Analysis of ALK, IDH1, IDH2 and MMP8 somatic mutations in differentiated thyroid cancers

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Abstract. Anaplastic lymphoma kinase (ALK), isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) and matrix metalloproteinase 8 (MMP8) gene mutations have been frequently reported in human cancers; however, to the best of our knowledge, they have not been specifically examined in differentiated thyroid cancers (DTCs). Therefore, the present study aimed to determine the somatic mutational frequencies of these genes in DTCs. Mutational analysis of the ALK (exons 23, 24 and 25), IDH1 (exon 4), IDH2 (exon 4), and MMP8 (all exons 1-10) was performed in 126, 271, 271 and 50 DTCs, respectively. All the indicated exons were PCR-amplified and the PCR products were directly sequenced by Sanger sequencing. The present study identified a high frequency (86%; 43/50) of MMP8 single nucleotide polymorphism (SNP) and also found some rare SNPs of this gene (S3C, T32I, L310P (86%; 43/50) of DTCs but no somatic mutation in IDH1, IDH2 and MMP8. Analyses of 414 DTCs from The Cancer Genome Atlas revealed rare ALK (1%) and MMP8 (0.24%) mutations and none in IDH1 and IDH2. Conversely, analyses of 117 aggressive thyroid cancers [84, poorly differentiated thyroid cancer (PDT); 33, anaplastic thyroid cancer (ATC)] from the Memorial Sloan Kettering Cancer Center cohort revealed ALK mutations in 3% of ATCs and fusions in 3.6% of PDTCs. IDH1 mutation was identified in 1.25% of PDTCs but not in ATC. IDH2 mutation was identified in 3% of ATCs but not in PDTC. The present study demonstrated that these genes are less frequently mutated in DTCs, but common in ATCs and PDTCs. It suggests that these genes serve a role in a small portion of DTCs and a more important role in ATCs and PDTCs and may serve as potential therapeutic targets in these subsets.

Introduction

Thyroid cancer is the most prevalent endocrine malignancy (1). Its incidence has been increasing over the past forty years in every part of the universe including Saudi Arabia (2). Mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3 kinase/AKT (PI3K/AKT) pathways are the two most genetically deregulated pathways in follicular cell-derived thyroid cancer. Albright activation of those vital signaling promotes uncontrolled cell division, proliferation, growth, invasion, and metastasis that collectively lead to thyroid tumorigenesis (3). Differentiated thyroid cancer (DTC) is the most commonly diagnosed type of thyroid cancer. DTC refers to papillary (PTC) and follicular thyroid cancer (FTC). Two other more aggressive subtypes of thyroid cancer include the poorly differentiated (PDTC) and anaplastic thyroid cancer (ATC) (4). Genetic alterations in DTC involve many genes and include RET/PTC, BRAF, RAS, PAX8/PPAR-γ, EGFR, EIF1AX, PPM1D, CHEK2, with a low prevalence of PIK3CA and PTEN genes (5-11). TERT promoter mutations have been demonstrated to be a major determinant of poor outcomes in DTCs (12-14).

High prevalence mutations of ALK (anaplastic lymphoma kinase), IDH1 (isocitrate dehydrogenase 1), IDH2 (isocitrate dehydrogenase 2), and MMP8 (matrix metalloproteinase 8) genes have been reported in diverse human cancers (15-17). ALK is a receptor tyrosine kinase that belongs to the insulin receptor subfamily. Initially, ALK has been identified as part of various oncogenic fusion genes (18,19). ALK mutations were found both in familial and sporadic neuroblastomas (6-14%). Most of the ALK mutations were identified within the catalytic domain of ALK. Some of its kinase domain mutations were demonstrated to be oncogenic. The ALK mutants F1174L and K1062M were shown to confer enhanced tyrosine kinase activity and promote cell transformation, focus formation, and tumor formation in nude mice (15).

