REVIEW

Differentiated Thyroid Cancer Genetic Mechanisms - Focus on Vitamin D Receptor and Methylenetetrahydrofolate Reductase Gene Polymorphisms

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Abstract

Thyroid cancer is the most frequent endocrine cancer representing 1-1.5% of all cancers. Approximately 90% of these are differentiated thyroid carcinomas (DTC) with a favorable prognosis and cure rate. DTC has recently witnessed an increase in incidence with a relatively stable mortality rate, mostly due to intensive screening. Despite being considered indolent and the 10-year survival rate being above 90%, local or distant recurrence can be observed in up to 20% of cases. Mutations in BRAF, RET, RAS, NTRK1, PAX8-PPARG are commonly found in DTC but studies show that genetic alterations with apparently no correlation to DTC might improve or aggravate prognosis.

Vitamin D deficiency and vitamin D receptor (VDR) gene polymorphisms is consider to be one of these factors, due to the fact that it exerts immunological and antineoplastic functions throught its antiproliferative and prodifferentiating actions. FokI gene polymorphism has been associated with later stage and negative prognosis in different studies. Also, polymorphisms of genes involved in folate metabolism (MTHFR, MTR, RFC1) may be incriminated in carcinogenesis, folate being an extremely important factor in DNA synthesis.

Studies suggest that through correction and avoidance of incriminated neoplastic agents, thyroid cancer incidence, evolution and prognosis might improve significantly. For this to be possible we need to be aware of the molecular pathways these environmental factors use to exert their carcinogenic effects.

Keywords: thyroid cancer, papillary, follicular, vitamin D, DTC, BRAF, VDR, MTHFR.

Rezumat

Cancerul tiroidian este cel mai frecvent cancer endocrin, reprezentând 1-1,5% din totalitatea cancerelor. Aproximativ 90% dintre acestea sunt carcinoame tiroidiene diferențiate (DTC) și au prognostic și rată de vindecare favorabile. Recent, datorită screening-ului extensiv, incidența DTC a crescut, însă acesta și-a menținut o rată de mortalitate constantă. Deși este considerat indolent și are o rată de supraviețuire la 10 ani de 90%, recidiva locală sau la distanță este descrisă în 20% din cazuri. Mutații ale genelor BRAF, RET, RAS, NTRK1, PAX8-PPARG sunt frecvente în DTC, însă studiile recente sugerează că modificări genetice necorelate specific cu DTC pot ameliora sau agrava prognosticul acestuia.

Deficitul de vitamina D și polimorfismele genei receptorului de vitamina D (VDR) sunt considerați astfel de factori datorită rolului imunologic și antineoplazic desfășurat prin acțiunile antiproliferative și de prodiferențiere ale vitaminei D. Polimorfismul FokI a fost corelat cu un stadiu mai avansat și prognosticul negativ în DTC. De asemenea, polimorfismele genelor implicate în metabolismul folaților (MTHFR, MTR, RFC1) pot fi incriminate în carcinogeneză, folatul fiind un factor esențial în sinteza ADN.

Studiile sugerează că incidența, evoluția și prognosticul cancerului tiroidian pot fi ameliorate semnificativ prin corectarea și evitarea agenților neoplazici. Ca acest lucru să fie posibil, trebuie studiate căile moleculare prin care acești factori le utilizează pentru exercitarea efectelor carcinogenice.

Cuvinte cheie: cancer tiroidian, papilar, folicular, vitamina D, DTC, BRAF, VDR, MTHFR.

