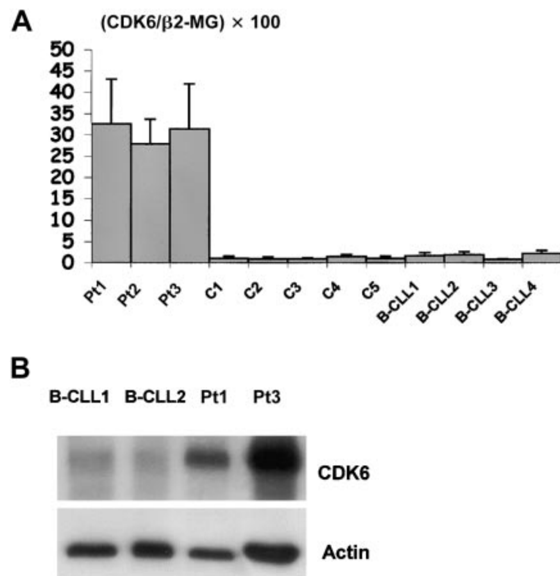

Figure 1. Overexpression of CDK6. (A) Quantitative real-time PCR was performed using the LightCycler system with FastStart DNA Master SYBR Green I kit according to the manufacturer's instructions (Roche Diagnostics, Mannheim, Germany). Primers from the *CDK6* gene are as follows: forward (5′-AGAAGAAGACTGGCCTAGAG) and reverse (5′-TGGAAGTATGGGTGAGACAGG). Primers from the β-2-microglobulin (*β2-MG*) gene used to evaluate correcting target molecule amounts are as follows: forward (5′-CCAGCAGAGAATGGAAAGTC) and reverse (5′-GATGCTGCTTACATGTCTCG). Amplification of specific transcripts was confirmed by a melting curve analysis. For PCR calibration, a serial 10-fold dilution series for each gene (ranging from 10^6 to 10 copies) was amplified and the assay was found to be linear over at least 5 orders of magnitude (*CDK6* slope, −3.47; *β2-MG* slope, −3.43). The absolute copy amount of each sample was obtained by the mean of the following ratio: ([copy number of the *CDK6* gene]/[copy number of *β2-MG*]) × 100. (B) Total cellular proteins were analyzed by Western blotting using anti-CDK6 antibody (Santa Cruz Biotechnology, Santa Cruz, CA). The same blot was probed for actin to control for equal protein loading. In all samples, tumor cells represented more than 80% of the nucleated cells. RNA and protein were extracted from bone marrow or blood samples from patients with the *CDK6* gene rearrangement (FIO, DUP, LAN), from patients with B-CLL without the *CDK6* gene rearrangement (B-CLL1 to B-CLL4), and from normal mononuclear peripheral blood cells (C1 to C5).

observed in a case of B-CLL. The t(7;14) involved the immunoglobulin heavy chain (*IgH*) locus in 14q32 and a transposon-like element located 29 kbp upstream of the *CDK6* coding sequence.[4] However, no material was available for CDK6 expression analysis in this case. To analyze CDK6 involvement in B-CLL, we have examined the status of the *CDK6* gene in bone marrow or blood samples from a further 8 adult patients with B-CLL carrying cytogenetic abnormalities (deletions, duplications, or translocations) involving bands 7q21 to 7q22 (1.5% of our B-CLL series).

B-CLL diagnosis was established according to the World Health Organization Classification of Tumours and was characterized by positivity for CD5 and CD23 expression. All the tumor samples were screened, where possible, by Southern blot, fluorescence in situ hybridization (FISH), reverse-transcriptase real-time quantitative polymerase chain reaction (RT-RQ-PCR), and immunoblotting. In 3 patients, involvement of the *CDK6* gene was found by at least 2 different methods. The relevant clinical, biologic, and molecular data for these patients are given in Table 1. FISH and Southern blot analysis showed that an interesting common feature was that in all 3 cases the *CDK6* gene was rearranged by reciprocal translocations involving either *Igκ*, *Igλ*, or *IgH* locus and not with only the *Igκ* light chain locus as has previously been reported in other series.[2,3]

Marked overexpression of CDK6 was observed in all 3 patients with *CDK6* rearrangement compared with normal peripheral blood lymphocytes and with B-CLL without *CDK6* involvement (Figure 1). This indicates that FISH and CDK6 expression analysis can be successfully used to identify patients with *CDK6* involvement. As previously reported,[2] CDK6 overexpression can result from aberrant variable-diversity-joining (VDJ) or variable-joining (VJ) recombination, leading to the juxtaposition of the *CDK6* gene with the Ig gene enhancer during B-cell differentiation. In addition, our findings demonstrate the involvement of the *CDK6* gene in B-CLL, underlying that those abnormalities are not restricted to SMZL.

