THE ROLE OF MOLECULAR TESTING IN THYROID TUMORS

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Thyroid cancer is the most common endocrine gland cancer. In the last few decades, the molecular diagnostics for thyroid tumors have been widely researched. It is one of the few cancers whose incidence has increased in recent years from microcarcinomas to common, large forms, in all age groups, from children to the elder people. Most researches focus on the genetic basis, since our current knowledge of the genetic background of various forms of thyroid cancer is far from being complete. Molecular and genetic research has several main directions: firstly, differential diagnosis of thyroid tumors, secondly, the prognostic value of detected mutations in thyroid cancer, and thirdly, targeted therapy for aggressive or radioactive iodine-resistant forms of thyroid cancer. In this review, we wanted to update our understanding and describe the prevailing advances in molecular genetics of thyroid cancer, focusing on the main genes associated with the pathology and their potential application in clinical practice.

KEYWORDS: molecular diagnostics; molecular genetic markers; thyroid tumors; thyroid cancer; targeted therapy.

РОЛЬ МОЛЕКУЛЯРНОЙ ДИАГНОСТИКИ ПРИ ОПУХОЛЯХ ЩИТОВИДНОЙ ЖЕЛЕЗЫ

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Рак щитовидной железы является наиболее распространенным онкологическим заболеванием эндокринных желез. Молекулярная диагностика при опухолях щитовидной железы широко исследуется в последние несколько десятилетий. Это один из немногих видов рака, заболеваемость которым в последние годы возросла от микрокарцином до распространенных форм большого размера, во всех возрастных группах, от детей до пожилых людей. Большинство исследований сосредоточено на исследовании генетической основы, поскольку наши современные знания о генетическом фоне различных форм рака щитовидной железы далеки от полноты. Молекулярно-генетические исследования имеют несколько основных направлений: во-первых, дифференциальная диагностика опухолей щитовидной железы, во-вторых, прогностическое значение выявленных мутаций при раке щитовидной железы, в-третьих, таргетная терапия при агрессивных или резистентных к радиоактивному йоду формах рака щитовидной железы. В данном обзоре мы хотели обновить наше понимание и описать преобладающие достижения молекулярной генетики рака щитовидной железы, сосредоточившись на основных генах, связанных с патологией, и возможности их применения в клинической практике.

КЛЮЧЕВЫЕ СЛОВА: молекулярная диагностика; молекулярно-генетические маркеры; опухоли щитовидной железы; рак щитовидной железы; таргетная терапия.

METHODS OF SEARCH AND SELECTION OF PUBLICATIONS FOR THE REVIEW

The review manuscript used scientific publications from the MEDLINE international database and clinicaltrials.gov. The search was performed from March 2020 to June 2020. The following filters were used in the search: date of publication from 2010 to present; keywords: molecular testing, thyroid tumors, thyroid cancer, target therapy, *RET, RET/PTC, BRAF, PAX8-PPARγ, KRAS, NRAS, HRAS, CTNNB1, TERT, GNAS, PTEN, EIF1AX, TP53, PIK3CA, AKT1, TSHR,* search queries: diagnosis of thyroid neoplasms, mutations in thyroid cancer, diagnosis of thyroid tumors, molecular testing of thyroid nodules, diagnosis and management of thyroid nodules, advanced thyroid cancers, mutations in indeterminate thyroid nodules, targeted therapy, radioactive iodine refractory thyroid cancer.

INTRODUCTION

Due to the high prevalence of modern diagnostic methods, the detection rate of thyroid tumors is quite high, for example, about 50% of 60 years old patients have thyroid nodules [1]. Moreover, although the prevalence of the disease depends on the study population and the methods used for detection, early diagnosis of malignancy remains the priority for clinicians. The prevalence of thyroid cancer is about 5% of thyroid tumors [2]. Fine-needle aspiration biopsy (FNA) is the standard diagnostic procedure for thyroid lesions of more than 1 cm [3]. However, it has several

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limitations such as ultrasonography (US) control of the procedure conducted by experienced professionals to ensure accurate target penetration, and further examination by an experienced pathologist as cellular features can be difficult to interpret. Approximately 20-30% of FNA results are classified as «undetermined» and belong to the categories III–V as per the Bethesda classification [4–5]. Among surgically resected cytologically «undetermined» thyroid tumors, approximately 15–30% of cases are malignant. As a result, most of the resected tumors are benign and do not require such radical treatment. This is vitally important for the patient since unnecessary surgery is associated with subsequent hormone therapy and lifelong endocrinologist follow-up and can lead to postoperative complications and a decrease in the quality of life. Besides, another important issue is the expensiveness of surgical treatment and postoperative follow-up [6]. Thus, there is currently a need for preoperative diagnostic methods that will help reduce the cases of irrational surgical treatment by determining the lesion type.

Thyroid cancer is the most common endocrine cancer demonstrating an approximately double increase in overall incidence over the last 25 years. Thyroid cancer accounts for 2% of all cancers [7]. Thyroid cancer is the sixth most common cancer in women, who are three times more likely to develop it compared to men. About 2% of cases occur in children and adolescents. Overall, the 5-year survival rate of patients with thyroid cancer is 98%. However, survival depends on many factors, such as the specific pathomorphological type of thyroid cancer and the stage of the disease [8].

