observed by G-banded cytogenetic analysis. The remaining 4 cases had cytogenetically visible rearrangements of 11q23, which have not yet been fully characterized. Unfortunately, the follow-up time on these 10 cases is too short to be informative.

Clearly, 11q23/*MLL* translocations are a recurrent feature of both adult and childhood T-ALL. Although most major 11q23 translocations have been reported in T-ALL, the results from this study and a recent international collaboration[9] suggest that t(11;19) may be associated with T-ALL. Traditionally, 11q23 abnormalities have been associated with a poor outcome,[10,11] however, recent studies suggest that the worst prognosis is restricted to older adults and infants.[3,9] Assessing the prognostic significance of 11q23/*MLL* abnormalities within the context of T-ALL, which is also an indicator of poor prognosis and itself not independent of age,[12] will be difficult. Furthermore, the prognostic relevance of different 11q23 translocations has yet to be determined. Therefore, we would strongly recommend screening all subtypes of ALL at all ages for 11q23/*MLL* abnormalities.

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References

1. Moorman AV, Hagemeijer A, Charrin C, Rieder H, Secker-Walker LM. The translocations, t(11;19)(q23;p13.1) and t(11;19)(q23;p13.3): a cytogenetic and clinical profile of 53 patients. Leukemia. 1998;12:805-810.
2. Lillington DM, Young BD, Martineau M, Berger R, Moorman AV, Secker-Walker LM. The t(10;11)(p12;q23) translocation in acute leukemia: a cytogenetic and clinical profile of 20 patients. Leukemia. 1998;12:801-804.
3. Johansson B, Moorman AV, Haas OA, et al. Hematologic malignancies with t(4;11)(q21;q23)—a cytogenetic, morphologic, immunophenotypic, and clinical study of 183 cases. Leukemia. 1998;12:779-787.
4. Martineau M, Berger R, Lillington DM, Moorman AV, Secker-Walker LM. The t(6;11)(q27;q23) translocation in acute leukemia: a laboratory and clinical study of 30 cases. Leukemia. 1998;12:788-791.
5. Swansbury GJ, Slater R, Bain BJ, Moorman AV, Secker-Walker LM. Hematological malignancies with t(9;11)(p21–22;q23)—a laboratory and clinical study of 125 cases. Leukemia. 1998;12:792-800.
6. Harrison CJ, Cuneo A, Clark R, et al. Ten novel 11q23 chromosomal partner sites. Leukemia. 1998;12:811-822.
7. Hayette S, Tigaud I, Maguer-Satta V, et al. Recurrent involvement of the MLL gene in adult T-lineage acute lymphoblastic leukemia. Blood. 2002;99:4647-4649.
8. Harrison CJ, Martineau M, Secker-Walker LM. The Leukaemia Research Fund/United Kingdom Cancer Cytogenetics Group karyotype database in acute lymphoblastic leukaemia: a valuable resource for patient management. Br J Haematol. 2001;113:3-10.
9. Pui CH, Gaynon PS, Boyett JM, et al. Outcome of treatment in childhood acute lymphoblastic leukaemia with rearrangements of the 11q23 chromosomal region. Lancet. 2002;359:1909-1915.
10. Secker-Walker LM, Prentice HG, Durrant J, Richards S, Hall E, Harrison G. Cytogenetics adds independent prognostic information in adults with acute lymphoblastic leukaemia on MRC trial UKALL XA: MRC Adult Leukaemia Working Party. Br J Haematol. 1997;96:601-610.
11. Chessells JM, Swansbury GJ, Reeves B, Bailey CC, Richards SM. Cytogenetics and prognosis in childhood lymphoblastic leukaemia: results of MRC UKALL X. Br J Haematol. 1997;99:93-100.
12. Hann I, Vora A, Harrison G, et al. Determinants of outcome after intensified therapy of childhood lymphoblastic leukaemia: results from Medical Research Council United Kingdom acute lymphoblastic leukaemia XI protocol. Br J Haematol. 2001;113:103-114.

