Clinical characteristics and significance of DICER1 mutation in advanced Differentiated thyroid cancer

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Abstract

Background

Up to 2/3 distant metastatic patients have poor prognosis, though most patients with DTC generally carry a favorable clinical outcome with the current standard treatments. The discovery of gene alterations profoundly expands our understanding into tumor oncogenic mechanism. As one of key component of microRNA processing pathway, DICER1 mutation in DTC has been described with increasing frequency, while its clinical significance as well as role in DTC progression is still needed to be investigated.

Method

A total of 313 FFPE samples from patients with DTC who underwent total thyroidectomy between 2011 and 2022 were collected and targeted next-generation sequencing was performed by ThyroLead panel with 26 genes included. The clinicopathological characteristics and response to radioiodine therapy, particularly radioiodine-refractory(RAIR) status of patients with DICER1 point mutation were retrospectively analyzed.

Results

A total of 313 DTC patients with intermediate or high risk features were included in our cohort. The overall prevalence of DICER1 mutation was 7.3% (23/313) in DTC patients with a mean age at diagnosis of 36.6±15.3 years old. Comparing with DICER1 mutation-negative, larger primary tumor (P=0.008) and more aggressive pathological type (P<0.001) was observed in DICER1 mutation-positive patients. Comparing with no mutation wild type group, more advanced AJCC T staging occurred in DICER1 mutation alone group (Ⅲ/Ⅳ occurrence 100% versus 42.9% (P=0.04)) though no difference between which in terms of primary tumor size (P=0.002) and pathological type (P=0.01). Of note, 69.6% (16/23) DICER1 mutated patients were identified coexisted with other active genetic alterations implicated with aggressive DTC evolvement, including BRAF, TP53, TERT promoter mutations, and RET fusions. Among these DICER1 co-mutation patients, except for the features of larger primary tumor size(P=0.04) aggressive pathological type (P<0.0001), worse response to RAI therapy was also observed, indicating the possible synergistic role of DICER1 mutation in promoting the progression of DTC, even loss of RAI avidity, and poor response to RAI therapy.

Conclusion

In this Chinese DTC cohort with high risk features, DICER1 mutation was revealed to associate with large tumor size, aggressive pathological type, and advanced TNM staging. The more common coexistence of DICER1 mutation and aggressive genetic events such as BRAF, TP53, and TERT mutations suggested it might play synergistic role in promoting the progression of DTC, even loss of RAI avidity, and poor response

to RAI therapy.

Keywords: differentiated thyroid cancer, DICER1 mutation, next-generation sequencing

Introduction

Thyroid cancer is the third most common cancer in women in China and the incidence rate continues to rise[1] . Differentiated thyroid cancer (DTC) accounts for more than 90%, includes papillary, follicular, Hürthle cell and poorly differentiated thyroid cancer[2, 3]. Despite DTC usually have excellent prognosis with existing therapy include surgery, radioactive iodine therapy and active surveillance, but 5-10% of cases developed metastatic disease, less than 5% of all DTC will progress to radioiodine-refractory differentiated thyroid cancer(RAIR-DTC), which have a poor prognosis with a 10-year survival rate is only 10%[4, 5].

The significant advance in thyroid cancer-related genes improved the understanding of molecular mechanism and disease course, such as $BRAF^{V600E}$ and TERT promoter mutations, which were proved to be related to aggressive thyroid cancer characteristics, tumor recurrence and patient mortality, even loss of radioiodine avidity[3, 6-8]. But some genetic alterations such as DICER1 mutation occurring in DTC whose association with disease progression are not yet well known.

DICER1 is a RNase III-type enzyme and a key component of microRNA processing pathway, which cleaves miRNA precursors to mature miRNAs and suppresses gene expression by preventing translation and/or promoting mRNA degradation[9, 10]. The effect of DICER1 on miRNAs processing machinery DICER1 is also proved essential for thyroid function[11]. Since DTC was first described in DICER1 syndrome in 2014, direct association between both germline and somatic DICER1 mutation and increased risk for DTC was supported by subsequent reports[12-14].

