Clinicopathological and Molecular Genomic Features of Monomorphic Epitheliotropic Intestinal T-Cell Lymphoma in The Chinese Population: A Study of 20 Cases

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Abstract

Background

Monomorphic epitheliotropic T-cell lymphoma (MEITL) is an aggressive non-Hodgkin lymphoma with a high fatality rate. This study was aimed to explore the clinicopathological and molecular genetic features of MEITL in the Chinese population.

Methods

A retrospective analysis was performed based on the clinical manifestations and pathological features of 20 Chinese MEITL. 9 cases with paired diseased-normal tissues were also analyzed for molecular information by whole-genome sequencing.

Results

There were 14 men and 6 women with a median age of 62.5 (28–81) years. 17(17/20) lesions were located in the jejunum or ileum; 13(13/20) cases had ulcers or perforations. Microscopically, except for 1(1/20) case of pleomorphic cells, the monomorphic, middle-sized tumor cells infiltrating into the intestinal epithelial and peripheral intestinal mucosa recess could be seen in the other 19 cases. Immunohistochemistry showed that most of the tumor cells in MEITL were positive for CD3(20/20), CD8(17/20), CD43(19/20), and CD56(15/20), but negative for CD5(20/20). The most frequently mutated genes of these Chinese cases were *STAT5B* (4/9) and *TP53* (4/9), not *SETD2*(2/9). *ZDBF2* mutations (4/9) were also detected for the first time with a high mutated frequency. We demonstrated that mutations of JAK-STAT pathway-related genes and the amplification of Chromosome 9q appeared at the same time in most cases(5/9).

Conclusions

The clinicopathological features were consistent with that in previous western studies, but a novel mutated gene, *ZDBF2*, was found in this study. A combination of gene mutations involved in the JAK-STAT pathway and the amplification of Chr9q may be used as one of the molecular features of MEITL diagnosis, especially when morphological and immunohistochemical evidence is insufficient.

Introduction

Monomorphic epitheliotropic intestinal T-cell lymphoma (MEITL) is an aggressive intestinal non-Hodgkin lymphoma originating from T cells, which has been classified as a separate category from enteropathy-associated T-cell lymphoma (EATL) in the 2016 WHO classification of hematopoietic and lymphoid tissues[1]. MEITL is more common in Asia with monomorphic CD3 + CD8 + CD56 + tumor cells and lacks

clinical evidence of celiac sprue[2]. Frequent mutations in *SETD2*, which encodes a non-redundant H3K36-specific trimethyltransferase, have been found in most MEITL cases in several studies, and the JAK/STAT pathway is also reported to be the most commonly mutated signaling pathway[3–5].

Due to its low incidence, and a lack of researches on large-scale cases, the clinicopathological and molecular characteristics of MEITL in the Chinese population have not been fully clarified. The present study performed a retrospective analysis based on clinicopathological features of 20 MEITL cases, nine of which also underwent whole-genome sequencing for molecular genetic information, to provide a basis for clinical diagnosis and treatment and supplement the genetic landscape of MEITL in the Chinese population.

Materials And Methods

Sample selection

The use of samples in this study was in accordance with the Declaration of Helsinki and approved by the Ethics Committee of The First Affiliated Hospital of Nanjing Medical University (2020-SR-169).

Patients were eligible for study inclusion if they: 1) were comprehensively diagnosed with MEITL based on pathological morphology, imaging examination, serological indicators, and clinical manifestations. 2) had complete clinical data. 3) had undergone surgical resection between 2013 and 2019.

Fifteen MEITL cases from The First Affiliated Hospital of Nanjing Medical University and 5 MEITL cases from Nanjing Jinling Hospital met the inclusion criteria. Hematoxylin-eosin staining and immunohistochemical slides were retrieved and re-evaluated by two pathologists (Z. Zhang and C. Chen). Nine cases with enough paired diseased-normal tissues were selected for whole-genome sequencing.

Patients' general information, clinical manifestations, pathological morphology, immunohistochemical results, and molecular genetic features were collected for subsequent analysis.

Immunohistochemistry

Immunohistochemical staining was performed on 3µm thick formalin-fixed paraffin-embedded tissue sections using Ventana automated (BenchMarker) stainer (Tucson, Roche, USA) according to the manufacturer's instructions. Standard immunohistochemical studies were performed using the following antibodies: CD3, CD5, CD8, CD43, CD56, TIA-1, Granzyme B, perforin, Ki-67. All antibodies and EBER probes were ready to use and bought from Maxim biotechnology development co. LTD (China).

