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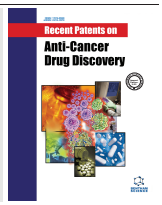

Epigenetics in Medullary Thyroid Cancer: From Pathogenesis to Targeted Therapy

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Abstract: ***Background:*** Medullary thyroid carcinoma (MTC) originates from the parafollicular C cells of the thyroid gland. Mutations of the RET proto-oncogene are implicated in the pathogenesis of MTC. Germline activating mutations of this gene have been reported in about 88–98% of familial MTCs, while somatic mutations of RET gene have been detected in about 23-70% of sporadic forms. Although these genetic events are well characterized, much less is known about the role of epigenetic abnormalities in MTC.

Objective: The present review reports a detailed description of epigenetic abnormalities (DNA methylation, histone modifications and miRNA profile), probably involved in the pathogenesis and progression of MTC.

Methods: A systematic review was performed using Pubmed and Google patents databases.

Results: We report the current understanding of epigenetic patterns in MTC and discuss the potential use of current knowledge in designing novel therapeutic strategies through epigenetic drugs, focusing on recent patents in this field.

Conclusion: Taking into account the reversibility of epigenetic alterations and the recent development in this field, epigenetic therapy may emerge for clinical use in the near future for patients with advanced MTC.

Keywords: DNA methylation, epigenetics, epigenetic therapy, histone modifications, medullary thyroid cancer, microRNA.

1. INTRODUCTION

Medullary thyroid carcinoma (MTC) is a malignant neoplasm of the parafollicular C cells and it accounts for about 5-10% of thyroid tumors. Most MTCs occur in a sporadic form (70-80% of cases). The other 20-30% is inherited as three familial forms: multiple endocrine neoplasia type 2A (MEN 2A), multiple endocrine neoplasia type 2B (MEN 2B), and familial MTC not associated with MEN (FMTC) [1].

Mutations of the RET proto-oncogene are implicated in the pathogenesis of MTC. Germline activating mutations of this gene have been reported in about 88-98% of familial MTCs, while somatic mutations of RET gene have been detected in about 23-70% of sporadic forms [2]. While these genetic events are well characterized, much less is known about the role of epigenetic abnormalities in this tumor.

Epigenetic mechanisms play an essential role in normal development and cell differentiation. Disruption of epigenetic processes can lead to altered gene function and malignant cellular transformation [3-5]. Besides genetic lesions, epigenetic alterations regarding DNA methylation, histone modifications and non-coding RNAs, play a role in all three steps of tumorigenesis [6]: initiation, promotion, and progression [7]. It is important to emphasize that, unlike genetic abnormalities, epigenetic changes are reversible with promising applications in the field of epigenetic therapy in cancer [8].

In this review, we report the current understanding of epigenetic patterns in MTC, the potential use of this knowledge in designing novel therapeutic strategies and a focus on recent patent applications in this field.

2. EPIGENETIC MODIFICATIONS IN MTC

2.1. DNA Methylation

DNA methylation involves the addition of a methyl group to DNA residues and plays an important role in regulating gene expression. DNA methylation is most commonly

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observed on the fifth carbon of the cytosine's pyrimidine ring in a phosphodiester-linked cytosine and guanine (CpG) dinucleotide context [9]. CpG islands are regions with a high frequency of CpG sites and are found in about 70% of human gene promoters. These islands are usually unmethylated when the genes are expressed, suggesting that DNA methylation is a general transcriptional mechanism for gene silencing [10]. DNA methylation is regulated by a family of DNA methyltransferases DNMTs: DNMT1, DNMT2, DNMT3A, DNMT3B, and DNMT3L. DNMT1 preferentially promotes methylation of hemimethylated DNA and is responsible for copying DNA methylation patterns to the daughter strands during DNA replication [11]. Little is known about DNMT2 that is characterized by an RNA methyltransferase activity, while DNMT3A and DNMT3B perform *de novo* methylation during development with the support of the DNMT3L [12].