Depending on the cells the tumor was originated from, there are several types and histological subtypes of thyroid cancer, and each of them has different characteristics and prognosis. Well-differentiated thyroid cancer (WDTC) originates from follicular cells and occurs in approximately ~95% of all cases. WDTC is divided into four groups: papillary thyroid cancer (PTC) accounting for more than 85% of cases, follicular thyroid cancer (FTC) — ~10% of all cases, poorly differentiated thyroid cancer (PDTC) — only 1–1.5% of all cases, and anaplastic thyroid cancer (ATC) (<1%). The latter two types of thyroid cancer are more aggressive compared to PTC and FTC [9]. 10% of all WDTC cases develop within the first two decades of life [10]. About 5% of them are diagnosed as familial, and the remaining 95% are sporadic.

Besides, about ~5% of thyroid cancer originates from parafollicular cells being a medullary type of thyroid cancer (MTC) [10]. About 75% of all metastases are considered sporadic, and the remaining 25% belong to hereditary syndromes known as multiple endocrine neoplasia type 2 (MEN2). MEN2 includes three clinically distinct types: MEN2A, MEN2B, and familial MTC. Familial non-medullary thyroid cancer is quite rare (only 3–9% of all cases). Moreover, only 5% of familial forms are included in specific syndromes: Cowden, Gardner, Werner, Li–Fraumeni, McCune–Albright, Carney, or DICER1 [11, 12]. Recent molecular genetic studies have identified some genes associated with both familial and sporadic forms of thyroid cancer. And since the role of the identified mutations has not been fully established, additional studies with a large number of observations are required for knowledge extension [11–15]. Therefore, in this review, we wanted to summarize current knowledge about the molecular basis of thyroid cancer and identify the genes that can affect its development.

MAIN MOLECULAR MARKERS FOR DIFFERENTIAL DIAGNOSIS OF THYROID TUMORS

Thyroid cancer is characterized by molecular modifications, such as activating/inactivating mutations, rearrangements, deletions, and frequency changes in the genes responsible for cell proliferation, differentiation, and apoptosis [16]. Several major signaling pathways are involved in thyroid carcinogenesis. All inter- and intracellular signals are recorded by receptors with the following processing through a precisely coordinated cascade of signaling pathways that direct the functioning of nuclear proteins. The key aspect in the signal transduction functioning is the control and regulation of gene expression. The cell responds to incoming signals, integrates them, and converts them into the desired cellular response by activating or suppressing the activity of certain genes. Any dysregulation or imbalance of signaling processes lead to serious consequences both for the individual cell and for the entire body. Moreover, above all, this applies to key cellular processes: proliferation, differentiation, and apoptosis. Thus, dysregulation is often caused by mutations in proto-oncogenes or tumor suppressor genes, leading to cell malignancy and carcinogenesis. Tumor growth and thyroid cancer progression are closely related to somatic point mutations in the *BRAF, RAS*, and *RET* genes. These mutations contribute to the activation of mitogen-activated protein kinase (MAPK) and phosphoinositide-3-kinase (PI3K) signaling pathways that play a critical part in thyroid cancer development (Figure 1). Once the expression of genes modifies, the development of prostate cancer begins, with an increase in cell proliferation, their unlimited growth and loss of differentiation, activation of angiogenesis, and invasion. The WNT signaling pathway is a signaling cascade that involves tumor suppressor proteins necessary for complete embryonic development, cell phenotype maintenance, and differentiation. P53 and p73 signaling pathways of the tyrosine kinase receptor (RTK) are also involved in the multistage process of cellular interaction in thyroid cancer, and modulate angiogenesis, proliferation, and differentiation [10, 17]. Changes in all these cascades can be linked by different mechanisms, including genetic and epigenetic modifications in pathway receptors and effectors [18].

Table 1 shows the most significant genetic modifications associated with thyroid tumors, including their location, the type of modifications, and the origin of mutations.

Somatic mutations of the *RET* gene

The *RET* gene (Rearranged During Transfection) encodes one of the tyrosine kinase receptors on the molecule cell surface, which is involved in the transmission of cellular signals from a family of glial cell-line derived neurotrophic factors that transmit signals for cell growth and differentiation [20].

Somatic point mutations of the *RET* gene are identified in 40–50% of sporadic MTC and are associated with a worse prognosis for the patients [21].

The *RET* (*RET/PTC*) rearrangements in papillary thyroid carcinoma (PTC) seem to be an early event in carcinogenesis and are detected in 10–20% of patients with PTC.

