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Significance of kinetic detection of the PML-RARα fusion gene in children with acute promyelocytic leukemia

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Abstract: Objective To study the significance of kinetic detection of the PML-RARα fusion gene in the treatment of childhood acute promyelocytic leukemia (APL). **Methods** Ten children with APL were involved in this study. They were administered with all-trans retinoic acid (ATRA) alone or with ATRA plus other chemotherapeutic drugs during remission induction, consolidation and maintenance treatments and were followed up. The bone marrow samples were regulary collected for morphological evaluation and PML-RARα fusion gene detection (by RT-PCR assay). **Results** The median follow-up time was 42 months (ranging from 14 to 156 months). The estimated 5-year disease-free survival rate was 56.0 ±18.5 %. Clinical complete remission (CR) was obtained in 9 cases (90 %) and 1 case died on the 4th day after ATRA treatment. Of the 9 cases, 4 cases relapsed 14 - 42 months after CR and 5 cases remained continuous CR. Among the 4 cases with relapse, the PML-RARα fusion gene was positive in 2 cases and it converted into positive in another 2 cases. Of the 5 cases with continuous CR, 3 cases with a positive PML-RARα fusion gene 24, 42 and 36 months after CR were given salvage therapy. After the salvage treatment, the 3 cases had negative PML-RARα fusion gene. **Conclusions** A continuous negative PML-RARα fusion gene may be associated with long-term disease-free survival. The kinetic detection of PML-RARα is important for modifying treatment and for preventing hematological relapse in the treatment of APL.

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Key words: Acute promyelocytic leukemia; All-trans retinoic acid; PML-RARα fusion gene; Child

儿童急性早幼粒细胞白血病 PML-RARα 融合基因跟踪检测的临床意义

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[**摘　要**] **目的**　探讨跟踪检测急性早幼粒细胞白血病(APL)特有的 PML-RARα 融合基因在发现 APL 早期复发和指导 APL 临床治疗方面的意义。**方法**　10 例 APL 患儿用全反式维甲酸(ATRA)和(或)其它化疗药物进行诱导缓解、巩固治疗和维持治疗,并进行随访。在病程的不同阶段采集骨髓标本进行形态学检查,并应用 RT-PCR 方法检测 PML-RARα 融合基因。**结果**　随访时间为 14～156 月(中位时间 42 月),5 年无病生存率为 56.0 % ± 16.5 %。10 例 APL 患儿完全缓解(CR)率为 90 %,早期死亡 1 例。9 例 CR 病人中 4 例在 CR 后 14～42 月复发,4 例在连续完全缓解 4～5 年后已停药,停止治疗时间为 18～96 月。1 例 CR,仍在继续治疗中。9 例 CR 患儿中,8 例在病程中 PML-RARα 转为阴性,1 例持续阳性。4 例复发病人中,2 例复发前持续阳性,2 例在病程中由阴性转为阳性。5 例仍生存的患儿中,1 例在病程中 PML-RARα 由阴性转为阳性,2 例分别在持续完全缓解 36 和 42 月仍呈阳性,这 3 例患儿经治疗干预后均转阴,且长期生存。**结论**　对 APL 患儿跟踪检测 PML-RARα 可早期发现分子复发,及时干预治疗可避免血液学复发。

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[**关　键　词**] 急性早幼粒细胞白血病;全反式维甲酸;PML-RARα 融合基因;儿童

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Acute promyelocytic leukemia (APL) is a specific type of acute myeloid leukemia characterized by the morphology of blast cell, by t(15;17) translocation which fuses the PML gene on chromosome 15 with the retinoic acid receptor (RAR)α gene on chromosome 17 to be the PML-RARα fusion gene, and by specific coagulopathy. The introduction of all-trans retinoic acid (ATRA) and arsenic trioxide (As_2O_3) improved prognosis of APL compared to standard chemotherapy with anthracyclines and cytarabine[1,2]. More than 90 % complete remission (CR) rates of APL have been achieved by chemotherapy combining with ATRA. However, a proportion of patients with CR will eventually relapse and die of recurrent leukemia. Much research has demonstrated that a positive PML-RARα after the consolidation treatment is a strong predictor of subsequent hematologic relapse, whereas repeated negative results were associated with long-term survival in a majority of patients[3]. This study aims to investigate the therapeutic effect of ATRA combining with other chemotherapeutic drugs and the significance of kinetic PML-RARα fusion gene detection in children with APL.

Subjects and methods

Subjects

Ten children with APL and hospitalized between 1989 and 1998 were enrolled in this study. There were 7 males and 3 females with ages ranging from 6 to 12 years old (the average age was 9). Their initial white blood cell (WBC) count of them was 1.6 - 27 $\times 10^9$/L. APL was dignosed using the criteria of the French-American-British (FAB) cooperative study group. Two cases were classified as microgranular or variant (M3v) APL on morphologic examination. Hematologic CR and hematologic relapse were defined according to the criteria of diagnosis and the therapeutic effect for blood disease[4]. Before ATRA treatment, the PML-RARα fusion gene was positive in 7 children. The deadline for follow-up was by the end of December, 2002.