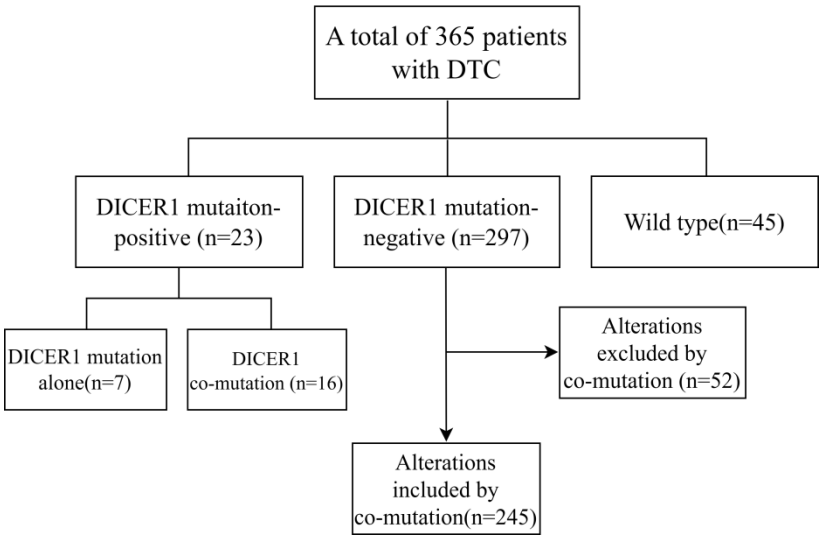
In DTC, DICER1 mutation has been reported in PTC, FTC, and PDTC with small sample size[15-17]. Pathogenic somatic DICER1 mutation is rare with a prevalence of 0.5% in adult PTCs identified by The Cancer Genome Atlas Program while other studies shows much higher prevalence[18, 19]. In pediatric DTC, DICER1 mutation are obviously more common than in adults[15, 17]. Interestingly, previous studies reported that DTCs with DICER1 mutation mostly show an indolent behavior and may represent a class of low-risk malignancies, while recently several researches manifested the opposite findings which indicated DICER1 mutation associated with aggressive clinicopathologic characteristics in children and rendered the possible underlying mechanism [15, 16, 20, 21].

Considering rarity of DICER1 mutation and its varying association with clinical pathology, the role of DICER1 mutation in DTC remains to be clarified. In this Chinese DTC cohort with advanced features, we aim to identify clinical and pathological features of patients with DTC harboring DICER1 mutation and to explore correlation between DICER1 mutation and aggressive clinicopathologic behaviors, particularly response to RAI/RAI non-avidity condition.

Materials and Methods

Study subjects

Our study consisted of 313 FFPE tumor tissue samples from patients with DTC admitted at the Peking Union Medical College Hospital between 2011 and 2022, and samples were collected from 313 patients include 255 adult patients and 58 pediatric patients under the age of 20 years. Detailed clinical and pathological data, including surgery, pathological examination, ^{131}I treatment, and other data (thyroid function level, imaging results, etc.) from all patients were collected. The off-site data were obtained through previous medical record with the consent of the patients when data was incomplete. The total cohort was divided into patients harbored DICER1 mutation (DICER1 mutation-positive), alterations excluded DICER1 mutation (DICER1 mutation-negative), and wild type groups. We subdivide DICER1-mutation positive patients into DICER1 mutation alone (DICER1 mutation-alone) and patients with DICER1 mutation coexisted with other alterations (DICER1 co-mutation). The alterations in DICER1 mutation-negative were consistent with DICER1 co-mutation group. The study was approved by the Hospital Ethics Committee of Peking Union Medical College Hospital. The approval number was __ and all patients provided written informed consent.(这里面 exclude 那部分需要细化说清楚)



Related Definitions（咱们还涉及到 intermediate to high risk 还需要复发风险分层的介绍 为什么有一些 co-mutation 就没有被排除？）

Pathological stage was identified according to American Joint Committee on Cancer (AJCC) 8th TNM Classification system for DTC. RAIR-DTC is defined as four basic situations based on 2015 American Thyroid Association Guidelines: (i) the malignant/metastatic tissue does not ever concentrate RAI(no uptake outside the thyroid bed at the first therapeutic WBS), (ii) the tumor tissue lose the ability to concentrate RAI after previous evidence of RAI-avid disease(in the absence of stable iodine contamination), (iii) iodine is concentrated in some lesions but not in others, and (iv) metastatic disease progresses despite significant concentration of RAI. The response to therapy were categorized as excellent response (ER), indeterminate response (IDR), biochemical incomplete response (BIR) and structural incomplete response (SIR) according to 2015 ATA Guidelines[2]. Variants are classified into pathogenic (P), likely pathogenic (LP), variant of uncertain significance (VUS), likely

benign and benign five categories based on American College of Medical Genetics and Genomics (ACMG) guidelines[22]. Missense located in DICER1 hotspots (e.g.E1705, D1709, G1809, D1810, E1813) or credibly reported as pathogenic in at least one publication are classified as pathogenic[22, 23].