Immunohistochemistry results were scored according to the intensity of staining and the percentage of positive cells. The IHC results of all cases were interpreted by two pathologists using a double-blinded method, and the Ki-67 index only evaluated the percentage of positive cells.

Whole-genome sequence

Genomic DNA was firstly extracted from selected FFPE surgical samples of 9 MEITL cases using QIAamp DNA FFPE tissue kit (Qiagen). To prepare materials for constructing the DNA library, we fragmented 2µg DNA using Covaris M220 sonication system (Covaris), followed by end-repairing, A-tailing, and adaptor ligation and purification by KAPA Hyper Prep Kit (KAPA Biosystems). What's more, the selected DNA fragments needed to be further amplified and purified before the Illumina Rapid Capture Extended Exome Kit (Illumina Inc.) could be used for exome enrichment. Finally, captured libraries were submitted to the Illumina Hiseq 2500 platform (Illumina Inc.) for further sequencing.

Sample quality control criteria: 1. DNA extracted from each sample $\geq 50\text{ng}$ with A260/280 ranged from 1.8 to 2.0; 2. DNA in the pre-library $> 100\text{ng}$; 3. Q30 (the percentage of bases with mass value ≥ 30) $> 75\%$; 4. mean sequencing depth of 150× for tissues and 60× for negative control tissues.

Sequence analysis and Pathway enrichment analysis

Paired-end sequencing data after quality control was aligned to the human reference genome hg19 using Burrows-Wheeler Aligner (BWA-MEM)[6], followed by sorting, PCR-duplicate removal, base quality score recalibration(BQSR) using Samtool v1.6, Picard v1.119 (<http://picard.sourceforge.net/>) and Genome Analysis Toolkit (GATK)[7], respectively. Single nucleotide variants (SNV) and germline mutations were identified by a useful variant caller – VarDict-1.5.4[8], and Scalpel (<http://scalpel.sourceforge.net>) was an effective algorithm for mining insertion and deletion mutations[9]. SNVs and indels identified were excluded if present $> 1\%$ frequency in the 1000 Genomic project. The annotations of all SNVs and indels were acquired using vcf2maf-1.6.16. Meanwhile, the CNV kit was performed for analysis of copy number variants (CNV) at the chromosome level.

We performed Gene Ontology (GO, <http://www.geneontology.org/>) and Kyoto Encyclopedia of Genes and Genomes (KEGG, <https://www.kegg.jp/>) analysis to explore the biological processes and pathways associated with MEITL.

Prediction of mutations on protein stability

Polyphen2 (Polymorphism Phenotyping v2), SIFT (Sorting intolerant of tolerant substitution) are used to predict the possible impact of an amino acid substitution on protein structure by generating a score based on each sequence. The default score threshold of SIFT is currently set at 0.05 for binary classification (deleterious: < 0.05 ; tolerated: ≥ 0.05) with a balanced accuracy of 76.99% (<http://sift.jcvi.org/>)[10]. The score in Polyphen2 ranges from 0 to 1, and the smaller the score, the less likely it is to damage protein stability. According to the score, mutations are divided into benign (0-0.452), possibly damaging (0.447–0.909) and probably damaging (0.909-1) (<http://genetics.bwh.harvard.edu/pph2/>)[11].

Results

Clinical findings

There were 14 men and 6 women with a median age of 62.5(range, 28–81 years). Among all cases, 17(17/20) lesions were located in the jejunum or ileum, 2(2/20) in the colon, and 1(1/20) in both ileum and colon. Patients had common gastrointestinal symptoms, including anemia (19/20), abdominal pain (17/20), and intestinal perforation (10/20). Weight loss (8/20), abdominal mass (7/20), fever (6/20), bloating (6/20), diarrhea (3/20), and intestinal obstruction (3/20) were observed in less than half of the cases. All patients had no evidence of celiac disease or inflammatory bowel disease.

Pathological features in MEITL

Seventeen jejunoileal specimens, 1 ileocolic specimen, and 2 colorectal specimens were selected for examination. Grossly, thirteen (13/20) cases showed single/multiple ulcers and perforations, and the mucosal folds around the rupture disappeared, part of the serous membrane had fibrinopurulent exudates. Seven (7/20) cases developed solid masses of size ranging from 3.2cm*2cm*1.3cm to 10cm*4cm*1cm with grayish-white and reddish cut surface, infiltrating the full- thickness of the intestinal wall.

