Correlation of mismatch repair deficiency with clinicopathological features and programmed death-ligand 1 expression in thyroid carcinoma

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Abstract
Background Mutations in DNA mismatch repair (MMR) genes associated with thyroid carcinoma (TC) have rarely been reported, especially in East Asian populations.

Methods We examined tumor tissue from a cohort of 241 patients diagnosed with TC between 2008 and 2020. MMR proteins were detected using tissue microarray-based immunohistochemistry in order to identify MMR-protein-deficient (MMR-D) and MMR-protein-intact (MMR-I) tumors. We retrospectively summarized the clinicopathologic characteristics of patients with MMR-D TC, measured the expression of PD-L1, and recorded overall survival (OS) and other clinical outcomes.

Results In our cohort, there were 18 (7.5%) MMR-D (MLH1, MSH2, MSH6, and PMS2) patients, including 12 with papillary TC (PTC) (6.7%), 2 with poorly differentiated TC (PDTC) (4.7%), and 4 with anaplastic TC (ATC) (22.2%). Half of them (9/18) showed a specific deletion in MSH6, and 6 of them also carried variants in the MSH6 and PMS2 gene. Survival was significantly better in patients with MMR-D ATC than in those with MMR-I tumors ($p = 0.033$). Four of the 18 MMR-D patients (22%) were found to be PD-L1 positive. Their OS was much shorter than that of PD-L1-negative patients.

Conclusions MMR-D and PD-L1 positivity appear to be associated with clinicopathological characteristics and prognosis in TC. The results indicate that MMR status may have important prognostic significance in TC. Therefore, immune checkpoint inhibitors that target the PD-1/PD-L1 pathway may be a treatment option for TCs.

Keywords Mismatch repair · Thyroid carcinoma · PD-L1

Introduction
Thyroid cancer (TC) is one of the most common malignancies of the endocrine system, with the fifth-highest prevalence among women in the United States, and its incidence continues to increase rapidly [1]. Although most TCs follow an indolent clinical course, the metastasis rate of differentiated TC (DTC) remains very high. Further, poorly differentiated TC (PDTC) and anaplastic TC (ATC) are very aggressive tumors and are often likely to result in death [2, 3]. For most patients, there is a lack of effective treatment. Therefore, effective immunotherapies or alternative treatments are urgently required.

Mismatch repair (MMR) is a high-fidelity mechanism that allows the maintenance of fidelity in DNA replication and mediates DNA damage-related signal transduction [4]. MMR deficiency is characterized by a decrease or increase in
nucleotide repeats, which can lead to the evasion of apoptosis, development of malignant mutations, and tumorigenesis [5, 6]. MMR mainly involves four major proteins: MLH1, MSH2, MSH6, and PMS2 [7]. Latham et al. demonstrated that an MMR-protein-deficient (MMR-D)/microsatellite instability (MSI) status is associated with Lynch syndrome, which is an autosomal-dominant syndrome that predisposes individuals to cancer [8]. Lynch syndrome leads to an 80% lifetime risk of cancer development (multiple types) and patients with this condition therefore require life-long surveillance [9, 10]. The MMR status confers a variety of distinct biological characteristics to solid tumors. For example, patients with MMR-D/MSI-H colorectal carcinoma (CRC) have a more favorable stage-adjusted prognosis than those with MMR-proficient tumors, although MMR-D/MSI-H is associated with a poor prognosis after 5-FU-based adjuvant treatment in cases of surgically resected CRC [11]. MMR genes are known to play a role in the occurrence and development of TC [12–19]. Although pembrolizumab has received FDA approval for the treatment all advanced MMR-D solid tumors [20], data on the prevalence and prognostic significance of MMR-D/MSI-H in TC remains limited, especially for PDTC, ATC, and locally advanced (LA) PTC.

