Analysis of mutational signatures in exomes from B-cell lymphoma cell lines suggest APOBEC3 family members to be involved in the pathogenesis of primary effusion lymphoma

Primary effusion lymphoma (PEL) is a rare large B-cell neoplasm particularly affecting immunodeficient hosts with an increased incidence in young or middle-aged males infected with the HIV.[1] The clinical outcome of patients with PEL is unfavorable with a median survival of <6 months.[1] PEL has been closely associated with human herpes virus 8 (HHV8, previously called Kaposi sarcoma herpesvirus) infection.[1] In some cases a coinfection of HHV8 with the Epstein–Barr Virus (EBV) has been described.[1] HHV8 encodes various genes homologous to cellular genes that have proliferative and anti-apoptotic functions.[2] Although HHV8 is supposed to be a major driver of PEL, it alone is not sufficient for a full-blown lymphomagenesis.[2] PEL usually shows complex karyotypes with many chromosomal aberrations.[3] This chromosomal complexity might be driven by the viral infection and lead to genetic alterations cooperating with HHV8 in PEL lymphomagenesis.[4]

The human innate immune defense against exogenous viruses comprises cytidine deaminases of the ApoB mRNA-editing catalytic subunit (APOBEC) family that are able to edit DNA and/or RNA by deaminating cytosins to uracil.[5] The APOBEC family comprises APOBEC1, APOBEC3 (including APOBEC3A-D and APOBEC3F-H), APOBEC4 and activation-induced cytidine deaminase (AID). The APOBEC3 proteins are implicated in natural defense against a variety of viruses associated with lymphomagenesis including human T-cell leukemia virus and EBV.[5] However, mitochondrial and nuclear DNA can also be targeted by APOBEC3 proteins.[6] APOBEC3 activity has been suggested to contribute to tumorigenesis by introducing somatic mutations through $C>U$ deamination.[7] The specificity of APOBEC activity in modifying DNA can be detected as a mutational signature in cancer genomes when analyzing whole-exome and whole-genome sequencing data.[8] Similarly, footprints of other mutational processes can also be traced in cancer genomes. Indeed, in a recent analysis of somatic mutations in 7042 cancers derived from 30 different classes of tumors, we extracted 27 distinct signatures of mutational processes including a signature attributed to the APOBEC family of cytidine deaminases.[8] Like in other tumor entities, this signature was detected in whole-genome sequencing data from mature B-cell lymphomas including Burkitt (BL), follicular and diffuse large B-cell lymphomas (DLBCL).

To further characterize the spectrum of mutational processes in mature aggressive B-cell lymphomas and to identify potential experimental model systems for the analyses of these processes, we analyzed whole-exome sequencing data of 41 mature aggressive B-cell lymphoma cell lines (4 PEL, 12 BL and 25 nonBL including germinal center B-cell like (GCB)- and activated B-cell like (ABC)-DLBCL and primary mediastinal B-cell lymphoma). Exome sequencing was performed within the Cell Lines Project at The Welcome Trust Sanger Institute (http://cancer.sanger.ac.uk/cancergenome/projects/cell_lines/). The mutational data entering the present analysis are available through the COSMIC database (http://cancer.sanger.ac.uk/cancergenome/projects/cell_lines/).

The number of mutations in the analyzed exomes ranged from 67 to 9367 (median: 256) whereby the number of mutations did not differ significantly between the cell line entities (median PEL: 696, range PEL: 283–1126; median BL: 232, range BL: 130–9367; median nonBL: 231, range nonBL: 67–1681). The assignment of mutational signatures of each cell line was based on the 27 recently described distinct consensus mutational signatures.[8]

Comparable to our recently published data on primary biopsies from BL and nonBL,[8] the mutational signatures 1 (associated with aging), 2 (associated with activity of APOBEC family members), 5 (unknown association) and 9 (associated with immunoglobulin gene hypermutation) dominated the landscape of mutation signatures in the lymphoma cell lines (Figure 1a). A notable exception was the BL cell line DG-75, in which 41.3% of mutations were assigned to signature 6 that is known to be associated with mismatch repair deficiency (summarized in Figure 1a in 'Other'). In line with this finding, the microsatellite data of DG-75, available at the COSMIC database, show a high microsatellite instability that might be caused by the lack of the MSH2 DNA mismatch repair protein.[9]