Mutations of the IDH1 gene were frequently detected at high frequency in secondary glioblastomas (70%). IDH1 plays a key role within the Krebs cycle and produces α-ketoglutarate (α-KG) by catalyzing the oxidative decarboxylation of isocitrate. The IDH activity is exclusively dependent on

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nicotinamide adenine dinucleotide phosphate (NADP+) which is catalyzed by IDH1 to produce NADPH that is involved in controlling oxidative damage of the cell (20). The IDH1 mutations were shown to occur mainly in the hotspot arginine at codon R132 (R132H/S/C/G). The IDH2 mutations were identified in codon 172 and tumors without mutations in IDH1 often harbor mutations in the analogous amino acid arginine (R) at 172 of the IDH2 gene. All the codon R132 IDH1 mutants have been shown to have decreased enzymatic activity (16).

The MMPs are calcium-dependent zinc-containing proteolytic enzymes that play a vital role in the extracellular environment particularly in degrading the extracellular matrix (ECM) and non-matrix protein. Essentially, they are involved in morphogenesis, wound healing, tissue repair, and remodeling (21). MMP8 gene mutations have been frequently reported in melanoma (17). The majority of MMP8 mutations have been identified in exon 2. This gene was characterized as a tumor-suppressor as the wild-type MMP8 could inhibit cell proliferation on soft-agar, cell invasion, and tumor formation in the immunocompromised nude mice. Various mutations (S50F, P78S, K87N, and G104R) found in the MMP8 gene were demonstrated to be invasive and tumorigenic (17).

Common mutations of the ALK, IDH1, IDH2, and MMP8 genes have previously been found in anaplastic thyroid cancers (22-25). Furthermore, these genes were also implicated in the activation of MAPK, PI3K/AKT, and metabolic pathways to promote proliferation and invasion (15-21). However, the rates of ALK, IDH1, IDH2, and MMP8 mutations have not been examined specifically in differentiated thyroid cancer (DTC), particularly in Saudi Arabia, where the incidence of the DTC is within the top two cancers in Saudi women. Given the important role of these genes in anaplastic thyroid cancers and a prominent role in activating thyroid cancer-associated MAPK, PI3K/AKT pathways, we aimed to determine whether ALK, IDH1, IDH2, and MMP8 genes could carry somatic mutations in DTCs.

Materials and methods

Tumor samples and DNA extraction. Unselected malignant thyroid tumor tissues which were fixed in formaldehyde and embedded in paraffin, dissected using a microtome, selected without normal cell contamination, deparaffinized with xylene and subjected to genomic DNA isolation. This formalin-fixed paraffin-embedded (FFPE) DTC samples with a total of 126 for ALK, 271 for IDH1 and IDH2, and 50 for MMP8 were used in this study. Non-cancer samples consisting of 17 multinodular goiters (MNGs) were also included for mutational analysis of the above-mentioned genes. Somatic mutations of each of these genes were analyzed at different time periods caused to have a different cohort with variable sample size. Our inclusion criterion is to consider only malignant thyroid tumors and hence samples we obtained after confirming malignancy by the pathologist in a particular period of study. Therefore, these samples were unselected and used in an unbiased manner for each study. In Saudi population, the aggressive thyroid cancer subtypes (ATCs and PDTCs) are rare. Therefore, we did not use these as exclusion criteria. Baseline demographic and clinical characteristics for each cohort are listed in Table I. This research work was approved (RAC-2130015) by the Institutional Review Board (IRB) of King Faisal Specialist Hospital and Research Centre (KFSH & RC), Riyadh, Saudi Arabia. Samples were carefully examined by an experienced pathologist (H.A.) and dissected with ~10-micron thickness from FFPE tissue. Genomic DNA was isolated from the FFPE tissue by a commercially available kit (Gentra Puregene; Qiagen) per the manufacturer's instruction as previously described (26).

