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REVIEW ARTICLE

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Review of the biologic and clinical significance of genetic mutations in angioimmunoblastic T-cell lymphoma

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Angioimmunoblastic T-cell lymphoma (AITL) is an age-related malignant lymphoma, characterized by immune system-dysregulated symptoms. Recent sequencing studies have clarified the recurrent mutations in ras homology family member A (*RHOA*) and in genes encoding epigenetic regulators, tet methyl cytosine dioxygenase 2 (*TET2*), DNA methyl transferase 3 alpha (*DNMT3A*) and *isocitrate dehydrogenase 2*, *mitochondrial* (*IDH2*), as well as those related to the T-cell receptor signaling pathway in AITL. In this review, we focus on how this genetic information has changed the understanding of the developmental process of AITL and will in future lead to individualized therapies for AITL patients.

KEYWORDS
angioimmunoblastic T-cell lymphoma, epigenetic regulator, multistep and multilineage tumorigenesis, ras homology family member A, T-cell receptor-signaling

1 | INTRODUCTION

Recent progress in next-generation sequencing has provided emerging evidence of characteristic genetic abnormalities in angioimmunoblastic T-cell lymphoma (AITL). In this review, we provide insight into how the biologic and clinical aspects of AITL are linked to its genetic features.

1.1 | Angioimmunoblastic T-cell lymphoma belongs to a nodal T-cell lymphoma with T follicular helper phenotype

Angioimmunoblastic T-cell lymphoma (AITL) is a subtype of malignant lymphoma. Together with nodal peripheral T-cell lymphomas (PTCL) with T follicular helper (TFH) phenotype and follicular T-cell lymphoma (FTCL), AITL belongs to *nodal T-cell lymphoma with TFH phenotype*, a newly proposed entity in the 2016 revised WHO classification.[1] Follicular helper T cells, a subset of helper T cells, reside mainly in the follicles to support B-cell survival, proliferation, maturation and migration.[2] The TFH phenotype is determined by expression of 2 or 3 markers that are expressed both in normal follicular helper T cells and in tumor cells: CD279/programmed death-1 (PD1) and inducible T-cell costimulator (ICOS), T-cell coinhibitory and costimulatory molecules; CD10, a membrane metalloendopeptidase; B-cell lymphoma 6 protein (BCL6), a key transcription factor for TFH development; C-X-C motif chemokine ligand 13 (CXCL13) and c-x-c motif chemokine receptor 5 (CXCR5), a chemokine and chemokine receptor; and signaling lymphocyte activation molecule (SLAM)-associated protein (SAP), an adaptor protein for SLAM family receptors.[1] Some gene mutations are commonly found in diseases categorized into nodal T-cell lymphomas with TFH phenotype, and AITL-specific mutations have been identified. (Note: See the Section below, "1.4.")

1.2 | Angioimmunoblastic T-cell lymphoma is an age-related lymphoma, presenting with symptoms of immune system dysregulation

The incidence of AITL increases with age, with the median age at onset reported to be 59-65 years.[3] The prevalence of AITL in elderly

individuals may be tightly linked to the age-related premalignant mutations in AITL. (Note: See the Section, "1.9.") AITL patients display generalized lymphadenopathy, a characteristic symptom of malignant lymphomas. Furthermore, the symptoms suggestive of immunologic hyperactivation are also present in AITL: fever, rash, Coombs test-positive hemolytic anemia and polyarthritis.[3] Again, these immune system-related symptoms may be attributable to genetic events involving multiple components of T-cell receptor (TCR) signaling pathways. (Note: See the Section below, "1.4.")

