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BRIEF METHOD

Establishment and Characterization by Gene Expression Profiling of a New Diffuse Large B-Cell Lymphoma Cell Line, EJ-1, Carrying t(14;18) and t(8;14) Translocations

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This report describes a new diffuse large B-cell lymphoma cell line, EJ-1, established from the malignant ascites of a patient with a diagnosis of de novo transformed lymphoma. This cell line was characterized using standard immunophenotypic and cytogenetic techniques as well as gene expression profiling. The EJ-1 cell line demonstrated a profile of mature B cell and different cytogenetic alterations, including both t(14;18) and t(8;14), as well as del(7)(q31q32). Moreover, the use of gene expression profiling has allowed us to identify genes that link this new established cell line to other similar lymphoma cell lines in a biologically relevant subgroup. EJ-1 cells represent a useful model for studies focusing on the biology of transformed lymphoma.

The cell line was established from a 43-year-old white HIV-1–negative woman who was referred to MD Anderson Cancer Center with a diagnosis of stage IV transformed diffuse large B-cell lymphoma in February 2002. She initially presented with abdominal pain, for which a work-up revealed extensive mesenteric and retroperitoneal adenopathy with large ascites. Cytologic analysis of the ascitic fluid showed a composite population of follicular small-cleaved cells and large cells with Burkitt-like features. Bone marrow biopsy showed 20% involvement by small-cleaved cells. The patient was started on a chemotherapy regimen (HyperCVAD, Methotrexate, and AraC) in combination with Rituximab, to which she responded initially. However, after the fifth cycle of chemotherapy, the disease rapidly progressed and the patient died a month later.

The cells were separated by Ficoll-Paque Plus density gradient centrifugation (Amersham Biosciences, Piscataway, New Jersey) and cultured at a density of 4×10^6/ml in RPMI 1640 medium supplemented with 20% heat-inactivated fetal calf serum (FCS), 2 mM L-glutamine, 0.05 mM 2-β-mercaptoethanol, and antibiotics (Penicillin 100 μg/ml, Streptomycin 100 μg/ml). The cultures were incubated at 37° C in a humidified atmosphere of 5% CO_2, and fresh media was added twice weekly. No additional cytokines or feeder cells were used. After 4 weeks in culture, the cells started to grow in suspension as small clusters and proliferated rapidly with a doubling time of 24 hours.

The morphology of EJ-1 cells was identical to the population of large B lymphoma cells identified at diagnosis. They are large cells, with convoluted nuclear contours, vesicular nuclear chromatin, and multiple nucleoli. The cell surface markers analysis of EJ-1 cells demonstrated a profile of mature B cell positive for CD10, CD19, CD20, CD22, CD45, CD79b, sIgM, and light chain lambda and negative for CD3, CD5, CD11c, CD23, CD25, and CD103. Tumor cells showed a high proliferation index, with 80% of the cells Ki67-positive. The absence of integrated genome of EBV was demonstrated by PCR. The cell line was also found to be negative for HHV8 by Southern blot hybridization.

Chromosomal analysis of EJ-1 cells at 8 weeks of culture revealed a hyperdiploid karyotype: 49, XY, del(5)(q31q35), del(7)(q31q32), t(8;14)(q24.1;q32), del(12)(q23q24,3), and t(14;18)(q32;q21.3) (Fig. 1). Bothe translocations t(14;18) and t(8;14) were confirmed by FISH analysis. The significance of other cytogenetic abnormalities seen in the EJ-1 cell line, such as del(5)(q31q35) and del(12)(q23q24,3), remains to be established in the context of B-cell

