Review Article

Update on recurrent mutations in angioimmunoblastic T-cell lymphoma

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Abstract: Angioimmunoblastic T-cell lymphoma (AITL) is a subtype of peripheral T cell lymphoma (PTCL), defined by genetic alterations that induce abnormal immune activity and inflammatory disorders. Through recent discoveries using genomic studies, the identification of various recurrent mutations has provided greater insight and changed our understanding of the molecular genetics of the disease. By acknowledging these recurrent mutations and their affected pathways, the diagnosis, prognosis, treatment, and survival of AITL can be improved. In this review, we summarize the known recurrent mutations present in the molecular pathogenesis of AITL by emphasizing the effects of mutations on signaling pathways and genes, as well as the multistep process of AITL development.

Keywords: AITL, RHOA, TET2, DNMT3A, IDH2, T-cell receptor signaling

Introduction

Angioimmunoblastic T-cell lymphoma (AITL) is characterized by partial or total architectural effacement of lymph nodes, with prominent hyperplasia of high endothelial venules and follicular dendritic cells. The disease induces inflammation and has a physical resemblance to T follicular helper (Tfh) cells; however, it is far more aggressive and is known to cause neoplastic activity in the lymphatic system [1, 2], which contributes to the disease’s microenvironment. Moreover, EBV infections are typically associated and detected in most AITL cases, with EBV-positive cells composed predominantly of reactive B cells instead of neoplastic T cells. AITL is also a rare disease, accounting for ~20% of PTCL and 2% of all non-Hodgkin lymphomas (NHL) [3], and elderly adults usually experience the greatest severity [1]. Although the survival rate of the disease has had little to no improvement throughout the past two decades, with ~30% 5-year survival [3], new studies have helped identify the essential molecular pathologic gene alterations that induce the disease.

The use of genomic profiling in recent studies has identified the major recurrent mutations present in the Ras superfamily proteins, epigenetic regulator genes, and relevant signaling pathways, which can drive the pathogenesis of the disease [1] by affecting the specification and differentiation of Tfh cells [2].

RHOA G17V mutations

The Ras homolog family member A (RHOA) gene encodes a small GTPase protein that regulates various cell-surface receptors involved in cytoskeleton remodeling, cell morphology, migration, signaling, proliferation, and survival [1, 4]. The RHOA protein is controlled by GEF and GAP, where the proteins can switch the GTPase from a GTP-activated state to a GDP-inactivated state and vice versa [1, 4-6, 10]. In AITL, frequent recurrent missense mutations in RHOA account for ~50-70% of cases [1, 4, 6-10, 20, 31], with predominant RHOA G17Val mutations present in 91% of all RHOA mutations [7]. Furthermore, the RHOA G17V mutation often co-occurs with mutations in epigenetic regulators (TET2, DNMT3A, IDH2), so that 94% of RHOA G17V positive cases contain at least one mutation in these genes [1] (Table 1).

The RHOA G17V mutation contributes to AITL pathogenesis by the expression of a dominant-
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Table 1. Frequency of recurrent mutations in AITL

