IDH2 mutations are frequent in angioimmunoblastic T-cell lymphoma.


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Rob A. Cairns 1,*, Javeed Iqbal 2,*, François Lemonnier 3,4,*, Can Kucuk 5, Laurence De Leval 5, Jean-Philippe Jais 6, Marie Parrens 7, Antoine Martin 8, Luc Xerri 9, Pierre Brousset 10,11, Li Chong Chan 12, Wing-Chung Chan 2, Philippe Gaulard 3,4, Tak W. Mak 1,2

1 Campbell Family Institute for Breast Cancer Research University Health Network, 620 University avenue, suite 706, Princess Margaret Hospital, Toronto, ON,CA
2 Department of Pathology and Microbiology University of Nebraska Medical Center, Omaha, NE 68198,US
3 Institut Mondor de Recherche Biomédicale INSERM : U955, Université Paris XII - Paris Est Créteil Val-de-Marne, IFR10, 51 Av Maréchal de Lattre de Tassigny, 94010 Créteil cedex, FR
4 Service d'anatomie et cytologie pathologiques [Mondor] Assistance publique - Hôpitaux de Paris (AP-HP), Hôpital Henri Mondor, Université Paris XII - Paris Est Créteil Val-de-Marne, 51 Av Maréchal de Lattre de Tassigny, 94000 Créteil, FR
5 Institute of Pathology Centre Hospitalier Universitaire Vaudois, 25 rue du Bagnon 1011 Lausanne, CH
6 NAMADES, Nouvelles Approches Méthodologiques pour l'aide à la décision et à la stratégie en santé Université Paris V - Paris Descartes : EA4472, Hôpital Necker 149 rue de Sèvres 75743 Paris cedex 15, FR
7 Service de pathologie [Bordeaux] CHU Bordeaux, Groupe hospitalier Pellegrin, Université Victor Segalen - Bordeaux II, place Aurélie Rabat-Léon 33000 Bordeaux, FR
8 Service d'Anatomie et de Cytologie Pathologiques [Avicenne] Assistance publique - Hôpitaux de Paris (AP-HP), Hôpital Avicenne, Université Paris XIII - Paris Nord, 125 rue de Stalingrad 93000 Bobigny, FR
9 Département de biopathologie Institut Paoli-Calmettes, Bd Sainte-Marguerite 13009 Marseille, FR
10 CRCT, Centre de recherche en cancérologie de Toulouse INSERM : U1037, Hôpital Purpan, place du Docteur Baylac BP3028 31024 Toulouse Cedex 3, FR
11 Service d'anatomie pathologique et histologie-cytologie [Toulouse] CHU Toulouse, Hôpital de Rangueil, Université Paul Sabatier - Toulouse III, 1, avenue du Professeur Jean Poulbès - TSA 50032 - 31059 Toulouse cedex 9, FR
12 Department of Pathology University of Hong Kong, Queen Mary Hospital compound, HK

* Correspondence should be addressed to: Tak Mak <mak@uhnres.utoronto.ca>
# These authors contributed equally to the work

Abstract

Mutations in isocitrate dehydrogenase 1 (IDH1) and isocitrate dehydrogenase 2 (IDH2) occur in most grade 2 and 3 gliomas, secondary glioblastomas, and a subset of acute myelogenous leukemias, but have not been detected in other tumor types. The mutations occur at specific arginine residues, and result in the acquisition of a novel enzymatic activity that converts 2-oxoglutarate to D-2-hydroxyglutarate. This study reports IDH1 and IDH2 genotyping results from a set of lymphomas which included a large set of peripheral T-cell lymphomas (PTCL). IDH2 mutations were identified in approximately 20% of angioimmunoblastic T-cell lymphomas (AITL), but not in other PTCL entities. These results were confirmed in an independent set of AITL patients, where the IDH2 mutation rate was approximately 45%. This is the second common genetic lesion identified in AITL after TET2, and extends the number of neoplastic diseases where IDH1 and IDH2 mutations may play a role.