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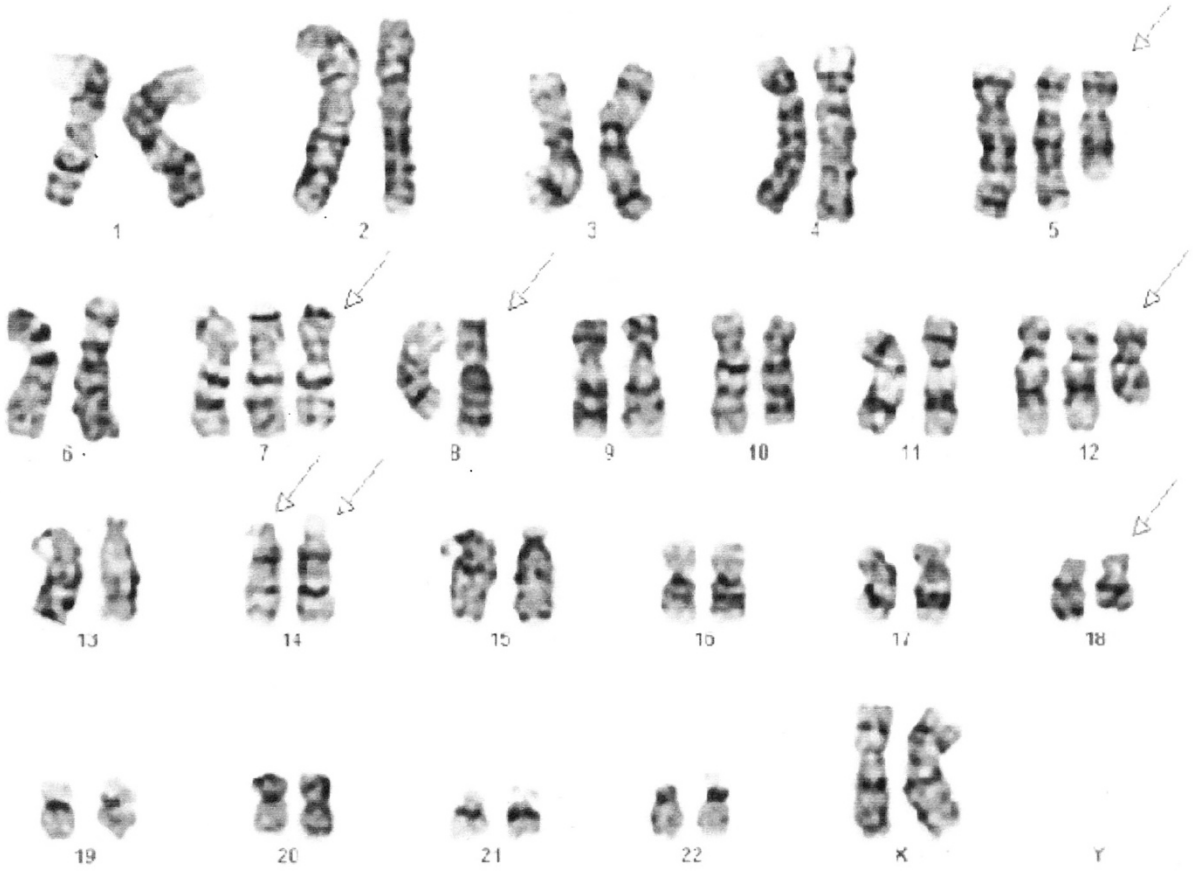

Figure 1.
Karyotype analysis. A representative G-banded karyotype of the EJ-1 cell line. The karyotype presented includes del(5)(q31q35), del(7)(q31q32), t(8;14)(q24.1;q32), del(12)(q23q24.3), and t(14;18)(q32;q21.3). Cytogenetic abnormalities are indicated by the *arrows*.

lymphoma. However, 7q is a commonly deleted region in mature B-cell lymphoid malignancies (Hernandez et al, 1997, Oscier et al, 1996). The deleted region has been mapped and spans about 5 cm and is located between bands 7q31 and 7q32, between loci D7S685 and D7S514 (Gruszka-Westwood et al, 2003). Deletion 7q has been reported to be associated with a subset of small cell lymphomas, including some cases with only few other detectable karyotypic abnormalities, suggesting this region might be involved in the early phase of lymphomagenesis (Dascalescu et al, 1999). However, other series suggested that del 7q is more frequently associated with progression or transformation of small cell lymphoma (Offit et al, 1995).