1.3 | Massive infiltration of accessory cells occurs in angioimmunoblastic T-cell lymphoma

Various immune cells, including nontumor reactive T cells, B cells (some of which are infected by Epstein–Barr virus [EBV]), eosinophils and macrophages, invade AITL tissues.[3] Moreover, the blood vessels are markedly increased and often surrounded by AITL tumor cells. In addition, follicular dendritic cells (FDC) are also prominently present near the tumor cells and blood vessels.[3] As mentioned above, AITL tumor cells resemble cytokine-producing and chemokine-producing TFH cells.[4] Cytokines and chemokines released from TFH-like tumor cells may recruit immune cells, blood vessels and FDC into AITL tissues, and activate them to further produce cytokines and chemokines. This positive circuit of cytokines and chemokines may exacerbate the trafficking of these cells into AITL tissues. For instance, the CXCL13 and its receptor CXCR5 network is thought to promote recruitment of B cells and FDC as well as tumor cells into AITL tissues.[3] Vascular endothelial growth factor (VEGF), a cytokine that promotes angiogenesis, is expressed in both tumor and vascular endothelial cells.[5] Cytokine-producing helper T17 (Th17) cells as well as CD8-positive T cells are also enriched in AITL tissues.[6] Mast cells in AITL tissues function as producers of VEGF, to recruit endothelial cells,[7] and of interleukin-6 (IL-6), to proliferate Th17 cells.[8]

Although the cytokine and chemokine circuit originating from TFH-like tumor cells may account for the massive infiltration of immune cells into AITL tissues, novel genetic evidence indicated that tumor-infiltrating cells may not be entirely attributable to the reactive process.[9] The infiltrating B cells in AITL tissues had gene mutations distinct from those found in tumor cells.[9] The genetic events in tumor-infiltrating cells may synergize with the cytokine-and chemokine-mediated reactions to produce the pathologic features of AITL. (Note: See the Section below, "1.10.")

1.4 | Ras homology family member A, epigenetic regulators, and T-cell signaling molecules are the main players in the genetic abnormalities of angioimmunoblastic T-cell lymphoma

Recent genetic studies identified recurrent mutations in ras homolog family member A (*RHOA*) (50%-70%)[10-12] and in genes encoding the epigenetic regulators, tet methyl cytosine dioxygenase 2 (*TET2*) (47%-83%),[10,13] DNA methyltransferase 3 alpha (*DNMT3A*) (20%-30%)[10,11,14] and isocitrate dehydrogenase 2, mitochondrial (*IDH2*) (20%-45%),[10,15] as well as the components of the TCR signaling pathways, phospholipase C gamma 1 (*PLC γ*) (14%),[16] *CD28* (9%-11%),[16,17] FYN protooncogene, Src family tyrosine kinase (*FYN*) (3%-4%)[11,16] and vav guanine nucleotide exchange factor 1 (*VAV1*) (5%)[16] in AITL (Table 1).

Almost all the *RHOA* mutations found in AITL were p.G17V (G17V *RHOA* mutations).[10-12] G17V *RHOA* mutations were commonly observed in the other nodal T-cell lymphomas with TFH phenotype: 57%-62% of nodal PTCL with TFH phenotype[10,14] and 60% of FTCL,[14] while they were quite rare in other diseases. In contrast, *TET2* and *DNMT3A* mutations were found in a broad range of hematologic malignancies,[18] and even in healthy elderly individuals.[19,20] (Note: See the Section below, "1.9.") Among T-cell lymphomas, *TET2* mutations were more prevalent in nodal T-cell lymphomas with TFH phenotype than those without TFH phenotype (nodal PTCL with TFH phenotype vs FTCL vs PTCL without the TFH phenotype: 64% vs 75% vs 17%).[14] *IDH2* mutations were also found in myeloid malignancies. However, *IDH2* mutations were not detectable in the other T-cell lymphomas,[10,15] even those with the TFH phenotype,[14] suggesting that *IDH2* mutations may provide AITL with its specific pathologic features. The mutations involving components of the TCR signaling pathways were commonly observed in nodal PTCL with TFH phenotype,[16] although *CD28* mutations were specific to AITL.[17] Notably, the AITL genome exhibited a specific combination of these mutations: the *RHOA*-mutated samples also had *TET2* mutations, while a part of the *RHOA* and *TET2*-mutated samples had *IDH2* mutations.[10] These combinations may have a synergistic effect on oncogenesis.