Microscopically, most tumor cells were monomorphic medium-sized cells with round dark nuclei and pale cytoplasm(Fig. 1A), spreading to adjacent areas and infiltrating the full-thickness of the intestinal wall. Only in one case, the tumor cells were atypical and polymorphic, along with large nuclei, obvious nucleoli, increased mitotic activity and nuclear fragmentation(Fig. 1B), infiltrating the fibrous connective adipose tissue outside the lamina propria, rather than the epithelium and lamina propria. Multinuclear cells and megakaryocytes were also seen. Epitheliotropic pattern(Fig. 1C) was demonstrated in 12(12/20) cases, with obvious neoplastic necrosis at the perforation and scattered acute and chronic inflammatory cells in the interstitium. Villus atrophy, crypt hyperplasia, and increased lymphocytes in the crypt and surface epithelium were found in adjacent mucosae(Fig. 1D). Lymph node involvements were histologically confirmed in 3 (3/20) cases.

Tumor cells in all 20 cases were strongly positive for CD3(Fig. 1E), while CD43(19/20) (Fig. 1F), CD8(17/20) (Fig. 1G), and CD56(15/20) were positive for partial tumor cells. B cell markers, like CD20(5/20) (Fig. 1H) and CD79α(6/20), were positive in scattered tumor cells; whereas CD5 was negative in all cases. The Ki-67 index was generally high ranging from 60–90%. Interestingly, more than half of these cases expressed cytotoxic markers including TIA-1(19/20), Granzyme B(12/20), and perforin(11/20). Moreover, all cases were EBER-ISH negative. (Fig. 1 and Table 1)

Table 1
The pathological features of 20 MEITL cases.

	n(%) (total = 20)
Gross	
Multiple ulcers	2(10)
Perforation	13 (65)
Mass	5(25)
Morphology	
Cell morphology	
Monomorphic	19(85)
Polymorphic	1(5)
Infiltration depth	
Full-thickness	18(90)
Mucosa and submucosa	2(10)
Epitheliotropic pattern	
Present	12(60)
Absent	8(40)
Neoplastic necrosis	
Present	11(55)
Absent	9(45)
Mitosis	
Present	20(100)
Absent	0(0)
Inflammation	
Present	20(100)
Absent	0(0)
Lymph node involvements	
Present	3(15)
Absent	17(85)

	n(%) (total = 20)
Crypt changes in adjacent mucosae	
Present	20(100)
Absent	0(0)
Immunohistochemistry	
CD3+	20(100)
CD5+	0(0)
CD8+	17(85)
CD43+	19(95)
CD56+	15(75)
TIA-1+	19(95)
GranB+	12(60)
perforin+	11(55)
EBER+	0(0)
CD20+	5(25)
CD79α+	6(30)
Ki-67 index	60%-90%

Molecular genetic findings

There were 1987 somatic mutations with a median of 220 (range 19-1577) mutations per patient (average of 1.82 per Mb), typically consisting of missense mutations(Fig. 2A), and the mutated spectrum showed a predominance of C > T and G > A transitions(Fig. 2B). 66.7% of cases had mutations in genes of the JAK-STAT signaling pathway which was known as a major oncogenic mechanism in T cell lymphomas, including *STAT5B* mutations (4/9), *JAK3* mutations (3/9), and *STAT5A* mutations (2/9) (Fig. 2C). Interestingly, the *STAT5A* mutation and *STAT5B* mutation were found to be mutually excluded, although either could activate the JAK-STAT pathway. The four *STAT5B*-mutated cases harbored three distinctive missense variants, including the common N642H mutation(rs938448224) and two mutations —Y665F(COSM1716592) and A766V(COSM8916674) which were only reported in COSMIC(Fig. 3A). *TP53* variants were discovered in 4 of 9 cases, and all were located in the DNA-binding domain(Fig. 3B). Two of them were frameshift mutations, V147Afs*19 and I232Sfs*15, which were expected to confer critical changes in protein structure, and the other two missense mutations (R248Q and G105C) were predicted to be deleterious with a damaging effect on the protein by SIFT and Polyphen2 algorithms. Four

of 9 cases harbored *ZDBF2* mutations, frameshift mutation K1726Nfs*5(rs745875253) in 3 cases, and missense mutation A564S in 1 case. Only 2 (2/9) cases had *SETD2* mutations.

KEGG and GO enrichment analysis revealed that mutated genes expressed higher in MEITL were enriched for the JAK-STAT signaling pathway and DNA damage system(Fig. 2C).

Copy number analysis derived from WGS data identified multiple regions of frequent gains and losses(Fig. 2D). The most significant chromosome copy number variation of MEITL was the amplification of 9q, accounting for 77.8% of all cases. Gains of 7q(4/9), 19q(3/9), 1q(3/9) and losses of 7p(4/9), 18p(3/9) and 8p(3/9) were also frequent in MEITL(Fig. 2D). The changes of gene copy number were consistent with that of chromosome arms. Tumor-associated genes on Chr9q such as *FANCC*, *NTRK2*, *SYK*, and *NOTCH1* were amplified; amplification of *BRAF*, *EZH2*, *MET*, *CDK6* and *HGF* located on Chr7 were also detected. Five(5/9) cases had both the amplification of Chr9q and mutations of genes of the JAK-STAT pathway.

