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Epigenetic Alterations in well-Differentiated Thyroid Cancer

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Abstract

Genetic and epigenetic alterations have been associated with cancer development and progression. Important epigenetic modifications include posttranslational histone changes, covalent modifications of DNA bases and non-coding RNAs. Well-differentiated thyroid carcinomas comprise papillary and follicular carcinomas, the most common subtypes. Methylation of thyroid-specific genes, including NIS and TSHR, genes involved in PI3K and MAPK pathways, as well as the tumor suppressor genes RASSF1A and PTEN and altered expression of microRNAs have been frequently reported in literature. Novel technologies using genome-wide approaches have contributed in revealing new epigenetically regulated genes involved in the molecular biology of thyroid cancer. The comprehensive analysis of genetic mutations, changes in DNA methylation, microRNA expression and posttranslational histone changes in thyroid carcinoma is critical to understand the crosstalk between these alterations and gene regulation that contributes to the development of diagnostic and prognostic tools, as well as new therapeutic strategies. The focus of this review is to present and discuss the current knowledge of molecular epigenetic data and their impact on thyroid carcinoma.

Keywords: DNA methylation; MicroRNA expression; Histone PTMs; Thyroid carcinoma; Genome-wide methylation

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Introduction

Thyroid cancer (TC) is the most common endocrine gland malignancy, its incidence having increased over the last few decades [1]. Well-differentiated carcinomas are derived from follicular cells and represent the majority of thyroid carcinomas [2], of which, papillary (PTC) and follicular (FTC) thyroid carcinomas are the most common histological subtypes representing 80-85% and 10-15% of cases, respectively [3].

Genetic and epigenetic alterations have been described as associated with thyroid carcinogenesis. Among the genetic alterations, mutations in effectors of the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K-AKT) pathways have been frequently described. Rearrangements involving the *RET* gene (such as *RET/PTC*), *RAS* and *BRAF* mutations have been reported in more than 70% of PTC cases [3, 4]. The most common genetic alteration is the *BRAFV600E* mutation (40-45% of PTC), which leads to a constitutive activation of *BRAF* and subsequent activation of downstream signaling transduction pathway.

In addition to genetic features, epigenetic events have been investigated in an attempt to understand other mechanisms involved in well-differentiated thyroid carcinoma. Changes in the DNA methylation pattern in genes involved in DNA damage repair, cell cycle control, tumor suppression and normal thyroid function have also been described [5-7].

In this review, the most relevant epigenetic alterations in well-differentiated thyroid cancer have been described, focusing the discussion on DNA methylation and microRNA, particularly for papillary thyroid carcinoma.

Epigenetic alterations as a mechanism of gene regulation

Epigenetics is defined as the study of inherited alterations in gene regulation that do not involve changes in the DNA sequence [8,

9]. The term "epigenetic" was first described by Waddington [10] as "the causal mechanisms between genes and their products, bring about phenotypic effects". Cytosine DNA methylation along with chromatin modification [11] and regulation by noncoding RNAs [12] are considered effector mechanisms of epigenetic control of gene expression.

The most studied epigenetic mechanism is DNA methylation, which involves the addition of a methyl group to the 5' position of cytosine, predominantly in the context of CpG dinucleotides [13]. These CpGs are non-randomly distributed across the genome, including in gene promoter, gene body and intergenic region, as well as in repetitive sequences (satellite DNA and retrotransposons) [14, 15]. CpG dinucleotide rich regions are known as CpG islands, which are present in approximately 60% of human genes [13, 16, 17].

Hypermethylation of CpG islands in promoter regions is a well-recognized epigenetic event in tumorigenesis [18]. Abnormal DNA methylation gains frequently occur in specific genes, which can be associated with loss of gene expression (reviewed by 19). Global loss of DNA methylation is associated with gene activation, loss of genomic imprinting and activation of DNA repetitive elements, resulting in enhanced chromosomal instability and consequently contributing to tumorigenesis [20, 21].

Alterations in microRNA expression patterns also play an important role in cancer development [22, 23]. MicroRNAs (miRNAs) are small non-coding RNAs that control the expression of their target genes, causing either degradation or inhibition of translation [24, 25]. In tumor cells, microRNAs may function as oncogenes or tumor suppressor genes [26]. As a result, modulation of many cellular pathways and biological process, such as those involved in embryogenesis, development timing, proliferation, apoptosis, angiogenesis, cell differentiation and other cancer-associated processes, may occur [27, 28]. Similarly to classical genes, microRNAs can be subjected to epigenetic alterations including DNA methylation [29].

Additional disruptions of epigenetic mechanisms, such as histone modification patterns have also been reported in thyroid cancer. Histones are proteins involved in the packaging of eukaryotic DNA, comprised of chromatin. In addition to chromatin being an essential structural component, it is critical for gene regulation, DNA replication and repair, and chromosome segregation [30, 31]. Post-translational modification (PTM) of histone tails includes acetylation, methylation, phosphorylation, ribosylation, sumoylation and ubiquitination [32]. Depending on the position and type of modification that occurs in the histone, there is either activation or suppression of gene transcription. [33].

Epigenetic Alterations in Thyroid Cancer

DNA methylation

Gene promoter hypermethylation and inativation of tumor supressor genes are important mechanisms in thyroid cancer [34-37]. Hu et al. [38] reported that aberrant methylation gains in promoter regions of the *TIMP3, DAPK*, *SLC5A8* and *RARß2* genes are associated with the *BRAFV600E* mutation and aggressiveness in papillary thyroid carcinoma. In PTC, similar results were reported for the *RARß2* and *TIMP3* genes, as well as an inverse correlation between *RASSF1A* methylation and the presence of the *BRAF* mutation [39].

RASSF1A promoter methylation is one of the most common epigenetic events in human cancers, leading to gene silencing [40]. *RASSF1A* promotor methylation is frequently described in follicular thyroid lesions including hyperplasia, adenoma and FTC [41, 42]. Moreover, a high frequency of the *RASSF1A* methylation has been reported in both benign thyroid lesions (BTL; 93%) and PTC (76%) [43]. *RASSF1A* hypermethylation has been suggested as an initial event associated with the development of TC [41, 44].

Mutation or down-expression of *PTEN*, a tumor suppressor gene, is frequently reported as involved in thyroid tumorigenesis. The encoded protein acts as an antagonist of the PI3K pathway [45]. Aberrant methylation of the *PTEN* promoter has been reported principally in follicular adenoma and carcinoma (83% and 84%, respectively) when compared to PTC (47%) [46]. The *PTEN* hypermethylation has been associated with genetic alterations of the PI3K/AKT pathway, suggesting that gene inactivation caused by methylation may stimulate signaling pathways already activated by mutations and thus contributing to the progression of thyroid cancer [47].

Changes in the methylation patterns of other genes involved in the initiation or transduction of signaling in the PI3K/Akt and MAPK pathways have also been described as associated with TC **(Figure 1).** The tumor suppressor gene *RASAL1* (*RAS protein activator like-1*) encodes a protein that acts as a negative modulator of the PI3K and MAPK pathways. Reduced expression of this gene mediated through CpG methylation has been described for several tumors with aberrant increased activity of the RAS signaling pathway [48].

In thyroid cancer, 13 known negative modulators of the RAS pathway (*NF1*, *SPRY1*, *SPRY2*, *SPRED1*, *SPRED2*, *RKIP*, *DUSP5*, *DUSP6*, *TSC1*, *TSC2*, *LKB1, RASAL1, DAB2IP)* were screened using a panel of 12 human-derived thyroid carcinoma cell lines [49]. The only downexpressed genes identified in the tumor cell lines were *DAB2IP* and *RASAL1*. Of note, *RASAL1* expression was absent in tumor cells, which was in stark contrast to normal tissue and normal thyroid cell lines. The *RASAL1* gene was predominantly hypermethylated in FTC and ATC compared with BTL and PTC. Thus, based on these findings, *RASAL1* alterations were suggested as affecting the regulation of the PI3K pathway over the MAPK pathway, acting as a major tumor suppressor gene in thyroid carcinoma [49].

