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PRAD1, a candidate *BCL1* oncogene: Mapping and expression in centrocytic lymphoma

(*D11S287E*/chromosome 11q13/cyclin/parathyroid/breast cancer)

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ABSTRACT **Rearrangement of the *BCL1* (B-cell lymphoma 1) region on chromosome 11q13 appears to be highly characteristic of centrocytic lymphoma and also is found infrequently in other B-cell neoplasms. Rearrangement is thought to deregulate a nearby protooncogene, but transcribed sequences in the immediate vicinity of *BCL1* breakpoints had not been identified. *PRAD1*, previously designated *D11S287E*, was identified on 11q13 as a chromosomal breakpoint region rearranged with the parathyroid hormone gene in a subset of parathyroid adenomas; this highly conserved putative oncogene, which encodes a novel cyclin, has been linked to *BCL1* and implicated also in subsets of breast and squamous cell neoplasms with 11q13 amplification. We report pulsed-field gel electrophoresis data showing *BCL1* and *PRAD1* to be no more than 130 kilobases apart. PRAD1 mRNA is abundantly expressed in seven of seven centrocytic lymphomas (Kiel classification), in contrast to 13 closely related but noncentrocytic lymphomas. Three of the seven centrocytic lymphomas had detectable *BCL1* DNA rearrangement. Also, two unusual cases of CLL with *BCL1* rearrangement overexpressed *PRAD1*, in contrast to five CLL controls. Thus, *PRAD1* is an excellent candidate "*BCL1* oncogene." Its overexpression may be a key consequence of rearrangement of the *BCL1* vicinity in B-cell neoplasms and a unifying pathogenetic feature in centrocytic lymphoma.**

Several B-cell malignancies have been shown to have characteristic chromosomal translocations that result in juxtaposition of DNA containing known or potential cellular oncogenes with DNA containing genes for immunoglobulin chains. These rearrangements are thought to deregulate the normal expression of the protooncogene by placing it under the influence of sequences that regulate the active, tissue-specific transcription of immunoglobulin DNA and thus contribute to the neoplastic phenotype.

For two of the common B-cell lymphoma translocations, t(8;14) and t(14;18), the DNA translocated into the immunoglobulin heavy chain gene region of chromosome 14 contains *MYC* and *BCL2* (B-cell lymphoma 2 gene), respectively (1–4). Another rearrangement associated with B-cell neoplasia is t(11;14) (5–10). In the translocations examined, chromosome 11q13 was rearranged with the immunoglobulin heavy chain locus on chromosome 14q32 (11); the DNA near the breakpoint on 11q13 was originally named the B-cell leukemia/lymphoma 1 (*BCL1*) locus (12, 13). It has been assumed that rearrangement of *BCL1* deregulates a protooncogene that lies nearby; however, until very recently no transcribed sequence in the vicinity of *BCL1* had been identified. Thus, the identity of the putative *BCL1* region oncogene and the consequences of *BCL1* rearrangement remain uncertain.

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Clonal *BCL1* rearrangement is found only occasionally in a variety of B-cell tumors, notably B-cell CLL, plasma cell myeloma, and diffuse large-cell lymphomas (DLCL) (14–19). In contrast, however, *BCL1* rearrangement has recently been observed in 30–50% of centrocytic lymphomas (20, 21) and intermediate lymphocytic lymphomas or lymphocytic lymphomas of intermediate differentiation (IDL) (19, 22). These lymphoma subtypes, from different lymphoma classifications, frequently overlap (23–25). These results, indicating that *BCL1* rearrangement is characteristic of a particular subtype of low–intermediate grade B-cell lymphoma, suggest that *BCL1* rearrangement may activate a gene whose abnormal expression is pathogenetically related to that subtype.

PRAD1 (for parathyroid adenomatosis) is a highly conserved putative oncogene on chromosome 11q13 that encodes a cyclin and has been implicated in parathyroid tumorigenesis (26–28). A subset of parathyroid adenomas bear clonal rearrangements of the parathyroid hormone (PTH) gene, and *PRAD1* (then identified as *D11S287*) was originally isolated as the breakpoint-adjacent DNA in such a tumor (26). *PRAD1*, or *D11S287E*, is dramatically overexpressed in these adenomas, probably driven by active, tissue-specific regulatory elements of the misplaced *PTH* gene. *PRAD1* may also be important in the pathogenesis of a subset of breast and squamous cell cancers that contain DNA amplification in 11q13 (29) and has been physically linked to *BCL1* (29, 30).

We considered the possibility that *PRAD1* might be the pathogenetically relevant *BCL1* region oncogene in lymphoid neoplasia and report that (*i*) the *BCL1* breakpoint locus and *PRAD1* are tightly linked, no more than 130 kilobases (kb) apart, and (*ii*) two unusual CLLs with *BCL1* rearrangement overexpressed *PRAD1*; seven of seven centrocytic lymphomas equally abundantly expressed *PRAD1*, in contrast to related lymphomas not associated with translocation t(11;14) or *BCL1* rearrangement. Therefore, *PRAD1* is an excellent candidate for being the *BCL1* region gene whose dysregulation contributes to lymphoid neoplasia.