Frequent germline mutations were also detected. *CADM1* germline variant c*161del was documented in 5 out of 9 cases, with the highest frequency, followed by MPP7 C.952 + 816T > G(4/9), RNF145 c.-40 + 1137A > G(4/9), PACRG c.613 + 6010T > G(3/9), CD9 c.538-159C > T(3/9), TMPRSS4 c.*169G > A(3/9).

Discussion

The present study analyzed the clinicopathological and molecular genetic characteristics of 20 Chinese patients with MEITL. In line with the previous studies[12, 13], men were more susceptible than women with a ratio of 2:1. MEITL patients commonly displayed primary symptoms of abdominal pain, accompanied by systemic symptoms such as fever, anemia, and weight loss. Complications such as intestinal perforation are also common. MEITL mostly occurred in the small intestine, but could also inflect the whole GI tract[14]. Previous studies reported colon and stomach as primary sites that also involved mesenteric nodules or other distant organs including liver, spleen, lung, bone, or skin[15, 16]. Skin involvement was rare as it only occurred in 5% of all patients and may present as erythema multiforme skin disease or skin pigmentation[17].

The histological findings and immunohistochemistry of our patients were consistent with those reported in most literature[12, 13, 18, 19], with one exception. The monomorphic and small to medium-sized tumor cells in most cases were positive for CD3, CD8, CD43, CD56, and negative for CD5, with round nuclei, obvious nucleoli, and pale cytoplasm. Increased mitotic activity, frequent necrosis, and epitheliotropic pattern could also be seen. While the outlier case showed cellular pleomorphism and lymph node involvement and had a longer overall survival time after CHOP chemotherapy than other patients. We hypothesized that cellular pleomorphism is related to prognosis, but due to the number of our cohort, the possibility of accidental occurrence cannot be ruled out.

WGS results of nine cases showed the genetic landscape of Chinese population. Mutation-induced abnormal activation of the JAK-STAT pathway together with chromatin remodeling and DNA damage

control pathways were pervasive in diverse T cell malignancies and were reported to have an important role in oncogenesis by regulating cell proliferation, survival, differentiation, and immune response[20]. As expected, protein-coding genes involved in the JAK-STAT pathway (*STAT5B*, *JAK3*, *STAT5A*), histone modifiers (*SETD2*, *CREBBP*), and DNA damage (*TP53*) were recurrently mutated. The mutation frequencies of these genes, except *SETD2*, were similar to those in previous reports[3–5, 21]. *SETD2* is an important epigenetic gene encoding histone methyltransferase SETD2 protein which is involved in gene transcription extension and mismatch repair, and the inactivation of *SETD2* may contribute to cancer development[22]. *SETD2* was found to be the most significant recurrently mutated gene and mutated in up to 60% of patients with MEITL in previous studies reported by Tomita[5], Moffitt[4], and Roberti[3]. But in our study only 2 patients had *SETD2* mutations (2/9), showing a lower incidence. Since Tomita's study was based on Asian patients, we ruled out the interference of ethnic heterogeneity, and we guessed it may be caused by the small sample size and environmental factors.

STAT5B was the most frequently mutated gene in our study. As a member of the STAT family, *STAT5B* plays a key regulatory role in the pathogenesis of various disease drivers, including BCR/ABL[23] and NPM-ALK[24], and expresses at a high level in hematological malignancies[25]. There have revealed multiple mutation hot-spots within SH2 and C-terminal domains in *STAT5B*, among which N642H has been discovered in various hematological malignancies of T cell origin including T-cell acute lymphoblastic leukemia(T-ALL), T-cell prolymphocytic leukemia(T-PLL), and MEITL[26]. Patient with a *STAT5B* N642H variant has a risk of relapse, drug resistance and poor outcome[27]. N642H was detected in two of four *STAT5B*-mutated cases in our study and predicted to be probably damaging by Polyphen-2. Two novel variants Y665F and A776V had also been found, but needed more research to explore their functions.

