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 and K460T) in DTCs but no somatic mutation in *ALK*, *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 (PDTC); 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.

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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. Aberrant 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%). IDH 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

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-indicated 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_005896.2), *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 cBioPortal for Cancer Genomics (www.cbioportal.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

Table I. Characteristics of DTCs used for mutational analysis of *ALK*, *IDH1, IDH2* and *MMP8* genes.

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.

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 259 position, 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

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) P693S (FV PTC); *ALK-STRN* (CPTC); *ALK-EML4* (CPTC) *GTF2IRD1-ALK* (CPTC)	4/414 (1.0)
		IDH1	(0)	0/414 (0.0)
		IDH2	(0)	0/414 (0.0)
		MMP8	(1) A444S (CPTC)	1/414 (0.2)
MSKCC	ATC	*ALK*	(1) K1079N	1/33 (3.0)
		IDH1	(0)	0/33 (0.0)
		IDH2	(1) T435M	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.

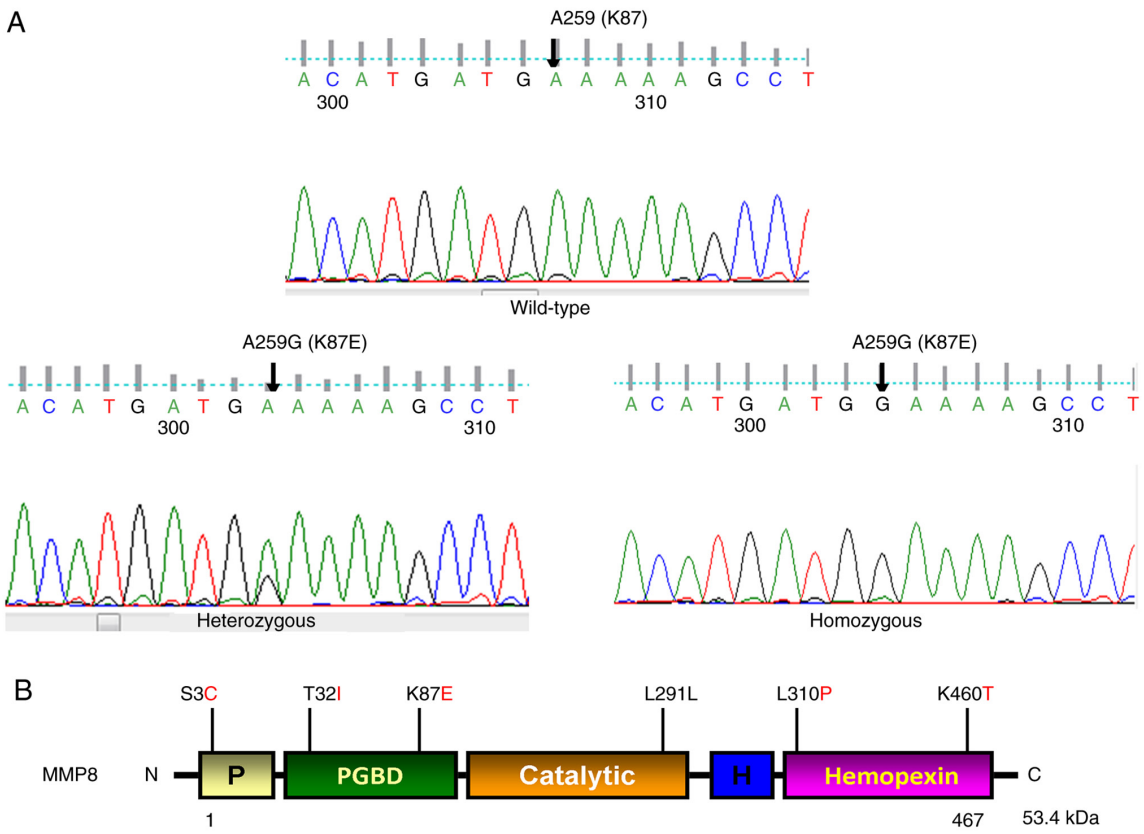

Figure 1. Identification of *MMP8* genetic variants. (A) Chromatopherogram of *MMP8* SNPs. Sequencing results are shown with a representative wild-type and mutated (heterozygous and homozygous) sense sequence chromatopherogram of the frequently detected SNP (A259G) in exon 2 of the *MMP8* gene. The *MMP8* 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.

