needle aspiration (FNA) histologies. Dissimilar to this, in advanced thyroid cancer, molecular markers permit targeted treatment of a modified signaling cascade. Determining causal mutation of targeted kinase receptors in advanced thyroid cancer can depict a promising treatment strategy with mutation-targeted tyrosine kinase inhibitors to reduce progression and eradicate mutation effects when conventional methods fail to manage. This review will focus on the molecular landscape and discuss the impact of molecular markers on the prognosis, treatment, and surveillance of differentiated and anaplastic thyroid cancer.

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Corresponding author:

Abbas Hussein Saleh Hussein, General Surgeon, Ahalia Hospital, Mussaffah, Abu Dhabi.

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Introduction

As per the GLOBOCAN 2018 reports, thyroid cancer accounts for 3% of all novel cancer management cases globally and, thus, ranks at ninth position concerning prevalence [7]. In addition, with an estimate of around 40,000 deaths yearly, mortality rates remain comparably low and stable. However, it is one of the largest growing cancer entities globally and its prevalence has increased three times over the past 30 years from 5.0 to 15.3 per 10,000 individuals in the United States [21]. Various reports in the literature discuss this epidemiological progression [17]. However, few authors also depict a contributing increase in prevalence [28], the major reason for the progression observed to be an outcome of overdiagnosis and over-surveillance of differentiated tumors. This could be accredited to substantial improvements in diagnostic methods, specifically highly sensitive ultrasound, computed tomography scan (CT scan), and fine needle aspiration. The consequent continuous investigation for clinically insignificant outcomes influences prevalence and is an important factor where recent healthcare systems become under a substantial cost burden [29].

Hence, it is important to determine molecular markers that can differentiate between benign and malignant cancers, prognosis, pathophysiology, relapse, and

novel therapeutic targets. With the advancement of next-generation sequencing technologies and genetic expression microarrays have given a better understanding of genetic modifications and molecular features in cancer. This delivers a potential for the progression of personalized cancer management where patients are diagnosed according to their personal tumor characteristics, with a potential to improve patient outcomes, rather than a general treatment strategy for all [36]. The progressive importance of molecular markers and pathways in treatment strategies for thyroid cancer was also further explored by the current American Association of Endocrine Surgeons guidelines [33].

The subtypes of thyroid cancer can be subdivided into differentiated, poorly differentiated, and anaplastic thyroid cancer histologically. Since the development of the BRAF (V600E) mutation, several genetic and molecular markers in thyroid cancer have been determined, and complemented a supplementary layer to it [35]. However, the complete mutational burden is lower than in most other tumor entities, the progressive significance of molecular modifications was highlighted by the Cancer Genome Atlas (TCGA), which reported genetic modifications in around 97% of studied cancers [26]. However, the TCGA analysis only reported papillary thyroid cancer, consequent reports have reported that various markers are responsible for its progression and differentiation into more advanced cancer subtypes. By regulating the genetic expression of known oncogenic markers, tumor suppressor genes, and signaling pathways, that play a crucial role in cellular migration, differentiation, invasion, and epithelial transition [8].

In the present review, we will outline the molecular landscape of cell-derived thyroid cancer and discuss its impact on the management and treatment of different types of thyroid cancer. In addition, we will be focusing on the emergence of novel therapeutic molecular marker strategies, which have the potential to make a huge impact on personalized thyroid cancer care and management, thereby lowering the risk of recurrence and overtreatment.

The molecular landscape of thyroid cancer

Understanding the molecular landscape of thyroid cancer has advanced progressively with the current genomic developments in thyroid cancer, which has allowed us to discover novel targets for cancer management. The genetic modifications identified in the TCGA illustrate specific patterns mostly emphasizing two important signaling pathways namely mitogen-activated protein kinase signaling pathway (MAPK), and phosphatidylinositol-3-kinase (PI3K/AKT) signaling pathways [16]. The genetic deregulations of transcription factors and kinases specific to such pathways lead to consequent activation that progresses and activates tumorigenic pathways and thereby progression of thyroid cancer. In the further sections, we will be discussing molecular modification in thyroid cancer.

