

FLT3 mutations in acute myeloid leukemia cell lines

H Quentmeier, J Reinhardt, M Zaborski and HG Drexler

DSMZ – German Collection of Microorganisms and Cell Cultures, Department of Human and Animal Cell Cultures, Braunschweig, Germany

Internal tandem duplications (ITD) and D835 point mutations of the receptor tyrosine kinase (RTK) FLT3 are found in a high proportion of cases with acute myeloid leukemia (AML). These genetic aberrations may lead to the constitutive activation of the receptor, thus providing the molecular basis for a persisting growth stimulus. We have screened 69 AML-derived cell lines for FLT3 mutations. Four of these cell lines showed ITD of the FLT3 gene, none carried a D835 point mutation. Two cell lines (MUTZ-11 and MV4-11) expressed exclusively the mutated allele, the other two cell lines (MOLM-13 and PL-21) displayed a mutated and the wild-type version of the gene. Although mutationally activated FLT3 is supposed to substitute for the stimulatory signal of a growth factor, one of these cell lines (MUTZ-11) was strictly cytokine-dependent. FLT3 transcripts were found in all four cell lines, but the constitutively phosphorylated receptor protein was clearly detectable only in cell line MV4-11, possibly explaining why MUTZ-11 cells were growth-factor dependent. Thus, not all FLT3 ITD-positive cells express high levels of the active receptor protein, a finding that might be of relevance for a possible future application of a kinase inhibitor as therapeutic agent. It had been described that STAT-5 phosphorylation was part of the FLT3 signalling chain and that STAT-5 molecules were constitutively phosphorylated in FLT3 ITD-positive cells. Although we observed the constitutive phosphorylation of STAT-5 molecules in FLT3-mutant cells, FLT3 ligand (FL) did not induce STAT-5 phosphorylation in FLT3 wild-type cells. These results suggest that the signalling mechanisms of the mutated FL receptor differ at least to some extent from those conferred by wild-type FLT3. In conclusion, (1) not all cells with FLT3 ITD express significant amounts of the mutated receptor protein; (2) signals downstream from wild-type and mutant FLT3 receptors are not 100% identical; and (3) MV4-11 represents a model cell line for FLT3 ITD signalling.

Leukemia (2003) 17, 120–124. doi:10.1038/sj.leu.2402740

Keywords: AML; cell lines; FLT3; leukemia; signal transduction

Introduction

FLT3 (fms-like tyrosine kinase), a member of the type III receptor tyrosine kinase (RTK) family, is expressed in multipotential hematopoietic stem cells and progenitors, but also in blast cells of most patients with acute myeloid leukemia (AML)^{1–3} and in AML-derived cell lines.^{4,5} The cognate ligand (FL) induces growth in a substantial proportion of FLT3-positive AML cells.^{5,6} We have shown that leukemic cell lines may express FLT3 and FL simultaneously, suggesting that autocrine or paracrine stimulation might cause proliferation of these cells.⁴ Recently, it has also been shown that FLT3 mutations may provide a growth stimulus for AML cells. Small internal tandem duplications (ITD) in the juxtamembrane part of the FLT3 gene in patients with AML have been reported by Nakao *et al.*⁷ FLT3 ITD were found in about 20% of AML cases.^{7–11} These FLT3 gene mutations were shown to be associated with an unfavorable prognosis.^{9,11,12} The exact location and length of the ITD varied from patient to patient, but the transcripts

always stayed in frame.^{8,10} It was reported that ITD led to the activation of the receptor through constitutive dimerization.¹³ Expression of FLT3 ITD in heterologous systems induced factor-independent growth and resistance to radiation-induced apoptosis.^{14,15} In addition, class III RTK inhibitors abolished the transformation mediated by constitutively active FLT3.^{16,17} These observations suggest that FLT3 ITD may be involved in the pathogenesis of AML. Recently, it was proposed that FLT3 D835 point mutations, found in 7% of AML patients, likewise may contribute to the low survival rates of the affected patients.^{18,19}

Most of these studies have been performed with primary tumor cells or in heterologous cell systems. Until now, only a relatively small number of AML-derived cell lines has been tested for expression of FLT3 mutations.^{8,20} As continuous cell lines have proven to be valuable tools in many experimental settings, we set out to screen a panel of 69 AML-derived cell lines for FLT3 ITD and D835 mutations. Here, we designate model cell lines for studies of FLT3 mutant and wild-type receptors. Our results indicate that there are differences between the signalling cascades stimulated by wild-type and mutated FLT3 receptors.