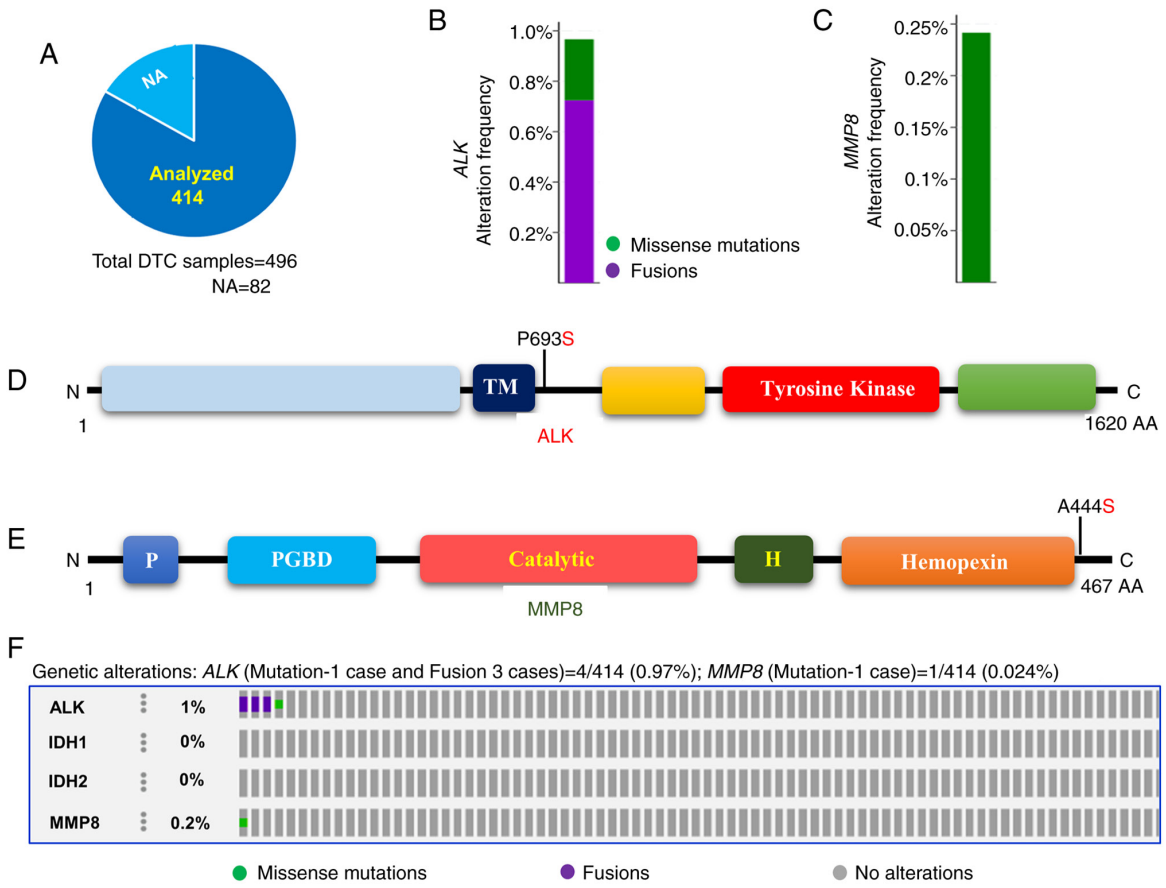

Figure 2. Prevalence of *ALK*, *IDH1*, *IDH2* and *MMP8* mutations in DTCs. (A) Pie chart of DTCs. The chart indicates the type and number of tumor samples analyzed from the DTCs of TCGA. (B) Histogram showing the genetic alterations of *ALK* in DTCs. The bar indicates both the mutation and fusion of *ALK* in 0.97% of DTCs. (C) Histogram showing the somatic mutations of the *MMP8* gene. The bar indicates the somatic mutation of *MMP8* (0.24%) in DTCs. (D) Mutation tab. Schematic illustration showing ALK protein and its respective domains with indicated mutation. (E) Mutation tab. Schematic diagram shows MMP8 protein and its respective domains with indicated mutation. The frequently mutated residue is depicted in the diagram. (F) OncoPrint tab. The tab indicates the *ALK*, *IDH1*, *IDH2* and *MMP8* mutations across the DTCs (TCGA). ALK, anaplastic lymphoma kinase; TM, transmembrane domain; Tyrosine kinase, tyrosine kinase domain; IDH1, isocitrate dehydrogenase 1; IDH2, isocitrate dehydrogenase 2; MMP8, matrix metalloproteinase 8; P, propeptide; PGBD, proteoglycan binding domain; catalytic, catalytic domain; Hemopexin, hemopexin-like domain; DTC, differentiated thyroid cancer; TCGA, The Cancer Genome Atlas; NA, not available.

