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REVIEW ARTICLE

Altered Epigenetic Mechanisms in Thyroid Cancer Subtypes

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Abstract Thyroid carcinoma (TC) is the most frequent malignant neoplasm of the endocrine system. Molecular methods for diagnosis of invasive thyroid disease can be effectively adopted. Epigenetic factors play an important role in the diversity patterns of gene expression and the phenotypic and biological characteristics of TC subtypes. We aimed to review epigenetic changes in the main subtypes of TC, along with a presentation of the methods that have examined these changes, and active clinical trials for the treatment of advanced TCs targeting epigenetic changes. A literature analysis was performed in MEDLINE using PubMed, Elsevier, and Google Scholar for studies published up to 2016, using the keywords: "Epigenetic alterations" OR "Epigenetic changes", "thyroid cancers", "papillary thyroid cancer", "medullary thyroid cancer", "follicular thyroid cancer", and "anaplastic thyroid cancer", which resulted in 310 articles in English. All related abstracts were reviewed and studies were included that were published in English, had available full text, and determined the details of the methods and materials associated with the epigenetic patterns of TC and its subtypes (100 articles). Analysis of epigenetic alterations in TC subtypes helps to identify pathogenesis and can play an important role in the classification and diagnosis of tumors. Epigenetic mechanisms, especially aberrant methylation of DNA and microRNAs (miRs), are likely to play an important role in thyroid tumorigenesis. Further studies are required to elucidate the role of histone modification mechanisms in TC development.

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Key Points

Disorders in mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3-kinase (PI3K-AKT) signaling pathways via DNA methylation and miRs regulation are the most common epigenetic modifications in well-differentiated thyroid carcinomas (TCs) [papillary TC and follicular TC].

Genome-wide analysis of DNA methylation patterns leads to the recognition of genes related to tumors with distinct subtypes and mutational status.

Aberrant promoter hypomethylation occurs in undifferentiated subtypes (poorly differentiated TC [PDTC] and anaplastic TC) more than hypermethylation.

Histone acetylation levels can change in thyroid cells by neoplastic transformation and hormonal stimulation.

Deregulation of some miRs causes progression of PDTC and in medullary TC is probably an early event in C cell carcinogenesis.

1 Introduction

Although thyroid carcinoma (TC) is quite a rare cancer, its incidence is increasing [1] and it is the most common endocrine malignancy, with about 1–5% of women and less than 2% of men affected worldwide. Thyroid cancers arising from follicular epithelial cells, which comprise about 95% of all thyroid tumors, have been categorized as (1) well-differentiated TC (WDTC), including both papillary TC (PTC; 80%) and follicular TC (FTC; 10–15%); (2) poorly differentiated TCs (PDTC; 0.5–7%); and (3) anaplastic TC (ATC; 1–2%) [2]. Medullary TC (MTC), a less common tumor among TCs, arises from parafollicular calcitonin-producing cells.

Generally, cancer as a genetic disease is caused by specific changes in genes that control cell function, especially their growth and division. Some common genetic alterations in TC and its subtypes consist of gene point mutations, copy-number changes, and translocations which dominantly occur in protein-coding genes involved in classical signaling pathways of cell proliferation and survival [3]. These pathways include mitogen-activated protein kinases (MAPK)/extracellular signal-regulated kinases (ERK), phosphatidylinositol-3-kinase (PI3K-AKT), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), Ras association domain family member 1 (RASSF1)-macrophage stimulating 1 (MST1)-forkhead box O3 (FOXO3), WNT-β-catenin signaling pathway, hypoxia-inducible factor 1-α (HIF1a) pathway, and the thyroid-stimulating hormone receptor (TSHR) signaling pathway [3]; among these, two main pathways, the MAPK/ERK and the PI3K-AKT, play a remarkable role in TC pathogenesis [4]. The most effective molecular variations which disturb the aforementioned pathways have been identified and four of the well-known ones are in the *RET*, *BRAF*, *RAS*, and paired box 8 (*PAX8*) genes [5]. The *RET* rearrangement is found in approximately 28.6% of TCs; *BRAF* mutation is found in 32.4% of PTC cases [6]; and the point mutation in *RAS* as a component of MAPK/ERK signaling pathway is frequently seen in cases of PDTC or ATC. *RAS* mutations occur in 20–50% of follicular cancers. Another chromosomal translocation including fusion of the promoter region of the *PAX8* gene and the coding region of the peroxisome proliferator-activated receptor-γ (*PPARγ*) gene occurs in 35% of FTC cases [7].

Along with genetic variations, environmental factors are reported as a powerful determinant for cancer incidence, and mortality, lifestyle (diet and physical inactivity), and chemical exposure are the most important factors based on the literature [8]. Environmental factors have no effect on gene sequences and they exert their effects through rearrangement of gene expression. These alterations are known as epigenetic changes; different types of epigenetic alterations in TCs have been identified and their important role in the diversity patterns of gene expression and phenotypic and biological characteristics of TCs have been confirmed; understanding each of these changes could be vital before starting any therapeutic approach. In this review, we discuss briefly the mechanism of epigenetic modifications and their role in the pathogenesis of TC and its main subtypes along with a presentation of the methods that have been used to examine these changes. We also introduce some active clinical trials for the treatment of advanced TCs using epigenetic changes. Lastly, we deliberate on and clarify whether there is any difference in epigenetic mechanisms in TC subtypes or not?

2 Methods

2.1 Data Sources

A literature analysis was performed in MEDLINE using PubMed, Elsevier, and Google Scholar to search for studies published up to 2016, using the keywords: "Epigenetic alterations" OR "Epigenetic changes", "thyroid cancers",

"papillary thyroid cancer", "medullary thyroid cancer", "follicular thyroid cancer", and "anaplastic thyroid cancer".

2.2 Study Selection

Using the keywords mentioned, 310 articles in English were obtained. All related abstracts were reviewed and studies were included that were published in English, had available full text, and determined the details of the methods and materials associated with the epigenetic patterns of TC and its subtypes (100 articles).

3 Results

3.1 Epigenetics of Thyroid Cancer Subtypes

3.1.1 Papillary Thyroid Carcinoma

PTC accounts for over 85–90% of thyroid malignancies [9]. With the advent of diagnostic techniques and molecular methods of detecting aggressive disease, the nature of PTC has been better elucidated in the last decade [10].