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutation Frequency</th>
<th>Co-occurrence frequency with other mutation</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>RHOA</td>
<td>50%-70%</td>
<td>At least one mutation in TET2, DNMT3A, IDH2 (94%)</td>
<td>[1, 4, 6-10, 20, 31]</td>
</tr>
<tr>
<td>IDH2</td>
<td>20%-45%</td>
<td>TET2 (70%-90%)</td>
<td>[2, 7, 8, 12, 15, 20]</td>
</tr>
<tr>
<td>TET2</td>
<td>47%-82%</td>
<td>DNMT3A (70%-100%)</td>
<td>[2, 7, 12, 17, 20]</td>
</tr>
<tr>
<td>DNMT3A</td>
<td>20%-40%</td>
<td>TET2 (70%-100%)</td>
<td>[1, 7, 8, 12, 20]</td>
</tr>
<tr>
<td>FYN</td>
<td>2.8%-4%</td>
<td>Unknown</td>
<td>[6, 9, 20]</td>
</tr>
<tr>
<td>FYN-TRAFL3IP2</td>
<td>Unknown</td>
<td>Unknown</td>
<td>[25]</td>
</tr>
<tr>
<td>CD28</td>
<td>4%-12%</td>
<td>Unknown</td>
<td>[6, 7, 9, 11, 20, 26]</td>
</tr>
<tr>
<td>CTLA4-CD28</td>
<td>38% (Without CD28 mutation)</td>
<td>Unknown</td>
<td>[8]</td>
</tr>
<tr>
<td>PLOC1</td>
<td>11.1% -14.1%</td>
<td>Unknown</td>
<td>[6, 9, 20]</td>
</tr>
<tr>
<td>CARD11</td>
<td>2.8%</td>
<td>Unknown</td>
<td>[9]</td>
</tr>
<tr>
<td>TNFRSF21</td>
<td>3.36%</td>
<td>Unknown</td>
<td>[7]</td>
</tr>
<tr>
<td>CCND3</td>
<td>3.36%</td>
<td>Unknown</td>
<td>[7]</td>
</tr>
<tr>
<td>SAMS1N</td>
<td>Unknown</td>
<td>Unknown</td>
<td>[5]</td>
</tr>
<tr>
<td>ITK-SYK</td>
<td>Unknown</td>
<td>Unknown</td>
<td>[8, 26, 28, 29]</td>
</tr>
<tr>
<td>VAV1</td>
<td>4.7%-5.6%</td>
<td>Unknown</td>
<td>[6, 9, 20]</td>
</tr>
<tr>
<td>VAV1-STAT2</td>
<td>8.2% (Without RHOA G17V)</td>
<td>Unknown</td>
<td>[6]</td>
</tr>
<tr>
<td>STAT3</td>
<td>2.8%-13%</td>
<td>RHOA (~10%)</td>
<td>[9, 30]</td>
</tr>
<tr>
<td>GAPDH</td>
<td>Unknown</td>
<td>Unknown</td>
<td>[31]</td>
</tr>
</tbody>
</table>

AITL, angioimmunoblastic T-cell lymphoma; RHOA, ras homolog gene family; IDH2, isocitrate dehydrogenase 2; TET2, tet methylcytosine dioxygenase 2; DNMT3A, DNA methyltransferase 3 alpha; FYN, FYN proto-oncogene; FYN-TRAFL3IP2, FYN proto-oncogene-adapter protein CIKS; CD28, cluster of differentiation 28; ICOS-CD28, inducible T-cell costimulatory-cluster of differentiation 28; CTLA4-CD28, cytotoxic T-lymphocyte-associated protein 4-cluster of differentiation 28; PLCO1, phosphoinositide phospholipase C; CARD11, caspase recruitment domain family member 11; TNFRSF21, death receptor 6; CCND3, cyclin D3; SAMS1N, SH3 domain and nuclear localization 1; ITK-SYK, interleukin 2 inducible T-cell kinase-spleen tyrosine kinase; VAV1, vav guanine nucleotide exchange factor 1; VAV1-STAT2, vav guanine nucleotide exchange factor 1-signal transducing adaptor family member 2; STAT3, signal transducer and activator of transcription 3; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

negative protein, as the mutant protein can interfere with the activity of the wild-type RHOA [4, 9, 10]. Through biochemical and cellular assays, RHOA G17V is found to sequester and obstruct GEF proteins in RHOA signaling [10] by affecting the glycine residue at position 17 of RHOA, which is crucial for GTP binding [6]. Although the RHOA G17V gene is the most frequent mutation, in vitro assay studies have identified RHOA K18N as a possible gain of function mutation in AITL; however, it accounts for >3% of cases [9]. Moreover, the mutations S26R and C20W have also been identified in AITL using mutation profiling; nevertheless, both mutations are infrequent, affecting about 1% of cases [7].

Additionally, the RHOA G17V mutation can further enhance Tfh differentiation and induce AITL through interactions with epigenetic regulators. In a murine study, the researchers generated Tet2 knockout mice infected with Rhoa G17V to identify the relationship between the two genes [4]. The results demonstrated a synergistic effect between Tet2 and Rhoa G17V, because increased clinical, cellular, and transcriptional characteristics of AITL with increased VEGF-A, IL6, and TNFα levels further augmented Tfh signature genes [4]. Moreover, in Tet2 knockout mice with Rhoa G17V mutation, a reduction of FoxO1 expression is recognized, which down regulates Fas, an apoptotic gene, and Foxp3 gene, causing CD4+ T cells to exhibit an inflammatory phenotype [17]. The RHOA G17V mutation and IDH2 R172 mutation also have good oncogenic synergy, as the expression of both mutations illustrates features of standard AITL with Tfh immunophenotype, cle-
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ar cells, high endothelial vessels, and follicular dendritic cell proliferation [7]. In addition to their synergistic effect, both RHOA G17V and IDH2 R172 mutations are found exclusively in neoplastic T-cell clones and PD1 cell fractions [2, 7].