**PCR amplification and sequencing.** Exons 23, 24, and 25 of the ALK gene were amplified in 126 DTCs and exon 4 of the IDH1 and IDH2 gene was amplified in 271 DTCs. Exons 1-10 of the MMP8 gene were amplified in 50 DTCs. Primers (sense and antisense) and PCR conditions for the ALK, IDH1, IDH2, and MMP8 gene amplification were used as exactly described before (15-17). The amplified PCR products (amplicons) were directly sequenced using the BigDye terminator v3.1 cycle sequencing ready reaction kit (Applied Biosystems; Thermo Fisher Scientific, Inc.). All the identified genetic alterations were ascertained in both sense and antisense sequencing. The related sequencing results were analyzed against the appropriate gene. GeneBank accession no: ALK (NM_005296.2), IDH1 (NM_002168.2), IDH2 (NM_002168.2), and MMP8 (NM_002424.2).

Analyses of the mutational rates of ALK, IDH1, IDH2, and MMP8 genes in differentiated thyroid cancers (DTCs). The TCGA data comprising 496 DTCs (well differentiated papillary thyroid carcinoma) were analyzed for the mutational frequencies of ALK, IDH1, IDH2, and MMP8 genes. Mutations/deletions were included and copy number variations (CNVs) were omitted in this study (9).

Analyses of the mutational rates of ALK, IDH1, IDH2, and MMP8 genes in aggressive thyroid cancers (PDTC and ATC). The next-generation sequencing data of 117 aggressive thyroid cancer samples [84 poorly differentiated (PDTC) and 33 anaplastic thyroid cancer (ATC)] from the MSKCC cohort were analyzed in this study (27). We excluded CNVs while only the mutations/deletions were included. TCGA and MSKCC data were analyzed with the tools incorporated within the eBioPortal for Cancer Genomics (www.ebiportal.org).

**Statistical analysis.** In this study, various basic statistical analyses, including percentage, histogram and median, were performed using GraphPad Prism (v8.0.2; GraphPad Software, Inc.).

Results

No somatic mutations were identified in the examined exons of ALK, IDH1, IDH2, and MMP8 genes in DTCs and MNGs (Table II). Mutations of the ALK (exons 23, 24, and 25), IDH1, IDH2 (exon 4), and MMP8 (all exons 1-10) genes were analyzed by PCR amplification followed by direct Sanger sequencing. We selectively analyzed the indicated exons because they harbored the majority of the reported mutations in these genes. Although no somatic mutation was found in MMP8, as illustrated in Fig. 1A and B, we found five previously reported non-synonymous single nucleotide polymorphisms (SNPs) in this gene [S3C (rs17099450), 1/50 (2%), [T32I (rs3765620), 4/50 (8%) in exon 1, [K87E (rs1940475) 43/50 (86%)] in exon 2, [L310P (rs61753779) 1/50 (2%) in
exon 7, and [K460T (rs35866072) 9/50 (18%)] in exon 10, and a synonymous SNP [L291L (rs61753779) 2/50 (4%)] in exon 6 of the *MMP8* gene. We also observed a high rate of [86% (43/50)] heterozygous/homozygous A>G transition at nucleotide position 259, resulting in codon 87 changing from AAA to GAA, lysine to glutamic acid (K87E) in exon 2 of *MMP8*. At nucleotide position 259, we observed GG in 48% (24/50), AG in 38% (19/50), and AA in 14% (7/50). The allele frequency of A=0.14 in this study and it varies greatly (A=0.25-0.499) across world regions (https://www.ncbi.nlm.nih.gov/snp/rs1940475). All these SNPs were documented in the SNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/).