To the editor:

Megakaryocytes from chronic myeloproliferative disorders show enhanced nuclear bFGF expression

Chronic myeloproliferative disorders (CMPDs) comprising chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), and chronic idiopathic myelofibrosis (IMF) differ in their potential for resulting in bone marrow fibrosis. In IMF, fibrosis usually starts developing in close vicinity to clusters of proliferating and enlarged atypical megakaryocytes.[1] Megakaryocyte-derived basic fibroblastic growth factor (bFGF) has been implicated in the pathogenesis of bone marrow fibrosis in IMF.[2,3] Different isoforms of bFGF exist and stimulate target cells via membrane receptor or nuclear binding.[4] Distinct biologic functions, depending on the subcellular location of the bFGF species generated, have been described, whereby the nuclear isoform is associated with growth and proliferation.[5]

In an immunohistochemical study of bone marrow trephines, all or at least a considerable proportion of megakaryocytes in PV (n = 10), ET (n = 10), and IMF (n = 19), including the prefibrotic stage (n = 10), exhibited a strong nuclear bFGF expression exceeding that of all other bone marrow cells except endothelium. By contrast, megakaryocytes in CML (n = 10) and controls with reactive megakaryocytic hyperplasia (n = 10) showed no or merely a weak nuclear positivity that did not exceed that of the precursors of the myeloid or erythroid lineage (Figure 1). Fewer than 10% of the non-CML CMPD cases did not exhibit nuclear labeling of megakaryocytes, and a nuclear staining similar to that observable in a minority of lower-expressing CMPD cases occurred exceptionally in one of the reactive control cases.

Because the enhanced nuclear decoration of megakaryocytes could be due to either uptake of bFGF secreted by other bone marrow cells[4,5] or to autocrine expression, we investigated bFGF mRNA in megakaryocytes by real-time reverse transcriptase–polymerase chain reaction (RT-PCR). Total RNA was extracted and reversely transcribed[6] from 50 to 100 megakaryocytes per case isolated by laser-microdissection (P.A.L.M., Wolfratshausen, Germany) from tissue sections of bone marrow.[7] After linearity of PCR amplification over a broad concentration range and equal efficiencies for all primers/probe systems could be shown, relative quantification of bFGF and β-glucuronidase (β-GUS) mRNA was performed in 2 independent runs using the ΔΔCT–method.[8] Megakaryocytes from PV, ET, and prefibrotic and fibrotic IMF displayed exaggerated bFGF mRNA levels compared to megakaryocytes from normal or reactive controls and CML ($P < .0001$, respectively). Interestingly, the highest amount of bFGF mRNA was found in megakaryocytes from PV and not in those derived from fibrotic IMF (Figure 2).

We conclude that nuclear overexpression of bFGF characterizes megakaryocytes from PV, ET, cellular and fibrotic IMF and, in most instances, discriminates these diseases from CML and reactive controls. The increased presence of nuclear-detectable bFGF is most likely due to growth factor production by

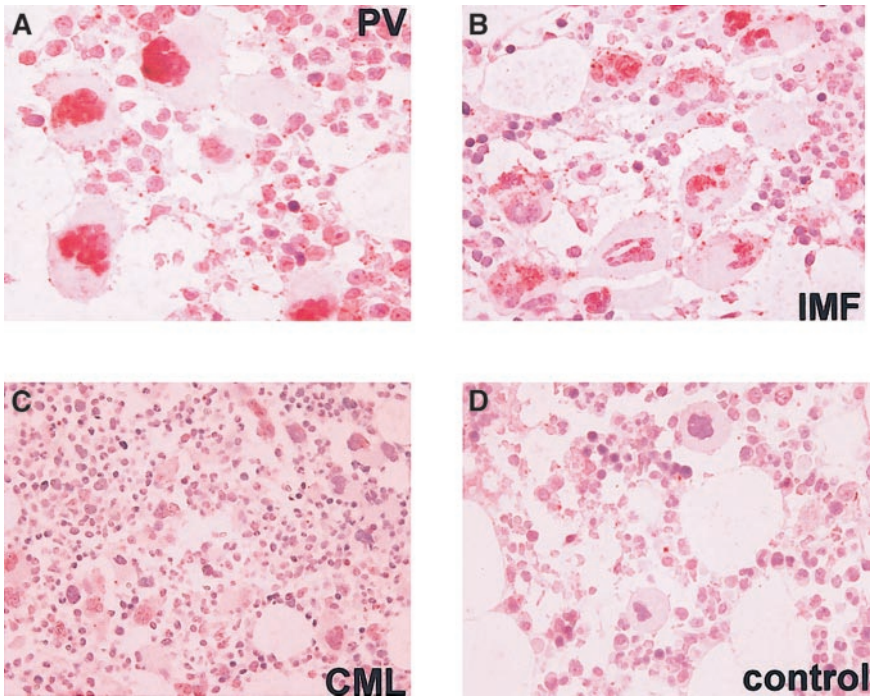

Figure 1. Immunohistochemical detection of bFGF expression. Immunohistochemical detection of bFGF expression in bone marrow biopsies from (A) polycythemia vera (PV), (B) prefibrotic stage of idiopathic myelofibrosis (IMF), (C) chronic myeloid leukemia (CML), and (D) reactive changes. A strong nuclear labeling exceeding that of all other bone marrow cells can be observed in PV and IMF (A,B). A considerably weaker and faint staining not highlighting the megakaryocyte nuclei occurred in CML and reactive controls (C,D). Magnification (A,B), × 400; (C), × 200; and (D), × 300 (polyclonal antibody #AF-233-NA, R&D Systems, Minneapolis, MN, diluted 1:50).