Through different signaling pathways, RHOA G17V can enhance Tfh differentiation and lineage specification. The expression of RHOA G17V can instigate the specification and proliferation of Tfh cells by overexpression of ICOS while increasing PI3K and MAPK signaling [4]. The costimulatory molecule ICOS is expressed on activated CD4+ T cells and helps promote the Tfh phenotype and stabilize BCL6 [8], a transcription repressor that suppresses essential genes in Th1, Th2, Th17, and Th17 cell lineages deviation [4]. The increase in ICOS and BCL6 is especially evident in a murine study done with Rhoa G17V expressing mice, where there was an increase in PD1, CXCR5, ICOS, and BCL6, which are all Tfh markers [4]. Moreover, the mice expressed heightened PI3K and MAPK signaling with increased and prolonged ERK1/2 and AKT pathway activation, as well as S6 phosphorylation [4]. The results of the murine study demonstrated that the activation of ICOS, PI3K, and MAPK signaling pathways through the Rhoa G17V mutation can augment the proliferation of Tfh cells and secretion of proinflammatory cytokines [4].

In AITL, the most prevalent ras superfamily protein mutation is RHOA G17V. The mutation augments proinflammatory cytokines, upregulates specific signaling pathways, and downregulates important genes in other T helper cells, thus promoting Tfh specification and differentiation.

IDH2 R172 mutation

Isocitrate dehydrogenase 2 (IDH2) gene typically encodes enzymes that convert isocitrate into alpha-ketoglutarate (2-oxoglutarate, aKG) in the mitochondria [1, 2, 8]. The IDH2 R172 mutation can create neo-function enzymes and promote the production of (R/D)-2-hydroxyglutarate (2-HG) from alpha-ketoglutarate, which can elevate the amount of 5-methylcytosine (5-mC) and reduce the levels of 5-hydroxymethylcytosine (5 hmC) from the TET family, and decrease the quantity of KDM Jumonji domain C from the Jumonji family [1, 7, 8, 11-13]. The inhibition of both the TET and Jumonji families will impair the hypoxic response, collagen maturation, ectopic histone, and DNA demethylation, which can affect cell fate at specific stages of progenitor or stem cell differentiation, thus promoting lymphomagenesis [1, 2, 7, 11, 13]. Additionally, the Warburg effect will arise with 2-HG outcompeting aKG, as it disturbs the citric acid cycle and alters the metabolic and epigenetic factors in T cells [11]. As a result, the possibility of tumorigenesis and an aberrant genome is further increased due to 2-HG impeding DNA repair enzymes [14]. The Warburg effect occurs as tumor cells produce significantly more lactate and less ATP from oxidative phosphorylation, despite utilizing large amounts of glucose, which supports the anaerobic conditions of tumor cells [13].

The IDH2 R172 mutation contributes to pathogenesis by expressing both dominant and gain of function effects on the wild-type allele, with IDH2 missense change at R172K/M/G/V/S/T being the most common IDH2 mutations that are associated with the most significant increase of 2-HG in AITL [13, 15]. Unlike AML and other PTCL diseases, AITL does not express IDH1 R132 or IDH2 R140 mutations, as the disease exclusively expresses the IDH2 R172 mutation [5, 7, 12, 15], which is not known to associate with an improvement in the survival rate of AITL [12]. Moreover, the IDH2 R172 mutation is identified in ~20-45% [2, 7, 8, 12, 15, 20] of AITL cases alongside epigenetic regulator TET2. These mutations have a ~70%-90% chance of co-occurrence [1].