Analyses of TCGA data revealed rare mutations of the ALK and *MMP8* and no mutation of *IDH1* and *IDH2* genes in DTCs. To test whether our results corroborate TCGA data, which is mostly derived from the Western population, we analyzed TCGA data of DTCs (6). As shown in Fig. 2A, although 496 samples were included in the study, only 414 samples included data on the *ALK, IDH1, IDH2,* and *MMP8* genes. Genetic alterations of ALK were found in 1% (4/414) of DTCs. Of 4 *ALK*-altered cases, 1 follicular variant papillary thyroid cancer (FV PTC) case had a mutation (ALK, P693S) and the other 3 conventional PTCs (CPTCs) had *ALK* fusions (*ALK*-STRN, *ALK*-EML4, and *GTF2IRD1*-ALK). Only one *MMP8* mutation (MMP8, A444S) was observed (in CPTC) out of 414 DTC samples (0.24%). No *IDH1* and *IDH2* gene mutations were identified in the DTCs of TCGA (Fig. 2B-F; Table II). These findings suggest that only a small subset of DTCs harbors *ALK* and *MMP8* genetic alterations but not *IDH1* and *IDH2* mutations.

Analyses of aggressive thyroid cancers (PDTC and ATC) showed common somatic mutations in *ALK, IDH1,* and *IDH2* genes but not in the *MMP8* gene. We found a low rate of *ALK,* *IDH1,* *IDH2,* and *MMP8* mutations in DTCs including our study and TCGA. We, therefore, analyzed the next-generation sequencing data of 84 PDTC and 33 ATC from the MSKCC cohort to examine whether these mutations play any role in

| Characteristic | ALK | IDH1 and IDH2 | MMP8 |
|---------------|-----|---------------|-----|
| Number of patients (%) | 126 (100.0) | 271 (100.0) | 50 (100.0) |
| Sex, n (%) | | | |
| Male | 23 (18.3) | 62 (22.9) | 15 (30.0) |
| Female | 89 (70.6) | 209 (77.1) | 31 (62.0) |
| N/A | 14 (11.1) | 0 (0.0) | 4 (8.0) |
| Age, years | | | |
| Range | 11-71 | 9-75 | 21-75 |
| Median age | 42 | 42 | 48 |
| Tumor types, n (%) | | | |
| PTC | | | |
| CPTC | 70 (55.6) | 142 (52.4) | 31 (62.0) |
| FV PTC | 24 (19.0) | 67 (24.7) | 10 (20.0) |
| TC PTC | 16 (12.7) | 29 (10.7) | 1 (2.0) |
| DSV PTC | 2 (1.6) | 4 (1.5) | 1 (2.0) |
| CCV PTC | 2 (1.6) | 4 (1.5) | 1 (2.0) |
| OV PTC | 1 (0.8) | 5 (1.8) | 1 (2.0) |
| HCC | 1 (0.8) | 3 (1.1) | 1 (2.0) |
| FTC | 4 (3.2) | 7 (2.6) | 0 (0.0) |
| N/A | 6 (4.8) | 6 (2.2) | 4 (8.0) |
| Tumor size, n (%) | | | |
| >4 cm | 16 (12.7) | 54 (19.9) | 11 (22.0) |
| 1-4 cm | 89 (70.6) | 196 (72.3) | 32 (64.0) |
| <1 cm | 6 (4.8) | 14 (5.1) | 3 (6.0) |
| N/A | 15 (11.9) | 7 (2.6) | 4 (8.0) |
| TNM stage, n (%) | | | |
| <I-II | 92 (73.0) | 211 (77.9) | 32 (64.0) |
| III-IV | 19 (15.0) | 57 (21.0) | 14 (28.0) |
| N/A | 15 (11.9) | 3 (1.1) | 4 (8.0) |

N/A, not available; ALK, anaplastic lymphoma kinase; IDH1, isocitrate dehydrogenase 1; IDH2, isocitrate dehydrogenase 2; MMP8, matrix metalloproteinase 8; PTC, papillary thyroid cancer; CPTC, conventional PTC; FV, follicular variant; TC, tall cell; DSV, diffuse sclerosing variant; CCV, columnar cell variant; OV, oncocytic variant; HCC, Hürthle cell carcinoma; FTC, follicular thyroid cancer.
Table II. Prevalence of ALK, IDH1, IDH2 and MMP8 gene somatic mutations in DTCs, ATCs and PDTCs.