MAPK-signaling pathway

The MAPK-signaling pathway is important for cellular development, differentiation, and progression. The pathway is commenced on the cellular surface by mitogenic stimuli which is a growth factor, that binds to tyrosine kinase receptor and phosphorylates the mitogen-activated protein kinase, and thereby downstream regulates the extracellular signal-regulated kinase (ERK). Phosphorylated signal-regulated kinase leads to the activation of transcription factors targeting genes for inducing cell cycle entry and suppressing negative regulation of the cell cycle [6]. The MAPK-signaling pathway is regulated in various steps by enzymes and factors, for epigenetic modification can lead to subsequent active mechanisms, uncontrollable growth, and cellular proliferation, marking tumorigenesis. However, alterations in vital indicators of the MAPK-signaling pathway are present in almost 70% of all thyroid

cancers.

A single point variation at T1799A in codon 600 in the *BRAF* oncogene, encoding serine and threonine kinase in the MAPK signaling pathway, causes BRAF (V600E) mutation [10]. This mutation leads to active BRAF kinase which is independent of its upstream targeted gene, *RAS*, further leading to increased ERK activation. However, the mutant BRAF (V600E) kinase is 500 times more active than its wild type, and its variation is considered one of the significant originating events in the tumorigenesis of thyroid cancer [13]. In addition, the BRAF (V600E) mutation is also responsible for activating the nuclear kappa beta factor (NFκB) mechanism which is crucial for inflammatory processes and activated during tumorigenesis.

Moreover, the development of telomerase reverse transcriptase (TERT) promoter mutations in thyroid cancer, illustrates an important incident in the thyroid cancer field, and has expanded its role in modifying the impact of driver mutations such as BRAF. The discovered mechanism of TERT promoter variation is to develop de novo binding elements of the ETS-transcription family of factors including GABPA, which causes telomere progression [3]. However, the ETS-transcription factors are activated by MAPK signaling pathways, which affect the concomitant BRAF (V600E) and TERT mutations, and are thereby associated with aggressive forms of thyroid cancer.

PI3K/AKT signaling pathway

The PI3K/AKT signaling pathway mainly regulates processes such as apoptosis, cellular proliferation, cell cycle development, cellular integrity, and ATP regulation, and leads to AKT activation [3]. One of the important targets of the AKT signaling pathway is the *RAS* gene, a G-protein anchored to the inner cellular surface and located upstream of BRAF kinase and activated while bound to GTP. The variation in the *RAS* gene downregulates GTPase and causes it to an active GTP-bound state. Mutations in the RAS gene can activate both MAPK and AKT signaling pathways due to its location inside the cellular surface and adjacent to tyrosine kinase receptors. However, it is observed that RAS variations generally activate AKT signaling pathways in thyroid tumorigenesis [30]. However, the *RAS* mutation is pre-malignant and other variations are required to cause tumorigenesis. Moreover, *PTEN* mutations activate the AKT signaling pathway, which is the molecular foundation for follicular thyroid cancer occurrence in Cowden disorder. Variations in the PI3K3CA gene are also common during follicular thyroid cancer but are inferiorly differentiated in thyroid cancer and anaplastic thyroid cancer occurrence [30].

Genetic translocations

The genomic translocations could lead to tumorigenic chromosomal rearrangements. The most usual group of translocations in thyroid cancer is *RET/PTC*, discovered by [14]. It should be noted that it does not include a single translocation but a combination of distinct translocations including multiple genes along with *RET*. For instance, the commonly occurring translocations are *RET/PTC1* and *RET/PTC3* which illustrate translocation between the *RET* gene and *CCD6* gene and *RET* gene and *NCOA4* gene, respectively. The *RET/PTC* translocations happen as a mixture of genetic recombination between the 3' tyrosine kinase of RET and the 5' partner gene due to nuclear spatial propinquity. As RET is a proto-oncogene, any recombination of mutation during activation could lead to a tumor. Any rearrangement in RET/PTC gene encodes oncogenic processes including tyrosine kinase receptors, causing activation of MAPK and PI3K-AKT processes. The *NTRK1, ALK,* and *PPARG* are recombinant oncogenes where oncogenic fusion modifies the transcription of targeted genes, predisposing for tumorigenesis in thyroid cancer.