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INTRODUCTION

Thyroid cancer is considered to be one of the most common endocrine malignancy with a continuously increasing incidence but a relatively stable death rate in the past years[1]. Although it was originally thought that overdiagnosis was the sole responsable for this, recent studies show that a true increase of thyroid cancer occurence and severity is possible. Recently, partially due to COVID-19 pandemic and the delays of treatment, an increasing number of large tumors, extrathyroidal invasion and distant metastases was noticed. Despite the indolent feature of thyroid cancer, and especially DTC, some patients suffered tumor progression and possibly other genetic alterations which can worsen the prognosis during the pandemic lockdown[2,3]. Studies show that post-lockdown patients were more likely to have multiple lesions (31.2% vs 36.5%), extrathyroidal extension (65.5% vs 72.2%) and lymph node metastases (37.7% vs 45%)[4].

Cancer in itself is considered to be a response of one or more genetic alterations and studying this genetic changes could lead to treatment and prognosis improvement. Thyroid cancer is divided by its histological desciption into four main types: differentiated thyroid carcinomas which include the papillary (PTC) and follicular (FTC) types, medullary thyroid carcinoma (MTC) and undifferentiated or anaplastic thyroid carcinomas (ATC)[5]. The vast majority of cases (85% of DTC) are attributed to PTC where the histologic pattern is represented by papillae which consist of layers of neoplastic thyroid cells lining a fibrous core with certain nuclear features and other specific changes according to its subtype (classic, follicular variant, tall cell variant and others). FTC represents the rest of DTC being identified by its follicular organization and absence of PTC nuclear features. Anaplastic thyroid cancer, the most aggressive type represents 2% of cases but almost 50% of deaths thus targeted therapies are much needed. MTC is a type of cancer arising from the C cells or parafollicular of the thyroid gland representing 4-10% of all thyroid cancers. It can be sporadic but also part of multiple endocrine neoplasia (MEN) genetic syndromes [6,7].

Risk factors for follicular-derived thyroid cancer have become of extreme interest worldwide. Beside the well-known risk factors such as gender, age, hormonal status, family history, cervical irradiation, obesity, alchool, more molecular and genetic mechanisms have been studied [8]. Genetic mutations and translocations in genes such as BRAF, RET, RAS, NTRK1 are already known and studies in PTC. RAS cascade and its three proteins (H-RAS, N-RAS, K-RAS), PAX8-PPARG, PTEN/PI3K/AKT, IDH1 and THRB are major mutations found in FTC. Many of this mutations have been also described in ATC along with loss of TP53 tumous suppressor gene function[6,9].

The role of vitamin D as an immunomodulator in different cancer pathways has lead to the conclusion that vitamin D throught VDR may play a significant prognostic role in DTC. Therefore, increasing evidence of the antiproliferative, antiinflammatory and prodifferentiation properties of vitamin D suggest a link between DTC, vitamin D status and VDR gene polymorphisms with the hope of further targeted adjuvant therapies[10].

Moreover, genetic polymorphisms involved in folate metabolism are considered to be related to carcinogenesis due to folate role in DNA synthesis and repair processes, which can lead to occurence of different types of cancer including thyroid cancer[11,12].

MATERIAL AND METHOD

We conducted a review of literature using online databases PubMed and Embase using the search criteria: differentiated thyroid cancer, papillary, follicular, vitamin D, VDR, polymorphism, MTHFR.

RESULTS AND DISCUSSIONS

Vitamin D and thyroid cancer

Vitamin D deficiency is a health issue worldwide, with an estimation that 50% of the population suffers from it. Due to the fact that research shows possible correlations between vitamin D levels and cancer, heart disease, bone loss, autoimmune disease, type-2 diabetes, depression and neurological disease, it is now considered to be an independent risk factor for total mortality in the general population [13]. Vitamin D metabolism is based on skin synthesis under ultraviolet exposure thus converting 7-dehydrocholesterol to previtamin D3 which generates vitamin D3. In the liver, vitamin D is enzimatically converted to 25-hydroxyvitamin D (25OHD), which in turn is converted into its biologically active form, 1**α**,25-dihydoxyvitamin D (1**α**25OH2D) in the kidneys by the enzyme 25(OH) D-1**α**-hydroxylase (CYP27B1)[14]. Vitamin D exerts

its actions through vitamin D receptor (VDR) which is considered to be uniquitous throughout the body. Binding of 1**α**25OH2D to VDR generates a nuclear complex which has regulatory effects on gene transcription thus explaining the antiproliferative, antiinflammatory and proapoptotic effects[1]. All this data suggest that vitamin D can regulate the entire tmorigenesis process including the distant metastsis consisting of cellular proliferation and differentiation, aptoptosis, angiogenesis, autophagy[15].