1.5 | Diagnostic impact of G17V ras homology family member A mutations

As mentioned above, G17V *RHOA* mutations were commonly identified in nodal T-cell lymphomas with the TFH phenotype,[10,14] although they were also observed in a few cases of adult T-cell leukemia/lymphoma

TABLE 1 Recurrent gene mutations in AITL

	Frequencies (%)	References
RAS superfamily		
RHOA	50-70	10-12
Epigenetic regulators		
TET2	47-83	10,13
DNMT3A	20-30	10,11,14
IDH2	20-45	10,15
TCR signaling pathway		
PLCγ	14	16
CD28	9-11	16,17
FYN	3-4	11,16
VAV1	5	16

AITL, angioimmunoblastic T-cell lymphoma; DNMT3A, DNA methyltransferase 3 alpha; FYN, FYN protooncogene, Src family tyrosine kinase; IDH2, isocitrate dehydrogenase 2, mitochondrial; PLCγ, phospholipase C gamma 1; RHOA, ras homolog gene family, member A; TCR, T-cell receptor; TET2, tet methylcytosine dioxygenase 2; VAV1, vav guanine nucleotide exchange factor 1.

(ATLL),[21] which can be distinguished by its integration of human T-lymphotropic virus (HTLV)-1. Therefore, G17V *RHOA* mutations serve as a genetic indicator to detect nodal T-cell lymphomas with the TFH phenotype. The tumor ratio is sometimes low because of the prominent reactive cells, which makes it difficult to detect G17V *RHOA* mutations by direct sequencing. It is reported that the allele-specific PCR (AS-PCR) assay is an easy-to-use method to detect G17V *RHOA* mutations.[22] The positive and negative concordance rates between AS-PCR and amplicon-based deep sequencing were as high as 95%.[22] G17 *RHOA* mutations were also detectable in cell-free DNA, enabling their application in noninvasive diagnostic testing of AITL.[23]

1.6 | Oncogenic roles of ras homology family member A mutations are under investigation

RHOA is a small guanine nucleotide triphosphate (GTP)-binding protein. RHOA mediates fundamental biologic processes, including cell mortality, adhesion, the cell cycle and cytokinesis. While the functions of RHOA in peripheral T cells have not been fully elucidated, the conditional deletion of the *RhoA* gene under the control of the *CD2* or *Lck* promoters resulted in severe defects in thymocyte development in mice.[24] RHOA carries out a switch-like function by making a round trip between the guanine nucleotide diphosphate (GDP)-bound inactive state and the guanine nucleotide triphosphate (GTP)-bound active state.[25] The 17th glycine of RHOA is located at a position essential for binding to GTP.[10] In a Rhotekin pull-down assay to detect GTP-bound RHOA, the G17V RHOA mutant was not bound to GTP,[10-12] indicating that the G17V RHOA mutant does not mediate classical RHOA signaling. Curiously, the p.K18N mutant existing in a few AITL samples had higher GTP-binding capacity.[16] Therefore, the oncogenic roles of the G17V RHOA mutant in AITL development may not be due to the disruption of classical RHOA signaling. Rather, the existence of mutations at a single amino acid strongly suggests that the G17V mutant acquires a specific oncogenic role. Recently it was reported that the G17V mutant activated TCR pathway through direct binding to VAV1, an essential mediator of the TCR pathway.[26] Together with the frequent mutations in TCR pathway, aberrant activation of TCR pathway by the G17V RHOA mutant may be a clue for AITL development. (See the Section, "1.8.")

Other *RHOA* mutations are reported in diffuse-type gastric carcinoma,[27] Burkitt lymphoma[28] and ATLL.[21] The most frequent *RHOA* mutations in gastric carcinoma and Burkitt lymphoma were the p.Y42C and p.R5Q mutations,[27,28] while the p.C16R mutations were the most frequent in ATLL.[21] Whether these various *RHOA* mutations share common downstream molecules essential for oncogenesis remains to be elucidated.