Epigenetic Regulation

Epigenetic alterations of the genome and genetic expression involve histone modifications, and DNA and miRNA methylation. Such epigenetic markers impact the central dogma of DNA transcription, RNA translation, and protein synthesis inside the cell. Epigenetic alterations are common during human oncology and are also observed in thyroid cancer [38]. Methylation of DNA of promoter positions generally leads to genetic transcription suppression. In microarray-based genome-wide DNA methylation reports of thyroid cancer, done by [30]. reveal that similarity to sequencing reports shows low-frequency mutation in parathyroid cancer along with low frequency of mutation in DNA methylation [20]. In contrast, anaplastic thyroid cancer displays a large frequency of DNA methylation modifications ten times higher than parathyroid cancer [5].

miRNA involvement in thyroid cancer has been reported in the literature (He et al., 2005). In addition, long non-coding RNA (lncRNA) involvement in thyroid cancer has recently been developed by various scientists. Deregulated lncRNAs are actively involved in the epigenetic modification of genetic transcription and are drawn in in the suppression of tumors and other tumorigenic functions [19].

Targeted treatment strategies for thyroid cancer

Thyroid nodules are very common among adults, however, the cancer frequency among individuals with thyroid nodules is less. Accordingly, thyroid cancer screening is not recommended generally. Ultrasound is considered the initial strategy for detecting malignancy and suspected nodules are detected through Fine Needle Aspiration (FNA). The usual limitation of FNA is indeterminate thyroid nodules. Moreover, the malignancy frequency ranges between 10-30%, with pathological examination needed to gain final insights (Ha et al., 2018). The molecular imaging techniques can successfully contribute to improving the preoperative analysis of indeterminate thyroid nodules.

Molecular Imaging of thyroid nodules

Scintigraphy of thyroids done using either pertechnetate or sodium iodine is the specific diagnostic method for detecting thyroid nodules functioning autonomously and eradicating malignancy with even lower TSH values. In addition, molecular imaging with FDG (fluorodeoxyglucose) might aid in distinguishing benign from malignant indeterminate thyroid nodules [12].

Upstream targeting tumoral molecular landscape

Targeting HER 2 and HER 3 in MAPK pathway

HER 2 and HER 3 are tyrosine kinase inhibitors and members of epidermal growth factors. Upon activation, they downstream activate the MAPK cascade. Clinical reports suggest that targeting these receptors with multiple kinase inhibitor drugs represents an efficient treatment strategy for breast cancer where these receptors are present in abundance. A process determined in BRAF mutants treated by Vemurafenib causes overexpression of HER 2 and 3 receptors and consequent activation of MAPK pathways [31]. Thus, recent clinical efforts targeting the HER receptor's role are assessing their significance in thyroid cancer occurrence. For instance, NCT01947023 is a clinical phase I trial evaluating Lapatinib (HER 2 and 3 inhibitors) in combination with Dabrafenib (inhibits BRAF) in patients with advanced thyroid cancer (ASCO, 2017). The researchers considered 15 patients with BRAF (V600E) variations, 13 patients had differentiated thyroid cancer and 2 had anaplastic thyroid cancer before multiple kinase inhibitor therapy was administered in 9 patients out of a total of 15 patients. Dabrafenib (150 mg) was administered in all patients two weeks before Lapatinib was given. The dosage of Lapatinib was increased gradually (750mg, 1250mg, and 1500mg). The partial drug

response was reported as 60% and only a single patient out of total patients (n=15) developed dose-limiting toxicity. In another clinical trial, the HER inhibitor Neratinib is being evaluated for its impact on solid tumors harboring any variation in thyroid cancer.

Downstream targeting tumoral molecular landscape

The growing evidence showing the involvement of AKT pathways in thyroid cancer pathogenesis and escape from traditional targeted therapies causes a closer look into multiple components of this pathway including the mTOR (mammalian target of rapamycin) component which when inhibited led to cellular proliferation suppression.

Two important mTOR suppressors significant during clinical trial data have been approved by the FDA for clinical usage for other cancers namely Temsirolimus in advanced kidney cancer and Everolimus for advanced pancreatic, breast, and renal carcinoma (Populo et al., 2012). Given the clinical benefits of these drugs and certainly mTOR activation as a therapeutic target in thyroid cancer, such targets are being examined for advanced thyroid cancer. Initial outcomes of phase II thyroid cancer clinical trial give positive hope. For instance, the NCT01025453 clinical phase II trial assessed the effect of Temsirolimus with Sorafenib in 37 thyroid cancer patients. Out of the total 37 patients, 8 patients responded partially, 21 patients had stable disease and only one patient developed progression while the remaining 7 patients were unassessable. Another clinical phase II trial NCT01141309 evaluates the combination of Everolimus and Sorafenib in 41 advanced thyroid cancer patients. In this cohort, 21 patients showed partial response, 14 patients had stable disease, and 3 patients had progressive disease. The cohort showed a better response in terms of toxicity assessment when compared to Sorafenib monotherapy, specifically in the differentiated thyroid cancer group.