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To the editor:

Evaluating the relevance of the platelet transcriptome

A recent article in *Blood* by Gnatenko et al[1] globally profiled mRNA expression in platelets. Using microarray analysis, the authors reported that approximately 2000 transcripts (13%-17% of probed genes) are present in unstimulated platelets isolated from healthy human subjects and concluded that evaluating the platelet transcriptome will be useful for identifying proteins that regulate normal and pathologic platelet and megakaryocyte functions. We have also found similar transcript profiles in platelets with different microarray strategies.[2,3]

An accompanying commentary voiced similar conclusions, but also suggested that the most frequent platelet mRNAs detected by microarray are well-known leukocyte or red cell messages, heightening suspicion that their abundance may be due to contamination by these classes.[4] These 3 messages were thymosin β_4, neurogranin, and β-globin.[1] Thymosin β_4 and neurogranin protein are also found in platelets,[1,5] however, which is consistent with observations that platelets express gene products that are also present in other cell lineages.[2,3,6,7] Moreover, Gnatenko et al[1] took rigorous

measures to account for contributions by leukocytes and red blood cells, and provided persuasive evidence that the thymosin β_4 and neurogranin transcripts are derived from platelets. The significance of detecting β-globin mRNA in their preparations still needs to be resolved, and if platelet-derived, will likely require confirmation by in situ detection methods. However, Gnatenko and colleagues indirectly addressed this issue by isolating total RNA from whole blood and concluded that the globin transcripts observed in their platelet preparations were not supported by erythrocyte contaminant estimates.[1]

Based on serial analysis of gene expression (SAGE) results, which preferentially targets abundant mRNAs, the commentary also underscored the author's conclusions that the vast majority of messages in platelets are mitochondrially derived.[1,4] We do not argue this point. Indeed, mitochondrial RNAs are continuously transcribed, in contrast to other platelet transcripts, and generate multiple polyadenylated transcripts from individual genes accounting for their enhanced detection by SAGE.[1] An important point, however, is that the mitochondrial genome encodes only 13 mRNAs and 2 rRNAs.[1] Platelets contain over 2000 individual mRNA species,[1-3] including well-known messages for the α_{IIb} and β_3 integrin subunits,[8,9] that were not detected by SAGE.[1] Thus, although they are more abundant, mitochondrially derived transcripts represent a minute fraction ($< 0.01\%$) of the mRNA species pool present in human platelets.[1-3] There are numerous examples in which the identification of nonmitochondrial mRNAs in platelets has generated important physiologic insights regarding the characterization and functional significance of corresponding proteins.[1-3,6,7,9,10]

Although the total RNA in human platelets is at least 100-fold less than in leukocytes,[10] platelet mRNAs are diverse, polyadenylated, distributed in a fashion that is influenced by cytoskeletal and RNA-binding proteins, and differentially translated in response to outside-in signals.[1-3,6-8,10] In addition to mitochondrial transcripts, many others are basally present.[1-3,6-10] Characterization of the platelet transcriptome, and the proteins that it encodes, will undoubtedly increase our understanding of platelet and megakaryocyte physiology and behavior in health and disease.

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To the editor:

Does cytogenetic mosaicism in $CD34^+CD38^{low}$ cells reflect the persistence of normal primitive hematopoietic progenitors in myeloid metaplasia with myelofibrosis?

Myeloid metaplasia with myelofibrosis (MMM) is a rare chronic myeloproliferative disorder characterized by myelofibrosis, extramedullary hematopoiesis, and absence of BCR-ABL rearrangement.[1,2]

Myeloproliferation is considered clonal and fibrosis, reactive.[2] Hierarchic level, primary mechanism, and/or gene alteration responsible for the malignant clone remain unknown. Here, we analyzed the clonality of $CD34^+$ cells ($CD34^+$), and questioned the hierarchic level of the disease and the origin of karyotypically normal $CD34^+$.

Karyotypes were performed on white blood cells (WBCs) and on immunomagnetically selected circulating $CD34^+$ (purity, $\geq$ 97%) from 23 patients as described.[3-5] According to previous reports,[1,6] 34.8% (8/23) of patients exhibited a high proportion of cytogenetic abnormal WBCs (nearly 100%). $CD34^+$ carried the same cytogenetic aberrations as WBCs in patients 13, 17, 19, and 33 (Table 1), but $CD34^+$ abnormal cell percentages were heterogeneous: 100% abnormal metaphases in patients 13, 17, and 19; 33% in patient 33; and 0% in patient 57.

This mosaicism could be due to normal residual $CD34^+$ whose proliferation is inhibited by unknown mechanism(s).[4] Normal WBC and $CD34^+$ karyotypes in the other 14 patients strengthen the alternative hypothesis that the primitive genetic lesion remains cryptic and that karyotypic alterations occur secondarily. Mosaicism present in several $CD34^+$ karyotypes and absent in WBCs also raises the question of the hierarchic level of the clonal event in MMM. The 6-fold higher proportion of $CD34^{high}CD38^{low}$ cells in MMM than in normal blood (25% vs 4%) suggested that the clonal myeloproliferation derives from primitive hematopoietic progenitors.[3] Therefore, cytogenetic and fluorescence in situ hybridization (FISH) studies were performed on $CD34^+CD38^{low}$, $CD34^+CD38^{high}$, $CD3^+$, and $CD19^+$ sorted cells (99% pure) in patient 19. FISH confirmed the reciprocal translocation with monoallelic 13q14 deletion (13q14−) (lsi D13S319 probe; Vysis, Downers Grove, IL).

Interestingly, in patient 19, 13q14− (FISH) was detected only in about 80% of freshly $CD34^+CD38^{low}$- and $CD34^+CD38^{high}$-sorted subpopulations (Figure 1A-B). This percentage increased after 7-day and 14-day cultures in the progeny of $CD34^+CD38^{low}$