Whereas the distribution of mutational signatures was widely comparable between BL and nonBL cell lines, PEL cell lines constituted a notable exception in that their mutation signature is dominated by signature 2. A median of 80.8% (range: 26.9–93.6%) of all mutations in the PEL cell lines could be assigned to this signature, which is related to overactivity of members of the APOBEC family. In contrast, the BL and nonBL cell lines showed a median contribution of signature 2 of 8.1% and 7.7%, respectively, which was significantly lower than in PEL cell lines ($P=0.027$ and $P=0.026$, respectively). The contribution of signature 2 to the mutational load in primary biopsies of BL and nonBL was 4.3% and 5.0%, respectively, and, hence, similar to that of these cell lines (Figure 1b). Thus, there was no evidence for a change in the signature 2 contribution through tissue culture artifacts. This suggests that the data on the contribution of signature 2 to the mutational load from lymphoma cell lines are representative also for primary lymphoma biopsies, which is important as (likely due to the scarcity of the disease) we neither ourselves had access to primary samples of PEL suitable for genome sequencing nor any genome sequences from primary PEL biopsies have yet been made available in the public domain to analyze the mutational signatures. Overall, the data suggest that the observed high contribution of signature 2 in PEL cell lines might be related to the tumor biology of PEL.

To identify which members of the *APOBEC* gene family might be contributing to the APOBEC signature observed in PEL cell lines, we mined gene expression data of five PEL cell lines (BC-1, BC-2, BC-3, BC-5 and BCBL-1)[10] with regard to APOBEC family members. PEL cell lines showed about 20-fold higher expression of the APOBEC3B mRNA and an ~8-fold higher expression of the APOBEC3C mRNA in comparison with the other APOBEC family members (Figure 2a). In contrast, these data also showed that the PEL cell lines, as compared with other cell lines from B-cell malignancies, did not exhibit a high expression of AID. To verify these findings and validate the data in a larger cohort, we quantified the mRNA levels for APOBEC3B and APOBEC3C in B-cell lymphoma cell lines by quantitative PCR. In line with the above mentioned gene expression data, we could show an increased