In addition, the abundance of AKT and mTOR deformities in anaplastic thyroid cancer spots this pathway as an effective target for such patients. NCT02289144 is a clinical trial objecting to evaluate the impact of Ribociclib (targeting retinoblastoma variation) and Everolimus in malignant anaplastic thyroid cancer patients. The basis for targeting retinoblastoma in such patients is the abundance of intact retinoblastoma in anaplastic thyroid cancer patients [1]. However, more clinical trial study outcomes are awaited to suggest novel treatment strategies for anaplastic thyroid cancer patients since retinoblastoma is not considered a therapeutic target in these patients.

Methods to detect molecular modifications in thyroid cancer

Fine Needle Aspiration (FNA)

The fine needle aspiration (FNA) method is recently the most stable diagnostic strategy for thyroid nodules and represents the definite diagnosis of benign and malignant lesions in most cases, whereas 10-40% of all FNA samples are detected as indeterminate for malignant tumors [15]. Owing to the limited definite diagnosis, most patients with indeterminate tumors undergo surgical treatment, however, only 10-20% of surgically removed tumors are cancerous. The patients with indeterminate FNA cytology and malignant cancers are not efficiently treated, as most of them undergo thyroid lobectomy and thyroidectomy.

Molecular diagnosis of FNA samples may improve the precision of cytological diagnosis of thyroid cancers. The most studied accumulated variation is BRAF mutation which has been reported in prospective and retrospective studies [24; 32]. In addition to BRAF mutation, various studies have suggested the probability of the detection of *RET*, *TRK,* and *RAS* variations in thyroid samples. However, the largest diagnostic effect can be gained by examining FNA samples for a cohort of

variations rather than for a single mutation. Recent literature reports have suggested the diagnostic utility of molecular diagnosis for a cohort of mutations including *BRAF, RAS, RET,* and *PAX8.* A study examined 470 successive FNA samples from thyroid cancer that were examined and generated 32 variations which were strong predictors of malignant diagnosis after surgery [32]. This report suggested that examining the mutation panel was helpful in predicting thyroid nodules with indeterminate cytology. In this group, the positive mutational status had 100% precision for depicting malignancy risk while the negative mutation status depicted benign nodules. Moreover, such a study represented that molecular examination reduced the false-negative frequency of cytology from 2-0.8%. in addition to BRAF mutation, *RAS* mutation also appeared to be of higher diagnostic value among FNA samples, as it depicted an 87-100% probability of malignancy. Interestingly, the RAS variations were determined in tumors, which are hard to diagnose cytologically alone, that is variants of papillary and follicular carcinoma. The diagnostic utilization of molecular markers has been reported effectively in Management Guidelines for patients with thyroid nodules and differentiated thyroid cancer, by the American Thyroid Association [11]. These guidelines suggested that using molecular markers including *BRAF, RAS, RET,* and *PAX8* for elucidating FNA cytology helps in guiding patient care and management.

Peptide receptor radionuclide therapy

The radioactive iodine treatment for thyroid cancer in patients has been applied since 1946. Currently, a combination of therapy and diagnosis using radiolabelled somatostatin analogs has proven to be effective for managing somatostatin receptor tumors. The Lu^{177}-labelled somatostatin analogs binding receptors are the most promising radioactive therapy in clinical practice. Radioactive therapy might be considered as a potential therapy for differentiated thyroid cancer treatment with high expression of somatostatin receptor owing to efficient safety features and therapeutic value.

