Case Report
Discordant intracellular and plasma D-2-hydroxyglutarate levels in a patient with IDH2 mutated angioimmunoblastic T-cell lymphoma

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Abstract: Objectives: Angioimmunoblastic T-cell lymphoma (AITL) is an aggressive peripheral T-cell lymphoma with mutations in genes encoding isocitrate dehydrogenase1 and 2 (IDH1 and IDH2). Mutant IDH generates the oncometabolite D-2-hydroxyglutarate (D-2HG). We report the first case of discordant intracellular and plasma D-2HG levels in a patient with IDH2 R172S mutated AITL. Methods: An 87-year-old woman was diagnosed with AITL in the groin lymph node by morphologic and immunophenotypic analyses, and molecular studies by DNA sequencing. D-2HG was measured in both tumoral tissue and in pre-treatment plasma by liquid chromatography-tandem mass spectrometry. Results: While D-2HG was markedly elevated in the tissue sample, its level in plasma was normal. We discuss this discordant D-2HG result within the context of previously reported discordant 2HG results in other IDH mutated tumors, and its implication for using circulating D-2HG as a biomarker of IDH mutation. In addition, this case also harbored mutations in RHOA, TET2, and TP53. The molecular pathogenesis is briefly discussed. Conclusion: While our case suggests that circulating D-2HG is not a reliable marker of IDH mutation in AITL, more cases need to be studied to arrive at a definite conclusion.

Keywords: Angioimmunoblastic T-cell lymphoma, D-2-hydroxyglutarate, IDH mutation, RHOA mutation, TET2 mutation

Introduction
Angioimmunoblastic T-cell lymphoma (AITL) is a peripheral T-cell lymphoma, accounting for 1-2% of non-Hodgkin lymphomas. AITL commonly presents at advanced clinical stage with generalized lymphadenopathy, frequent involvement of the liver, spleen, skin and bone marrow, and a poor overall prognosis [1]. In addition, patients often experience tumor-associated immunodeficiency, which precludes the use of higher-intensity chemotherapeutic regimens due to an increased risk of infection and autoimmune complications. Although the molecular pathogenesis of AITL has not been well-characterized, gene expression profiling has proposed the cell of origin as follicular helper T-cells, which may explain the observed immunosuppressive effects due to T-cell cytokine dysregulation. Furthermore, molecular studies of AITL have identified mutations in several genes including TET2 (Ten-Eleven Translocation methylcytosine dioxygenase 2), DNMT3A (DNA (cytosine-5)-Methyl Transferase3 Alpha), RHOA (RasHomolog gene family, member A), and the focus of this report, IDH2 (Isocitrate Dehydrogenase 2) [2-4].

Somatic heterozygous mutations in IDH1 and IDH2 have been identified in a number of cancers including acute myeloid leukemia (AML), glioma, chondrosarcoma, intrahepatic cholangiocarcinoma, and AITL [5]. IDH1 and IDH2 respectively encode cytoplasmic/peroxisomal isocitrate dehydrogenase 1 (IDH1) and mitochondrial isocitrate dehydrogenase 2 (IDH2), which catalyze the oxidative decarboxylation of isocitrate to α-ketoglutarate (α-KG). IDH muta-
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Mutations mainly involve a single amino acid substitution at an arginine residue (R132 in IDH1, or R140 or R172 in IDH2). These mutations result in loss of function for oxidative decarboxylation of isocitrate to α-KG, and confer an increase in gain-of-function enzymatic activity facilitating reduction of α-KG to the oncometabolite D-2-hydroxyglutarate (D-2HG) [5]. While large accumulations of D-2HG in tumor cells have been demonstrated in cases of AML and glioma with IDH1 or IDH2 mutations, the level of D-2HG in peripheral blood varies from normal to significantly elevated in patients with IDH-mutated tumors [6-10] and is the focus of this case report and review.

Here, we describe the first case of AITL with markedly increased intracellular D-2HG as a consequence of an IDH2 R172S mutation, but without increase in plasma D-2HG level. We discuss this case within the context of previously reported discordant 2HG results in AML and solid tumors, and its clinical implication for using plasma/serum D-2HG as a biomarker of IDH mutation. In addition, this case also harbored mutations in RHOA, TET2, and TP53. The molecular pathogenesis relevant to these mutations is briefly discussed.

Case report

Clinical history

An 87-year-old woman with a distant history of breast cancer, status/post lumpectomy and radiation therapy, presented with right lower extremity edema, right groin tenderness, frequent night sweats, and pruritus. A physical examination and ultrasound identified enlarged lymph nodes in the right groin and left axilla. A needle core biopsy of a right groin lymph node showed atypical T cells, prompting an

Figure 1. AITL infiltrates in the lymph node with a striking low power appearance of collections of lymphoma cells with clear cytoplasm distinct from adjacent areas of small, mature lymphocytes (A). Lymphoma cells are intermediate in size with ovoid nuclei, variably distinct nucleoli, and abundant clear to pale cytoplasm, frequently surrounding high endothelial venules (B), and express CD3 (C) and CD10 (D) (original magnification, hematoxylin and eosin stain ×100 and 500, respectively (A, B); immunohistochemistry ×100 and 500, respectively (C, D)).
Excisional biopsy. No bone marrow biopsy was performed. Positron emission tomography/computed tomography identified [18] F-fluorodeoxyglucose (FDG) uptake concerning for lymphoma in enlarged bilateral pelvic, retroperitoneal, inguinal, femoral, and cervical lymph nodes; left cervical subcutaneous tissue; and T8 vertebral body.