MATERIALS AND METHODS

Pulsed-Field Gel Electrophoresis (PFGE). DNA samples for PFGE were prepared from peripheral blood leukocytes and digested with *Not* I, *Mlu* I, *Sal* I, *Ksp* I, *Nru* I, *Nae* I, *Xho* I, and *Sfi* I by standard techniques (31). Three different pulsed-field gels were prepared to resolve fragments in the range of 4–400 kb, 240 kb to 1 Mb, and 500 kb to 2 Mb

Abbreviations: CLL, chronic lymphocytic leukemia; DLCL, diffuse large-cell lymphomas; PFGE, pulsed-field gel electrophoresis; DSCL, diffuse small cleaved-cell lymphoma; MTC, major translocation cluster; BCL1, B-cell lymphoma 1; PRAD1, parathyroid adenomatosis; PTH, parathyroid hormone; IDL, lymphocytic lymphoma of intermediate differentiation.

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according to manufacturer's instructions (Bio-Rad). Fragment sizes were estimated by interpolation based on the migration of *Saccharomyces cereviseae*, λ phage concatamer, and *Hin*dIII-digested λ phage size standards.

Tissue Samples. *Selection.* We sought samples of lymphoma tissue in which *BCL1* had a relatively high likelihood of having detectable rearrangements and of lymphoma tissues that would serve as controls, in which *BCL1* had a low or negligible chance of being rearranged. Consecutive cases of centrocytic lymphoma, as defined by morphologic and immunophenotypic criteria in the Kiel classification (23), for which adequate frozen tissue remained for DNA and RNA analysis were selected for the study. In the modified Rappaport classification (24, 25), six of these cases met the histologic criteria for intermediate lymphocytic lymphoma and one case would have been classified as poorly differentiated lymphocytic lymphoma. All cases were classified as diffuse, small, cleaved-cell lymphoma (DSCL) in the "Working Formulation," since, as originally described, this category is considered to contain by definition all cases of centrocytic lymphoma of the small-cell type (32). All cases expressed the pan-B-cell antigen CD20 and monotypic immunoglobulin. Control tissues represented various types of low- and intermediate-grade B-cell tumors with morphologic features similar to centrocytic lymphoma. These included: five cases of small lymphocytic lymphoma (all were lymph nodes or spleen tissue from patients with CLL); four of these five cases were $CD5^+$; two cases of DSCL that did not fit the Kiel criteria defining centrocytic lymphoma; both could be classified as IDL in the modified Rappaport criteria. One of these cases (4265) had an admixture of so-called centrocyte-like cells or monocytoid B cells, consistent with the recently described entity called low-grade B-cell lymphoma of mucosa-associated lymphoid tissue (33) or monocytoid B-cell lymphoma (34, 35), and the other (3270) was unclassifiable in the Kiel classification, having a mixture of small lymphocytes and small cleaved cells with extensive noncaseating granulomatous reaction. Other control tissues included: one case of follicular small cleaved-cell lymphoma, two follicular lymphoma cell lines (FL-1412 and FL-18) (36), two pre-B-cell lines [697 (37) and REH (38)], and a case of B-cell DLCL involving the spleen.

Preparation. At the time of surgical excision, tissue blocks were frozen in liquid nitrogen and stored at −70°C as part of the standard protocol for pathological evaluation of lymphoma.

Immunohistologic studies. Acetone-fixed cryostat sections from each case were stained with an avidin–biotin–horseradish peroxidase method (Vectastain, Vector Laboratories) as previously described, with aminoethylcarbazole as a substrate and hematoxylin as the counterstain. All cases were stained with antibodies to immunoglobulin heavy and light chains (DAKO, Carpinteria, CA), anti-CD20 (B1), anti-CD10 (J5) (Coulter), anti-CD5 (Leu-1), and anti-CD3 (Leu-4) (Becton Dickinson). See Table 1 for results of many of the immunophenotyping studies.

Northern Analysis. Total RNA was isolated by the guanidinium isothiocyanate/cesium chloride method, electrophoresed on a denaturing formaldehyde/agarose gel, and transferred to nitrocellulose or nylon filters (31). Hybridization conditions were similar to those used for Southern filters. Blots were washed in 0.015 M NaCl/0.0015 M sodium citrate, pH 7, at 60°C. The 28S ribosomal RNA oligonucleotide (5′-CAA GAT CTG CAC CTG CGG CGG CCT CCA CCC-3′) (39) was end-labeled with [^{32}P]ATP (31) and used to control the amount of high molecular weight RNA present in each lane. Hybridization and washing were at 42°C in low salt concentrations, and band intensities on autoradiograms were measured by densitometry (31).