Material and methods

Cell culture

The continuous cell lines were either taken from the stock of the cell bank (DSMZ – German Collection of Microorganisms and Cell Cultures)²¹ or were generously provided by the original investigators. The cell lines were cultivated according to the protocols described previously.^{21,22} The following cell lines were tested for FLT3 ITD and D835 point mutations: (1) AML-derived cell lines: AML-193 (derived from patient with AML FAB M5), AS-E2 (M6), CHRFB-288-11 (M7), CMK (M7), CMY (M7), CTS (M1), CTV-1 (M5), ELF-153 (M7), EOL-1, F-36EGM (M6), GDM-1 (M4), GF-D8 (M1), HEL (M6), HL-60 (M2), HML-2 (M7), HNT-34 (M4), HU-3 (M7), IMS-M1 (M5), JOSK-I (derivative of U-937, M5), JOSK-M (derivative of U-937, M5), K-051 (M2), KASUMI-1 (M2), KASUMI-3 (M0), KG-1, KG-1a (derivative of KG-1), KMOE-2 (M6), KP-MO-TS (M5), M-07e (M7), MARIMO (M2), MB-02 (M7), ME-1 (M4), MEGAL (M7), MKPL-1 (M7), ML-1 (M4), ML-2 (sister of ML-1, M4), MML-1 (M1), M-MOK (M7), MOLM-13 (M5), MOLM-14 (sister of MOLM-13, M5), MOLM-16 (M0), MONO-MAC-1 (M5), MONO-MAC-6 (sister of MONO-MAC-1, M5), MR-87, MUTZ-2 (M2), MUTZ-3 (M4), MUTZ-8 (M4), MUTZ-11 (M4), MV4-11 (M5), NB-4 (M3), NOMO-1 (M5), OCI-AML-1 (M4), OCI-AML-2 (M4), OCI-AML-3 (M4), OCI-AML-4 (M4), OCI-AML-5 (M4), OCI-AML-6 (M4), OCI-M1 (M6), OCI-M2 (M6), OHN-GM, OMA-AML-1 (M4), PL-21, PLB-985 (derivative of HL-60, M2), SIG-M5 (M5), SKM-1 (M5), SKNO-1 (M2), TF-1 (M6), THP-1 (M5), TK-1B (M4), TSU-1621MT (M4), U-937 (M5), UCSD/AML-1, UF-1 (M3), UG-3 (M5), UT-7 (M7), YNH-1 (M1), X-376, ie 69 cell lines plus seven sister cell lines or derivatives; (2) CML-derived cell lines: AR-230, EM-2, EM-3

Correspondence: H Quentmeier, DSMZ – German Collection of Microorganisms and Cell Cultures, Mascheroder Weg 1 B, D-38124 Braunschweig, Germany; Fax: +49-531-2616.150

Received 31 May 2002; accepted 12 July 2002

(sister of EM-2), GM/SO, JK-1, K-562, KBM-7, KOPM-28, KU-812, KYO-1, LAMA-84, LAMA-87 (derivative of LAMA-84), MC-3, MEG-01, MOLM-1, MOLM-6, NALM-1, SPI-801 (derivative of K-562), SPI-802 (derivative of K-562), YOS-M, ie 16 cell lines plus four sister cell lines or derivatives. All cell lines were free of contamination with mycoplasmas and were harvested in the logarithmic growth phase with a viability exceeding 85% as determined by trypan blue dye exclusion.

Growth factors, antibodies and inhibitors

The recombinant human (rh) versions of the various cytokines were employed, unless otherwise indicated. Granulocyte-macrophage colony-stimulating factor (GM-CSF) and rabbit anti-STAT-5B antiserum were obtained from R&D Systems (Wiesbaden, Germany). Rabbit anti-FLT3 antiserum was purchased from Santa Cruz (Heidelberg, Germany). Mouse anti-STAT-5 monoclonal Ab (mAb) was obtained from Dianova (Hamburg, Germany). Rabbit anti-phospho-STAT-5A/B antiserum and mouse anti-phosphotyrosine mAb 4G10 were purchased from Biomol/Upstate Biotechnology (Hamburg, Germany). FL was a generous gift of Dr SD Lyman (Immunex, Seattle, WA, USA).

[³H]-thymidine uptake

Assays of ³H-thymidine incorporation were done as follows: 2.5×10^4 cells (in 100 μ l) were seeded in triplicate in 96-well flat-bottom microtiter plates after washing and resuspension in growth factor-free medium. Effectors were added as $2 \times$ concentrated solutions in a 100 μ l volume. For the last 3 h of the incubation period, 1 μ Ci ³H-thymidine (Amersham Pharmacia Biotech, Freiburg, Germany) was added to each well. Cells were harvested on glass fiber filters with a multiple automatic sample harvester, and radioactivity was determined in a liquid scintillation counter.