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

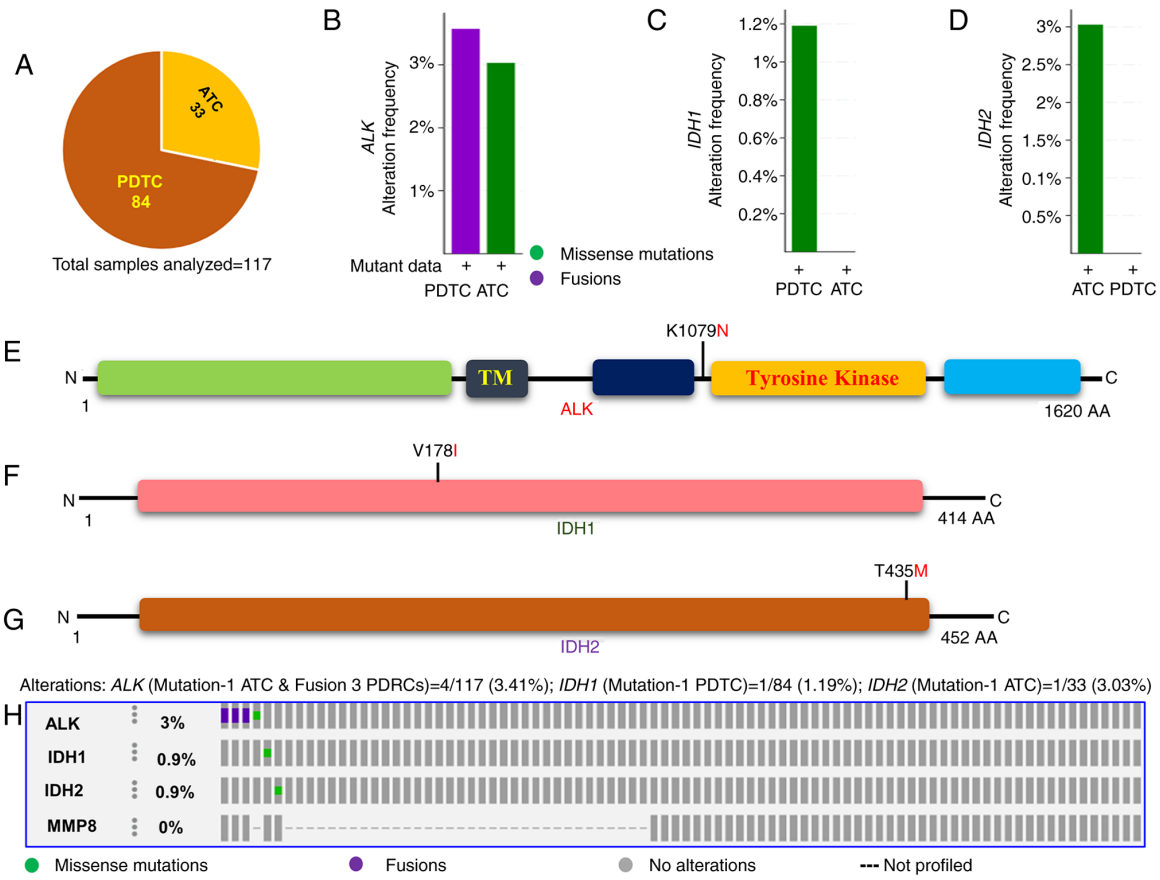

Figure 3. Prevalence of *ALK*, *IDH1*, *IDH2* and *MMP8* mutations in aggressive thyroid cancers (PDTC and ATC). (A) Pie chart of aggressive thyroid cancers. The chart displays the type of tumors, the number of samples in each type and the total number of aggressive thyroid cancer samples analyzed. (B) Histogram showing the genetic alterations of *ALK* in PDTC and ATC. The bar indicates the overall frequency of *ALK* fusions and mutations in PDTC (3.6%) and ATC (3.03%), respectively. (C) Histogram indicating the mutation rate of *IDH1*. The bar indicates 1.2% *IDH1* mutations in PDTC. (D) Histogram showing the mutation rate of *IDH2*. The bar indicates 3.03% *IDH2* mutations in ATC. (E) Mutation tab. The schematic diagram displays ALK protein and its domains indicating the position of the detected mutation. (F) Mutation tab. The schematic diagram shows IDH1 protein and indicating the position of the detected mutation. (G) Mutation tab. The schematic diagram shows IDH2 protein indicating the position of the detected mutation. (H) OncoPrint tab. The tab indicates the *ALK*, *IDH1*, *IDH1* and *MMP8* mutations across the aggressive thyroid cancers, PDTC and ATC. Each row represents a particular gene and each column displays a tumor sample. The non-synonymous mutations are indicated as green square plots on the columns. ALK, anaplastic lymphoma kinase; IDH1, isocitrate dehydrogenase 1; IDH2, isocitrate dehydrogenase 2; MMP8, matrix metalloproteinase 8; PDTC, poorly differentiated thyroid cancer; ATC, anaplastic thyroid cancer.

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 were 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 $BRAF^{V600E}$ mutation (10). Two BRAF inhibitors (vemurafenib and dabrafenib) are in clinical use. The vemurafenib is used for $BRAF^{V600E}$ mutated thyroid cancer with radioactive iodine-refractory phenotype and the dabrafenib along with tramatenib, a MEK inhibitor is used for $BRAF^{V600E}$-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=thca_tcga_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 (RAC-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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