Both wild-type IDH2 and IDH2 R172 mutations can induce AITL through different signaling pathways, in which abnormal genome changes are associated with IDH2 R172 mutation. However, in comparison to other PTCL diseases like ATLL, ALCL, and PTCL-GATA3, IDH2 R172 mutation AITL has a >10% rate of chr11, chr19, chr22q, and chr7/7q gain with no chr1q gain and del-10p, which indicates a minimally aberrant genome [11]. Nevertheless, the co-occurrence of both 43% chromosome 5 (Chr5) gain and 23% chromosome 21 (Chr21) gain [2] is identified in the AITL genome with IDH2 R172 mutation, in which the chromosomes have cooperative effects through pleiotropic effects and epigenetic mutations [11]. Moreover, the Chr5 gain from the IDH2 R172 mutation upregulates MAPK9, IL4, IL13, and genes linked to...
T-cell differentiation, T-cell activation, and cell cycle regulation [11]. On the other hand, there is an enrichment in the NF-κB pathway and the PI3K-AKT-mTOR pathway in wild-type IDH2 AITL cases, since targeted deletion of phosphatases that are negative regulators is recognized using DAVID [11]. The IDH2 R172 mutation can also co-occur with the TET2 mutation, upregulating THF-inducing genes of IL6, IL12, IL21, IL23, and ICOS, while downregulating TH1 (STAT1, CXCR3, IL2), TH2 (IL10RA), and TH17 (IL17RA) associated genes in AITL [12]. Furthermore, the IDH2/TET2 double-mutation enhances MYC targets, FOXP3 targets, EZH2 targets, VEGF-A signaling, and TGF-B signaling while reducing FOXO3 target genes, IFN signaling, IL17 signaling, and TP53, a gene involved with cell division [12].

The IDH2 R172 mutation can induce hypermethylation and hypomethylation on DNA gene promoters [2, 12]. The mutation mainly induces hypermethylation on promoters and CpG islands, which are regulatory regions known to downregulate gene transcription that are used to promote intergenic regions and gene body hypomethylation [12]. Moreover, in AITL IDH2 R172 mutation cases, there are 46 hypermethylated and 9 hypermethylated genes that significantly affect normal CD4+ T cell genes through epigenetically downregulating TH1 positive regulators (PF11, PHF11), T-cell receptor signaling negative regulators (DGKA, PTPN7, SIT1), and cell surface marker genes (CD7, IL10RA) [8, 12]. The IDH2 R172 mutation can also significantly augment the trimethylation of Histone H3 [2, 12]. In a Jurkat cell study, IDH2 mutation infested Jurkat cells have increased levels of H3K27me3, H3K4me3, H3K9me3, which suggest the disruption effects of 2-HG on histone lysine demethylases, KDM6A, and KDM6B demethylation on H3K27me3 [12]. The abnormal repression of genes by H3K27me3 can cause promoter hypermethylation, thus manifesting as transcriptional silencing [12]. Moreover, the IDH2 mutation was shown to instigate hypermethylation of SMAD7 and SMURF2 [12], two negative regulators of the TGF-β signaling pathway, preventing T-helper cell differentiation [16].

In AITL, the most common isocitrate dehydrogenase mutation is IDH2 R172. The mutation affects the cell fate of HSCS cells at specific stages of differentiation through epigenetic changes in hypermethylation and hypomethylation on gene promoters and CpG islands, which promote Thf differentiation and suppress T-helper cell differentiation.

**TET2/DNMT3A mutations**

Ten-eleven translocation 2 (TET2) gene encodes the enzyme 2-oxoglutarate-dependent dioxygenase [1, 12, 22], which catalyze the oxidation reaction of 5-methylcytosine (5 mC) into 5-hydroxymethylcytosine (5-hmC), and then further into 5-carboxylcytosine (5caC) and 5-formylcytosine (5FC) [2, 17]. The TET2 mutation can affect the development of hematopoietic stem and progenitor cells (HSCS) through augmentation of the self-renewal process and differentiation towards certain myeloid lineages [1, 2, 8, 17]. However, further mutations are needed in order to develop malignant transformation [17]. Additionally, TET2 mutations can affect B cells, as the mutation can induce germinal center (GC) hyperplasia by preventing GC B cells from exiting the GC light zone [2].

The TET2 mutation accounts for ~70-80% of AITL cases [2, 7, 12, 20] and co-occurs with RHOA mutation in ~60%-70% of cases [17]. The TET2 mutations are mainly nonsense alterations and frameshift changes [2, 7], exhibiting loss of function effects. They are distributed randomly throughout the TET2 gene affecting the overall structure, function, and catalytic activity [7]. Furthermore, the TET2 mutation can enrich the IFN response [12] and promote BCL6, a transcription factor essential for Thf phenotype, expression through hypermethylation of CpG islands in intron 1 of BCL6 [2, 8, 18]. The upregulation of BCL6 expression encourages the derivation of Thf cells and enhances the possibility of future malignant transformation when further cooperative mutations are obtained [18]. In clonal hematopoietic cells carrying the TET2 mutation, the cells can acquire NOTCH1 mutations during B cells differentiation, further augmenting the effect of the TET2 mutations [2]. Moreover, this interaction is seen in EBV-infected B cells, which are often associated with AITL [2].

