Real-Time RT-PCR for the Detection and Quantification of AML1/MTG8 Fusion Transcripts in Patients with t(8;21) Positive AML

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Abstract. AML1/MTG8 was quantified relative to the expression of the GAPDH housekeeping gene by real-time RT-PCR in 22 patients with t(8;21) positive AML at initial diagnosis and in seven of these patients also during/after chemotherapy and allogeneic bone marrow transplantation. Real-time PCR was able to specifically detect and quantify AML1/MTG8 over a 5 log range. The detection limit for t(8;21) positive cells was a dilution of 1:10^5. The AML1/MTG8 expression varied considerably among the 22 AML patients at initial diagnosis with a ratio AML1/MTG8:GAPDH of 0.5135±0.536 (range 0.1 to 2.14, median 0.318). In six patients with t(8;21) positive AML a marked decline of AML1/MTG8 could be induced by chemotherapy. These patients are in ongoing complete hematological remission (CR) with a constant low-level AML1/MTG8 expression. In another patient, a rapid rise of AML1/MTG8 transcripts could be detected in CR after allogeneic bone marrow transplantation and the patient relapsed ten weeks later. In conclusion, real-time RT-PCR is a suitable approach for the quantification of AML1/MTG8 transcripts in the monitoring of AML-patients with t(8;21) during/after chemotherapy and can provide data of prognostic relevance.

1 Introduction

The translocation t(8;21)(q22;q22) is one of the most common structural chromosomal aberrations in patients with acute myeloblastic leukemia (AML). It fuses the 5'-part of the AML1-gene on chromosome 21 to the almost complete MTG8 gene on chromosome 8. The AML1 gene encodes for a transcription factor essential for normal hematopoiesis, whereas the function of MTG8 is still unknown [1]. In all t(8;21) positive patients a constant AML1/MTG8 fusion mRNA can be detected by reverse transcriptase polymerase chain reaction (RT-PCR) [2,3]. The presence of a t(8;21) is associated with a high complete remission rate. However, the impact of this chromosomal aberration on long-term prognosis remains controversial [4-7]. RT-PCR for AML1/MTG8 can be used as a sensitive tool for the detection of residual t(8;21) positive cells in these patients [8]. However, in several studies using RT-PCR for the detection of minimal residual disease most of the patients remained positive for AML1/MTG8 even in long-term complete hematological remission [8-10]. Preliminary data using quantitative competitor PCR suggest, that quantification of the AML1/MTG8 transcripts might be able to detect patients with a high risk of relapse [11,12]. We used a novel quantitative PCR assay, real-time PCR, for the prospective quantification of AML1/MTG8 transcripts in patients with t(8;21) positive AML at initial presentation and after intensive induction and consolidation chemotherapy.

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2 Patients and Methods

2.1 Generation of an AML1/MTG8 standard plasmid

A 338bp DNA-fragment spanning the AML1/MTG8 fusion site was generated by 35 cycles of standard PCR with cDNA from the t(8;21) positive Kasumi-1 cell line as described [3]. This fragment was cloned into the pCR2.1 vector. After UV-quantification, this plasmid was used for the generation of the AML1/MTG8 standard-curves.

2.2 Real-Time PCR for AML1/MTG8

The probe and primers for the AML1/MTG8 real-time PCR (table 1) were designed with the PRIMER-EXPRESS” software (Perkin-Elmer). FAM (6-carboxyfluorescein) was used as the reporter and TAMRA (6-carboxy-tetramethyl-rhodamine) as the quencher dye. The reaction was carried out in 50μl with 1 x Taqman buffer A, 8,5mmol MgCl, nucleotides 200μmol, probe 150nmol, primers 300 nmol each, 0,25U AmpliTaq-Gold Polymerase (Perkin Elmer) and 1μl cDNA-template. The reaction conditions were 95°C 10min and then 40 cycles of 15s 95°C followed by 60s 60°C. A serial dilution of Raji-cDNA with a defined number of GAPDH copies from 10° to 10² was used for the generation of a standard curve.

2.4 Kasumi-1 dilution series

Kasumi-1 cells positive for t(8;21) were serially diluted in t(8;21) negative HL60 cells from 1:0 to 1:10⁵. Total cellular RNA was extracted from 10⁷ cells of this dilution series using the Trizol-method (GibcoBRL). cDNA was synthesised for 1 hour at 37°C using 2μg RNA, random primers (1μmol/l) and murine Moloney virus reverse transcriptase in a total volume of 20μl.

2.5 Detection of AML1/MTG8 transcripts by real-time PCR in patient samples

After informed consent was given, bone marrow samples were taken from 22 patients with t(8;21) positive AML at initial diagnosis (≥80% blasts in the bone marrow). Prior to analysis, the mononuclear cells were enriched by a Ficoll-Isopaque gradient (1,077g/ml). RNA-extraction, cDNA-synthesis and real-time PCR for AML1/MTG8 were performed as described above. AML1/MTG8 was quantified relative to the GAPDH expression in the samples analysed. In seven of the patients also bone marrow samples at various time points during/after chemotherapy were analysed to detect minimal residual disease. These patients were treated with two cycles of induction chemotherapy consisting of standard dose AraC, VP16 and idarubicine. After that, they received a first consolidation cycle of intermediate dose AraC (1g/m², 8 doses) and daunorubicin. For late consolidation, the patients were treated with high dose AraC (3g/m², 12 doses) and daunorubicine [5].

Table 1. Primers and probe for AML1/MTG8 real-time PCR

<table>
<thead>
<tr>
<th>Primer or Probe</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>AML1-Primer</td>
<td>5’-AATCACAGTGGAGTGGGCCC-3’</td>
</tr>
<tr>
<td>MTG8-Primer</td>
<td>5’-TGCGTCTTCACATCCACAGG-3’</td>
</tr>
<tr>
<td>AML1/MTG8-probe</td>
<td>5’-FAM-CTGAGAAGCACTCCACAATGCCAGACT-TAMRA-3’</td>
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