The Programmed cell death-1 (PD-1) and programmed cell death-ligand 1 (PD-L1) axis is a crucial immune pathway, and an increase in PD-L1 levels in tumor cells or T cell death-ligand 1 (PD-L1) axis is a crucial immune path-way, and an increase in PD-L1 levels in tumor cells or tumor-infiltrating lymphocytes causes T-cell attenuation [21]. Diaz et al., found that one biomarker of the response to anti-PD-1 therapy is the tumor mutational burden (TMB), whereas the colorectal cancer subtype showing MMR-D has a significantly lower TMB and a poor response to these agents [11, 22]. In recent years, anti-PD-1/PD-L1 therapy has been found to be promising for treating advanced-stage ATC and may be a potential therapeutic strategy to treat advanced medullary TC (MTC) [23, 24].

According to reports, PD-L1 expression in the tumor microenvironment can predict the prognosis of patients with MMR-D tumors and their response to immunotherapy [25].

The present study is the first to examine the relationship between MMR and LA and dedifferentiated TC in a large cohort. The study attempted to identify the clinical features of MMR-D in papillary TC (PTC), PDTC, and ATC. The aim of the study was to explore the correlation between MMR and PD-L1 expression and to clarify the clinical outcomes of MMR-D tumors.

**Materials and methods**

**Patients and samples**

Between 2009 and 2020, a total of 243 consecutive patients were diagnosed with TC (180 PTC, 43 PDTC, and 18 ATC) and received surgical treatment at the Fudan University Shanghai Cancer Center (FUSCC). Patient information, including sex, age, histologic type, tumor size, extra-thyroidal extension, presence of lymphovascular invasion, tumor-node-metastasis (TNM) stage, and lymph node status, was obtained from pathology reports. According to the 8th AJCC system, LA PTC is defined as PTC showing obvious invasion of crucial structures. All patients provided written informed consent.

**TMA construction and IHC staining**

Formalin-fixed, paraffin-embedded (FFPE) blocks obtained from primary tumor specimens were used to construct a tissue microarray (TMA). Four-micron-thick sections were cut from representative tissue to create a 1.5-mm tissue core for the TMA. TMA sections were stained using hematoxylin-eosin and then processed using IHC based on the manufacturer’s instructions (Runnerbio Company, Shanghai, China). Briefly, the slides were incubated with the following primary antibodies: anti-MLH1 rabbit monoclonal antibody (1:250, ab92312), anti-MSH2 rabbit monoclonal antibody (1:1000, ab227941), anti-MSH6 rabbit monoclonal antibody (1:500, ab92471), anti-PMS2 rabbit monoclonal antibody (1:100, ab110638), anti-PD-L1 rabbit monoclonal antibody (1:200, ab205921), and anti-PD-1 rabbit monoclonal antibody (1:500, ab137132). The slides were then incubated at 4 °C overnight. Then, they were washed with PBS and probed with a secondary antibody for 2 h at 37 °C. The slides were then incubated with DAB and hematoxylin and dried. IHC-stained slides were screened using a KF-PRO-120 Digital Pathology Slide Scanner and viewed under a K-Viewer System, version 1.5.3.1 (Konfoong Biotech), and results were separately recorded by two experienced pathologists who were blinded to the patients’ clinical data.

Based on previous studies, the loss of MLH1, MSH2, MSH6, and PMS2 expression in tumor cells was defined as complete loss of nuclear expression. In contrast, the internal control used showed normal positive nuclear staining for these proteins [12].

PD-1/PD-L1 expression was evaluated based on the combined positive score (CPS), which was calculated as follows:

$$
CPS = \frac{\text{No. of PD-L1 positive tumor cells (tumor cells, lymphocytes, and macrophages)}}{\text{Total no. of viable tumor cells}} \times 100
$$

As described by Kulangara et al., who developed the CPS system, a tumor cell was considered PD-L1-stained if complete or partial membrane PD-L1 staining was observed and an immune cell was considered stained if it featured any PD-1/PD-L1 staining (membrane/cytoplasm). The specimen was defined as PD-L1-positive if the CPS was >1 [26]. Furthermore, patients with positive PD-L1 staining were
separated into low (1 ≤ CPS < 5), moderate (5 ≤ CPS < 20), and high (CPS ≥ 20) expression groups.