MESH Keywords Aged; Female; Gene Frequency; Genotype; Humans; Immunoblastic Lymphadenopathy; enzymeology; genetics; pathology; Isocitrate Dehydrogenase; genetics; Kaplan-Meier Estimate; Lymphoma, T-Cell; enzyymology; genetics; pathology; Lymphoma, T-Cell, Peripheral; enzyymology; genetics; pathology; Male; Mutation; Mutation Rate; Prognosis

Introduction

Heterozygous isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) mutations at specific active site arginine residues occur in most low-grade gliomas, secondary glioblastomas, and in some acute myeloid leukemias (AML)[1,3,4]. These mutations alter IDH enzymatic function, resulting in the conversion of 2-oxoglutarate to the rare metabolite D-2-hydroxyglutarate (D-2-HG), which accumulates to high levels in cells and tissues[4,5]. D-2-HG may act as an oncometabolite, driving tumor progression by interfering with 2-oxoglutarate-dependent enzymes that affect hypoxia signalling (prolyl hydroxylases), histone methylation, and DNA methylation (TET2)[6,7]. Despite extensive genotyping, IDH1 and IDH2 mutations have not been identified in significant proportions of other neoplasms[3,8,9].

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Peripheral T-cell lymphomas (PTCL) are non-Hodgkin lymphomas with a diverse presentation, histology, therapy response, and outcome[10]. Since this group of diseases has not been comprehensively assessed for the presence of IDH mutations, patient samples from three independent groups were genotyped to determine whether these mutations are present.

**Methods**

**Patients and clinical data**

Lymphoma and leukemia DNA samples from frozen tissue were provided by a multicentric T-cell lymphoma consortium (Tenomic) and the University of Hong Kong (UHK). Diagnosis was confirmed by a panel of pathologists to ensure consistent classification of PTCL according to the WHO classification. A complimentary set of DNA from 22 angioimmunoblastic T-cell lymphomas (AITL) was provided by the University of Nebraska Medical Center (UNMC) Lymphoma Tumor Bank[11]. These AITL patients were identified by histopathologic assessment and confirmed by gene expression profiling[11].

As there is no standard therapy for AITL, patients received heterogeneous treatment. AITL samples with greater than 50% estimated tumor cell content were prioritized (Table S1). All patients provided informed consent in accordance with the Declaration of Helsinki.

**IDH1 and IDH2 Genotyping**

For Tenomic and UHK patients, IDH1 R132 and IDH2 R172 and R140 genotypes were determined at the Analytical Genetics Technology Centre at the University Health Network (Toronto, Canada) using a Sequenom MassARRAY™ platform (Sequenom, San Diego, CA) as previously described[5]. Positive results were confirmed by Sanger sequencing of the mutated region. In the UNMC patients, IDH1 and IDH2 genotype was determined by Sanger sequencing of all exons and subsequently by Sequenom (Genomic Core facility, UNMC, Omaha, NE). Sequenom genotyping is more sensitive and specific than Sanger sequencing, with the ability to detect a mutation in 10% of the input DNA, which is an advantage for samples with stromal contamination[12].

**Statistical Analysis**

Fisher’s exact text and the Wilcoxon rank sum test were used to test for differences between IDH2wt and IDH2 mutant patients. Estimates of overall and progression-free survival were calculated using the Kaplan-Meier method, and compared using the log-rank test.

**Results and Discussion**

Lymphoma samples from the Tenomic consortium and HKU were genotyped to determine whether mutations were present in IDH1 at R132 and in IDH2 at R172 and R140 (Table 1). This set of samples included a large group of PTCL. Although other studies have suggested that IDH1/IDH2 mutations are not present in lymphomas and leukemias other than AML[3,9], PTCL has not been comprehensively studied.