One of the most important antineoplastic mechanisms of calcitriol is the inhibition of malignant cells proliferation by blocking the cell cycle in G_0/G_1 phase through p53 which is a tumor suppressor factor thus increasing the expression of $p21^{Waf/Cip1}$ and $p27^{Kip1}$ with antiproliferative effects and reducing CDK2 cyclin-dependent kinase activity and pRb retinoblastoma protein which is also a tumoral suppressor[15,16]. Vitamin D enhances the expression of p73 inducing tumor apoptosis. It controls the activity of growth factors involved in the tumor process such as insulin-like growth factor binding protein-3 (IGFBP-3), which inhibits cell proliferation by increasing $p21^{Waf/Cip1}$[15,16,17]. Calcitriol also induces cell differentiation by various mechanisms involving the regulation of signaling pathways: **β**-catenin, Jun-N-terminal kinase (JNK), phosphatidyl inositol 3-kinase, nuclear factor **κ**B (NF-**κ**B) and forkhead box transcription factors O3/4 (FoxO3/4) which triggers the transcription of target genes involved in cell cycle blocking [15-17]. NF-**κ**B is a family of transcription factors with ubiquitous presence in cells that are important regulators of the innate immune response and inflammation. Unlike normal cells, malignant cells have high levels of active NF-**κ**B. Calcitriol directly modulates the activity of NF**κ**B in many cell types including lymphocytes, fibroblasts, and peripheral monocytes and reduces the production of proinflammatory and angiogenic cytokines by inhibiting the nuclear translocation of the p65 subunit of NF**κ**B[18].

Other incriminated mechanism are based on inducing cellular apoptosis by disrupting mitochondrial function, releasing cytochrome and producing reactive oxygen species, inhibiting the expression of antiapoptotic molecules such as Bcl2 (B-cell lymphoma 2) and stimulating proapoptotic ones such as Bax (Bcl2-associated X protein), Bad (Bcl2 associated cell death agonist), Bak (Bcl-2 homologous antagonist/killer), caspases and other proapoptotic proteins[19, 20]. Vitamin D also protects DNA damage induced by oxidative stress through antioxidant activity and stimulates DNA repair processes, thus preventing genetic mutations at the onset of the tumor process[15-18]. Calcitriol reduces the ability of malignant cells to invade and metastasize by inhibiting angiogenesis and controlling molecules involved in this process such as: plasminogen activation system (PA), metalloproteinases (MMP), decreased tenascin C expression, an extracellular matrix protein that promotes growth, invasion and angiogenesis, increased expression of cadherin E (tumor suppressor gene whose expression is inversely correlated with metastatic potential)[20, 21]. It has anti-inflammatory action, chronic inflammation being involved in tumorigenesis through reactive oxygen species and cytokines. Calcitriol inhibits the pathway of prostaglandins involved in the proinflammatory process by suppressing the expression of cyclooxygenase 2 (COX2), the receptors of these prostaglandins and the release of inflammatory interleukins such as IL6, IL10 and TNF-**α**[17-20]. Angiogenesis representsmthe formation of new blood vessels from adjacent vascularization, having a crucial role in tumor extension and metastasis. Among the most important proangiogenic factors are VEGF and prostaglandins. Calcitriol is thought to be a potent inhibitor of tumor angiogenesis by inhibiting the action of VEGF and endothelial cell proliferation [20-22].