### Evaluation of MSI

Eighteen MMR-D FFPE specimens were tested for MSI. Testing was conducted with capillary electrophoresis in combination with fluorescence multiplex PCR (Mircroread Genetics Technology Co., Ltd, Beijing, China) using both tumors and corresponding normal tissues. Testing was performed for a set of 6 mononucleotide repeat markers (BAT25, BAT26, NR21, NR24, NR27, and MONO27), amelogenin, and 2 pentanucleotide-repeat loci (Penta C and Penta D). Semiquantitative expression in tumors was

| Patient clinicopathological characteristics (N = 241) | Clinicopathological features | Overall cohort (n = 241) | MMR-D (n = 18) | MMR-I (n = 223) | p Value |
|-----------------------------------------------------|-----------------------------|-------------------------|---------------|----------------|---------|
| Age, yr                                             | Median (range)              | 56 (16–87)              | 54 (19–67)    | 56 (16–87)     | 0.886   |
| Sex                                                 | Male                        | 115 (47.7%)             | 7 (38.9%)     | 108 (48.4%)    | 0.593   |
|                                                     | Female                      | 126 (52.3%)             | 11 (61.1%)    | 115 (51.6%)    |         |
| Laterality                                          | Unilateral                  | 181 (75.1%)             | 17 (94.4%)    | 164 (73.5%)    | 0.05    |
|                                                     | Bilateral                   | 60 (24.9%)              | 1 (5.6%)      | 59 (26.5%)     |         |
| Tumor size, cm                                      | ≤2                           | 98 (40.7%)              | 11 (61.1%)    | 87 (39.0%)     | 0.098   |
|                                                     | 2.1–4                       | 87 (36.1%)              | 6 (33.3%)     | 81 (36.3%)     |         |
|                                                     | >4                           | 56 (23.2%)              | 1 (5.6%)      | 55 (24.7%)     |         |
| Multifocal                                          | NO                           | 163 (67.6%)             | 15 (83.3%)    | 147 (65.9%)    | 0.21    |
|                                                     | YES                          | 78 (32.4%)              | 3 (16.7%)     | 76 (34.1%)     |         |
| Lymphovascular invasion                             | NO                           | 220 (91.3%)             | 18 (100%)     | 202 (90.8%)    | 0.379   |
|                                                     | YES                          | 21 (8.7%)               | 0             | 21 (9.2%)      |         |
| Lymph node metastasis                               | CLNM **Negative**           | 90 (37.3%)              | 5 (27.8%)     | 85 (40.8%)     | 0.383   |
|                                                     | CLNM **Positive**           | 151 (62.7%)             | 13 (72.2%)    | 138 (61.9%)    |         |
|                                                     | LLNM **Negative**           | 100 (41.5%)             | 9 (50%)       | 91 (40.8%)     | 0.446   |
|                                                     | LLNM **Positive**           | 141 (58.5%)             | 9 (50%)       | 132 (59.2%)    |         |
| AJCC TNM classification                              | I/II **Negative**           | 72 (29.9%)              | 7 (38.9%)     | 65 (29.1%)     | 0.631   |
|                                                     | I/II **Positive**           | 58 (24.1%)              | 3 (16.7%)     | 55 (24.7%)     |         |
|                                                     | IV                           | 111 (46.1%)             | 8 (44.4%)     | 103 (46.2%)    |         |
| ETE                                                 | YES                          | 152 (63.1%)             | 11 (61.1%)    | 141 (63.2%)    | 0.858   |
|                                                     | NO                           | 89 (36.9%)              | 7 (38.9%)     | 82 (36.8%)     |         |
| HT                                                   | YES                          | 30 (12.4%)              | 5 (27.8%)     | 25 (11.2%)     | 0.094   |
|                                                     | NO                           | 211 (87.6%)             | 13 (72.2%)    | 198 (88.8%)    |         |
| PD-L1 expression                                    | YES                          | 50 (20.7%)              | 4 (22.2%)     | 46 (20.6%)     | 0.771   |
|                                                     | NO                           | 191 (79.3%)             | 14 (77.8%)    | 177 (79.4%)    |         |