Lin et al. [50] proposed that *MIG6* acts as a tumor suppressor gene in PTC, negatively regulating the MAPK pathway. Promoter methylation was associated with *MIG6* down-expression and high levels of EGFR and ERK phosphorylation. In a functional analysis, the increased expression of *MIG6* inhibited proliferation and Mig-6 knockdown promoting the invasion of PTC cells [50]. More recently, Lee et al. [51] investigated the DNA methylation pattern of MAPK signal-inhibiting genes. The promoter regions of

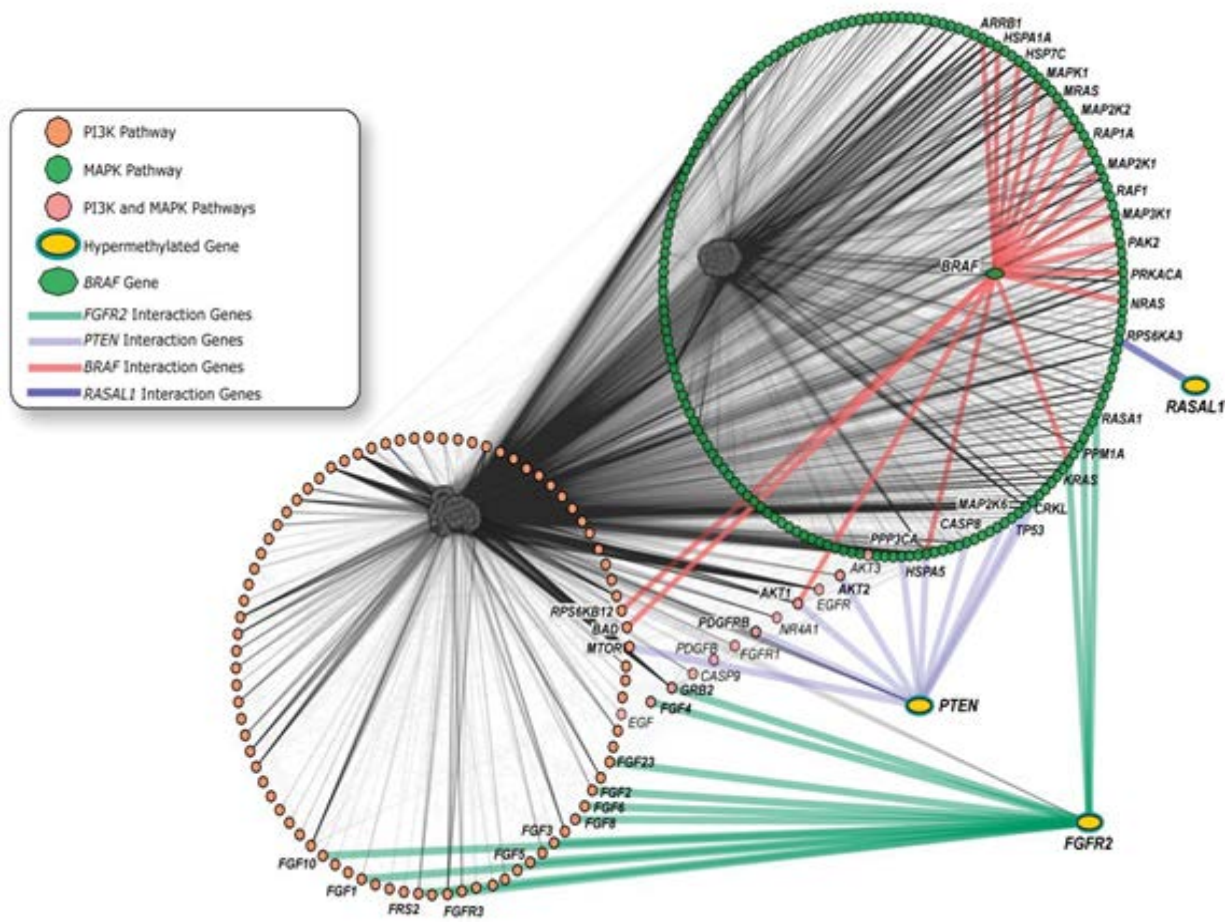

Figure 1 PI3K and MAPK protein network analysis (NAViGaTOR version 2.3). The interaction of proteins codified by PTEN, RASAL1 FGFR2 hypermethylated genes (in yellow) with several proteins of the PI3K and MAPK pathways in thyroid cancer. BRAF protein presents high level of protein interactions. Lines correspond to protein- protein interactions.

DUSP4 and *DUSP6* and the genes expressed in the cell lines TPC1, WRO82-1 and XTC were identified as unmethylated. In contrast, the TPC1 cell line and tumor tissues (63 of 76 cases) revealed the methylation of *SERPINA5*, in addition to an association with the *BRAF* mutation.

By using cDNA transfection in the WRO thyroid cell line, Kondo et al. [52] demonstrated that restoration of *FGFR2-IIIb* isoform expression was able to interfere in BRAF phosphorylation and decrease MAPK activation. Treatment with 5'-aza-deoxycytidine restored *FGFR2-IIIb* expression in WRO and TPC-1 thyroid cell lines, suggesting that methylation is a mechanism that affects *FGFR2* regulation, consequently being involved in MAPK control [52].

The interaction between the PI3K and MAPK pathways has been well established, with epigenetic control of key factors of these pathways adding an additional layer of complexity. Overall, these data indicate that aberrant methylation of critical tumor suppressor genes may play a pivotal role in thyroid carcinogenesis.

In addition to the tumor suppressor genes, mismatch repair genes have also been described as hypermethylated in thyroid carcinoma. Guan et al. [53] analyzed the methylation pattern of 23 DNA repair genes in PTC samples. The authors observed methylation gains in *MLH1*, *PCNA* and *OGG1* (21%, 13% and 5%, respectively). No difference was observed for *MLH1* and *MGMT* methylation patterns in PTC, FTC and benign follicular thyroid adenomas [54]. However, samples with mutations in **BRAF**, **IDH1** and **NRAS showed decreased MLH1 expression.**

The normal function of thyroid cells requires a complex regulatory mechanism [55] for example, the *TSHR*, *SLC26A4* and *NIS* genes involved in the uptake of iodide and thyroid hormone synthesis present aberrant methylation in their promoter regions. *TSHR* gene hypermethylation has been reported in 59% of PTC and 47% of FTC, while in normal and BTL samples it was unmethylated [56]. The *SLC26A4* gene encodes the pendrin protein, an anion exchanger responsible for the efflux of iodide in thyrocytes [57]. Progressive hypermethylation of this gene has been described in a variety of lesions from adenomas to FTC, while a similar methylation pattern was seen in PTC and ATC [58].

Although Stephen et al. [59] evaluated a limited number of cases; they observed methylation of the sodium/iodide symporter *(NIS)* gene in PTC (11 cases), FTC (2 cases) and BTL (3 cases) when compared with normal tissue (5 cases). *NIS* gene promoter methylation was also reported in cold thyroid nodules, associated to mRNA down-expression in 50% of hypermethylated samples [60].