1.7 | Mutations in epigenetic regulators

TET2 encodes a methylcytosine, dioxygenase, to convert methylation cytosine (mC) to hydroxymethylcytosine (hmC), formylcytosine (fC) and carboxylcytosine (CaC).[29] These modified cytosines function as intermediates of the passive or active demethylating process, and as epigenetic marks.[29] Nonsense and frameshift mutations were distributed throughout the entire TET2 protein, while missense mutations almost always existed at the C-terminal catalytic domain in AITL, as in myeloid malignancies.[10,13] This distribution of mutations indicates that *TET2* mutations are loss-of-function mutations. *DNMT3A* encodes a DNA methyltransferase, which methylates nonmethylated CpG. *DNMT3A* mutations were distributed across the entire protein. Hotspot p.R882 mutations accounted for approximately 15% of *DNMT3A* mutations in AITL,[10] while they accounted for more than half of the mutations in myeloid malignancies.[30] The p.R882H DNMT3A mutant was shown to have reduced methyltransferase activity and also to dominant-negatively inhibit wildtype *DNMT3A* by interference with homotetramer formation.[31] *DNMT3A* and *TET2* mutations were sometimes seen together in AITL[32] as well as in myeloid malignancies, although the epigenetic effects were opposite. The synergistic effects of *TET2* and *DNMT3A* loss on AITL development were shown using a mouse model[33] (Note: See the Section below, "1.11.") In AITL, *IDH2* mutations were exclusively present at the p.R172 position,[10,15] while both p.R140 and p.R172 *IDH2* mutations and p.R132 *IDH1* mutations were seen in myeloid malignancies.[34] Under physiologic conditions, IDH enzymes convert isocitric acid to α-ketoglutaric acid (α-KG) in an NADP+-dependent manner. α-KG functions as an intermediate metabolite of the tricarboxylic acid (TCA) cycle and also as a substrate in enzymes that are not included in the TCA cycle. *IDH* mutants have been shown to aberrantly produce D-2-hydroxyglutarate (D-2-HG), a so-called oncometabolite. D-2-HG inhibits the α-KG-dependent enzymatic activity of dioxygenases, including the TET family of proteins and Jumonji-C histone demethylases.[34] As mentioned above, *IDH2* and *TET2* mutations coexist in AITL samples, suggesting that TET proteins other than TET2 or Jumonji-C histone demethylases may be the main targets of the oncometabolite. In fact, it was shown that both DNA methylation and histone H3K27 methylation were more prominent in AITL samples with *TET2* and *IDH2* mutations than in those with *TET2*/without *IDH2* mutations.[35]

Hypomethylating reagents are currently in clinical use for myelodysplastic syndrome. These reagents tend to be more effective in *TET2*-mutated cases than in *TET2* wildtype cases. Highly prevalent *TET2* mutations in AITL suggest that AITL may also respond to these hypomethylating reagents. In fact, several AITL cases effectively treated with azacytidine have been reported.[36,37]

1.8 | T-cell receptor-related mutations

Upon TCR stimulation, CD28 functions as a costimulatory molecule to support full and sustained T-cell activation. Subsequently, FYN, a Src kinase, is activated, resulting in further phosphorylation of downstream molecules (ie PLCγ and VAV1). VAV1, known as a GEF protein, also functions as an adaptor to facilitate and activate the TCR proximal signaling complex, involving PLCγ and SLP-76. PLCγ catalyzes phosphatidylinositol 4, 5-bisphosphate ($PI_{(4,5)}P_2$) into inositol-1, 4, 5-trisphosphate (IP3) and diacylglycerol (DAG), leading to intracellular signal transduction through calcium mobilization and

activation of protein kinase C (PKC). Activating mutations were observed in these players participating in TCR signaling.

CD28 mutations were accumulated at 2 hotspots, p.D124 and p.T195.[16,17] The p.D124 mutant was shown to have higher affinity for the ligands CD80 and CD86,[17] while the p.T195 mutant had higher affinity for the intracellular adaptor proteins GADS/GRAP2 and GRB2.[17,38] The *CTLA4-CD28*[39] and *ICOS-CD28* fusion genes[17] have also been described. *FYN* mutations found in the SH2 domain and C terminus were activating mutations, presumably through the disruption of the intramolecular inhibitory interaction between the SH2 domain and the C terminus.[11] *PLCγ* mutations were found in several functional motifs, including the PI-PLC, SH2, SH3 and C2 domain. *PLCγ* mutations were also shown to be activating mutations,[16] although the biologic consequence of these mutations has yet to be clarified. *VAV1* mutations were found at several hotspots,[16] although the oncogenic mechanisms of these mutations remain unclear. In addition, the C-terminal portion of VAV1, participating in intramolecular inhibition, was recurrently deleted by 2 distinct mechanisms: an alternative splicing mechanism resulting from in-flame deletion of the N-terminal site of the CSH3 domain[40] and formation of fusion genes with several distinct partners.[40,41]