Southern Hybridization. Extraction of high molecular weight DNA, restriction enzyme digestion, and Southern blotting were performed as described (40).

Probes. BCL1 probe b is a 2.3-kb *Sac* I–*Sac* I genomic DNA fragment described previously (13). The PRAD1 probes were a partial PRAD1 cDNA containing the full coding region (insert of λ phage P1-4 in ref. 28); and the insert of plasmid pDY-12, a 500-base-pair (bp) genomic fragment (27), here called probe D, located ≈15 kb upstream of the first exon of *PRAD1*. These probes were random-primed and labeled with [^{32}P]dATP (41).

RESULTS

PFGE analysis of the *BCL1* and *PRAD1* regions (Fig. 1) showed that both PRAD1 probe D and BCL1 probe b hybridized to the same (comigrating) genomic DNA fragments in restriction digests with *Not* I (370 kb), *Mlu* I (700, 620, and 185 kb), *Sal* I (620 and 580 kb), *Ksp* I (620 and 150 kb), *Nru* I (1200 kb), and *Nae* I (130 kb). Several enzymes produced fragments that did not comigrate: an 80-kb *Sal* I fragment detected by PRAD1 was not seen with BCL1; *Sfi* I generated a 15-kb fragment detected with PRAD1 and a 55-kb fragment detected with BCL1; *Xho* I gave a series of fragments in the 50- to 185-kb range detected with both probes, but a 10-kb fragment detected with PRAD1 only. Thus, *PRAD1* normally lies no more than 130 kb from the *BCL1* breakpoint locus and could be considerably closer. Previous data had indicated that *PRAD1* was telomeric to *BCL1* on chromosome 11q13 (29), as would be expected for the area's relevant oncogene (12, 13). Furthermore, BCL1 probe b appears to be located upstream or 5′ relative to *PRAD1*'s

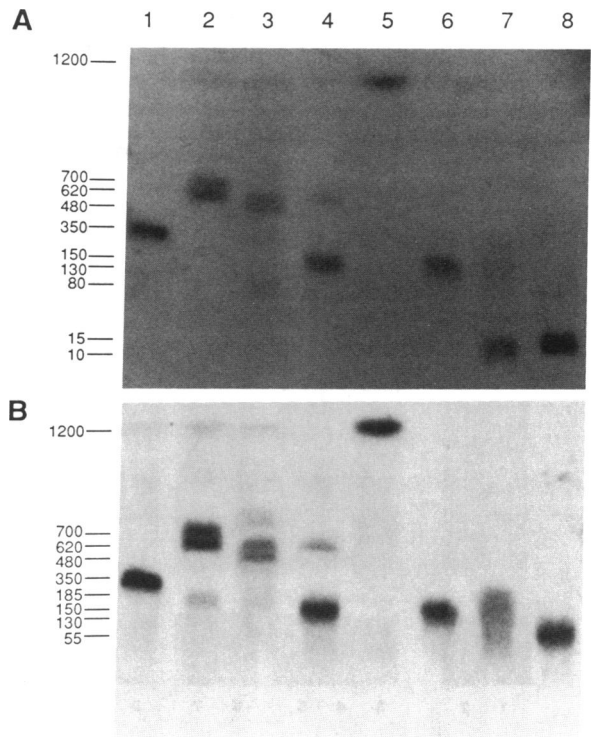

FIG. 1. PFGE analysis of *PRAD1* (*A*) and *BCL1* (*B*). Lanes: 1, *Not* I digest; 2, *Mlu* I digest; 3, *Sal* I digest; 4, *Ksp* I digest; 5, *Nru* I digest; 6, *Nae* I digest; 7, *Xho* I digest; 8, *Sfi* I digest. *A* is probed with PRAD1 probe D, and *B*, with BCL1 probe b. Fragment sizes in kb were estimated from three separate pulsed-field gels, which in combination gave good resolution of fragments in the 4-kb to 2-Mb range (see text). Discrepancies with fragment sizes reported previously in abstract form (30) reflect the improved accuracy of the current estimates based on additional PFGE data.

transcriptional orientation, based on the presence of fewer bands comigrating with *BCL1* when a far 3′ PRAD1 cDNA probe was used (not shown) instead of the 5′ PRAD1 probe D.

We hypothesized that *PRAD1*, if it is the *BCL1* area oncogene, would be transcriptionally activated in all B-cell tumors with detectable *BCL1* rearrangement and also in some tumors without detectable *BCL1* rearrangement (because breakpoints may be heterogeneous, and some activating rearrangements will likely escape detection on Southern blots hybridized with the original *BCL1* breakpoint probes). We obtained total RNA from seven centrocytic lymphomas, two CLLs known to contain a *BCL1* rearrangement (CLLs 271 and 1386) (12, 13), and numerous tissues from lymphoma types representing a similar stage of B-cell differentiation but in which *BCL1* rearrangement occurs rarely or not at all (see *Materials and Methods*).