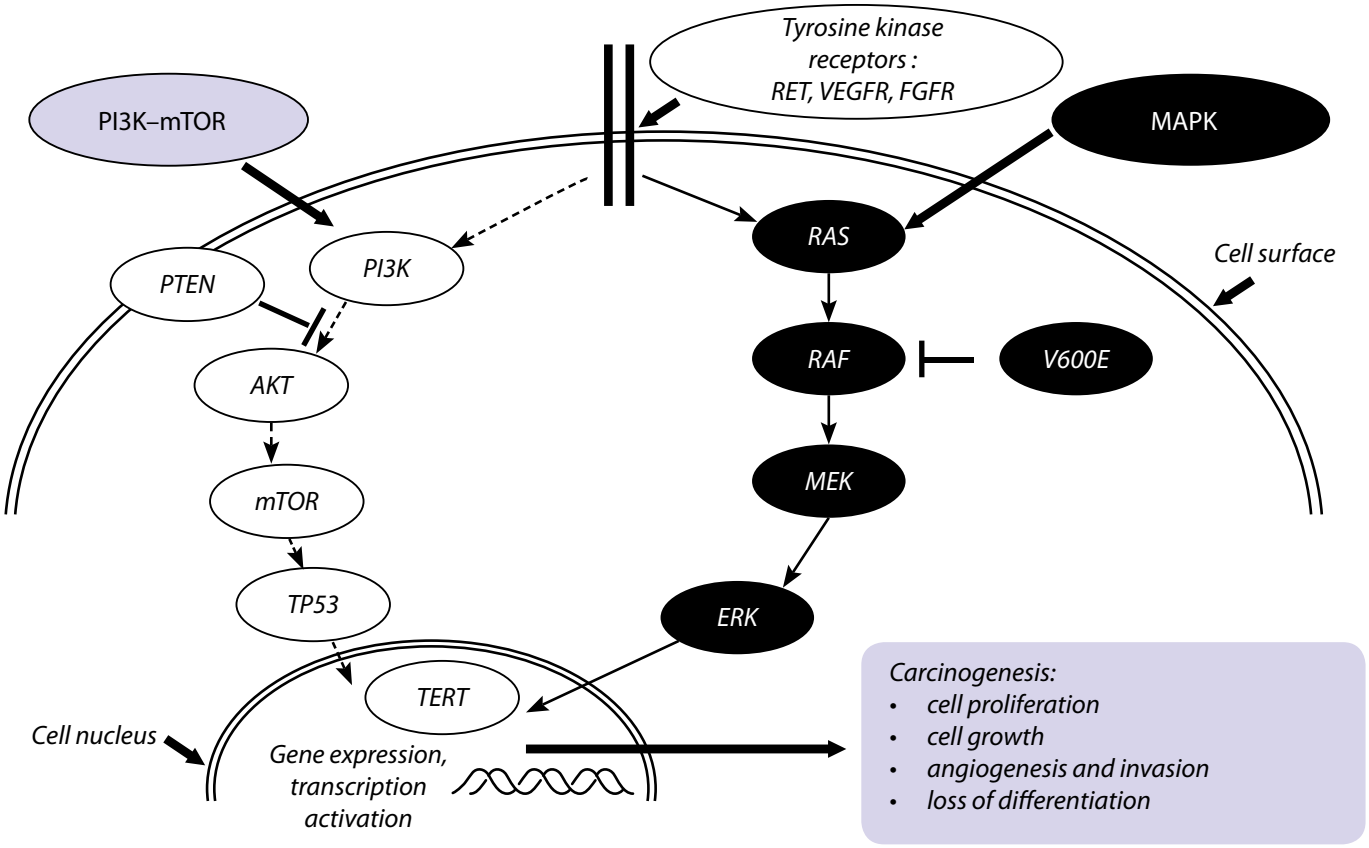

Figure 1. Key molecular signaling pathways of PI3K-mTOR and MAPK involved in the development of thyroid cancer.

RET/PTC rearrangements are associated with sporadic and radiation-induced PTC [20]. More than 10 main *RET/PTC* oncoproteins have been studied: *RET/PTC1, RET/PTC2, RET/PTC3, RET/PTC4, RET/PTC5, RET/PTC6, RET/PTC7, RET/PTC8, RET/PTC9, RET/ELKS, RET/PCM1, RET/RFP RET/HOOK3,* the most common are rearrangements of *RET/PTC1* (70%) and *RET/PTC3* (up to 30%). They are the most studied molecular events in PTC and are crucial in the assessment of thyroid lesions with undetermined FNA results [15].

Somatic mutations of the *BRAF* gene

The *BRAF* gene encodes a serine-threonine kinase activating the effectors of the MAPK signaling pathway [15]. Mutations in the *BRAF* gene are associated with carcinogenesis, as the normal activation of this pathway controlled by growth factors and hormones regulates cell preservation and proliferation, while impaired stimulation of this pathway may lead to excessive cell proliferation and false apoptosis resistance.

Mutations in the *BRAF* gene are identified in 30–67% of patients with PTC. The most common mutation in the *BRAF* gene is the *BRAFV600E* mutation (p.Val600Glu, widely known as V600E), which is detected in 95% of cases being a risk biomarker in PTC [24]. It is one of the mutations with high kinase activity, like other, less common mutations Glu586Lys, Val600Asp, Val600Lys, Val600Arg, Lys601Glu, etc.

The incidence of malignant tumors in *BRAF*-positive FNA samples is 99.8% [28]. Undetermined FNA samples demonstrate the *BRAF* mutation in 15–39% of cases. Hence, the detected *BRAF* V600E mutation can significantly improve the accuracy of preoperative diagnosis of PTC [5].