Although the genetic evidence suggests that activation of TCR signaling by gene mutations may play a role in the symptoms and progression of AITL, it has not been exactly proven by in vivo experiments. Cyclosporin A, a calcineurin inhibitor that blocks TCR signaling, is widely used for the treatment of immune system-mediated diseases.[42] Cyclosporin A as a single reagent[43] or with other immunosuppressive reagents[44] was shown to effectively ameliorate the progression and symptoms of AITL. The effectiveness of cyclosporine A supports the hypothesis that activation of TCR signaling may actually contribute to AITL progression and that it can be a candidate pathway in targeted therapies.

1.9 | Age-related mutations may precede angioimmunoblastic T-cell lymphoma

Hematologic malignancies are classified according to their normal counterparts; that is, normal cells sharing the characteristics of tumor cells. Furthermore, they had previously been thought to originate from their normal counterparts; for example, AITL had been thought to originate from its normal counterpart, TFH cells. However, we now believe that at least some hematologic malignancies including AITL may originate from immature blood cells.[18] The *TET2* and *DNMT3A* mutations detected in tumor cells were also recognized in the tumor-free peripheral blood cells,[45] bone marrow cells[10,32,45] and hematopoietic progenitors[32,45] of AITL patients. Some patients simultaneously or serially developed both AITL and myeloid malignancies. Identical *TET2* and *DNMT3A* mutations were reported to be present in both diseases, suggesting that both diseases originate from premalignant cells harboring these mutations.[32,37] When a nationwide survey was conducted to examine the cooccurrence of myeloid and lymphoid malignancies, 72 cases were identified: 45 cases having the diseases simultaneously and 27 cases sequentially.[46] Whether the multiple diseases in these cases actually had common ancestors remains to be elucidated.

Finally, somatic mutations were also detected even in healthy individuals.[19,20] Mutation frequencies were reportedly increased with age: by 5% for those in their 60s, by 10% to 15% for those in their 70s, and by 10% to 25% for those in their 80s.[19,20] Somatic mutations were also detected in 95% of individuals aged 50-60 years when the detection sensitivity was set at 0.0003 variant allele frequencies (VAF).[47] The most frequently mutated genes in the healthy individuals were *DNMT3A*, *TET2* and *ASXL1*.[19,20] Although these mutations were first found in hematologic malignancies, they may be defined as *age-related mutations*. The status of having somatic mutations without any evidence of hematologic diseases is called clonal hematopoiesis of indeterminate potential (CHIP).[48] CHIP was related to a high incidence of blood cancers and inferior overall survival.[19,20] The presence of premalignant mutations in AITL patients suggests that CHIP may precede AITL in most cases. However, the actual incidence rate of AITL caused by CHIP has not been determined. Indeed, because of its rarity, it would be tough to determine the incidence rate.

TET2 and *DNMT3A* mutations themselves in premalignant cells may not be sufficient to induce AITL development. Multiple *TET2* mutations were frequently observed in AITL tissues,[10] while *TET2* mutations were heterozygous in CHIP as well as in myeloid malignancies. When the distribution of *TET2* mutations were examined in 19 AITL/PTCL samples using laser microdissection followed by targeted sequencing, 10 samples had 2 distinct *TET2* mutations, while 6 had one *TET2* mutation.[9] Although both mutations were determined as premalignant mutations in 5 of the samples, the 5 samples had 1 mutation as a premalignant mutation and the other as a tumor-specific mutation.[9] These observations suggest that the profound defect in TET2 function may skew premalignant cells into tumor cells. In addition, *RHOA* and *IDH2* mutations were detected in tumor cells,[9] suggesting that acquisition of these mutations together with preexisting *TET2* and *DNMTA* mutations leads to AITL development.