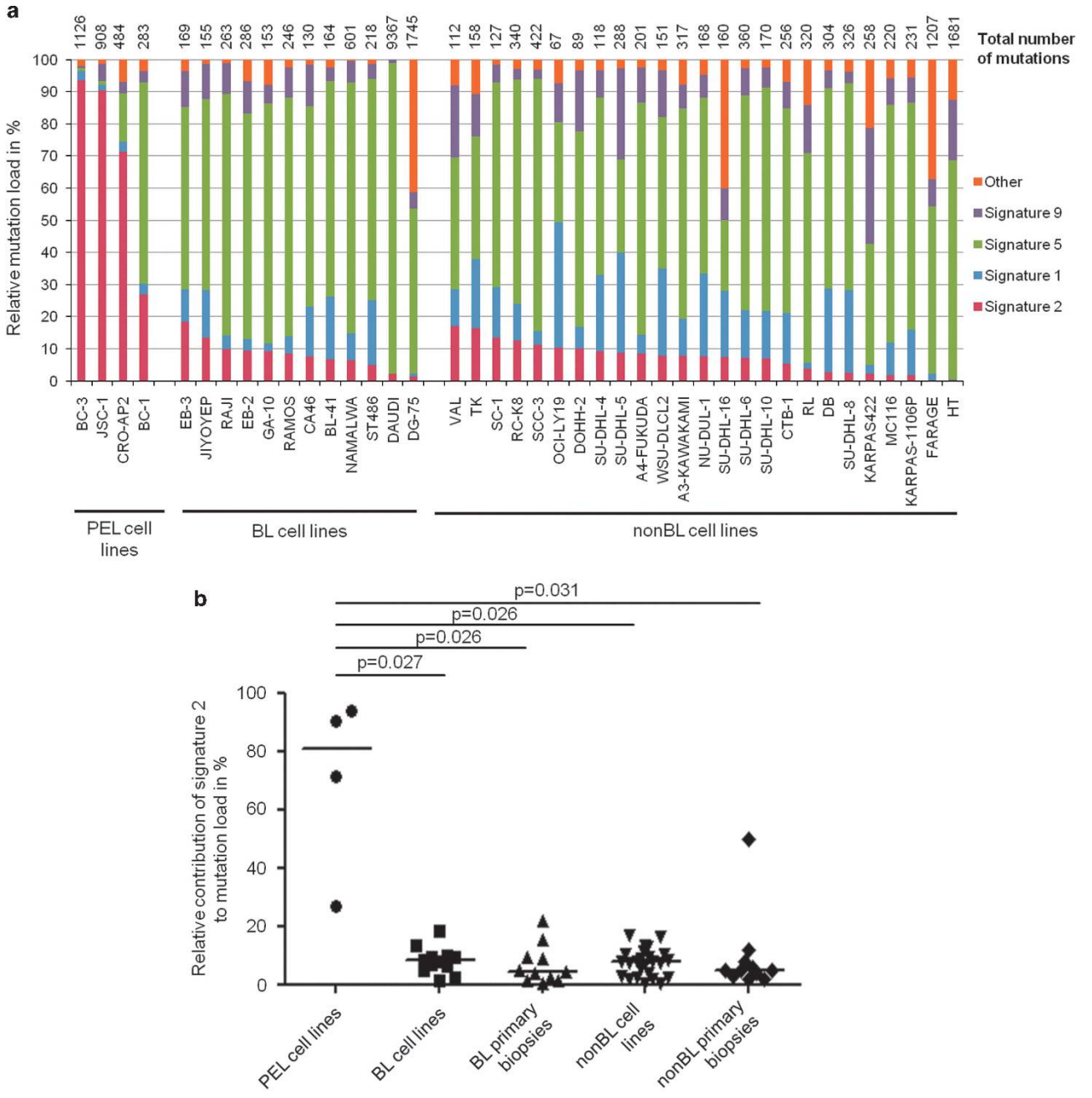

Figure 1. Mutational signatures in mature aggressive B-cell lymphoma cell line. (**a**) Relative contribution of the different mutational signatures to overall mutational load detected by exome sequencing of 4 PEL, 12 BL and 25 nonBL cell lines. The total number of mutations detected by exome sequencing is given at the top per cell line. Cell lines are sorted within each entity with regard to the contribution of signature 2 (red), which is associated with overactivity of the *APOBEC* gene family. Signature 1 (blue) is associated with aging, signature 5 (green) has a so far unknown association and signature 9 (purple) is assigned to immunoglobulin gene hypermutation machinery. 'Other' (orange) summarizes signature 6 associated with DNA mismatch repair and the unassigned signatures 17 and 19. The dominating signature in PEL cell lines is the signature 2, which is distinct from the BL and nonBL cell lines that are dominated by signatures 1 and 5. Exceptions with >20% mutations contributed by 'other' signatures are SU-DHL-16, FARAGE and KARPAS422, which also show high mutation signature related to others (35.6% signature 17, 31.7% signature 15 and 20.9% signature 17, respectively), as well as DG-75 in which 41.3% of the mutations are associated with signature 6. (**b**) Relative contribution of signature 2 (APOBEC activity associated) to the mutational load in the 4 PEL cell lines in comparison to that in 12 BL and 25 nonBL cell lines, as well as to that in primary biopsies from 11 BL and 11 nonBL.[8] The PEL cell lines have a significant higher mutation signature 2 than BL ($P=0.027$) and nonBL cell lines ($P=0.026$), as well as BL ($P=0.026$) and nonBL ($P=0.031$) primary biopsies analyzed by whole-genome sequencing. Bars indicate median.