Fig. 2 shows that expression of the normal-size 4.5-kb *PRAD1* transcript in all seven centrocytic lymphomas and in the *BCL1*-rearranged CLLs was dramatically greater than in any other B-cell tissue, including the noncentrocytic diffuse small cleaved-cell lymphomas and other CLLs. There was some heterogeneity in the level of *PRAD1* expression in the centrocytic lymphomas, with a 2-fold variation within the group. However, the centrocytic lymphomas expressed *PRAD1* 16- to 37-fold more than the average comparison tissue and 10- to 23-fold more compared with the noncentrocytic tissue with the highest level of expression. *PRAD1* expression in CLLs 271 and 1386 was of a similar magnitude to that in the centrocytic lymphomas. Two bands of smaller size, which may represent alternatively processed *PRAD1* or *PRAD1*-related mRNAs, may also be seen as minor hybridizing products; the smaller band is especially prominent in CLLs 271 and 1386.

The group of centrocytic lymphomas we studied appeared to be representative of those described in the literature with regard to both their immunophenotype (Table 1) and their high incidence of *BCL1* rearrangement. Three of seven cases (43%)

Table 1. Histologic and immunophenotypic profiles

Cases	sIg	CD5	CD10	*BCL1* rearr.	*PRAD1* overexpr.
Centrocytic lymphomas					
3233	ML	+	−	−	+
4487	ML	+	−	−	+
3093	MK	+	−	−	+
4628	MDK	+	−	−	+
4911	MK	+	−	+	+
3923	MDK	+	−	+	+
4706	ML	−	−	+	+
CLL–BCL1 rearr.					
271	MK	NA	NA	+	+
1386	MK	+	NA	+	+
Noncentrocytic DSCL					
3270	L	−	−	−	−
4265	ML	−	−	−	−
CLL–BCL1 unrearr.					
4290	MDL	+	−	−	−
4690	MDK	+	−	−	−
5511	MK	+	−	−	−
5523	ML	+	−	−	−
4312	MK	−	−	−	−
Follicular lymphoma					
4564	MK	−	−	−	−
1412	GL	NA	NA	NA	−
18	GK	NA	NA	NA	−
Other					
Pre-B-cell					
697	M	−	NA	NA	−
REH	−	NA	+	−	−
DLCL	−	NA	NA	−	−

sIg, surface immunoglobulin; M, IgM; G, IgG; D, IgD; L, λ light chain; K, κ light chain; NA, not available; unrearr., unrearranged; rearr., rearrangement; overexpr., overexpression. Plus indicates that surface markers, rearrangement, or overexpression were detected; minus indicates that they were not detected.

