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Advances in Brief

Expression of Fibroblast Growth Factor Receptors in Human Leukemia Cells[1]

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Abstract

We have previously cloned from K562 leukemia cells two novel fibroblast growth factor receptors (FGFR-3 and FGFR-4; J. Partanen *et al.*, EMBO J., *10:* 1347-1354, 1991). Here we have analyzed the mRNA expression of four different FGFRs, including the two novel genes in human leukemia cell lines. We show FGFR-1, FGFR-3, and FGFR-4 mRNAs in several leukemia cell lines at levels similar to those in solid tumor cell lines. Ligand cross-linking experiments indicate that K562 cells have receptors binding acidic FGF but not basic FGF. Expression of FGFRs in leukemia cells may reflect their presence on normal hematopoietic precursor cells or induction during leukemogenesis or cell culture.

Introduction

Several of the currently known protein tyrosine kinase genes encode transmembrane receptors for polypeptide growth factors and hormones, such as epidermal growth factor, insulin, platelet-derived growth factor, and FGFs[3] (1). The receptors of some hematopoietic growth factors are also tyrosine kinases; these include the macrophage-colony-stimulating factor receptor, which is the protooncogene c-*fms*-encoded protein (2) and another protooncogene product, c-*kit*, a receptor for a hematopoietic stem cell growth factor (3). We used the polymerase chain reaction to detect members of the tyrosine kinase family expressed in K562 human chronic myeloid leukemia cells (4). Two of the novel genes obtained were found to belong to the FGFR family (4, 5), which at present contains five members. These are FGFR-1 [*flg* (6)], FGFR-2 [*bek* (6-8)], FGFR-3 and FGFR-4 from the K562 leukemia cells (4, 5), and FGFR-5 (*flg*-2), recently isolated from a human keratinocyte cDNA library (9). These receptors are 56-93% identical with each other and share several structural features, such as glycosylated immunoglobulin-like loops in their extracellular domains. Our earlier analyses indicated that in addition to the K562 cells, the FGFR-3 and FGFR-4 mRNAs are expressed in the Dami megakaryoblastic leukemia cells (4). We have therefore studied the expression of these novel genes along with the two previously characterized FGFR genes in human leukemia cell lines representing different hematopoietic lineages.

Materials and Methods

Cells and Growth Factors. The leukemia cell lines K-562, Dami, MOLT-4, HL-60, KG-1, HEL, U937, Jurkat, JOK-1, ML-2, and RC-2A were grown in RPMI 1640 supplemented with 10% fetal calf serum and antibiotics. The K562 cells were induced to megakaryoblastoid differentiation by 48 h treatment with 2 nM 12-*O*-tetradecanoyl-phorbol-13-acetate. The Tera-2 teratocarcinoma, RD rhabdomyosarcoma, and A549 lung carcinoma cell lines, obtained from the American Type Culture Collection (Rockville, MD), were grown as suggested by the supplier. Recombinant human aFGF was a generous gift from Dr. Ralf Petterson (Ludwig Institute for Cancer Research, Stockholm), and bFGF was a gift from Dr. Laura Bergonzoni (Farmitalia Carlo Erba, Milan).

Extraction and Analysis of RNA. Polyadenylated RNA was extracted from the cell lines as described (10), and 5 μg of each sample were electrophoresed in agarose gels containing formaldehyde and blotted to nylon membranes using standard conditions. The inserts of cDNA clones HE6 and HE8 [FGFR-4 and FGFR-3 (11)] as well as CD115 and CD116 coding for the extracellular domains of FGFR-1 and FGFR-2, respectively (kindly donated by Drs. C. Dionne and M. Jaye, Rhone-Poulenc Rorer, King of Prussia, PA), were labeled by the random priming method and hybridized to the blots. The human glyceraldehyde 3-phosphate dehydrogenase cDNA or β-actin oligonucleotide probes were used as internal controls for the amounts of RNA on the filters.

