Brief report

Regions of acquired uniparental disomy at diagnosis of follicular lymphoma are associated with both overall survival and risk of transformation

Derville O’Shea,1 Ciarán O’Riain,1 Manu Gupta,2 Rachel Waters,2 Youwen Yang,1 David Wrench,1 John Gribben,1 Andreas Rosenwald,3 German Ott,4,5 Lisa M. Rimsza,6 Harald Holte,6 Jean-Baptiste Cazier,1,7 Nathalie A. Johnson,8 Elias Campo,9 Wing C. Chan,10 Randy D. Gascoyne,1 Bryan D. Young,1 Louis M. Staudt,11 T. Andrew Lister,1 and Jude Fitzgibbon1

1Centre for Medical Oncology, Barts and The London School of Medicine, London, United Kingdom; 2Centre for Statistics in Medicine, Oxford University, Oxford, United Kingdom; 3Institute of Pathology, University of Würzburg, Würzburg, Germany; 4Department of Clinical Pathology, Robert-Bosch-Krankenhaus, Stuttgart, Germany; 5Department of Pathology and Arizona Cancer Center, University of Arizona, Tucson; 6Department of Oncology, Cancer Clinic Norwegian Radium Hospital, Rikshospitalet, Oslo, Norway; 7Bioinformatics and Biostatistics, Cancer Research UK, London, United Kingdom; 8Department of Pathology and Division of Medical Oncology, British Columbia Cancer Agency, Vancouver, BC; 9Department of Pathology and Hospital Clinic, Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain; 10Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha; and 11Metabolism Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD

Acquired homozygosity in the form of segmental acquired uniparental disomy (aUPD) has been described in follicular lymphoma (FL) and is usually due to mitotic recombination. SNP array analysis was performed with the use of the Affymetrix 10K 2.0 Gene-chip array on DNA from 185 diagnostic FL patients to assess the prognostic relevance of aUPD. Genetic abnormalities were detected in 118 (65%) of 182 patients. Number of abnormalities was predictive of outcome; more than 3 abnormalities was associated with inferior overall survival (OS; \( P < .03 \)). Sites of recurrent aUPD were detected on 6p (n = 25), 16p (n = 22), 12q (n = 17), 1p36 (n = 14), 10q (n = 8), and 6q (n = 8). On multivariate analysis aUPD on 1p36 correlated with shorter OS (\( P = .05 \)). aUPD on 16p was predictive of transformation (\( P = .03 \)) and correlated with poorer progression-free survival (\( P = .02 \)). aUPD is frequent at diagnosis of FL and affects probability of disease transformation and clinical outcome.

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Introduction

Follicular lymphoma (FL) is the most common indolent lymphoma and has marked heterogeneity in outcome.1-3 With frequent transformation to aggressive lymphoma affecting dramatically on overall survival (OS),4,5 the genomic hallmark of FL is t(14;18)(q32;q21) with many secondary abnormalities described,6,7 although few have confirmed prognostic value.6,8,9 The target genes remain largely unidentified.

Single nucleotide polymorphism (SNP) arrays allow detection of copy-neutral loss of heterozygosity (LOH) undetectable by previous methods, termed acquired uniparental disomy (aUPD). We and others have described aUPD in several hematologic malignancies,10-11 including FL.12,13 It is due to mitotic recombination or nondisjunction and may render a cell homozygous for a preexisting abnormality leading to clonal selection. Both FL series profiled to date have shown frequent, nonrandom regions of aUPD; however, its prognostic implication has not been addressed. We aimed to clarify the incidence of aUPD in 185 diagnostic FL patients and correlate this with clinical outcome.

Methods

Material and Methods

Patient Information

Tumor-extracted DNA from 185 untreated patients with FL presenting between 1974 and 2001 was obtained through the Lymphoma/Leukemia Molecular Profiling Project (LLMPP). Clinical data were available in 169 (93%) of 182 cases (Table S1, available on the Blood website; see the Supplemental Materials link at the top of the online article), transformation data were available for 141 cases, and 39 patients transformed to aggressive lymphoma. Research ethics committee approval was obtained from the London School of Medicine before initiation of the study.

