RESEARCH ARTICLE

Lack of Associations of the MDM4 rs4245739 Polymorphism with Risk of Thyroid Cancer among Iranian-Azeri Patients: a Case-Control Study

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Abstract

Background and Aim: MDM4, a negative regulator of the p53 tumor suppression pathway, has been demonstrated to be overexpressed in a variety of human cancers. Research has revealed that the rs4245739 A>C polymorphism of MDM4 in the 3′-untranslated region makes it a miR-191 target site, leading to lower MDM4 expression. This study aimed to detect if the rs4245739 single nucleotide polymorphism (SNP) impacts on thyroid cancer (TC) development in Iranian-Azeri patients. Materials and Method: Blood samples were taken from 232 healthy controls and 130 TC patients of Iranian-Azeri ethnicity. For genotyping, Tetra-ARMS PCR was performed. SPSS for Windows (version 22.0, IBM SPSS Inc., USA) and the SHEsis online software were used for data analysis. Results: Alleles of MDM4 rs4245739 SNP demonstrated no significant different in frequencies between patients and controls (p>0.05). Additionally, genotypes of MDM4 rs4245739 SNP did not increase or decrease TC risk in patients compared with healthy subjects. Conclusion: Considering the lack of any observed association between the MDM4 rs4245739 polymorphism and TC, we conclude no significant role in the pathophysiology of the disease.

Keywords: Thyroid cancer- MDM4- SNP- rs4245739

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Introduction

A common disorder in the endocrine system, TC is a fast-growing malignancy, which is classified mainly into 4 groups of follicular thyroid carcinoma (FTC), papillary thyroid carcinoma (PTC), anaplastic thyroid cancer (ATC), and medullary thyroid cancer (MTC) (Mazzaferri, 1999). PTC is responsible for almost 80% of TC cases, however, FTC with 10%-15% occurrence, stands on the second rank (Faam et al., 2015). In spite of diet changes and improved health care services, along with economic development, an increment has been seen in the TC incidence (Davies and Welch, 2006). Like other malignancies, environmental and genetic factors have been observed to play role in the etiopathology of TC (Pierotti et al., 1998). To enumerate environmental contributing factors, there are lesions in thyroid (Galanti et al., 1995), thyroid-stimulating hormone (TSH) and its receptors (Boelaert, 2009), estrogen (Sakoda and Horn-Ross, 2002), malfunction in iodine uptake (Chow et al., 2003), ionizing radiation (Chow et al., 2003), neck ultrasound (Zheng et al., 1996), social and cultural factors (Pujo et al., 1996), and other disease (Aschebrook-Kilfoy et al., 2011; Kitahara et al., 2012).

On the other side, several investigations have documented association of genetic factors of oncogenes like CCND1, BRAF, MYC, RAS, and RET with TC (Motoi et al., 2000; Bièche et al., 2001; Fukushima et al., 2003; Rusinek et al., 2011; Huang and Yang, 2015). Furthermore, the genome-wide association studies (GWAS) emphasize on the direct genetic association of SNPs with TC risk (Maillard et al., 2015; Pereda et al., 2015). Therefore, studies showing novel potential SNPs associated with TC are worthwhile approaches to investigate the role of genetic variations in TC.

Mouse double minute 2 homolog (MDM2), a major negative regulator of tumor suppression pathway of p53, acts through direct binding to p53, which leads to ubiquitination and then degradation of P53 (Landers et al., 1997). Mdm4 p53 binding protein homolog (MDM4) which structurally has homology with MDM2, can collaborate with MDM2 to inhibit p53 activity when cell responses to DNA damage (Wade et al., 2010). Furthermore, MDM4 can interact with MDM2 protein and inhibit degradation of MDM2 (Linares et al., 2003). MDM4 becomes phosphorylated in response to DNA damages, which results in the shift from the degradation

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of p53 to the degradation of MDM4. The consequence is stabilization of p53 and cell cycle suppression (Wang et al., 2007). It has been shown that transgenic mice overexpressing MDM4 develop spontaneous carcinogenesis, which makes it more clear that MDM4 may act like an oncogene in vivo (Xiong et al., 2010). The rs4245739 A>C SNP, in the 3'-untranslated region (3'-UTR) of MDM4 has been shown as a putative target site for miR-191 (Wynendaele et al., 2010). miR-191 can selectively bind to C allele contained MDM4 mRNA but not A allele contained MDM4 mRNA, culminating in a lower level expression of the C allele contained MDM4 mRNA. This observation explains the significantly increased expression of MDM4 mRNA and protein in MDM4 rs4245739 A allele carriers in malignancies (McEvoy et al., 2012).