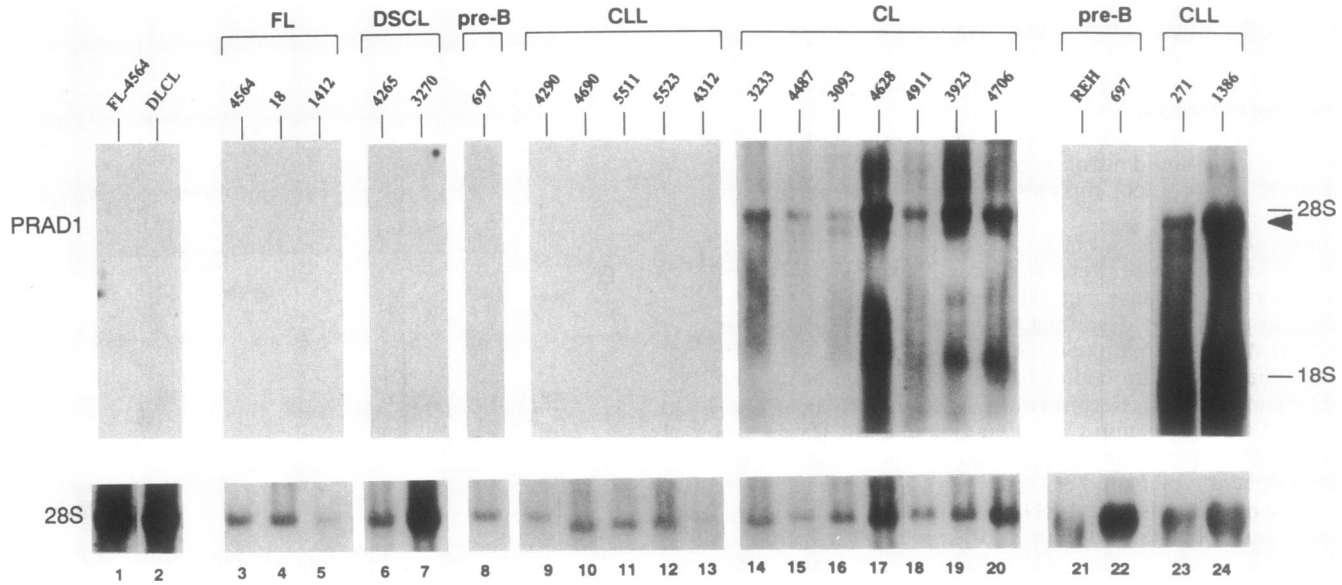

FIG. 2. Expression of PRAD1. Control tissues were loaded in lanes 1–13, 21, and 22 and are labeled above each lane. Lanes 14–20 contain RNA from the seven cases of centrocytic lymphoma (CL), case numbers being above each lane. Lanes 23 and 24 contain RNA from the *BCL1*-rearranged CLLs 271 and 1386, respectively. Lanes 1 and 2 are from one gel, lanes 3–20 are from a second gel, and lanes 21–24 are from a third gel. Duplicates of follicular lymphoma (FL) 4564 (gels 1 and 2) and the pre-B-cell line 697 (gels 2 and 3) were loaded to permit direct comparisons between the three gels. Approximately 7 μg of total RNA were loaded into each lane. The filters were probed first with the PRAD1 probe. Arrowhead indicates the location of the normal 4.5-kb PRAD1 transcript, which is distinct from the 28S marker. (*Upper*) Two-day exposure of lanes 1 and 2 and a 3-day exposure of lanes 3–24, showing bands in lanes 14–20. Longer exposures show faint bands in lanes 2, 7, 12, 21, and 22, representing the single case of DLCL, one noncentrocytic DSCL, one *BCL1*-unrearranged CLL, and both examples of pre-B-cell lymphoma (not shown). (*Lower*) Same filters probed with a 28S rRNA oligonucleotide probe to control for the amount of high molecular weight RNA in each lane. Lanes: 1 and 2, 2-hr exposure; 3–20, 1-hr exposure; 21–24, 2-day exposure.

had DNA rearrangements detectable with BCL1 probe b (Fig. 3). As expected from the reported rarity of such rearrangements in CLL, DLCL, and follicular lymphomas (15, 19, 43), no *BCL1* rearrangements were detectable in any of the noncentrocytic tissues for which DNA was available (10 of 13 specimens). In addition, none of these tissues nor the centrocytic lymphomas contained DNA rearrangements detectable with the PRAD1 cDNA probe (data not shown). No comigration of any of the rearranged *BCL1* bands with the immunoglobulin heavy-chain joining region (J_H) bands was present (not shown), consistent with previous findings of such comigration in only a fraction of centrocytic lymphomas (20, 21) or IDL (19). Thus, the *BCL1* rearrangements in these lymphomas may not be due to a t(11;14) translocation or the chromosome 14 breakpoint may not involve the immunoglobulin J_H region.

DISCUSSION

Rearrangement of *BCL1*, which occurs rarely in a variety of B-cell tumors and occurs frequently only in centrocytic lymphomas, has been postulated to activate a nearby oncogene on chromosome 11q13 although, until recently, no transcribed sequences had been found in its vicinity. *PRAD1* is a putative oncogene on 11q13 recently found to be transcriptionally activated by clonal DNA rearrangement in a subset of parathyroid adenomas (26, 27). Bale *et al.* (30) and Lammie *et al.* (29) have shown that the *BCL1* region and *PRAD1* are within 240 kb of each other. Using electrophoresis conditions that improve resolution in this size range, we have narrowed the distance between the two loci to no more than 130 kb. Even this maximal separation would be close enough to permit a chromosome rearrangement at *BCL1* to deregulate *PRAD1* gene expression (reviewed in refs. 44 and 45). Further, we have demonstrated abundant *PRAD1* expression in two unusual CLLs with translocation t(11;14) and *BCL1* rearrangement and in all centrocytic lymphomas examined, but in no lymphomas of other histologic subtypes selected to include B-cell tumors that morphologically resemble centrocytic lymphoma and are known to infrequently or never contain *BCL1* rearrangement or t(11;14). These data support the hypothesis that transcriptional activation of *PRAD1* may be a basic and consistent molecular finding in centrocytic lymphoma, probably resulting from *BCL1* region rearrangements, not all of which are currently detectable.

In addition, our results provide some justification for the recognition of centrocytic lymphoma as a more specific biological entity than either IDL or DSCL. Three overlapping entities exist in three different lymphoma classifications, termed variously IDL (Rappaport), DSCL (Working Formulation), and centrocytic lymphoma (Kiel). Of nine cases classified in the Working Formulation as DSCL, eight of which could be classified as IDL, only the seven that fulfilled the Kiel classification criteria for centrocytic lymphoma overexpressed *PRAD1*. Considerable controversy exists over the optimal method of subclassifying diffuse low- and intermediate-grade B-cell lymphomas; assessment of *PRAD1* expression and *BCL1/PRAD1* area rearrangement may ultimately provide a biologically meaningful basis to aid in this classification.