Disruption of epigenetic processes can lead to altered gene function and malignant transformation. Global DNA hypomethylation plays a significant role in tumorigenesis and occurs at various genomic sequences including repetitive elements, retrotransposons, CpG poor promoters, introns and proto-oncogenes [3]. This leads to genomic instability and activation of growth-promoting genes. In addition, site-specific hypermethylation contributes to tumorigenesis by silencing tumor suppressor genes [3]. New evidence suggests that epigenetic abnormalities, along with genetic alterations, are implicated in MTC tumorigenesis and progression. Indeed, aberrant DNA methylation of several oncogenes and tumor suppressor genes has been reported in thyroid tumors [13], including MTC [14, 15].

The possibility that RET has a role in the pathogenesis of MTC not only by genetic but also through epigenetic mechanisms cannot be excluded. Interestingly, the expression of human RET protooncogene is controlled by a promoter harboring several transcription factor binding sites and rich in CpG islands [16]. A preliminary study, investigating the DNA methylation profile at the Ret locus, identified a significantly lower degree of methylation for this protooncogene in MTC cells compared with normal thyroid tissue [17, 18]. However, a larger study is necessary to confirm this finding.

Rodríguez-Rodero *et al.* [19] analyzed the DNA methylation profile in thyroid tumors through the genome-wide 27 K Infinium Methylation Array. They found that 490 CpG (corresponding to 393 genes) and 148 CpG (corresponding to 131 genes) were respectively hypomethylated and hypermethylated in MTC compared with normal thyroid tissue. Interestingly, two oncogenes were frequently hypomethylated: insulin like 4 (INSL4), belonging to the insulin and IGF family, was hypomethylated in 60% of MTC; and developmental pluripotency associated-2 (DPPA2), involved in the maintenance of the active epigenetic status of several genes, was hypomethylated in 30% of MTC. These findings suggest that promoter demethylation might be a frequent mechanism of INSL4 and DPPA2 oncogenes activation in MTC. Hypomethylation of the membrane-associated protein 17 (MAP17) promoter was detected in 38% of MTC. Interestingly, MAP17-overexpressing TT cells, a human MTC cell line, were associated with increased number of colonies, cell growth, and migration capability, suggesting the potential oncogenic property of MAP17 in MTC and its involvement in MTC carcinogenesis through epigenetic events [19].

The tumor suppressor gene RASSF1A (RAS association domain family protein 1A gene), which is epigenetically inactivated [20], in a variety of human tumors, resulted hypermethylated in 40-80% of MTC [21, 22]. This gene encodes a protein similar to the RAS effector proteins. The inactivation of RASSF1A has been associated with increased tumour cell proliferation through accelerated cell cycle progression and resistance to apoptosis signals. Thus, it may be an event in the pathogenesis of MTC [21, 22].

Macià *et al.* [23] investigated the role of the epigenetic modifications of Spry1, which belongs to Sprouty family of genes composed of four members in mammals (Spry1-4) [24]. Spry proteins have been shown to be activators or inhibitors of receptor tyrosine kinase signalling, depending on the cellular context or the type of receptor tyrosine kinase. Spry1 was expressed at very high levels in normal thyroid C-cells and it has been identified as a candidate tumour suppressor gene in MTC. Indeed expression of Spry1 reduced cell proliferation of TT cells. In MTC, SPRY1 promoter was frequently methylated and its expression was consequently decreased. This process may represent another mechanism involved in MTC tumorigenesis. Indeed, the targeted deletion of Spry1 caused C-cell hyperplasia, a precancerous lesion preceding MTC, in young adult mice. The tumour suppressor activity of Spry1 does not appear to be related to inhibition of RET signalling, but to the induction of cellular senescence [23].

A global dysregulation of epigenetic machinery appears to be involved in the progression of MTC. The expression of several DNMT (DNMT1, DNMT3A and DNMT3B) resulted higher in MTC with lymph node and distant metastases (N1 M1) compared to localized MTC (N0 M0) [25].