| Cohort   | Thyroid cancer subtypes | Genes               | Alterations (mutations/fusions) | Prevalence [altered cases/total cases analyzed (%)] |
|----------|-------------------------|---------------------|--------------------------------|-----------------------------------------------|
| Current study | DTC                     | ALK (0)             | 0/126 (0.0)                     |
|           |                         | IDH1 (0)            | 0/271 (0.0)                     |
|           |                         | IDH2 (0)            | 0/271 (0.0)                     |
|           |                         | MMP8 (0)            | 0/50 (0.0)                      |
| TCGA      | DTC                     | ALK (4)             | 4/414 (1.0)                     |
|           |                         | IDH1 (0)            | 0/414 (0.0)                     |
|           |                         | IDH2 (0)            | 0/414 (0.0)                     |
|           |                         | MMP8 (1)            | 1/414 (0.2)                     |
| MSKCC     | ATC                     | ALK (1)             | 1/33 (3.0)                      |
|           |                         | IDH1 (0)            | 0/33 (0.0)                      |
|           |                         | IDH2 (1)            | 1/33 (3.0)                      |
|           |                         | MMP8 (0)            | 0/33 (0.0)                      |
| PDTC      | ALK (3)                 | EML4-ALK; STRN-ALK; CCDC149-ALK | 3/84 (3.6)                     |
|           | IDH1 (1)                | V178I               | 1/84 (1.2)                      |
|           | IDH2 (0)                |                     | 0/84 (0.0)                      |
|           | MMP8 (0)                |                     | 0/84 (0.0)                      |

TCGA, The Cancer Genome Atlas; MSKCC, Memorial Sloan Kettering Cancer Center; DTC, differentiated thyroid cancer; CPTC, conventional papillary thyroid cancer; FV PTC, follicular variant PTC; ATC, anaplastic thyroid cancer; PDTC, poorly differentiated thyroid cancer; ALK, anaplastic lymphoma kinase; IDH, isocitrate dehydrogenase; MMP8, matrix metalloproteinase 8; STRN, striatin; EML4, echinoderm microtubule-associated protein-like 4; GTF2IRD1, general transcription factor II-I repeat domain-containing protein 1; CCDC149, coiled-coil domain-containing protein 149.

Figure 1. Identification of MMP8 genetic variants. (A) Chromatophereogram of MMP8 SNPs. Sequencing results are shown with a representative wild-type and mutated (heterozygous and homozygous) sense sequence chromatopherogram of the frequently detected SNP (A259G) in exon 2 of the MMP8 gene. The MMP8 gene is localized on chromosome 11q22.3. (B) Schematic diagram of MMP8 protein. Various domains of MMP8 indicating a synonymous (L291L) and non-synonymous SNPs (S3C, T32I, K87E, L310P and K460T) identified in differentiated thyroid cancers. MMP8, matrix metalloproteinase 8.
the more aggressive subtypes of thyroid cancer. As shown in Fig. 3A, B, E and H, the **ALK** mutation (K1079N) was found in 3% (1/33) of ATC and various fusions (**EML4-ALK**, **STRN-ALK**, and **CCDC149-ALK**) were identified in 3.6% (3/84) of PDTC. The **IDH1** (V178I) and **IDH2** (T435M) mutations were found in 1.2% (1/84) of PDTC and 3% (1/33) of ATC, respectively (Fig. 3C, D and F-H; Table II). No **IDH1** and **IDH2** mutations were found in ATC and PDTC, respectively. None of the ATC and PDTC samples harbored **MMP8** mutations (Fig. 3H). These results suggest that, unlike the **MMP8** gene, the **ALK**, **IDH1**, and **IDH2** are likely to have a role in aggressive subtypes of thyroid cancer (ATC and PDTC).