Khan et al [61] described an association between *TSHR*

promoter hypermethylation and the *BRAFV600E* mutation. *TSHR* hypermethylation was detected in 73.3% of *BRAF*-mutated tumors (12 PTC, 2 FTC and 1 with an unusual histology). The presence of *BRAF* mutation was also related to *NIS* down-expression, mediated by *DNMT1*. In 30 PTC samples, Choi et al. [62] found significant *NIS* mRNA and protein down-expression in cases harboring the *BRAFV600E* mutation, leading the authors to suggest that high *DNMT1* expression was associated with epigenetic silencing of *NIS*.

The findings described herein reveal that several genes with altered methylation patterns have been found in well-differentiated thyroid carcinoma. However, these DNA methylation studies are mostly restricted to candidate gene approaches. Only recently have large-scale studies addressing the gene methylation profile in thyroid cancer, most of which included PTC samples, been reported. To our knowledge, epigenetic profiles have been reported in six studies involving thyroid carcinomas **(Table 1)**. In 2011, Hou *et al*. [63] assessed the global epigenetic alterations associated with the *BRAFV600E* mutation in two PTC cell lines (BCPAP and OCUT1) using shRNA knockdown. This strategy allowed the authors to describe 10 hypo- and 59 hypermethylated genes, respectively, in both cell lines following *BRAF* knockdown, revealing *BRAFV600E* as the driving force behind these genes becoming hyper or hypomethylated.

Global DNA methylation analysis has been applied to identify molecular signatures in different subtypes of thyroid cancer. Rodríguez-Rodero et al. [64], using the 27K platform (Illumina) on a small number of samples (2 PTC, 2 FTC, 2 ATC, 2 medullary thyroid carcinomas -MTC, 2 normal samples and 4 cell lines), identified an epigenetic signature for PTC and FTC (262 and 352 hypermethylated genes and 13 and 21 hypomethylated genes, respectively). Comparing these findings with the alterations found in MTC and ATC revealed 155 and 210 hypermethylated genes and 13 and 21 hypomethylated genes exclusive to PTC and FTC, respectively. The authors identified several potential tumor suppressor genes (*ADAMTS8* and *HOXB4* in PTC and *ZIC1* and *KISS1R* in FTC) and oncogenes (*INSL4* and *DPPA2* for MTC and *TCL1B* and *NOTCH4* in ATC) confirmed as altered by methylation in thyroid carcinoma.

A similar platform (27K platform, Illumina) was used by Kikuchi et al. [65] and Mancikova et al [66] to describe the differential methylation profiles of TC. In the first study, 14 PTC samples were investigated according to *BRAF* and *RAS* mutations. Mutated cases were more frequently methylated (nine of 11 samples), with six hypermethylated genes (*HIST1H3J*, *POU4F2*, *SHOX2*,

Table 1 Genome-wide methylation analysis in thyroid carcinomas.

Reference	Samples	Hypomethylated Genes or Probes	Hypermethylated Genes or Probes	Platform	Validation	Integrative analysis* (samples)
[63]	2 PTC cell lines *BRAF* V600E knockdown	10 genes	59 genes	12K Human CpG-island Array chip (Microarray Center, Toronto, ON, Canada)	QMSP in the same cell lines	No
[64]	2 PTCs 2 FTCs 2 ATCs 2 MTCs 2 NT 4 cell lines	PTCs - 13 probes FTCs - 21 ATCs - 280 MTCs - 328 PTC and FTC cell line – 25 probes MTC and ATC cell line – 598 probes	PTCs – 262 probes FTCs – 352 ATCs – 86 MTCs -131 PTC and FTC cell line – 334 probes MTC and ATC cell line – 198 probes	Infinium HumanMethylation27k (Illumina)	Pyrosequencing independent samples	GEO data
[65]	14 PTCs 10NT	PTCs - 0 gene	PTCs - 25 genes in 3 or more samples	Infinium HumanMethylation27k (Illumina)	Pyrosequencing independent samples	No
[66]	42 PTCs 5 fvPTC 18 FTCs 18 FA	PTCs – 51 genes FTCs – 77 FA – 9	PTCs – 31 genes FTCs – 416 FA – 83	Infinium HumanMethylation27k (Illumina)	Bisulfite sequencing in test and validation set	Yes (31)
[67]	29 PTCs 7 rPTCs 15 fvPTC 8 NT	Classic PTC – 2582 probes rPTCs – 2796 fvCPT – 405	Classic PTC – 255 probes rPTCs – 1023 fvCPT – 164	Infinium HumanMethylation450k (Illumina)	-	No
[68]	324 PTCs 99 fvPTCs 35 tcvPTC 9 uPTCs 22 PTCs no annotated	4 groups: 2 groups enriched with *H/K/NRAS* mutations including most of fvPTCs cases 2 groups enriched for classical and tcvPTCs and *BRAF* mutations		Infinium HumanMethylation450k (Illumina)	-	Yes (482)

PTC – Papillary thyroid carcinoma; FTC – Follicular thyroid carcinoma; AF – Follicular adenoma; CMT – Medullary thyroid carcinoma; fvPTC – follicular-variant of papillary thyroid carcinoma; rPTCs – Recurrent papillary thyroid carcinoma; uPTC - Uncommon papillary thyroid carcinoma variants; tcvPTC – Tall cell variant of papillary thyroid carcinoma; NT – Normal tissue; QMSP - Quantitative methylation-specific PCR.* Integrative analysis with mRNA expression data.

PHKG2, *TLX3* e *HOXA7*) being validated by pyrosequencing and presenting down-expression (except *SHOX2*) in at least one PTC cell line (TPC1, KTC1, and KTC3). Following treatment with 5-aza-2´deoxycytidine and/or trichostatin A, expression of these genes was restored in the cell lines [65]. In the second study, 83 tumors (47 PTC, 18 FTC and 18 adenomas) were investigated for methylation, with the results being integrated with transcript expression data from 31 samples [66]. The authors reported methylation profiles according to histological subtype and with the *BRAF* and *RAS* mutation.

Ellis et al., using a more robust platform (450K platform, Illumina) on 51 cases of PTC, reported a global hypomethylation compared to normal tissue (91% of differentially methylated sites had a methylation loss) [67]. The follicular variant showed a distinct methylation profile in comparison with the classical variant. Differences between the methylation profiles according to *BRAF*, *RAS* and *RET*/*PTC* mutation (3.6 fold more differentially methylated compared with wild-type tumors) were also described.

Recently, a comprehensive analysis of the genome in more than 400 PTC samples from the TCGA project [68] revealed the involvement of several alterations associated with the development and progression of the disease. Analysis of genomic variants, transcript expression and DNA methylation revealed potential molecular drivers. Unsupervised clustering analysis with methylation data revealed four clusters of PTC classified as follicular, methylated CpG Islands, Classical 1 and Classical 2. In addition, two meta-clusters were identified according to the *BRAFV600E* and *RAS* mutations. The *RAS* mutation samples were detected in two different clusters, one with a lower number of methylation changes and the other with a large number of CpG hypermethylated in CpG islands and CpG shore regions, termed Meth-follicular and Meth-CpG Island cluster, respectively. The clusters named Classical 1 and 2 were enriched for classical and tall cell PTCs and *BRAF* mutations showing low levels of methylation regions outside the CpG Islands that would normally be methylated.

These results revealed changes in the methylation pattern of genes involved in DNA damage repair, and associated them with the normal function of thyrocytes. These recent approaches, as demonstrated by Ellis et al. [67], show that global hypomethylation is an important mechanism associated with PTC development. Moreover, these studies revealed the involvement of *CpG island shores* (regions with a low density of CpGs mapped at 2kb *up* or *downstream* of CpGs islands) and *shelves* (2 to 4kb *up* or *downstream* of *CpG island shores*) that have been reported as associated with gene expression regulation [69, 70].