Somatic mutations of the *RAS* gene

RAS genes (*H-, N-, K-RAS*) encode cytoplasmic proteins involved in intracellular signal transmission from growth factor receptors. They play an important role in differentiation, cell growth, and migration. Localization of *RAS* mutations is most often found in exon 3 (codons 59 and 61), less often – in exon 2 (codons 12 and 13) or 4 (codons 117 and 146) [10, 44]. *RAS* point mutations can be detected in follicular adenoma (FA), FTC (40–53%), PTC (0–20%), FTC (17–25%), PDTC and ATC (20–60%) [23–25]. *NRAS* exon 3 (codon 61) mutations were detected in follicular tumors four times more often than in PTC, being the second most common point mutation after the *BRAF* V600E mutations with 8.5% incidence [24]. Thus, *RAS* mutations are associated with follicular tumors with a potential transition from preinvasion to true malignancy, whether it is FTC, PTC, or follicular PTC, which is the most difficult type to diagnose with an FNA biopsy [5].

Somatic mutations of the *PAX8-PPARγ* fusion protein

The PAX8-PPARγ fusion protein is a product of chromosomal translocation. Somatic mutations of *PAX8-PPARγ* are FTC-associated and are identified in 30–40% of cases [45].

Somatic mutations of the *TERT* gene

The *TERT* gene encodes a catalytic subunit of telomerase reverse transcriptase, which plays a key part in maintaining telomere length. The most common *TERT* mutation is C228T, less common – C250T. *TERT* point mutations were not detected in benign thyroid tumors and MTC. Their incidence in WDTC is low (10%) [46, 47], but it is quite high in PDTC

Table 1. The most significant genetic modifications associated with thyroid tumors.

Gene	Localization (chromosome (Chr))	Type of modifications	Origin of mutations	Disease	Source
RET	Chr 10	*RET/PTC* rearrangement	Somatic	PTC	[19, 20]
		Point mutations	Somatic	Sporadic MTC	[15]
			Germinal	MEN2A, MEN2B and familial MTC	[21, 22]
BRAF	Chr 7	V600E mutation (p.Val600Glu)	Somatic	PTC	[15, 23]
		Point mutations	Somatic	ATC	
RAS	*NRAS* Chr 1 *KRAS* Chr 12 *HRAS* Chr 11	Point mutations	Somatic	FA, FTC, PTC, follicular PTC, PDTC, and ATC	[23, 24, 25]
PTEN	Chr 10	Insertions, deletions, fusions	Germinal	Cowden syndrome 1	[15, 26]
PIK3A	Chr 3	Point mutations	Somatic	PTC	[27]
			Germinal	Cowden syndrome 5	[28]
AKT1	Chr 14	Point mutations	Germinal	Cowden syndrome 6	[29]
TERT promoter	Chr 5	Point mutations, including a combination with *BRAF* and *RAS* mutations	Somatic	ATC and severe non-medullary TC	[30, 31]
TP53	Chr 17	Point mutations	Somatic	ATC and PDTC	[32, 33]
			Germinal	Li–Fraumeni syndrome	[34]
MET	Chr 7	Point mutations	Somatic	MTC	[35]
ALK	Chr 2	Gene rearrangements	Somatic	PTC, ATC, and PDTC	[36]
CTNNB1	Chr 3	Point mutations	Somatic	PTC	[15]
JAK3	Chr 19	Point mutations	Somatic	FTC	[37]
CHEK2	Chr 22	Deletions and point mutations	Germinal	Li–Fraumeni syndrome 2	[38]
APC	Chr 5	Point mutations	Somatic	PTC	[15, 39]
			Germinal	Gardner syndrome	
GNAS	Chr 20	Point mutations	Somatic	Colloid nodular goiter, FA	[40]
TSHR	Chr 14	Point mutations	Somatic	FA	[41]
EIF1AX	Chr X	Point mutations	Somatic	FA, FTC, PDTC	[42]
NTRK1/3	Chr 15	Point mutations and chromosomal rearrangements	Somatic	PTC	[43]

(40%) and ATC (up to 73%) [5, 30, 31]. The detection of this mutation with an FNA biopsy can significantly improve the preoperative diagnosis of more aggressive forms of thyroid cancer.

Somatic mutations of the *EIF1AX* gene

The *EIF1AX* gene encodes a cytoplasmic protein involved in translation. The most common *EIF1AX* mutations occur in exons 2, 5, and 6. Mutations of the *EIF1AX* gene can be found in thyroid cancer, more often in PTC and ATC, but also benign neoplasms such as FA. Several other cases of thyroid cancer were associated with a combination of mutations in the *EIF1AX* and *RAS* genes. Simultaneous detection of mutations in the *EIF1AX* and *RAS* genes in follicular thyroid tumors clearly indicates the malignancy of the tumor, which can help in the diagnosis of thyroid tumors with undetermined FNA results [42].

Other significant mutations in thyroid cancer

Rearrangements involving the anaplastic lymphoma kinase (*ALK*) gene and striatin (*STRN*) activate ALK kinase inducing carcinogenesis. Such a fusion may be a therapeutic target for patients with highly aggressive types of thyroid cancer [48].

The *NTRK* gene belongs to the tyrosine kinase receptor encoding group. The *NTRK* gene rearrangements lead to the activation of the MAPK signaling pathway. The prevalence of *NTRK* rearrangements is approximately 1–5% in PTC and is more frequent in patients with a history of radiation exposure. Besides, the rearrangement of *ETV6-NTRK3* can be detected only in follicular PTC; together with *STRN-ALK*, they are recurrent and can not be present in benign lesions, which is useful for the differential diagnosis of thyroid tumors [49, 50].