The DNA methyltransferase 3A (DNMT3A) gene encodes for de novo DNA methyltransferase enzyme that catalyzes the reaction of cytosine to 5-methylcytosine (5-mC), as well as regulat-
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The DNMT3A mutations account for ~20%-40% of AITL cases [1, 7, 8, 12, 20] with mutations characterized by nonsense changes, missense changes, substitution at essential splicing sites, and frameshift indels [7]. The most frequent missense DNMT3A mutation is at R882 (R882C, R882H, R882P), affecting the MTase-binding domain [7, 12]. Moreover, the R882 mutation can exhibit both dominant negative and loss of function effects on wild-type DNMT3A by diminishing the ~80% of DNA methylation activity in vitro [19, 21, 22], which can cause specific CpG hypomethylation [12, 23]. The DNA hypomethylation will upregulate the genes associated with multipotency while downregulating early differentiation factors, thus expanding the number of HSCs cells rather than determining the cell fate [8, 19].

In AITL, the two epigenetic gene mutations of TET2 and DNMT3A R882 can promote HSCs self-renewal, respectively. Moreover, both mutations have a synergistic effect, inducing self-renewal and clonal hematopoiesis of HSCs cells; however, further mutations are required in order for HSCs cells to commit to malignant Tfh cell lineage.

TCR pathway mutations

The T-cell receptor (TCR) signaling pathway is essential for T-cell activation, apoptosis, cytoto-
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kine production, and proliferation [1]. The factors regulating the signaling pathway such as cytokines, chemokines, and interactions between co-stimulatory and co-inhibitory molecules, can easily be affected by mutations. This can influence and induce PTCL development [1, 8]. In AITL, mutations in the TCR signaling pathway components are identified to occur in ~50% of patients [9, 24], as TCR stimulation is vital to Tfh cell specification [2].

The FYN proto-oncogene (FYN), a src tyrosine kinase, is a crucial component in TCR stimulation, since the kinase alongside LCK can trigger T-cell activation [1, 10]. Generally, the FYN mutations can impede the intramolecular inhibitory mechanisms between the FYN SH2 domain and the FYN C-terminal Tyr531 phosphosite, enhancing tyrosine kinase signaling and promoting AITL pathogenesis [10, 24]. In human AITL, FYN mutations account for 2.8%-4% [6, 9, 20] of cases with increased kinase activity caused by a mutation in S186L of the FYN SH2 domain and mutations in T524fs and Q527X of FYN C terminal domain Tyr531 [9]. Further mutations were identified in a Rat1A cell study, with L174R and R176 mutations in the SH2 domain of FYN and Tyr531His affecting the C-terminal domain Tyr531, where enhanced FYN activation is present [10]. Another recurrent gene identified in AITL is the FYN-TRAF3IP2 gene fusion, which can dysregulate and induce abnormal NF-κB signaling pathways during TCR activation [25]. In response to TCR stimulation, the FYN-TRAF3IP2 will upregulate the canonical NF-κB signaling through the direct recruitment of TRAF6 while downregulating the non-canonical NF-κB signaling by not interacting with CARD11, BCL10, or MALT1 paracaspase [25].