1.10 | Clonal evolution in tumor-infiltrating B cells

As mentioned above, the massive infiltration of immune cells into AITL tissues is partly due to the cytokine and chemokine storm, beginning from the cytokine and chemokine production from tumor cells and being amplified by the tumor-infiltrating inflammatory cells. At the same time, it is well known that rearrangement of immunoglobulin (*Ig*) genes in addition to that of *TCR* genes is found in 0% to 40% of AITL samples,[3] suggesting that B cells as well as T-lineage tumor cells proliferate clonally in AITL tissues. EBV infection observed in 66% to 86% of cases[49–51] may partly explain the clonal expansion of B cells. Notably, AITL and B-cell lymphomas simultaneously cooccur as composite lymphomas, or serially during the disease course in up to 20% of AITL patients.[52,53] Although EBV may account for oncogenic mechanisms of EBV-positive B-cell lymphomas, EBV is negative in a substantial proportion of B-cell lymphomas.[52–54]

TET2-and *DNMT3A*-mutated premalignant cells may be differentiated into tumor-infiltrating B cells as well as tumor cells. In fact, when the distribution of these mutations was examined in AITL tissues using laser microdissection followed by targeted sequencing, *TET2* mutations were detected even in B cells as well as in tumor cells in 15 of the 16 cases, and *DNMT3A* mutations were also found in both B cells and tumor cells in 4 of the 7 cases (Figures 1 and 2).[9] Remarkably, B-cell specific mutations were also identified.[9] In particular, all 3 *NOTCH1* mutations exhibited B-cell-specific distribution (Figures 1 and 2). These observations suggest that B cells residing in AITL tissues may have undergone clonal selection.

1.11 | Angioimmunoblastic T-cell lymphoma mouse model mimicking the human angioimmunoblastic T-cell lymphoma genome

The impact of genetic events on AITL development can be examined using mouse models. As mentioned above, loss-of-function *TET2* mutations were highly frequent in AITL.[10] It was reported that TFH cells were gradually increased and finally T-cell lymphomas with the *TFH* phenotype developed at long latencies in *Tet2* gene-trap mice.[55] The lymphoma cells exhibited increased methylation at the transcriptional start site (TSS) regions, gene bodies and CpG islands, and decreased hydroxymethylation at the TSS regions.[55] In particular, the negative regulatory region of *BCL6* encoding a fate-determinant of TFH cells was highly methylated in lymphoma cells.[55] Decitabine treatment results in demethylation of the loci, accompanying downregulation of

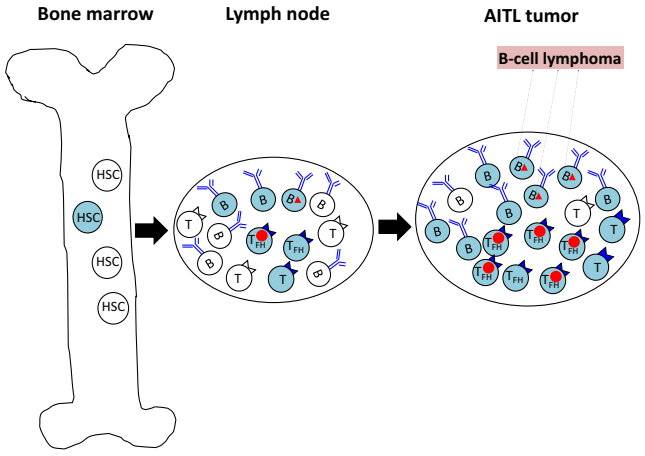

FIGURE 2 Multistep and multilineage tumorigenesis in angioimmunoblastic T-cell lymphoma (AITL). The blue cells show cells that acquired *TET2/DNMT3A* mutations. The circles indicate *RHOA/IDH2* mutations, and the triangles, *NOTCH1* mutations. Hematopoietic stem/progenitor cells (HSC/HSPC) acquire *TET2/DNMT3A* mutations and become premalignant cells. These cells can be differentiated into both T and B cells. Acquisition of *RHOA/IDH2* mutations in T cells leads the cells to transform into AITL tumor cells. In contrast, acquisition of *NOTCH1* mutations in B cells may lead the cells to transform into B-lymphoma cells