Covalent Cross-Linking. K562 cells and COS-7 cells transfected with the FGFR-4 and FGFR-1 expression vectors (11) were washed twice with binding buffer (Dulbecco's modified minimal essential medium containing 0.1% gelatin and 50 mM HEPES, pH 7.5). About 25 ng of ^{125}I-aFGF, labeled by the chloramine T iodination method, was added in the binding buffer on ice and incubation continued for 90 min. The cells were washed once with binding buffer and twice with phosphate-buffered saline and incubated for 20 min at 4°C in phosphate-buffered saline containing 0.3 mM of the covalent cross-linking agent disuccinimidyl suberate. The cells were then washed once with 10 mM HEPES, pH 7.5, 200 mM glycine, 2 mM EDTA, and once with phosphate-buffered saline; lysed in 20 mM HEPES, pH 7.5, 150 mM NaCl, 1% Triton X-100, 10% glycerol, 1.5 mM $MgCl_2$, 1.0 mM EDTA, and 1 mg/ml aprotinin; and centrifuged for 10 min at 10,000 × *g*. Aliquots of the supernatants were boiled in an equal volume of the electrophoresis sample buffer and analyzed in 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Results

The expression of FGFR mRNAs in the cell lines was analyzed by Northern blotting and hybridization with specific cDNA probes. As can be seen from Fig. 1, the *FGFR-4* gene is expressed as an approximately 3.0-kilobase mRNA in K562 erythroleukemia and Dami megakaryoblastic leukemia cells. In addition, the FGFR-4 probe weakly detects a 4.8-kilobase mRNA in these cells (see also Fig. 2) and in HEL erythroleukemia cells. The most prominent RNA band hybridizing to the FGFR-3 probe is 4.5 kilobases in size, and additional, weaker bands of about 4.8 and 7 kilobases are seen in RNA from the K562, Dami, and HEL cells. No FGFR-2 mRNA was found in any of the leukemia cells studied (data not shown). Interestingly, the HL-60 promyelocytic leukemia and KG-1 myeloid leukemia cells, largely devoid of the FGFR-3 and FGFR-4 mRNAs, and the MOLT4 T-cell leukemia cells, which contained no FGFR-4 and only low levels of FGFR-3, showed expression of the 4.4-

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[3] The abbreviations used are: FGF, fibroblast growth factor; aFGF, acidic FGF; bFGF, basic FGF; FGFR, FGF receptor; cDNA, complementary DNA; HEPES, *N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulfonic acid).

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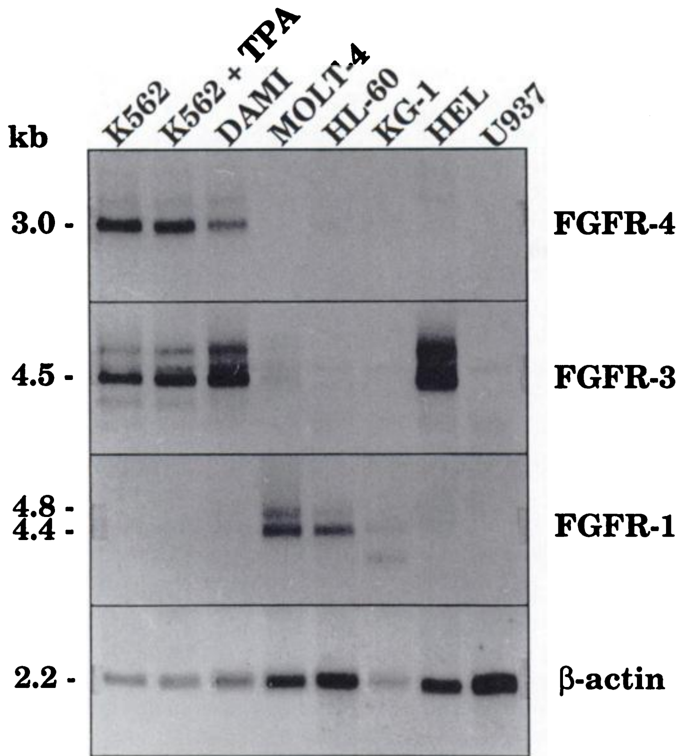

Fig. 1. Expression of the mRNAs for FGFR family members in human leukemia cell lines. Northern analysis of 5 μg of polyadenylated RNA from the cell lines (*top*) hybridized sequentially with the cDNAs for FGFR-1–4. The FGFR-2 mRNA was not expressed by any of the cells (data not shown). *Bottom*, Hybridization with a β-actin probe as an internal control for the amounts of RNA.