Genetic analysis by NGS

Primary and metastatic DTC samples were collected and ThyroLead panel (Topgen, China) which covers the exonic region of 18 genes (HRAS, KRAS, NRAS, CDC73, CDKN1B, DICER1, IDH1, MEN1, MTOR, PIK3CA, PTEN, TP53, TSHR, CTNNB1, GNAS, PAX8, AKT1, EIF1AX), a 1000-bp region in the promoter region of TERT, and both exonic and intronic region for 7 fusion oncogenes (BRAF, RET, NTRK1/2/3, ALK and PPARG) was used. DNA was isolated using QIAamp DNA kit (Qiagen, Dusseldorf, Germany). Sequence libraries were prepared using KAPA HyperPlus Library Preparation Kit (KAPA, Roche, Switzerland) and ThyroidLead panel probe was used to conduct library capture to get pooled libraries[24]. Pooled libraries were checked using Agilent 2100 Bioanalyzer (Aglient, America) and subsequently sequenced using the NextSeq 2000 system (Illumina, America). Adapter sequences and low-quality reads (with more than 40% of base failed Q25; reads < 70bp; and low-complexity reads) were removed by Fasp. The reads were aligned to reference human genome hg19 with BWA-MEM using default parameters. The variant calling process and indel re-alignment were performed using Sentieon TnSeq. Variation annotation was performed according to databases such as COSMIC, 1000g, ESP, ExAC, gnomAD and ClinVar with VEP and Annovar. After quality control, samples with uneven depth of coverage, low de-duplicated depth or aberrant insert sizes were discarded in the next analysis.

Statistical Analysis

All analyses were performed using SPSS software for Windows (version 26.0) and GraphPad Prism 8.0. Differences in continuous variables were compared using 2-tailed Student's *t* test or 1-way ANOVA with Bonferroni's multiple-comparison test or Mann-Whitney *U* test. Categorical variables were compared using Chi-square test or Fisher's exact test. The *P* values are illustrated in the figures and legends. A P value of less than 0.05 was considered statistically significant.

Results

DICER1 Mutation identified in DTCs

In 313 cases of DTC, DICER1 mutation were identified in 23/313 (7.3%), other thyroid cancer-related alterations were identified in 245/313 (78.3%) and no mutation was identified in 45/313 (14.4%). According to ACMG guidelines and current information, among patients with DICER1 mutation, 43.5% (10/23) likely pathogenic mutation and 56.5% (13/23) variants of uncertain significance (VUS) were identified in DTC with DICER1 mutation. In known likely pathogenic DICER1 mutation, 8/10 (80%) patients carried somatic RNase IIIb hotspot mutation (p.E1705, p.D1709, p.G1809, p.D1810, p.E1813,p.D1713), which have been reported before [10].

Among DICER1 mutation identified, 30.4%（7/23）had DICER1 mutation only

and 69.6% (16/23) of which were found coexisting with other alterations. Among the coexisting mutations above 15%'s prevalence, BRAF, TERT promoter, TP53 mutations and RET fusions occurred with ?%,?% ?% and ?% respectively, indicating synergistic tumor-driven role of DICER1 mutation.