Other pathways in which vitamin D interferes with neoplastic activity is based on the VDR expression in different tissues. VDR cellular expression is reduced during the dedifferentiation and tumoral progression processes and increased nuclear VDR expression is associated with a reduction of tumoral progression risk in malignant disease[23]. Similar to VDR, CYP27B1 expression is inversely correlated with tumor progression suggesting that local calcitriol production may be important for cancer prevention. Studies show that the cytokines IL-6 and TNF-**α** reduce the expression of this gene in certain cancers, contributing to a decrease in its level during tumor proliferation[15-17, 24, 25]. The involvement of VDR gene polymorphisms in different types of cancer has been intensively studied, the most important associations being found in breast cancer (BsmI, FokI), prostate cancer (FokI), malignant melanoma (FokI), renal carcinoma (TaqI), ovary (FokI, ApaI) and bladder (FokI)[26].

In terms of thyroid action, vitamin D deficiency and VDR gene polymorphisms have been associated with both autoimmune diseases and thyroid cancer.

The mechanisms involved in this correlation are similar to those of other cancers, but with some peculiarities, especially the blockade of cell proliferation by 1.25 (OH) 2D by inhibiting c-MYC (protooncogene) expression and stimulating p27 release causing an increase in the number of cells in G0-G1 phase of the cell cycle and decreased Ki67 expression. In addition, calcitriol has the ability to increase cell adhesion by stimulating fibronectin, a relationship mediated by the PTEN/PI3K pathway. As with other cancers, the anti-proliferative action of vitamin D is essential in DTC[27-29].

Preclinical studies have shown discontinuation of malignant cell growth in differentiated thyroid carcinoma after administration of pharmacological doses of 1.25OH2D or its analogues[30]. Thus, in vitro, calcitriol and an analog of it suppressed malignant cell migration and invasion by blocking the epithelial-mesenchymal transition, increasing cadherin expression by blocking F-actin formation and destabilizing the structure. Also in vitro, administration of calcitriol reduced tumor size, accentuated cell differentiation and caused the accumulation of p27 in thyroid malignant cells thus preventing metastasis[28-31]. The two main theories that vitamin D correlates with differentiated thyroid carcinoma are based on epidemiological studies that have shown significantly lower serum levels of 25 (OH) vitamin D in neoplastic patients compared to control groups, but also the presence of VDR gene polymorphisms which increase the predisposition to the development of this type of cancer or which, on the contrary, provide protection against it[27-30]. Many studies showed a significantly higher rate of malignancy in thyroidectomized patients with a deficient level of vitamin D compared to those with normal serum levels thus suggesting that vitamin D may represent a potential adjuvant factor in the evolution and appearance of thyroid carcinoma and correlated the tumor diameter with the value of vitamin D, thus, vitamin D-deficient patients are considered to have larger, more aggresive tumors (stages T3/4) and risk of metastasis is higher [32,33].

The second hypothesis incriminated in the correlation of vitamin D with DTC concerns the involvement of VDR gene polymorphisms. Polymorphisms are one of the most common variations in the human genome. Those present in genes involved in the process of DNA repair, in the regulation of the cell cycle, metabolism and immunity, are associated with increased susceptibility to cancer. Polymorphisms in the promoter area affect gene expression by altering the binding of transcription factors, DNA methylation and histone changes. Polymorphisms in the exonal region influence cancer predisposition by suppressing gene transcription and translation, and those in the intron region disrupt RNA binding and function[34-36]. The most researched polymorphisms of the VDR gene are FokI (rs2228570), BsmI (rs1544410), ApaI (rs7975232), TaqI (rs731236) and Cdx2 (rs11568820).

The main research regarding this correlation concluded that the AA and FF alleles of the ApaI and FokI polymorphisms and the tABF haplotype provide protection against susceptibility to follicular carcinoma, while the Tabf haplotype appears to be correlated with increased risk of FTC[37,38]. The TT genotype of FokI is associated with T3/T4 stages of thyroid carcinoma, extrathyroid invasion, multifocality and a tumor diameter ≥ 10 mm[39].