Mutations of the *PIK3* gene are activating ones and usually occur in exon 9 and 20 hot spots. As with somatic mutations of the *PTEN* gene, they were identified in FTC and ATC [5]. When *PTEN* mutations are inherited, patients with Cowden syndrome have an increased risk of FTC [5].

TR53 is a tumor suppressor that plays an important role in cell cycle regulation and DNA repair. Point mutations of *TP53* are detected in 50–80% of ATC and PDTC or advanced stages of thyroid cancer [5].

Mutations of the *CTNNB1* gene (beta-catenin) activate the WNT signaling pathway. *CTNNB1* exon 3 mutations occur in more than 60% of ATC cases [5].

The accumulation of several oncogenic changes in ATC is equivalent to an increased level of differentiation and aggressiveness [51]. The role of p53 in thyroid carcinogenesis is well known, but the role of the rest of the p53 family in thyroid carcinogenesis requires further studies. More and more evidence suggests that such members of a gene family contribute to multifocal types of thyroid cancer, and also, they can be used as therapeutic targets [52].

Somatic mutations of the *TSHR* gene can often be found in autonomously functioning thyroid nodules but they are also identified in thyroid cancer [5]. Therefore, this marker can only be used together with other markers of thyroid tumors.

GNAS is a gene encoding the alpha subunit of heterotrimeric G protein complexes. Mutations of the *GNAS* gene are mainly found in benign hyperplastic nodules and FA. Therefore, it may be concluded that an isolated *GNAS* mutation can be a marker of benign tumors [5].

Besides, it should be noted that various mutually exclusive molecular modifications might be associated with specific stages of the disease or with different histological types of thyroid cancer [53]. Figure 2 shows the most relevant genetic modifications involved in carcinogenesis in different histological types of thyroid cancer. Besides, considering the mutation rate and their role in carcinogenesis, it is possible to imagine the process of thyroid carcinogenesis from PTC and FTC to PDTC and ATC. Table 2 shows the incidence of the main genetic modifications in different histological types of thyroid cancer. Significant variations in the mutation prevalence are due to both the type of the patient groups in a study and tumor heterogeneity.

Differential diagnosis of thyroid tumors requires the study of the main and most common molecular markers. As we have discussed earlier, the most characteristic oncogenic mutations for PTC are mutations in the following genes: *BRAF* (V600E substitution), *RAS*, and *RET/PTC* gene rearrangements. The most characteristic mutations for FTC are the ones that show *PAX8/PPARγ* rearrangements, *RAS* mutations, and *PTEN*-inactivating mutations or deletions. *PTEN* and *CTNNB1* mutations, and *TP53* inactivation are common for ATC [15]. However, their isolated detection will not be sufficiently sensitive and specific and will not have positive and negative prognostic significance (PPV and NPV) for diagnosis.

According to clinical guidelines, single-gene testing today is quite limited: according to the guidelines of the American Thyroid Association in 2015 [57] and the American Association of Clinical Endocrinologists in 2016, it is possible to consider molecular genetic testing in undetermined FNA results (diagnostic categories III and IV as per the Bethesda classification). Recommendations include the analysis of *BRAF, RET/PTC, PAX8/PPRγ* and, additionally, *RAS* mutations [58]. According to the Russian Clinical Guidelines in 2017, differential diagnosis of thyroid tumors requires to consider genetic testing for *BRAF* mutations and other markers (*RET/PTC, PAX8/PPARγ, RAS, TERT*, etc.) in undetermined FNA results (diagnostic categories III, IV, and V as per the Bethesda classification) [3].

Since 2010, molecular genetic panels have been used and studied. In addition to point mutations, they include the expression of the most common tumor oncogenes and microRNAs [59]. There are 4 main commercially available tests today. Table 3 contains general information about them including name, type of response, test type, and the study parameters, such as sensitivity, specificity, NPV, PPV, and price. However, despite the affordability of these tests and relatively high PPV and NPV, the most credible scientific associations are not ready to include such tests in their recommendations [6]. This is because today there is no data on the prognostic value of the selected treatment strategy of a patient in compliance with the test result.

Therefore, despite a significant number of detected genes for potential preoperative diagnosis, before including molecular genetic testing in routine clinical practice, additional data is required.