Interest in *PRAD1* as a likely oncogene has been fueled by the convergence of several independent lines of evidence. *PRAD1* was discovered initially because it was clonally rearranged adjacent to the transcriptionally active *PTH* gene in a subset of parathyroid adenomas (26, 46). This rearrangement is analogous to the juxtaposition and overexpression of *MYC* or *BCL2* with transcriptionally active immunoglobulin genes in B-cell lymphoid tumors (reviewed in ref. 1). *PRAD1*, normally expressed in parathyroid tissue, is dramatically overexpressed in the tumors with a rearrangement of the *PTH* gene and the *PRAD1* region (27). In addition, *PRAD1* mapped to chromosome 11q13 (26) and was found to be consistently amplified in the 15–20% of breast and squamous cell cancers in which the *BCL1-INT2-HST1* region of 11q13 is amplified (29). *PRAD1* expression was easily detectable in these tumors, in contrast with the generally observed lack of expression of the other oncogenes. Thus, *PRAD1* has been implicated as a candidate cellular oncogene, whose overexpression (secondary to rearrangement or amplification) may contribute to tumorigenesis in several tissues. The abundant expression of the normal-size PRAD1 mRNA, and perhaps of alternatively processed variants, in every centrocytic lymphoma and the rare *BCL1*-rearranged CLLs is consistent with the gene's hypothesized role as a direct-acting oncogene. The normal function of the *PRAD1* gene product has not yet been clarified. However, PRAD1 cDNA encodes a cyclin (28), which may play a role in regulating key phase transitions in the cell cycle (28, 47–49).

Apparent overexpression of *PRAD1* in centrocytic lymphomas without detectable *BCL1* rearrangement may be due to variation in the chromosome 11q13 translocation breakpoint. Only about half of these breakpoints may be close enough to the original *BCL1* locus to be seen on Southern blots hybridized with a *BCL1* major translocation cluster (MTC) DNA probe. Consistent with this interpretation, two tumors with breakpoints up to 63 kb away from the MTC have been reported (16, 50); in addition, three centrocytic lymphomas that appeared unrearranged with *BCL1* MTC probes revealed rearrangements when probed with a DNA fragment located 15 kb from the MTC (21). Thus, it seems likely that as additional probes become available, an increasingly large proportion of centrocytic lymphomas will prove to have 11q13 rearrangements in this general region. Deregulation leading to overexpression of *PRAD1* may be the unifying functional consequence of all such rearrangements.

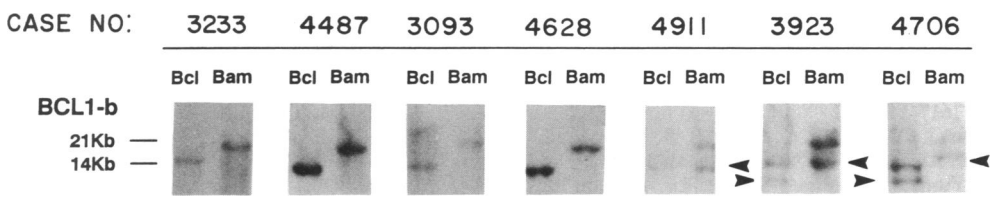

FIG. 3. *BCL1* rearrangements in centrocytic lymphomas. Genomic DNA was isolated from each centrocytic lymphoma and digested separately with the enzymes *Bcl* I and *Bam*HI. Southern blots were probed with BCL1 probe b. In this resulting autoradiogram, the positions of the normal-size *Bcl* I band (14 kb) and *Bam*HI band (21 kb) are indicated by lines. Rearranged bands in DNA from tumors 4911, 3923, and 4706 are indicated with arrowheads. In tumor 4911, the size of the rearranged *Bam*HI band is 14.5 kb; in tumor 3923, the sizes of the rearranged *Bcl* I and *Bam*HI bands are 10 kb and 13 kb, respectively; in tumor 4706, the sizes of the rearranged *Bcl* I and *Bam*HI bands are 10.5 kb and 14.5 kb, respectively. In lymphoma 4911, with a rearrangement detected only in the *Bam*HI digest, the *BCL1* region breakpoint may lie outside the 14-kb *Bcl* I fragment, or the rearranged and normal *Bcl* I fragments may be of equal size. Although some rearranged bands from different tumors were similar in size, it is unlikely that they represent restriction fragment length polymorphisms (RFLPs) normally found in the human genome. A recent examination of 120 specimens for RFLPs at the *BCL1* locus found only one example of a *Bcl* I polymorphism and no *Eco*RI, *Bam*HI, *Hin*dIII, *Sst* I, *Pvu* II, or *Bgl* II RFLPs (42).

