

Genetic and Epigenetic of Medullary Thyroid Cancer

Fatemeh Khatami¹ and Seyed Mohammad Tavangar^{*1,2}

¹Chronic Diseases Research Center, Endocrinology and Metabolism Population Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran; ²Department of Pathology, Dr. Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran

Received 19 September 2017; revised 25 October 2017; accepted 28 October 2017

ABSTRACT

Medullary thyroid carcinoma (MTC) is an infrequent, calcitonin producing neuroendocrine tumor and initiates from the parafollicular C cells of the thyroid gland. Several genetic and epigenetic alterations are collaterally responsible for medullary thyroid carcinogenesis. In this review article, we shed light on all the genetic and epigenetic hallmarks of MTC. From the genetic perspective, *RET*, *HRAS*, and *KRAS* are the most important genes that are characterized in MTC. From the epigenetic perspective, Ras-association domain family member 1A, telomerase reverse transcriptase promoter methylations, overexpression of histone methyltransferases, *EZH2* and *SMYD3*, and wide ranging increase and decrease in non-coding RNAs can be responsible for medullary thyroid carcinogenesis.

Keywords: Thyroid carcinoma, Genetic markers, Proto-oncogene

Corresponding Author: Seyed Mohammad Tavangar

Department of Pathology, Dr. Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran;
Tel. : (+98-21) 84902187; Fax: (+98-21) 88633078; E-mail: Tavangar@ams.ac.ir

INTRODUCTION

Medullary thyroid carcinoma (MTC) is a rare neuroendocrine tumor that originates from the parafollicular cells (C cells) and produces calcitonin^[1]. Approximately, a quarter of MTCs are genetic in nature; they are caused by a mutation in the rearranged during transfection (*RET*) proto-oncogene, a receptor tyrosine kinase gene, which can undergo oncogenic activation through both cytogenetic rearrangement and activation of point mutation. REven though MTC is mostly sporadic (70-80%), some hereditary patterns can be seen in 20-30% of cases: these are classified as familial MTC (FMTC) with autosomal dominant trait^[2-5]. High serum concentration of calcitonin and carcinoembryonic antigen is regularly regarded as MTC markers in blood^[6-8]. It is common knowledge that cancer is the result of genetic changes accumulated in a manner that disturbs the normal homeostatic stability between cell proliferation and cell death^[9,10]. In addition to genetic changes, epigenetic events have been considered as key indicators of carcinogenesis. Research on epigenetics

has become gradually noticeable with the aim of understanding the role of epigenetic mechanisms in the abnormal events leading to cancer^[11-13]. In fact, previous studies on cancer suggest that genetic and epigenetic alterations are two sides of the same coin responsible for morphological changes occurring during cancer progression^[12,14-16]. Moreover, the notion that early-stage cancer is not as systematically aggressive as late-stage cancer is based on the finding that gene expression profiles is alike in early-stage cancer and fully metastatic cancer^[17-19]. Thus, both genetic and epigenetic events correspond to several steps of carcinogenesis. In this review, we summarize current concepts on genetic and epigenetic changes associated with MTC and then discuss their potential relevance as biomarkers for cancer detection, diagnosis, and prognosis.

Hallmarks of genetic MTC

A mutation is a stable modification in the DNA sequence of a given gene, which may alter the normal gene function^[20]. Mutations can occur anywhere, from a single DNA building block (base pair) to a large

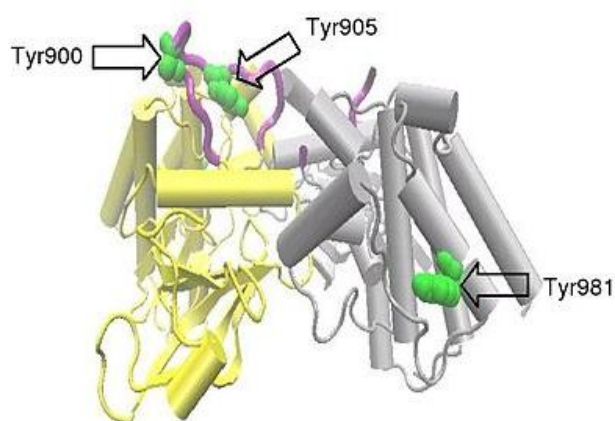


Fig. 1. A RET dimer formed between two protein molecules, each spanning amino acids 703-1012 of the RET molecule and covering RET intracellular tyrosine kinase domain. One protein molecule, molecule A, is shown in yellow and the other, molecule B, in grey. The activation loop is colored purple and selected tyrosine residues in green. Part of the activation loop from molecule B is absent. mRET proto-oncogene with three main domains: an N-terminal extracellular domain with four cadherin-like repeats and a cysteine-rich region, a hydrophobic transmembrane domain, and a cytoplasmic tyrosine kinase domain. Phosphorylation of Tyr981 and the additional tyrosines Tyr1015, Tyr1062, and Tyr1096 are not covered by the above structure, though these have been shown to be important for initiation of the intracellular signal transduction processes^[36].

segment of a chromosome, including multiple genes^[20]. Some of the mutations are heritable, i.e. they are inherited from a parent and are present throughout a person's life in virtually every cell in the body. These mutations are called germline mutations since they are present in the parent's egg or sperm cells (germ cells)^[20,21]. Other group of mutations are acquired mutations (or somatic): These happen only at a particular time during a person's life and are present only in certain cells in the body^[20]. These mutations can be caused by environmental factors, such as ultraviolet radiation from the sun or can occur if an error takes place in DNA replication during cell division. Acquired mutations in somatic cells (cells other than sperm and egg cells) cannot be passed to the next generation^[22-25].