The P53 pathway malfunctions have pivotal roles in mammary tumorogenesis. Considering the role of the p53- MDM4 pathway in the preventing of tumor development, we hypothesized that rs4245739 A>C polymorphism of MDM4 might be involved in TC pathogenesis. In the present survey, 130 patients with TC and 232 healthy controls were recruited to investigate the association of MDM4 rs4245739 polymorphism with the risk of TC.

Materials and Methods

Subjects

In order to perform the genetic analysis, a total of 480 individuals, which comprised of 130 unrelated TC patients and 232 healthy individuals with no history of cancer, were recruited. Patients with histologically confirmed for TC, were chosen from Imam Reza Hospital, Tabriz, Iran. Clinical data of the cases was collected from 2014 till 2015 according to documentary files of patients diagnosed through physician and pathologist. All study patients were native of Azerbaijan to elicit valid results of race-related associations. Additionally, healthy subjects had the same race with the patients and were age and sex-matched with patients. The local Ethical Committee of Tabriz University approved the study protocol and written informed consent was collected through all subjects. Five ml of venous blood was taken from each patient and control through EDTA-containing venoject tubes. Afterwards, genomic DNA was extracted from peripheral blood through salting-out approach.

Genetic analysis

In the present study, Tetra-primer ARMS-PCR assay for allele and genotype detection was carried out. The primers were designed using Primer3Plus tool (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) and were blasted in NCBI website: http://www.ncbi.nlm.nih.gov/tools/primer-blast/ (Table 1). The allele-specific (inner) primer was designed in opposed directions and, in combination with the common primers, can simultaneously amplify both the mutant allele and wild type alleles in a single-tube PCR. PCR-amplified DNA samples were electrophoresed through Agarose gel (2%). After that, a number of PCR products was assayed randomly by DNA sequencing in order to confirm if the results determined by T-ARMS-PCR were in concordance with those that determined by sequencing.

Genotyping was performed by Tetra-primer ARMS PCR using the Taq-PCR Master Mix (Cat. No: 5200350-0050, Lot. No: 13C18, amplicon, Denmark) and the thermocycler PCR System (SensoQuest, Germany). Each reaction mixture contained a total volume of 26.4μl (master mix 10μl, forward and reverse inner primer 1μl each, forward and reverse outer primer 0.2μl each, and H2O 12μl). The thermocycler conditions were: 95°C for 5 minutes, then 35 cycles of 95°C for 30 seconds, 56°C for 30 seconds, and 72°C 40 seconds and final extension of 72°C for 10 minutes.

Statistical analysis

Genotype and allelic distribution between case and control groups were implemented by Fisher’s exact test. Moreover, the odds ratio (OR) and 95% confidence interval (CI) were calculated. The genotype distributions of MDM4 rs4245739 SNP were tested for deviation from Hardy-Weinberg equilibrium in the control group. The Bonferroni correction test was exerted in multiple statistical testing (i.e. p-value was set to <0.01) to recognize a statistically significant result, adjusting the multiple comparison, and controlling the false discovery rate (FDR) (Benjamini and Hochberg, 1995). Also, several parts of statistical analysis were performed using the SPSS for Windows (version 22.0, IBM SPSS Inc., USA). Additionally, the SHEsis online software was exerted for analyzing the genotype and Hardy-Weinberg equilibrium (Yong and Lin, 2005).