*CLNM* central lymph node metastasis, *LLNM* lateral lymph node metastasis, *AJCC* American Joint Committee on Cancer, *HT* Hashimoto’s thyroiditis, *ETE* extrathyroidal extension, *PD-L1* programmed cell death-ligand 1, *MMR* mismatch repair, *MMR-D* MMR-protein-deficient, *MMR-I* MMR-protein-intact
calculated according to NCI guidelines [27] and compared with the expression in normal tissue for each patient.

**Statistical analysis**

All statistical analyses were conducted using the SPSS v24.0 software (IBM SPSS, Inc, Armonk, NY). Categorical variables were denoted as frequency (percentage) and compared using Chi-square tests or Fisher’s exact tests (if appropriate). Continuous variables were expressed as median value (range) and compared using 2-sample Student’s t-tests. All reported p values were two-tailed, with a significance level of 0.05.

**Results**

**Patient characteristics and IHC patterns of the DNA MMR proteins**

The clinical characteristics of the 241 patients who met the inclusion criteria and were enrolled for TMA analysis are shown in Table 1. MMR-D was detected in 1.1% of PTC patients, 4.7% of PDTC patients, and 22.2% of ATC patients (Fig. 1). Accordingly, a cohort of 18 patients with MMR-D tumors was obtained.

In this MMR-D cohort, six cases (33.33%) showed paired MSH6 and PMS2 losses, one case showed MLH1 and MSH2 losses, one case showed MLH1 and PMS2 losses, and one case showed MSH2 and MSH6 losses. In nine cases (50.00%), only MSH6 loss was observed (Table 2). Cases of MMR-D and MMR-I are depicted in Fig. 2.

**MSI testing in 18 patients with MMR-D**

We performed MSI detection for 18 cases of MMR-D: one case showed MSI-H, two cases showed MSI-L, and nine cases showed microsatellite stability (MSS); in six cases, analysis could not be performed owing to limited DNA availability. High MSI instability was detected with both NR21 and Mono27 mononucleotide markers. BAT26 and NR21 variants were detected in two other cases of MMR-D (Table 2), and none of the patients had Lynch syndrome or a related family history.

**Association between MMR expression and overall survival (OS) in TCs**

MMR-D (N = 18) and MMR-protein-intact (MMR-I) patients (N = 223) (Table 1) were compared. For each patient enrolled in this study, at least one follow-up conversation or visit was performed. Moreover, survival data for 234 of 241 patients’ (97.1%) was obtained through these follow-ups. The maximum follow-up duration was 176 months. During the follow-up, there were 49 deaths (20.9%); of the patients who died, 12 had PTC, 23 had PDTC, and 14 had ATC. The mortality rates for PDTC and ATC were relatively high, reaching 53.5% (23/43) and 77.8% (14/18), respectively.

Moreover, 4 of 4 (100%) MMR-D ATC patients and 10 of the 14 (71.4%) MMR-I ATC patients died. However, MMR-D ATC patients tended to have better OS than their MMR-I counterparts (p = 0.032; Fig. 3). Univariate Cox analysis showed that PD-L1 expression (HR = 1.04, 95% CI: 1.02–1.07, p < 0.001) and lateral lymph node metastasis (LLNM) (HR = 2.902, 95% CI: 1.068–7.891, p = 0.037) were independent predictors of OS in PTC and PDTC, respectively. Extrathyroidal extension (HR = 3.69, 95% CI: 1.045–13.02, p = 0.04) and MMR-D (HR = 0.183, 95% CI: 0.038–0.873, p = 0.033) appeared to be independent predictors of OS in ATC and were included in the subsequent multivariate Cox regression analysis (Table 3).

