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The expression of Toll-like receptors in patients with acute myeloid leukemia treated with induction chemotherapy

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ABSTRACT

Toll-like receptors play an important role in the host defense against microorganisms. TLRs are mainly expressed in human immune-related cells, such as monocytes, neutrophils, macrophages, dendritic cells, T cells, B cells and NK cells. The expression or up-regulation of TLRs has been demonstrated in some tumors and tumor cell lines but the role of TLRs in pathogenesis and development of acute leukemias remains unclear.

The aim of this study was to evaluate the expression of TLR2, TLR4 and TLR9 and their significance as prognostic factors in patients with acute leukemias treated with induction chemotherapy.

103 patients with newly diagnosed acute myeloid leukemia (AML) were evaluated (47 females and 56 males). The median age of patients was 51 years. Using quantitative reverse transcriptase PCR, the mRNA expression of genes TLR2, TLR4 and TLR9 was measured.

The mRNA expression of TLR2 and TLR4 was significantly higher in patients with NR than in patients with CR and CRi. We especially observed that mRNA expression of TLR2 and TLR4 was significantly higher in patients with myelomonocytic and monoblastic acute leukemia than in patients with other types of AML. The mRNA expression of TLR2 and TLR4 was higher in AML patients than in healthy individuals, although there was no statistically significant difference. Patients with higher mRNA expression of TLR2 and TLR4 had significantly shorter OS than patients with lower mRNA expression of TLR2 and TLR4. Multivariate analysis showed that mRNA expression of TLR2 and the age of patients were independent factors associated with treatment response.

Our results suggest that TLRs could be an independent prognostic factor for response rate after induction therapy in patients with acute myeloid leukemias.

1. Introduction

Toll-like receptors (TLRs) are a family of pattern recognition receptors. TLRs play an important role in the host defense against microorganisms. TLRs are mainly expressed in human immune-related cells, such as monocytes, neutrophils, macrophages, dendritic cells, T cells, B cells and NK cells. Their effect is connected with the secretion of cytokines and chemokines that recruit immune cells and help prevent the expansion of microbes [1]. The expression or up-regulation of TLRs has been demonstrated in some tumors and tumor cell lines. Strong expression of TLR4 and TLR5 was detected in patients with gastric carcinoma [2]. Breast cancer cells and human lung cancer cells demonstrated expression of TLR4 and TLR9 [3–5]. TLR4 may initiate the production of cytokines such as TNF-β, VEGF and proangiogenic chemokine IL-8 in lung cancer cell lines [5].

However, the exact function of TLRs on cancer cells remains unknown. Only few reports explored the potential role of TLRs in hematological malignancies. The plasma cells isolated from patients with multiple myeloma (MM) showed higher expression of TLRs than plasma cells from healthy donors [6]. In MM cells the expression of TLR1, 4, 7 and 9 was found [7]. In B-cell chronic lymphocytic leukemia cells (B-CLL) the expression of TLR7, 8 and 9 occurred [8].

The role of TLRs in pathogenesis and development of acute leukemias remains unclear.

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The aim of our study was to evaluate the expression of TLR2, TLR4 and TLR9 and their significance as prognostic factors in patients with acute myeloid leukemias.

2. Materials and methods

103 patients with newly diagnosed acute myeloid leukemia (AML) were examined (47 females and 56 males). The median age of patients was 51 years (range: 18–85). The diagnosis was performed according to the WHO criteria for AML. There were 57 patients (55%) with acute myeloid leukemias (minimally differentiated, without maturation and with maturation) and 46 patients (45%) with acute myelomonocytic and monoblastic leukemia. All patients were treated with induction chemotherapy including anthracyclines and arabinoside cytosine. 17 patients (17%) had favorable cytogenetic/molecular risk, 32 (31%) patients had intermediate cytogenetic/molecular risk and 34 patients (33%) had poor cytogenetic/molecular risk.

The healthy control group included 20 age-matched individuals (9 females and 11 males). Bone marrow samples in all leukemia patients were taken before induction therapy. Mononuclear cells from AML patients and healthy control group were prepared by Ficoll density gradient separation. We detected CD34+ cells and the purity was confirmed by flow cytometry. Using quantitative reverse transcriptase PCR, the mRNA expression of genes TLR2, TLR4 and TLR9 was measured. To establish the expression of TLRs transcripts in CD34+ cells, mRNAs were isolated with Trizol and cDNAs were prepared with Moloney murine leukemia virus reverse transcriptase. The PCR was performed using ampli-Taq DNA polymerase with denaturation, annealing and elongation. PCR products were separated in agarose gel. The relative quantitation was indicated by cycle threshold (Ct) values. The Ct value of the target genes was normalized (ΔCt) to the Ct value of the GUS gene of the samples.