Recently, numerous investigations of epigenetic modifications in PTC have mainly focused on candidate tumor suppressor genes and genes known for their role in thyroid function. Numerous studies have assessed the DNA methylation status of several tumor suppressor genes (*RAPβ2* [22%], *SLC5A8* [33%], *DAPK* [34%], *TIMP3* [53%], *DKK3* [38.8%], *DACT2* [64.6%], *Mig-6* [79%], *XAF1* [35.7%]) in the tissues from PTC patients and in the PTC cell lines [11–16]. Other studies results showed that *ADAMTS8*, *HOXB4*, *ZIC1*, *KISS1R*, *INSL4*, *DPPA2*, *TCL1B*, and *NOTCH4* genes were frequently regulated by aberrant methylation in PTC and FTC [17]. Some investigations also showed that *RASSF1* was methylated in 80, 78, 70, and 62% of MTC, undifferentiated TC (UDTC), FTC, and PTC, respectively [18], and $p16^{INK4A}$ was methylated in 33, 44, 50, 75, 85, and 13% of follicular adenoma (FA), PTC, FTC, PDTC, UDTC, and non-tumorous tissue, respectively [19]. *SLC26A4* was methylated in 44, 46, 71, 71, and 100% of BA, FTC, PTC, ATC, and cell lines, respectively, using DNA sequencing assays [20]. Moreover, *Rap1GAP* promoter region was methylated in 9–45% of PTCs [16] (Table 1). Mancikova et al. [21] performed a genome-wide DNA methylation profile in a large WDTC series including 83 tumor samples and eight normal adjacent tissues. They observed 89 hypermethylated CpGs (83 genes) in FA, 460 (416 genes) in FTC, and 39 (31 genes) in PTC, and found that follicular tumors had higher levels of methylation, which seemed to accumulate in a progressive manner along the tumorigenic process from adenomas to carcinomas [21]. In a study by Lee et al. [22], DNA methylation of MAPK signal-inhibiting genes was investigated. The results revealed that the Serpin family A member 5 (*SERPINA5)* promoter was methylated in 82.9% of PTC samples ($n = 76$). The methylation level was positively correlated with the presence of a *BRAF* mutation [22]. One of the most important translational contributions has been presented in the field of PTC in The Cancer Genome Atlas (TCGA) research project is the BRAF-like and RAS-like phenotypes. BRAF-like PTCs are mostly shown in classical structure of tumors such as classic and tall cell variants and mainly in PTCs with solid progress such as the RET/PTC-driven solid variant, while RAS-like PTCs are revealed in follicular-variant PTCs (FVPTCs) [23, 24]. In a large cohort of almost 496 PTCs, the PTC samples were subdivided into BRAF-like and RAS-like groups depending on the exome and RNA sequencing, proteomic profiles, and epigenetic changes in the analysis of the TCGA. Based on the DNA methylation levels, the investigators defined four groups, two of them improved by a *H/K/N RAS*-mutated FVPTC and two developed by *BRAF*-mutated classical and tall cell PTC. The TCGA classical/tall cell PTC-enriched cluster was distinct by the low levels of methylation in CpG normally methylated outside of islands by *BRAF*-mutated tumors. The authors indicated that BRAF-like PTCs specially activate the MAPK pathway, while RAS-like PTCs signal through MAPK in addition to the PI3K pathway [24]. In another study, Smith et al. [25] investigated DNA methylation using locus-specific non-quantitative methods in PTC samples including E-cadherin (*ECAD)*, *TSHR*, *BRAF*, solute carrier family 5 member 8 (*SLC5A8*), *RASSFI*, tissue inhibitor of metalloproteinases 3 (*TIMP3*), sodium/iodide symporter (*NIS*), death-associated protein kinase (*DAPK*), ataxia telangiectasia mutated (*ATM*), retinoid acid receptor β2 (*RARβ2*), apical iodide transporter (*AIT*), and *MAPK* signal-inhibiting genes. The results revealed promoter hypermethylation of *TSHR*, *RARβ2*, *NIS-L*, *ATM*, and *ECAD* in 34–59, 22, 22, 50, and 56% of patients with PTC, respectively [25]. Ellis et al. conducted a study of genome-wide methylation patterns in 51 PTC patients based on histological subtype and tumor genotype using advanced genome-wide methylation bead chips. They analyzed altered methylation patterns by stage, recurrence, histological subtype of tumor, and tumor genotype and demonstrated that PTC was globally hypomethylated compared with the normal thyroid, with 2837 differentially methylated CpG sites [26]. Ishida et al. [27] surveyed 39 lesions of PTC using quantitative methylation-specific polymerase chain reaction (qMSP) to assess hypermethylation in multiple genes implicated in regulatory processes such as $p16^{INK4a}$ and $p14^{ARF}$, generated by the cyclin-dependent kinase inhibitor 2A (*CDKN2A*) gene; they

Table 1 DNA methylation prevalence of some proto-oncogenes and tumor suppressor genes in thyroid cancer

Genes	Full name	Function	DNA methylation prevalence	Sample size	Methods used	References
Tumor suppressor						
RASSF1	Ras association domain family member 1	*RASSF1A* localizes to microtubules and promotes their stabilization	80% of MTC, 78% of UDTC, 70% of FTC, 62% of PTC	1 PDTC, 5 MTC, 10 FTC, 9 UDTC, 13 PTC, and 9 cell lines	MSP	[18]
$p16^{INK4A}$	Cyclin-dependent kinase 4 inhibitor A	*P16* plays an important role in cell cycle regulation	33% of FA, 44% of PTC, 50% of FTC,75% of PDTC, 85% of UDTC, and 13% of non-tumorous tissue	18 FA, 18 FTC, 16 PTC, 12 PDTC, 13 UDTC, and 15 tumor-free tissues	MSP	[19]
SLC26A4	Solute carrier family 26 member 4	*E*ncodes pendrin, which is an antiporter anion exchanger protein	44% of BA, 46% of FTC,71% of PTC,71% of ATC, and 100% of cell lines	64 tumors and 6 thyroid cell lines	BSSQ and MSP	[20]
ADAMTS8	A disintegrin and metalloproteinase with thrombospondin motifs 8	A potential tumor suppressor that disrupts angiogenesis	18% of PTC	24 PTC, 7 FTC, 4 goiters, 6 normal, and 4 cell lines	Illumina infinium methylation arrays	[17]
HOXB4	Homeobox B4	A sequence-specific transcription factor	18% of PTC			
ZIC1	Zic family member 1	Transcription factor activity, sequence-specific DNA binding	67% of FTC			
KISS1R	Kisspeptins receptor	Endogenous ligand of the G-protein coupled receptor	17% of FTC			
THRβ	Thyroid hormone receptor β	A nuclear receptor protein	34% of PTC, 81% of FTC, and 25% of FA	29 PTC, 27 FTC, and 20 FA	BSSQ and MSP	[49]
			90% of WDTC	17 PTC and 4 FTC	MSP	[50]
RAP1GAP	RAP1 GTPase activating protein	Regulation pathway and development angiotensin activation of ERK	9–45% of PTC	7 BA, 40 HN, 49 FA, 27 FTC, 78 PTC, and 3 ATC	MSP	[16]
Oncogene						
TSHR	Thyroid-stimulating hormone receptor	Provides instructions for making a protein, known as a receptor, that attaches (binds) to the thyroid-stimulating hormone	59% of PTC and 47% of FTC	39 PTC, 15 FTC, 8 BA, and thyroid tumor cell lines	BSSQ and MSP	[51]
INSL4	Insulin-like 4	Belongs to the insulin and IGF family	60% of MTC	24 PTC, 7 FTC, 4 goiters, 6 normal, 26 MTC, 9 ATC, and 4 cell lines	Illumina infinium methylation arrays	[17]
DPPA2	Developmental pluripotency-associated 2	Binds to target gene promoters including *NKX2-5* and *SYCE1*	30% of MTC			
TCL1B	T cell leukemia/lymphoma 1B	An oncogene frequently activated by reciprocal translocations	64% of ATC			
NOTCH4	Neurogenic locus notch homolog 4	A member of the notch family, which plays a role in a variety of developmental processes	45% of ATC			
MAP17	Membrane-associated protein 17	A membrane-associated protein that is overexpressed in human tumors	38% MTC and 33% ATC			
TTF-1/ NKX2-1	Thyroid transcription factor-1	A homeodomain containing transcriptional factor identified in thyroid	60% of UC and 50% of the cell lines	11 normal, 7 PTCs, 10 UTCs, and 8 cell lines	MSP	[75]

