Epigenetic modifications in human thyroid cancer (Review)

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Abstract. Thyroid carcinoma is the most common endocrine malignancy of the endocrine organs, and its incidence rate has steadily increased over the last decade. Over 95% of thyroid carcinoma is derived from follicular cells that have a spectrum of differentiation to the most invasive malignancy. The molecular pathogenesis of thyroid cancer remains to be clarified, although activating the *RET*, *RAS* and *BRAF* oncogenes have been well characterized. Increasing evidence from previous studies demonstrates that acquired epigenetic abnormalities participating with genetic alteration results in altered patterns of gene expression/function. Aberrant DNA methylation has been established in the CpG regions and microRNAs (miRNAs) expression profile recognized in cancer development. In the present review, a literature review was performed using MEDLINE and PubMed with the terms 'epigenetic patterns in thyroid cancer [or papillary thyroid carcinoma (PTC), follicular thyroid carcinoma (FTC), medullary thyroid cancer (MTC), anaplastic thyroid cancer (ATC)]', 'DNA methylation in thyroid cancer (or PTC, FTC, MTC, ATC)', 'miRNA expression in thyroid cancer (or PTC, FTC, MTC, ATC)', 'epigenetic patterns in cancer' and the current understanding of epigenetic patterns in thyroid cancer was discussed.

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1. Introduction

Thyroid carcinoma is the most common endocrine malignancy and accounts for ~1% of all types of human cancer, with a rapid incidence rate reported worldwide (1). Over 95% of thyroid carcinomas are derived from follicular epithelial cells (2). They have been traditionally classified as well-differentiated thyroid carcinoma, including papillary (80%) and follicular thyroid carcinoma (PTC and FTC, respectively) (10-15%) (3). By contrast, poorly differentiated (2,4) and anaplastic thyroid carcinoma account for 1-2% of thyroid malignancies. Medullary thyroid carcinoma (3%; MTC) is a malignancy of parafullicular C cells that are derived from neural crest and occurs in sporadic (75%) and hereditary (25%) types (5). This wide spectrum of progression has been closely linked with the pattern of cumulative genetic and epigenetic alterations, which are correlated with tumor differentiation, metastasis and invasion (6). In thyroid carcinoma, the majority of genetic alterations initiate their functions through activating metabolic pathways. Constitutive activation of the mitogen-associated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathway leads to tumorigenesis and promotes cell division (7). Activation of this pathway is a common and important mechanism in the initiation and progression of human cancers. Genetic defects in the *RET*/*PTC*, *BRAF* and *RAS* genes are associated with thyroid tumorigenesis. The prevalence of activating mutations in the *RAS* gene are dependent on the tumor histology. For instance, certain studies showed that *RAS* mutations are more frequent in FTC than PTC (8). *RET* proto-oncogene is responsible for encoding a cell membrane receptor tyrosine kinase (9). Ligands of this kinase have been reported as belonging to the glial-cell-line derived neurotropic factor family that causes receptor dimerization upon binding, leading to autophosphorylation of tyrosine residues and initiation of the MAPK/ERK pathway signaling cascade (10). *RET* functional deficiency results in Hirschsprung's disease; however, an increase in its activities is associated with numerous types of human cancer, including MTC (11,12). Concurrent *RET*/*PTC* and *BRAF* mutations have been reported in PTC (7,13). The *BRAF V600E* mutation, which is the sporadic form of these mutations, is restricted to papillary, anaplastic and poorly differentiated thyroid carcinoma (14,15). The objective of

the present study was to review the current understanding of epigenetic patterns in thyroid cancer.