The reversible nature of epigenetic changes that occur in cancer renders them ideal targets for therapeutic interventions. DNA methylation inhibitors were among the first epigenetic drugs proposed as cancer targeted therapy. 5-aza-2'-deoxycytidine and 5-aza-cytidine, both DNMT inhibitors, are currently used for treatment of hematologic malignancies. Unfortunately, clinical trials with DNMT inhibitors in solid tumors have not shown the same results. Both 5-aza-2'-deoxycytidine and 5-aza-cytidine are active in hematologic malignancies at lower (less toxic) doses than are required for demethylation in solid tumors. At high doses these compounds are associated to severe toxicity mainly related to myelosuppression, due to the chemical instability and their incorporation in the genome. The development of novel inhibitors, that do not rely on DNA incorporation for activity, has the potential to greatly increase the clinical use of DNMT inhibitors [26]. Andrew *et al.* recently patented a new approach to inhibit DNMT activity, using DNMT trapping oligonucleotides. These oligonucleotides are designed specifically as trapping suicide oligonucleotides, which capture DNMTs. They are not incorporated into genomic DNA, reducing or eliminating the main cause of dose-limiting toxicities of existing epigenetic drugs (US20150038548) [27].

We have recently showed a potent *in vitro* antitumor activity of 5-aza-2'-deoxycytidine in MTC cell lines (TT and MZ-CRC-1), mainly through cell cycle arrest [28]. Further studies are required to confirm the antitumor activity of demethylating agents in MTC, in particular for these new DNMT trapping oligonucleotides. It would be also interesting to focus on establishing clinically relevant combinations of DNMT inhibitors and conventional therapies in MTC. Indeed, there are several evidences supporting a role of DNMT inhibitors as efficient chemo- and radiosensitizers in solid tumors [26]. According to several recent patents, demethylating agents potentiate also the antitumor activity of immunomodulatory agents (WO2014128245, WO2015035112) [29, 30], somatostatin analogues and dopamine agonists (WO2007114697) [31].

2.2. Histone Modifications

Human genomic DNA is packed into nucleosomes, consisting of approximately 150 base pairs of DNA wrapped around a histone octamer [32]. A gene can be transcribed only if the chromatin structure allows regulatory proteins to bind the relevant portion of the DNA [33]. Several histone post-translational modifications, such as acetylation, methylation, phosphorylation and ubiquitination [34], can modulate chromatin structure and transcriptional activity. Acetylation of histones is the key mechanism of chromatin modification and has been correlated with transcriptionally active chromatin, whereas histone methylation can be linked to either repression or activation of transcription depending on the context and extent of methylation [35, 36]. Histone deacetylases (HDAC) and histone acetyltransferases exert opposing enzymatic activities that modulate the degree of acetylation of the histones. In the same way, histone methylation is regulated by two families of enzymes with opposing catalytic activities: lysine methyltransferases and demethylases. These enzymes regulate gene expression, cellular differentiation and survival [37].

Aberrant pattern of histone modifications appears to be another relevant hallmark of MTC. A relative high expression of EZH2 and SMYD3 genes occurred in MTCs with a more aggressive behavior and a worst clinical outcome [25]. However, expression of EZH2 and SMYD3 did not correlate significantly with mutational status of RET and RAS [25]. EZH2 induces trimethylation of histone H3 lysine 27 (H3K27me3) and determines transcriptional repression in genes involved in cell growth, adhesion, and transformation [38]. SMYD3 encodes a histone methyltransferase involved in the growth of various human cancers [39] and its overexpression is correlated to metastatic cancer progression [40]. A small but significant increase of the histone demethylase KDM5B and the histone deacetylase 7 (HDAC7) was also observed in the group of tumors with local and distant metastases (N1, M1) compared to less aggressive MTCs (N0, M0) [25].