ATC anaplastic thyroid carcinoma, *BA* benign adenomas, *BSSQ* bisulfite sequencing, *ERK* extracellular signal-regulated kinases, *FA* follicular adenomas, *FTC* follicular thyroid carcinoma, *HN* hyperplastic nodules, *MSP* methylation-specific polymerase chain reaction, *MTC* medullary thyroid carcinoma, *PDTC* poorly differentiated thyroid carcinoma, *PTC* papillary thyroid carcinoma, *UDTC* undifferentiated thyroid carcinoma, *WDTC* well-differentiated thyroid carcinoma

Table 2 Histone modification investigations in thyroid cancer

Name	Modification	Tumor type	Sample size	Methods used	References
HDAC2	Acetylation	From BA to PTC, FTC, and ATC	5 Cell lines	HDAC activity assay	[30]
HDAC1, HDAC2, and HDAC3		NT, PTC, FTC, and ATC	4 Cell lines	ChIP	[77]
Lysine at positions 9–14 of H3 histone (H3K9–K14ac)		FA, PTC, FTC, UC, and NT	10 FA, 30 PTC, 9 FTC, 6 UC, 6 NT, and 5 cell lines	IHC	[79]
Lysine 18 of H3 histone (H3K18ac)		NT and UC			
Lysine 12 of H4 histone (H4K12ac)		FA and NT			
Histone H3 lysine 9 (H3-lys9)		UC and PTC	11 Normal, 7 PTCs, 10 UTCs, and 8 cell lines	ChIP	[75]
Lysine residues H3K9/14, H3K18, total H4, and H4K16	Deacetylation	PTC	2 cell lines	ChIP	[31]

ATC anaplastic thyroid cancer, *BA* benign adenomas, *ChIP* chromatin immune precipitation, *FA* follicular adenoma, *FTC* follicular thyroid cancer, *HDAC* histone deacetylase, *IHC* immunohistochemistry, *NT* normal tissues, *PTC* papillary thyroid cancer, *UC* undifferentiated carcinoma

suggested that hypermethylation in $p16^{INK4a}$ may be linked to tumor growth but not to tumor development, while alterations in $p14^{ARF}$ may contribute to the induction of chronic inflammation-related PTCs.

Studies of histone modifications associated with PTC are very limited. Recently, Brest et al. [28] reported that two inhibitors of histone deacetylation, trichostatin A and vorinostat, induced overexpression of miR-129-5p, histone acetylation, and apoptosis in cancer cell lines and primary cultures of ATC and PTC. The chromobox 7 (*CBX7*) gene encodes a protein of 28.4 kDa the level of which progressively decreased in proportion to the malignant grade and neoplasias stage [29]. To elucidate the function of *CBX7* in thyroid carcinogenesis, Federico et al. [30] investigated the correlation between *CBX7* protein and histone deacetylase 2 (HDAC2) using a proteomic analysis. They confirmed that *CBX7* interacts with the HDAC2 protein and is able to inhibit its activity. Moreover, they indicated that both these proteins bind to the *ECAD* promoter and that CBX7 upregulates *ECAD* expression; they showed that the expression of *CBX7* increased the acetylation status of the histones H3 and H4 on the *ECAD* promoter [30]. Histone acetylation increased at the *NIS* promoter in human thyroid cancer cells harboring a homozygous *BRAF* V600E mutation and suggesting that *BRAF* V600E normally maintains histone in a deacetylated state at the *NIS* promoter [31] (Table 2).

Many studies have been conducted to identify the regulation of miRs in PTC and their target genes; it was shown that miR-221 and -222 reduced the *P27* expression in the protein levels, miR-146a decreased *PKCε* expression and increased apoptosis, downregulation of miR-181b caused the upregulation of cylindromatosis (*CYLD*) at the protein levels, and miR-195 directly regulated *ZNF367* expression and regulated cellular invasion [32–35] (Table 3). Cahill et al. [36] investigated the effect of *BRAF* V600E mutation on transcription and post-transcriptional regulation in PTC by measuring the expression levels of 160 miRs in a set of cell lines using Stem-looped TaqMan™ reverse transcription PCR (RT-PCR). They indicated that 15 miRs were detected to be upregulated and 23 miRs were downregulated. Of these, miR-200a, -200b, and -141 were upregulated and miR-127, -130a, and -144 were downregulated. Moreover, miR-323 and -302b in the *BRAF*-mutated cell line were downregulated [36]. Song et al. [37] investigated the biological role of miR-96 in PTC cell lines K1 and two pore segment channel 1 (TPC1) and found miR-96 to be upregulated in PTC specimens in comparison to normal tissues by miRs microarray and quantitative RT-PCR analysis. In addition, forkhead box O1 (*FOXO1*), which plays a role in myogenic growth and differentiation, may be a potential target of miR-96. The expression of *FOXO1* had an inverse correlation with expression of miR-96 in PTC specimens, suggesting that miR-96 could promote proliferation and inhibit apoptosis in PTC cell lines and thus may play an oncogenic role in PTC [37]. Dual specificity phosphatase 6 (*DUSP6*) negatively regulates members of the MAPK superfamily (MAPK/ERK, stress-activated protein kinases/c-Jun N-terminal protein kinases (SAPKs/JNKs), p38). In their study, Gu et al. [38] examined the expression of miR-145 in human PTC and its potential function and found that the overexpression of miR-145 inhibited TPC1 cellular growth by targeting *DUSP6* [38]. In another study, with the aim of identifying a subset of differentially expressed miRs between aggressive