At this time, we cannot exclude at least two alternative hypotheses. Another *BCL1* region gene instead of (or in addition to) *PRAD1* may be deregulated by rearrangement and critical for tumorigenesis; if so, the abundant *PRAD1* expression might be pathogenetically irrelevant, whether induced by the rearrangement or normally present at a narrow window of lymphoid differentiation. Detection of *PRAD1* transcriptional activation in parathyroid neoplasia with direct clonal *PRAD1* rearrangement argues against this alternative, however, and the absence of transcribed sequences within the 63-kb length of DNA telomeric to the *BCL1* MTC (16, 50) further diminishes this possibility. Also, *PRAD1* could be the relevant oncogene, but activating mechanisms other than *BCL1/PRAD1* region translocation may be involved in some tumors. Clarification of these issues must await a more detailed dissection of the *BCL1/PRAD1* genomic region, plus direct assessment of tumorigenicity of the *PRAD1* gene product.

PRAD1 has been implicated as a putative cellular oncogene in subsets of parathyroid adenomas, breast and squamous cell cancers, and now should be considered a good candidate for being the actual *BCL1* region oncogene in lymphoid neoplasia.

Note Added in Proof. It appears that at least one case of CLL with t(11;14) studied here (no. 1386), from which the *BCL1* breakpoint locus was originally cloned, may in fact be a centrocytic lymphoma with initial leukemic presentation rather than CLL (51); this strengthens the association of abundant *PRAD1* expression with this histologic subtype.