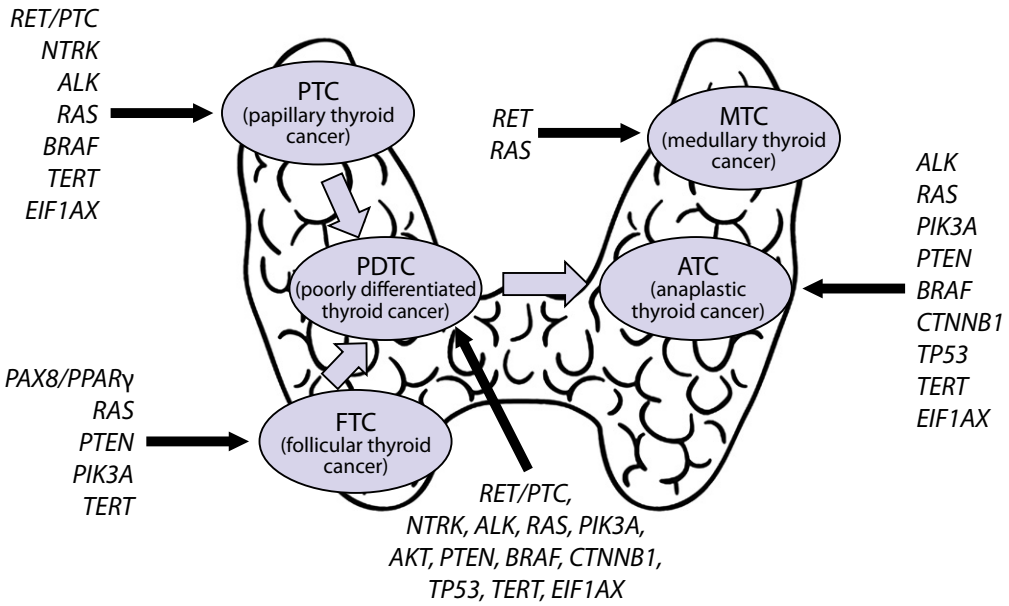

Figure 2. Main genetic modifications involved in carcinogenesis in different histological types of thyroid cancer.

PROGNOSTIC VALUE OF IDENTIFIED MOLECULAR MARKERS IN THYROID CANCER

The second trend in a study of the molecular profile of thyroid tumors is the evaluation of a relationship of identified genetic changes with clinical and pathological features of the disease. This is extremely important, since it may lead to the development of criteria for accurate prognosis that will facilitate the choice of an optimal treatment strategy for such patients and the development of a prognostic genetic marker-oriented risk stratification [66]. This, in turn, will allow to justify both organ-preserving surgery and more aggressive surgical interventions in the treatment of thyroid cancer, including indications for total thyroidectomy, preventive central lymph node dissection (level VI), RIT, and suppressive therapy.

So, mutations of the *RET* gene are associated with a more aggressive course of the disease, a larger tumor size at diagnosis, invasion, and an increased risk of lymph node metastases and distant metastases [15, 20, 21]. According to the clinical guidelines for the management of MEN2 patients, it is recommended to perform early genetic screening in individuals from risk groups to identify germinal *RET* mutations that are associated with the worst prognosis [15].

RET/PTC1 or *RET/PTC3* rearrangements are detected in about 20% of children with PTC. *RET/PTC* rearrangements were detected in benign thyroid lesions. So a high prevalence of *RET/PTC* was detected in patients with Hashimoto's thyroiditis [15].

Somatic mutations in the *BRAF* gene were more often associated with a high risk of relapse, aggressive course of the disease, lymph node metastases and extrathyroid spread, and increased mortality [67, 68]. But isolated mutations in the *BRAF* gene have a quite low specificity with high sensitivity, so they are difficult to use in the risk assessment for relapse and mortality.

There are no mutations in the *BRAF* gene in benign thyroid nodules, but they can be detected in 1/3 of ATC cases [22, 23].

Somatic mutations in the *RAS* gene are the second most common after *BRAF*, and their prognostic value is contradictory since the detection of *RAS* mutations in the thyroid gland does not determine the degree of malignancy. These mutations occur in all pathomorphological types of thyroid lesions, from benign tumors to ATC. At the same time, the frequency of detection of *RAS* mutations in PDTC and ATC is higher than in other types of thyroid cancer; and several studies have confirmed the clinical significance of the association of *RAS*

Table 2. Incidence of the main genetic modifications in different histological types of thyroid cancer [5, 30, 54–56].

Histological type of thyroid cancer	PTC	FTC	ATC	PDTC	MTC	FA
Prevalence of mutations, %	*BRAF*: 30–67 *RET/PTC*: 10–20 *RAS*: 10–20 *TERT:* 9 *NTRK*: 1–5 *EIF1AX:* 1	*RAS*: 40–50 *PAX8/PPARγ*: 30–40 *TERT*: 14–36 *TP53*: 22	*TP53*: 50–80 *TERT*: 33–73 *CTNNB1*: >60 *BRAF*: 20–40 *RAS*: 20–40 *EIF1AX:* 10 *ALK:* 4	*TERT*: 40 *EIF1AX:* 10 *ALK:* 9	*RET/PTC*: 40–50 *RAS*: 25	*RAS*: 20-40

Table 3. Main commercially available molecular genetic panels and their characteristics.