Study criteria. The terms 'epigenetic patterns in thyroid cancer [or PTC, FTC, MTC, anaplastic thyroid cancer (ATC)]', 'DNA methylation in thyroid cancer (or PTC, FTC, MTC, ATC)', 'microRNA (miRNA) expression in thyroid cancer (or PTC, FTC, MTC, ATC)', and 'epigenetic patterns in cancer' were used in the MEDLINE and PubMed search for studies published between 1970-2014. All the abstracts were reviewed. The studies published in English were included if appropriately designed. The studies of abstracts meeting the criteria were subsequently reviewed to identify the details of the materials associated with the epigenetic patterns of cancer, in particular DNA methylation and miRNAs expression in thyroid cancer. The strategy used to search for studies was developed with the assistance of a research librarian at the Jundishapour University of Medical Science (Ahvaz, Iran).

Study selection. The following criteria were considered as essential for a study to qualify for inclusion in the present review: i) Correct cross-sectional study design involving case-control; and ii) review studies by a permanent scholar. All the studies were initially potential candidates for inclusion; however, they were excluded if they lacked appropriate study design.

2. Epigenetic pattern in cancers

Epigenetic mechanisms are essential for normal cell development and the maintenance of tissue-specific gene expression patterns in mammals (16). However, epigenetic modifications can result in inappropriate activity or inhibition of various signaling pathways, leading to cancer. According to previous studies, epigenetic modification is reported in numerous types of cancers, in addition to a number of genetic variations (17-20). Epigenetic patterns include the covalent modification of chromatin, DNA cytosine methylation, non-coding RNAs expression and nucleosome remodeling (21). Aberrant DNA methylation is associated with gene expression and plays an important role in tumorigenesis (22). Hypomethylation leads to genomic instability and activation of proto-oncogenes through a variety of mechanisms, which contribute to cancer development and progression. However, hypermethylation is associated with gene silencing, particularly tumor suppressor genes, and it is considered to be the hallmark of cancers (23). The ability of hypermethylation is well recognized; however, the mechanism through which genes are targeted for hypermethylation is unclear. Further understanding of how specific genomic regions are targeted for hypermethylation will potentially result in the design of additional therapeutic regions.

Another epigenetic modification is the miRNA expression profile. In a previous study the expression profile of miRNAs in tumors was compared to the associated normal tissues, indicating wide-spread changes in the expression level (24). Since miRNAs regulate the expression of numerous genes that are involved in the transcriptional regulation, cell proliferation and apoptosis, alteration in their expression can promote tumorigenesis. miRNAs may function as either tumor suppressors or oncogenes, depending on their effect on the target genes. Various mechanisms, including chromosomal abnormalities, transcription factor binding and epigenetic alteration, are important in miRNA expression.

3. Epigenetic modification in thyroid cancers

DNA methylation. Aberrant DNA methylation of tumor suppressor genes and proto-oncogenes are common in thyroid tumors, and it occurs in a number of other human tumors. Certain specific tumor suppressor genes in the thyroid are *PTEN*, *RASSF1A*, *TIMP3*, *SLC5A8*, *DAPK*, *RAPβ2* and *RAP1GAP* (Table I).

PTEN was identified as a tumor suppressor gene, which is mutated in a large number of cancers. This gene encodes the phosphatidylinositol-3, 4, 5-triphosphate 3-phosphatase protein. *PTEN* negatively regulates the AKT/PKB signaling pathway and is involved in the regulation of cell cycle, opposing cell growth and rapid division (25,26). Aberrant DNA methylation in this gene is mostly reported in PTC and FTC (27).

The *RASSF1A* gene encodes a protein that is similar to the RAS effector protein (28). The altered expression of this gene is associated with cancer, and aberrant DNA methylation has been identified as an important mechanism in the inactivation of this gene (29,30). In contrast to FTC, only a small proportion of PTC harbored the aberrant methylation of *RASSF1A*, which may have a critical role in thyroid tumorigenesis, independent of the BRAF/MAPK kinase (MEK) MAPK pathway (30).