**Correlation between MMR-D and PD-1/PD-L1 expressions in TCs**

Based on IHC staining, we observed that 50 patients from the overall cohort (20.7%) showed positive expression for PD-L1 in tumor tissues (13, 11, and 26 showing low, medium, and high expression, respectively). Of the 4 (22.2%) PD-L1-positive MMR-D patients, 1 and 3 showed medium and high
| Patient ID | Pathological type | Age | MMR status | Gene          | MSI status    | Initial treatment received               | Recurrence (Y/N) |
|------------|-------------------|-----|------------|---------------|--------------|------------------------------------------|------------------|
| 1          | PTC               | 56  | MMR-D      | MLH1/PMS2     | MSI-L NR21   | Surgery alone                            | N                |
| 2          | LA                | 55  | MMR-D      | MLH1/MSH2     | MSS          | Surgery and I131                         | Y                |
| 3          | ATC               | 67  | MMR-D      | MSH2/MSH6     | NA           | Surgery and radiotherapy                 | Y                |
| 4          | PTC               | 48  | MMR-D      | MSH6/PMS2     | MSS          | Surgery alone                            | N                |
| 5          | PTC               | 24  | MMR-D      | MSH6/PMS2     | MSS          | Surgery alone                            | N                |
| 6          | PTC               | 19  | MMR-D      | MSH6/PMS2     | MSS          | Surgery alone                            | N                |
| 7          | PTC               | 53  | MMR-D      | MSH6/PMS2     | NA           | Surgery alone                            | N                |
| 8          | ATC               | 63  | MMR-D      | MSH6/PMS2     | MSS          | Surgery and radiotherapy                 | Y                |
| 9          | PTC               | 49  | MMR-D      | MSH6/PMS2     | MSI-H NR21.Mono27 | Surgery alone                  | N                |
| 10         | PTC               | 50  | MMR-D      | MSH6          | MSI-L BAT26  | Surgery alone                            | Y                |
| 11         | PDTC              | 65  | MMR-D      | MSH6          | NA           | Surgery, I131, Chemo and radiotherapy   | Y                |
| 12         | PDTC              | 47  | MMR-D      | MSH6          | NA           | Surgery alone                            | N                |
| 13         | PTC               | 61  | MMR-D      | MSH6          | MSS          | Surgery alone                            | N                |
| 14         | PTC               | 45  | MMR-D      | MSH6          | MSS          | Surgery alone                            | N                |
| 15         | PTC               | 47  | MMR-D      | MSH6          | NA           | Surgery alone                            | N                |
| 16         | ATC               | 59  | MMR-D      | MSH6          | MSS          | Surgery alone                            | Y                |
| 17         | ATC               | 64  | MMR-D      | MSH6          | MSS          | Surgery alone                            | Y                |
| 18         | PTC               | 63  | MMR-D      | MSH6          | NA           | Surgery alone                            | N                |

MMR-D MMR-protein-deficient, PTC papillary thyroid carcinoma, LA locally advanced, PDTC poorly differentiated thyroid carcinoma, ATC anaplastic thyroid carcinoma, MSI microsatellite instability, MSS microsatellite stability, NA not available.
expression of PD-L1, respectively. PD-1 positive expression accounts for only one case. Representative images showing PD-L1 expression are shown in Fig. 4.

We observed that 4 of the 18 MMR-D patients (22%) were PD-L1-positive, and their OS was much shorter than that of PD-L1-negative patients. Moreover, their OS was associated with their CPS values. In the log-rank test, significant differences in OS were observed between PD-L1-negative and -positive patients, and a significant difference was also observed among the negative/low, moderate, and high expression groups (both $p < 0.001$) (Fig. 5).

**Discussion**

In this study, we identified MMR-D TCs and investigated their mutation profiles, clinicopathological features, and prognostic significance. Previous studies have shown that the MMR-D phenotype occurs not only in hereditary non-polyposis CRC but also in endometrial cancer and breast cancer [28–30]. Mutations in MMR genes have been reported in approximately 14% of ATC cases [12]. We examined the rate of MMR-D tumors in patients with PTC (6.7%), PDTC (4.7%), and ATC (22.2%), and our findings were in line with the results of two prior studies that evaluated the genetic landscape of TCs [12, 31].