ITD analysis of the FLT3 gene

Previous studies showed that the location of ITD of the FLT3 gene was restricted to exons 14 and 15 according to the revised nomenclature.²³ Therefore, genomic PCR amplification was performed using the previously described primers; 14 forward: 5'-GCA ATT TAG GTA TGA AAG CCA GC-3' and 15 reverse: 5'-CTT TCA GCA TTT TGA CGG CAA CC-3'.¹⁰ High molecular weight DNA was extracted from the cell lines as previously described.²⁴ PCR was performed using 100 ng of genomic DNA in a total volume of 50 μ l with a DNA thermal cycler (Perkin Elmer Cetus, Heidelberg, Germany) under the following conditions: 30 s at 95°C for denaturation, 30 s at 56°C for annealing, and 2 min at 72°C for extension. The amplified PCR products were electrophoresed in 2.2% agarose gels. Sequencing was performed using the Taq DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems, Weiterstadt, Germany) and an Applied Biosystems model 373A automated DNA sequencer.

ITD analysis of the FLT3 mRNA

First-strand cDNA was synthesized using a reverse transcriptase preamplification system kit (Super Script; Invitrogen,

Karlsruhe, Germany) following the manufacturer's instructions. Reverse transcriptase (RT)-PCR was performed with the same primers and under the same conditions as described above.

Immunoprecipitation and Western blot analysis

Analysis of FLT3 and STAT-5 phosphorylation was done as follows: 1×10^7 cells/analysis were washed free of growth factor-containing medium and cultured overnight in factor-free and serum-free medium (0.1% BSA) prior to stimulation with FLT3 (200 ng/ml in 1 ml), or GM-CSF (100 ng/ml in 1 ml). After stimulation, the cells were pelleted and washed with ice-cold phosphate-buffered saline (PBS). Cells were lysed with 250 μ l lysis buffer (50 mM Hepes, pH 7.4, 10% glycerol, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 1 mM EGTA, 1 mM Na orthovanadate, 25 mM NaF, 2 μ g/ml aprotinin, 5 μ g/ml leupeptin, 20 μ M antipain, 20 μ M pepstatin, 10 mM NaF, 0.2 mg/ml PMSF). Two μ g anti-STAT-5B or anti-FLT3 Abs were added to the precleared lysates. After 1 h rotation (4°C) the immunoprecipitates were collected by adding protein A sepharose (45 min). The immunoprecipitates were washed twice with ice-cold lysis buffer, twice with 0.5 M LiCl, 100 mM Tris-HCl pH 8.0, and boiled for 10 min in 35 μ l SDS sample buffer containing 15% glycerol, 125 mM Tris-HCl pH 6.8, 5 mM EDTA, 2% SDS, 0.1% bromophenol blue and 1% β -mercaptoethanol. The samples (10 μ l for STAT-5 or FLT3 detection; 20 μ l for phosphotyrosine detection) were subjected to SDS-gel electrophoresis on polyacrylamide gels using the Protean II chamber (BioRad, München, Germany). The samples were separated on a 7% gel and electroblotted on to nitrocellulose membranes (Trans-Blot Transfer Medium; BioRad). The membranes were labeled overnight with the respective Abs, and specific bands were visualized with the biotin/streptavidin-horseradish peroxidase system (Amersham) in combination with the 'Renaissance Western Blot Chemoluminescence Reagent' protocol (Du Pont, NEN, Bad Homburg, Germany).

Analysis of FLT3 D835 point mutations

We amplified parts of exon 20 of the FLT3 gene by genomic PCR using the previously described primers; 20 forward: 5'-CCG CCA GGA ACG TGC TTG-3' and 20 reverse: 5'-GCA GCC TCA CAT TGC CCC-3'.¹⁸ Exons were named according to the new nomenclature by Abu-Duhier *et al*.²³ Oligonucleotides were obtained from Invitrogen. PCR conditions were as described for the analysis of the ITD mutations. Amplified PCR products were digested with *EcoRV*, and subjected to electrophoresis on a native 10% polyacrylamide gel, stained with ethidium bromide, and observed under UV light.