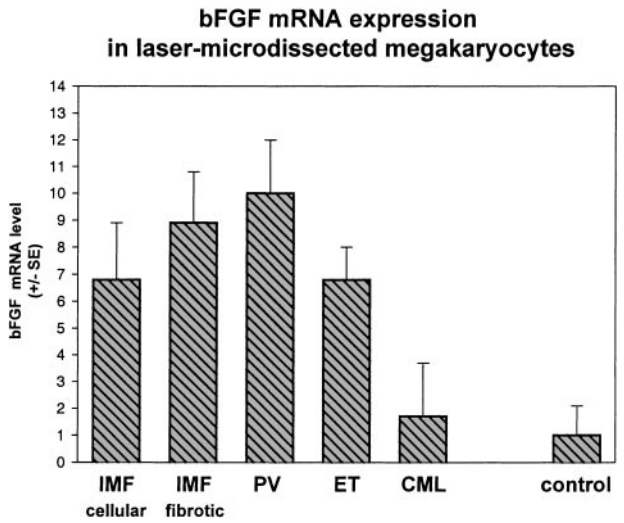

Figure 2. bFGF expression in laser-microdissected megakaryocytes. In microdissected megakaryocytes, overexpression of bFGF mRNA was found in PV (× 10, $P < .0001$), in fibrotic IMF (× 8.9, $P < .001$), in prefibrotic IMF (× 6.8, $P < .009$), and in ET (× 6.8, $P < .0001$), compared to control megakaryocytes. In CML, megakaryocytes exhibited moderately higher bFGF mRNA levels compared to controls without significance, but significant differences occurred in comparison to PV ($P < .0001$), fibrotic IMF ($P < .002$), and prefibrotic IMF and ET ($P < .04$ and $P < .001$, respectively). Real-time RT-PCR with bFGF forward primer 5′-CCCGGCCACTTCAAGGA; bFGF reverse primer 5′-TGGATGCGCAGGAAGAAG and bFGF probe 5′-CCCAAGCGGCTGTACTGCAAAAACG. β-Glucuronidase (β-GUS) forward primer 5′-CTCATTTGGAATTTTGCCGATT, β-GUS reverse primer 5′-AGTGAAGATCCCCTTTTTA, and β-GUS probe 5′-TGAACAGTCACCGACGAGAGTGCTGG. Transcript levels are depicted as multiples of the amount found in reactive control cases (= 1).

megakaryocytes in an autocrine fashion. bFGF could primarily be involved in the exaggerated proliferation of megakaryocytes in CMPD rather than in direct induction of fibroblasts, as has been suggested earlier.[2,3] It cannot be excluded, however, that an enhanced turnover of megakaryocytes in IMF deliberates more bFGF from disintegrating cells, which then might lead to a paracrine stimulation of fibroblasts.[9]

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References

1. Georgii A, Buesche G, Kreft A. The histopathology of chronic myeloproliferative diseases. Baillieres Clin Haematol. 1998;11:721-749.
2. Martyre M-C, Le Bousse-Kerdiles M-C, Romquin N, et al. Elevated levels of basic fibroblast growth factor and platelets from patients with idiopathic myelofibrosis. Br J Haematol. 1997;97:441-448.
3. Tefferi A. Myelofibrosis with myeloid metaplasia. N Engl J Med. 2000;342:1255-1265.
4. Delrieu I. The high molecular weight isoform of basic fibroblast growth factor (FGF-2): an insight into an intracrine mechanism. FEBS Lett. 2000;468:6-10.
5. Mason IJ. The ins and outs of fibroblast growth factors. Cell. 1994;78:547-552.
6. Bock O, Kreipe H, Lehmann U. One-step extraction protocol for the extraction of RNA from archival biopsies. Analyt Biochem. 2001;295:116-117.
7. Lehmann U, Bock O, Glöckner S, Kreipe H. Quantitative molecular analysis of laser-microdissected paraffin-embedded human tissues. Pathobiology. 2001; 68:202-208.
8. Giulietti A, Overbergh L, Valckx D, Decallonne B, Bouillon R, Mathieu C. An overview of real-time quantitative PCR: applications to quantify cytokine gene expression. Methods. 2001;25:386-401.
9. Nugent MA, Iozzo RV. Fibroblast growth factor-2. Int J Biochem Cell Biol. 2000; 32:115-120.