MTC has been described in two forms: sporadic and hereditary/familial. About one-fourth of MTC patients have one of three different syndromes, which are FMTC, multiple endocrine neoplasia type 2A (MEN 2A), or type 2B (MEN 2B). Around a half of the patients with MEN 2A or MEN 2B develop pheochromocytomas^[26-30]. Moreover, 25% of patients with MEN 2A will possibly develop primary hyperparathyroidism^[28,31], while patients with MEN 2B develop marfanoid habitus and mucosal/intestinal

ganglioneuromatosis^[32]; patients with just FMTC individually develop MTC^[29,32]. In fact proto-oncogene RET germline mutations is presented in 90% of patients with hereditary MTC (FMTC, MEN 2A, or MEN 2B)^[33]. Thus, the entire hereditary syndromes are attributed to the same disease-causing gene^[34,35]. RET proto-oncogene is a tyrosine kinase receptor coding gene, and it is an element of the glial cell line-derived neurotrophic factor (GDNF) family which are classified as extracellular signaling molecules^[36]. Human RET gene with 21 exons is localized on chromosome 10 (10q11.2)^[37,38]. Like other tyrosine kinase receptors, RET is able to motivate several signaling pathways, including RAS/extracellular signal-regulated kinase (ERK), phosphatidylinositol 3-kinase (PI3K)/AKT, p38 mitogen-activated protein kinase (MAPK), and c-Jun N-terminal kinase (JNK) pathways^[30,39-44]. The typical splicing of the RET gene results in three different isoforms. The C-terminal region in RET51, RET43, and RET9 have 51, 43, and 9 amino acids, respectively^[45]. As shown in Figure 1—which is premised on the protein data bank code 2IVT—all RET protein isoforms can be subdivided into three main domains: an N-terminal extracellular domain with four cadherin-like repeats and a cysteine-rich region, a hydrophobic transmembrane domain, and a cytoplasmic tyrosine kinase domain that is divided through the 27 amino acids insertion^[36,46].

Glial cell line-derived neurotrophic factor and some other related molecules like neurturin, artemin, and persephin trigger an intracellular signaling pathway through a unique multi-component receptor systems including glycosyl-phosphatidylinositol-anchored co-receptor in addition to RET tyrosine kinase^[37,40,47-49]. These neurotrophic factors support the survival of many neurons, including the central motor dopamine

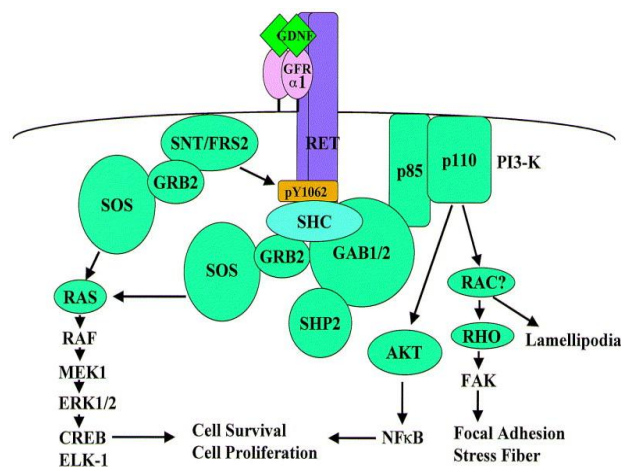


Fig. 2. Intracellular signaling pathways mediated by activated RET^[55].

neurons; they also cause peripheral autonomy^[50-52] more than renal development and facilitate regulation of spermatogonia differentiation (Fig. 2)^[53,54].

Unfortunately, unlike hereditary MTC, the etiology of sporadic MTC has not been completely elucidated. This is despite the fact that 75% of all MTC patients being sporadic without any family history of MTC or incidence of any other MEN 2-specific disease. Recently, some additional mutations, such as *HRAS* and *KRAS* mutations, have been found to be more appropriate diagnostic markers than *RET* for MTC. This observation has been suggested on the basis of high-throughput mutation profiling study (Fig. 3)^[56-59]. In few cases of sporadic MTC, a deletion of codons Glu632 and Leu633 of *RET* proto-oncogene was identified. These mutations activate the *RET* gene more effectively than the Cys634Arg missense mutation. They also induce stable dimer formation in the absence of ligand^[60,61].

In more aggressive phenotypes of sporadic MTC, the *M918 TRET* mutation has been found in around 30 to 50% of cases^[62]. Actually, the somatic *RET* mutation (*M918T*) is associated with stage of the disease and persistence of the disease after total thyroidectomy because it makes the chance of recurrence and metastasis greater than before and reduces chances of free survival^[62,63]. A connection between the presence of this somatic mutation with the more advanced pathological TNM stage has also been identified^[64-67].

Hallmarks of epigenetic MTC

The word 'epigenetics' refers to covalent modification of DNA, protein, or RNA, resulting in changes in the function and/or regulation of DNA without modification of their original sequences.

In some cases, epigenetic modifications can be stable and can pass on to future generations; mostly, however, they are vigorous modifications in response to environmental stimuli^[68]. The major mechanisms responsible for epigenetic regulation are DNA methylation, histone modifications, and non-coding RNAs^[59,69-72]. The role of epigenetics in MTC is largely defined as hypermethylation of CpG islands in the promoter region of Ras-association domain family member 1A (*RASSF1A*)^[73,74] and telomerase reverse transcriptase (*TERT*) genes^[75], overexpression of histone methyltransferases like *EZH2* and *SMYD3*^[76,77], and microRNAs (miRNAs) expression profile (Fig. 4)^[78-84].

In spite of the fact that *RASSF1A* gene promoter hypermethylation is linked to more aggressive thyroid cancers, CpG island methylation of tumor-associated genes—such as *p16*, *TSHR*, *MGMT*, and *PTEN*—have not shown any significant degree of hypermethylation in MTC^[73,85-87]. Nevertheless, the existence of methylation in the promoter region of *TERT* gene and consequent variation of DNA copy numbers within a huge cohort study of MTC cases have been documented^[75]. In fact, telomerase is a protein responsible for keeping and fixing telomeres of the chromosomes. Its activation by *TERT* has been increased frequently in many types of cancers, including MTC^[86]. Wang and his colleagues^[75] have reported that *TERT* gene hypermethylation is related to high DNA copy number, and MTC patients with higher *TERT* methylation have lower chances of survival^[75].