Results

FLT3 internal tandem repeats

FLT3 ITD have been described in about 20% of AML cases.⁷⁻¹¹ We have screened a panel of AML and CML cell lines for this mutation by genomic and RT-PCR: 76 AML-derived cell lines (including seven sister cell lines or subclones) and 20 CML-derived cell lines (including four sister cell lines or subclones) were analyzed. No CML-derived cell line, but four

of the AML-derived cell lines (plus one sister cell line) exhibited an ITD. In two of the cell lines (MUTZ-11 and MV4-11), only the mutated allele was expressed, the other lines (PL-21, MOLM-13 and sister cell line MOLM-14) expressed a mutated and the wild-type allele (Figure 1a). In contrast to our studies, PL-21 had been described earlier to express only the wild-type allele.⁸ The mutation found in this cell line appears to be of special interest as the ITD begins in intron 14 and duplicates parts of the preceding exon and the exon/intron transition. Sequencing of the RT-PCR product showed that the mutated allele in cell line PL-21 was transcribed, that a 7 bp insertion preceded the duplication, and that the second exon/intron transition was used as splice-donor site (Figure 1b and c). In the other three cell lines, the ITD was located in exon 14, the mutation did not lead to a shift in the reading frame (legend to Figure 1), but resulted in an exchange of the first amino acid of the repeats (MOLM-13: Val to Phe; MV4-11: Tyr to His; MUTZ-11: Val to Leu). Our results differ from those of earlier studies with respect to the exact localization of the 21 bp ITD in MOLM-13 cells.⁸

FLT3 protein expression

Immunoprecipitation analysis revealed striking differences in the levels of FLT3 protein expression of the cell lines with ITD mutations. Of these, only cell line MV4-11 clearly expressed the FLT3 protein (Figure 2). Supporting the hypothesis that ITD activates the receptor, the FLT3 protein was constitutively phosphorylated, ie also without stimulation by its cognate ligand (Figure 2). As wild-type control, we used MUTZ-2 and OCI-AML-5 cells. These cells grew upon stimulation with FL and expressed the FLT3 protein.^{5,20} The receptor, non-phosphorylated in unstimulated cells, was activated upon stimulation with FL (Figure 2, data not shown for MUTZ-2).

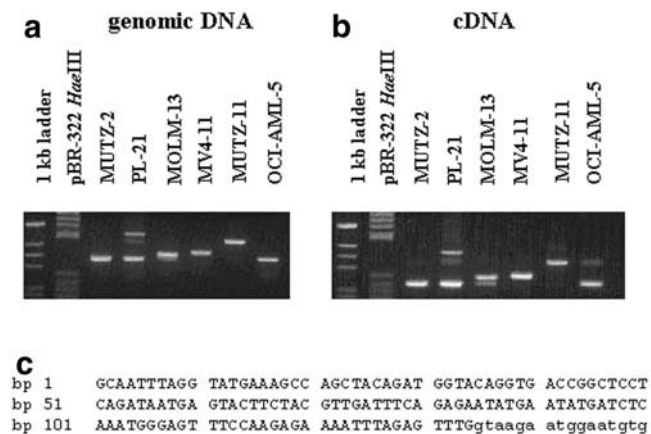


Figure 1 FLT3 ITD in AML-derived cell lines. Four of 69 AML-derived cell lines expressed FLT3 ITD, as assessed by genomic PCR (a) and RT-PCR (b). Cell lines MUTZ-2 and OCI-AML-5 were included as wild-type controls. Localization of the ITD was determined by sequencing (c); MOLM-13: the 21 bp preceding bp 92 were repeated; MV4-11: 30 bp were repeated after bp 98; MUTZ-11: 87 bp were repeated after bp 126; PL-21: 119 bp were repeated after bp 149. Note: in cell lines MOLM-13, MV4-11 and MUTZ-11, ITD were in exon 14, leading to the exchange of one amino acid, but the reading frame was not altered. ITD in cell line PL-21 spanned the exon/intron 14 transition and was preceded by a 7 bp insertion (TCAAATC). Intron sequence is in lower case letters.

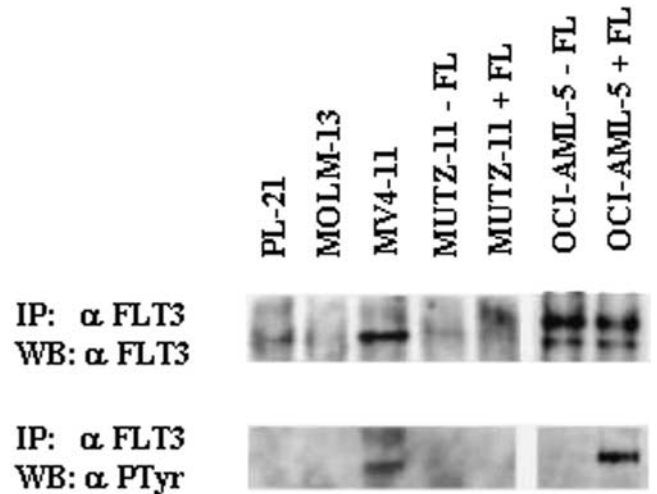


Figure 2 FLT3 phosphorylation in AML-derived cell lines. MV4-11 is the only ITD-positive cell line clearly expressing the constitutively phosphorylated receptor. Wild-type FLT3 receptor molecules expressed in OCI-AML-5 cells are phosphorylated upon stimulation with FL (200 ng/ml, 10 min).