**Discussion**

The **ALK**, **IDH1**, **IDH2**, and **MMP8** gene mutations were recurrently reported in human cancer. Particularly, point mutations of these genes were demonstrated to be major therapeutic targets and important prognostic markers. However, to date, the prevalence of somatic mutations of these genes has never been examined in DTC from Saudi Arabia, a highly consanguineous society with high prevalence of thyroid cancer. We, therefore, studied somatic point mutations of these genes in samples of DTC from this population.

In this study, we found five non-synonymous SNPs (S3C, T32I, K87E, L310P, and K460T) and a synonymous SNP (L291L) of the **MMP8** gene. We found no **ALK**, **IDH1**, **IDH2**, and **MMP8** gene mutations in DTC and benign goiters. The **MMP8** SNP, S3C (rs17099450), has been shown to be one of the significant genetic determinants of allergic sensitization to cockroach allergens in children. However, the role of this SNP in cancer is not fully investigated (28). The **MMP8** SNP K87E (rs1940475) has been shown to have a differential effect on human cancers. For example, rs1940475 was reported to be associated with an enhanced risk of bladder cancer in never smokers while it was shown to protect from the invasive type in former smokers of this malignancy (29). Moreover, rs1940475 has been reported to be significantly associated with a higher risk for recurrence, reduced overall survival, recurrence-free survival, and disease-free survival in gastric adenocarcinoma (30). Further, this SNP was described to have a reduced risk for basal cell carcinoma (BCC) while exhibited no effect in squamous cell carcinoma (SCC) and melanoma (31). Conversely, a recent meta-analysis of several
MMP8 polymorphisms showed that the K460T (rs35866072) and K87E (rs1940475) variants were not significantly associated with cancer susceptibility (32). Nevertheless, this SNP was not substantially studied despite its higher prevalence in PTCs of the Western population (80.6%) (8) and DTCs in our study (86%) suggesting that a future study with a large number of samples is warranted to discover the role of this SNP in thyroid cancer. The MMP8 SNP K460T (rs35866072) was shown to have no association with cancer risk including leukemia, head and neck, lung, breast, and bladder (33). The importance of other rare MMP8 SNPs (rs3765620 and rs61753779) remains unknown in cancers including thyroid cancer.

Besides, while our study revealed no mutations in ALK, IDH1, IDH2, and MMP8 genes in DTCs, we comprehensively analyzed the TCGA data from a completely distinct ethnic background, a large Western cohort for DTCs (n=414). Consistent with our data, we found a rare incidence of ALK (0.97%) and MMP8 (0.24%) genetic alterations and no incidence of IDH1 and IDH2 mutations. Our study and TCGA data result collectively suggest that somatic mutations of the ALK, IDH1, IDH2, and MMP8 are uncommon in DTCs and hence play a pivotal role in a small portion of DTC pathogenesis.

Aggressive subtypes of thyroid cancers including PDTC and ATC are generally rare yet they are deadly. Notably, ATC has <5 months of median survival from the initial detection (3). Therefore, we inquisitive whether these genes play any role in aggressive thyroid cancers (PDTC and ATC). Analysis of ALK, IDH1, IDH2, and MMP8 gene mutations in 117 aggressive thyroid cancers from MSKCC data revealed 3% of ALK genetic alterations including 3% mutations in ATC and 3.6% fusions in PDTC. Consistently, two previous studies also independently showed identification of ALK somatic mutations in ATCs ~10% (22,23) which is much higher than the currently analyzed aggressive thyroid cancer data (3%) from MSKCC (27).