Table 3 A concise list of microRNAs expression and their target genes in thyroid carcinoma subtypes

miRs	Location	Description	TC subtype	Sample size	Methods used	Notable target genes in thyroid cells	References
miR-221 and -222	Xp11.3	Oncogenic miR	PTC	2 cell lines	Western blotting and qRT-PCR	*P27*	[32]
miR-146a	5q34	Involved in the feedback system of the classical NF-κB signal pathway		1 cell line	Luciferase reporter assay	*PKCε*	[33]
miR-181b	9q33.3	Played a role in regulating cellular growth, migration, invasion, and apoptosis		10 PTC, adjacent normal tissues, and 3 cell lines	miRNA array, luciferase reporter assays, qRT-PCR, and western blotting	*CYLD*	[34]
miR-197	1p13.3	MiR-197 and its target gene may be the novel molecular markers to differentiate malignant (FTCs) from benign (FAs)	FTC	4 cell lines	miRNA-chip, microarray, and qRT-PCR	*ACVR1*, *TSPAN3*	[55]
miR-346	10q23.2	MiR-346 participates in the transformation of follicular tumors from benign to malignant status				*EFEMP2*, *CFLAR*	
miR-20a	13q31.3	Plays a role as a tumor suppressor in TC cells	ATC	8 ATC, 22 DTC, 24 benign, 11 normal thyroid tissues, and 4 cell lines	qRT-PCR, western blotting, and genome-wide mRNA expression array	*LIMK1*	[80]
miR-618	12q21.31	Downregulation in TC cell lines	ATC	2 cell lines	Western blotting and qRT-PCR	*XIAP*	[81]
			TCs	20 TC, 15 normal, and 1 cell line		*PI3K/Akt pathway*	[83]
miR-10a	17q21.32	Important for tumor development	MTC	15 MTC (10 with and 5 without *RET* mutations)	miRNA array profiling and qRT-PCR	*MDM4*, *NCOR2*	[98]
miR-375	2q35	Regulate genes post-transcriptionally by inhibiting translation and/or causing mRNA degradation				*YAP1*, *SLC16a2*	
miR-455	9q32	Involved in post-transcriptional regulation of gene expression by affecting both the stability and translation of mRNAs					
miR-195	17p13.1	Regulates cellular invasion	TCs	105 human adrenocortical, 47 thyroid tissues (8 normal, 39 PTC), 68 tissues (19 normal adrenal medulla, 28 benign, 21 malignant pheochromocytoma), and 4 cell lines	IHC, western blotting, and qRT-PCR	*ZNF367*	[35]
miR-145	5q32	A master regulator of TC growth	TCs	16 Normal, 21 PTC, 15 ATC/PDTC, and 3 cell lines	Western blotting and qRT-PCR	*AKT3*	[82]

ATC anaplastic thyroid cancer, *DTC* differentiated thyroid cancer, *FA* follicular adenoma, *FTC* follicular thyroid cancer, *IHC* immunohistochemistry, *miRNA* microRNA, *mRNA* messenger RNA, *MTC* medullary thyroid cancer, *NF-κB* nuclear factor-κB, *PDTC* poorly differentiated thyroid cancer, *PTC* papillary thyroid cancer, *qRT-PCR* quantitative reverse transcription, *TC* thyroid cancer

and non-aggressive PTC, Yip et al. [39] used the miRs array to report that miR-146b, -221, -222, -155, and -31 were upregulated and miR-1, -34b, -130b, and -138 were downregulated [39]. Cahill et al. [40], using microarray analysis in ten pairs of PTC specimens, revealed a group of genes differentially expressed between normal thyroid cell lines and those harboring a *RET/PTC1* rearrangement; they showed that 21 miRs overexpressed and 14 underexpressed in these cell lines when compared to the normal thyroid. Microarray analysis of PTC showed that several genes were directly or indirectly regulated by the miR-221 and the use of a bioluminescence imaging system confirmed downregulation of homeobox B5 (*HOXB5*) by endogenous or exogenous miR-221 [41]. Pallante et al. [42], using significance analysis of microarrays (SAM), reported that miR-221, -222, and -146b were overexpressed and led to their target tyrosine-protein kinase kit (*KIT*) gene being downregulated; this gene encodes the human homolog of the proto-oncogene c-kit and is a type 3 transmembrane receptor for mast cell growth factor (MGF) [42]. Similarly, other lines of evidence have also identified the roles of miR-221, -222, and -181b overexpression in thyroid cell transformation and the contribution of miR-146b to tumor aggressiveness in PTC. MiR-221, -222, and -146 have high transcriptional regulation in PTC compared to normal tissues [43]. With the upregulation of these miRs, transcription of the *KIT* gene, which plays a role in the pathogenesis of TC, was decreased. However, there is a lack of data on the PTC pathogenesis, indicating the need for further studies of miR networks in PTC.

3.1.2 Follicular Thyroid Carcinoma

FTC classically accounts for 10–32% of thyroid malignancies. Traditionally, FTC has been classified as minimally invasive (MI-FTC) and widely invasive (WI-FTC) [44]. In the clinical setting, FTC poses a special diagnostic challenge due to the morphological and molecular similarities to the benign FA. Although different molecular profiles have been proposed to improve preoperative diagnosis, the accurate preoperative diagnosis of FTC, especially MI-FTC, continues to be a challenge.