The cluster of differentiation 28 (CD28), a member of the immunoglobulin subfamily, is a central co-stimulatory molecule for TCR-mediated activation such as cytokine production and T-cell proliferation upon ligand binding and TCR stimulation [1, 26]. After ligand binding, CD28 can mediate and enhance T-cell activation, NFAT, and NF-κB nuclear translocation, T-cell survival, cell cycling, and proliferative cytokine production through various signaling pathways [26]. Mutations affecting the CD28 gene can hyperactivate the CD28 signaling, which causes aberrant T-cell activation and induces PTCL development [26]. In AITL, CD28 amplification accounts for 4%-12% of cases [6, 7, 9, 11, 20, 26], with T195P/I changes affecting the cytoplasmic domain and D124E/V alterations affecting the extracellular domain [1, 7, 9, 26]. Mutations at T195 residue illustrated increased affinity for GADS/GRAP2 intracellular adapter proteins, and mutations at D124 residue demonstrated increased affinity in CD28 ligand binding [5, 26]. The T195 mutation can upregulate CD226, and TNFα expression, so that CD226 can outcompete TIGHT to bind to CD155 and TNFα can induce NF-κB activation and IkB kinase phosphorylation [26]. The D124 mutation demonstrated increased ligand affinity to CD86 compared to CD80; however, both CD86 and CD80 bindings to CD28 still showed similar NF-κB stimulation [26]. Furthermore, D124 mutations can increase the binding affinity of CD28 to ICOS to form an ICOS-CD28 fusion protein, driven by ICOS promoters due to high ICOS expression in AITL [26]. The fusion protein can further augment CD28 expression in T-cells [26]. The T195 mutation together with the D124 mutations can result in augmented signal transduction and ligand-receptor interaction [1, 7], with enhanced NF-κB activity from TCR/CD28 activation in vitro [9]. The CD28 can also combine with CTLA4, an inhibitory signal for T cells, to form the fusion protein CD28-CTLA4, transforming CTLA4 into a stimulatory signal for T-cell activation [5]. The CD28-CTLA4 can increase downstream signaling of CD28 through phosphorylation of PI3K and MAPK signaling pathways, which can augment the survival and proliferation of T cells [8]. Moreover, the CD28-CTLA4 protein is mutually exclusive with other CD28 mutations, accounting for 38% of AITL cases [8], and it has possible oncogenic cooperation with the RHOA mutation [5].