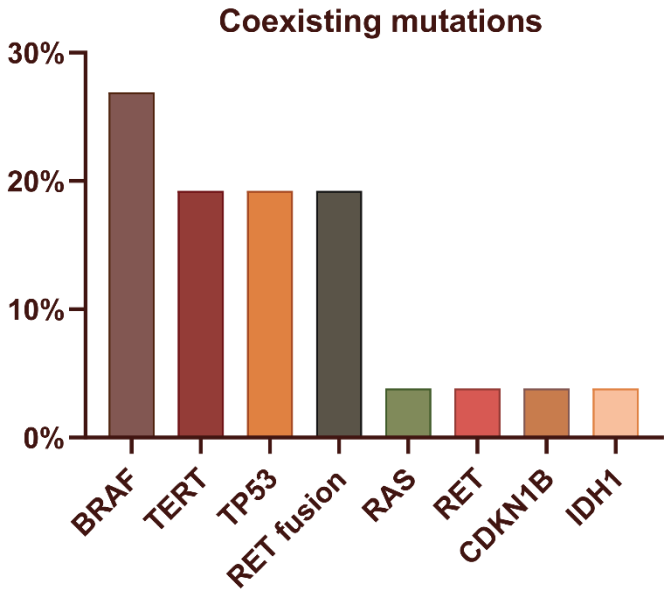


Figure1. Distribution of mutations coexisting with DICER1

Clinical and pathological characteristics in patients with DICER1 Mutation

The clinical and pathological data of patients with DICER1 mutation and its subgroups were summarized in Table 1. DICER1 mutation occurred with a female to male ratio of 1.3:1, and the mean age at diagnosis was 36.6±15.3 years (range 8-57 years). From the perspective of age distribution, 21.7% (5/23) were under 21 years old and 78.3%(18/23) were above 21 years old 这个地方更有意义的数据应该是 DICER1 在儿童 DTC 和成人 DTC 中的分别的发生率（such as 儿童 DICER1/儿童 DTC 总数）. DICER1 mutation occurred in 13/23 (56.5%) PTCs, 5/23 (21.7%) FTCs, and 4/23 (17.4%) PDTCs.

Of the patients with mutant DICER 1, 82.6% (19/23) with distant metastases 78.3%(?/?) with lymph node metastases, 65.2%(?/?) with extrathyroidal extension and 39.1%(?/?) with intravascular invasion? were noted. Sites of distant metastases included 11 cases of lung metastasis, 2 cases of lung with bone metastasis, and 6 cases of other sites, almost all cases affecting lungs (18/19). Sites of distant metastases included lung (18/19) metastasis, lung and bone (2/?), and other sites such as ???(?/?).

Radioactive iodine (RAI) therapy administered in 87% (20/23) patients with the median cumulative RAI dose of 289.1±229.1 mCi. In terms of response to radioiodine therapy among these patients，45%(9/20) had structural progression and 10%(2/20) had biochemical progression(?/?). The median follow-up was 35.1 months (range 12.5-113.3 months), 88.9% patients with structural progression and 100% patients with biochemical progression were identified as RAIR-DTC till last follow-up. Additionally, 82.6% of DICER1-mutated patients were identified as SIR, 13% as

BIR and 4.4% as ER, respectively in the latest evaluation. Totally, 47.8% (11/23) received targeted therapy due to disease progression. ？？

Table 1 Clinicopathologic characteristics of DICER1 mutation-positive DTCs

Clinicopathologic characteristics	all (n=23)	DICER1 mutation alone (n=7)	DICER1 co-mutation (n=16)
Age (year)	36.6±15.3	29.1±15.2	39.9±14.6
Age group (<21/21-55/>55), n	5/15/3	2/5/0	3/10/3
Gender (Male/Female), n	10/13	2/5	8/8
Number of surgery (time)	2（1-3）	2.3±0.8	1.5（1-2）
Tumor size (cm)	3.4±1.9	3.1±2.2	3±1.4
Histology (PTC/FTC/PDTC), n, NA=1	13/5/4/1	3/2/1/1	10/3/3/0
Extrathyroidal invasion (yes/no), n, NA=7	15/1/7	5/1/1	10/0/6
Vascular invasion (yes/no), n, NA=5	9/9/5	2/4/1	7/5/4
T classification (T1+T2/T3+T4), n, NA=6	5/12/6	0/4/3	5/8/3
N classification (N0/N1a/N1b, n, NA=1	4/3/15/1	2/0/5/0	2/3/10/1
Distant metastases (yes/no), n	19/4	5/2	14/2
Site of metastases (lung/lung&bone/other), n	11/2/6	3/1/1	8/1/5
RAI therapy (yes/no), n	20/3	5/2	15/1
Number of RAI therapy (time)	2（1-3）	1.1±1.1	2.3±1.4
Cumulative RAI dose (mCi)	289.1±229.1	171.4±155.1	340.6±240.9
Outcome of RAI therapy (progression/remission), n, NA=5	11/7/5	1/3/3	10/4/2
RAIR (yes/no/NA), n	11/8/4	2/5/0	9/3/4
$Tg^{+}I^{-}$ (yes/no/NA), n	1/21/1	2/6/1	1/15/0
Follow-up period (month)	35.1（12.5-113.1）	30.8（10.7-178.0）	45.9（12.9-110.8）
Last efficacy evaluation (ER/SIR/BIR), n	1/19/3	1/5/1	2/14
Targeted therapy (yes/no), n	11/12	2/5	9/7