Immunotherapy

The association between thyroid cancer and the immune system has been explored largely owing to the concomitance of papillary thyroid cancer and Hashimoto thyroiditis. The dysregulation in the immune system and response partially correlates with differentiated thyroid cancer oncogenesis and development involving activation of immunosuppressors such as tumor-connected macrophages, immune checkpoints such as programmed death ligand-1 (PD-L1), and cytotoxic associate T-lymphocyte-4 (CTLA-4). PD-L1 was actively expressed in 50% of papillary thyroid cancers [3]. This percentage increased to 61% in differentiated thyroid cancer and 70% in advanced papillary thyroid cancer, and 75% in anaplastic thyroid cancer [4]. On the basis of these outcomes, the immunotherapeutic strategies involving immune checkpoint inhibitors may manage thyroid cancer effectively. The immune checkpoint inhibitors involve two major classes: those targeting CTLA-4 namely tremelimumab and ipilimumab, and targeting PD-L1 namely pembrolizumab, nivolumab, and atezolizumab. The immune checkpoint inhibitors augment the effector T cells reduce the regulation of suppressor cells and establish immune surveillance from which malignant cells can easily be removed. However, reports on immune checkpoint inhibitors for treating radioactive iodine refractory-differentiated thyroid cancer are still limited.

Surgical treatment for differentiated thyroid cancer and Preoperative staging

Conventionally, total thyroidectomy was performed in most anaplastic thyroid cancer patients, even though the American Thyroid Association standards endorse lobectomy for individuals with intra-thyroid low-risk differentiated thyroid cancer [18]. Cervical lymph nodes occur in 20-50% of patients with differentiated thyroid cancer, and neck surgery dissection decreases the risk of tumor relapse. Prophylactic neck segmentation may progress control for invasive tumors, but it is dejected for

reduced-risk differentiated thyroid cancer because potential morbidities are not justified by a significant clinical advantage. Pre-operative neck ultrasound generally suffices for planning surgery; however, supplementary cross-sectional imaging and magnetic resonance imaging are reserved for patients with locally advanced disease or for those who are at higher risk of establishing distant metastases. CT scan with [^{18}F] FDG could be performed pre-operatively in aggressive differentiated thyroid cancer and anaplastic thyroid cancer. [25]. retrospectively analyzed 60 patients with lower-risk differentiated thyroid cancer who underwent CT scans before thyroidectomy. The authors reported very low sensitivity (10%) in elucidating lymph node metastasis with high specificity (90%) [25]. Few other studies compared the precision of positron emission tomography with that of neck ultrasound, and CT scans. It was observed that the specificities of all positron emission tomography, ultrasound, and CT scans are very high in evaluating both central and lateral neck regions. Although, the sensitivities are low (<50%) for all three methods. The total diagnostic precision of [^{18}F] FDG positron emission tomography tends to be higher for lateral in comparison to central lymph nodes [9].

Postoperative Radioiodine therapy for differentiated thyroid cancer

The total thyroidectomy followed by ^{131}I management has continued to achieve an efficient diagnosis in multiple differentiated thyroid cancer patients. The ^{131}I therapy for differentiated thyroid cancer is based on the principle of sodium iodide symporter articulating thyroid cells with differentiated thyroid cancer having the capability of confining circulating ^{131}I. after surgical treatment, the risk of structural disorder relapse and persistence is evaluated using three-layer stratification (low, medium, and high) as recommended by the American Thyroid Association [18]. The objective of therapeutic ^{131}I administration after total thyroidectomy is delineated based on the standard guidelines as follows [2].

1. Adjuvant treatment for irradiating suspected but neoplastic cell sites in low and medium-risk patients, as elucidated by histopathological features, thus decreasing disease relapse.
2. Treating known diseases for managing recurrent disorders in patients with determined metastatic disease.
3. Remnant excision to remove normal thyroid tissue fragments in low-risk patients, hence safeguarding minimal triglyceride levels, and facilitating follow-up.

Generally, two strategies for ^{131}I treatment planning and delivery are approved in clinical practices: a method based on the clinical and pathological features and institutional procedures, and a strategy integrating post-operative radioiodine functional imaging. However, no pieces of evidence are available to permit recommending a single method over others and choice is based on local factors, including clinician and patient preferences.