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1. Croce, C., Tsujimoto, Y., Erikson, J. & Nowell, P. (1984) *Lab. Invest.* **51,** 258–267.
2. Tsujimoto, Y., Finger, L., Yunis, J., Nowell, P. & Croce, C. (1984) *Science* **226,** 1097–1099.
3. Cleary, M. & Sklar, J. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 7439–7443.
4. Bakhshi, A., Jensen, J., Goldman, P., Wright, J., McBride, O., Epstein, A. & Korsmeyer, S. (1985) *Cell* **41,** 899–906.
5. Fukuhara, S., Rowley, J., Variakojis, D. & Golomb, H. (1979) *Cancer Res.* **39,** 3119–3128.
6. Nowell, P., Shankey, T., Finan, J., Guerry, D. & Besa, E. (1981) *Blood* **57,** 444–451.
7. Van den Berghe, H., Vermaelen, K., Louwagie, A., Criel, A., Mecucci, C. & Vaerman, J. (1984) *Cancer Genet. Cytogenet.* **11,** 381–387.
8. Yunis, J., Oken, M., Theologides, A., Howe, R. & Kaplan, M. (1984) *Cancer Genet. Cytogenet.* **13,** 17–28.
9. Melo, J., Brito-Babapulle, V., Foroni, F., Robinson, D., Luzzatto, L. & Catovsky, D. (1986) *Int. J. Cancer* **38,** 531–538.
10. Weisenburger, D., Sanger, W., Armitage, J. & Purtilo, D. (1987) *Blood* **69,** 1617–1621.
11. Erikson, J., Finan, J., Tsujimoto, Y., Nowell, P. & Croce, C. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 4144–4148.
12. Tsujimoto, Y., Yunis, J., Onorato-Showe, L., Erikson, J., Nowell, P. & Croce, C. (1984) *Science* **224,** 1403–1406.
13. Tsujimoto, Y., Jaffe, D., Cossman, J., Gorham, J., Nowell, P. & Croce, C. (1985) *Nature (London)* **315,** 340–343.
14. Selvanayagam, P., Goodacre, A., Strong, L., Saunders, G. & Barlogie, B. (1987) *Proc. Am. Assoc. Cancer Res.* **28,** 19 (abstr.).
15. Ince, C., Blick, M., Lee, M., Pathak, S., Vadhan-Raj, S., Selvanayagam, P., Gutterman, J. U. & Cabanillas, F. (1988) *Leukemia* **2,** 343–346.
16. Rabbitts, P. H., Douglas, J., Fischer, P., Nacheva, E., Karpas, A., Catovsky, D., Melo, J. V., Baer, R., Stinson, M. A. & Rabbitts, T. H. (1988) *Oncogene* **3,** 99–103.
17. Koduru, P., Offit, K. & Filippa, D. (1989) *Oncogene* **4,** 929–934.
18. Paquette, R. L., Berenson, J., Lichtenstein, A., McCormick, F. & Koeffler, H. P. (1990) *Oncogene* **5,** 1659–1663.
19. Medeiros, L. J., Van Krieken, J. H., Jaffe, E. S. & Raffeld, M. (1990) *Blood* **76,** 2086–2090.
20. Williams, M., Westermann, C. & Swerdlow, S. (1990) *Blood* **76,** 1387–1391.
21. Williams, M., Meeker, T. & Swerdlow, S. (1991) *Blood* **78,** 493–498.
22. Rimokh, R., Berger, F., Cornillet, P., Wahbi, K., Roualt, J.-P., Ffrench, M., Bryon, P.-A., Gadoux, M., Gentilhomme, O., Germain, D. & Magaud, J.-P. (1990) *Genes Chromosomes Cancer* **2,** 223–226.
23. Tolksdorf, G., Stein, H. & Lennert, K. (1980) *Br. J. Cancer* **41,** 168–182.
24. Jaffe, E., Bookman, M. & Longo, D. (1987) *Hum. Pathol.* **18,** 877–880.
25. Lardelli, P., Bookman, M., Sundeen, J., Longo, D. & Jaffe, E. (1990) *Am. J. Surg. Pathol.* **14,** 752–763.
26. Arnold, A., Kim, H., Gaz, R., Eddy, R., Fukushima, Y., Byers, M., Shows, T. & Kronenberg, H. (1989) *J. Clin. Invest.* **83,** 2034–2040.
27. Rosenberg, C., Kim, H., Shows, T., Kronenberg, H. & Arnold, A. (1991) *Oncogene* **6,** 449–454.
28. Motokura, T., Bloom, T., Kim, H. G., Juppner, H., Ruderman, J. V., Kronenberg, H. M. & Arnold, A. (1991) *Nature (London)* **350,** 512–515.
29. Lammie, G. A., Fantl, V., Smith, R., Schuuring, E., Brookes, S., Michalides, R., Dickson, C., Arnold, A. & Peters, G. (1991) *Oncogene* **6,** 439–444.
30. Bale, A. E., Wong, E. & Arnold, A. (1990) *Am. J. Hum. Genet.* **47,** A3 (abstr.).
31. Sambrook, J., Fritsch, D. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), 2nd Ed., Chap. 3, Section 10, 2–5; Chap. 4, Section 9, 1–8.
32. Non-Hodgkin's Lymphoma Classification Project (1982) *Cancer* **49,** 2112–2135.
33. Isaacson, P. & Spenser, J. (1987) *Histopathology* **11,** 445–462.
34. Sheibani, K., Swartz, W., Nadamanee, A. & Winberg, C. (1988) *Cancer* **62,** 1531–1538.
35. Cogliatti, S., Lennert, K., Hansmann, M. & Zwingers, T. (1990) *J. Clin. Pathol.* **43,** 619–625.
36. Ohno, H., Doi, S., Fukuhara, S., Nishikori, M., Uchino, H. & Fuji, H. (1985) *Jpn. J. Cancer Res.* **76,** 563–566.
37. Findley, H. W., Cooper, M. D., Kim, T. H., Alvarado, G. & Ragab, A. H. (1982) *Blood* **60,** 1305–1309.
38. Greaves, M. & Janossy, G. (1978) *Biochim. Biophys. Acta* **516,** 192–230.
39. Gonzalez, I., Gorski, J., Campen, T., Dorney, D., Erickson, J., Sylvester, J. & Schmickel, R. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 7666–7670.
40. Arnold, A., Cossman, J., Bakhshi, A., Jaffe, E. S., Waldmann, T. A. & Korsmeyer, S. J. (1983) *N. Engl. J. Med.* **309,** 1593–1599.
41. Feinberg, A. & Vogelstein, B. (1983) *Anal. Biochem.* **132,** 6–13.
42. Selvanayagam, P., Strong, L., Saunders, G. & Barlogie, B. (1990) *Nucleic Acids Res.* **18,** 1665.
43. Rechavi, G., Katzir, N., Brok-Simoni, F., Holtzman, F., Mandel, M., Gurfinkel, N., Givol, D., Ben-Bassat, I. & Ramot, B. (1989) *Leukemia* **3,** 57–60.
44. Cole, M. D. (1986) *Annu. Rev. Genet.* **20,** 361–385.
45. Cotter, F. E. (1990) *Br. J. Haematol.* **75,** 449–453.
46. Arnold, A., Staunton, C., Kim, H., Gaz, R. & Kronenberg, H. (1988) *N. Engl. J. Med.* **318,** 658–662.
47. North, G. (1991) *Nature (London)* **351,** 604–605.
48. Xiong, Y., Connolly, T., Futcher, B. & Beach, D. (1991) *Cell* **65,** 691–699.
49. Matsushime, H., Roussel, M. F., Ashmun, R. A. & Sherr, C. J. (1991) *Cell* **65,** 701–713.
50. Meeker, T., Grimaldi, J., O'Rourke, R., Louie, E., Juliusson, G. & Einhorn, S. (1989) *Blood* **74,** 1801–1806.
51. Raffeld, M. & Jaffe, E. (1991) *Blood* **78,** 259–263.