On this basis, histone methyltransferases and HDACs appear to be promising targets for MTC therapy. Novel histone methyltransferase inhibitors, also specific for EZH2, with potential antitumor activity have been recently patented (US9175331, WO2015152437) [41, 42]. Indeed, molecular targeting of specific histone methyltransferase is emerging as a new direction for cancer therapy. However, the antitumor efficacy of histone methyltransferase inhibitors has not yet been tested in MTC. On the other hand, the antitumor activity of HDAC inhibitors has been widely studied in the last years [43]. Several HDAC inhibitors, suberoylanilide hydroxamic acid (SAHA), m-carboxycinnamic acid bis-hydroxamide (CBHA) and belinostat (PXD101), suppressed *in vitro* cell proliferation of TT cells [37, 44]. The HDAC inhibitors suberoyl bis-hydroxamic acid (SBHA) and valproic acid (VPA) have been shown to inhibit the expression of neuroendocrine markers (chromogranin A and calcitonin) and the growth of MTC cells, both *in vitro* [45, 46] and *in vivo*, through the activation of the Notch1 signaling pathway and the induction of apoptosis [47]. The Notch1 signaling pathway plays an important role in cellular differentiation and proliferation, acting as a tumor suppressor and its overexpression in MTC cells is associated with growth inhibition [48, 49]. Also Notch3 acts as a tumor suppressor in human MTC. AB3, a novel class I HDAC inhibitor, inhibited *in vitro* MTC proliferation and expression of neuroendocrine markers (chromogranin A and ASCL1) through the activation of Notch3 signaling [50].

To date, the FDA has approved three HDAC inhibitors: vorinostat (SAHA), romidepsin and belinostat, for cutaneous/peripheral T-cell lymphoma [3]. Other HDAC inhibitors are in different stages of clinical trials for the treatment of hematologic malignancies and solid tumors [51].

A Phase II study assessed objective response to vorinostat in patients with advanced thyroid cancer (3 out of 19 with MTC). No patient achieved a partial or complete response. Median duration of therapy in patients with differentiated thyroid cancer was 17 weeks, whereas in MTC patients it was 25 weeks [52]. Although this study suggested that vorinostat alone is not effective in metastatic thyroid carcinoma, there remains the question of whether combination therapy including HDAC inhibitors may be effective. In fact, few preclinical studies have demonstrated that HDAC inhibitors potentiate the antitumor activity of several compounds in MTC. SAHA strongly sensitized TT cells to doxorubicin inducing cell death [37]. Combination therapy with HDAC inhibitors (SBHA or VPA) and lithium chloride exhibited potent antiproliferative effects and suppressed hormonal secretion in TT cells [53] through concomitant upregulation of Notch1 and inhibition of glycogen synthase kinase-3ß (GSK-3ß) pathway [48]. All this represents the rationale for clinical studies of HDAC inhibitors [54] in combination with other anticancer therapies, in an effort to improve the clinical outcome of patients with aggressive MTC. In future studies it would be extremely useful to evaluate the antitumor activity of HDAC inhibitors in combination with tyrosine kinase inhibitors [55] taking into consideration that: 1) in several tumors this combination showed a synergistic antitumor activity *in vitro* (US9101579, WO2015054197) [56-59]; 2) treatment of advanced MTC was recently improved with the approval of two tyrosine kinase inhibitors (vandetanib and cabozantinib) [60, 61]. Interestingly, new chimeric HDAC inhibitors merged with protein tyrosine kinase inhibitors [62] have been recently synthesized. These chimeric compounds retain both selective pharmacological activities, postulating a broader activity

spectrum and less likelihood of drug resistance in cancer patients [63].

On the basis of the strategy of creating multifunctional drugs, a novel series of compounds capable of simultaneously inhibiting HDACs and promoting the release of nitric oxide, has been recently synthesized and patented for the treatment of cancer (CN104356087) [64]. These compounds showed additive antitumor activity via HDAC inhibition and nitric oxide release in several tumors [65] and may represent a new potential tool in the therapy of MTC, taking into consideration that a very strong anti-tumor activity of nitric oxide donors has been previously observed in MTC animal models [66, 67].