Future perspectives and challenges

Over the last few decades, understanding the underlying molecular pathways for thyroid differentiation and determination of important causal genes have led to the development of multiple novel radionuclide imaging strategies. A few variations such as EIF1AX and IDH1 and other signaling processes involving the differentiation process are still unclear, the significance of which needs to be explored. Various reports suggested that processes such as acetylation, glycosylation, methylation, and ubiquitination are closely associated with epigenetic oncogenesis [28]. There are also reports available in the literature suggesting proteomic evaluation in thyroid oncogenesis [37]. However, the underlying molecular mechanisms are still unknown. Moreover, clinical analysis of functional imaging of differentiated thyroid cancer has represented the importance of disease management and diagnosis, and further studies

relevant to molecular targeted therapies are needed for better diagnosis and therapeutics of thyroid cancer in the future.

The advent of novel targeted therapy has unquestionably delivered us with more treatment options for advanced thyroid cancer. The emergent outcomes of phase II trials of Temsirolimus and Everolimus are expected to provide new insights into treatment options for thyroid cancer. However, how to utilize these weapons is of clinical importance, as whether to use them at an early stage of treatment or at completion of the current standard treatment mode. The timing of initiation with novel agents is of utmost significance. More explorations and examinations are required to address these issues. In the future, properly designed clinical trial studies will help us to better understand the comparative efficiency of novel agents. It is of utmost significance to determine an effective sequential and combined treatment method for minimizing resistance to inactive drugs in long-term clinical practice for advanced thyroid cancer management.

Conclusions

The complete management of thyroid cancer is promising and advantageous yet its treatment in patients remains challenging. Thyroid cancer is a heterogeneous disorder driven by various molecular modifications. Over the last decade, advancements in understanding the tumorigenic alterations and signaling mechanisms have helped researchers and clinicians to early determine and diagnose advanced thyroid cancer patients. The outcomes of molecular modifications involving epigenetic modifications, DNA methylation, and post-translational modifications also modify the therapeutic strategy for advanced thyroid cancer. Moreover, emerging molecular imaging studies provide more potential for diagnosing and treating advanced thyroid cancer. The targeted therapies, immunotherapy, and radionuclide therapy are making robust progress in the personalized management of advanced thyroid cancer. Future examinations and clinical consequences are guaranteed to achieve more targeted therapies efficiently and choose specific candidate patients who might benefit and improve advanced thyroid cancer treatment modes.

References

1. Anwar F, et al. Retinoblastoma Expression in Thyroid Neoplasms. Mod Pathol. 2000, 13(5):562–9.
2. Avram A.M., Zukotynski K., Nadel H.R., Giovanella L.M. Management of differentiated thyroid cancer: The standard of care. J. Nucl. Med. 2022; 63:189–195. doi: 10.2967/jnumed.121.262402.
3. Bai Y, Niu D, Huang X, Jia L, Kang Q, Dou F, et al. PD-L1 and PD-1 expression are correlated with distinctive clinicopathological features in papillary thyroid carcinoma. Diagn Pathol (2017) 12 (1):72. doi: 10.1186/s13000-017-0662-z
4. Bastman JJ, Serracino HS, Zhu Y, Koenig MR, Mateescu V, Sams SB, et al. Tumor-infiltrating T cells and the PD-1 checkpoint pathway in advanced differentiated and anaplastic thyroid cancer. J Clin Endocrinol Metab (2016) 101(7):2863–73. doi: 10.1210/jc.2015-4227
5. Bisarro Dos Reis M., Barros-Filho M.C., Marchi F.A., Beltrami C.M., Kuasne H., Pinto C.A.L., Ambatipudi S., Herceg Z., Kowalski L.P., Rogatto S.R. Prognostic classifier based on genome-wide DNA methylation profiling in well-differentiated thyroid tumors. J. Clin. Endocrinol. Metab. 2017;102:4089–4099. doi: 10.1210/jc.2017-00881.
6. Braicu C., Buse M., Busuioc C., Drula R., Gulei D., Raduly L., Rusu A., Irimie A., Atanasov A.G.,

Slaby O., et al. A comprehensive review on mapk: A promising therapeutic target in cancer. Cancers. 2019;11:1618. doi: 10.3390/cancers11101618.