Although epigenetic alterations based on genome-wide methods have been described in well-differentiated thyroid carcinoma, a better understanding of the molecular alterations is necessary to guide the development of meaningful markers for early diagnosis, prognosis and prediction of response to therapies.

MicroRNA expression in thyroid cancer

In well-differentiated thyroid carcinomas, mainly PTC, numerous miRNAs have been described as having altered expression **(Table 2).** The miRNA signature based on expression levels has been used to differentiate distinct variants in PTC and FTC cases. In PTC, up-regulation of miR-21, 146b, 155, 181a, 181b, 221 and 222, among others, have been described [71-75]. In particular, the expression levels of miR-21, 146b, 181b, 221 and 222 were able to distinguish PTC from follicular adenoma and multinodular goiter [76]. Differences in miRNA expression levels were described in the classical variant (miR-146b) and tall cell variant (miR-146b, -21 and -222) when compared with the follicular variant of PTC [76].

Alterations in microRNA expression were also associated with the mutational status of PTC. Up-regulation of miR-221 and miR-222 was demonstrated in samples with the *BRAF* and *RAS* mutations, while overexpression of miR-146b was observed in cases carrying the *RAS* mutation [75]. The miR-221 expression observed in cases with the *BRAF* mutation was also associated with extra thyroidal invasion, lymph node metastasis and advanced stage of disease [77]. Recently, using deep-sequencing, Mancikova et al. [78] found down-expression of miR-7 and -204 in PTC harboring the *BRAF*V600E mutation.

To understand the role of microRNAs in thyroid carcinogenesis, many studies have focused on functional assays to identify the targets of these molecules. In PTC cell lines, miR-221 was associated with control of cell proliferation [72]. In addition, miR-221 and -222 potentially regulate the cell cycle targeting the *CDKN1B* transcript, which encodes for the $p27^{kip1}$ protein [79]. The authors showed that these miRNAs targeted the 3'UTR of *CDKN1B* resulting in reduced protein levels, regulating thyroid cell proliferation.

miR-181b, also up-regulated in PTC, directly targets the *CYLD* gene, which is associated with apoptosis [80]. High expression levels of miR-21 were associated with cell proliferation and invasion, revealing a negative correlation with *PDCD4* gene expression [81]. Using *in silico* analysis, Jazdzewski et al. [82] showed that the 3'UTR of the *THRβ* gene contains binding sites for several microRNAs with aberrant expression in PTC. The authors used the CAKI-2 cell line (derived from renal carcinoma) and demonstrated a direct interaction between *THRβ* and miR-21 and -146a. Down-expression of this gene was detected following transfection assays with miR-21, -146a and -221.

In FTC, Weber et al. [83] reported up-regulation of miR-192, miR-197, miR-328 and miR-346 when compared with follicular adenoma. Overexpression of miR-197 and -346 was associated with cell proliferation. In addition, down-expression of two predicted targets of each miR (miR-197: *ACVR1* and *TSPAN3*; miR-346: *EFEMP2* and *CFLAR*) was found. Up-regulation of miR-187, -224, -155, -222, and -221 were observed in conventional follicular thyroid carcinoma (cFTC), whereas in oncocytic follicular thyroid carcinoma (oFTC) up-regulation of miR-187, -221, -339, -183, -222, and -197 was found when compared with hyperplastic nodules. The authors also analyzed a panel of miRs in a small number of fine-needle aspiration biopsies (FNAB), observing high accuracy in detecting cancer samples [75]. A higher expression of miR-96, -182, -183, -221, -222, -449a and -874 and lower expression of miR-542, -574, -455, and -199a were found in oFTC and cFTC when compared with normal tissue. miR-885 was revealed as a potential novel marker for oFTC [84].

Table 2 MicroRNAs aberrantly expressed in well-differentiated thyroid carcinoma.

Reference	Samples	miRNA		Association
		Over expression	Down expression	
[72]	30 PTC 10 NT	miR-221, miR-222, miR-213, miR-220, miR-181b	let-7f-1, miR-142-as, miR-140-as, miR-199a-1, miR-151	PTC compared with normal tissue
[73]	10 PTC 10 MNG	miR-221, miR-222, miR-21, miR-31, miR-172, miR-34a, miR-213, miR-181b, miR-223, miR-224, miR-181a	miR-218, miR-300, miR-292-as, miR-345, miR-30c	PTC compared with MNG
[74]	10 PTC 10 FA	miR-146b, miR-222, miR-221	-	PTC compared with FA miR-146b consistently overexpressed in PTC compared with non-PTC tumors (32 PTC, 24 FA, 11 HD, 2 FTC, 5NT)
[71]	15 PTC 15 NT	miR-146, miR-221, miR-222, miR-21, miR-155, and miR-181a	mir-24-1, mir-9-3, mir-219-1, mir-138-1, mir-138-2, mir-345, mir-26a-1	PTC compared with normal tissue
[84]	44 PTC 8 NT	let-7i-3p, miR-146-3p, miR-146-5p, miR-15a-3p, miR-181a-2-3p, miR-21, miR-221, miR-222, miR-222-5p, miR-34a, miR-34a-3p, miR-375, miR-542-5p, miR-551b, miR-99b-3p	miR-1179, miR-138, miR-144-5p, miR-199b-5p, miR-204, miR-219-5p, miR-451	fvPTC (17) compared to normal tissue
[93]	23 FTC 20 FA 4 NT	miR-192, miR-197, miR-328, miR-346	-	FTC compared with FA
[94]	24 FA 24 FTC 15 PTC 8 ATC 6 fvPTC	miR-191	miR-191	Down-expressed in FA, FTC and fvPTC compared with NT, Up-expressed in PTC
[95]	10 FTC 10 FA 10 NT	-	let-7a	FA and FTC compared with NT
[96]	12 PTC 4 NT	**miR-146b, miR-221, miR-222, miR-155**, miR-31	miR-153, miR-448, miR-325, miR-382, miR-495, **miR-34b, miR-1**, miR-144, miR-367, **miR-130b**, **miR-138**	PTC compared with NT **In bold,** miR differentially expressed in aggressive PTC compared with non-aggressive PTC
[97]	2 FA 4 OTT 2 PTC 8 NT	miR-125a-5p, miR-143, miR-23b, miR-425, miR-623, and miR-654 –3p	miR-1225–3p, miR-1238, miR-135a, miR-150, miR-494, miR-663, miR-801, miR-923	Comparison between tumor and normal tissues
[75]	54 BTL 7 PTCs 1 FTC	miR-187, miR-221, miR-222, miR-146b, miR-155, miR-224, miR-197	-	Tumors vs. hyperplastic nodules in FNA samples
[98]	12 PTC 12 NT	miR-21*, miR- 203	-	PTC *BRAF*V600E compared with PTC *BRAF* WT

PTC – Papillary thyroid carcinoma; FTC – Follicular thyroid carcinoma; ATC - Anaplastic thyroid carcinoma; AF – Follicular adenoma; fvPTC – follicular-variant of papillary thyroid carcinoma; NT – Normal tissue; oTT – Oncocytic thyroid tumors, either benign or malignant; HD - Hyperplastic nodules; MNG - Proliferative multinodular goiter; BTL – Benign thyroid lesions.

Despite altered microRNA expression being well established in cancer, the molecular mechanisms underlying the regulation of these miRNAs are not completely understood. In general, miRNAs are mapped in regions involved in amplification and deletion as well as in breakpoints associated with other structural alterations, such as translocations [85, 86]. Moreover, similarly to genes that encode for proteins, microRNAs are transcribed by genes that are targets for epigenetic events, which also act by modulating gene expression.

