Brief report

Deregulated expression of the *Myc* cellular oncogene drives development of mouse "Burkitt-like" lymphomas from naive B cells

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Chromosomal translocations juxtaposing immunoglobulin (Ig) and *MYC* genes are the hallmarks of human Burkitt lymphoma (BL), with deregulated MYC expression being a critical factor in pathogenesis. By inserting an intact mouse *Myc* gene into the mouse genome, proximal to the Ig enhancer Eμ, the effect of a precise mimic of the major t(8;14) translocation of human endemic BL (eBL) could be investigated. Knock-in mice developed IgM-positive B-cell tumors, with most being typical of eBL by histology and immunophenotype, including expression of the germinal center (GC)–associated protein, BCL6. Unlike eBL, however, analysis of Ig V_H sequences revealed no significant level of somatic mutation. Thus, constitutive expression of *Myc* in the knock-in mice is apparently able to induce "Burkitt-like" lymphomas before antigen stimulation and formation of a GC. In contrast, human eBL development occurs in a GC or post-GC site with a likely contribution to pathogenesis from Epstein-Barr virus (EBV) and other epigenetic factors. (Blood. 2005;105:2135-2137)

Introduction

Reciprocal chromosomal translocations that activate the cellular oncogene *MYC* are characteristic of human Burkitt lymphoma (BL).[1] In endemic BL (eBL) the most common translocation is t(8;14)(q24;q32), which recombines *MYC* at 8q24 with the immunoglobulin heavy-chain gene locus, *IGH,* at 14q32.[1,2] Breakpoints usually locate in the joining gene region of the *IGH* locus, J_H, and in the 5′-flank of *MYC*. The resulting exchanges allocate the intact *MYC* to the intronic heavy-chain enhancer, Eμ, in opposite transcriptional orientation. Deregulated *MYC* expression as a result of the chromosomal rearrangements is widely accepted as an initiating step in the development of BL.

The mechanism by which the t(8;14) translocation deregulates the expression of *MYC* and promotes the malignant transformation of B lymphocytes is not well understood.[3] We recently created a mouse model of eBL t(8;14) translocation, designated iMyc$^{E\mu}$, by inserting a single copy of a histidine-tagged mouse *Myc* gene (*Myc*^{His}) into the intervening region between J_H4 and Eμ in opposite transcriptional orientation to *Igh*.[21] The inserted *Myc* included the noncoding first exon with an active P1/P2 natural promoter, a 5′-flank containing the normal transcription-regulatory region, and a short stretch of 3′-untranslated region harboring the *Myc* major polyadenylation site. This is a precise reconstruction of the translocation breakpoint region on human der(14) and mimics the defining translocation in eBL more accurately than other models. The heterozygous iMyc$^{E\mu}$ mice showed high incidence of various forms of B-cell neoplasms, with 35% of mice developing Burkitt-like lymphomas (BLLs) with a typical histologic appearance. It was considered, therefore, that the iMyc$^{E\mu}$ transgene could provide a useful system for studying the promoting role of deregulated *Myc* expression in BLL tumor development. However, we show here that, unlike human eBLs that arise from somatically mutated, postgerminal center (post-GC) B cells,[4] V_H sequences in BLL showed insignificant levels of somatic mutation. This study defines more closely the cellular origin of mouse BLL and highlights the influence of other factors, likely to include Epstein-Barr virus (EBV) and other infections, in the pathogenesis of human eBL.

Study design

Generation and characterization of iMyc$^{E\mu}$ mice

The iMyc$^{E\mu}$ mice showed an increased *Myc* expression in B220^{low}IgM^{-} pro-B/pre-B cells, transitional B220^{low}IgM^{+} B cells, and mature B220^{hi}IgM^{+} bone marrow B cells. Constitutive *Myc* expression led to B-cell neoplasms with an overall incidence of 70% by 21 months of age, of which BLL formed the major group (50%). In contrast to other models,[5] mice had normal B-cell numbers, normal levels of serum immunoglobulin M (IgM)/IgG, and were able to respond to T-cell–dependent antigens.[4]

Sequence analysis of expressed V_H genes

Total RNA extraction, cDNA synthesis, and V_H gene amplification and sequencing were performed as previously described.[6] Sequence analysis was performed using the IgBLAST program (http://www.ncbi.nlm.nih.gov/igblast).

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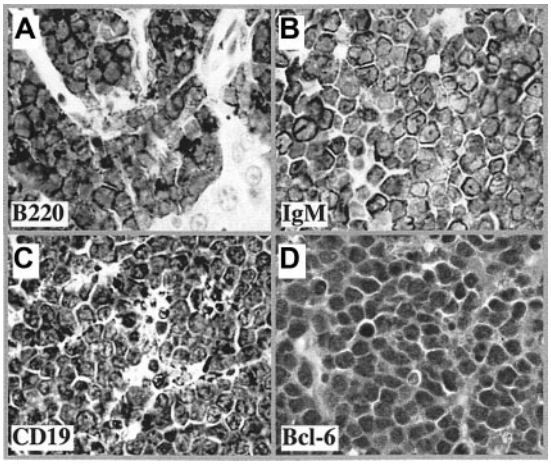

Figure 1. Immunophenotype of mouse BLL. Shown are representative tissue sections of lymphomas after immunostaining with antibody to B220 (A), IgM (B), CD19 (C), and Bcl-6 (D), followed by horseradish peroxidase (HRP)–conjugated secondary antibody (1:200 dilution). The images were captured by a Zeiss Axiophot microscope equipped with a DC330E CCD camera (Dage MTI, Michigan City, IN) and Scion Image software (Scion, Frederick, MD). The original magnification of the objective lens was 40 ×, and its numerical aperture was 0.75. Scion Image software (Scion, Frederick, MD) was used to capture images.