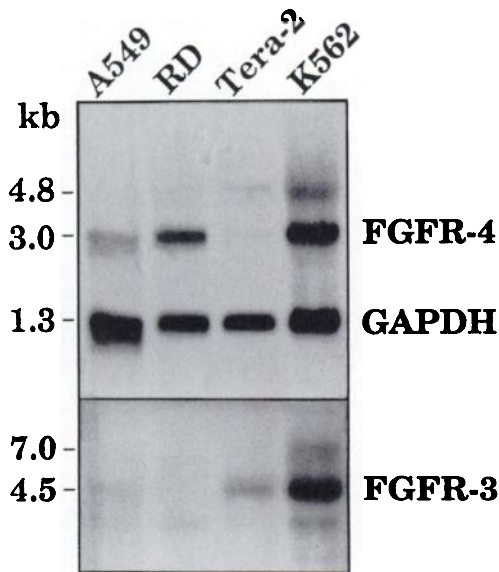

Fig. 2. Comparison of FGFR-3 and FGFR-4 mRNA levels between K562, Tera-2 teratocarcinoma, RD rhabdomyosarcoma, and A549 lung carcinoma cell lines. Northern blotting and hybridization analysis was performed as in Fig. 1. Hybridization with the glyceraldehyde 3-phosphate dehydrogenase cDNA was performed as an internal control for RNA loading.

and 4.8-kilobase FGFR-1 mRNAs. In addition, the FGFR-1 probe hybridized to a 2.8-kilobase RNA expressed in the KG-1 cells. The identity of this RNA is unknown, but it should be noted that several differentially spliced forms of FGFR-1 mRNA have been shown previously (12, 13).

The levels of FGFR-3 and FGFR-4 expression were compared between K562 cells and the solid tumor cell lines A549 (lung carcinoma), RD (rhabdomyosarcoma), and Tera-2 (teratocarcinoma). The results show that the FGFR-3 RNA is expressed at higher levels in the leukemia cells and that the 3.0-kilobase FGFR-4 RNA is expressed at equally high levels in the K562 and RD cells (Fig. 2).

Scanning of the autoradiographs was used to quantitate the expression of FGFR RNA relative to the amount of cellular polyadenylated RNA on the filters. Expression levels for the leukemia cell lines shown in Fig. 1, plus additional leukemia cell lines, were divided into three groups according to the amount of specific mRNA, and these results are given in Table 1.

In order to show that the FGFR mRNAs were translated into receptor polypeptides, the K562 cells which contained abundant FGFR-4 and FGFR-3 RNA were chosen for covalent cross-linking analysis with ^{125}I-aFGF. COS cells transfected with either FGFR-4 or FGFR-1 expression vectors (11) were used as controls. The results are shown in Fig. 3. *Lane 2* shows that the K562 cells contain an aFGF-binding polypeptide comigrating with the high-molecular-weight polypeptide in samples from FGFR-4 transfected COS cells (Fig. 3, *Lane 6*). The polypeptides expressed in FGFR-1-transfected COS cells show a different mobility (Fig. 3, *Lane 7*). The binding specificity of the K562 cell polypeptide is demonstrated by the fact that its labeling is completely competed off by a 50-fold excess of unlabeled aFGF (Fig. 3, *Lane 3*). On the other hand, a 50-fold excess of unlabeled bFGF, without or with heparin, caused only a very minor decrease in the binding of the labeled aFGF (Fig. 3, *Lanes 4* and *5*). This suggests that the receptor expressed in K562 cells does not bind bFGF. As a control, labeled aFGF was completely competed by bFGF in COS cells transfected with FGFR-1, which has been shown to bind both aFGF and bFGF (6). These results confirm that the K562 cells express FGF receptors, probably mostly FGFR-4, which we have previously shown to bind aFGF but not bFGF (11), and suggest that despite the presence of mRNA coding for FGFR-3, significant numbers of these receptors are not expressed on the cell surface.