PLCy1, CARD11, TNFRSF21, CCND3, SAMSN1, ITK-SYK, and VAV1 are all genes heavily involved in the pathogenesis of AITL [5]. The phospholipase C, gamma 1 (PLCG1/PLCy1) is downstream of TCR signaling that hydrolyzes diacylglycerol and inositol 1, 4, 5-triphosphate from phosphatidylinositol 4,5-bisphosphate [5]. Mutations affecting the PLCy1 gene will hyperactivate the NFAT signaling and increase TCR signaling. In AITL, PLCy1 mutations account for 11.1%-14.1% of cases [6, 9, 20], with most mutations being missense changes and gain of function [7, 10]. The mutations E47K, R48W, S342G, S345F, S520F, E730K, G869E,
E1163K, and D1165G/H are identified in PLCγ1, contributing to the activation of MALT1 and NFAT in vitro [5, 7, 9]. The caspase recruitment domain family member 11 (CARD11) is a downstream scaffolding protein for PLCγ1, required for NF-κB activation by CD28/TCR. InAITL, CARD11 mutations account for 2.8% of cases with the missense alterations F176C, S547T, and F902C, all promoting NF-κB activity [9]. The necrosis factor receptor superfamily member 21 (TNFRSF21/DR6) induces cell apoptosis through NF-κB and JNK pathways. The TNFRSF21 mutation accounts for 3.36% ofAITL cases [7]. A frameshift deletion in the death domain of TNFRSF21 can inactivate the protein product, which causes hyperproliferation of CD4+ T cells and deviation towards TH2 phenotype following TCR stimulation [5]. The cyclin D3 (CCND3) gene regulates CDK kinases and the cell cycle. InAITL, CCND3 mutations account for 3.36% of cases with one frameshift indel, two nonsense mutations, and P284S alteration, a mutation hotspot, at the C-terminus of the Cyclin-C domain [7]. The mutations can affect the phosphorylation motif in cyclin D3 carboxyl terminus for polyubiquitination, phosphorylation, and proteasome-mediated degradation of D-type cyclin, which can promote CCND3 stabilization and mediate its drive for cell proliferation [5, 7]. The SH3 domain and nuclear localization signals 1 (SAMSN1) is a cell proliferation negative regulator [5]. A stop codon mutation upstream of the SH3 domain will cause a loss of function change to the gene, thus stimulating cell proliferation [5]. The interleukin 2-inducible T-cell kinase (ITK) gene encodes tyrosine kinase specific to T cells that regulate T-cell differentiation and proliferation [27, 28]. Chromosomal translocations of the ITK gene on chromosome 5 at (5;9) (q33;q22) and SYK gene on chromosome 9 can form ITK-SYK fusion proteins [5, 25, 27, 29]. The fusion protein triggers antigen-independent phosphorylation of TCR proximal proteins, contributing to malignant T cell growth [29]. However, the fusion protein is extremely rare in AITL, and more prevalent in follicular variant of PTCL-NOS patients [5, 25, 27]. Instead, there is a 38% ITK gene gain and 14% SYK gene gain in AITL patients [27, 29]. The vav guanine nucleotide exchange factor 1 (VAV1) helps promote PLCγ1 phosphorylation [6], mediates the signaling cascade after TCR engagement as a GEF-independent adaptor [2], and works as a GEF for RHOA, CDC42, and RAC1 [9]. InAITL, VAV1 mutation accounts for 4.7%-5.6% of cases [6, 9, 20], with missense alterations, D797G and Y826S affecting the second SH3 domain [9]. The D797G mutation helps promote oncogenic signaling through cell-cell contact deregulation [9]. The RHOA G17V mutation can also affect the TCR signaling pathway by using the adaptor function of the VAV1 protein [6]. The RHOA G17V mutation does so by interfering with tyrosine phosphorylation of VAV1, affecting both the GEF-independent and -dependent function of VAV1 protein [2]. VAV1 protein is generally phosphorylated at Tyr160 and Tyr142 by TCR engagement and recruits Src tyrosine kinases associated with FYN and LCK [6]. However, RHOA G17V can induce Tyr174 hyperphosphorylation, thereby increasing TCR signaling through the recruitment of the two Src tyrosine kinases [2, 6]. A study done with RHOA G17V Jurkat cells demonstrated that VAV1 binds to G17V RHOA both in the presence or absence of TCR stimulation; albeit, TCR stimulation can accelerate RHOA G17V phosphorylation on VAV1 [6]. Moreover, the interaction between RHOA and VAV1 promotes VAV1 adapter function, thus augmenting the TCR signaling cascade and assisting TCR signaling complex formation [6]. The protein VAV1 can enhance the phosphorylation of PLCγ1 as an adaptor in the PLCγ1-SLP76-LAT complex [6]. As a result, PLCγ1 phosphorylation can promote NFAT and calcium-calmodulin (CaM), both of which are essential for T-cell differentiation and activation, through inositol 1, 4, 5-triphosphate and second messenger diacylglycerol [6]. The results of the Jurkat cell study demonstrated more significant PLCγ1 phosphorylation with TCR stimulation in RHOA G17V Jurkat cells [6]. Moreover, the study also identified a VAV1-STAP2 gene fusion protein found exclusively in 8.2% of AITL patients lacking the RHOA mutation [6]. The fusion protein causes Tyr174 hyperphosphorylation and increases PLCγ1 phosphorylation even in the absence of TCR-stimulation. However, TCR stimulation does promote greater phosphorylation of VAV1-STAP2 and increases its activity [6]. Furthermore, VAV1 mutation and RHOA G17V mutation are mutually exclusive [6].
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Figure 2. Flow chart of the effect of AITL recurrent mutations on different signaling pathways and proteins. The mutation RHOA G17V increases PI3K and MAPK signaling, as well as the level of PD1, CXCR5, ICOS, and BCL6, which are all Tfh signature genes. Through the PI3K and MAPK signaling, there is an increase in ERK1/2, signaling, AKT signaling, and S6 phosphorylation, and together these changes augment the proliferation of Tfh cells and secretion of proinflammatory cytokines. Moreover, RHOA G17V can co-occur with either TET2 or IDH2 R172 to increase Tfh signature genes, inflammatory phenotypes, and AITL like features. The RHOA G17V mutation also affects the VAV1 protein, which causes a downstream signaling cascade, leading to increased TCR signaling through FYN and LCK and an increase in NFAT and CaM through PLCy1. The IDH2 R172 mutation increases 2-HG, which disrupts KDM6A and KDM6B, thus increasing the level of H3K27me3, H3K4me3, and H3K9me, manifesting as transcriptional silencing. Moreover, the mutation by itself or co-occurring with TET2 promotes Tfh cell differentiation and suppresses T-helper cell differentiation through various signaling pathways and cytokines. The IDH2 mutation also has increased chromosome 5 and 21 gain, augmenting genes related to T-cell differentiation, activation, and cell cycle regulation. The TET2 mutation and DNMT3A mutation together activate the oncogenic notch signaling pathway while individually promoting mechanisms and genes used during self-renewal of HSCS cells. In mutations affecting the TCR signaling pathway, the two mutations in CD28 and FYN increase TCR signaling activity, respectively. The
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FYR mutation augments kinase activity and NF-kB signaling pathways, while the CD28 mutation augments NF-kB activation and IκB phosphorylation. Furthermore, both proteins can form fusion proteins with FYR manifesting as FYR-TRAF3IP2 and CD28 becoming either ICOS-CD28 or CD28-CTLA4. The FYR fusion proteins influence the NF-kB signaling pathway, while the CD28 fusion proteins increase CD28 signaling. The PLCγ1 mutation is a common mutation present in the AITL TCR signaling pathway, where it increases NFAT signaling, MALT1, and TCR signaling. Another mutation in the TCR signaling pathways is CARD11, where it increases NF-kB activity. The mutations of TNFRSF21, CCND3, and SAMS1N, all augment cell proliferation to a certain degree, whereas TNFRSF21 is known to induce CD4+ T cell proliferation and deviation toward a TH2 phenotype. The fusion protein ITK-SYK increases the activation antigen-independent phosphorylation of TCR proximal proteins. The VAV1 mutation increases the oncogenic signaling pathway through cell-cell contact deregulation. Moreover, the VAV1 gene forms a VAV1-STATp2 fusion protein, which increases the phosphorylation of PLCy1. In mutations affecting the JAK/STAT signaling pathway, there is an increase in STAT protein activation, and mutations occurring in the STAT3 protein increase myeloid malignancies in mice. With a GAPDH mutation, the constitutive activation of the canonical NF-kB pathway augments the non-canonical NF-kB pathway, leading to enhancement of Tfh development.