Results

Allele frequency

The allele of MDM4 rs4245739 (A/C) polymorphism was detected in 78.4% of the patients and healthy individuals. Furthermore, the C allele also showed an equal distribution between patients and controls (21.6% each). Hence, there was not any significant difference in the A and C allele frequencies of rs4245739 SNP between

| SNP     | Target                          | Sequence                         | Amplicon Size | Tm (°C) |
|---------|---------------------------------|----------------------------------|---------------|---------|
| MDM4 A/C| Forward inner primer (A allele) | 5’GTAGTACGAACATATAATGAATTTATCCA3’ | 232           | 56      |
|         | Reverse inner primer (C allele) | 5’ATTTTCAATAATGTTGTAAGTGACCG3’   | 275           | 56      |
|         | Forward outer primer            | 5’ACAGAGACAGATACAGAAAAACATGGG3’  | 450           | 56      |
|         | Reverse outer primer            | 5’ACCTAATCTGTAACCTGACTGCTGATA3’  | 427           | 56      |
p=0.92, OR=1.02, 95% CI: 0.62-1.63). Finally, there was no significant difference in respect of CC genotype distribution between the patient and the control groups (4.9% vs. 5.2%, respectively; p=0.92, OR=0.94, 95% CI: 0.32-2.75).

Demographic specifications and genotype distribution
Specifications of the study subjects according to genotype distribution are listed in Table 2. The patients with the mean age of 38.9±3.8 were age matched with healthy controls with that of 37.4±4.3, and did not show significant genotype distribution in respect of age. Tumor size of patients was 2.43±0.32 cm, and the mean tumor size of different genotypes were almost equal and the patient and the control groups (p=0.99, OR=0.99, 95% CI: 0.67-1.49 vs. p=0.99, OR=1.001, 95% CI: 0.67-1.49, respectively).

Genotype frequency
Distributions of the rs3129882 (A/C) polymorphism genotypes in the healthy group disclosed no evidence of deviation from Hardy–Weinberg’s equilibrium. The AA genotype was observed to be the most common genotype, which was represented in 61.8% of the patients and 62.1% of the controls (p=0.92, OR=0.98, 95% CI: 0.61-1.59). Although the frequency of the AC genotype in the patients was higher than the control group, this difference was not significant (33.3% vs. 32.8%, respectively; p=0.92, OR=1.02, 95% CI: 0.62-1.63). Finally, there was no significant difference in respect of CC genotype distribution between the patient and the control groups (4.9% vs. 5.2%, respectively; p=0.92, OR=0.94, 95% CI: 0.32-2.75).

Table 2. Association of Clinical Manifestations of TC Patients with Three Genotypes of MDM4

| Clinical properties | Total | AA Genotypes | AC Genotypes | CC Genotypes | P value |
|--------------------|-------|--------------|--------------|--------------|---------|
| Age                | 38.9±3.8 | 37.7±4.1 | 38.8±2.1 | 40.1±3.1 |         |
| Tumor Size (cm)    | 2.43±0.32 | 2.14±0.14 | 2.27±0.14 | 2.91±0.35 |         |
| Malignancy         |         |             |             |             | 0.034   |
| Benign             | 24 (23.5%) | 8 (33.3%) | 9 (37.5%) | 7 (29.16%) |         |
| Malignant          | 78 (76.47%) | 24 (30.76%) | 26 (33.3%) | 28 (35.9%) |         |
| Tumor Dimension    |         |             |             |             | 0.193   |
| T1                 | 40 (39.22%) | 13 (32.5%) | 14 (35%) | 13 (32.5%) |         |
| T2                 | 35 (34.31%) | 10 (28.57%) | 13 (37.14%) | 12 (34.28%) |         |
| Other              | 27 (26.47%) | 9 (33.3%) | 11 (40.74%) | 7 (25.92%) |         |
| Grade of Tumor     |         |             |             |             | 0.241   |
| I                  | 63 (61.76%) | 23 (36.5%) | 18 (28.57%) | 22 (34.92%) |         |
| II                 | 22 (21.57%) | 7 (31.81%) | 9 (40.9%) | 6 (27.27%) |         |
| III                | 17 (16.67%) | 5 (29.41%) | 8 (47.06%) | 4 (23.52%) |         |
| Involved Lobe      |         |             |             |             | 0.093   |
| Left               | 35 (34.31%) | 14 (40%) | 10 (28.57%) | 11 (31.42%) |         |
| Right              | 43 (42.16%) | 17 (39.53%) | 12 (27.90%) | 14 (32.55%) |         |
| Both               | 24 (23.53%) | 10 (41.66%) | 8 (33.3%) | 5 (20.83%) |         |
| Lymph Node Involvement |    |             |             |             | 0.059   |
| N0                 | 47 (42.16%) | 19 (40.42%) | 16 (34.04%) | 12 (25.53%) |         |
| N1                 | 36 (35.29%) | 15 (41.6%) | 13 (36.11%) | 8 (22.2%) |         |
| N1a                | 10 (9.28%) | 4 (40%) | 4 (40%) | 2 (20%) |         |
| N1b                | 4 (3.92%) | 2 (50%) | 1 (25%) | 1 (25%) |         |
| NX                 | 5 (4.90%) | 3 (60%) | 1 (20%) | 1 (20%) |         |
| Pathology          |         |             |             |             | 0.063   |
| PTC                | 26 (25.49%) | 10 (38.46%) | 8 (30.76%) | 8 (30.76%) |         |
| FTA                | 20 (19.16%) | 7 (35%) | 8 (40%) | 5 (25%) |         |
| FTC                | 4 (3.92%) | 2 (50%) | 2 (50%) | 0 (0%) |         |
| HCC                | 3 (2.94%) | 1 (33.3%) | 2 (66.6%) | 0 (0%) |         |
| MTC                | 2 (1.96%) | 1 (50%) | 0 (0%) | 1 (50%) |         |
| PTC (classical)    | 28 (27.45%) | 10 (35.71%) | 11 (39.28%) | 7 (25%) |         |
| PTC (folicular)    | 7 (6.86%) | 4 (44.4%) | 2 (22.2%) | 3 (33.3%) |         |
| PTC (metastatic)   | 3 (2.94%) | 1 (33.3%) | 1 (33.3%) | 1 (33.3%) |         |
| Follicular Adenoma | 6 (5.88%) | 3 (50%) | 1 (16.6%) | 2 (33.3%) |         |
| Hurthle Cell Adenoma | 3 (2.94%) | 2 (66.7%) | 1 (33.3%) | 0 (0%) |         |