Our cohort contained some cases wherein two MMR proteins were deficient (MLH1 and PMS2, MLH1 and MSH2, MSH2 and MSH6, MSH6 and PMS2), and our results showed a higher proportion of MSH6 and PMS2 loss. A study based on sporadic and hereditary colorectal and endometrial carcinomas showed that heterogenous MSH6 loss that is unrelated to Lynch syndrome can be associated with somatic instability within a polycytosine tract in an MSH6 exon [32]. Latham et al. noted that 20% (2/10) of small bowel adenocarcinoma (SBA) patients harbored PMS2 germline mutations, and suggesting that these mutations drove the malignancy [33]. Sugano highlighted that homozygous mutations in the PMS2 gene were related to early-onset gastrointestinal adenocarcinoma (<20 years), multiple adenomatous polyps, childhood brain lymphoma/leukemia, and neurofibromatosis type 1 [34, 35].

Several reports have demonstrated the discordance among MLH1/PMS2/MSH2/MSH6 immunohistochemistry, microsatellite testing, and tumor MLH1/PMS2/MSH2/MSH6 sequencing results. For example, in one study, while some lesions showed a loss of nuclear MSH2/MSH6 staining on tumor IHC, they also showed MSS [36].
Table 3: Univariate and multivariate Cox analysis of prognostic factors for overall survival in 241 all cohort