A group of FTC is associated with the *RAS* gene mutation, whereas the *RAS* mutation-negative FTC group often features gene rearrangements of *PPARγ*, most commonly the *PAX8-PPARγ* fusion. *RAS* and *PAX-PPARγ* together explain up to 80% of FTC cases [45]. The tumor-suppressive *RAS* effector, *RASSF1A*, one of the two major isoforms of the putative tumor suppressor gene *RASSF1*, contain a *RAS* association domain, and plays a role in the regulation of cell cycle and apoptosis [46]. Stephen et al. [47] examined the promoter methylation status of the Caspase 8 (*CASP8*), *CDKN2A*, death associated protein kinase 1 (*DAPK1*), estrogen receptor 1 (*ESR1*), *NIS*, *RASSF1*, and *TIMP3* genes in a cohort of FTCs including 26 Hurthle and 27 Classic subtypes using qMSP; they found that *RASSF1* was significantly methylated in classic tumor tissue as compared to Hurthle. Moreover, extra thyroidal extension was found to be associated with *DAPK1* and *ESR1* methylation, suggesting that the methylation status of *RASSF1*, *DAPK1*, and *ESR1* might molecularly distinguish the TC subtypes and enhance classification and early detection of TC [47]. Besides, no-expression of a tumor-suppressor gene, phosphatase and tensin homolog (*PTEN*), a phosphatase that blocks the PI3K/AKT signaling pathway, has also been implicated in the development of FTC. The detection of *PTEN* promoter hypermethylation in about 50% of PTCs and almost 100% of FTCs suggests that it might be involved in the thyroid tumorigenesis [48]. Moreover, some studies showed that hypermethylation of the *TRβ* gene was evaluated in 34, 81, 25, and 30% of PTCs, FTCs, follicular thyroid adenomas (FTAs), and thyroid tumor cell lines, respectively. The expression level of the *THRB* gene was repressed by hypermethylation of the *THRB* promoter in TCs. Furthermore, *TSHR* gene promoter was methylated in 59 and 47% of PTC and FTC, respectively [49–51] (Table 1).

In various tumor types, the levels of acetylation at several histone residues are associated with clinical aggressiveness [52, 53]. However, information on histone modification in FTC is quite limited.

Several reports have addressed dysregulated miRs expression in FTC and it has been suggested that miRs analysis might help distinguish FTC from FTA. Colamaio et al. [54] recently assessed the expression of miR-142-3p in a large panel of FTA and FTC and evaluated its effect on thyroid cell proliferation and target expression by qRT-PCR; they observed that miR-142-3p is downregulated in FTAs, FTCs, and FVPTC. Moreover, this miR was able to downregulate the expression of absent, small, or homeotic-like (*ASH1L*) and mixed-lineage leukemia 1 (*MLL1*) genes by direct and indirect mechanisms, respectively. It has been demonstrated that downregulation of miR-142-3p has a role in thyroid tumorigenesis by regulating *ASH1L* and *MLL1* [54]. In 2006, Weber et al. [55] investigated expression of miRs and their target genes. Four miRs (miR-192, -197, -328, and -346) were overexpressed in FTCs compared to FAs (Table 3). Nikiforova et al. [56] compared miRs expression profiles in 60 surgically removed thyroid neoplastic and non-neoplastic samples and in 62 fine-needle aspiration (FNA) samples by RT-PCR using the TaqMan™ MicroRNA Panel. They found a distinctive expression pattern associated with FTC: miR-155, -187, -221, -222, and -224 resulted in being highly overexpressed in conventional FTCs, while miR-183, -187, -197, -221, -222, and -339 were overexpressed in the FTC oncocytic

variants [56]. Wojtas et al. [57] conducted a miR-microarray study to identify miRs differentially expressed between FTCs and their surrounding tissues, and revealed that miR-146b, -183, and -221 were upregulated, whereas miR-199b was downregulated in FTCs. They also studied the influence of these miRs on cell proliferation, cell cycle, apoptosis, and migration in some FTC cell lines, and functional characterization suggested an impact of miR-183 and -146b in FTC development. Furthermore, overexpression of miR-183 significantly repressed apoptosis. MiR-199b and -221 had no significant effects on proliferation, cell cycle, apoptosis, or migration in cell lines. It seemed that miR-146b and -183 might influence FTC development through the induction inhibition of migration and apoptosis [57]. Jikuzono et al. [58] conducted another study on 34 patients with MI-FTC through an extensive analysis of miR expression, and results indicated that miR-221/222, -10b, and -92a profiles in MI-FTC significantly increased metastasis. Despite much progress over recent years, there is still a need for clarification of the molecular and biological relationship of the different benign thyroid neoplasias to each other and to TCs, in particular FTC.

3.1.3 Poorly Differentiated Thyroid Carcinoma

The prevalence of PDTC is about 0.5–7% of all TCs and the mean age of diagnosis is around 60.6 years [59]. PDTC shows intermediate aggressive clinical features between WDTC and ATC [60, 61]. With the advent of genetic/epigenetic analysis, epigenome data on PDTCs are lacking. Further, some dysregulated gene expressions associated with epigenetic modifications are grouped based on their implicated roles in the overall dedifferentiation process in PDTCs [62, 63]. Currently, treatment for PDTC is still challenging and surgery is a current therapeutic choice.

Ogasawara et al. [64] investigated the methylation status of the promoter region of *SERPINB5* (serine [or cysteine] proteinase inhibitor, clade B [Ovalbumin], member 5), a mammary serine protease inhibitor (*Maspin*), in six human ATC cell lines, 17 PDTCs, and 13 ATCs [64]. They found promoter hypomethylation in 41% of PDTCs and 62% of ATCs. Moreover, some investigations indicated the *RASSF1* and $p16^{INK4A}$ methylation status in PDTC [18, 19], which is presented in Table 1.

Studies on histone methyltransferases (HMTs) in PDTCs are very limited; however, recent transcriptome analysis showed that expression of HMTs (lysine methyltransferase 2A (KMT2A), KMT2C, KMT2D, and SET domain containing 2 (SETD2)) was increased more in ATC than in PDTC [65].

Although numerous dysregulated miRs and their target genes have been identified in TCs, very few were found in PDTCs. To date, some miRs such as miR-23, -26a, -125b, -130b, -139-5p, -150, -193a-5p, -219-5p, -451, -455-3p, -886-3p, let7c have been found to be downregulated and others such as miR-15a-3p, -125a-5p, -129, -146b, -182, -183, -187, -221, -222, -339 upregulated in PDTC [56, 66–70].

3.1.4 Anaplastic Thyroid Carcinoma

Compared to other TCs, ATC is the least common but the most aggressive cancer with a median survival rate of 3–5 months. It is thought to develop from existing PTC and FTC, has an extremely high proliferative rate, and can quickly invade neck structures and metastasize to other organs; more importantly, it is resistant to radioiodine treatment [71].