Epigenetic control through histone methyltransferases in more aggressive forms of MTC have recently been investigated by Sponziello *et al.*^[76]. In fact, the platform of epigenetic regulatory factors and their

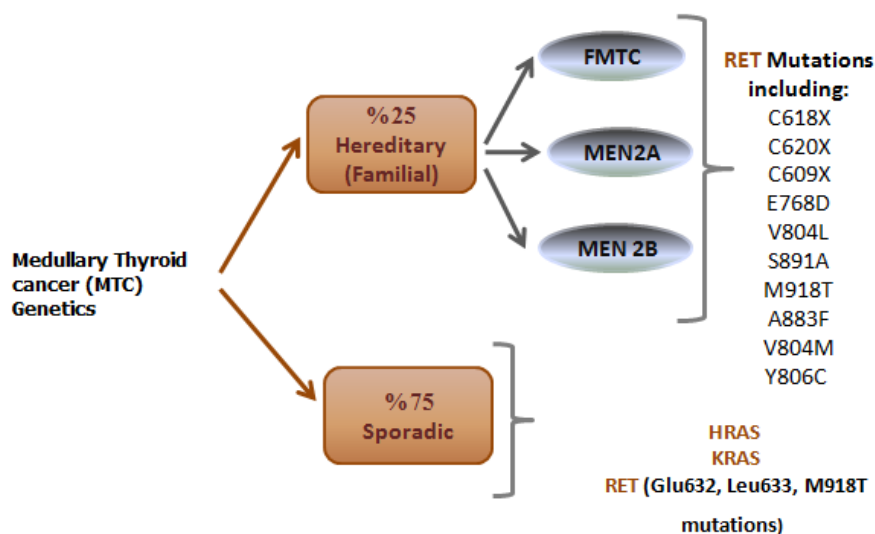


Fig. 3. Genetics of medullary thyroid cancer.

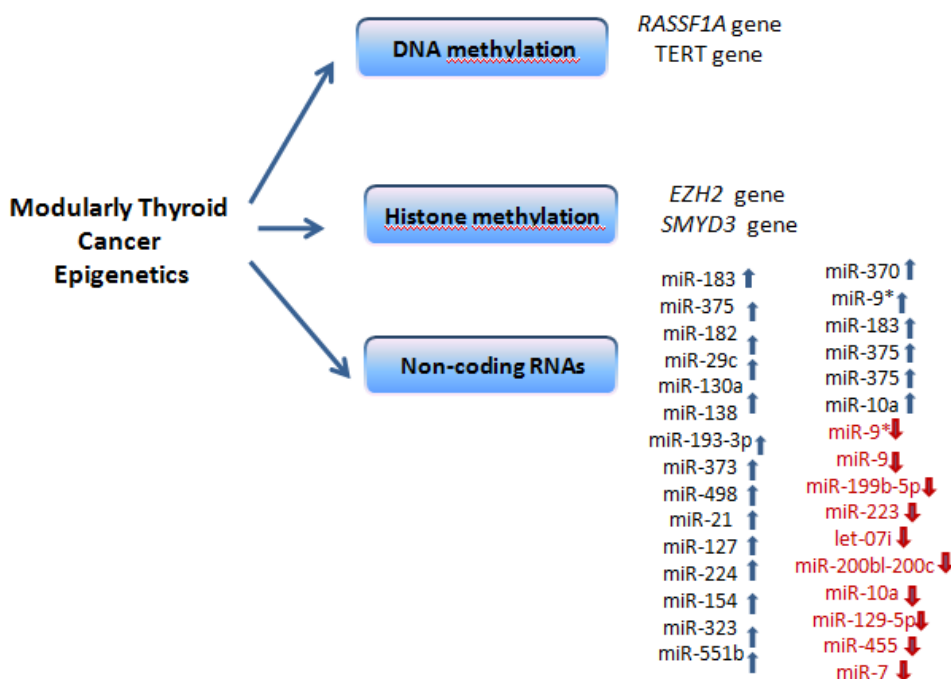


Fig. 4. Epigenetics of medullary thyroid cancer.

mRNA levels profiling in a big cohort of MTC tissues has revealed the fact that overexpression of two histone methyltransferases, *EZH2* and *SMYD3*, is connected with higher risk of metastases, disease consistency, and finally death of patients: These can be prognostic biomarkers for MTC^[76,88]. Remarkably, gene expression profile was free of *RET* or *RAS* mutations.

The most common mutation is related to the *RET* mutation in genetic alteration of MTC tumorigenesis. The transcriptional activity of *RET* is controlled by epigenetic processes as well. It has been demonstrated that in colorectal cancer CpGisland methylation of *RET* gene promoter is a potential prognostic marker for stage II of the disease^[89,90]. Patients with considerable hypermethylated *RET* have worse overall survival compared to those with unmethylated *RET* promoter^[91]. Significantly, *RET* expression is regulated by a transcription factor, homeobox B5^[92], which is related to the multi-species conserved sequence in the primary intron of the *RET* gene in addition to the higher level of *RET* transcription. Another regulating

mechanism of *RET* transcription level is acetylation because in human neuroblastoma cells with a low *RET* mRNA level, histone deacetylase inhibitor and sodium butyrate cause hyper acetylation and increase the transcription of *RET* gene^[93].

MiRNAs are small non-coding RNAs with the lengths of 20-23 nucleotides; they are classified as epigenetic modifiers^[94]. MiRNAs are non-protein-coding RNAs that alter gene expression through mRNA translation inhibition or by means of the target molecule degrading^[94,95]. In reality, mutations or abnormal expression of miRNAs are more often related to the pathogenesis of a wide range of cancers because they affect both tumor suppressors and oncogenes^[96]. In spite of the fact that several studies have highlighted the role of miRNA profiling of MTC and its malignancy (Table 1), due to difficulty in obtaining normal C cells, none of the existing literature has compared miRNA profiles between MTC and normal C cells^[97].