| Variables                                      | Univariate analysis |      |      | Multivariate analysis |      |      |
|-----------------------------------------------|---------------------|------|------|-----------------------|------|------|
|                                               | HR (95% CI)         | P    |      | HR (95% CI)           | P    |      |
| Male vs female                                |                     |      |      |                       |      |      |
| PTC                                           | 1.243 (0.39–3.967)  | 0.713|      |                       |      |      |
| PDTC                                          | 1.054 (0.447–2.484) | 0.904|      |                       |      |      |
| ATC                                           | 1.126 (0.354–3.58)  | 0.84 |      |                       |      |      |
| Age < 65 y vs ≥ 65 y                          |                     |      |      |                       |      |      |
| PTC                                           | 1.468 (0.291–7.393) | 0.642|      |                       |      |      |
| PDTC                                          | 0.516 (0.182–1.464) | 0.214|      |                       |      |      |
| ATC                                           | 1.00 (0.98–1.03)    | 0.741|      |                       |      |      |
| Tumor size (cm)                               |                     |      |      |                       |      |      |
| PTC                                           | 0.923 (0.111–7.696) | 0.941|      |                       |      |      |
| PDTC                                          | 1.256 (0.144–10.922)| 0.836|      |                       |      |      |
| ATC                                           | 0.706 (0.24–2.076)  | 0.527|      |                       |      |      |
| Extrathyroidal extension (Yes vs no)          |                     |      |      |                       |      |      |
| PTC                                           | 0.718 (0.215–2.389) | 0.589|      |                       |      |      |
| PDTC                                          | 1.603 (0.653–3.936) | 0.303|      |                       |      |      |
| ATC                                           | 3.69 (1.045–13.024)| 0.042|      | 2.07 (0.553–7.758)    | 0.3  |      |
| Concomitant HT (Yes vs no)                    |                     |      |      |                       |      |      |
| PTC                                           | 1.135 (0.144–8.922)| 0.904|      |                       |      |      |
| PDTC                                          | 1.257 (0.271–5.819)| 0.77 |      |                       |      |      |
| ATC                                           | 0.891 (0.191–4.162)| 0.884|      |                       |      |      |
| Multifocal (Unifocality vs Multifocality)     |                     |      |      |                       |      |      |
| PTC                                           | 4.413 (0.568–34.302) | 0.156|      |                       |      |      |
| PDTC                                          | 0.776 (0.504–1.193)| 0.248|      |                       |      |      |
| ATC                                           | 0.59 (0.177–1.968) | 0.391|      |                       |      |      |
| Lymphovascular invasion (Yes vs no)           |                     |      |      |                       |      |      |
| PTC                                           | 0.342 (0.042–2.774) | 0.315|      |                       |      |      |
| PDTC                                          | 1.794 (0.632–5.094)| 0.272|      |                       |      |      |
| ATC                                           | 1.61 (0.86–3.03)   | 0.205|      |                       |      |      |
| Laterality (Bilateral vs unilateral)          |                     |      |      |                       |      |      |
| PTC                                           | 1.844 (0.403–8.439)| 0.43 |      |                       |      |      |
| PDTC                                          | 1.967 (0.813–4.763)| 0.134|      |                       |      |      |
| ATC                                           | 0.29 (0.057–1.48)  | 0.137|      |                       |      |      |
| Lymph node metastasis (CLNM)                  |                     |      |      |                       |      |      |
| PTC                                           | 0.783 (0.235–2.611) | 0.691|      |                       |      |      |
| PDTC                                          | 2.458 (0.814–7.419)| 0.111|      |                       |      |      |
| ATC                                           | 1.433 (0.459–4.47) | 0.536|      |                       |      |      |
| Lymph node metastasis (LLNM)                  |                     |      |      |                       |      |      |
| PTC                                           | 0.137 (0.018–1.063)| 0.057|      |                       |      |      |
| PDTC                                          | 2.902 (1.068–7.891)| 0.037|      |                       |      |      |
| ATC                                           | 0.946 (0.307–2.92) | 0.923|      |                       |      |      |
Several studies have shown that MSH6 somatic mutations can be present in cases of both positive and negative MSH6 IHC staining [37, 38]. In this study, we observed MSI in three cases. It is worth noting that one of the included tumors was assessed for MSI-H/MMR-D status and two were assessed for MSI-L/MMR-D status. The other 11 cases showed MSS. The loss of MLH1/PMS2 and MSH6 expression can be due to germline or somatic DNA variants in MLH1 or due to MLH1 promoter hypermethylation. MSH2/MSH6 expression loss occurs owing to germline or somatic DNA variants. Further, solitary MSH6 loss results from germline or somatic DNA variants, and in MMR genes, the inactivation of these repair mechanisms may also be caused by epigenetic modifications. In particular, promoter hypermethylation and silencing by microRNAs have been reported to affect the function of the DNA MMR gene MLH1 [33, 39]. Santos et al. demonstrated that the diagnostic MSI-panel that was used in this study correlates very well with MMR status in CRC but not as much in cases of endometrial carcinoma and other carcinomas [40]. The major differences in the detection frequencies of MMR-D/MSI in variable thyroid neoplasms can be explained, with some studies illustrating a complete absence [41]. The solution of this problem might also be development of MSI panels that are dedicated to a particular cancer entity.

In the study by Shia et al., MSH6 and PMS2 were found to be obligate binding partners of MSH2 and MLH1, respectively. This study showed that these proteins are not expressed in the absence of their partner proteins. Thus, the loss of MSH6 expression can indicate deficits in both MSH6 and MSH2, whereas PMS2 loss can reflect both PMS2 and MLH1 deficits. The loss of both MSH2 and MSH6 protein expression despite the preservation of these genes on germline testing may indicate an underlying EPCAM deletion [42].

Moreover, it is known that MMR protein loss typically corresponds to MLH1 promoter hypermethylation and MSI within small portions of tumors [43]. The advantage of IHC is the ability to detect MMR-D cases that can potentially be missed by MSI testing, specifically for MSH6 mutations, which tend to cause weak or no MSI in endometrial carcinomas [44].