In 2011, Kunej et al. [87] reported that more than one hundred miRNAs were regulated by epigenetic mechanisms. The first epigenetically regulated microRNA described was miRNA-127 in bladder carcinoma cells [88]. Following this discovery, several studies demonstrated alterations in the methylation pattern of microRNAs in gastric carcinoma [89], lung adenocarcinoma [90], gliobastoma [91] and colon cancer [92]. Thyroid carcinoma is a relatively unexplored field, with only one study published by the TCGA group thus far, which revealed that microRNA genes were controlled by epigenetic mechanisms [68]. miR-21, miR-146b, and miR-204 were aberrantly methylated in PTC and associated with altered expression of these microRNAs.

Taken together, the majority of studies have revealed miRNAs that are able to distinguish PTC and FTC from normal tissues, some of which present the potential to refine or strengthen strategies for diagnosis and management of TCs. However, the combined knowledge of the silenced miRNA genes associated

with CpG island hypermethylation and those with high expression regulated by DNA hypomethylation are still an unexplored field in thyroid carcinoma.

Histone modification in thyroid cancer

Among the aberrant post-translational modifications in histones described in thyroid cancer, higher acetylation levels of lysine 9-14 (H3K9-14ac) and 18 (H3K18ac) of the H3 histone have been associated with early events in thyroid cancer progression. Puppin et al. [99] reported that levels of H3K18ac in non-differentiated TC were lower compared to FA, PTC and FTC, suggesting a switch off of this marker in the transition to more aggressive subtypes. In addition, high levels of H4K12ac were only found in FA.

The hypothesis of histone hypoacetylation of the *NIS* gene promoting its regulation and down-expression in papillary BHP 2-7 cells was reported by Kogai et al. [100]. The authors treated the cell line with histone deacetylase (HDAC) inhibitor, trichostatin A (TSA) where no restoration of NIS expression or increased iodide accumulation by BHP cells was observed. On the other hand, Puppin et al. [101] demonstrated that histone acetylation controls the *NIS* promoter. The treatment of two papillary (BCPAP and TPC1) and two anaplastic (ARO and FRO) cell lines with TSA and Na butyrate (NaB) induced the expression of the *NIS* mRNA without modifying the expression of the thyroid-specific transcription factors *TTF-1*, *TTF-2*, *PAX8*, and *HEX*.

The potential of the aberrant histone PTM for treatment has been investigated. Lin et al. [102] evaluated a deacetylase inhibitor PXD101 as a potential treatment for thyroid cancer. Using cell lines derived from papillary, follicular, anaplastic and medullary carcinomas, the authors reported that PXD101 was efficient in inhibiting cell proliferation in all cell lines (particularly ATC cell lines), and suppressing HDACs in PTC (BHP7-13), FTC (WRO82-1) and ATC (8505C) cells depending on the dose used. The drug also induced apoptosis and double-strand DNA damage, as well as ROS accumulation (except in PTC cells), while inhibiting the RAS/RAF/ERK and PI3K/mTOR pathways (in ATC cell).

A phase I study with suberoylanilide hydroxamic acid (SAHA) was conducted in patients with advanced thyroid cancer. Kelly et al. [103] reported that HDAC inhibition promotes a partial response in patients with refractory PTC. Woyach et al. [104] conducted a phase II study with 16 patients with differentiated TC and 3 with medullary thyroid carcinoma receiving oral Vorinostat for 2 weeks followed by 1 week without therapy. Unfortunately, the drug was not effective for patients to achieve either partial or complete response at the dose tested.

Thus, future investigations on histone acetylation and other histone PTMs may be important for defining innovative therapeutic strategies for the treatment of thyroid cancer.

Methylation and clinical implications

Despite the significant number of epigenetic alterations described for thyroid cancer, reports of useful molecular prognostic markers for clinical practice are rare.

A strong association of DNA methylation in the *TIMP3, DAPK*, *SLC5A8* and *RARß2* genes and classical and PTC tall-cell variants, in addition to tumor aggressiveness was demonstrated by Hu et al. [38]. Guan et al. [53] reported a *MLH1* methylation associated with lymph node metastasis and *BRAFV600E* mutation in PTC [53]. *Rap1GAP* promoter hypermethylation and/or loss of heterozygosity were described in FA, PTC, FTC and ATC samples and its loss of expression was correlated with invasive and aggressive forms of thyroid cancer [105].

Several genes regulated by methylation have been described as associated with tumor recurrence. A recent study demonstrated that hypermethylation at specific sites of *RUNX3* was capable of predicting recurrence in patients with PTC [106]. The methylation levels at CpG sites -1397, -1406, -1415 and -1417 were able to predict prognosis with high specificity (85.1 to 95.7%), with negative predictive values of 86.1 to 88.9%, yet with low sensitivity (30.0 to 50.0%) [106]. The recurrence was associated with unmethylated promoter regions of the *TSHR* gene in a study of 32 patients with PTC [107]. Recently, Mancikova et al. [66] reported that methylation levels of *EI24* and *WT1* were associated with risk of recurrence in patients with PTC and FTC. A high percentage of recurrence-free survival was observed in patients with unmethylated genes [66].

Pathological grade, TNM stage and lymph node metastasis have been used to compare the methylation pattern. A significant association was described with methylation of the *FHIT* promoter regions in 42 PTCs and 23 FTCs [108]. The authors also observed a difference in methylation pattern according to gender (higher in female) and histological classification (higher in FTC).

Conclusion

As highlighted in this review, alterations in DNA methylation, microRNA expression and aberrant histone PTMs play a role in the development of thyroid carcinoma. Most cases of DNA methylation reported in the literature were designed using analysis of strategies in the promoter region of candidate genes. There is still a very limited number of data obtained from global analysis, and even less so for miRNA methylation and histone PTMs. Therefore, a complete understanding of these alterations in TC remains to be clarified. Recent reports addressing the gene methylation profiles in appropriate sample numbers have contributed in revealing important drivers for aberrant methylation in these tumors. Two lines of evidence point out the relevance of microRNAs in TC. Firstly, recurrently altered expression in miRNAs that regulate several TC-associated transcripts and, secondly, the recent discovery of miRNA methylation. From this perspective, future studies are required to better explain the mechanisms involved in the process of thyroid carcinogenesis. Future exploratory research should focus on characterization of aberrant DNA methylation, microRNA and histone PTMs, and how this knowledge could contribute to the clinical management of well-differentiated thyroid carcinomas, in addition to identifying new targets for therapy.

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Disclosure Summary

The authors have nothing to disclose.

References

1 Siegel RL, Miller KD, Jemal A (2015) Cancer statistics. CA Cancer J Clin 65: 5-29.

2 Elisei R, Pinchera A (2012) Advances in the follow-up of differentiated or medullary thyroid cancer. Nat Rev Endocrinol 8: 466-475.

3 Xing M (2013) Molecular pathogenesis and mechanisms of thyroid cancer. Nat Rev Cancer 13: 184-199.

4 Nikiforov YE, Nikiforova MN (2011) Molecular genetics and diagnosis of thyroid cancer. Nat Rev Endocrinol 7: 569-580.

5 Czarnecka K, Pastuszak-Lewandoska D, Migdalska-Sek M, Nawrot E, Brzezinski J, Dedecjus M, et al. (2011) Aberrant methylation as a main mechanism of TSGs silencing in PTC. Front Biosci (Elite Ed) 3:137-157.

6 Schagdarsurengin U, Richter AM, Hornung J, Lange C, Steinmann K, Dammann RH (2010) Frequent epigenetic inactivation of RASSF2 in thyroid cancer and functional consequences. Mol Cancer 9: 264.