Ten miRNAs were shown to have different expression pattern between sporadic MTC and

Table 1. A list of suggesting microRNA (miRNA) in medullary thyroid cancer

A signature of increased and decreased miRNA associated with MTC

Increasing miRNA	miR-183, miR-375, miR-182, miR-29c, miR-130a, miR-138, miR-193-3p, miR-373, miR-498, miR-21, miR-127, miR-224, miR-154, miR-323, miR-551b, miR-370, miR-9, miR-183, miR-375, miR-375, miR-10a
Decreasing miRNA	miR-199b-5p, miR-223, let-07i, miR-200b-200c, miR-10a, miR-129-5p, miR-455, and miR-7, miR9

hereditary MTC^[78]. In correlation with clinical outcomes, high levels of *miR-183* and *miR-375* were linked to the lateral lymph node and distant metastases^[78]. Significantly, similarity in miRNA profiles of *miR-183*, *miR-375*, and *miR-9-3p* (*miR-9*) between primary tumor tissues and lymph node metastasis tissues was observed^[79]. This was further to the role of *miR-9-3pin* in regulation of autophagy^[80]. More than that, a comparison of miRNA profiling between primary and metastatic forms of MTC highlighted 10 deregulated miRNAs^[81-83,98]. There is possibility that the constitutive activation of RET, as a crucial occurrence in MTC tumor genesis, is regulated through epigenetic mechanisms like miRNAs^[81,99,100].

From genetic point of view, *RET* mutations in codons 609 (*C609X*), 618 (*C618X*), 620 (*C620X*), 786 (*E768D*), 804 (*V804L*), 819 (*S891A*), 918 (*M918T*), 833 (*A883F*), 804 (*V804M*), 806 (*Y806C*), 632 (*Glu632*), 633 (*Leu633*), and 918 (*M918T*) as well as *HRAS*, and *KRAS* mutations are the most important mutations that cause medullary thyroid carcinogenesis. From epigenetic perspective, *RASSF1*, *TERT* promoter methylations, histone methyltransferases (*EZH2* and *SMYD3*) overexpression, and wide ranging increase and decrease of non-coding RNAs contribute to medullary thyroid carcinogenesis.

ACKNOWLEDGMENTS

This article is a part of a larger project granted by the Iranian organization of the National Institute for Medical Research Development (NIMAD, grant number: 957222).

CONFLICT OF INTEREST. None declared.

REFERENCES

- Alford EM, Hu MI, Ahn P, Lamont JP. Cancer Management: Thyroid and Parathyroid Cancers. 13th ed. Cancer Network: 2011.
- Leboulleux S, Baudin E, Travaglini JP, Schlumberger M. Medullary thyroid carcinoma. *Clinical endocrinology (Oxf)* 2004; **61**(3): 299-310.
- Haghpahan V, Soliemanpour B, Heshmat R, Mosavi-Jarrahi AR, Tavangar SM, Malekzadeh R, Larijani B. Endocrine cancer in Iran: based on cancer registry system. *Indian journal of cancer* 2006; **43**(2):80-85.
- Larijani B, Mohagheghi MA, Bastanagh MH, Mosavi-Jarrahi AR, Haghpahan V, Tavangar SM, Bandarian F, Khaleghian N. Primary thyroid malignancies in Tehran, Iran. *Medical principles and practice* 2005; **14**(6): 396-400.
- Larijani Mohammad Bagher A, Shirzad M, Mohagheghi SMA, Haghpahan V, Mousavi Jarahi SAR, Tavangar SM, Vassigh AR, Hosseinnzhad A, Bandarian F, Baradar Jalili R. Epidemiologic analysis of the Tehran Cancer Institute data system registry (TCIDSR). *Asian Pacific journal of cancer prevention* 2004; **5**(1): 36-39.
- Fragu P. Calcitonin's fantastic voyage: From hormone to marker of a genetic disorder. *Gesnerus* 2007; **64**(1-2): 69-92.
- Barbet J, Champion L, Kraeber-Bodéré F, Chatal JF, Group GS. Prognostic impact of serum calcitonin and carcinoembryonic antigen doubling-times in patients with medullary thyroid carcinoma. *The journal of clinical endocrinology and metabolism* 2005; **90**(11): 6077-6084.
- Haddadi-Nezhad S, Larijani B, Tavangar SM, Nouraei SM. Comparison of fine-needle-nonaspiration with fine-needle-aspiration technique in the cytologic studies of thyroid nodules. *Endocrine pathology* 2003; **14**(4): 369-373.
- Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000; **100**(1): 57-70.
- Elenbaas B, Spirio L, Koerner F, Fleming MD, Zimonjic DB, Donaher JL, Popescu NC, Hahn WC, Weinberg RA. Human breast cancer cells generated by oncogenic transformation of primary mammary epithelial cells. *Genes and development* 2001; **15**(1): 50-65.
- Jones PA, Baylin SB. The fundamental role of epigenetic events in cancer. *Nature reviews genetics* 2002; **3**(6): 415-428.
- Feinberg AP, Ohlsson R, Henikoff S. The epigenetic progenitor origin of human cancer. *Nature reviews genetics* 2006; **7**(1): 21-33.
- Momeny M, Sabourinejad Z, Zarrinrad G, Moghaddaskho F, Eyvani H, Yousefi H, Mirshahvaladi S, Poursani EM, Barghi F, Poursheikhani A, Dardaee L, Bashash D, Ghazi-Khansari M, Tavangar SM, Dehpour AR, Yaghmaie M, Alimoghaddam K, Ghavamzadeh A, Ghaffari SH. Anti-tumour activity of tivozanib, a pan-inhibitor of VEGF receptors, in therapy-resistant ovarian carcinoma cells. *Scientific reports* 2017; **7**: 45954.
- You JS, Jones PA. Cancer genetics and epigenetics: two sides of the same coin? *Cancer cell* 2012; **22**(1): 9-20.
- Zingg J-M, Jones PA. Genetic and epigenetic aspects of DNA methylation on genome expression, evolution, mutation and carcinogenesis. *Carcinogenesis* 1997; **18**(5): 869-882.
- Nasseri-Moghaddam S, Malekzadeh R, Sotoudeh M, Tavangar M, Azimi K, Sohrabpour AA, Mostadjabi P, Fathi H, Minapoor M. Lower esophagus in dyspeptic Iranian patients: A prospective study. *Journal of gastroenterology and hepatology* 2003; **18**(3): 315-321.
- Schedin P, Elias A. Multistep tumorigenesis and the microenvironment. *Breast cancer research* 2004; **6**(2): 93-101.
- Tavangar SM, Monajemzadeh M, Larijani B, Haghpahan V. Immunohistochemical study of oestrogen receptors in 351 human thyroid glands. *Singapore medical journal* 2007; **48**(8): 744-747.