TIMP3 is a tissue inhibitor of metalloproteinase, which inhibits the growth, angiogenesis, invasion and metastasis of several tumors (31). This gene has been reported to be hypermethylated in thyroid cancer (32,33) and is associated with extra thyroidal invasion and lymph node metastasis (33). The *RAP1GAP* gene encodes a type of GTPase-activating protein that downregulates the activity of the RAS-related protein. *RAP1GAP* is implicated in the regulation of mitogenic and oncogenic pathways in thyroid cells (34,35).

RAP1 has an important role in the regulation of the ERK-dependent pathway and activation of the BRAF-MEK-ERK pathway (36-38). The immunohistochemistry assay data showed the decreased expression of *RAP1GAP* gene in PTC (39), which was associated with its proliferation and invasion in thyroid cancer cell lines (40). Additionally, DNA hypomethylation has an important role in tumorigenesis; however, its role is not well understood. In this regard, Rodríguez-Rodero *et al* (41) aimed to determine the global patterns of aberrant DNA methylation in thyroid cancer using DNA methylation arrays. The study identified 262 and 352 hypermethylated and 13 and 21 hypomethylated genes in PTC and FTC, respectively. In addition, 280 and 393 hypomethylated genes and 86 and 131 hypermethylated genes were identified, which were determined in anaplastic and MTC, respectively. Among these genes, four oncogenes (*INSL4*, *DPPA2*, *TCL1B* and *NOTCH4*) were frequently regulated by hypomethylation.

Furthermore, a member of the serine protease inhibitor superfamily, mammary serine protease inhibitor (*Maspin*), which is encoded by the *SERPINB5* gene, is a unique tumor suppressor gene, as it has a variety of biological behavior and function. The expression of this gene is regulated by epigenetic

Table I. DNA methylation prevalence of thyroid-related genes in thyroid cancers.

Genes	Function	DNA methylation prevalence	Author (year)	(Refs.)
Tumor suppressor				
PTEN	*PTEN* is involved in the regulation of cell cycle and preventing cells from growing and dividing rapidly	50% of PTC, 100% of FTC	Alvarez-Nuñez *et al* (2006)	(27)
RASSF1A	*RASSF1A* localizes to microtubules and promotes their stabilization	30% of thyroid cancers	Xing *et al* (2004)	(30)
TIMP3	Tissue inhibitor of metalloproteinase	53% of PTC	Hu *et al* (2006)	(33)
SLC5A8	Sodium solute symporter family	33% of PTC	Hu *et al* (2006)	(33)
DAPK	Ca/calmodulin-dependent ser/thr kinase protein	34% of PTC	Hu *et al* (2006)	(33)
RAPβ2	Negative regulator of cell growth	22% of PTC	Hu *et al* (2006)	(33)
RAP1GAP	RAP1GTPase-activating protein	72% of PTC, 38% of FTC	Zuo *et al* (2010)	(56)
Oncogenes				
INSL4	Belongs to the insulin and IGF family	60% of MTC	Rodríguez-Rodero *et al* (2013)	(41)
DPPA2	Developmental pluripotency-associated 2	30% of MTC	Rodríguez-Rodero *et al* (2013)	(41)
TCL1B	An oncogene frequently activated by reciprocal translocations	64% of ATC	Rodríguez-Rodero *et al* (2013)	(41)
NOTCH4	A member of notch family, which plays a role in a variety of developmental processes	45% of ATC	Rodríguez-Rodero *et al* (2013)	(41)
Maspin	A member of serine protease inhibitor superfamily	100% of WDTC, 38% of UDTC	Ogasawara *et al* (2004)	(42)
Thyroid specific				
NIS	Sodium/iodide symporter	53.8% of thyroid cancers	Stephen *et al* (2011)	(57)
Tg	Thyroglobulin molecule	NA	NA	
TPO	Thyroid peroxidase	NA	NA	
TSHR	Thyroid stimulating receptor	59% of PTC 47% of FTC	Xing *et al* (2003), Eze *et al* (2011)	(43) (45)

PTC, papillary thyroid cancer; FTC, follicular thyroid cancer; MTC, medullary thyroid cancer; ATC, anaplastic thyroid cancer; WDTC, well-differentiated thyroid carcinoma; UDTC, undifferentiated thyroid carcinoma; NA, not available.

modification in a cell-type-specific manner. For the first time, Ogasawara *et al* (42) examined the DNA methylation status in the promoter region of *Maspin*, indicating that the over-expression of this gene, as a result of DNA hypomethylation, is closely associated with morphological dedifferentiation in thyroid cancers.