Cell signalling

Recently it has been reported that phosphorylation of the transcription factor STAT-5 was part of the signalling chain induced by FL, eventually leading to cell growth.²⁵ STAT-5 phosphorylation was also observed in a high percentage of AML blasts, and mutationally activated FLT3 was suggested to be a possible cause for activation of this transcription factor.^{14,26} However, there is still an ongoing debate about the role of STAT-5 in FLT3 signalling. One report claims that stimulation of wild-type FLT3 does not result in STAT-5 phosphorylation at all,¹⁴ while others indicate that STAT-5A and STAT-5B molecules may be diversely activated downstream of wild-type and constitutively active receptors.^{25,27} Most of these studies have been performed in heterologous expression systems. Using AML-derived cell lines, we found that MV4-11 cells expressing the mutated FLT3 receptor protein exhibited low levels of constitutively phosphorylated STAT-5 molecules (data not shown). However, FL did not induce STAT-5 phosphorylation in FLT3 wild-type cells, as assessed by Ab recognizing STAT-5B (Figure 3) and STAT-5A/B (data not shown). These observations support the notion that STAT-5 phosphorylation is not an obligatory step in FLT3 wild-type signalling but that it may be a consequence of the FLT3 ITD.¹⁴

Although not activating STAT-5, FL induced phosphorylation of p42/44 MAPK and S6 in cells with wild-type FLT3 (data not shown). These data support previous results showing that FL stimulates ERK and PI3 kinase pathways.^{28,29}

FLT3 D835 point mutations

D835 point mutations of the FLT3 gene have been described in 7% of AML cases.^{18,19} Like ITD, they lead to the constitutive activation of the FLT3 receptor tyrosine kinase.¹⁸ We have screened 76 AML-derived cell lines (including seven sister cell lines or subclones) and 20 CML-derived cell lines (including four sister cell lines or subclones) for FLT3 D835 mutation. To detect mutations at D835, we took advantage of the fact that the gene sequence affected exhibited a GATATC *Eco*RV restriction site.

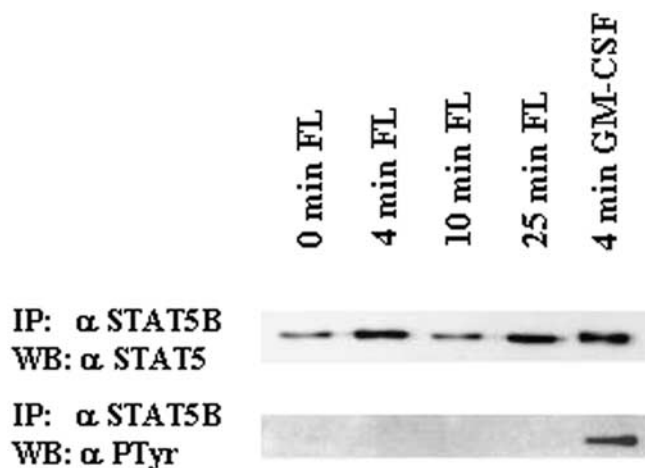


Figure 3 STAT-5 phosphorylation in OCI-AML-5 cells. FL (200 ng/ml, 10 min) does not induce phosphorylation of STAT-5 molecules in FLT3 wild-type cells. Proteins were immunoprecipitated with anti-STAT-5B antiserum. Immunoblots with anti-STAT5A/B antiserum showed identical results. As positive control, cells were stimulated with GM-CSF (100 ng/ml, 10 min).

Thus, products of the PCR amplifying exon 20 were digested with *EcoRV* and subjected to PAGE. The *EcoRV* site was not changed in any of the 96 cell lines tested (data not shown).

Discussion

FLT3, like KIT and other class III RTKs, is expressed in hematopoietic stem cells and progenitors. FL induces growth of many AML cells, suggesting that FLT3 signalling is important not only for normal hematopoiesis but also for the proliferation of leukemic cells.³⁰ Recently, it has been demonstrated that short duplications of the juxtamembrane part of FLT3 lead to the constitutive activation of the receptor and confer a persisting growth stimulus to the cells.^{13,14} ITD occur in about 20% of AML cases,^{7–11} and the affected patients have a decreased chance of cure.^{9,11,12} However, constitutive activation of the FLT3 is not restricted to patients with ITD.³¹ A second FLT3 mutation, a point mutation at D835, likewise contributes to the activation of the receptor.¹⁸ FLT3 D835 mutations have been found in 7% of AML cases.^{18,19} Now, the search is on for a specific and effective inhibitor of the mutationally activated FLT3 for a possible therapeutic intervention.^{16,17}

Most of the studies on FLT3 and FLT3 mutations have been performed using primary cells or transgenic cell systems. However, continuous tumor-derived cell lines are of great advantage in many experimental settings, for example, in cell signalling studies. In primary cell systems, healthy bystander cells may express receptors of the cytokines studied and thus directly or indirectly influence the results of the experiments. The monoclonal origin of cell lines averts these artefacts. Until now, there has been no search for FLT3 aberrations on a large panel of AML-derived cell lines. It was our aim to find cell lines that can be used as model systems for FLT3 signalling studies, expressing the wild-type or mutated forms of the receptor.