19. Amoli MM, Yazdani N, Amiri P, Sayahzadeh F, Haghpanah V, Tavangar SM, Amirzargar A, Ghaffari H, Nikbin B, Larijani B, Mostaan LV, Bazzaz JT. HLA-DR association in papillary thyroid carcinoma. *Disease markers* 2010; **28**(1): 49-53.
20. Loewe L. Genetic mutation. *Nature education* 2008; **1**(1): 113.
21. Greenman C, Stephens P, Smith R, Dalgliesh GL, Hunter C, Bignell G, Davies H, Teague J, Butler A, Stevens C, Edkins S, O'Meara S, Vastrik I, Schmidt EE, Avis T, Barthorpe S, Bhamra G, Buck G, Choudhury B, Clements J, Cole J, Dicks E, Forbes S, Gray K, Halliday K, Harrison R, Hills K, Hinton J, Jenkinson A, Jones D, Menzies A, Mironenko T, Perry J, Raine K, Richardson D, Shepherd R, Small A, Tofts C, Varian J, Webb T, West S, Widaa S, Yates A, Cahill DP, Louis DN, Goldstraw P, Nicholson AG, Brasseur F, Looijenga L, Weber BL, Chiew YE, DeFazio A, Greaves MF, Green AR, Campbell P, Birney E, Easton DF, Chenevix-Trench G, Tan MH, Khoo SK, Teh BT, Yuen ST, Leung SY, Wooster R, Futreal PA, Stratton MR. Patterns of somatic mutation in human cancer genomes. *Nature* 2007; **446**(7132): 153-158.
22. Harper PS. Practical Genetic Counselling 7th Edition: CRC Press; 2010.
23. Erickson RP. Somatic gene mutation and human disease other than cancer: An update. *Mutation research* 2010; **705**(2): 96-106.
24. Miller BF, Keane CB. Encyclopedia and Dictionary of Medicine, Nursing, and Allied Health. 7th ed. Elsevier: 2017.
25. Tavangar SM, Sharifabrizi A, Soroush AR. Her-2/neu over-expression correlates with more advanced disease in Iranian colorectal cancer patients. *Medical science monitor* 2005; **11**(3): CR123-CR126.
26. Romei C, Pardi E, Cetani F, Elisei R. Genetic and clinical features of multiple endocrine neoplasia types 1 and 2. *Journal of oncology* 2012; **2012**: Article ID 705036.
27. Wells SA Jr, Pacini F, Robinson BG, Santoro M. Multiple Endocrine Neoplasia Type 2 and Familial Medullary Thyroid Carcinoma: An Update. *The Journal of clinical endocrinology and metabolism* 2013; **98**(8): 3149-3164.
28. Melmed S, Polonsky K, Larsen PR, Kronenberg H. Williams Textbook of Endocrinology. 12th ed. Elsevier: 2011.
29. Orth DN, Kovacs W, DeBold C. The adrenal Cortex. In: Wilson JD, Foster DW eds. Williams Textbook of Endocrinology. Philadelphia: Saunders; 1992.
30. Khatami F, Tavangar SM. Current diagnostic status of pheochromocytoma and future perspective: A mini review. *Iranian journal of pathology* 2017; **12**(3): 313-322.
31. Giusti F, Tonelli F, Brandi ML. Primary hyperparathyroidism in multiple endocrine neoplasia type 1: when to perform surgery? *Clinics* 2012; **67**(Suppl 1): 141-144.
32. Cetin D, Unübol M, Soyder A, Güney E, Coşkun A, Ozbaş S, Unsal A, Erkuş M. Coexistence of multiple endocrine neoplasia type 2B and chilaiditi sign: A case report. *Case reports in endocrinology* 2012; **2012**: 360328.
33. Lodish MB, Stratakis CA. RET oncogene in MEN2, MEN2B, MTC, and other forms of thyroid cancer: molecular genetics and therapeutic advances. *Expert review of anticancer therapy* 2008; **8**(4): 625-632.
34. Hofstra RM, Landsvater RM, Ceccherini I, Stulp RP, Stelwagen T, Luo Y, Pasini B, Höppener JW, van Amstel HK, Romeo G, Lips CJM, Buys HCM. A mutation in the RET proto-oncogene associated with multiple endocrine neoplasia type 2B and sporadic medullary thyroid carcinoma. *Nature* 1994; **367**(6461): 375-376.
35. Donis-Keller H, Dou S, Chi D, Carlson KM, Toshima K, Lairmore TC, Howe JR, Moley JF, Goodfellow P, Wells SA Jr. Mutations in the RET proto-oncogene are associated with MEN 2A and FMTC. *Human molecular genetics* 1993; **2**(7): 851-856.
36. Knowles PP, Murray-Rust J, Kjør S, Scott RP, Hanrahan S, Santoro M, Ibáñez CF, McDonald NQ. Structure and chemical inhibition of the RET tyrosine kinase domain. *Journal of biological chemistry* 2006; **281**(44): 33577-33587.
37. Takahashi M, Ritz J, Cooper GM. Activation of a novel human transforming gene, ret, by DNA rearrangement. *Cell* 1985; **42**(2): 581-588.
38. Ceccherini I, Bocciardi R, Luo Y, Pasini B, Hofstra R, Takahashi M, Romeo G. Exon structure and flanking intronic sequences of the human RET proto-oncogene. *Biochemical and biophysical research communications*. 1993; **196**(3):1288-1295.
39. Feng L, Wang CY, Jiang H, Oho C, Dugich-Djordjevic M, Mei L, Lu L. Differential signaling of glial cell line-derived neurotrophic factor and brain-derived neurotrophic factor in cultured ventral mesencephalic neurons. *Neuroscience* 1999; **93**(1): 265-73.
40. Soler RM, Dolcet X, Encinas M, Egea J, Bayascas JR, Comella JX. Receptors of the glial cell line-derived neurotrophic factor family of neurotrophic factors signal cell survival through the phosphatidylinositol 3-kinase pathway in spinal cord motoneurons. *Journal of neuroscience* 1999; **19**(21): 9160-9169.
41. Murakami H, Iwashita T, Asai N, Shimono Y, Iwata Y, Kawai K, Takahashi M. Enhanced phosphatidylinositol 3-kinase activity and high phosphorylation state of its downstream signalling molecules mediated by ret with the MEN 2B mutation. *Biochemical and biophysical research communications* 1999; **262**(1):68-75.
42. Lattayer S, Klein-Hitpass L, Khandanpour C, Zwanziger D, Poeppel T, Schmid K, Führer D, Moeller LC. A 6-base pair in frame germline deletion in exon 7 of RET leads to increased RET phosphorylation, ERK activation, and MEN2A. *The journal of clinical endocrinology and metabolism* 2016; **101**(3): 1016-10122.
43. Hong DS, Cabanillas ME, Wheler J, Naing A, Tsimberidou AM, Ye L, Busaidy NL, Waguespack SG, Hernandez M, El Naggar AK, Bidyasar S, Wright J, Sherman SI, Kurzrock R. Inhibition of the