Name	Afirma GEC/GSC	ThyroSeq2/3	Rosetta Reveal	ThyGenX/ThyramiR
Approach	Gene expression analysis (RNA)	Mutation analysis (DNA)	MicroRNA expression analysis	Mutation analysis (DNA), microRNA expression analysis
Test type	Exclude	Confirm/exclude	Confirm	Confirm/exclude if both tests were performed
Response	Benign/ possibly malignant	Negative / positive	Benign/ possibly malignant	Negative / positive
Mutations analyzed	No	ThyroSeq2: *BRAF, KRAS, HRAS, NRAS,* and *RET/PTC1, RET/PTC3, PAX8/PPARγ*, (*TRK) rearrangements* ThyroSeq3 expanded: *PIK3CA, TP53, TSHR, PTEN, GNAS, CTNNB1, AKT1, RET*	No	*BRAF, HRAS, KRAS, NRAS, PIK3CA*, and *PAX8/PPARγ, RET/PTC1, RET/PTC3 rearrangements*
Test parameters	NPV 94%, PPV 37%, sensitivity 90%	ThyroSeq2: NPV 96%, PPV 83%, sensitivity 90%, specificity 93% ThyroSeq3: NPV 97%, PPV 66% (given the prevalence of thyroid cancer 28%), sensitivity 94%, specificity 82%	NPV 91%, PPV 59%, sensitivity 85%, specificity 72%	NPV 94%, PPV 74%, sensitivity 89%, specificity 85%
Price [58]	$6400	$4056	$3700	$5675
Data source	[60, 61]	[62, 63]	[64]	[65]

mutations with the risk of distant metastases and reduced survival rate [15].

Besides, a combination of *RAS* and *TERT* mutations was associated with a more aggressive course of the disease, a higher risk of relapse, and mortality [30].

Mutations of the *TERT* gene are associated with aggressive characteristics of the thyroid tumor: extrathyroid spread, large tumor size, lymph node metastases, and distant metastases, a more severe TNM stage, as well as tumor recurrence and mortality; with a more aggressive course of thyroid cancer [5, 31]; aggressive types of thyroid cancer: PDTC, ATC. There were no mutations of the *TERT* gene in benign thyroid tumors.

The combination of *BRAFV600E* and *TERT* mutations has a strong synergistic effect on PTC aggressiveness, increased risk of relapse, and mortality of patients, whereas, when detected separately, such an effect is significantly less [47].

Since the detection of the *EIF1AX* mutation occurs in both thyroid cancer and benign lesions, isolated use of this marker is difficult. However, simultaneous detection of *EIF1AX* and *RAS* mutations in follicular thyroid tumors clearly indicates the malignancy of the tumor. In addition, detection of the *EIF1AX* gene mutation in ATC is a predictor of the most aggressive course of the disease [42].

Detection of *TP53* and *CTNNB1* mutations occurs in a more aggressive course of WDTC and in PDTC and ATC. Detection of *PTEN, PIK3CA, AKT1* mutations is also ATC-associated [5].

Thus, it is possible to use some molecular genetic modifications in clinical practice as indicators of tumor malignancy. Since they are associated with a more aggressive course of the disease, the doctor can use the most aggressive treatment strategy. These modifications include: *TERT, RET, BRAF* (especially combined with *TERT*), *TP53, CTNNB1, PTEN, PIK3CA, AKT1*. The *GNAS* mutation can be a marker of benign tumors, and therefore the treatment strategy can be minimally aggressive or even limited to follow-up. Because some other molecular genetic changes can be detected in benign and in malignant tumors, it is possible to use them as additional markers. Combined with malignancy-indicating mutations,

Table 4. Targeted drugs for aggressive forms of thyroid cancer.

Tyrosine kinase inhibitors	Target tyrosine kinase	Target patient population	Source
Multi-target inhibitors			
Anlotinib	VEGFR 2-3, FGFR 1-4, PDGFR-α/β, c-KIT, RET	MTC	[78–79]
Axitinib	VEGFR1-2-3, PDGFR-β, c-KIT	Common types of TC	[67, 80]
Dovitinib	FGFR, VEGFR	Common types of TC	[81]
Cabozantinib	MET, VEGFR-2, RET	MTC **(FDA approved)**	[82–84]
Imatinib	ABL, c-KIT, PDGFR	ATC, MTC	[67, 85]
Lenvatinib	VEGFR 1-2-3, FGFR 1-2-3-4, PDGFR-α, RET, c-KIT	RIT-resistant WDTC **(FDA approved)**	[86–88]
Motesanib	VEGFR 1-2-3, PDGFR, RET, c-KIT	Common types of WDTC, MTC	[67]
Pazopanib	VEGFR 1-2-3, PDGFR-α/β, c-KIT, FGFR 1-3-4	RIT-resistant WDTC, ATC, MTC	[89–91]
Sorafenib	VEGFR 1-2-3, RET, RAF, PDGFR-β, c-KIT, FLT3	RIT-resistant WDTC **(FDA approved)**	[92–93]
Sunitinib	VEGFR 1-2, c-KIT, RET, PDGFR-β, FLT3	RIT-resistant WDTC, common types of WDTC, MTC	[94–96]
Vandetanib	RET, VEGFR 2-3, c-KIT, EGFR	MTC **(FDA approved)**	[97–99]
Mono-target inhibitors			
Apatinib	VEGFR-2	RIT-resistant WDTC	[100–101]
Dabrafenib + Trametinib	BRAF + MEK	*BRAFV600E* in ATC, PTC	[102–104]
Dabrafenib + Lapatinib	BRAF + HER2/3	*BRAFV600E* in common types of WDTC	[105]
Selumetinib	MEK-1/2, BRAF, RAS	RIT-resistant WDTC	[106]
Vemurafenib	BRAF	*BRAFV600E* in RIT-resistant WDTC, common types of TC	[107]
Tipifarnib	HRAS	RIT-resistant WDTC	[66]
Ceritinib	ALK	ATC	[50, 108, 109]
Crizotinib	ALK	ATC	[110–111]
Entrectinib	NTRK (TRK, ROS1, ALK)	Common types of TC	[112–114]
Larotrectinib	NTRK (TRK)	Common types of TC	[115–116]
LOXO-195	NTRK (TRK)	Common types of TC	[115]
Buparlisib	PI3K	RIT-resistant WDTC	[117]
Everolimus	mTOR	RIT-resistant WDTC, MTC	[118–120]
Everolimus + Pasireotide	mTOR + PI3K (Somatostatin analogue)	Common types of MTC, RIT-resistant WDTC	[121, 122]
Temsirolimus	mTOR	RIT-resistant WDTC	[123]
Sirolimus	mTOR	RIT-resistant WDTC	[124]
Efatutazone + Paclitaxel	PPAR agonist	ATC	[125]