Discussion

These experiments indicate that at least three of the five different members of the FGFR family are expressed and

Table 1 *Expression of FGFR mRNAs in leukemia cell lines*

Polyadenylated RNA was extracted from the cells, run in agarose gels, and after Northern blotting hybridized sequentially to the specific FGFR probes and either the β-actin or GAPDH probes. Scanning of the autoradiographs was used to quantitate the specific signals relative to the β-actin or GAPDH signals.

	mRNA for			
Leukemias	FGFR-4	FGFR-3	FGFR-2	FGFR-1
K562 (erythroid)	+++[a]	+++	–[b]	–
K562+TPA (megakaryoblastic)	+++	+++	–	–
Dami (megakaryoblastic)	++	+++	–	–
HEL (erythroid)	+	+++	–	–
HL-60 (promyelocytic)	–	–	–	++
KG-1 (myeloid)	–	–	–	+
ML-2 (myeloid)	+	–	–	+
RC-2A (monocytic)	–	–	–	–
U937	–	–	–	–
Jurkat (T-cell)	++	+	–	+++
MOLT4 (T-cell)	–	+	–	++
JOK-1 (B-hairy cell)	+++	–	–	–

[a] FGFR RNA expression was classified into three levels presented by +, ++, and +++.
[b] –, no RNA detected.

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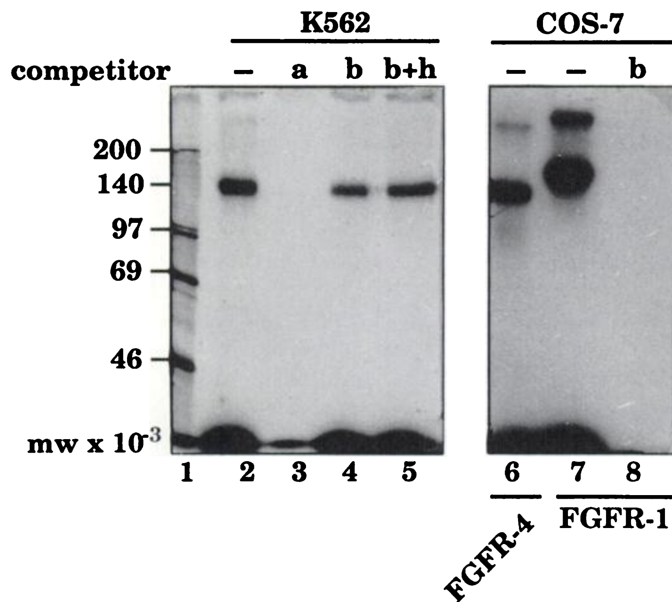

Fig. 3. Covalent cross-linking of aFGF and bFGF to K562 cells. Cross-linking was performed as described in "Materials and Methods." An autoradiogram of the SDS-PAGE is shown. *Lane 1*, molecular weight marker; *Lane 2*, K562 cells cross-linked to ^{125}I-aFGF; *Lane 3*, cross-linking of ^{125}I-aFGF to K562 cells in the presence of a 50-fold excess of unlabeled aFGF; *Lane 4*, 50-fold excess of unlabeled bFGF without and with (*Lane 5*) heparin. *Lanes 6*, *7*, and *8*, control experiments with COS cells transfected with FGFR-4 and FGFR-1 expression vectors and cross-linked to ^{125}I-aFGF. *Lane 8*, cross-linking in the presence of a 50-fold excess of bFGF.

differentially regulated in human hematopoietic cells. At present, seven FGFs are known (14, 15). aFGF and bFGF are the best characterized of these ligands. They have similar but very complex effects, depending on the responder cell type; they can induce mitogenesis, participate in angiogenesis, support cell survival, and either induce or inhibit cellular differentiation.