Pathological, immunophenotypic, molecular and oncometabolite studies

Histological sections of the excisional biopsy revealed fragments of a lymph node and fibro-adipose tissue. The lymph node had a thickened fibrous capsule, and its architecture was effaced by a diffuse infiltrate of lymphoma cells with frequent mitoses. The lymphoma cells were intermediate in size with ovoid nuclei, variably distinct nucleoli, and abundant clear to pale cytoplasm. The background stroma contained many high endothelial venules frequently surrounded by lymphoma cells, resulting in a striking low power appearance of collections of lymphoma cells with clear cytoplasm distinct from adjacent areas of small, mature lymphocytes. A few scattered immunoblasts, eosinophils, and plasma cells were present in the background. By immunohistochemistry, the lymphoma cells showed reactivity for CD3, CD4, CD5, CD10, PD-1, and BCL-6, but they were negative for CD8, TIA-1, ALK-1, CD20, CD30, and CD21. CD20 and CD30 highlighted the immunoblasts. CD21 revealed expanded follicular dendritic cell meshworks rimmed by abundant CD20-positive, small B lymphocytes. In situ hybridization for EBV encoded small RNA (EBER) was negative (Figure 1). Flow cytometric analysis performed by 4-color FACSCalibur and evaluated by cluster analysis using Paint-A-Gate (BD Biosciences, San Jose, CA, USA) revealed a 17% population of medium to large-sized neoplastic T cells with the following immunophenotype: CD2(+) , CD3(+), CD4(+), CD5(+), CD7(-), CD8(-), CD10(+), CD19(-), CD20(-), CD30(-), CD38(+) , and CD45(+), in a background of 19% small mature T-lymphocytes and 45% mature polytypic B-lymphocytes (Figure 2).

Conventional cytogenetic studies revealed a normal female karyotype. Molecular studies performed by massively parallel DNA sequencing revealed wild type IDH1 and ac.G516T (p. R172S) IDH2 mutation with a mutant allele frequency (MAF) of 8.0%. Mutations in TET2 (M376fs1, M1333fs6, MAF 8.0% for both mutations), RHOA (G17V, MAF 7.0%) and TP53 (L35F, a variant of unknown significance, MAF 53.0%) were also identified.

Samples of a suspension made from a portion of the excised lymph node and peripheral blood plasma were assayed for D-2-hydroxyglutarate.

Figure 2. AITL immunophenotypic analysis by 4-color flow cytometry. Neoplastic cells (in red) are intermediate in size and express CD2, CD3, CD4, CD5, CD10, CD38 and CD45, and lack CD7, CD8, CD19 and CD30. Normal T cells are in green.
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![Graph showing D-2-hydroxyglutarate levels](image)
(D-2-HG) and L-2-hydroxyglutarate (L-2-HG). Briefly, the extracted metabolites were derivatized with (+)-diacetyl-L-tartaric anhydride and analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS), as previously described [11]. In the neoplastic cells, D-2-HG was markedly increased (4,532 ng/mg protein), while L-2-HG was not increased (2.7 ng/mg protein) with a ratio of D-2-HG/L-2-HG of 1.679 (Figure 3). In the plasma sample, D-2-HG (74 ng/ml) and L-2-HG (38 ng/ml) were within their normal reference ranges (18-263 ng/ml for D-2-HG, 6-147 ng/ml for L-2-HG), with a ratio of D-2-HG/L-2-HG of 1.94.

Discussion

Mutations in IDH1 and IDH2 have been described in hematologic and non-hematologic malignancies including AML and glioma [5] and more recently IDH2 R172 mutations were described in AITL with a prevalence of approximately 30% [4]. We describe the first case of AITL with an expected elevated intracellular D-2-HG as a result of IDH2 R172S mutation, extending the number of neoplastic diseases where IDH mutations generate this oncometabolite. Surprisingly, the peripheral blood plasma, analyzed at the time of pre-therapy active disease, did not show an increase in D-2-HG, in contrast to a generally strong association of circulating 2HG with IDH mutation status in AML [10, 12].

It is important to note that there are two enantiomers of 2HG: D-2HG and L-2HG. They are normal endogenous metabolites that can be oxidized back to α-KG by their specific 2-hydroxyglutarate dehydrogenases (D-2HGDH and L-2HGDH). IDH mutations only produce the D enantiomer. The mechanisms by which D-2-HG is released from tumor cells into the circulation are not completely understood. Possible ways include passive diffusion through the cell membrane, active efflux by a membrane bound transporter, or release from cells undergoing necrosis. Whatever the mechanism, increased levels of circulating D-2HG have indeed been demonstrated in patients with IDH-mutated AML [10, 12], suggesting that levels of plasma/serum D-2HG may be useful to predict IDH mutations at diagnosis, as well as evaluate minimal residual disease and relapse. On the other hand, plasma/serum D-2HG levels in patients with IDH-mutated solid tumors are widely variable.