- Ras/Raf/MEK/ERK and RET kinase pathways with the combination of the multikinase inhibitor sorafenib and the farnesyltransferase inhibitor tipifarnib in medullary and differentiated thyroid malignancies. *The journal of clinical endocrinology and metabolism* 2011; **96**(4): 997-1005.
44. Ouyang B, Knauf JA, Smith EP, Zhang L, Ramsey T, Yusuff N, Batt D, Fagin JA. Inhibitors of Raf kinase activity block growth of thyroid cancer cells with RET/PTC or BRAF mutations *in vitro* and *in vivo*. *Clinical cancer research* 2006; **12**(6): 1785-1793.
 45. Myers SM, Eng C, Ponder BA, Mulligan LM. Characterization of RET proto-oncogene 3' splicing variants and polyadenylation sites: a novel C-terminus for RET. *Oncogene* 1995; **11**(10): 2039-2045.
 46. Arighi E, Borrello MG, Sariola H. RET tyrosine kinase signaling in development and cancer. *Cytokine and growth factor reviews* 2005; **16**(4): 441-467.
 47. Van Weering DH, Medema JP, Van Puijenbroek A, Burgering BM, Baas PD, Bos JL. Ret receptor tyrosine kinase activates extracellular signal-regulated kinase 2 in SK-N-MC cells. *Oncogene* 1995; **11**(11): 2207-2214.
 48. Xing S, Furminger TL, Tong Q, Jhiang SM. Signal transduction pathways activated by RET oncoproteins in PC12 pheochromocytoma cells. *Journal of Biological Chemistry* 1998; **273**(9): 4909-4914.
 49. Hayashi H, Ichihara M, Iwashita T, Murakami H, Shimono Y, Kawai K, Kurokawa K, Murakumo Y, Imai T, Funahashi H, Nakao A, Takahashi M. Characterization of intracellular signals via tyrosine 1062 in RET activated by glial cell line-derived neurotrophic factor. *Oncogene* 2000; **19**(39):4469-4475.
 50. De Vita G, Melillo RM, Carlomagno F, Visconti R, Castellone MD, Bellacosa A, Billaud M, Fusco A, Tschlis PN, Santoro M. Tyrosine 1062 of RET-MEN2A mediates activation of Akt (protein kinase B) and mitogen-activated protein kinase pathways leading to PC12 cell survival. *Cancer research* 2000; **60**(14): 3727-3731.
 51. Paratcha G, Ledda F, Baars L, Couplier M, Besset V, Anders J, Scott R, Ibañez CF. Released GFR α 1 potentiates downstream signaling, neuronal survival, and differentiation via a novel mechanism of recruitment of c-Ret to lipid rafts. *Neuron* 2001; **29**(1): 171-84.
 52. Kajbafzadeh AM, Payabvash S, Salmasi AH, Monajemzadeh M, Tavangar SM. Smooth muscle cell apoptosis and defective neural development in congenital ureteropelvic junction obstruction. *Journal of urology* 2006; **176**(2): 718-723.
 53. Costantini F. GDNF/Ret signaling and renal branching morphogenesis: From mesenchymal signals to epithelial cell behaviors. *Organogenesis* 2010; **6**(4): 252-262.
 54. Waxman S. Molecular Neurology. 1st ed. USA: Academic Press; 2007.
 55. Takahashi M. The GDNF/RET signaling pathway and human diseases. *Cytokine growth factor reviews* 2001; **12**(4): 361-373.
 56. Simbolo M, Mian C, Barollo S, Fassan M, Mafficini A, Neves D, Scardoni M, Pennelli G, Rugge M, Pelizzo MR, Cavedon E, Fugazzola L, Scarpa A. High-throughput mutation profiling improves diagnostic stratification of sporadic medullary thyroid carcinomas. *Virchows archiv* 2014; **465**(1): 73-78.
 57. Moura MM, Cavaco BM, Leite V. RAS proto-oncogene in medullary thyroid carcinoma. *Endocrine-related cancer* 2015; **22**(5): R235-R252.
 58. Romei C, Cosci B, Renzini G, Bottici V, Molinaro E, Agate L, et al. RET genetic screening of sporadic medullary thyroid cancer (MTC) allows the preclinical diagnosis of unsuspected gene carriers and the identification of a relevant percentage of hidden familial MTC (FMTC). *Clinical endocrinology (Oxford)* 2011; **74**(2): 241-247.
 59. Tabriz HM, Adabi K, Lashkari A, Heshmat R, Haghpanah V, Larijani B, Tavangar SM. Immunohistochemical analysis of nm23 protein expression in thyroid papillary carcinoma and follicular neoplasm. *Pathology research and practice* 2009; **205**(2): 83-87.
 60. Marsh DJ, Learoyd DL, Andrew SD, Krishnan L, Pojer R, Richardson AL, Delbridge L, Eng C, Robinson BG. Somatic mutations in the RET proto-oncogene in sporadic medullary thyroid carcinoma. *Clinical endocrinology (Oxford)* 1996; **44**(3): 249-257.
 61. Bongarzone I, Vigano E, Alberti L, Mondellini P, Uggeri M, Pasini B, Borrello MG, Pierotti MA. The Glu632-Leu633 deletion in cysteine rich domain of Ret induces constitutive dimerization and alters the processing of the receptor protein. *Oncogene* 1999; **18**(34): 4833-4838.
 62. Elisei R, Cosci B, Romei C, Bottici V, Renzini G, Molinaro E, Agate L, Vivaldi A, Faviana P, Basolo F, Miccoli P, Berti P, Pacini F, Pinchera A. Prognostic significance of somatic RET oncogene mutations in sporadic medullary thyroid cancer: a 10-year follow-up study. *The journal of clinical endocrinology and metabolism* 2008; **93**(3): 682-687.
 63. Van Veelen W, De Groot J, Acton D, Hofstra R, Höppener J, Links T, Lips CJ. Medullary thyroid carcinoma and biomarkers: past, present and future. *Journal of internal medicine* 2009; **266**(1): 126-140.
 64. Dvorakova S, Vaclavikova E, Sykorova V, Vcelak J, Novak Z, Duskova J, Ryska A, Laco J, Cap D, Kodetova D, Kodet R, Krskova L, Vlcek P, Astl J, Vesely D, Bendlova B. Somatic mutations in the RET proto-oncogene in sporadic medullary thyroid carcinomas. *Molecular and cellular endocrinology* 2008; **284**(1-2): 21-27.
 65. Saffar H, Sanii S, Emami B, Heshmat R, Panah VH, Azimi S, Tavangar SM. Evaluation of MMP2 and caspase-3 expression in 107 cases of papillary thyroid carcinoma and its association with prognostic factors. *Pathology research and practice* 2013; **209**(3): 195-199.
 66. Sanii S, Saffar H, Tabriz HM, Qorbani M, Haghpanah V, Tavangar SM. Expression of matrix metalloproteinase-2, but not caspase-3, facilitates distinction between benign and malignant thyroid follicular neoplasms. *Asian Pacific journal of cancer prevention* 2012; **13**(5): 2175-2178.