The gene expression patterns of ATC are more complex and not very distinct. There have been a multitude of genetic alterations associated with ATC, very often causing dysfunction in the ERK1/2-MEK1/2 and PI3K-AKT signaling pathways [72]. Keller et al. [73] investigated whether the methylation status of the *galectin-3* gene is a candidate molecular marker for thyroid malignancy; to do this they examined the methylation state of a large genomic region encompassing the *galectin-3* transcriptional start site in 50 patients, including five normal thyroid, three goiter, 39 PTC, and three ATC cases utilizing high-resolution melt (HRM) analyses. Results showed that five CpGs were differentially methylated among the samples, suggesting that the average methylation state of the five CpGs clearly distinguished cancer from the non-neoplastic thyroid tissues [73]. Hou et al. [74] examined *PTEN* methylation and its relationship with genetic alterations in the PI3K/AKT pathway in various types of thyroid tumors using q MSP. They found that *PTEN* methylation was progressively higher from benign thyroid adenoma to FTC and to aggressive ATC, which harbored activating genetic alterations in the PI3K/AKT pathway corresponding to a progressively higher prevalence. The association of *PTEN* methylation was seen with phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) alterations and *RAS* mutations in the PI3K/AKT pathway, within each of the three types of thyroid tumors; in contrast, they found no such a relationship for the tumor suppressor gene *RASSF1A* [74]. Genome-wide promoter methylation status indicated that hypomethylated genes (280 in ATC and 393 in MTC) were more than aberrantly hypermethylated genes (86 in ATC and 131 in MTC) [17]. It seems that thyroid transcription factor-1 (*TTF-1*) is a homeodomain containing transcriptional factor identified in thyroid, and DNA methylation of this gene was shown in 60% of the undifferentiated carcinomas (UCs) and 50% of the cell lines [75] (Table 1).

Studies on the associations between histone modifications and ATC are very limited; however, Borbone et al. [76] showed that *EZH2* directly contributed to transcriptional silencing of the *PAX8* gene and differentiation in ATC tissues. Moreover, in another study, they found that HDACs 1 and 2 overexpressed in ATCs compared with normal cells or benign tumors [77]. Smith et al. [78] found that histone methylation was increased to alter the expression of $p16^{INK4A}$, *DAPK*, ubiquitin C-terminal hydrolase L1 (*UCHL1*), *O*-6-methylguanine-DNA methyltransferase (*MGMT*), *TSHR*, *PTEN*, and melanoma antigen gene (MAGE) family member A4 (*MAGE-A4*) genes in ATC cell lines [78]. Kondo et al. [75] demonstrated that acetylation of H3-lys9 was positively correlated with *TTF-1* expression in TC cells. A study on the protein levels of HDACs 1, 2, and 3 showed that HDACs 1 and 2 upregulated in ATCs compared to benign tumors and HDAC inhibitors induced apoptosis [75]. Moreover, another study indicated that an acetylated level of H3K9–K14ac was significantly higher in FA, PTC, FTC, and UC than in normal tissues and the level of H4K12 acetylation was higher in thyroid adenoma than in TCs [79]. Also, oncoproteins *RET/PTC*, *RAS*, and *BRAF* increased levels of H3K9–K14 and H3K18 acetylation [75] (Table 2).

In addition, miRs also can alter the gene expression of ATC cells by targeting expression of essential genes. Investigations have indicated that miR-20a significantly inhibited TC cell proliferation and invasion and targeted the *LIMK1* gene. MiR-618 inhibited ATC by suppressing the *XIAP* gene in ATC cells. Moreover, overexpression of miR-145 inhibited the PI3K/AKT pathway and directly targeted *AKT3* [80–83] (Table 3). Recently, Fuziwara et al. [84] showed that a new group of miRs, such as miR-146b, -221, and -222, were upregulated in ATC and also in PTC and FTC, indicating that the overexpression of these miRs was essential in maintaining tumorigenesis. On the other hand, they mentioned that some specific miRs, such as those of the miR-200 and -30 families, were downregulated in ATC; these are important negative regulators of cell migration, invasion, and epithelial-to-mesenchymal transition (*EMT*), processes that are over-activated in ATC [84]. Esposito et al. [85] reported that miR-30d is significantly downregulated in human ATC, and this is believed to be an important event in thyroid cell transformation. Additionally, Zhang et al. [86] demonstrated that miR-30d has a critical role in modulating the sensitivity of ATC cells to cisplatin; they also showed that miR-30d could negatively regulate the expression of beclin 1 (*BECN1*) and lead to suppression of the cisplatin-activated autophagic response that protects ATC cells from apoptosis, suggesting that miR-30d might be exploited as a potential target for therapeutic intervention in the treatment of ATC [86].

3.1.5 Medullary Thyroid Carcinoma

MTC, which arising from parafollicular C cells, encompasses about 3–10% of all TCs, and occurs in both familial and sporadic forms. Approximately 20% of patients develop distant metastases, which makes MTC an incurable disease, responsible for up to 13.4% of all TC mortality [87]. The RAS–RAF–MEK–MAPK–ERK and PI3K–AKT–mTOR (mammalian target of rapamycin) pathways are the most important signaling pathways in MTC and play a crucial role in vital cell processes such as cell survival, growth, proliferation, and differentiation [88, 89]. Through these pathways the product of the *RET* oncogene plays an essential role in cell survival, proliferation, and apoptosis. Thus, mutations in *RET* are considered to be an important factors in the diagnosis and treatment of MTC. Mutations of the *RET* gene can be inherited and are observed in virtually all patients who develop MTC; in addition, somatic mutations have been detected in sporadic cases of the disease [90–95].

Schagdarsurengin et al. [96] evaluated the DNA methylation pattern of 17 tumor-related genes in TC cell lines, primary tumors (PTC, FTC, UTC, and MTC), FA, and goiters using MSP and validated by expression analysis. Their results showed that three of these genes, *RASSF1A* (83%), *TSHR* (33%), and *ERβ* (20%), were methylated in MTC [96].

Although histone modifications have been described in various malignancies, there are not enough data on the pattern of expression of epigenetic regulators in MTCs and their relationship with the genotype and phenotype of the tumors. Sponziello et al. [97] revealed the expression profile of more aggressive disease with increased levels of Enhancer of zeste 2 polycomb repressive complex 2 subunit (*EZH2*) and SET and myeloid, Nervy, and deformed epidermal autoregulatory factor-1 (MYND) domain containing 3 (*SMYD3*) gene expression, which did not correlate with the mutational status of *RET* or *RAS* genes. Thus, messenger RNA (mRNA) expression of HMT *EZH2* and *SMYD3* may demonstrate useful prognostic biomarkers, modifying the most appropriate follow-up and timing of therapeutic approaches in MTC [97].