PTC, papillary thyroid carcinoma; FTA, follicular thyroid adenoma; FTC, follicular thyroid carcinoma; HCC, hurthle cell carcinoma; MTC, medullary thyroid cancer

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Table 3. Allele and Genotype Distribution of MDM4 in TC Cases and Healthy Controls

| dbSNP  | Alleles/Genotypes | Case (n=102) N(%) | Control (n=232) N(%) | P     | Adj. P* | OR (95% CI) |
|--------|-------------------|------------------|---------------------|-------|---------|-------------|
|        | C                 | 44 (21.6%)       | 100 (21.6%)         | 0.996 | 0.99    | 1.001       |
|        | A                 | 160 (78.4%)      | 364 (78.4%)         | 0.993 | 0.99    | (0.6707-1.4939) |
|        | MDM4 rs4245739    |                  |                     |       |         |             |
|        | CC                | 5 (4.9%)         | 12 (5.2%)           | 0.917 | 0.92    | 0.945       |
|        | AC                | 34 (33.3%)       | 76 (32.8%)          | 0.918 | 0.92    | (0.3241-2.7559) |
|        | AA                | 63 (61.8%)       | 144 (62.1%)         | 0.957 | 0.92    | 0.9872      |
| HWE    |                   | 0.88             | 0.63                |       |         |             |

*Adjusted p-value for multiple testing using Benjamini-Hochberg method; OR, odds ratio; CI, confidence interval

Discussion

Several studies in the past two years have pointed on the pivotal importance of MDM4 in human cancers. It has been shown that MDM4 is overexpressed in roughly 10–20% of over 800 various tumors such as lung, colon, stomach, and breast cancers (Eischen and Lozano, 2014). The incidence of TC has been observed more in females than in males (Akslen et al., 1990) and is raising with aging (Albores-Saavedra et al., 2007). Several therapies have been implemented for treatment of TC like endocrine and radioactive therapies, surgery, and chemotherapy; nonetheless the outcomes were not satisfying (Khoyvahi et al., 2015). GWAS revealed a role of genetic variants in the etiopathology of TC (Gudmundsson et al., 2009). Furthermore, interleukin 17 Receptor A (IL17RA) polymorphisms have been associated with both the development of PTC and bilaterality of involved sides in Korean population (Lee et al., 2015); the expression level of mir-149-5p was related to local progression and the susceptibility of PTC in Chinese patients (Wei et al., 2014). Chen et al., demonstrated that esophageal cancer-related gene 4 (ECRG4) had a role in the regulation of cell cycle in PTC cells through transiting them from the G1 phase to G2, resulting in tumor growth development (Chen et al., 2015). In this study, in order to make the results more reliable, standard confirmed safeguards were employed to decrease possible biases. All TC patients and healthy individuals were selected from a population native of the same geographic region (Iranian-Azeri race). Through present case-control study, we contemplated a hypothesis to examine if there is a potential association between MDM4 rs4245739 polymorphism and the TC risk.