Expression of FGFRs in leukemia cells or normal hematopoietic precursor cells has not been previously reported. Normal lymphoblasts were recently reported to contain FGFR-3 transcripts (16). The high levels of FGFR-3 and FGFR-4 mRNA observed in leukemia cell lines in culture may either reflect expression in a subset of normal hematopoietic cells or represent aberrant expression induced by leukemogenesis or *in vitro* growth. A recent report suggests that FGFs can have a permissive role for primitive hematopoietic cell colony formation in culture (17), and a low concentration of bFGF has been shown to stimulate myelopoiesis, especially the formation of neutrophil granulocytes and their precursors, in long-term human bone marrow culture (18). It is possible that FGFs are produced by bone marrow stromal cells, and they could have local effects in the hematopoietic microenvironment, including the extracellular matrix, leading to direct or indirect effects on blood cell production or function.

The finding of the highest levels of FGFR-3 and FGFR-4 mRNA in the erythroid and megakaryoblastic cell lines suggests that their expression may be typical of leukemias in these lineages. We have shown FGFR-4 mRNA in the liver and spleen of human fetuses but have not been able to detect FGFR expression in unfractionated normal bone marrow or in the bone marrow mononuclear cell fraction by Northern hybridization analysis (11).[4] This may be due to the heterogeneity of the cell population analyzed, with possibly only some types of cells expressing the receptor mRNAs, which remain thus undetected in the RNA from these mixed cell preparations. This hypothesis is supported by the finding of FGFR-4 clones during the screening of a human bone marrow cDNA library.[5] Clearly, *in situ* hybridization experiments are needed to specify possible hematopoietic cells expressing the different FGFRs.

Several genes involved in the regulation of hematopoietic cell growth and differentiation have been mapped to the 5q chromosomal arm, where we have localized also the *FGFR-4* gene [bands q33-qter (19)]. Deletions of the 5q arm including some hematopoietic growth factors and receptors are frequently seen in myelodysplastic syndromes, secondary acute myeloid leukemias, and a subset of *de novo* acute myeloid leukemias (20). We are currently exploring the possible role of the *FGFR-4* gene in translocations of the distal end of chromosome 5 (21, 22).

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References

1. Heldin, C-H., and Westermark, B. Platelet-derived growth factor: mechanism of action and possible *in vivo* function. Cell Regul., *1:* 555–566, 1990.
2. Sherr, C. J. Colony-stimulating factor-1 receptor. Blood, *75:* 1–12, 1990.
3. Witte, O. Steel locus defines new multipotent growth factor. Cell, *6:* 5–6, 1990.
4. Partanen, J., Mäkelä, T. P., Alitalo, R., Lehväslaiho, H., and Alitalo, K. Putative tyrosine kinases expressed in K-562 human leukemia cells. Proc. Natl. Acad. Sci. USA, *87:* 8913–8917, 1990.
5. Keegan, K., Johnson, D. E., Williams, L. T., and Hayman, M. J. Isolation of an additional member of the fibroblast growth factor receptor family, FGFR-3. Proc. Natl. Acad. Sci. USA, *88:* 1095–1099, 1991.
6. Dionne, C. A., Crumley, G., Bellot, F., Kaplow, J. M., Searfoss, G., Ruta, M., Burgess, W. H., Jaye, M., and Schlessinger, J. Cloning and expression of two distinct high-affinity receptors cross-reacting with acidic and basic fibroblast growth factors. EMBO J., *9:* 2685–2692, 1990.
7. Hattori, Y., Odagiri, H., Nakatani, H., Miyagawa, K., Naito, K., Sakamoto, H., Katoh, O., Yoshida, T., Sugimura, T., and Terada, M. K-*sam*, an amplified gene in stomach cancer, is a member of the heparin-binding growth factor receptor genes. Proc. Natl. Acad. Sci. USA, *87:* 5983–5987, 1990.
8. Houssaint, E., Blanquet, P. R., Champion-Arnaud, P., Gesnel, M. C., Torriglia, A., Courtois, Y., and Breathnach, R. Related fibroblast growth factor receptor genes exist in the human genome. Proc. Natl. Acad. Sci. USA, *87:* 8180–8184, 1990.
9. Avivi, A., Zimmer, Y., Yayon, A., Yarden, Y., and Givol, D. Flg-2, a new member of the family of fibroblast growth factor receptors. Oncogene, *6:* 1089–1092, 1991.
10. Sambrook, J., Fritsch, E. F., and Maniatis, T. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1989.
11. Partanen, J., Mäkelä, T. P., Eerola, E., Korhonen, J., Hirvonen, H., Claesson-Welsh, L., Huebner, K., and Alitalo, K. FGFR-4, a novel fibroblast growth factor receptor with a distinct expression pattern. EMBO J., *10:* 1347–1354, 1991.
12. Johnson, D. E., Lee, P. L., Lu, J., and Williams, L. T. Diverse forms of a receptor for acidic and basic fibroblast growth factors. Mol. Cell. Biol., *10:* 4728–4736, 1990.
13. Hou, J., Kan, M., McKeehan, G., Adams, P., and McKeehan, W. J. Fibroblast growth factor receptors from liver vary in three structural domains. Science (Washington DC), *251:* 665–668, 1991.
14. Burgess, W. H., and Maciag, T. The heparin-binding (fibroblast) growth factor family of proteins. Annu. Rev. Biochem., *58:* 575–606, 1989.