7 Galrao AL, Sodre AK, Camargo RY, Friguglietti CU, Kulcsar MA, Lima EU, et al (2013) Methylation levels of sodium-iodide symporter (NIS) promoter in benign and malignant thyroid tumors with reduced NIS expression. Endocrine 43: 225-229.

8 Schübeler D, Elgin SC (2005) Defining epigenetic states through chromatin and RNA. Nat Genet 37: 917-918.

9 Sawan C, Vaissière T, Murr R, Herceg Z (2008) Epigenetic drivers and genetic passengers on the road to cancer. Mutat Res 642: 1-13.

10 Waddington CH (2012) The epigenotype. 1942. Int J Epidemiol 41: 10-13.

11 Sharma S, Kelly TK, Jones PA (2010) Epigenetics in cancer. Carcinogenesis 31: 27-36.

12 Lopez J, Percharde M, Coley HM, Webb A, Crook T (2009) The context and potential of epigenetics in oncology. Br J Cancer 100: 571-577.

13 Jones PA (2012) Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nat Rev Genet 13: 484-492.

14 Park YJ, Claus R, Weichenhan D, Plass C (2011) Genome-wide epigenetic modifications in cancer. Prog Drug Res 67: 25-49.

15 Stirzaker C, Taberlay PC, Statham AL, Clark SJ (2014) Mining cancer methylomes: prospects and challenges. Trends Genet 30: 75-84.

16 Bernstein BE, Meissner A, Lander ES (2007) The mammalian epigenome. Cell 128: 669-681.

17 Laird PW (2003) The power and the promise of DNA methylation markers. Nat Rev Cancer 3: 253-266.

18 You JS, Jones PA (2012) Cancer genetics and epigenetics: two sides of the same coin? Cancer Cell 22: 9-20.

19 Baylin SB, Jones PA (2011) A decade of exploring the cancer epigenome - biological and translational implications. Nat Rev Cancer 11: 726-734.

20 Berdasco M, Esteller M (2010) Aberrant epigenetic landscape in cancer: how cellular identity goes awry. Dev Cell 19: 698-711.

21 Esteller M (2008) Epigenetics in cancer. N Engl J Med 358: 1148-1159.

22 Feinberg AP (2007) Phenotypic plasticity and the epigenetics of human disease. Nature 447: 433-440.

23 Taby R, Issa JP (2010) Cancer epigenetics. CA Cancer J Clin 60: 376-392.

24 Krol J, Loedige I, Filipowicz W (2010) The widespread regulation of microRNA biogenesis, function and decay. Nat Rev Genet 11: 597-610.

25 Choi JD, Lee JS (2013) Interplay between Epigenetics and Genetics in Cancer. Genomics Inform 1: 164-173.

26 Kasinski AL, Slack FJ (2011) Epigenetics and genetics MicroRNAs en route to the clinic: progress in validating and targeting microRNAs for cancer therapy. Nat Rev Cancer 11: 849-864.

27 Chhabra R, Saini N (2014) MicroRNAs in cancer stem cells: current status and future directions. Tumour Biol 35: 8395-8405.

28 Hayes J, Peruzzi PP, Lawler S (2014) MicroRNAs in cancer: biomarkers, functions and therapy. Trends Mol Med 20: 460-469.

29 Davis-Dusenbery BN, Hata A (2010) Mechanisms of control of microRNA biogenesis. J Biochem 148: 381-392.

30 Mellor J (2006) Dynamic nucleosomes and gene transcription. Trends Genet 22: 320-329.

31 Rothbart SB, Strahl BD (2014) Interpreting the language of histone and DNA modifications. Biochim Biophys Acta 1839: 627-643.

32 Kouzarides T (2007) Chromatin modifications and their function. Cell 128: 693-705.

33 Li B, Carey M, Workman JL (2007) The role of chromatin during transcription. Cell 128: 707-719.

34 Huang Y, de la Chapelle A, Pellegata NS (2003) Hypermethylation, but not LOH, is associated with the low expression of MT1G and CRABP1 in papillary thyroid carcinoma. Int J Cancer 104: 735-744.

35 Lal G, Padmanabha L, Smith BJ, Nicholson RM, Howe JR, O'Dorisio MS, et al (2006) RIZ1 is epigenetically inactivated by promoter hypermethylation in thyroid carcinoma. Cancer 107: 2752-2759.

36 Yin DT, Wu W, Li M, Wang QE, Li H, Wang Y, et al (2013) DKK3 is a potential tumor suppressor gene in papillary thyroid carcinoma. Endocr Relat Cancer 20: 507-514.

37 Qiang W, Zhao Y, Yang Q, Liu W, Guan H, Lv S, et al (2014) ZIC1 is a putative tumor suppressor in thyroid cancer by modulating major signaling pathways and transcription factor FOXO3a. J Clin Endocrinol Metab 99: E1163-1172.

38 Hu S, Liu D, Tufano RP, Carson KA, Rosenbaum E, Cohen Y, et al (2006) Association of aberrant methylation of tumor suppressor genes with tumor aggressiveness and BRAF mutation in papillary thyroid cancer. Int J Cancer 119: 2322-2329.

39 Brait M, Loyo M, Rosenbaum E, Ostrow KL, Markova A, Papagerakis S, et al. (2012) Correlation between BRAF mutation and promoter methylation of TIMP3, RARβ2 and RASSF1A in thyroid cancer. Epigenetics 7: 710-719.

40 Pfeifer GP, Dammann R (2005) Methylation of the tumor suppressor gene RASSF1A in human tumors. Biochemistry (Mosc) 70:576-83.

41 Xing M, Cohen Y, Mambo E, Tallini G, Udelsman R, Ladenson PW, et al (2004) Early occurrence of RASSF1A hypermethylation and its mutual exclusion with BRAF mutation in thyroid tumorigenesis. Cancer Res 64: 1664-1668.

42 Brown TC, Juhlin CC, Healy JM, Prasad ML, Korah R, Carling T (2014) Frequent silencing of RASSF1A via promoter methylation in follicular thyroid hyperplasia: a potential early epigenetic susceptibility event in thyroid carcinogenesis. JAMA Surg 149: 1146-1152.

43 Mohammadi-asl J, Larijani B, Khorgami Z, Tavangar SM, Haghpanah V, Kheirollahi M, et al. (2011) Qualitative and quantitative promoter

hypermethylation patterns of the P16, TSHR, RASSF1A and RARβ2 genes in papillary thyroid carcinoma. Med Oncol 28: 1123-1128.

44 Nakamura N, Carney JA, Jin L, Kajita S, Pallares J, Zhang H, et al (2005) RASSF1A and NORE1A methylation and BRAFV600E mutations in thyroid tumors. Lab Invest 85: 1065-1075

45 Brzezianska E, Pastuszak-Lewandoska D (2011) A minireview: the role of MAPK/ERK and PI3K/Akt pathways in thyroid follicular cell-derived neoplasm. Front Biosci (Landmark Ed) 16: 422-39.

46 Alvarez-Nuñez F, Bussaglia E, Mauricio D, Ybarra J, Vilar M, Lerma E, et al (2006) PTEN promoter methylation in sporadic thyroid carcinomas. Thyroid 16: 17-23.

47 Hou P, Ji M, Xing M (2008) Association of PTEN gene methylation with genetic alterations in the phosphatidylinositol 3-kinase/AKT signaling pathway in thyroid tumors. Cancer 113: 2440-2447.

48 Jin H, Wang X, Ying J, Wong AH, Cui Y, Srivastava G, et al. (2007) Epigenetic silencing of a Ca(2+)-regulated RasGTPase-activating protein RASAL defines a new mechanism of Ras activation in human cancers. Proc Natl Acad Sci USA 104: 12353-12358.