expression of APOBEC3B (PEL vs BL ns, PEL vs DLBCL; $P=0.0009$) and a significantly increased expression of APOBEC3C in the 9 PEL cell lines in comparison with 12 BL and 10 DLBCL cell lines (PEL vs BL $P=0.003$, PEL vs DLBCL; $P=0.0009$) (Figures 2b and c). In addition, we compared the gene expression profiles from primary biopsies of AIDS-associated PEL with those of primary biopsies of AIDS-associated B-cell lymphomas.[11,12] These data showed that PEL tumors have a significantly higher APOBEC3B expression than

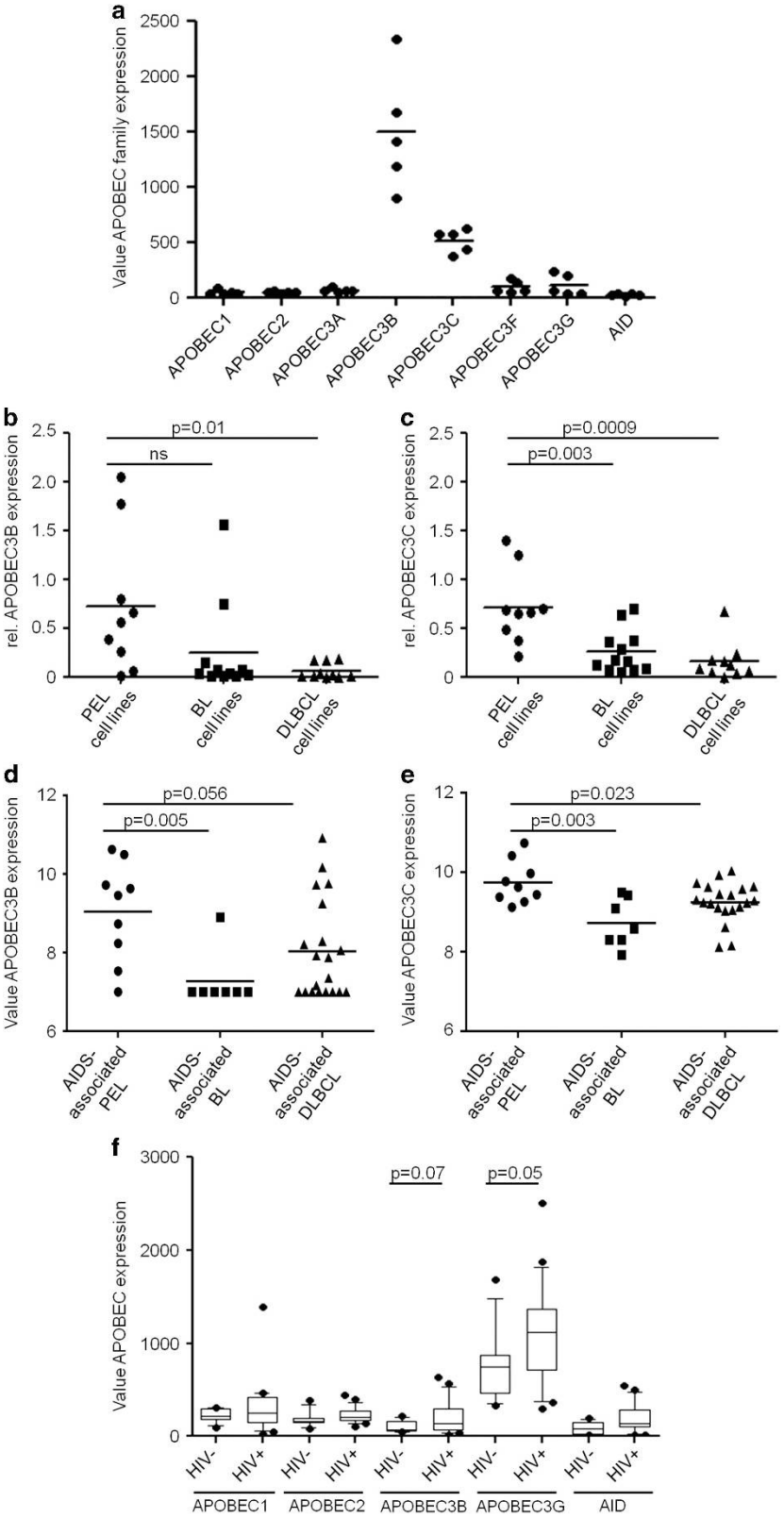

Figure 2. Transcript levels of genes encoding APOBEC proteins in B-cell lymphoma cell lines and primary B-cell lymphomas. (**a**) Gene expression analysis of APOBEC family members using the gene expression profiles of five PEL cell lines (BC-1, BC-2, BC-3, BC-5 and BCBL-1) published by Fan *et al*.[10] Depicted are the normalized gene expression values for every cell line and the mean for every gene. Bars indicate means. Relative expression levels of (**b**) APOBEC3B and (**c**) APOBEC3C in 9 PEL, 12 BL and 10 DLBCL cell lines and their means determined using quantitative PCR. Bars indicate means. Gene expression analysis of (**d**) APOBEC3B and (**e**) APOBEC3C in AIDS-associated B-cell lymphomas using published gene expression profiles.[11,12] Bars indicate means. (**f**) *APOBEC* gene expression analysis in peripheral blood mononuclear cells of 12 HIV-seronegative and 22 HIV-seropositive patients published by Ockenhouse *et al*.[14]

AIDS-associated BL ($P = 0.005$) and DLBCL ($P = 0.056$). Furthermore, the expression of APOBEC3C is significantly higher in AIDS-associated PEL than in AIDS-associated BL ($P = 0.003$) and DLBCL ($P = 0.023$). Interestingly, some of the AIDS-associated BL and DLBCL show also a higher expression of the *APOBEC3B* gene (Figures 2d and e).

To address whether the increased expression of APOBEC3B and APOBEC3C is due to recurrent rearrangement or copy-number variation, we analyzed the available karyotype (http://www.dsmz.de/) and array-CGH data (http://cancer.sanger.ac.uk/cancergenome/projects/cell_lines/) of the PEL cell lines. Although structural alterations affecting 22q were observed in some of the cell lines, there was no evidence for a consistent change (Supplementary Table S1). Moreover, in the exome data of the four PEL cell lines, no mutations within the *APOBEC* family members were detected.