[4] E. Armstrong, S. Vainikka, J. Partanen, J. Korhonen, and R. Alitalo, unpublished observations.

[5] A. Polvi, E. Armstrong, S. Vainikka, J. Partanen, J. Korhonen, and R. Alitalo, unpublished observations.

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15. Olwin, B. B. Heparin-binding growth factors and their receptors. Cytotechnology, *2:* 351–365, 1989.
16. Thompson, L. M., Plummer, S., Schalling, M., Altherr, M. R., Gusella, J. F., Housman, D. E., and Wasmuth, J. J. A gene encoding a fibroblast growth factor receptor isolated from the Huntington disease gene region of human chromosome 4. Genomics, *11:* 1138–1142, 1991.
17. Gabbianelli, M., Sargiacomo, M., Pelosi, E., Testa, U., Isacchi, G., and Peschle, C. "Pure" human hematopoietic progenitors: permissive action of basic fibroblast growth factor. Science (Washington DC), *249:* 1561–1564, 1990.
18. Wilson, L. E., Rifkin, D. B., Kelly, F., Hannocks, M-J., and Gabrilove, J. L. Basic fibroblast growth factor stimulates myelopoiesis in long-term human bone marrow cultures. Blood, *77:* 954–960, 1991.
19. Armstrong, E., Partanen, J., Cannizzaro, L., Huebner, K., and Alitalo, K. Localization of fibroblast growth factor receptor-4 gene to chromosome region 5q33-qter. Genes Chromosomes Cancer, *4:* 1–5, 1991.
20. Huebner, K., Nagarajan, L., Besa, E., Angert, A., Lange, B. J., Cannizzaro, L. A., van der Berghe, H., Santoli, D., Finan, J., Croce, C. M., and Nowell, P. C. Order of genes on human chromosome 5q with respect to 5q interstitial deletions. Am. J. Hum. Genet., *46:* 26–36, 1990.
21. Fischer, P., Nacheva, E., Mason, D. Y., Sherrington, P. D., Hoyle, C., Hayhoe, F. G. J., and Karpas, A. A Ki-1 (CD30)-positive human cell line (Karpas 299) established from a high-grade non-Hodgkin's lymphoma, showing a 2;5 translocation and rearrangement of the T-cell receptor β-chain gene. Blood, *72:* 234–240, 1988.
22. Knuutila, S., Franssila, K., Lakkala, T., Kovanen, P., Peltomäki, P., and Tecrenhovi, L. A specific chromosomal abnormality in Ki-1 lymphoma. 10th International Human Gene Mapping Workshop, New Haven, Connecticut, USA, June 11–17, 1989. Cytogenet. Cell Genet., *51:* 1024, 1989.

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