To further characterize the EJ-1 cell line, we compared its gene expression profile with the profile of 28 cell lines representing 6 different subtypes of lymphoma. cDNA microarray expression profile analysis was performed with Clontech Human Cancer 1.2 cDNA nylon membrane (Clontech, Palo Alto, California). Radiolabeled cDNA probes were synthesized by reverse transcription from 5 μg of total RNA using manufacturer's protocol with P^{32}-dATP (Amersham Biosciences). The images were quantitated using the Image Quantification Software Array Vision from Imaging Research Inc. (Ontario, Canada). The signal intensities and the local background intensities were determined, and the background-subtracted signal intensities were used for analysis using the in-house program for microarray analysis (Baggerly et al, 2001). As a structure detection method, principal component analysis (PCA) was applied to this set of data, with six groups corresponding to six subtypes of lymphoma (Raychaudhuri et al, 2000).

PCA analysis revealed a clear separation of the different subtypes of lymphoma, and the EJ-1 cell line was found to cluster within a subgroup of six cell lines categorized as large B-cell lymphoma (Fig. 2). There were only two misclassified cases within the cluster corresponding to the Burkitt's lymphoma subtype. These two cases corresponded to an MCL cell line (L27; Fig. 2) and a DLCL cell line (L20; Fig. 2).

This analysis also revealed a total of 59 differentially expressed genes at the RNA level by two-fold or more that allowed discrimination among the six different subtypes of lymphoma cell lines. Among those genes, seven were found to be differentially expressed in the subgroup of large B-cell lymphoma and are known to have a role in cell invasion, cell cycle, or signal transduction. They included the Bruton's tyrosine kinase, the NGF-inducible antipro-