10K GeneChip assay

SNP array genotyping was performed with the use of the 10K 2.0 GeneChip (Affymetrix, Santa Clara, CA) as previously described.14 Signal intensity data were analyzed by GeneChip DNA analysis software, and GTTYPE was used for calling genotypes. GOLF, an in-house software was used for copy number (CN) and UPD estimation (http://bioinformatics.cancer-researchuk.org/~cazier01/). In the absence of germline controls the definition of aUPD was based on runs of consecutive homozygous markers defined as maximum 2 heterozygous calls in 50 consecutive SNPs. This was described by Gupta et al,15 and briefly this stringent criterion is based on 10K 2.0 data available from 90 independent germline samples from the ColoRectal Tumour Gene Identification (CORGI) study consortium giving a false-positive rate of 3.3%.16 CN was determined based on the log2 ratio of signal intensity from the lymphoma sample versus the pooled signal intensity of 10 unrelated control DNA samples. A ratio below 0.75 and above 1.25 on at least 3 consecutive SNPs was defined as a loss and a gain, respectively. The data were also analyzed with the use of Partek Genomics Suite (Partek, St Louis, MO). Sex chromosomes were excluded from analysis. SNP and gene annotations used National Center for Biotechnology Information (NCBI) genome build 35.


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Mutation analysis

Mutation screening was performed for the coding region of \( ID4, BRD2, HLA\ DQB1, SOCS1, CDK4, DYRK2, PTEN, \) and specific exons of \( NFkB2 \) (Table S2; protocols available on request).

Statistical analysis

The Fisher exact test and the Mann-Whitney \( U \) test were used to determine the association with clinical characteristics. OS was defined as the time from diagnosis to death or, for patients remaining alive, the time from diagnosis to last contact. Progression-free survival (PFS) was defined as the time from diagnosis to first clinical progression, transformation, or death from any cause or, for patients remaining alive and disease-free, the time from diagnosis to last contact. Transformation to aggressive lymphoma was defined histologically or by clinical criteria. Kaplan-Meier survival estimates were obtained, and the log-rank test was used to compare differences between the groups with UPD versus those without. Multivariate Cox regression was used to determine whether regions of recurrent abnormality remained independently predictive of PFS and OS after adjusting for the International Prognostic Index (IPI). Statistical significance was set at \( P \) values less than .05.

Results and discussion

This study of 185 diagnostic FL cases addresses the clinical effect of aUPD, using the well-characterized LLMPP cases.\(^\text{18}\) It confirms earlier reports that aUPD is frequent at diagnosis of FL, occurs nonrandomly at recurring chromosomal locations,\(^\text{12,13}\) and now shows that aUPD is clinically important. SNP genotype call rates
of 22 cases transformed to aggressive lymphoma (aUPD16p occurred in 22 (12%) of 182 cases (Table S4) and in 10 aUPD1p abnormalities, (B) progression-free survival by aUPD16p, and (C) overall survival by aUPD16p. Progression-free survival by number of abnormalities, (B) progression-free survival by aUPD1p, and (C) overall survival by aUPD1p were greater than 90% for 182 of 185 cases analyzed. Abnormalities, including CN and copy-neutral changes, were detected in 118 (65%) of 182 cases (Figure 1). The number of abnormalities ranged from 0 to 9 (median, 1) with median LOH size of 48 Mb (range, 3.4-241 Mb). The 20 cases with more than 3 abnormalities ranged from 0 to 9 (median, 1) with median LOH size of 48 Mb.

Correspondence: Derville O’Shea, Centre for Medical Oncology, Barts and The London School of Medicine, Charterhouse Square, London EC1M6BQ, United Kingdom; e-mail: derville.oshea@cancer.org.uk.

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Authorship
Contribution: D.O. designed the study, performed research, analyzed data, and wrote the paper; C.O., Y.Y., and D.W. performed research; M.G., R.W., J.G., B.D.Y., and J.B-C. analyzed data; A.R., G.O., L.M.R., H.H., N.A.J., E.C., W.C.C., and R.D.G. collected data; and L.M.S., T.A.L., and J.F. designed the study and wrote the paper.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Derville O’Shea, Centre for Medical Oncology, Barts and The London School of Medicine, Charterhouse Square, London EC1M6BQ, United Kingdom; e-mail: derville.oshea@cancer.org.uk.
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