Data are showed as mean ± standard deviation or median (interquartile range), RAI: radioactive iodine, PTC: papillary thyroid cancer, FTC: follicular thyroid cancer, PDTC: poorly differentiated thyroid cancer, RAIR: radioiodine refractory, $Tg^{+}I^{-}$: Tg increased but negative on ^{131}I Diagnostic Whole-body Scan, NA: not available

Association of DICER1 alteration and Clinicopathological Features of DTC

Firstly, we divided the entire cohort into alterations contain DICER1 (DICER1 mutation-positive, 23/313), alterations other than DICER1 (consistent with alterations co-occurred with DICER1, DICER1 mutation-negative, 245/313) and wild type (no genetic alteration identified) (45/313) three groups. DICER1 mutation-positive patients was older than wild type at diagnosis (36.6±15.3 vs. 28.3±15.4, *P*=0.038) (Figure 1A). had larger mean primary tumor than DICER1 mutation-negative and wild type group (?

vs ? vs? P=0.008, P=0.046, respectively) (Figure 1B). DICER1 mutant patients had higher occurrence of FTC and PDTC than DICER1 mutation-negative group (40.9% vs. 6.5%, P<0.001) and wild type group (40.9% vs. 8.9%, P=0.003) (Figure 1C).

Moreover, proportion of RAIR-DTC patients who received targeted therapy due to the disease progression in DICER1 mutation-positive group was obviously higher than other two groups (47.8% vs. 14.7%, P<0.001; 47.8% vs. 15.6%, P=0.004). These results indicated that thyroid cancer which harbored DICER1 mutation was significantly associated with larger primary tumor size and more aggressive pathological type, possibly related to worse clinical prognosis.put it into discussion

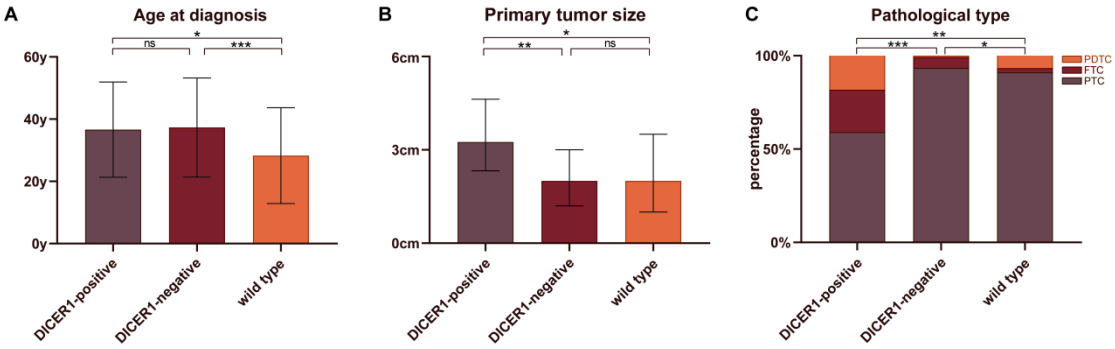


Figure 1. Comparison of clinical and pathological features among the 3 groups: DICER1 mutation-positive (n=23), DICER1 mutation-negative (n=245) and wild type (n=45). ns P>0.05, *P<0.05, **P<0.01, ***P<0.001. DICER1 mutation-positive, patients harbored DICER1; DICER1 mutation-negative, patients with alterations exclude DICER1; wild type, patients with no alterations.