The p53 pathway has been shown to be inactivated in almost all human cancers (Vousden and Lane, 2007). About half of human malignancies has been estimated to carry mutations in the TP53 gene itself, whereas the rest tumors with wild type TP53 have genetic alterations in other key regulatory genes in the p53 pathway (Marine and Jochemsen, 2004; Horn and Vousden, 2007). Genetic amplification of the MDM2 or MDM4 genes can result in the aberrant protein expression and suppression of the p53 response over the course of tumor development and progression (Marine et al., 2006; Wade et al., 2010). Furthermore, data suggests that polymorphisms at the MDM2 or MDM4 loci may contribute to the increased basal expression of these important p53 antagonists and increase cancer susceptibility (Bond et al., 2004; Atwal et al., 2009; Kulkarni et al., 2009). The MDM4 rs4245739 polymorphism in the 3’-UTR creates a potential target site for micro RNA-191 (Wynendaele et al., 2010). The miR-191 can selectively bind to MDM4-C allele mRNA. This may somewhat explain the observation of a significantly increased expression of MDM4-C allele mRNA and therefore, result in a decreased expression of MDM4-C allele mRNA. This may somewhat explain the observation of a significantly increased expression of MDM4 mRNA and protein in MDM4 rs4245739 A allele carriers of ovarian cancer and retinoblastoma (Wynendaele et al., 2010; McEvoy et al., 2012).

The interaction of MDM4 and p53 was evaluated by Liu et al. and they reported that functional MDM4 rs4245739 SNP, alone and in combination with p53 Arg72Pro genetic variant, had association with a significantly decreased risk of breast cancer in Chinese women. Meanwhile, they postulated that perhaps genetic...
variants modifying microRNA-mediated gene regulation approach in breast cancer risk and mark the potential role of genes in the p53 tumor suppressor pathway in the initial steps of affecting as well during cancer development (Liu et al., 2013). In our investigation, the alleles and genotypes of MDM4 rs4245739 SNP were not significantly frequent in patients in comparison to controls. Therefore, none of the alleles could affect the risk of TC in our population.

The potential risk for TC is underlying to several clinical characteristics which can supposedly affect contracting, disease level, or being healthy. Regarding to research proven clinical manifestation of breast cancer, we attempted to correlate the age of onset, tumor size, being benign or malignant tumor type, tumor dimension, grade of tumor, involved thyroid lobe, number of involved lymph nodes, and pathology of cancer with various genotypes in MDM4 rs4245739 SNP towards TC risk. It was seen that none of the clinical characteristics were associated with three genotypes of MDM4 rs4245739 SNP. We found no statistically significant association between genotype distribution and specific prognostic predictors and clinical features for the TC outcome. Therefore, at least according to our data, MDM4 rs4245739 A>C SNP did not impress the clinical features of the TC. It seems that long time following-up of the patient’s clinical changes will facilitate to elicit a more definitive conclusion pertaining to the influence of this polymorphism on TC manifestation.

All in all, to our best knowledge, this is the first study designed to assess the role of the MDM4 rs4245739 gene variant in a replicated case-control of Iranian-Azeri patients with TC. We could not identify significant differences in both allelic and genotype frequencies in the TC group in comparison to the control group. Last but not least, these results suggest the MDM4 rs4245739 (A/C) polymorphism may not be a risk factor for TC in Iranian-Azeri population and further studies are still needed to validate these data in other populations.

Disclosure of conflict of interest

None.

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References

Akslen L, Haldorsen T, Thoresen SO, et al (1990). Incidence of thyroid cancer in Norway 1970–1985. *Apmis*, 98, 549-58.