7. Bray F., Ferlay J., Soerjomataram I., Siegel R.L., Torre L.A., Jemal A. Global cancer statistics 2018: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin. 2018; 68:394–424. doi: 10.3322/caac.21492
8. Calabrese G., Dolcimascolo A., Caruso G., Forte S. Mir-19a is involved in the progression and malignancy of anaplastic thyroid cancer cells. Onco. Targets Ther. 2019; 12:9571–9583. doi: 10.2147/OTT.S221733.
9. Choi W.H., Chung Y.-A., Han E.J., Sohn H.S., Lee S.H. Clinical value of integrated [18F]Fluoro-2-Deoxy-d-glucose positron emission tomography/computed tomography in the preoperative assessment of papillary thyroid carcinoma: Comparison with sonography. J. Ultrasound Med. 2011;30:1267–1273. doi: 10.7863/jum.2011.30.9.1267.
10. Cohen Y., Xing M., Mambo E., Guo Z., Wu G., Trink B., Beller U., Westra W.H., Ladenson P.W., Sidransky D. Braf mutation in papillary thyroid carcinoma. J. Natl. Cancer Inst. 2003; 95:625–627. doi: 10.1093/jnci/95.8.625.
11. Cooper DS, Doherty GM, Haugen BR, et al. Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid 2019; 19:1167–1214
12. De Koster E.J., De Geus-Oei L.-F., Dekkers O.M., van Engen-van Grunsven I.V., Hamming J., Corssmit E.P.M., Morreau H., Schepers A., Smit J., Oyen W.J.G., et al. Diagnostic utility of molecular and imaging biomarkers in cytologically indeterminate thyroid nodules. Endocr. Rev. 2018;39:154–191. doi: 10.1210/er.2017-00133.
13. Fagin J.A. Challenging dogma in thyroid cancer molecular genetics--role of ret/ptc and braf in tumor initiation. J. Clin. Endocrinol. Metab. 2004; 89:4264–4266. doi: 10.1210/jc.2004-1426.
14. Fusco A., Grieco M., Santoro M., Berlingieri M.T., Pilotti S., Pierotti M.A., Della Porta G., Vecchio G. A new oncogene in human thyroid papillary carcinomas and their lymph-nodal metastases. Nature. 1987; 328:170–172. doi: 10.1038/328170a0.
15. Gharib H . Changing trends in thyroid practice: understanding nodular thyroid disease. Endocr Pract 2004;10:31–39
16. Giordano T.J. Follicular cell thyroid neoplasia: Insights from genomics and the cancer genome atlas research network. Curr. Opin. Oncol. 2016;28:1–4. doi: 10.1097/CCO.0000000000000248.
17. Ha E.J., Na D.G., Baek J.H., Sung J.Y., Kim J.-H., Kang S.Y. US fine-needle aspiration biopsy for thyroid malignancy: Diagnostic performance of seven society guidelines applied to 2000 thyroid nodules. Radiology. 2018; 287:893–900. doi: 10.1148/radiol.2018171074.
18. Haugen B.R., Alexander E.K., Bible K.C., Doherty G.M., Mandel S.J., Nikiforov Y.E., Pacini F., Randolph G.W., Sawka A.M., Schlumberger M., et al. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid. 2016;26:1–133. doi: 10.1089/thy.2015.0020
19. Hauptman N., Glavac D. Long non-coding rna in cancer. Int. J. Mol. Sci. 2013;14:4655–4669. doi: 10.3390/ijms14034655.