Association of DICER1 mutation alone and Clinicopathological Features of DTC

To explore the role of DICER1 mutation alone in the progression of DTC, patients was further compared between those only have DICER1 mutation (n= 7) with those no alteration (n=45). DICER1 mutation-alone group had larger tumor (? Vs ? P=0.002) (Figure 2A) , more aggressive pathological types (42.9% (3/7) versus 8.9% (4/45) ,P=0.01) (Figure 2B), and more advanced AJCC T staging (Ⅲ/Ⅳ occurrence 100% vs 42.9% (P=0.04) (Figure 2C). Except for the association between DICER1 mutation alone with larger primary tumor size and aggressive pathological types as observed before, it also showed that patients with DICER1 mutation alone had higher AJCC T classification (should be put into discussion).

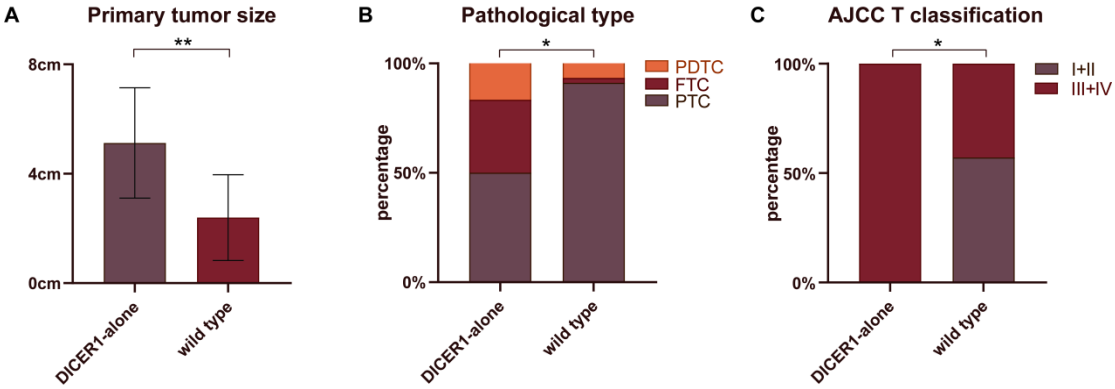


Figure 2. Comparison of clinical and pathological features between DICER1 mutation-alone group (n=7) and wild type group (n=45). *P<0.05, **P<0.01.

Comparison of coexistence of DICER1 alteration and alterations other than DICER1 on clinicopathological features of DTC

Furthermore, the difference of DICER1 co-mutation (n=16) and partner alterations other than DICER1(DICER1 mutation negative)(n=245) were compared. Again, we found DICER1 co-mutation group had larger primary tumor size and more aggressive pathological type and more progressive after RAI treatment than DICER1 mutation-negative group (Figure3). Among DICER1 co-mutation patients, more patients with N1b (62.5%10/16) and distant metastasis (87.5% 14/16) patients were observed. Of the DICER1 co-mutation patients who received RAI therapy(15/16), with a median follow up of ??, 87.5% (14/16) were identified as SIR and 12.5% (2/16) as BIR, 66.7% (10/15) presented progression after RAI therapy, 10 (66.7%) had progression after RAI therapy and 9 (60%) patients developed to RAIR status, suggesting worse outcomes in DICER1 co-mutation.

In terms of RAIR, difference can be observed when comparing DICER1 co-mutation and DICER1 mutation-negative (60% vs. 46.7%, P=0.056) and DICER1 mutation-alone (60% vs. 28.6%, P=0.074), which indicated DTC tend to be RAIR when harbor both DICER1 and other alterations.

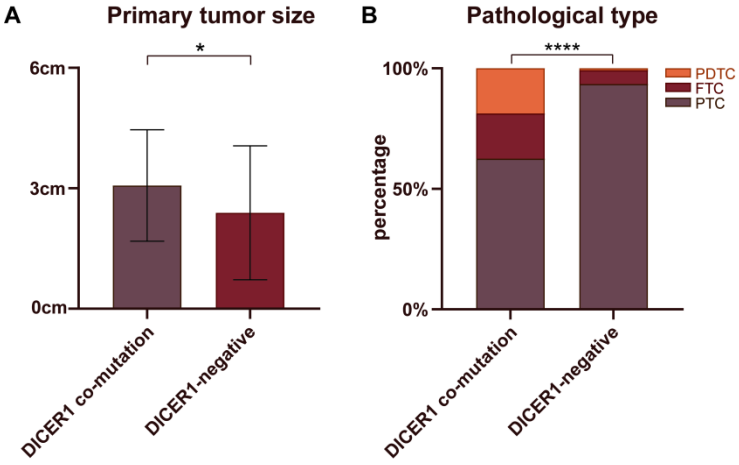


Figure 3. Comparison of clinical and pathologic features between DICER1 co-mutation group (n=16) and alterations without DICER1 mutation group (n=245). *P<0.05, ****P<0.0001.