In addition to tumor suppressor genes and oncogenes, the expression of thyroid specific genes is frequently absent in thyroid cancer. Although the molecular mechanisms underlying the silencing of these genes are not well understood, aberrant DNA methylation could be considered as an important mechanism. For instance, hypermethylation, which leads to the silencing of NIS and TSHR gene, is frequently reported (43). Loss or altered expression of thyroid-specific genes is associated with the progression and dedifferentiation of thyroid cells, resulting in various thyroid diseases (44,45). Therefore, aberrant methylation of these genes may be a pathogenesis or progression factor for thyroid cancers. Of note, this biological mechanism is associated with thyroid tumorigenesis, and the methylation pattern of these genes is also relevant to unsuccessful radioiodine therapy as the main medical treatment for this cancer (46).

miRNA expression profile. In normal or tumor cells with distinct biological properties, miRNAs identify the cell origin of different tumors; however, it remains unknown whether various tumors, which originate from the same cells, have different miRNA expression profiles. Consequently, thyroid tumors represent a suitable model for this study, as thyroid cancers encompass several tumors with different histology and degree of differentiation. Therefore, comparing the expression profile of miRNAs in normal and tumor thyroid cells may be a useful factor for diagnosis of thyroid malignancy (Table II).

miRNA expression in PTC. Previous studies that assessed the expression profile of miRNAs in PTC reported that the expression of *miR-146*, *miR-221*, *miR-222*, *miR-21* and *miR-181a* increased in PTC compared to normal thyroid cells. Particularly, *miR-146*, *miR-221* and *miR-222* showed a 9-11-fold

Table II. A summary of microRNAs (miRNAs or miRs) expression and their target gene in thyroid cancers.

miRNA	Location	Description	Notable target genes in thyroid cells	Author (year)	(Refs.)
miR-146a	5q34	*miR-146a* is involved in the feedback system of the classical NF-κB signal pathway in PTC	*PRKCE*	Zhang *et al* (2014)	(58)
miR-221	Xp11.3	Oncogenic microRNA	*P27*	Visone *et al* (2007)	(59)
miR-222	Xp11.3	Regulates p27 expression and thereby cell cycle	*P27*	Visone *et al* (2007)	(59)
miR-21	17q23.2 (54)	*miR-21* has an important role in oncogenic Ras-induced cell proliferation (55)	*PTEN*, *PDCD4*, *RhoB*	Meng *et al* (2007), Asangani *et al* (2008), Sabatel *et al* (2011)	(60) (61) (62)
miR-181a	1q32.1	*miR-181* has a potential role in differentiating PTC, and BRAF mutation may interact with *miR-181* in pathogenesis and prognosis of PTC	*THRB*	Jazdzewski *et al* (2011), Sun *et al* (2013)	(63) (64)
miR-197	1p13.3	*miR-197* and its target gene may be the novel molecular markers to differentiate malignant (FTCs) from benign (FAs)	*ACVR1*, *TSPAN3*	Marini *et al* (2011)	(65)
miR-346	10q23.2	*miR-346* participates in the transformation of follicular tumors from benign to malignant status	*EFEMP2*	Marini *et al* (2011)	(65)
miR-9	1q22	*miR-9* is significantly overexpressed in hereditary when compared to sporadic medullary thyroid tumor	-	Abraham *et al* (2011)	(52)
miR-10a	17q21.32	*miR-10a* is important for tumor development in MTC	*MDM4*, *NCOR2*	Hudson *et al* (2013)	(66)
miR-124a	*8p23.1*	*miR-124a* is upregulated in MTC	*CDK6*	Ajith (2013)	(67)
miR-127	14q23.2	*miR-127* is overexpressed in MTC samples carrying a wild-type RET than mutated RET, suggesting an oncogenic role for this miRNA	*BCL6*	Chen *et al* (2013)	(68)
miR-224	Xq28	*miR-224* upregulation was more detected in the early stage of MTC	-	Mian *et al* (2012)	(53)
miR-323	14q32.31	*miR-323* is upregulated in MTC	*BRAF*	Cahill *et al* (2007)	(69)

NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PTC, papillary thyroid cancer; FTC, follicular thyroid cancer; FA, follicular adenoma of thyroid gland; MTC, medullary thyroid cancer.

higher level in thyroid tumors. Deregulation expression of *miR-146b*, *miR-221* and *miR-222* may be the crucial component for initiation and development of PTC (47). The putative target of these miRNAs was suspected to be c-KIT, as a tyrosine kinase receptor that plays an important role in cell growth and differentiation (48). c-KIT is frequently expressed in benign thyroid adenomas and goiter; however its expression decreased to 60% in FTC and is completely absent in PTC and ATC.

miRNA expression in FTC. The expression level of *miR-192*, *miR-197*, *miR-328* and *miR-346* has been reported to be decreased in FTC compared to follicular adenoma of thyroid gland (FA) (49). These miRNAs are evidently specifically associated with FTC. The expression profile of *miR-197* and *miR-346* may be associated with transferring follicular tumors from a benign to malignant status. These miRNAs and their target genes may provide the novel molecular markers for differentiation of the malignant status FTC from the benign form (50). By contrast, assessing the role of *miR-221* and *miR-222* in thyroid carcinomas showed that these molecules are not associated with FTC (51).

miRNA expression in MTC. There are limited numbers of studies that evaluated the role of miRNAs in MTC. According to these aforementioned studies, miRNAs play a pivotal role in the biology of MTC and represent the important class of prognostic biomarker and therapeutic targets. *miR-9* has been determined as a specific biomarker in MTC and in sporadic MTC (sMTC). The expression of *miR-9* is known to be lower compared to heritable MTC. Overexpression of *miR-183* and *miR-375* have also been reported as important predictive biomarkers for lateral lymph node metastases (52). The result of one study that examined the association between miRNA expression and RET status in MTC, reported a significant overexpression of miRNA as follows: 4.2-Fold for *miR-21*, 6.7-fold for *miR-127*, 8.8-fold for *miR-154*, 6.6-fold for *miR-224*, 5.8-fold for *miR-323*, 6.1-fold for *miR-370*, 13-fold for *miR-9*, 6.7-fold for *miR-183* and 10.1-fold for *miR-375*. The upregulation of *miR-224* determined it as a prognostic biomarker and the lower level of *miR-127* was observed in sMTC that was carrying somatic *RET* mutation in comparison to sMTC, which was carrying a wild-type *RET* (53).

4. Conclusion

The epigenetic revolution during the last decades has challenged whether genetic codes are the key determinant for gene function. Studies in epigenetic patterns of cancer have demonstrated that genome packaging is as important as the genome by itself in regulating the essential cellular processes. Understanding the epigenetic alterations is required for molecular treatment design.

As in other types of cancer, the majority of genetic and epigenetic alterations is somatic, and assessing the epigenetic pattern in thyroid cancer revealed a critical role for these alterations in the classification and prognosis of tumors. The reversible epigenetic changes that occur in cancer result in the possibility of epigenetic therapy as an optional treatment. DNA methylation inhibitors were among the first epigenetic drugs proposed for use as cancer therapeutics. Since miRNAs are associated with cell proliferation, differentiation and invasion, these molecules and their biological target genes are considered as potential targets for tumor diagnosis and treatment.

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