Increased expression of VDR and CYP27B1 was found in PTC tissue compared to normal thyroid tissue. However, in PTC with lymph node metastases, VDR and CYP24A1 expression were decreased compared to PTC without lymph node extension, and in the case of anaplastic carcinoma, VDR expression is often lost, suggesting a local antitumor response of 1,25OH2D in thyroid cancer. The low expression of VDR, CYP24A1 and CYP27B1 was also demonstrated in the N1 stages of PTC compared to N0, CYP24A1 expression being correlated with advanced stages of PTC and BRAF V600E mutation. Moreover, some BRAF V600E inhibitors may inhibit the presence of the enzyme thereby stimulating the antiproliferative effects of calcitriol in thyroid cells[40-42].

The increasing incidence of aggressive thyroid carncers and global vitamin D deficiency especially post-pandemic but also the genetic variability of VDR gene polymorphisms, are the basis for the need for extensive population studies of the genetic pattern to initiate adjuvant therapy specific to thyroid cancer with the purpose of improving the cure rate, improving the prognosis or even preventing disease in at-risk population groups[43,44].

Folate metabolism and thyroid cancer

Folate is a water-soluble B vitamin which plays and important role in generating S-adenosylmethionine, the universal donor for DNA methylation and synthesis. Folate deficiency can be incriminated in the tumorigenesis process either by leading to aberrant DNA methylation which can genrate an

altered expression of tumor suppressor genes and protooncogenes or by causing imbalances within the nucleotide precursors, leading to DNA mutations and affecting its integrity and repairing processes. Epidemiologic studies have observed that lower folate level is associated with cancer of the uterus, colorectum, lung, esophagus, brain, breast and not in least, thyroid cancer[45,46]. Several genes of which the most important are methylenetetrahydrofolate reductase (MTHFR), methionin synthase (MTR), reduced folate carrier 1 (RFC1) and cystathionine **β**-synthase (C**β**S) regulate folate metabolism thus their genetic polymorphisms change enzymatic activity, resulting in DNA hypomethylation and genomic instability [47,48]. MTHFR is one of the key enzymes in folate metabolism. It catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate which is the methyl donor for the remethylation of homocysteine to methionine. Its gene is located on chromosome 1p36.3. MTHFR main polymorphisms are MTHFR C677T (rs1801133) which is associated with decreased activity of the MTHFR activity and A1298C (rs1801131) which leads to reduce MTHFR activity. The homozygous genotypes of MTHFR are associated with high homocysteine levels which can produce DNA hypomethylation and thus increased cancer frequence. The RFC1 enzyme is responsabile for folate absorbtion and transport and its gene is located on 21q22.3. substitution of adenin for guanine at nucleotide 80A>G (rs1051266) is the most common single nucleotide polymorphism in RFC1. It affects plasma folate and homocysteine levels alone or together with the C677T polymorphism in the methylenetetrahydrofolate reductase gene [47-50]. Regarding the MTR gene, the main polymorphism is represented by an adenine to guanine transition at 2756 position (rs1805087) which determines a substitution of aspartic acid with glycine with secondary higher activity, leading to more effective homocysteine remethylation and methionine production[5]. The C**β**S gene encodes cystathionine **β**-synthase which is involved primarily in the process that irreversibly metabolizes homocysteine to cystathione. Its polymorphism located in exon 8 consists of an insertion of 68 base pairs at nucleotide 844ins(68) and has been correlated with lower homocysteine and DNA methylation perturbation because of low levels of S-adenosylmethionine (SadoMet) which is the main methyl donor for methylation reactions[5,51]. Deficiencies in folate metabolism plays an important role as a risk factor for neural tube defects (NTD) and other birth defects, cardiovascular disease, pregnancy complications and some cancers[52].