Our study showed for the first time frequent *ZDBF2* mutations. *ZDBF2* is located on chromosome 2q33.3 and encodes a protein containing DBF4-type zinc finger domains, related to nucleic acid binding and zinc ion binding according to GO annotations[28]. So far, only Nasopalpebral Lipoma-Coloboma Syndrome[28] and Temple Syndrome[29], both of which are dominant genetic diseases, have been reported to be associated with *ZDBF2*. Since frequent frameshift mutation K1726Nfs*5(rs745875253) was discovered in our study and *ZDBF2* was reported to be imprinted and paternally expressed in lymphocytes[30], we speculate that *ZDBF2* is an important gene contributing to the MEITL mechanism and had been largely overlooked.

Abnormalities of Chr9q were frequently detected in non-Hodgkin lymphoma, especially loss of Chr9q[31]. Both Type-I EATL and MEITL were characterized by Chr9q gains[32], and its frequency could be up to 75% in MEITL[3, 33]. Our research had verified that amplification of Chr9q(7/9) was the most common chromosome copy number variant in MEITL, and multiple important oncogenes on Chr9q showed consistent amplifications. Five cases also had mutations of JAK-STAT pathway-related genes at the same time. Though recurrent Chr9q gains were reported in mantle cell lymphoma(MCL), its frequency was lower, and the characteristic mutated genes in MCL were not related to the JAK-STAT pathway. These

findings indicated that a combination of genetic mutations and Chr9q gains could be used as one of the molecular features of MEITL diagnosis.

There were some limitations in the current research. First, due to the small sample size, we have not yet analyzed the correlation between the genetic changes and clinicopathological manifestations. Second, the pathogenic mechanisms of the above genetic changes on MEITL have not been further studied. We will continue to collect Chinese MEITL cases to solve the above problems and verify our results.

Conclusions

Our work uncovers the clinicopathological and special molecular genetic characteristics of MEITL patients in the Chinese population. MEITL often occurs in elderly males and has non-specific digestive symptoms, with CD3⁺/CD8⁺/CD56⁺/CD5⁻ monomorphic medium-sized tumor cells and obvious epitheliotropic performance. Frequent mutations of *TP53*, *ZDBF2*, and genes affecting the JAK-STAT pathway, especially *STAT5B*, are common to be discovered. Combining the mutant genes involved in the JAK-STAT pathway and the amplification of Chr9q may be a powerful auxiliary tool for diagnosing MEITL.

Abbreviations

MEITL Monomorphic Epitheliotropic Intestinal T-cell Lymphoma

IHC Immunohistochemistry

WGS Whole-genome sequence

SNV single nucleotide variant

CNV copy number variant

GO Gene Ontology

KEGG Kyoto Encyclopedia of Genes and Genomes

MCL mantle cell lymphoma

Declarations

Ethics approval

The use of samples in this study was in accordance with the Declaration of Helsinki and approved by the [Ethics Committee](#) of The First Affiliated Hospital of Nanjing Medical University (2020-SR-169).

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Z. Zhang designed research; C. Chen, Y. Gong, Q. Xia, Q. Rao, X. Zhang collected data; C. Chen, Y. Gong, J. Zhang and B. Zhai analyzed data; Y. Shao, L. Zhu, X. Li and P. Ji conducted the experiments; C. Chen, Y. Gong and Y. Yang wrote the paper; Z. Zhang supported administratively; Z. Zhang revised the paper. All authors contributed to and have approved the final manuscript.

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Figures

Figure 1

Monomorphic epitheliotropic intestinal T-cell lymphoma A-H: $\times 10$ (A) monomorphic medium-sized tumor cells with round dark nuclei and pale cytoplasm; (B) atypical and polymorphic tumor cells, along with large nuclei, obvious nucleoli, increased mitotic activity and nuclear fragmentation; (C) epitheliotropic pattern; (D) villus atrophy, crypt hyperplasia, and increased lymphocytes in the crypt and surface

epithelium; (E) CD3+; (F) CD43+; (G) CD8+; (H) CD20+. (A-D: hematoxylin and eosin staining, E-H: IHC staining)

Figure 2

MEITL mainly consisted of missense mutations (A) and had a predominance of C>T and G>A transitions (B); (C) depicted the heat map and associated signal pathways of MEITL, and 66.7% of cases had mutations in genes of JAK-STAT signaling pathway, including STAT5B, JAK3 and STAT5A; (D) showed the major copy number variants in MEITL, and the most significant chromosome copy number variation was the amplification of 9q, followed by gains of 7q and losses of 7p.

Figure 3

Somatic alterations in STAT5B and TP53 identified in this study. (A) Four STAT5B-mutated cases harbored three distinctive missense variants, including the common N642H mutation(rs938448224) and two mutations—Y665F(COSM1716592) and A766V(COSM8916674); (B) Four TP53-mutated cases harbored two frameshift variants - V147Afs*19 and I232Sfs, and two missense variants - R248Q and G105C.