IDH1 was found in 1.2% of PDTC while no IDH1 mutation was detected in ATC. The IDH2 mutation was observed in 3.03% of ATC but not in PDTC. Interestingly, similar to ALK mutations, a high frequency of IDH1 mutations has previously been reported both in ATCs (11%) and undifferentiated thyroid cancers (33%) (24,25). The prevalence of IDH1 mutations in the previously reported
cases was relatively higher when compared with the current analysis of aggressive thyroid cancer (ATC and PDTC) data derived from MSKCC (27). The incidence of the ALK and IDH1 and IDH2 mutation in aggressive thyroid cancer cases varies among different studies, likely because of the selection of tumor tissue, tissue preservation, mode of tissue dissection, tumor tissue with normal cell contamination, number of samples, and sequencing methods that could greatly influence the incidence of mutation (34). Collectively, data from MSKCC and other previous studies strongly suggest that the ALK, IDH1, and IDH2 are likely to play an important role in the pathogenesis of some cases of aggressive thyroid cancers including ATCs and PDTCs. Consistent with a previous study (8), somatic mutation of the MMP8 was not found in the current analysis of aggressive thyroid cancer (PDTC and ATC) and this was also observed in both DTCs of our study and only one case in TCGA (9) suggesting that the MMP8 somatic mutations are rare in thyroid cancer regardless of its subtypes.

Moreover, the mutational incidence of DTC-specific genes including BRAF, TERT, RAS and PIK3CA, in the Saudi Arabian cohort was comparable to that of the incidence of the Western cases (10,11,35). The limitation of this study is a failure to analyze the ALK gene fusion which is considerably detected in thyroid cancers (9,27).

More than 85% of DTCs are treated and cured with surgical methods, radioactive iodine, and TSH suppression. About 50‑60% of DTCs harbor the BRAFV600E mutation (10). Two BRAF inhibitors (vemurafenib and dabrafenib) are in clinical use. The vemurafenib is used for BRAFV600E mutated thyroid cancer with radioactive iodine-refractory phenotype and the dabrafenib along with tramatenib, a MEK inhibitor is used for BRAFV600E‑mutated ATC (36). Rare DTC cases harboring ALK alterations may benefit from a range of first (crizotinib), second (alectinib and brigatinib), and third (lorlatinib) generation ALK inhibitors, and they are currently approved for ALK-positive non-small cell lung cancers (37). Similarly, IDH1-mutant cases could be treated with ivosidenib or recently developed vaccine targeting IDH1 mutants (38). Although MMP inhibitors were developed a few decades ago, their therapeutic impact on cancer was not that potent as expected in the beginning; yet, thyroid cancers with genetic alterations in MMPs are likely to benefit from BRAF inhibitors as they mediate signals downstream of BRAF (39). Given the availability of these therapeutic agents, tumors bearing these gene alterations may have the advantage of being treated precisely and more effectively.

In conclusion, both the data derived from our study and the TCGA revealed a rare incidence of ALK, IDH1, IDH2, and MMP8 gene mutations in DTCs and a higher prevalence of mutations of these genes in ATCs and PDTCs. The findings suggest that these mutations are rare and could play a role in the pathogenesis of a small subset of DTCs but are more likely to have a role in the aggressive tumor subtypes and may serve as potential therapeutic targets at least in a portion of PDTCs and ATCs.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. In addition, the mutation rate datasets generated and/or analyzed during the current study are available in the cBioPortal repository (https://www.cbioportal.org/study/summary?id=tca_pub and https://www.cbioportal.org/study/summary?id=thyroid_mskcc_2016).

Authors’ contributions

AKM and EQ performed the experiments and analyzed the data. AKM wrote the manuscript. HAH carefully identified and selected the samples and was involved in interpretation of data. ASA contributed to conception and design, critically reviewed the data and revised the manuscript. AKM and ASA confirm the authenticity of all the raw data. All the authors read and approved the final manuscript.

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The present study was approved (RA-C-2130015) by the Institutional Review Board of King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia. Written informed consent was obtained from all individual participants included in the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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