The APOBEC proteins have an important role in anti-viral immunity.[5] The hallmark of PEL tumors is infection with HHV8, although additionally infections with HIV or EBV can be detected. Therefore, it is reasonable to argue that an infection with one of the above-mentioned viruses can trigger the increased expression of the *APOBEC3B* and *APOBEC3C* genes observed in PEL cell lines. To address this issue, we analyzed published gene expression profiles where the influence of a viral infection on gene expression was studied. Although no differences in *APOBEC3* gene expression was found in blood vascular endothelial cells after HHV8 infection,[13] we cannot exclude that the expression of *APOBEC3* genes is indeed influenced by HHV8 infection specifically in the B-cell lineage. The expression of APOBEC3B and APOBEC3G was instead found to be increased in peripheral blood mononuclear cells upon HIV infection ($P = 0.07$ APOBEC3B and $P = 0.05$ APOBEC3G after HIV infection)[14] (Figure 2f). These data are in line with the observations by Li et *al*.[15] that the expression of APOBEC3B, APOBEC3F and APOBEC3G was increased in acutely infected HIV patients. Furthermore, AIDS-related BL or DLBCL tumors showed expression of APOBEC3B and APOBEC3C, which indicates that HIV might influence the expression of the *APOBEC3* genes. It is therefore possible that the persisting high expression of APOBEC3B and even APOBEC3C in the PEL tumors might reflect an altered gene expression program of the cell before it became immortalized.

In summary, our report shows a high burden of mutations with features of APOBEC induction and identifies *APOBEC3B* and *APOBEC3C* as highly expressed genes in PEL. Whether or not the signature identified is due to the almost invariable association of PEL with viral infection, we propose that the APOBEC-induced mutation load might be a driving force in the establishment and development of PEL.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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