49 Liu D, Yang C, Bojdani E, Murugan AK, Xing M (2013) Identification of RASAL1 as a major tumor suppressor gene in thyroid cancer. J Natl Cancer Inst 105: 1617-1627.

50 Lin CI, Du J, Shen WT, Whang EE, Donner DB, Griff N, et al. (2011) Mitogen-inducible gene-6 is a multifunctional adaptor protein with tumor suppressor-like activity in papillary thyroid cancer. J Clin Endocrinol Metab 96: E554-565.

51 Lee EK, Chung KW, Yang SK, Park MJ, Min HS, Kim SW, et al. (2013) DNA methylation of MAPK signal-inhibiting genes in papillary thyroid carcinoma. Anticancer Res 33: 4833-4839.

52 Kondo T, Zheng L, Liu W, Kurebayashi J, Asa SL, Ezzat S (2007) Epigenetically controlled fibroblast growth factor receptor 2 signaling imposes on the RAS/BRAF/mitogen-activated protein kinase pathway to modulate thyroid cancer progression. Cancer Res 67: 5461-5470.

53 Guan H, Ji M, Hou P, Liu Z, Wang C, Shan Z, et al. (2008) Hypermethylation of the DNA mismatch repair gene hMLH1 and its association with lymph node metastasis and T1799A BRAF mutation in patients with papillary thyroid cancer. Cancer 113: 247-255

54 Santos JC, Bastos AU, Cerutti JM, Ribeiro ML (2013) Correlation of MLH1 and MGMT expression and promoter methylation with genomic instability in patients with thyroid carcinoma. BMC Cancer 13: 79.

55 Kondo T, Asa SL, Ezzat S (2008) Epigenetic dysregulation in thyroid neoplasia. Endocrinol Metab Clin North Am 37: 389-400.

56 Xing M, Usadel H, Cohen Y, Tokumaru Y, Guo Z, Westra WB, et al. (2003) Methylation of the thyroid-stimulating hormone receptor gene in epithelial thyroid tumors: a marker of malignancy and a cause of gene silencing. Cancer Res 63: 2316-2321.

57 Royaux IE, Suzuki K, Mori A, Katoh R, Everett LA, Kohn LD, et al. (2000) Pendrin, the protein encoded by the Pendred syndrome gene (PDS), is an apical porter of iodide in the thyroid and is regulated by thyroglobulin in FRTL-5 cells. Endocrinology 141: 839-845.

58 Xing M, Tokumaru Y, Wu G, Westra WB, Ladenson PW, Sidransky D (2003) Hypermethylation of the Pendred syndrome gene SLC26A4 is an early event in thyroid tumorigenesis. Cancer Res 63: 2312-2315.

59 Stephen JK, Chitale D, Narra V, Chen KM, Sawhney R, Worsham MJ (2011) DNA methylation in thyroid tumorigenesis. Cancers (Basel) 3: 1732-1743.

60 Neumann S, Schuchardt K, Reske A, Reske A, Emmrich P, Paschke R (2004) Lack of correlation for sodium iodide symporter mRNA and protein expression and analysis of sodium iodide symporter promoter methylation in benign cold thyroid nodules. Thyroid 14: 99-111.

61 Khan MS, Pandith AA, Masoodi SR, Wani KA, Ul Hussain M, Mudassar S (2014) Epigenetic silencing of TSHR gene in thyroid cancer patients in relation to their BRAF V600E mutation status. Endocrine 47: 449-55.

62 Choi YW, Kim HJ, Kim YH, Park SH, Chwae YJ, Lee J, et al. (2014) B-RafV600E inhibits sodium iodide symporter expression via regulation of DNA methyltransferase 1. Exp Mol Med 46: e120.

63 Hou P, Liu D, Xing M (2011) Genome-wide alterations in gene methylation by the BRAF V600E mutation in papillary thyroid cancer cells. Endocr Relat Cancer 18: 687-697.

64 Rodríguez-Rodero S, Fernández AF, Fernández-Morera JL, Castro-Santos P, Bayon GF, Ferrero C, et al. (2013) DNA methylation signatures identify biologically distinct thyroid cancer subtypes. J Clin Endocrinol Metab 98: 2811-2821.

65 Kikuchi Y, Tsuji E, Yagi K, Matsusaka K, Tsuji S, Kurebayashi J, et al. (2013) Aberrantly methylated genes in human papillary thyroid cancer and their association with BRAF/RAS mutation. Front Genet 4: 271.

66 Mancikova V, Buj R, Castelblanco E, Inglada-Pérez L, Diez A, de Cubas AA, et al (2014) DNA methylation profiling of well-differentiated thyroid cancer uncovers markers of recurrence free survival. Int J Cancer 135: 598-610.

67 Ellis RJ, Wang Y, Stevenson HS, Boufraqech M, Patel D, Nilubol N, et al. (2014) Genome-wide methylation patterns in papillary thyroid cancer are distinct based on histological subtype and tumor genotype. J Clin Endocrinol Metab 99: E329-E337.

68 Cancer Genome Atlas Research Network. Integrated genomic characterization of papillary thyroid carcinoma. Cell 159: 676-690.

69 Irizarry RA, Ladd-Acosta C, Wen B, Wu Z, Montano C, Onyango P, et al. (2009) The human colon cancer methylome shows similar hypo- and hypermethylation at conserved tissue-specific CpG island shores. Nat Genet 41: 178-186.

70 Bibikova M, Barnes B, Tsan C, Ho V, Klotzle B, Le JM, et al. (2011) High density DNA methylation array with single CpG site resolution. Genomics 98: 288-295.

71 He H, Jazdzewski K, Li W, Liyanarachchi S, Nagy R, Volinia S, et al. (2005) The role of microRNA genes in papillary thyroid carcinoma. Proc Natl Acad Sci USA 102: 19075-19080.

72 Pallante P, Visone R, Ferracin M, Ferraro A, Berlingieri MT, Troncone G, et al. (2006) MicroRNA deregulation in human thyoid papillary carcinomas. Endocr Relat Cancer 13: 497-508.

73 Tetzlaff MT, Liu A, Xu X, Master SR, Baldwin DA, Tobias JW, et al. (2007) Differential expression of miRNAs in papillary thyroid carcinoma compared to multinodular goiter using formalin fixed paraffin embedded tissues.. Endocr Pathol 18: 163-173.

74 Chen YT, Kitabayashi N, Zhou XK, Fahey TJ 3rd, Scognamiglio T (2008) MicroRNA analysis as a potential diagnostic tool for papillary thyroid carcinoma. Mod Pathol 21: 1139-1146

75 Nikiforova MN, Tseng GC, Steward D, Diorio D, Nikiforov YE (2008) MicroRNA expression profiling of thyroid tumors: biological significance and diagnostic utility. J Clin Endocrinol Metab 93: 1600-1608.

76 Sheu SY, Grabellus F, Schwertheim S, Worm K, Broecker-Preuss M, Schmid KW (2010) Differential miRNA expression profiles in variants of papillary thyroid carcinoma and encapsulated follicular thyroid tumours. Br J Cancer 102: 376-382.

77 Zhou YL, Liu C, Dai XX, Zhang XH, Wang OC (2009) Overexpression of miR-221 is associated with aggressive clinicopathologic characteristics and the BRAF mutation in papillary thyroid carcinomas. Med Oncol 29: 3360-3366.

78 Mancikova V, Castelblanco E, Pineiro-Yanez E, Perales-Paton J, de Cubas AA, Inglada-Perez L, et al. (2015) MicroRNA deep-sequencing reveals master regulators of follicular and papillary thyroid tumors. Mod Pathol 28: 748-757.