Albores-Saavedra J, Henson DE, Glazer E, et al (2007). Changing patterns in the incidence and survival of thyroid cancer with follicular phenotype-papillary, follicular, and anaplastic: a morphological and epidemiological study. *Endocr Pathol*, 18, 1-7.

Ascheroor-Kilfoy B, Sabra MM, Brenner A, et al (2011). Diabetes and thyroid cancer risk in the national institutes of health-AARP diet and health study. *Thyroid*, 21, 957-63.

Atwal GS, Kirchhoff T, Bond EE, et al (2009). Altered tumor formation and evolutionary selection of genetic variants in the human MDM4 oncogene. *Proc Natl Acad Sci*, 106, 10236-41.

Benjamin Y, Hochberg Y (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Series B Stat Methodol*, 35, 289-300.

Bièche I, Frané B, Vidaud D, et al (2001). Analyses of MYC, ERBB2, and CCND1 genes in benign and malignant thyroid follicular cell tumors by real-time polymerase chain reaction. *Thyroid*, 11, 147-52.

Boelaert K (2009). The association between serum TSH concentration and thyroid cancer. *Endocr-Relat Cancer*, 16, 1065-72.

Bond GL, Hu W, Bond EE, et al (2004). A single nucleotide polymorphism in the MDM2 promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. *Cell*, 119, 591-602.

Chen J, Liu C, Yin L, et al (2015). The tumor-promoting function of ECRG4 in papillary thyroid carcinoma and its related mechanism. *Tumor Biol*, 36, 1081-9.

Chow S-M, Law SC, Au S-K, et al (2003). Changes in clinical presentation, management and outcome in 1348 patients with differentiated thyroid carcinoma: experience in a single institute in Hong Kong, 1960–2000. *Clin Oncol*, 15, 329-36.

Davies L, Welch HG (2006). Increasing incidence of thyroid cancer in the United States, 1973-2002. *JAMA*, 295, 2164-7.

Eischen CM, Lozano G (2014). The Mdm network and its regulation of p53 activities: a rheostat of cancer risk. *Hum Mutat*, 35, 728-37.

Fam A, Ghaffarí MA, Ghadirí A, et al (2015). Epigenetic modifications in human thyroid cancer (Review). *Biomed Rep*, 3, 3-8.

Fukushima T, Suzuki S, Mashiko M, et al (2003). BRAF mutations in papillary carcinomas of the thyroid. *Oncogene*, 22, 6455-7.

Galanti MR, Sparèn P, Karlsson A, et al (1995). Is residence in areas of endemic goiter a risk factor for thyroid cancer?. *Int J Cancer*, 61, 615-7.

Gudmundsson J, Sulem P, Gudbjartsson DF, et al (2009). Common variants on 9q22. 33 and 14q13. 3 predispose to thyroid cancer in European populations. *Nat Gen*, 41, 460-4.

Horn H, Voussen K (2007). Coping with stress: multiple ways to activate p53. *Oncogene*, 26, 1306-16.

Huang K-X, Yang F (2015). RET polymorphisms might be the risk factors for thyroid cancer. *Int J Clin Exp Pathol*, 8, 5793.

Itahara CM, Platz EA, Freeman LB, et al (2012). Physical activity, diabetes, and thyroid cancer risk: a pooled analysis of five prospective studies. *Cancer Causes Control*, 23, 463-71.

Kulkarni DA, Vazquez A, Hafty BG, et al (2009). A polymorphic variant in human MDM4 associates with accelerated age of onset of estrogen receptor negative breast cancer. *Carcinogenesis*, 30, 1910-5.

Landers JE, Cassel SL, George DL (1997). Translational enhancement of mdm2 oncogene expression in human tumor cells containing a stabilized wild-type p53 protein. *Cancer Res*, 57, 3562-8.

Lee YC, Chung J-H, Kim SK, et al (2015). Association between interleukin 17/interleukin 17 receptor gene polymorphisms and papillary thyroid cancer in Korean population. *Cytokine*, 71, 283-8.

Linares LK, Hengstermann A, Ciechanover A, et al (2003). HdmX stimulates Hdm2-mediated ubiquitination and degradation of p53. *Proc Natl Acad Sci*, 100, 12009-14.