Recently published data provide important information on miRs regulation in MTC and on their target genes; *NCOR2* and *MDM4* were validated as direct targets of miR-10a/b and *YAP1* and *SLC16a2* were downregulated by miR-375 in MTC compared with non-tumor thyroid tissues [98] (Table 3). Pallante et al. [67] summarized the main alterations in miRs expression that have been identified in thyroid neoplasias, and reported that miR-323, -370, -129, -137, -10a, -124a, -224, -127, -9, -154, -183, -375, and -21 were upregulated and, conversely, miR-9 was downregulated in MTC [67]. Mian et al. [99] investigated

Table 4 Clinical trial investigations and epigenetic drugs for the treatment of advanced thyroid carcinoma in patients aged ≥ 18 years (http://www.clinicaltrials.gov)

Drugs	Description	Testing phase	Title
Romidepsin	Potent histone deacetylase inhibitor	I	Depsipeptide to treat thyroid and other advanced cancers
Belinostat (PXD101)	Hydroxamate-type inhibitor of histone deacetylase		Study of PXD101 alone and in combination with 5-fluorouracil (5-FU) in patients with advanced solid tumors
			Study of oral PXD101 in patients with advanced solid tumors or lymphoma
Vorinostat (SAHA)	Inhibits histone deacetylase 1 and 3	II	Suberoylanilide hydroxamic acid in treating patients with metastatic and/or locally advanced or locally recurrent thyroid cancer
Romidepsin	Potent histone deacetylase inhibitor	II	A bicyclic depsipeptide and a potent histone deacetylase inhibitor from chromobacterium violaceum
Panobinostat (LBH589)	Hydroxamic acid analog histone deacetylase inhibitor from Novartis	II	Trial of LBH589 in metastatic thyroid cancer
Valproic acid	Histone deacetylase inhibitor	II	A Phase II trial of valproic acid in patients with advanced thyroid cancers of follicular cell origin
		II	Valproic acid (Depakote ER) in patients with advanced thyroid cancer
Decitabine	DNA methyltransferase inhibitor	II	Decitabine in treating patients with metastatic papillary thyroid cancer or follicular thyroid cancer unresponsive to iodine I 131

dysregulation of miRs in heredity and sporadic MTC patients to determine the relationship between miR profiles and outcomes in MTC; they found significant overexpression of several miRs, including miR-127, -154, -224, -323, -370, -183, -375, and -9, in MTC samples. Furthermore, recently, Duan et al. [100] suggested an important role of non-coding RNAs in related to the incidence of MTC. They showed that miR-129-5p reduced cell growth, induced apoptosis, and inhibited cell migration ability in MTC, by reducing the phosphorylation of AKT. Downregulation of these molecules has been observed in MTC [100].

3.2 Utility of Epigenetics in Thyroid Cancer

Most therapies target specific genes, proteins, or the tissues that contribute to cancer growth and survival, and act selectively on cancer cells with a particular genetic dysfunction. These potential treatments are under assessment for clinical application in aggressive TC [101]. In addition, researchers are looking for new combinations of chemotherapy and other treatments.

Epigenetic therapy is being studied in order to stimulate genes irregularly silenced in cancer by epigenetic mechanisms and to reverse epigenetic alterations, and as such it may soon emerge for clinical use in patients with advanced TCs. Epigenetic drugs mainly target two mechanisms of epigenetic modifications, acetylation and DNA methylation, which control transformed cells' differentiation and proliferation. The role of HDAC inhibitors has been assessed in numerous hematologic malignancies and solid tumors involving TC in clinical trials sponsored by the National Cancer Institute (NCI) [102] (Table 4). Some of these agents exert their effect via activation of proapoptopic signaling, while others, such as radioactive iodine (I-131), are being used in combination with conventional chemotherapy drugs [103]. Studies have shown that deacetylation inhibitors and demethylating agents are suitable in the treatment of TCs; some of these drugs have been examined in metastatic radioiodine-refractory advanced TC and the lists of these drugs have been reported [104, 105]. Numerous clinical trials using drugs with an epigenetic mode of action, including romidepsin, belinostat, vorinostat, panobinostat, valproic acid, and decitabine, have been completed (http://www.clinicaltrials.gov) (Table 4).

Romidepsin (depsipeptide) is currently used to treat cutaneous T cell lymphoma (CTCL) in subjects who have been treated with at least one other medication. It is a cyclic peptide that inhibits HDAC activity. There are currently two clinical studies ongoing at the NCI: the first is a phase I trial of romidepsin in patients with the thyroid neoplasms and other advanced cancers, testing safety and tolerability; the second is a phase II trial in radioiodine-refractory metastatic non-medullary TCs (stage IV FTC and PTC, recurrent TC, and Hurthle cell variants) to determine the anti-tumor activity of the drug.

Belinostat is used to treat peripheral T cell lymphoma that has not improved or has returned after treatment with other medications. There are currently two phase I clinical trials underway at the NCI, one is looking at advanced solid tumors and the other one solid tumors and lymphoma.

Vorinostat (suberoylanilide hydroxamic (SAHA)) is a hydroxamic acid that binds directly to the HDAC catalytic site and inhibits deacetylase enzymatic activity. A phase II study was completed in patients with insular TC, recurrence TC, stage II/IV FTC and PTC, and MTC. As a result of no complete or partial responses in these patients, vorinostat was not approved for treatment of advanced TCs [106].

Panobinostat (LBH589), which is used to treat multiple myeloma, is a hydroxamic acid with potent inhibitory activity. In vitro treatment of three ATC cell lines (BHT-101, CAL-62, and 8305C) with panobinostat resulted in impairment of cell viability, inhibition of colony formation, cell cycle arrest, and apoptosis induction [107]. A phase II trial of panobinostat was completed in patients with metastatic MTC and radioactive iodine-resistant DTC. There are currently no reported results for this trial.

Valproic acid is a short-chain fatty acid with anticonvulsant properties that is used in the treatment of epilepsy. The mechanism of its therapeutic activity is not well understood, although it may act by increasing the γ-aminobutyric acid level in the brain or by altering the properties of voltage-dependent sodium channels [108]. However, deacetylase inhibitors (DCI) activity, anti-proliferation, and pro-differentiation in various solid tumors has been reported [109, 110]. Two trials using valproic acid have been completed. A phase II study in patients with advanced TCs of follicular origin were treated with liothyronine sodium agent, however, according to the response evaluation criteria in solid tumors (RECIST), no partial or complete response was shown and valproic acid had an effect on radioiodine uptake in these patients [111]. Another phase II in patients was conducted in thyroglobulin positive/radioiodine unresponsive patients with advanced TCs of follicular origin; currently, no data are available on the results of this trial.

Decitabine, or 5-aza-2′-deoxycytidine, is a cytidine antimetabolite analog with potential antineoplastic activity. Decitabine acts as a nucleic acid synthesis inhibitor and inhibits DNA methyltransferase, causing hypomethylation of DNA and intra-S-phase arrest of DNA replication [112]. A phase II trial of decitabine has been completed in patients with metastatic PTC or FTC (stages IVA, IVB, and IVC) who are unresponsive to radioiodine, but no results of this trial are available to date.

Research on epigenetic modifications in TCs must continue, but hopefully once the final results of these trials become available, we may have more effective treatments for these tumors. On the other hand, using miRs for treatment of cancers, as both a target and a tool, could be a new interesting and promising therapy; however, although several preclinical studies underline this possibility, no clinical trial has yet been undertaken.