The numerous mutations present in the different components of the TCR signaling pathways induce aberrant NF-kB signaling pathways and T-cell activation, and they hyperactivate TCR signaling, thus increasing malignant T cell growth. As a result, the mutations mainly affect Tfh cell specification and cell proliferation (Figure 2).

JAK/STAT signaling pathway mutations

The JAK/STAT signaling pathway has an essential role in Tfh cell development through STAT3/STAT4 signaling [1, 30]. Mutations affecting the JAK/STAT pathway can induce T-cell lymphomas by hyper-activating STAT proteins and releasing various cytokines [30]. In AITL, a gain of function STAT3 mutations occurs in 2.8%-13% [9, 30] of patients, with deletion mutation E616 and missense mutation S614R affecting the SH2 domain [30]. In a bone marrow transplantation murine study, the E616 mutation has demonstrated increased myeloid malignancy in mice with bone marrow transplantation [30]. Moreover, the mutations RHOA and STAT3 co-occur in ~10% of AITL cases [30]. Various mutations present in the JAK/STAT signaling pathways can hyperactivate STAT protein and release various cytokines, increasing the possibility of a myeloid malignancy.

Glyceraldehyde-3-phosphate dehydrogenase mutation

In a murine study done with plck-GAPDH mice, the overexpression of GAPDH induced initial constitutive activation of the canonical NF-kB pathway followed by the constitutive activation of the non-canonical pathway, which significantly enhanced Tfh development [31]. The non-canonical pathway in GAPDH overexpressed mice is mainly activated by PD1-PDL1 ligation, which is not known to trigger non-canonical pathways in human AITL patients [2, 31]. Moreover, PD1-PDL1 ligation can activate the NF-kB pathway in GC B cells, contributing to AITL-like development in mice with overexpression of GAPDH [2]. The overexpression of GAPDH in the murine study can constitutively activate NF-κB pathways, leading to the development of AITL-like symptoms in mice.

Conclusion

AITL is a disease affecting the germinal centers of secondary lymphoid tissues through the aggressive expansion of T-cell follicular helper cells. The recognition of new recurrent mutations helps improve the diagnosis and prognosis of AITL. Although the recurrent mutations mentioned above are not recognized as diagnostic and prognostic criteria for AITL, they may become molecular markers for integrated diagnosis in the future. Through new improvements in techniques including single-cell RNA-sequencing, ATAC sequencing, and genomic profiling studies, the underlying molecular genetics and pathophysiology of AITL can be further elucidated and identified. Based on novel and existing mutations of AITL, new targeted therapy and treatments can be developed to improve the overall and relative survival rate from the disease.

Disclosure of conflict of interest

None.

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