2.3 Non-coding RNA

Based on size, non-coding RNAs (ncRNAs) are grouped into two categories, long ncRNAs (> 200 nucleotides) and small ncRNAs (< 200 nucleotides) [68]. Several evidences suggest that long ncRNAs act at different levels of gene expression, such as chromatin remodelling, transcription, genome stability, post-transcriptional modifications and translation. Long ncRNAs constitute an important component of tumor biology, they have been found to be differentially expressed in cancer and their deregulated expression seems to be involved in cell malignant transformation [69]. Small ncRNA family includes microRNAs (miRNAs), small nucleolar RNAs (snoRNAs), small nuclear RNAs, piwi-interacting RNAs and small interfering RNAs [70], out of which miRNAs were most known and extensively studied in human cancers [71]. miRNAs act as negative regulators of the gene expression [33]. Endogenous miRNAs are important in the regulation of cell differentiation, proliferation, apoptosis and autophagy [72]. The link between miRNAs and epigenetics is bidirectional, whilst the expression of miRNAs is dictated by the methylation and acetylation status of DNA, on the other hand miRNAs regulate the expression of DNMTs and HDACs [72, 73]. Indeed, a large proportion of miRNA loci are associated with CpG islands giving strong basis to their regulation by DNA methylation [70]. This complicated network appears to form an epigenetics-miRNA regulatory circuit able to modulate the whole gene expression profile. Dysregulation of miRNA has been observed in several tumors, including MTC, suggesting a potential role in tumorigenesis and progression by regulating cell growth, cell death and migration through targeting transcription factors or signalling pathways (Table **1**).

Nikiforova *et al.* were the first to investigate miRNA expression in 2 patients with MTC. They showed that ten miRNAs (miR-323, miR-370, miR-129, miR-137, miR-10a, miR-124a, miR-224, miR-9, miR-154 and miR-127) were up-regulated in MTC compared to normal thyroid tissues [74-76].

Other studies have focused on the analysis of miRNAs expression in MTC by 'miRNA array profiling', revealing the dysregulation of several miRNAs [6, 17, 77]. Hudson *et al.* evaluated the expression of over 700 miRNAs in a cohort of 15 patients with MTC. They found significant overexpression of miR-375 and miR-10a, while miR-455 was less expressed compared to normal thyroid tissue. These date were validated in a larger cohort of 59 cases [6]. Interestingly, miR-375 is highly expressed in neuroendocrine cells [17] and seems to play opposite roles in cancer pathogenesis, either as oncogene or as a tumor suppressor [65, 78]. YAP1, a growth inhibitor that is known to be downregulated in neuroendocrine cell lung tumors by miR-375 [79], resulted to be also downregulated in this series of MTC [80]. Therefore, miR-375 mediated downregulation of YAP1 may be involved in tumor development of MTC.

In another work overexpression of miR-21, miR-127, miR-154, miR-224, miR-323, miR-370, miR183, miR375 and miR-9* has been found in MTC and c-cell hyperplasia compared to normal thyroid tissue [17]. The most pronounced increase was observed for miR-9*, while miR-21 upregulation in MTC samples was concomitant with a marked loss of nuclear programmed cell death 4 (PDCD4) protein. PDCD4 is a tumor suppressor gene involved in apoptosis, cell transformation, invasion, and tumor progression [81]. miR-21 has been reported to target the PDCD4 3'UTR, which negatively regulates its expression [82]. From all nine miRNAs, only for miR-127 has been observed a link with RET status, sporadic MTC carrying somatic RET mutations showed a lower upregulation of miR-127 than those with a wild-type RET [17].

miR-129-5p represents another miRNA probably involved in MTC tumorigenesis. It resulted down-regulated in human MTC tissues compared to normal adjacent thyroid

Table 1. miRNAs Potentially Involved in the Tumorigenesis and/or Progression of MTC and Related Targets.

miRNA	Description	Targets	References
miR-375	It is expressed in neuroendocrine cells and is important for tumor development	YAP1	[58]
miR-21	It promotes neoplastic cell trasformation by repressing tumor suppressor genes	PDCD4	[59]
miR-129-5p	It decreases cell growth, induces apoptosis and suppresses migration in MTC cells	AKT and RET	[64]
miR-183	Knock down of miR-183 reduced cell proliferation in TT cells	LC3B	[57]
miR-200	The down-regulation of miR-200 family confers a more aggressive phenotype to MTC cells	ZEB1, ZEB2, TGFβ1 and TGFβ2	[67]
miR-9-3p	It reduced cell viability through an arrest of cell cycle and significantly inhibits autophagy	Atg5, PIK3C3, mTOR and LAMP-1	[71]

and exerted tumor suppressor activity by inhibiting expression of RET, through a direct interaction with 3′UTR of RET, and by suppressing AKT phosphorylation. Interestingly, the overexpression of miR-129-5p through transfection in MTC cell lines (TT and MZ-CRC-1) inhibited cell proliferation and migration ability, and stimulated cell apoptosis [83].