Discussion

As many molecular alterations has been identified as new diagnostic and prognostic markers of thyroid cancer, clinical characteristics and prognostic value of many alterations such as BRAF, TERT mutation and RAS, NTRK fusions have been discussed before[25-27]. However, researches on DICER1 mutation remain very limited given its rarity. In this Chinese DTCs cohort mostly with locally advanced and metastatic features, we retrospectively explored the occurrence of DICER1 mutation, and its association with clinical, histological characteristics, as well as the RAI response

for the first time.

Different from indolent clinical behaviors reported in DICER1-mutated thyroid cancer before[15, 17, 20] , our study suggests DTC patients with DICER1 mutation may represent a subset of aggressive thyroid cancers. By comparing DICER1-positive, DICER1-negative, and wild type group, we found that DICER1 mutation is significantly associated with aggressive features such as larger primary tumor size and more aggressive pathological type. In order to avoid confounding bias due to other thyroid cancer-related alterations, we further compared DICER1-alone with wild type group. In addtion to same findings as above, it was showed that patients with DICER1 mutation alone had advanced AJCC T staging than those with wild type.

Interestingly, 69.6% of patients harbored DICER1 mutation were found to coexist with BRAF, TERT promoter, TP53 mutations and RET fusions, which was quite different from the known mutual exclusion between DICER1 and other alterations reported before[16, 20]. Although BRAF mutation is the most common among the co-mutations, mutations of TERT promoter and TP53, known late genetic events in DTC, also have been reported and was confirmed in this study[16]. Among DICER1-mutant cases, ?% TERT promoter and ?% TP53 mutations were identified. TERT and BRAF associated with RAIROne 46-year-old man had a double mutation and was found to progress to RAIR during follow-up.??

Considering the coexistence of DICER1 and other alterations is special and rare, we further analyzed the distinction between DICER1 co-mutation and DICER1 mutation-negative group. Except more aggressive clinicopathological behaviors, another promising finding was that more patients tended have disease progression after RAI therapy and advanced to RAIR-DTC in DICER1co-mutation group. The difference is near to statistical significance(P=0.056) and we believe the findings were clinically significant. Noticeably, the DICER1 co-mutation group was composed of BRAF, TP53, TERT promoter mutations, which were confirmed to have robust impact on clinical outcomes including disease recurrence and mortality[8, 28]. Moreover, many studies reported the correlation between these alterations and loss of radioiodine avidity[6, 7, 29]. These discoveries provide more evidence for strengthening our findings, suggesting that DICER1 mutation not only associate with clinicopathological features, but also being involved in the evolution of RAIR-DTC. TERT and BRAF??

Of note, DICER1 has been proved to act as a tumor suppressor, whose mutations decrease its expression, thus promoted proliferation, migration, and invasion of thyroid cancer by downregulating miRNA[11, 21, 30]. DICER1 also affects thyrocyte differentiation by regulating expression of key genes including Tg, TSHR, TPO even sodium iodide symporter (NIS), which is the cornerstone of RAI therapy[11, 31, 32]. Furthermore, DICER1 was confirmed to regulate transcription factors Nkx2-1 and PAX8 expression in thyroid tumor cells, which are essential for tumor differentiation, diagnosis and treatment[32, 33]. Thus the robust effect of DICER1 mutation on some clinicopathological behaviors we observed could be explained by above mechanism suggested and our research can provide strong clinical associated evidence for DICER1 being involved in the progression of thyroid cancer, as well as RAI-refractory status .

??? uncertain ??

It should be noted that our study had several limitations. First, the results were obtained from only Chinese patients. Second, survival outcomes were not collected completely due to limited follow up. Additionally, our study did not perform further study on the mechanism that how DICER1 mutation functions in progression of DTC.

To sum up, our work reveals some robust association between DICER1 mutation and aggressive clinical, pathological behaviors of DTC even worse response to RAI therapy . The exact role that DICER1 mutation plays in progression of DTC remains needed to be explored through?? study. DICER1 mutated DTCs require more attention and more research is needed to fully understand its effect.

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