these markers will be associated with increased aggressiveness of the disease (*KRAS, NRAS, HRAS, TSHR, EIF1AX*). The isolated determination of these mutations should not affect the treatment strategy in any way. However, despite the long-term experience in research of molecular testing, before it will have a significant effect on the indication for surgical intervention and the treatment strategy of a patient, additional data on its long-term results is required.

USE OF MOLECULAR MARKERS FOR TARGETED THERAPY IN THYROID CANCER

In this section, we would like to review the current potential of targeted therapy in thyroid cancer. The vast majority of patients with WDTC and a high risk of relapse show a good response to standard treatment, including surgery followed by RIT (based on ^{131}I) and hormone suppressive therapy [66]. Despite an overall good prognosis, distant metastases are already present at the time of diagnosis or develop at follow-up in 10–20% of patients with WDTC. Most of these patients have a good response to RIT with a 40% chance of complete and long-term response [16]. However, the remaining 60% show primary or acquired resistance to RIT and require other additional treatment options. A small proportion (<10%) of WDTC, as well as many types of MTC and almost all ATC, can not be cured with standard therapies [66]. Besides, as thyroid cancer progresses, an increase in the number of molecular modifications leads to variations in normal cell functions, resulting in resistance to RIT, which is due to impaired expression of the sodium-iodine transporter [10, 69, 70].

Since parafollicular cells are characterized by a lack of ^{131}I capture, the preferred therapy for localized MTC is thyroidectomy followed by hormone therapy. However, targeted therapy or, less frequently, chemotherapy are possible for locally advanced or metastatic forms of the disease [71].

ATC is characterized by rapid growth and loss of common functions of follicular cells, including iodine absorption, so ATC demonstrates the impaired function of the sodium iodide symporter and resistance to RIT. Thus, radiation therapy and chemotherapy are the only treatment options for this disease, even though the outcomes are quite bleak [72, 73]. In aggressive types of WDTC, MTC, ATC, PDTC, the 5-year survival rate is less than 50%, as opposed to ~98% of the 5-year survival rate in patients with iodine-sensitive WDTC.

Thyroid cancer is characterized by molecular modifications in the genes responsible for cell proliferation, differentiation, and apoptosis [74]. Therefore, in recent years, the discovery of thyroid cancer-specific molecular targets has led to studies of many targeted drugs for the treatment of aggressive thyroid cancer. However, the mechanisms of internal resistance of a lesion to targeted drugs, as well as the systemic toxicity of drugs, lead to a limited clinical benefit and require additional studies [66].

Tyrosine kinase inhibitors (TKI) are the main class of drugs for targeted therapy in thyroid cancer. TKI modify signaling pathways and modulate the processes of angiogenesis, proliferation, and differentiation. Table 4 demonstrates the main TKIs, their targets, and patient populations. There are completed studies for some of these molecules that have not shown their significant effect on the prognosis. Some other molecules are currently being studied. But only few tyrosine kinase-inhibiting molecules involved in cell proliferation, their survival, and angiogenesis have shown clinical efficiency [75]. Today four drugs are FDA-approved (Food and Drug Administration): sorafenib and lenvatinib for the treatment of RIT-resistant WDTC, and cabozantinib and vandetanib for the treatment of MTC [76, 77].

TKIs have shown significant benefits in survival in both RIT-resistant WDTC and MTC. These benefits were obtained with significant clinical and financial costs [126, 127]. While tyrosine kinase inhibitors in WDTC and MTC are currently being analyzed in additional studies, their use in ATC has been largely unsatisfactory. The combination of BRAF and MEK inhibitors, on the other hand, has led to a high response rate in this group of patients.

Therefore, in the last few years, there has been rapid progress in understanding the molecular mechanisms underlying thyroid carcinogenesis. Along with the identification of key genes that contribute to the development and progression of the disease, this has led to the initiation of several biological therapies, including the use of monoclonal antibodies and antibody-drug conjugates in addition to tyrosine kinase inhibitors [128–131].

CONCLUSION

Understanding the molecular genetic mechanisms of thyroid cancer development provides broad options for molecular diagnostics in a differential diagnosis, in predicting the course of a disease, and in the treatment of aggressive forms of thyroid cancer. And despite that molecular genetic studies are currently limited by low availability, high price, and lack of long-term results in clinical practice, their use can have a significant impact on the personalized treatment of patients with thyroid tumors.

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