A20, a negative regulator of NF-κB, has been implicated as a tumor suppressor gene in multiple types of B-cell lymphoma. AIDs-related lymphomas (ARLs) are high-grade B-cell lymphomas that are frequently associated with EBV infection. We examined a panel of ARLs for A20 alterations. FISH showed A20 deletion in 6 of 33 cases (18%). A20 mutations were found in 3 of 19 cases (16%), including 2 cases with deletions of the complementary allele. Immunohistochemistry showed the absence of A20 protein in 7 of 55 samples (13%). In contrast to reports in Hodgkin lymphoma in which EBV infection and A20 alteration are mutually exclusive, A20 inactivation was observed in both EBV⁺ and EBV⁻ cases. The EBV latent membrane protein 1, which activates NF-κB, was not expressed in 12 of 13 cases with A20 loss. In ARLs loss of A20 may be an alternative mechanism of NF-κB activation in the absence of latent membrane protein 1 expression. (Blood. 2011;117(18):4852-4854)

Methods

Patient samples

Sixty-eight formalin-fixed paraffin-embedded samples of ARL were collected from New York Presbyterian Hospital–Weill Cornell, the University of Siena, and the AIDS Malignancy Consortium. Cases were included if > 80% tumor cells were present and the diagnosis of B-cell lymphoma was confirmed. All samples were obtained with the approval of the institutional review boards at both institutions.

Tumor characterization

Immunohistochemistry was performed on tissue microarray to evaluate for the presence of CD10, CD20, CD3, PAX 5, Bcl-6, Bcl-2, BLIMP-1, FOXP-1, CD138, and MUM-1. EBV and Kaposi sarcoma herpes virus status were determined by in situ hybridization for EBV-encoded RNA (EBER) and immunohistochemistry for LMP-1, EBV nuclear antigen 2, and latency-associated nuclear antigen. Tissue diagnosis was made with the use of criteria from the World Health Organization. In cases of DLBCL, germinal center B-cell (GCB) versus non-GCB subtype was determined by the Hans algorithm.

Fluorescence in situ hybridization

FISH was performed with the use of the spectrum green-labeled A20 probe (provided by Dr Vandavalli, Columbia University) and the spectrum orange-labeled centromeric probe for chromosome 6 (Vysis/Abbott Molecular), following the standard protocols for paraffin FISH assay.

A20 mutation analysis

Direct sequencing was performed on the coding region and splice sites of A20. Genomic DNA was extracted from paraffin-embedded tissue (QIAGEN). DNA was amplified by PCR with the use of the primers and


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A20 ALTERATION IN AIDS-RELATED LYMPHOMA

Immunohistochemistry

Immunohistochemical staining of A20 was performed with the use of the Bond Max Autostainer (Leica Microsystems). Formalin-fixed paraffin-embedded tissue sections were baked and deparaffinized, followed by antigen retrieval with the use of the Bond Epitope Retrieval Solution 1 (ER1) at 99°C to 100°C for 30 minutes (Leica Microsystems). Sections were then subjected to sequential incubations with endogenous peroxidase block, primary antibody (Clone EPR2663, 1:50; Epitomics), postprimary antibody, polymer, diaminobenzidine, and hematoxylin for 5, 25, 15, 25, and 5 minutes, respectively (Bond Polymer Refine Detection Kit; Leica Microsystems). Finally, stained sections were dehydrated and mounted in Cytoseal XYL (Richard-Allan Scientific). Tumors with > 20% positivity were scored as positive.

Results and discussion

Fifty-six of 68 samples had sufficient tumor and interpretable results. The sample set included DLBCL, GCB (37%) and non-GCB (25%) types; Burkitt lymphoma (14%); plasmablastic lymphoma (9%); B-cell lymphoma, unclassifiable (13%); and polymorphic lymphoproliferative disorder (2%; Figure 1A). EBV was present in 37% of cases. Of 19 EBV/EBER+ cases, 4 were positive for LMP-1 with expression in > 90% of tumor cells. Two additional cases were heterogeneous with intermediate LMP-1 positivity consisting of LMP-1 expression in < 10% of tumor cells. The remaining cases were negative for LMP-1 (Figure 1B).