79 Visone R, Russo L, Pallante P, De Martino I, Ferraro A, Leone V, et al. (2007) MicroRNAs (miR)-221 and miR-222, both overexpressed in human thyroid papillary carcinomas, regulate p27Kip1 protein levels and cell cycle. Endocr Relat Cancer 14: 791-798.

80 Li D, Jian W, Wei C, Song H, Gu Y, Luo Y,et al. (2014) Down-regulation of miR-181b promotes apoptosis by targeting CYLD in thyroid papillary cancer. Int J Clin Exp Pathol 7: 7672-7680.

81 Zhang J, Yang Y, Liu Y, Fan Y, Liu Z, Wang X, et al. (2014) MicroRNA-21 regulates biological behaviors in papillary thyroid carcinoma by targeting programmed cell death 4. J Surg Res 189: 68-74.

82 Jazdzewski K, Boguslawska J, Jendrzejewski J, Liyanarachchi S, Pachucki J, Wardyn KA, et al. (2011) Thyroid hormone receptor beta (THRB) is a major target gene for microRNAs deregulated in papillary thyroid carcinoma (PTC). J Clin Endocrinol Metab 96: E546-53.

83 Weber F, Teresi RE, Broelsch CE, Frilling A, Eng C. (2011) A limited set of human MicroRNA is deregulated in follicular thyroid carcinoma. J Clin Endocrinol Metab 91: 3584-3591.

84 Dettmer M, Vogetseder A, Durso MB, Moch H, Komminoth P, Perren A, et al. (2013) MicroRNA expression array identifies novel diagnostic markers for conventional and oncocytic follicular thyroid carcinomas. J Clin Endocrinol Metab 98: E1-7.

85 Croce CM (2009) Causes and consequences of microRNA dysregulation in cancer. Nat Rev Genet 10: 704-714.

86 Zhang L, Huang J, Yang N, Greshock J, Megraw MS, Giannakakis A, et al. (2006) microRNAs exhibit high frequency genomic alterations in human cancer. Proc Natl Acad Sci USA 103: 9136-9141.

87 Kunej T, Godnic I, Ferdin J, Horvat S, Dovc P, Calin GA (2011) Epigenetic regulation of microRNAs in cancer: an integrated review of literature. Mutat Res 717: 77-84.

88 Saito Y, Liang G, Egger G, Friedman JM, Chuang JC, Coetzee GA,et al. (2006) Specific activation of microRNA-127 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells. Cancer Cell 9: 435-43.

89 Steponaitiene R, Kupcinskas J, Langner C, Balaguer F, Venclauskas L, Pauzas H5, et al. (2015) Link Epigenetic silencing of miR-137 is a frequent event in gastric carcinogenesis. Mol Carcinog.

90 Nadal E, Chen G, Gallegos M, Lin L, Ferrer-Torres D, Truini A, et al. (2013) Epigenetic inactivation of microRNA-34b/c predicts poor disease-free survival in early-stage lung adenocarcinoma. Clin Cancer Res 19: 6842-6852.

91 Chiou GY, Chien CS, Wang ML, Chen MT, Yang YP, Yu YL, et al. (2013) Epigenetic regulation of the miR142-3p/interleukin-6 circuit in glioblastoma. Mol Cell 52: 693-706.

92 Tanaka T, Arai M, Wu S, Kanda T, Miyauchi H, Imazeki F, et al. (2011) Epigenetic silencing of microRNA-373 plays an important role in regulating cell proliferation in colon cancer. Oncol Rep 26: 1329-1335.

93 Dettmer M, Perren A, Moch H, Komminoth P, Nikiforov YE, Nikiforova MN. (2013) Comprehensive MicroRNA expression profiling identifies novel markers in follicular variant of papillary thyroid carcinoma. Thyroid 23: 1383-1389.

94 Colamaio M, Borbone E, Russo L, Bianco M, Federico A, Califano D, et al. (2011) miR-191 down-regulation plays a role in thyroid follicular tumors through CDK6 targeting. J Clin Endocrinol Metab 96: E1915-24.

95 Colamaio M, Calì G, Sarnataro D, Borbone E, Pallante P, Decaussin-Petrucci M, et al. (2012) Let-7a down-regulation plays a role in thyroid neoplasias of follicular histotype affecting cell adhesion and migration through its ability to target the FXYD5 (Dysadherin) gene. J Clin Endocrinol Metab 97: E2168-78.

96 Yip L, Kelly L, Shuai Y, Armstrong MJ, Nikiforov YE, Carty SE, et al. (2011) MicroRNA signature distinguishes the degree of aggressiveness of papillary thyroid carcinoma. Ann Surg Oncol 18: 2035-41.

97 Jacques C, Guillotin D, Fontaine JF, Franc B, Mirebeau-Prunier D, Fleury A, et al. (2013) DNA microarray and miRNA analyses reinforce the classification of follicular thyroid tumors. J Clin Endocrinol Metab 98: E981-9.

98 Huang Y, Liao D, Pan L, Ye R, Li X, Wang S, et al. (2013) Expressions of miRNAs in papillary thyroid carcinoma and their associations with the BRAFV600E mutation. Eur J Endocrinol 168: 675-81.

99 Puppin C, Passon N, Lavarone E, Di Loreto C, Frasca F, Vella V, et al. (2011) Levels of histone acetylation in thyroid tumors. Biochem Biophys Res Commun 411: 679-83.

100 Kogai T, Hershman JM, Motomura K, Endo T, Onaya T, Brent GA. (2001) Differential regulation of the human sodium/iodide symporter gene promoter in papillary thyroid carcinoma cell lines and normal thyroid cells. Endocrinology 142: 3369-3379.

101 Puppin C, D'Aurizio F, D'Elia AV, Cesaratto L, Tell G, Russo D, et al. (2005) Effects of histone acetylation on sodium iodide symporter promoter and expression of thyroid-specific transcription factors. Endocrinology 146: 3967-3974.

102 Lin SF, Lin JD, Chou TC, Huang YY, Wong RJ (2013) Utility of a histone deacetylase inhibitor (PXD101) for thyroid cancer treatment. PLoS One 8: e77684.

103 Kelly WK, O'Connor OA, Krug LM, Chiao JH, Heaney M, Curley T, et al (2005) Phase I study of an oral histone deacetylase inhibitor, suberoylanilide hydroxamic acid, in patients with advanced cancer. J Clin Oncol 23: 3923-3931.

104 Woyach JA, Kloos RT, Ringel MD, Arbogast D, Collamore M, Zwiebel JA, et al. (2009) Lack of therapeutic effect of the histone deacetylase inhibitor vorinostat in patients with metastatic radioiodine-refractory thyroid carcinoma. J Clin Endocrinol Metab 94: 164-170.

105 Zuo H, Gandhi M, Edreira MM, Hochbaum D, Nimgaonkar VL, Zhang P, et al. (2010) Downregulation of Rap1GAP through epigenetic silencing and loss of heterozygosity promotes invasion and progression of thyroid tumors. Cancer Res 70: 1389-1397.

106 Wang D, Cui W, Wu X, Qu Y, Wang N, Shi B, et al. (2014) RUNX3 site-specific hypermethylation predicts papillary thyroid cancer recurrence. Am J Cancer Res 4: 725-37.

107 Smith JA, Fan CY, Zou C, Bodenner D, Kokoska MS (2007) Methylation

status of genes in papillary thyroid carcinoma. Arch Otolaryngol Head Neck Surg 133: 1006-1011

108 Yin DT, Wang L, Sun J, Yin F, Yan Q, Shen R, et al. (2010) Association of the promoter methylation and protein expression of Fragile Histidine Triad (FHIT) gene with the progression of differentiated thyroid carcinoma. Int J Clin Exp Pathol 3: 482-491.