Puppin *et al.* [84] demonstrated that mRNA levels of DICER, DGCR8, and XPO5, all genes involved in miRNA biogenesis, were significantly overexpressed in MTC harbouring RET mutations, particularly in the presence of RET634 mutation, but not RAS mutations. These data suggest that DICER, DCGR8, and XPO5 may represent novel targets for therapy in RET-mutated MTC aimed to restore the normal miRNA pattern [84].

Few studies supported a potential role of miRNA dysregulation also in MTC progression.

Through an array covering 1084 miRNAs, ten miRNAs were found to be differentially expressed between sporadic and familial MTC. Among these miRNAs, six (miR-199b-5P, miR-9, miR-9*, miR-223, let-7i, and miR-23a) were underexpressed, whereas four miRNAs (miR-182, miR-183, miR-375 and miR-551b) were overexpressed in sporadic MTC compared with hereditary MTC [76]. Overexpression of miR-183 [85] and miR-375 was associated with a more aggressive behaviour including residual disease, lymph node metastases, distant metastases, and higher mortality [6]. In this regard, downregulation of miR-183 reduced cell proliferation in TT cells in association with an increase in LC3B expression, probably via autophagy, indicating that miR-183 represents a potential therapeutic target for MTC [77, 86].

In another series of MTCs, high miR-224 levels significantly correlated with the absence of node metastases, lower stages at diagnosis and with a biochemically free status at the end of the follow-up [17].

MiRNA expression profiling was evaluated through an array with more than 1200 capture probes in matched metastatic and primary tumor samples. Five miRNA were downregulated (miR-7, miR-10a, miR-29c, miR-200b and miR-200c) and five were up-regulated (miR-130a, miR-138, miR-193a-3p, miR-373 and miR-498) in metastatic MTC tissues respect to primary MTC [87, 88]. Interestingly, the downregulation of the miR-200 family in MTC cells (TT and MZ-CRC-1) significantly increase migration and invasion potential of these cells, shifting from an epithelial to a mesenchymal and more aggressive phenotype, through repression of E-cadherin expression. In fact, the miR-200 family regulate the expression of E-cadherin through direct targeting of ZEB1 and ZEB2 mRNA and by enhancing the expression of tumor growth factor β (TGFβ)-2 and TGFβ-1. Concerning the other miRNAs differently expressed between matched metastatic and primary MTC samples, in several other cellular systems miR-7 regulates the expression of epidermal growth factor receptor (EGFR); miR-29c induces apoptosis in a p53-dependent manner; miR-130a, miR-138, miR-193a, miR-373 and miR-498 control several genes that modulate apoptosis, cell migration and angiogenesis. However, the real effects of these miRNAs have not yet been established in MTC cells [88].

These data suggest that expression of specific miRNAs seems to be involved in MTC carcinogenesis and progression, providing new insights to pathogenesis and very attractive therapeutic targets [89]. Indeed, unlike genetic changes, miRNA alterations can be corrected. The detrimental impact of upregulated miRNAs with a potential oncogenic role can be counteracted with specific inhibitors or antimiRNA, which are synthetic molecules able to prevent the selective binding of a specific miRNA to its target. Few antisense oligonucleotides, able to affect the activity of miR-375 and miR-21, have been recently constructed and patented (US8951984, US9181547) [90, 91].

On the other hand, restoration of expression for miRNA with a tumor-suppressor role represents another therapeutic approach in cancer through the use of synthetic miRNA mimics, miRNA precursors or pre-miRNA mimics, to be introduced in cancer cells by miRNA delivery systems [92]. Several preliminary studies showed the possibility to suppress the proliferation of MTC cells, at least *in vitro*, through the upregulation of miR-129-5p [83] and miR-9-3p [93] and the downregulation of miR-183 [77, 86].