Results and discussion

Cellular features of BLL

The insertion in the iMyc$^{E\mu}$ mice includes the complete set of correctly spaced *Igh* enhancers, designed to create the same complex promoter and enhancer interactions that govern the expression of the translocated *MYC* in human eBL.[7] Positioning within the *Igh* chromatin domain also presumably subjects the gene to the same higher-order regulatory influences, including those imposed by chromatin remodeling and positional effects in the interphase nucleus. Among 32 tumors, only 1 tandem mutation was found in the transgenic *Myc* cDNA.[4]

Tumors expressed Myc^{His} protein as diffuse cytoplasmic and speckled nuclear staining by immunocytochemistry. Presentation was commonly in the mesenteric lymph node, closely followed by Peyer patches, sites reminiscent of human sporadic BL and some cases of eBL.[7] Later stages exhibited generalized splenomegaly and lymphoadenopathy. Histologically, BLL cases were lymphoblastic B-cell lymphomas with a typical "starry sky" appearance. This is due to tingible body macrophages engulfing transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL)–positive apoptotic tumor cells—again similar to human BL.[7]

Tumor cells had a high proliferative index and expressed the B-cell markers B220, IgM, and CD19 (Figure 1A-C), consistent with mature B cells. However, most lacked the cytoplasmic lipid vacuoles characteristic of classic human BL. In parallel with human BL, mouse BLL also expressed the GC-associated protein, BCL6 (Figure 1D).

Analysis of Ig V_H gene sequences

Southern blot analysis revealed clonal B-cell rearrangements in 7 of 7 of the BLL cases (not shown). The Ig V_H genes were sequenced, with results summarized in Table 1. Strikingly, all were essentially unmutated. The V_H sequence of one tumor perfectly matched a germ-line gene of the SM7 V_H family. Five tumor sequences showed 100% identity to rearranged V_H segments in the IgBLAST database. They were also considered as unmutated because it is unlikely that rearranged V_H genes from different cells share exactly the same somatic mutations. The remaining tumor sequence (tumor "ARS") contained one base difference in comparison with its best-matched V_H gene. The status of 2 additional nucleotides in the tumor sequence could not be accurately established because of the ambiguity in the matched V_H.

Cell of origin

These data provide conflicting results regarding the placement of the cell of origin in the normal scheme of B-cell development. The BCL6 protein is required for GC formation in normal B cells and expressed at high levels in that site.[8] This is mirrored in human B-cell malignancies where BCL6 can be detected in GC tumors, particularly diffuse large cell lymphoma, and in eBL.[9]

By analogy, BCL6 protein in BLL of iMyc$^{E\mu}$ mice would point to a GC origin. One possible explanation is that deregulated *Myc* expression may impose GC features on B cells that have never seen a GC. This is analogous to the consequences of *Myc* overexpression in vitro that leads lymphoblastoid cells to adopt BL morphology and phenotype.[10,11] Although *Bcl6* is not known to be a direct target of *Myc,* it is possible that its expression could be up-regulated indirectly by aberrant *Myc* expression. The lack of somatic mutations in V_H genes in the current model is also characteristic of B-cell lymphomas developing in the previous λ–*Myc*-transgenic model[12] (our unpublished observations, June 2004), indicating a similar origin of these "Burkitt lymphomas" from pre-GC cells.

Our study emphasizes the value of V_H-gene data in adding to morphologic and immunophenotypic description of B-cell malignancies.[13] This designation can have significant clinical impact as is evident for chronic lymphocytic leukemia where tumors with unmutated or mutated Ig V genes have a completely different prognosis.[14,15] Somatic mutational analysis of V genes therefore is now a part of the molecular description of B-cell tumors.[13]

It is interesting that the cell of origin of human eBL differs from that in the mouse model. In eBL, it had been assumed that the

Table 1. V_H gene sequence analysis of BLL

Tumor designation	Best-matched V_H genes			D segment	J_H segment
	Family	Accession no.	Homology, %		
86	S107	AF045506	100	DSP2.9	J_H4
97	Q52	U88686	100	n.d.	J_H1
99	J558	X03088	100	DQ52	J_H2
103	J558	X03088	100	DSP2.2	J_H1
180	36-60	X63801	100	DFL16.1	J_H4
193	SM7	AC073563	100	DSP2.9	J_H3
ARS*	J558	J04547	99	DSP2.13	J_H2

n.d. indicates not determined.

*Sequence contains 2 ambiguous bases.

translocation and consequent up-regulation of *MYC* occurred during VDJ recombination,[16] but it is also possible that it occurs in the GC via somatic mutation events.[17] Most human eBL and sporadic BL (sBL) have somatically mutated V genes, often with ongoing mutational activity characteristic of a GC location.[4] Somatic mutation may also contribute to pathogenesis by introducing sites in the V-gene sequences for addition of oligosaccharides.[18] These are positively selected in eBL and could be important for maintenance and growth of tumor in the GC site.[18,19] Intense antigen stimulation associated with malaria and other infections would provide continuous drive on the immune system, a factor difficult to mimic in mice but which could influence tumorigenesis. A further major factor likely to influence development of eBL is EBV, associated with virtually all cases. EBV persists in normal B memory cells with a pattern of viral gene expression mimicking that of BL.[20] In B cells already bearing a translocation, the proliferative or antiapoptotic pressure from persisting EBV could render them vulnerable to tumor development at the memory stage. This multifactorial route to tumor development is difficult to model in mice, and correlations should be made cautiously.

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