20. Hou P., Liu D., Xing M. Genome-wide alterations in gene methylation by the braf v600e mutation in papillary thyroid cancer cells. Endocr. Relat. Cancer. 2011;18:687–697. doi: 10.1530/ERC-11-0212.
21. Howlader N., Noone A., Krapcho M., Miller D., Bishop K., Altekruse S.F., Kosary C.L., Yu M., Ruhl J., Tatalovich Z., et al., editors. Seer Cancer Statistics Review, 1975–2013. National Cancer Institute; Bethesda, MD, USA: 2015. [(accessed on 25 September 2023)]. Available online: https://seer.cancer.gov/archive/csr/1975_2013/.
22. Huang F.W., Hodis E., Xu M.J., Kryukov G.V., Chin L., Garraway L.A. Highly recurrent tert promoter mutations in human melanoma. Science. 2013; 339:957–959. doi: 10.1126/science.1229259.
23. Jo YS, Huang S, Kim YJ, et al. Diagnostic value of pyrosequencing for the BRAF V600E mutation in ultrasound-guided fine-needle aspiration biopsy samples of thyroid incidentalomas. Clin Endocrinol (Oxf) 2009; 70:139–144.
24. Kim J., Gosnell J.E., Roman S.A. Geographic influences in the global rise of thyroid cancer. Nat. Rev. Endocrinol. 2020; 16:17–29. doi: 10.1038/s41574-019-0263-x.
25. Kim B.S., Kim S.-J., Kim I.J., Pak K., Kim K. Factors associated with positive F-18 fluorodeoxyglucose positron emission tomography before thyroidectomy in patients with papillary thyroid carcinoma. Thyroid. 2012; 22:725–729. doi: 10.1089/thy.2011.0031.
26. Lawrence M.S., Stojanov P., Polak P., Kryukov G.V., Cibulskis K., Sivachenko A., Carter S.L., Stewart C., Mermel C.H., Roberts S.A., et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. Nature. 2013;499:214–218. doi: 10.1038/nature12213.
27. Li J, Zhan X. Mass spectrometry-based proteomics analyses of post-translational modifications and proteoforms in human pituitary adenomas. Biochim Biophys Acta Proteins Proteom (2021) 1869 (3):140584. doi: 10.1016/j.bbapap.2020.140584
28. Lim H., Devesa S.S., Sosa J.A., Check D., Kitahara C.M. Trends in thyroid cancer incidence and mortality in the United States, 1974–2013. JAMA. 2017; 317:1338–1348. doi: 10.1001/jama.2017.2719.
29. Lin J.F., Jonker P.K.C., Cunich M., Sidhu S.B., Delbridge L.W., Glover A.R., Learoyd D.L., Aniss A., Kruijff S., Sywak M.S. Surgery alone for papillary thyroid microcarcinoma is less costly and more effective than long term active surveillance. Surgery. 2020; 167:110–116. doi: 10.1016/j.surg.2019.05.078.
30. Liu Z., Hou P., Ji M., Guan H., Studeman K., Jensen K., Vasko V., El-Naggar A.K., Xing M. Highly prevalent genetic alterations in receptor tyrosine kinases and phosphatidylinositol 3-kinase/akt and mitogen-activated protein kinase pathways in anaplastic and follicular thyroid cancers. J. Clin. Endocrinol. Metab. 2008; 93:3106–3116. doi: 10.1210/jc.2008-0273.
31. Montero-Conde C, et al. Relief of feedback inhibition of HER3 transcription by RAF and MEK inhibitors attenuates their antitumor effects in BRAF mutant thyroid carcinomas. Cancer Discov. 2013;3(5):520–33.
32. Nikiforov YE, Steward DL, Robinson-Smith TM, et al. Molecular testing for mutations in improving the fine-needle aspiration diagnosis of thyroid nodules. J Clin Endocrinol Metabol 2009;94:2092–2098.

33. Patel K.N., Yip L., Lubitz C.C., Grubbs E.G., Miller B.S., Shen W., Angelos P., Chen H., Doherty G.M., Fahey T.J., 3rd, et al. The American Association of Endocrine Surgeons guidelines for the definitive surgical management of thyroid disease in adults. Ann. Surg. 2020; 271: e21–e93. doi: 10.1097/SLA.0000000000003580.
34. Pópulo H, Lopes JM, Soares P. The mTOR Signalling pathway in human cancer. Int J Mol Sci. 2012;13(2):1886–918
35. Prete A., Borges de Souza P., Censi S., Muzza M., Nucci N., Sponziello M. Update on fundamental mechanisms of thyroid cancer. Front Endocrinol. (Lausanne) 2020; 11:102. doi: 10.3389/fendo.2020.00102.
36. Sicklick J.K., Kato S., Okamura R., Schwaederle M., Hahn M.E., Williams C.B., De P., Krie A., Piccioni D.E., Miller V.A., et al. Molecular profiling of cancer patients enables personalized combination therapy: The i-predict study. Nat. Med. 2019;25:744–750. doi: 10.1038/s41591-019-0407-5.
37. Spartalis E, Kotrotsios K, Chrysikos D, Spartalis M, Paschou SA, Schizas D, et al. Histone deacetylase inhibitors and papillary thyroid cancer. Curr Pharm Des (2021) 27(18):2199–208. doi: 10.2174/1381612826666201211112234
38. Zafon C., Gil J., Pérez-González B., Jordà M. DNA methylation in thyroid cancer. Endocr. Relat. Cancer. 2019;26:R415–R439. doi: 10.1530/ERC-19-0093.