Genetic alterations in A20 were evaluated with the use of FISH and direct sequencing (Figure 2A-B). Monoallelic deletion of A20 was detected by FISH in 5 of 33 cases (15%). Biallelic deletion was detected in one case. Direct sequencing was performed in 19 samples with sufficient DNA. Point mutations were present in 3 of 19 cases (16%; supplemental Table 2). Nonsense mutations coding for a premature stop codon in exon 2 were seen in 2 cases. The third case had a missense mutation in exon 7 resulting in an amino acid change. Two of the 3 cases with A20 mutation also
had monoallelic deletion in the complementary allele, indicating biallelic alteration of the A20 gene.

Immunohistochemistry for A20 was performed and is described for the first time in this report (Figure 2C). Absence of A20 was shown in 7 of 55 cases (13%). In all cases negative for A20, the infiltrating normal cells stained positive. Included among the cases negative for A20 is the case with biallelic A20 deletion determined by FISH. Cases with A20 mutation or monoallelic deletion or both frequently retained reactivity toward A20. Immunohistochemistry may therefore be useful in identifying cases with complete loss of A20 but not helpful in cases in which A20 is present but altered.

In total 13 of 56 cases (23%) showed evidence of A20 inactivation by FISH, sequencing, immunohistochemistry, or a combination (supplemental Table 3). A20 inactivation was seen in most histologic subtypes, including Burkitt lymphoma (n = 3), DLBCL, GCB (n = 3), and non-GCB (n = 2) subtypes, and B-cell lymphoma, unclassifiable (n = 2). Three of 5 plasmablastic lymphomas (60%) had evidence of A20 loss (Figure 2D), but given the small sample size further investigation is necessary to determine whether A20 inactivation is more common in this lymphoma subtype.

A20 inactivation was seen in both EBV+ and EBV− cases. Interestingly, the EBV viral protein LMP-1, which activates NF-κB, was not expressed in 12 of 13 cases with A20 alteration (Figure 2E). One case with A20 inactivation had evidence of LMP-1 expression however in only 10% of tumor cells (Figure 1B). This is the first report to show A20 inactivation in EBV-associated lymphoma. This contrasts with previous studies in HL in which A20 inactivation and EBV infection were almost mutually exclusive.11 The EBV gene expression pattern differs in HL and ARL. In HL Reed Sternberg cells, EBV uniformly expresses LMP-1; however, in ARL viral gene expression is more heterogeneous and LMP-1 is frequently absent. Our data indicate that A20 may represent a tumor suppressor gene in a significant subset of ARLs and that A20 inactivation can be present in both EBV+ and EBV− cases. In EBV-related lymphoma the inactivation of A20 may be an alternative mechanism of NF-κB up-regulation in lymphoma cells in which LMP-1 is not expressed.

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Authorship

Contribution: L.G. performed sequence analysis, collected and analyzed data, and wrote the paper; S.M. and S.G. performed FISH analysis; G.B. performed sequence analysis and analyzed data; A.C., S.B., G.A., and L.L. collected and characterized samples; Y.F.L. performed immunohistochemistry for A20; W.T. performed the pathology evaluation, including immunohistochemistry; and E.C. designed the research and wrote the paper.

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References

11. Schmitz R, Hansmann ML, Bohle V, et al. A20 inactivation was seen in both EBV+ and EBV− cases. In EBV-related lymphoma the inactivation of A20 may be an alternative mechanism of NF-κB up-regulation in lymphoma cells in which LMP-1 is not expressed.

A20 (TNFAIP3) genetic alterations in EBV-associated AIDS-related lymphoma

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