67. Attaran SY, Omrani G, Tavangar SM. Lympho-epithelial-like intrathyroidal thymic carcinoma with foci of squamous differentiation. Case report. *APMIS* 1996; **104**(6): 419-423.
68. Bird A. Perceptions of epigenetics. *Nature* 2007; **447**(7143): 396-398.
69. Sharma S, Kelly TK, Jones PA. Epigenetics in cancer. *Carcinogenesis* 2010; **31**(1): 27-36.
70. Khatami F, Noorinayer B, Ghiasi S, Mohebi R, Hashemi M, Zali MR. Single nucleotide polymorphisms of DNA methyltransferase 1 gene and gastric cancer in Iranian patients: a case control study. *Iranian journal of cancer prevention* 2012; **1**(3): 111-118.
71. Khatami F, Noorinayer B, Mohebi SR, Ghiasi S, Mohebi R, Hashemi M, et al. Effects of amino acid substitution polymorphisms of two DNA methyltransferases on susceptibility to sporadic colorectal cancer. *Asian Pacific journal of cancer prevention* 2009; **10**(6): 1183-1188.
72. Khatami F, Mohebi SR, Ghiasi S, Haghghi MM, Safaee A, Hashemi M, Zali MR. Amino acid substitution polymorphisms of two DNA methyltransferases and susceptibility to sporadic colorectal cancer. *Gastroenterology and hepatology from bed to bench* 2009; **1**(3): 105-112.
73. Schagdarsurengin U, Gimm O, Hoang-Vu C, Dralle H, Pfeifer GP, Dammann R. Frequent epigenetic silencing of the CpG island promoter of RASSF1A in thyroid carcinoma. *Cancer research* 2002; **62**(13): 3698-3701.
74. Mohammadi-Asl J, Larijani B, Khorgami Z, Tavangar SM, Haghpanah V, Kheirollahi M, Mehdipour P. Qualitative and quantitative promoter hypermethylation patterns of the P16, TSHR, RASSF1A and RAR β 2 genes in papillary thyroid carcinoma. *Medical oncology* 2011; **28**(4): 1123-1128.
75. Wang N, Kjellin H, Sofiadis A, Fotouhi O, Juhlin CC, Bäckdahl M, Zedenius J, Xu D, Lehtiö J, Larsson C. Genetic and epigenetic background and protein expression profiles in relation to telomerase activation in medullary thyroid carcinoma. *Oncotarget* 2016; **7**(16): 21332-21346.
76. Sponziello M, Durante C, Boichard A, Dima M, Puppini C, Verrienti A, Tamburrano G, Di Rocco G, Redler A, Lacroix L, Bidart JM, Schlumberger M, Damante G, Russo D, Filetti S. Epigenetic-related gene expression profile in medullary thyroid cancer revealed the overexpression of the histone methyltransferases EZH2 and SMYD3 in aggressive tumours. *Molecular and cellular endocrinology* 2014; **392**(1-2): 8-13.
77. Haghpanah V, Shooshtarizadeh P, Heshmat R, Larijani B, Tavangar SM. Immunohistochemical analysis of survivin expression in thyroid follicular adenoma and carcinoma. *Applied Immunohistochemistry and molecular morphology* 2006; **14**(4): 422-425.
78. Abraham D, Jackson N, Gundara JS, Zhao J, Gill AJ, Delbridge L, Robinson BG, Sidhu SB. MicroRNA profiling of sporadic and hereditary medullary thyroid cancer identifies predictors of nodal metastasis, prognosis, and potential therapeutic targets. *Clinical cancer research* 2011; **17**(14): 4772-4781.
79. Gundara JS, Zhao JT, Gill AJ, Clifton-Bligh R, Robinson BG, Delbridge L, Sidhu SB. Nodal metastasis microRNA expression correlates with the primary tumour in MTC. *ANZ journal of surgery* 2014; **84**(4): 235-239.
80. Gundara JS, Zhao J, Gill AJ, Lee JC, Delbridge L, Robinson BG, McLean C, Serpell J, Sidhu SB. Noncoding RNA blockade of autophagy is therapeutic in medullary thyroid cancer. *Cancer medicine* 2015; **4**(2): 174-182.
81. Santarpia L, Calin GA, Adam L, Ye L, Fusco A, Giunti S, Thaller C, Paladini L, Zhang X, Jimenez C, Trimarchi F, El-Naggar AK, Gagel RF. A miRNA signature associated with human metastatic medullary thyroid carcinoma. *Endocrine-related cancer* 2013; **20**(6): 809-823.
82. Mian C, Pennelli G, Fassan M, Balistreri M, Barollo S, Cavedon E, Galuppini F, Pizzi M, Vianello F, Pelizzo MR, Girelli ME, Rugge M, Opocher G. MicroRNA profiles in familial and sporadic medullary thyroid carcinoma: preliminary relationships with RET status and outcome. *Thyroid* 2012; **22**(9): 890-896.
83. Hudson J, Duncavage E, Tamburrino A, Salerno P, Xi L, Raffeld M, Moley J, Chernock RD. Overexpression of miR-10a and miR-375 and downregulation of YAP1 in medullary thyroid carcinoma. *Experimental and molecular pathology* 2013; **95**(1): 62-67.
84. Duan L, Hao X, Liu Z, Zhang Y, Zhang G. MiR-129-5p is down-regulated and involved in the growth, apoptosis and migration of medullary thyroid carcinoma cells through targeting RET. *FEBS letters* 2014; **588**(9): 1644-1651.
85. Schagdarsurengin U, Gimm O, Dralle H, Hoang-Vu C, Dammann R. CpG island methylation of tumor-related promoters occurs preferentially in undifferentiated carcinoma. *Thyroid* 2006; **16**(7): 633-642.
86. Tavangar SM, Larijani B, Mahta A, Hosseini SMA, Mehrazine M, Bandarian F. Craniopharyngioma: A clinicopathological study of 141 cases. *Endocrine pathology* 2004; **15**(4): 339-344.
87. Sarmadi S, Izadi-Mood N, Sotoudeh K, Tavangar SM. Altered PTEN expression; a diagnostic marker for differentiating normal, hyperplastic and neoplastic endometrium. *Diagnostic pathology* 2009; **4**:41.
88. Joo LJS, Zhao JT, Gild ML, Glover AR, Sidhu SB. Epigenetic regulation of RET receptor tyrosine kinase and non-coding RNAs in MTC. *Molecular and cellular endocrinology* 2017; doi: 10.1016/j.mce.2017.03.014
89. Munnes M, Patrone G, Schmitz B, Romeo G, Doerfler W. A 5'-CG-3'-rich region in the promoter of the transcriptionally frequently silenced RET proto-oncogene lacks methylated cytidine residues. *Oncogene* 1998; **17**(20): 2573-2583.
90. Draht MX, Smits KM, Tournier B, Jooste V, Chapusot C, Carvalho B, Cleven AH, Derks S, Wouters KA, Belt EJ⁷, Stockmann HB, Bril H, Weijenberg MP, van den Brandt PA, de Bruïne AP, Herman JG, Meijer GA, Piard F, Melotte V, van Engeland M. Promoter CpG island methylation of RET predicts poor prognosis in stage II colorectal cancer patients. *Molecular oncology*