Our data also demonstrated that MMR-D may be associated with better disease prognosis, especially in stage IVB ATC [12] and stage III CRC [45]. Related studies have also demonstrated that the improvement in survival rate may be due to the enhanced anti-tumor immune response caused by an increased neoantigen load resulting from the hypermutation phenotype of MMR-D tumors [46]. Konishi et al. pointed out that MMR CRC with a better prognosis may have fewer mutations in p53 [12, 47] and more frequent mutations in the β-catenin, transforming growth factor β receptor type II genes [48], and the intense lymphocytic infiltrates observed in tumors [49]. The penetrance of MSH6 and PMS2 mutations in CRC has been controversial because these mutations do not significantly increase the risk of extracolonic malignancies [50].

Santos et al. demonstrated that combinations of alleles including MSH6 rs1042821 (e.g., XRCC3 rs861539, XPC rs2228001, CCNH rs2230641, MSH6 rs1042821, and ERCC5 rs2227869) are associated with thyroid cancer susceptibility. More precisely, they showed that DNA repair genes and their combinations are involved in DTC susceptibility [51].

Previous studies have reported PD-L1 expression in 6.1 and 40% of PTC cases, and 58.3% of DTC cases. Moreover, 30.3% of cases showed membranous PD-L1 expression and 82.3% showed cytoplasmic expression, and the positive rate of PD-L1 expression in ATC was reported to be 75, 22, and 23%, respectively, showing that PD-L1 expression in PTC correlates with a greater risk of recurrence and shortened disease-free survival [52–56]. We found that 22% of MMR-D patients were PD-L1-positive,
and OS analysis shows that a PD-L1-positive MMR-D phenotype was linked to a poor prognosis. Previous research has shown a positive rate of 14.4% among MTC patients. The five-year structural recurrence-free survival rate of patients with PD-L1-positive tumors has been reported to be nearly 28% lower than that of those with PD-L1-negative tumors [23]. MMR status is correlated with an increased neoantigen load, causing stronger immune responses and the upregulation of immune checkpoints, including PD-1/PD-L1. Hence, positive patients are generally more likely to obtain a survival benefit from ICIs [27, 57]. Although nivolumab has been approved by the FDA for the treatment of adult and pediatric patients (age ≥12 years) [58], a multicenter study that investigated the combination of nivolumab and ipilimumab in MMR-D/MSI-H CRC patients reported an overall response rate of 55% [59]. A more comprehensive assessment of MMR/MSI, PD-1/PD-L1 expression, TMB, and T-cell-inflamed gene expression profile (GEP) could help in improved identification of patients with higher odds of responding to ICIs in future clinical trials for advanced TCs.

There are some limitations to the present study. First, although the sample size was large in terms of TC cases, considering the retrospective nature of the study, further research is still warranted. Second, MMR-associated gene mutations may need to be further involved in the present study, including mutations in the GTPase (KRAS), KRAS proto-oncogene, and serine/threonine kinase (BRAF). Finally, it is necessary to further performed next-generation sequencing along with targeted gene sequencing or whole-exome/genome sequencing for cases with MMR IHC results to infer the MMR genotype status.

Overall, we have depicted the DNA MMR and PD-L1 expression patterns in TC patients in the present study. A comprehensive analysis of multiple markers could help in the development of optimal strategies for identifying patients with LA, PDTC, and ATC sensitive to immunotherapy in the near future.

Fig. 4 Representative immunohistochemistry images (200X) showing A negative PD-L1 expression of MMR-D patient 1 (CPS = 0); B moderate PD-L1 expression of (CPS = 15) patient 11; C high PD-L1 expression of (CPS = 96.5) patient 17. MMR-D MMR-protein-deficient, CPS combined positive score, PD-L1 programmed death-ligand 1.

Fig. 5 Kaplan–Meier survival plots presenting overall survival of A the overall cohort; B the MMR-D PD-L1-negative and PD-L1-positive groups. MMR-D MMR-protein-deficient, PD-L1 programmed cell death-ligand 1.
Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

Ethical approval This study was performed with the approval of the ethics committee of the Fudan University Shanghai Cancer Center.

Informed consent Informed consent was obtained from all individual participants included in the study.

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