3. CURRENT & FUTURE DEVELOPMENTS

The primary molecular mechanism underlying MTC tumorigenesis is the aberrant activation of RET signalling through gene mutations [94]. Epigenetic modifications appear to be involved together with the overactivation of the RET signalling pathway in the pathogenesis and progression of MTC. However, it is difficult to accurately estimate the epigenetic mechanisms that play a pivotal role in this tumour. In few studies the epigenetic profile of MTC cells has been compared with that of normal thyroid tissue, composed of follicular and parafollicular C cells. Because MTC originates from parafollicular cells, the differential expression between MTC tissues and their adjacent normal thyroid could be partially influenced by epigenetic differences in cellular populations of normal tissue. The application of cell enrichment techniques, like laser capture microdissection, could increase the specificity and sensitivity of cell-specific molecular profiling from tissue sections [95]. Future studies should better identify the epigenetic profile in MTC compared with that detected in normal parafollicular C cells, isolated through the use of laser capture microdissection.

Taking into account the reversibility of epigenetic alterations and the recent development in this field [32], epigenetic therapy may emerge for clinical use in the near future for patients with advanced MTC. Several new epigenetic tools and strategies, recently patented, such as DNMT trapping oligonucleotides, histone methyltransferase inhibitors specific for EZH2 and SMYD3, HDAC inhibitors with nitric oxide donor and antisense oligonucleotides counteracting the activity of miR-375 and miR-21, should be investigated in MTC (Fig. (**1**)).

In addition, the next efforts of research should be devoted towards: 1) the identification of epigenetic mechanisms potentially involved in drug resistance in MTC; 2) the development of novel chimeric molecules with combined tyrosine kinase inhibitor specific for RET and HDAC inhibitor activity. These molecules should combine the best characteristics

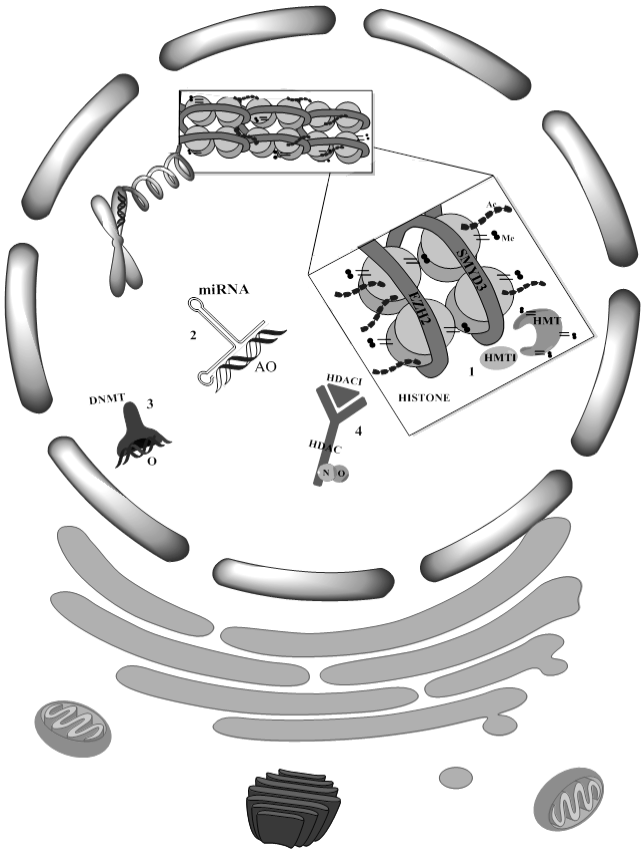

Fig. (1). Future perspectives in the epigenetic therapy of MTC: Histone methyltransferase (HMT) inhibitors (HMTI) specific for EZH2 and SMYD3 (1), antisense oligonucleotides (AO) counteracting the activity of miR-375 and miR-21 (2), DNMT trapping oligonucleotides (O) (3), HDAC inhibitors (HDACI) with nitric oxide (NO) donor (4). Me: Methyl group; Ac: Acetyl group.

of RET tyrosine kinase inhibitors and HDAC inhibitors, and exhibit a potent antitumor activity with a good tolerability to provide a clinical use in patients with MTC.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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