We tested 69 AML-derived cell lines (plus seven sister cell lines or subclones) for expression of the FLT3 mutations mentioned. Furthermore, we included 16 CML-derived cell lines (plus four sister cell lines or subclones). The CML-derived cell

lines did not show either mutation. While none of the AML-derived cell lines carried the FLT3 D835 point mutation, five cell lines (MOLM-13 and sister cell line MOLM-14, MUTZ-11, MV4-11, PL-21) showed FLT3 ITD. The percentage of cell lines with FLT3 mutations (5.8%) was clearly lower than the one reported for primary AML cases, suggesting that FLT3 mutations, in spite of the proliferative stimulus they may provide, are rather disadvantageous for immortalization of such cells. Two of the cell lines described here (MUTZ-11 and MV4-11) expressed the mutated version of the gene, but not the wild-type allele. The same observation has been made in 35% of patients with FLT3 ITD.³² Loss of heterozygosity has been shown to explain this phenomenon.³²

Sequencing located the ITD in MOLM-13, MUTZ-11 and MV4-11 cell lines to exon 14. The coding frame was not changed and the mutated versions of the gene were transcribed, as shown by RT-PCR. In cell line PL-21, a 7 bp insert preceded the ITD that was located in intron 14, repeating parts of exon 14 and spanning the transition to intron 14. Of the four cell lines with mutated FLT3 genes, only MV4-11 showed distinct protein expression of the receptor. These results, based on immunoprecipitation studies, might provide an explanation why cell line MUTZ-11 remained cytokine-dependent in spite of the mutated FLT3 gene: the mRNA of this gene was not translated into sufficient amounts of the active protein and could not provide for a growth stimulus. Although these findings do not exclude that the mutated receptor protein was expressed in the originating tumor cell, they still demonstrate that FLT3 ITD are not necessarily expressed as active proteins. This may be of clinical relevance, because the application of a kinase inhibitor can obviously contribute to the cure of the disease only when the kinase is expressed as active protein. Thus, detection of the constitutively activated receptor protein may prove necessary before application of any inhibitor.

Recently, it has been reported that phosphorylation of the transcription factor STAT-5 is an important element of FLT3 signalling.^{14,25} Accordingly, we found that STAT-5 molecules were constitutively phosphorylated in MV4-11 cells expressing the mutated FLT3 protein. However, although FL induced p42/44 and S6 phosphorylation in OCI-AML-5 cells, it did not induce STAT-5 activation. The OCI-AML-5 cell line expresses wild-type FLT3, the receptor becomes phosphorylated upon stimulation with FL, and the cytokine induces growth of these cells. Thus, our results suggest that STAT-5 phosphorylation is not obligatory for FL-induced proliferation.

In conclusion, we found that (1) 4/69 AML-derived cell lines (5.8%) showed an FLT3 ITD, but none of them carried an FLT3 D835 point mutation; (2) cell line MV4-11 is the only one of the FLT3 mutant cell lines clearly expressing the phosphorylated receptor protein, making it a model cell line for FLT3 ITD-related research; and (3) while FLT3 mutant cells exhibit a constitutive phosphorylation of STAT-5 molecules, FL does not induce phosphorylation of STAT-5A or STAT-5B molecules in the FLT3 wild-type cell line OCI-AML-5.

Acknowledgements

We thank Mrs Ina Kramer (DSMZ) for sequencing FLT3 ITD.

References

- 1 Birg F, Courcou M, Rosnet O, Bardin F, Pebusque MJ, Marchetto S, Tabilio A, Mannoni P, Birnbaum D. Expression of the FMS/KIT-