Excess folate intake also may be a risk factor considering that folic acid, found in fortified foods and supplements, is converted to tetrahydrofolate in the liver by dihydrofolate reductase (DHFR). It has been reported that more than 400 mcg of can saturate the DHFR enzyme, resulting in excess folic acid, which has been hypothesized as a potential mechanism for carcinogenesis. Also, just as folate deficiency or excess can influence cell replication, reduced enzymatic activity can alter metabolism and increase disease risk[53].

Regarding the importance of folate in nucleic acid metabolism, the most important step is the synthesis of S-adenosylmethionine (SAdoMet) and de novo deoxynucleoside triphosphate synthesis. Each of these two biosynthetic pathways is a means by which folate plays a major role in DNA metabolism. Methionine results from homocysteine in a reaction for which 5-methyltetrahydrofolate homocysteine methyltransferase (5-methylTHF) is both cofactor and substrate. Methionine is then converted to SadoMet in a reaction catalyzed by methionine adenosyl transferase. SAdoMet donates the methyl group for over 80 biological methylation reactions, including an array of reactions whereby specific sites within DNA and RNA become methylated. Although there are some alternative pathways which may compensate, dietary folate depletion alone is a sufficient perturbing force to diminish SAdoMet pools[46].

Folate metabolism, which is the base of larger set of transformations known as one-carbon (1C) metabolism, is a universal metabolic process that serves to activate and transfer 1C units for biosynthetic processes including purine and thymidine synthesis and homocysteine remethylation which is a rate-limiting step for DNA synthesis. Folate-mediated one-carbon metabolism (FOCM) ensures essential events for the growth and survival of proliferating cells. Nucleotide synthesis and DNA methylation are the biochemical bases of cancers that are highly dependent on FOCM. Studies revealed that FOCM is connected with redox homeostasis and epigenetics in cancer and folate metabolism enzymes, such as serine hydroxymethyltransferase 2 (SHMT2) and methylenetetrahydrofolate dehydrogenase 2 (MTHFD2), are associated with the development of cancers thus revealing their potential use in tumor-targeted therapy. Therefore, targeting metabolizing enzymes, especially SHMT2 and MTHFD2, could provide a a base for new cancer treatments [53,54].

Although studies confirm possible correlations between gene polymorphisms and different types of cancer, the relationship between them and thyroid cancer is still poorly understood. MTHFR 677C>T was associated with increased risk for thyroid cancer and MTR 2756A>G with tumor extension and aggressiveness. Studies also show increased risk of thyroid cancer for genotype GG of RFC1 polymorphism [5].

To summarize, folate can interfere in carcinogenesis including thyroid cancer both by deficiency and excess, leading to DNA methylation perturbancies with secondary alteration of tumor suppressor genes and causing imbalances in the nucleotide necessary thus affecting DNA integrity. Also, polymorphisms in the MTHFR, MTR, RFC1 and C**β**S genes are associated both with the risk for developing thyroid cancer and also with the aggressiveness and prognosis [5].

CONCLUSIONS

The increasing incidence of thyroid cancer cases and aggresive forms especially during this COVID-19 pandemic can be associated with overdiagnosis but we cannot afford to rely only on that assumption. A true increase of number of cases may be possible and we need to research a cause and solutions for it. Genetic plays an essential role in carcinogenesis but other molecular mechanisms are worth to be studied. Both vitamin D and folate deficiencies and genetic polymorphisms have been correlated with the risk of differentiated thyroid cancer with the hope of adjuvant targeted therapies to improve prognosis and lower complication or even death rate. There are still a number of factors which need to be taken into consideration like geographical and ethnic differences, genetic mutations and polymorphisms and molecular or epigenetic processes which influence enzyme activity with secondary alteration of substrate availability or even gene expression. Future research should aim understanding how gene polymorphisms influence carcinogenesis and if deficiency correction can influence prognosis of patients with thyroid cancer.

Compliance with ethics requirements: The authors declare no conflict of interest regarding this article. The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study.

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