- 2014; **8**(3): 679-688.
91. Draht MX, Smits KM, Jooste V, Tournier B, Vervoort M, Ramaekers C, Chapusot C, Weijenberg MP, van Engeland M, Melotte V. Analysis of RET promoter CpG island methylation using methylation-specific PCR (MSP), pyrosequencing, and methylation-sensitive high-resolution melting (MS-HRM): impact on stage II colon cancer patient outcome. *Clinical epigenetics* 2016; **8**: 44.
92. Zhu JJ, Kam MK, Garcia-Barceló MM, Tam PK, Lui VC. HOXB5 binds to multi-species conserved sequence (MCS+ 9.7) of RET gene and regulates RET expression. *International journal of biochemistry and cell biology* 2014; **51**: 142-149.
93. Puppo F, Griseri P, Fanelli M, Schena F, Romeo G, Pelicci P, et al. Cell-line specific chromatin acetylation at the Sox10-Pax3 enhancer site modulates the RET proto-oncogene expression. *FEBS letters* 2002; **523**(1-3): 123-127.
94. Winter J, Jung S, Keller S, Gregory RI, Diederichs S. Many roads to maturity: microRNA biogenesis pathways and their regulation. *Nature cell biology* 2009; **11**(3): 228-234.
95. Peschansky VJ, Wahlestedt C. Non-coding RNAs as direct and indirect modulators of epigenetic regulation. *Epigenetics* 2014; **9**(1): 3-12.
96. Singh P, Soon PS, Feige JJ, Chabre O, Zhao JT, Cherradi N, Lalli E, Sidhu SB. Dysregulation of microRNAs in adrenocortical tumors. *Molecular and cellular endocrinology* 2012; **351**(1): 118-128.
97. Nikiforova MN, Tseng GC, Steward D, Diorio D, Nikiforov YE. MicroRNA expression profiling of thyroid tumors: biological significance and diagnostic utility. *Journal of clinical endocrinology and metabolism* 2008; **93**(5): 1600-1608.
98. Lassalle S, Zangari J, Popa A, Ilie M, Hofman V, Long E, Patey M, Tissier F, Belléannée G, Trouette H, Catargi B, Peyrottes I, Sadoul JL, Bordone O, Bonnetaud C, Butori C, Bozec A, Guevara N, Santini J, Hénaoui IS, Lemaire G, Blanck O, Vielh P, Barbry P, Mari B, Brest P, Hofman P. MicroRNA-375/SEC23A as biomarkers of the in vitro efficacy of vandetanib. *Oncotarget* 2016; **7**(21): 30461-30467.
99. Hu Y, Wang H, Chen E, Xu Z, Chen B, Lu G. Candidate microRNAs as biomarkers of thyroid carcinoma: a systematic review, meta-analysis, and experimental validation. *Cancer medicine* 2016; **5**(9): 2602-2614.
100. Samimi H, Zaki Dizaji M, Ghadami M, Shahzadeh F, Khashayar P, Soleimani M, Larijani B, Haghpanah V. MicroRNAs networks in thyroid cancers: focus on miRNAs related to the fascin. *Journal of diabetes and metabolic disorders* 2013; **12**(1): 31.