Review of the literature for D-2HG accumulation in peripheral blood in patients with IDH-mutated tumors reveals several notable observations [6-10, 12, 13]. First, most studies reported total 2HG, rather than the IDH mutation specific D-2HG, but a study by Janin et al demonstrated that the ratio of D-2HG/L-2HG >2.5 increased specificity in detecting IDH mutations [10]. Second, the thresholds of plasma/serum 2HG for discriminating the presence of an IDH mutation are widely variable among these studies, which may partly reflect genetic/ethnic and/or dietary differences or even underlying subclinical inherited or acquired metabolic perturbations. Third, while there is a general association of circulating 2HG and IDH mutation status in AML, the correlation is not perfect. Absence of increase in circulating 2HG in patients with IDH-mutated tumors is more common in solid tumors, such as glioma. In IDH-mutated cholangiocarcinoma, serum 2HG was elevated but still approximately 10-fold lower than the levels seen in AML [6]. In our patient with IDH2 R172S mutated AITL with widespread disease as demonstrated by imaging studies, plasma D-2HG, total 2HG, and D-2HG/L-2HG ratio were all within the normal range. On the other hand, a small subset of AML patients had a high 2HG, yet no IDH mutation was identified by employing high sensitivity molecular assays and sequencing of the entire IDH1/2 coding regions for possible mutations beyond the hot spot [12]. These findings suggest that other genetic or biochemical events may cause 2HG elevation. Indeed, a recent study discovered a functional relationship between glutamine and D-2HG metabolism and the MYC pathway activation in breast cancer [14]. Wild-type IDH2 and phosphoglycerate dehydrogenase have also been suggested to catalyze reductive carboxylation of α-KG to D-2HG in vitro models of breast cancer [15, 16]. Elevated levels of L-2HG in clear cell renal
cell carcinoma were shown to be mediated at least in part by reduced expression of L2-hydroxyglutarate dehydrogenase [11]. However, D-2HG elevation in cancer has not yet been ascribed to a deficiency of D-2-hydroxyglutarate dehydrogenase.

Collectively, these data suggest that plasma/serum D-2HG measurements and determination of the D-2HG/L-2HG ratio may allow for rapid and accurate identification of IDH mutations in patients with AML and cholangiocarcinoma, but may not be useful in patients with glioma. The data on the utility of D-2HG to predict IDH mutation in AITL and breast adenocarcinoma is too limited to draw a conclusion, and large prospective studies are warranted to assess this possibility.

D-2HG may be a major oncogenic effector of IDH mutant activity, disrupting the function of multiple-KG dependent pathways including prolyl hydroxylases, histone demethylases, and ten-eleven translocation (TET) 5-methylcytosine hydroxylases [5]. This may induce global hypermethylation and further oncogenic events in co-operation with other gene mutations in AITL such as TET2 and the more recently identified RHOA. RHOA encodes ras homology GTPase that is involved in cellular migration and cell polarity in cancer cells. RHOA operates as a molecular switch that regulates a wide variety of processes through cycling between an active (GTP-bound) state and an inactive (GDP-bound) state. The RHOA G17V mutant may contribute to the pathogenesis of AITL through the inhibition of wild-type RHOA in a dominant-negative manner [3].

Mutations in RHOA occur at a low frequency, found in <1% of cancers including lymphomas. However, >50% of AITL cases carry RHOA inactivating mutations [3]. Remarkably, mutations in RHOA, TET2, and IDH2 are strongly correlated [3]. The vast majority of IDH2 mutations were confined to the cases also harboring RHOA and TET2 mutations, as in our case where the mutant allele frequencies of these mutations were similar. This is in contrast to AML, where IDH and TET2 mutations are usually mutually exclusive [17]. However, the predominant type of IDH mutation is different in AML versus AITL: IDH1R132H or IDH2R140Q mutations are usually seen in AML, while IDH2 R172 mutations characterize AITL. This suggests differential oncogenetic effects induced by different types of IDH mutations. From a diagnostic standpoint, the co-occurrence of mutations in RHOA, TET2, and IDH2 is predominantly seen in AITL but not in peripheral T-cell lymphoma, not otherwise specified [3]. Recognition of this highly specific mutation pattern could help to distinguish AITL from its morphologic mimics. Moreover, further research into the oncogenic molecular mechanisms of RHOA mutations will lead to the development of novel therapeutics, similar to the promising development of IDH inhibitors and DNA hypomethylating agents in combating the epigenetic modifications induced by IDH, TET2, and related gene mutations.

In conclusion, we report a case of IDH2-mutated AITL with high intracellular level of D-2HG without an increase in circulating D-2HG. While our case suggests that circulating D-2HG is not a reliable marker of IDH mutation in AITL, more cases need to be studied to arrive at a definite conclusion.

Disclosure of conflict of interest

None.

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