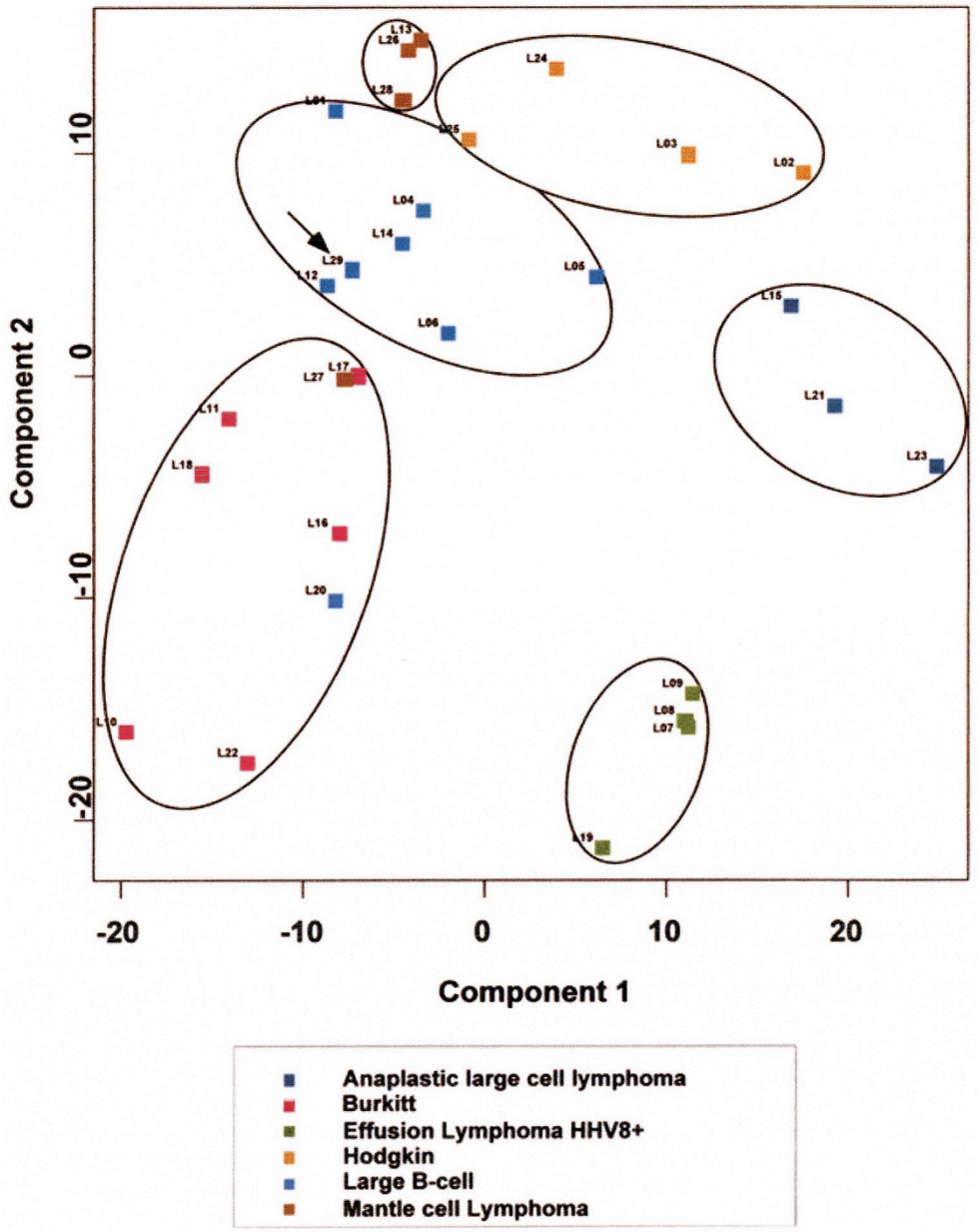

Figure 2.

PCA analysis. The PCA plot shows the grouping of samples that correlates with the histology. The cell lines included six Burkitt's lymphoma cell lines: CA46 (L10), Daudi (L11), Raji (L18), Ramos (L16), ST 486 (L22), and BCHN1 (L17); four Hodgkin lymphoma cell lines: L-428 (LO2), L-1236 (LO3), E (L24), and V (L25); three anaplastic large cell lymphoma cell lines: Karpas 299 (L15), SR 786 (L21), and SU-DHL-1 (L23); four HHV8 effusion lymphoma cell lines: BC-1 (L07), BC-2 (L08), BC-3 (L09), and BC-4 (L19); four mantle cell lymphoma: Mino (L28), M-1 (L13), JEKO (L27), and DB (SP)53 (L26); eight diffuse large cell lymphoma: MS (L01), JMcA (L04), JM-M2 (LO5), CJ (LO6), MC116 (L12), JM1 (L14), SKI-DLCL-1 (L20), and EJ-1 (L29). An *arrow* indicates the position of the EJ-1 cell line.

liferative protein PC3, the tissue-type plasminogen activator precursor (T-plasminogen activator), the metalloproteinase inhibitor 1 precursor (TIMP1), the metalloproteinase 11 (MMP11), the G1/S-specific cyclin D2 (CCND2), and the interferon-inducible protein 9–27. Moreover, the EJ-1 cell line expressed BCL-2 and c-MYC as expected, as well as a number of other genes such as *MCL1*, *TRAP1*, *Caspase 10*, *VGEF*, *HMG1*, *BCGF1*, *PCNA*, and *SOD1*. The scientific interest of EJ-1 cell line lies in its cluster of cytogenetic alterations, including both t(14;18) and t(8;14), as well as abnormalities such as the 7q31 breakpoint. The EJ-1 cell line and its gene expression profile will be provided to outside investigators upon request.

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