No mutations were observed in lymphoma subtypes, including 46 B-cell lymphomas and 66 Hodgkin lymphomas, except for angioimmunoblastic T-cell lymphoma (AITL), where 16/79 (20%) samples from the Tenomic consortium carried an IDH2 mutation. This is the second common mutation to be identified in AITL after TET2[13], and makes AITL the third disease where IDH1/IDH2 mutations have been identified in a significant proportion of patients. As has been observed in glioma and AML, all mutations were heterozygous. However, the spectrum of mutations observed in AITL was different. Unlike in glioma and AML, no IDH1 mutations were identified, and the IDH2 mutations were largely confined to alterations resulting in an R172 substitution (12 R172K, 2 R172G, 1 R172T, and 1 R140G).

To further validate these results, 22 AITL patients from UNMC were genotyped (Table 1). None of the patients carried IDH1 mutations, and in 10/22 cases (45%) IDH2 mutations were identified, confirming the results of the Tenomic data. Although this was an independent set of patients, and mutation detection was performed using Sanger sequencing of all exons, the mutation spectrum was consistent with that of the Tenomic patients, with the IDH2 mutations detected at R172 (4 R172K, 4 R172S, 1 R172T and 1 R172G). There were no other mutations found in IDH1 or IDH2. The higher rate of mutation in this smaller set of patients may reflect differences in patient selection defined by a gene expression signature[11] which may identify a more homogeneous group of patients that share the IDH2 mutation at higher frequency.

AITL is one of the three most common PTCL subtypes, along with anaplastic large cell lymphoma and PTCL not otherwise specified[14]. It normally presents as a systemic disease, with polyclonalopathy and a variety of immunologic abnormalities, and carries a poor prognosis[15]. Based on molecular marker expression (CD4, CD10, BCL6, PD1, CXCL13) and microarray profiling, AITL is thought to arise from follicular T-helper cells normally present in germinal centres[11,16,17]. The molecular pathogenesis and underlying genetic events driving AITL are largely unknown.
IDH2 mutations in AITL

The clinical features of IDH2 wildtype and mutant AITL patients were assessed. Although all clinical parameters were not available for each patient, there were no significant differences between the two groups in the Tenomice patients, except that the Direct Coombs test was less frequently positive in IDH2 mutant patients (Table S1). These results were consistent with the smaller UNMC patient group (data not shown). Upon review, there were no pathological differences between the groups. Furthermore, IDH2 status had no effect on progression-free or overall survival (Figure 1). This is at odds with the findings in glioma, where IDH1/IDH2 mutations predict for improved survival, but more consistent with AML, where there is no overall independent impact on outcome, although some studies show prognostic value when specific IDH mutations are combined with other prognostic markers[18]. Prognostic impact may be difficult to detect in AITL, as patients are acquired from multiple centers and receive heterogeneous treatment.

Although the current results suggest that IDH2 may not provide prognostic information in AITL, a better understanding of the mechanisms underlying IDH1/IDH2 driven tumor progression may lead to new opportunities for AITL treatment. Future measurement of D-2-HG, the metabolite produced by these mutant enzymes, may provide a useful biomarker of disease progression and response to therapy. In addition, small molecule inhibitors specific for the mutant IDH2 enzymes could represent important tools in the future management of AITL.

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Footnotes:

Authorship: R.A.C., J.I., L.X., L.C.C., W.C., P.B., P.G. and T.W.M. designed the research. R.A.C., J.I., C.K., L.D.L., M.P., F.L., L.X. and A.M. performed research and collected and analyzed the data. F.L., J.P.J., and J.I. performed statistical analysis. R.A.C. wrote the manuscript. The authors declare they have no competing conflicts of interest.

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Figure 1
Overall survival (A), and progression free survival (B) of AITL patients with wildtype (n=61) or mutant IDH2 (n=16) from the Tenomic consortium data set. Wildtype IDH2 patients were not significantly different from IDH2 mutant patients for either parameter.
<table>
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<th>IDH2R172</th>
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