Bcl6 expression.[55] Furthermore, human PTCL samples also had hypermethylation of the corresponding loci, especially when they had *TET2* mutations.[56] The impaired TET2 function may induce *BCL6* upregulation, resulting in the skewed differentiation toward TFH cells in both

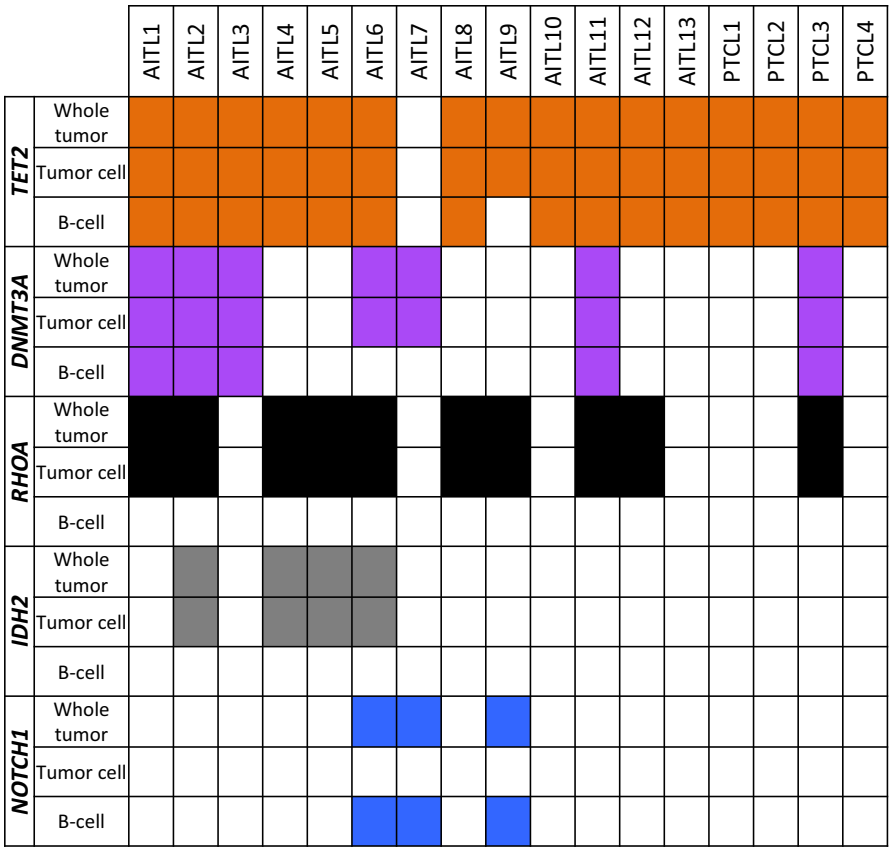

FIGURE 1 Distribution of common gene mutations in angioimmunoblastic T-cell lymphoma (AITL). The orange boxes show *TET2* mutations; the purple boxes, *DNMT3A* mutations; the black boxes, *RHOA* mutations; the gray boxes, *IDH2* mutations; the blue boxes, *NOTCH1* mutations; and the white boxes, no mutation

humans and mice. The synergistic effect of *TET2* and *DNMT3A* mutations on AITL development was proven using mice transplanted with *Tet2*-null hematopoietic stem/progenitor cells expressing genes transduced with R882H *DNMT3A* mutant cDNA.[33] The synergistic effect of *TET2* and G17V *RHOA* mutations, the most frequent combinations in human AITL, was also shown by mice transplanted with *Tet2*-null T cells expressing genes transduced with G17V *RHOA* mutant cDNA.[57]

2 | CONCLUSION

The biology of AITL has become gradually understood as a result of the *multistep and multilineage tumorigenesis* concept: premalignant cells having epigenetic mutations evolve into tumor and tumor-infiltrating cells through clonal selection of the mutated cells. The multistep and multilineage acquisition of mutations may contribute to the formation of the striking pathologic features of AITL. Concurrently, these characteristic gene mutations have begun to change the clinical approach to AITL. G17V *RHOA* mutations will be used in a clinical setting to assist diagnosis of AITL. This genetic information may lead to individualized therapies for AITL patients in future.

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CONFLICT OF INTEREST

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