- like gene FLT3 in human acute leukemias of the myeloid and lymphoid lineages. *Blood* 1992; **80**: 2584–2593.
- 2 Carow CE, Levenstein M, Kaufmann SH, Chen J, Amin S, Rockwell P, Witte L, Borowitz MJ, Civin CI, Small D. Expression of the hematopoietic growth factor receptor FLT3 (STK-1/FLK2) in human leukemias. *Blood* 1996; **87**: 1089–1096.
 - 3 Rosnet O, Bühring HJ, Marchetto S, Rappold I, Lavagna C, Sainy D, Arnoulet C, Chabannon C, Kanz L, Hannum C, Birnbaum D. Human FLT3/FLK2 receptor tyrosine kinase is expressed at the surface of normal and malignant hematopoietic cells. *Leukemia* 1996; **10**: 238–248.
 - 4 Meierhoff G, Dehmel U, Gruss HJ, Rosnet O, Birnbaum D, Quentmeier H, Dirks W, Drexler HG. Expression of FLT3 receptor and FLT3-ligand in human leukemia-lymphoma cell lines. *Leukemia* 1995; **9**: 1368–1372.
 - 5 Dehmel U, Zaborski M, Meierhoff G, Rosnet O, Birnbaum D, Ludwig WD, Quentmeier H, Drexler HG. Effects of FLT3 ligand on human leukemia cells. I. Proliferative response of myeloid leukemia cells. *Leukemia* 1996; **10**: 261–270.
 - 6 Stacchini A, Fubini L, Severino A, Sanavio F, Aglietta M, Piacibello W. Expression of type III receptor tyrosine kinases FLT3 and KIT and responses to their ligands by acute myeloid leukemia blasts. *Leukemia* 1996; **10**: 1584–1591.
 - 7 Nakao M, Yokota S, Iwai T, Kaneko H, Horiike S, Kashima K, Sonoda Y, Fujimoto T, Misawa S. Internal tandem duplication of the flt3 gene found in acute myeloid leukemia. *Leukemia* 1996; **10**: 1911–1918.
 - 8 Yokota S, Kiyoi H, Nakao M, Iwai T, Misawa S, Okuda T, Sonoda Y, Abe T, Kahsima K, Matsuo Y, Naoe T. Internal tandem duplication of the FLT3 gene is preferentially seen in acute myeloid leukemia and myelodysplastic syndrome among various hematological malignancies. A study on a large series of patients and cell lines. *Leukemia* 1997; **11**: 1605–1609.
 - 9 Kiyoi H, Naoe T, Nakano Y, Yokota S, Minami S, Miyawaki S, Asou N, Kuriyama K, Jinnai I, Shimazaki C, Akiyama H, Saito K, Oh H, Motoji T, Omoto E, Saito H, Ohno R, Ueda R. Prognostic implication of FLT3 and N-RAS gene mutations in acute myeloid leukemia. *Blood* 1999; **93**: 3074–3080.
 - 10 Kiyoi H, Naoe T, Yokota S, Nakao M, Minami S, Kuriyama K, Takeshita A, Saito K, Hasegawa S, Shimodaira S, Tamura J, Shimazaki C, Matsue K, Kobayashi H, Arima N, Suziki R, Morishita H, Saito H, Ueda R, Ohno R, and the Leukemia Study Group of the Ministry of Health and Welfare (Kohseisho). Internal tandem duplication of FLT3 associated with leukocytosis in acute promyelocytic leukemia. *Leukemia* 1997; **11**: 1447–1452.
 - 11 Meshinchi S, Woods WG, Stirewalt DL, Sweetser DA, Buckley JD, Tjoa TK, Bernstein ID, Radich JP. Prevalence and prognostic significance of Flt3 internal tandem duplication in pediatric acute myeloid leukemia. *Blood* 2001; **97**: 89–94.
 - 12 Rombouts WJC, Blokland I, Löwenberg B, Ploemacher RE. Biological characteristics and prognosis of adult acute myeloid leukemia with internal tandem duplications in the Flt3 gene. *Leukemia* 2000; **14**: 675–683.
 - 13 Kiyoi H, Towatari M, Yokota S, Hamaguchi M, Ohno R, Saito H, Naoe T. Internal tandem duplication of the FLT3 gene is a novel modality of elongation mutation which causes constitutive activation of the product. *Leukemia* 1998; **12**: 1333–1337.
 - 14 Hayakawa F, Towatari M, Kiyoi H, Tanimoto M, Kitamura T, Saito H, Naoe T. Tandem-duplicated FLT3 constitutively activates STAT5 and MAP kinase and introduces autonomous cell growth in IL-3-dependent cell lines. *Oncogene* 2000; **19**: 624–631.
 - 15 Mizuki M, Fenski R, Halfter H, Matsumura I, Schmidt R, Müller C, Grüning W, Kratz-Albers K, Serve S, Steur C, Büchner T, Kienast J, Kanakura Y, Berdel WE, Serve H. Flt3 mutations from patients with acute myeloid leukemia induce transformation of 32D cells mediated by the Ras and STAT5 pathways. *Blood* 2000; **96**: 3907–3914.