Liu J, Tang X, Li M, et al (2013). Functional MDM4 rs4245739 genetic variant, alone and in combination with P53 Arg72Pro polymorphism, contributes to breast cancer susceptibility.
Breast Cancer Res Treat, 140, 151-7.
Lkhoyaali S, Benhmida S, Ait EM, et al (2015). Targeted therapy in thyroid cancer: Towards a treatment card. *Pathol Biol, 63*, 1-6.

Maillard S, Damiola F, Clero E, et al (2015). Common variants at 9q22.33, 14q13.3, and ATM Loci, and risk of differentiated thyroid cancer in the French Polynesian population. *PLoS One, 10*, e0123700.

Marine J-C, Francoz S, Maetens M, et al (2006). Keeping p53 in check: essential and synergistic functions of Mdm2 and Mdm4. *Cell Death Differ, 13*, 927-34.

Marine J-C, Jochenssen AG (2004). Mdm and Mdm2: brothers in arms?. *Cell Cycle, 3*, 898-902.

Mazzafareri EL (1999). An overview of the management of papillary and follicular thyroid carcinoma. *Thyroid, 9*, 421-7.

McEvoy J, Ulyanov A, Brennan R, et al (2012). Analysis of MDM2 and MDM4 single nucleotide polymorphisms, mRNA splicing and protein expression in retinoblastoma. *PLoS One, 7*, e42739.

Motoi N, Sakamoto A, Yamochi T, et al (2000). Role of ras mutation in the progression of thyroid carcinoma of follicular epithelial origin. *Pathol Res Pract, 196*, 1-7.

Pereda CM, Lesueur F, Pertesi M, et al (2015). Common variants at the 9q 22.33, 14q13.3 and ATM loci, and risk of differentiated thyroid cancer in the Cuban population. *BMC Genet, 16*, 22.

Pierotti M, Vigneri P, Bongarzone I (1998). Rearrangements of RET and NTRK1 tyrosine kinase receptors in papillary thyroid carcinomas. In ‘Genes and Environment in Cancer’, Eds Springer, pp 237-47

Pujol P, Daures J-P, Nsakala N, et al (1996). Degree of thyrotropin suppression as a prognostic determinant in differentiated thyroid cancer. *J Clin Endocrinol Metab, 81*, 4318-23.

Rusinek D, Szpak-Ulczok S, Jarzab B (2011). Gene expression profile of human thyroid cancer in relation to its mutational status. *J Mol Endocrinol, 47*, 91-103.

Sakoda LC, Horn-Ross PL (2002). Reproductive and menstrual history and papillary thyroid cancer risk the San Francisco Bay area thyroid cancer study. *Cancer Epidemiol Biomarkers Prev, 11*, 51-7.

Vousden KH, Lane DP (2007). p53 in health and disease. *Nat Rev Mol Cell Biol, 8*, 275-83.

Wade M, Wang YV, Wahl GM (2010). The p53 orchestra: Mdm2 and Mdmx set the tone. *Trends Cell Biol, 20*, 299-309.

Wang YV, Wade M, Wong E, et al (2007). Quantitative analyses reveal the importance of regulated Hdmx degradation for p53 activation. *Proc Natl Acad Sci, 104*, 12365-70.

Wei W-J, Lu Z-W, Li D-S, et al (2014). Association of the miR-149 Rs2292832 polymorphism with papillary thyroid cancer risk and clinicopathologic characteristics in a Chinese population. *Int J Mol Sci, 15*, 20968-81.

Wynendaele J, Böhnke A, Leucci E, et al (2010). An illegitimate microRNA target site within the 3′ UTR of MDM4 affects ovarian cancer progression and chemosensitivity. *Cancer Res, 70*, 9641-9.

Xiong S, Pant V, Suh Y-A, et al (2010). Spontaneous tumorigenesis in mice overexpressing the p53-negative regulator Mdm4. *Cancer Res, 70*, 7148-54.

Yong Y, Lin H (2005). SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. *Cell Res, 15*, 97-8.

Zheng T, Holford TR, Chen Y, et al (1996). Time trend and age-period-cohort effect on incidence of thyroid cancer in Connecticut, 1935-1992. *Int J Cancer, 67*, 504-9.