4 Discussion

During the two last decades, the incidence of TC and its subtypes has increased, which is attributed to the genetic and environmental changes that create epigenetic modifications. Based on the evidence presented in this review considerable epigenetic changes in TC subtypes have been identified and more are being studied. Of these, most of have focused on follicular cell-derived carcinomas of the thyroid gland and WDTCs because of their prevalence and the availability of samples. Based on the studies' results, epigenetic mechanisms, especially aberrant DNA methylation and regulation of miRs, are suggested to play an important role in thyroid tumorigenesis [113, 114]. It seems that a general analysis of epigenetic alterations in TC subtypes is critical to understanding the relationship between these changes and gene regulation, and how this will contribute to, and inform, diagnosis, prognosis, and also the development of new therapeutic strategies.

The molecular basis for disease progression from WDTC to PDTC and/or ATC is debatable. WDTCs commonly demonstrate disturbed action of the MAPK and PI3K-AKT signaling pathways, or a compounding of the two [3]. These alterations occur via direct genomic/epigenomic mutations or indirectly via epigenetic deregulation. The overlap of dysregulated signaling pathways among these objects may support a concatenation of PDTC and ATC from WDTC [115]. Similarly, between WDTCs and PDTC/ATC there is the observation that the dysfunctions identified in the ERK1/2-MEK1/2 and PI3K-AKT signaling pathways are due to common genetic alterations, such as *BRAF* V600E [72, 116]. Downregulation of the MAPK signal-inhibiting genes may be regulated by DNA methylation, which was associated with a higher *BRAF* mutation rate in PTC [36, 117]. *BRAF* V600E can promote PTC tumorigenesis by altering the methylation, and hence the expression, of numerous important genes [118].

Generally, cell organization can be affected at three levels by epigenetic mechanisms: (1) direct regulation of the gene function (switching it on or off) or modulation of synthesis of specific proteins; (2) regulation of the topographic distribution and functions of proteins; and (3) regulation of cell differentiation by alteration of RNA translation into proteins [119].

DNA methylation may result in downregulation of tumor suppressor genes and overexpression of oncogenes. In the literature, most cases in which DNA methylation was investigated were considered using analysis in the candidate genes promoter region [7, 16, 64]. Genome-wide characterization of DNA methylation patterns of WDTCs shows that tumors with distinct subtypes and mutational status have unique methylation profiles, offering insight

into the biology underlying the heterogeneity and differential outcomes of the TCs [21, 26, 61, 118]. The prevalence of DNA methylation is different in TC subtypes; tumor suppressor genes silencing via this mechanism are most common in WDTCs while the frequencies of oncogene overexpression and activation are significantly higher in MTC, ATC, and PDTCs (Table 1). As ATC is the most aggressive subtype of TCs, it seems that the overexpression of oncogenes through their promoter methylation in the thyroid gland has the most adverse effects on thyroid tumorigenesis and this alteration may direct thyrocytes toward undifferentiated subtypes [120]. In addition, it is suggested that TC subtypes present differential promoter methylation signatures, and undifferentiated subtypes are characterized by aberrant promoter hypomethylation rather than hypermethylation [17]. Overall, systematic analysis of global methylation of tumors is likely to identify signaling pathways that lead to the development of cancer. DNA methylation is involved in WDTC development and has led to the identification of novel markers such as etoposide induced 2.4 (*EI24*) and Wilms Tumor 1 (*WT1*) which are associated with recurrence-free survival [21].

Most of the studies reviewed here have focused on histone modifications performed on different TC cell lines (Table 2). According to these findings, histone modifications occur in all subtypes of TC. However, due to the low number of studies and their small sample size, the data obtained on histone modifications cannot be generalized to the TC patients and more studies are required to confirm the relationship between these alterations and different TC subtypes. Nevertheless, data showed that treatment with HDAC inhibitors impaired Rap2 activity in both differentiated and anaplastic tumor cell lines [16]. HDAC inhibitors may provide a tractable approach to impair Rap activity in human tumor cells [121]. Neoplastic transformation and hormonal stimulation can modify levels of histone acetylation in thyroid cells.

Gene regulation by miRs is another important epigenetic modification in TC subtypes that has recently drawn researchers' attention. Study results suggest that the level of miR-222, -221, and -146a were significantly higher in PTC and non-toxic multinodular goiter (NMG) samples, and these molecules have been introduced as recurrence biomarkers in PTC [32, 33, 43]. In the FTC subtype, significant overexpression of miR-197 and -346 in FTC cell lines shows that a combination of FNA results with miR panels will result in an improvement in the discrimination between FTC and FA [55, 56]. Moreover, in PTC, miR-145, a master regulator of TC growth that mediates its effect via the PI3K/AKT pathway, may serve as an adjunct biomarker for PTC diagnosis [38, 82]. Analysis of distinct sets of miRs represents a useful tool to distinguish PDTC from pure PTC [69]. Additionally, the lack of deregulation of some miRs may select a subset of PTC prone to progression to PDTC. Downregulation of miR-20a and -618 shows that these molecules may play an important role as tumor-suppressor miRs in ATC [81, 83]. In addition, miR-10a, -375, and -455 are the most important small-interfering RNAs for tumor development and/or the reflect C cell lineage of MTC (Table 3). Therefore, dysregulation of miRs in MTC is probably an early event in C cell carcinogenesis [67, 98].

Treatment options have been proposed and implemented on the basis of the results obtained from research conducted on epigenetic alterations. Therefore, development of new therapeutic strategies based on targeting epigenetic changes, including inhibition of DNA methyltransferase and inhibition of HDAC, to restore the expression of tumor suppressor miRs or to blunt overexpressed oncogenic miRs may provide a new landscape for treatment of different types of cancers and particularly TCs. With the advent of advanced diagnostic techniques now, endocrine tumors are identified much more frequently than in the past. Preoperative diagnosis of thyroid nodules with molecular testing on FNA is now commonly considered to determine whether these nodules are benign or malignant without the need for diagnostic surgery. Moreover, analysis of epigenetic alterations in TC subtypes helps to identify pathogenesis and can play an important role in the classification and diagnosis of tumors [114]. Further studies are required to elucidate the effect of the histone modification mechanism as a potential issue relating to therapeutic aspects for TCs and also to verify the microarray analysis results (in the regulation of miRs). Given that epigenetic changes can be prevented by controlling the environmental conditions and that these changes are reversible, further studies are recommended in this field. Finding the most imperative environmental factors will be a major step towards the prevention and treatment of TCs.

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Compliance with Ethical Standards

Conflict of interest The authors (MZ, AZV, FA, FF, MMA, and MH) declare no conflicts of interest.

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