 - 16 Tse KF, Novelli E, Civin CI, Bohmer FD, Small D. Inhibition of FLT3-mediated transformation by use of a tyrosine kinase inhibitor. *Leukemia* 2001; **15**: 1001–1010.
 - 17 Levis M, Tse KF, Smith BD, Garrett E, Small D. A FLT3 tyrosine kinase inhibitor is selectively cytotoxic to acute myeloid leukemia blasts harboring FLT3 internal tandem duplication mutations. *Blood* 2001; **98**: 885–887.
 - 18 Yamamoto Y, Kiyoi H, Nakano Y, Suzuki R, Kodera Y, Miyawaki S, Asou N, Kuriyama K, Yagasaki F, Shimazaki C, Akiyama H, Saito K, Nishimura M, Motoji T, Shinagawa K, Takeshita A, Saito H, Ueda R, Ohno R, Naoe T. Activating mutation of D835 within the activation loop of FLT3 in human hematologic malignancies. *Blood* 2001; **97**: 2434–2439.
 - 19 Abu-Duhier FM, Goodeve AC, Wilson GA, Care RS, Peake IR, Reilly JT. Identification of novel FLT-3 Asp835 mutations in adult acute myeloid leukaemia. *Br J Haematol* 2001; **113**: 983–988.
 - 20 Serve H, Flesch K, Serve S, Fenski R, Berdel WE. Expression and function of Flt3/flk2 in human tumor cell lines. *Int J Oncol* 1999; **14**: 765–770.
 - 21 Drexler HG, Dirks W, MacLeod RAF, Quentmeier H, Steube KG, Uphoff CC. *DSMZ Catalogue of Human and Animal Cell Lines*, 8th edn. DSMZ: Braunschweig, Germany, 2001.
 - 22 Drexler HG. *The Leukemia-Lymphoma Cell Line Factsbook*. Academic Press: San Diego, CA, 2000.
 - 23 Abu-Duhier FM, Goodeve AC, Wilson GA, Care RS, Peake IR, Reilly JT. Genomic structure of human FLT3: implications for mutational analysis. *Br J Haematol* 2001; **113**: 1076–1089.
 - 24 Dirks W, MacLeod RAF, Jäger K, Milch H, Drexler HG. First searchable database for DNA profiles of human cell lines: sequential use of fingerprint techniques for authentication. *Cell Mol Biol* 1999; **45**: 841–853.
 - 25 Zhang S, Fukuda S, Lee Y, Hangoc G, Cooper S, Spolski R, Leonard WJ, Broxmeyer HE. Essential role of signal transducer and activator of transcription (Stat)5a but not Stat5b for Flt3-dependent signaling. *J Exp Med* 2000; **192**: 719–728.
 - 26 Birkenkamp KU, Geugien M, Lemmink HH, Kruijer W, Vellenga E. Regulation of constitutive STAT5 phosphorylation in acute myeloid leukemia blasts. *Leukemia* 2001; **15**: 1923–1931.
 - 27 Tse KF, Mukherjee G, Small D. Constitutive activation of FLT3 stimulates multiple intracellular signal transducers and results in transformation. *Leukemia* 2000; **14**: 1766–1776.
 - 28 Srinivasa SP, Doshi PD. Extracellular signal-regulated kinase and p38 mitogen-activated protein kinase pathways cooperate in mediating cytokine-induced proliferation of a leukemic cell line. *Leukemia* 2002; **16**: 244–253.
 - 29 Beslu N, LaRose J, Casteran N, Birnbaum D, Lecocq E, Dubreuil P, Rottapel R. Phosphatidylinositol-3' kinase is not required for mitogenesis or internalization of the Flt3/Flk2 receptor tyrosine kinase. *J Biol Chem* 1996; **271**: 20075–20081.
 - 30 Reilly JT. Class III receptor tyrosine kinases: role in leukaemogenesis. *Br J Haematol* 2002; **116**: 744–757.
 - 31 Fenski R, Flesch K, Serve S, Mizuki M, Oelmann E, Kratz-Albers K, Kienast J, Leo R, Schwartz S, Berdel WE, Serve H. Constitutive activation of FLT3 in acute myeloid leukaemia and its consequences for growth of 32D cells. *Br J Haematol* 2000; **108**: 322–330.
 - 32 Whitman SP, Archer KJ, Feng L, Baldus C, Becknell B, Carlson BD, Carroll AJ, Mrozek K, Vardiman JW, George SL, Kolitz JE, Larson RA, Bloomfield CD, Caligiuri MA. Absence of the wild-type allele predicts poor prognosis in adult de novo acute myeloid leukemia with normal cytogenetics and the internal tandem duplication of FLT3: a Cancer and Leukemia Group B study. *Cancer Res* 2001; **61**: 7233–7239.