The results were statistically analyzed using 'STATISTICA 8.0'. Statistical analysis was performed by means of Mann–Whitney's *U*-test and $p < 0.05$ indicated a significant difference. Overall survival (OS) was determined using Kaplan–Meier method. The long-rank test was used to compare the curves. To determine the independent factors of treatment response, multivariate logistic regression analysis was performed. $p < 0.05$ were accepted as statistically significant.

Clinical characteristics of patients are summarized in Table 1.

Table 1
Clinical data of patients with AML.

	103 patients
Gender	47 F/56 M
Median age	51 (range: 18–85)
Diagnosis	AML with minimally differentiated – 6 AML without maturation – 22 AML with maturation – 29 AML myelomonocytic – 34 AML monoblastic – 12
Cytogenetic/molecular risk	Favorable risk – 17 Intermediate risk – 32 Poor risk – 34

Table 2
Correlation between mRNA expression of TLRs and response to induction therapy.

	CR + CRi ($x \pm$ SD) $n = 67$	NR ($x \pm$ SD) $n = 36$	*p*
ΔCt TLR2	0.78 ± 0.82	1.31 ± 0.87	<0.01
ΔCt TLR4	0.28 ± 0.29	0.35 ± 0.35	<0.01
ΔCt TLR9	0.003 ± 0.002	0.003 ± 0.003	ns

n: number of patients; *x*: mean; ns: not significant; SD: standard deviation.

3. Results

In comparison to control group TLR2 and TLR4 mRNA expression was higher in AML patients than in healthy individuals, although there was no statistically significant difference (ΔCt TLR2 0.9 ± 0.85 versus 0.82 ± 0.87 and ΔCt TLR4 0.33 ± 0.23 versus 0.29 ± 0.32).

ORR (overall response rate) in the whole group was 65%. 60 patients (58%) with AML achieved complete remission (CR) after induction therapy, 7 patients (7%) achieved complete response with incomplete marrow recovery (CRi) and 36 patients (35%) had no response (NR). TLR2, TLR4 and TLR9 mRNA were expressed in all samples. The mRNA expression of TLR2 and TLR4 was significantly higher in patients with NR after induction therapy than in patients with CR and CRi. Moreover, we observed that mRNA expression of TLR2 and TLR4 were significantly higher in patients with myelomonocytic and monoblastic acute leukemia than in other types of AML.

The results are shown in Tables 2 and 3.

4. Survival

The median OS in all patients was 9.5 months (range: 1–102 months).

The results are shown in Fig. 1.

The median mRNA expression of TLR2 was 0.702 (Me = 0.702), first quartile was 0.345 (Q1 = 0.345), and third quartile was 1.016 (Q3 = 1.016).

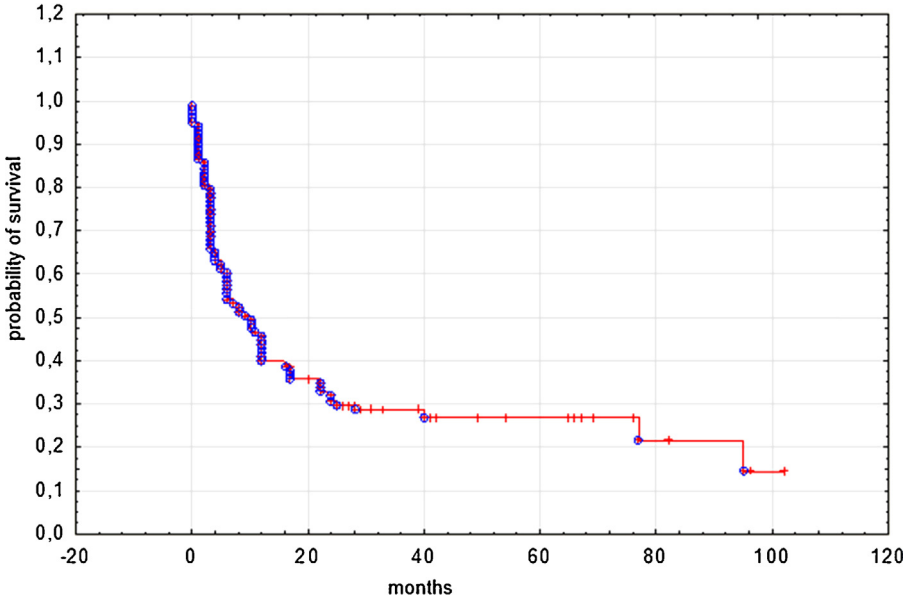

Fig. 1. The Kaplan–Meier estimate of OS in patients with AML.