Induction treatment and post-remission treatment

Oral ATRA was administered every 12 hours with daily dosage of 60 mg/m^2 until CR was achieved. CR was defined as 5 % blasts plus promyelocytes in a normal cellular marrow with a normal peripheral blood count and an absence of signs and symptoms of leukemia. The children in CR received 3 courses of consolidation treatment monthly with daunorubicin of 45 mg/m^2 for 3 days and cytarabine of 200 mg/m^2 for 7 days. During the continuation treatment following consolidation, homoharringtonine plus cytarabine were administered in the first month, daunorubicin plus cytarabine were administered in the fourth month, ATRA was administered in the second and fifth months, and 6-mercatopurine (6-MP) plus methotrexate (MTX) and low dose cytarabine were administered in the third and sixth months. Since 1998, during the second continuation treatment, 6MP plus MTX has been administered in the first month, ATRA in the second month and arsenic trioxide in the third month. When the ATRA syndrome occurred, the dose of ATRA was reduced or discontinued temporarily and 10 mg dexamethasone was administered every 12 hours. The same dose of dexamethasone was also administered in the cases whose WBC counts were greater than 5 $\times 10^9$/L, 10 $\times 10^9$/L and 15 $\times 10^9$/L on days 5, 10 and 15, respectively after being treated with ARTA.

Detection of PML-RARα fusion gene

The bone marrow samples were collected for morphological evaluation and PML-RARα fusion gene detection before treatment, after remission induction, after consolidation treatment, respectively, and then followed up every 3 to 6 months. The samples were processed for RNA extraction (by the one-step guanidinum-thiocyanate-phenol-chloroform method) The sequence of oligonucleotides used in this study were as follows: (a) retrotranscription, 5′TGGAGCTGCGCGGAAGAAGCCTTGCAG3; (b) first round: ①3′CTCACAGGCGCTGACCCA, ②5′CCGATGGCTTC GACGACGAGTTCA, ③5′GTCATAGGAAGTGAGGTCTTC. (c) second round: ①3′AGCCTCAGGACTTGTCCTGA, ②5′TTCAAGGTGCGCCTGCAGGA, ③5′GTCATAGGAAG TGAG-

GTC TTC. After the second round, the amount of 10 μl of the PCR mixture was run on a 2.5 % agarose gel, stained with ethidium bromide, and was then visualized with an ultraviolet lamp.

Statistical analysis

Disease-free survival (DFS) is defined as the time from the beginning of complete remission to relapse or death from any cause on patient. Analysis of DFS was performed with the Kaplan-Meier product-limit estimation.

Results

Results of therapy

Of the ten cases, 9 cases (90 %) achieved CR, with a CR median time of 37 days (ranging from 31 to 45 days), and 1 case died of cerebral hemorrhage on the 4th day after starting ATRA treatment. The median follow-up time was 42 months (ranging from 14 to 156 months). Four cases relapsed 14 - 42 months after CR and died, 4 cases survived without treatment for 18 - 96 months after they had CR for 4-5 years and 1 case was still in the continuation treatment. The estimated 5-year DFS rate was 56 ±16.5 %.

PML-RARα fusion gene and clinic outcome

The PML-RARα fusion gene was serially detected in 6 cases (cases 1 to 6) from initial diagnosis and in 3 cases (cases 7 to 9) from 10 to 36 months after CR. Of the 4 cases with relapse, the PML-RARα fusion gene was persistently positive in 1 case and converted to positive in another 2 cases which had a negative PML-RARα fusion gene between 12 to 18 months after CR. The PML-RARα fusion gene was persistently positive before relapse and then converted to negative as relapse in 1 case. This was confirmed to be acute monoblastic leukemia according to morphocytochemical characterization and immunophenotype examination. Three cases (cases 6, 8, and 9) with a positive PML-RARα fusion gene 24, 42 and 36 months after CR were given salvage therapy with idarubicin plus cytarabine (IA) for case 6 and arsenic trioxide for cases 8 and 9. After 6 months of completing salvage therapy, the positive PML-RARα fusion genes converted into negative and they were still in hematologic and molecular CR. See Figure 1.

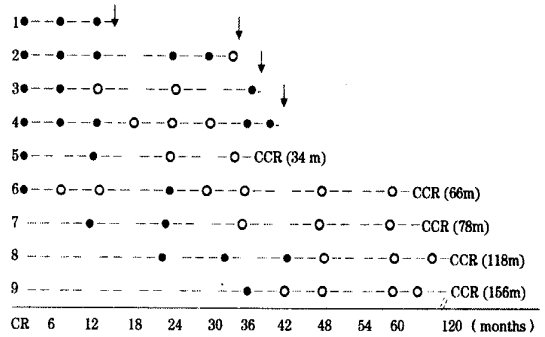

Figure 1 Results of PML/RARa fase gene and clinical outcome in 9 children with APL

PML-RARα positive; PML-RARα negative; ↓ Hematological relapse; CCR: continuous complete remission; m: month

Discussion

ATRA has been demonstrated to be an effective agent for inducing CR in APL[5]. The results in this study are in agreement with previous reports. However, the efficacy of maintenance therapy with ATRA alone after CR is undesirable, with only five months of median CR duration. It has been shown that chronic oral administration of ATRA resulted in a progressive decrease of plasma concentrations, with an increase of urinary elimination of its metabolite which suggests an increased catabolism of the drug. The increased catabolism might be because ATRA can induce cytochrome p450-like enzyme systems and/or increase cytoplasmic retinoic acid binding proteins levels in normal tissues, which could clear the drug from plasma[6,7]. A combination of ATRA with chemotherapeutic agents is thought to be more effective in the maintenance treatment of APL than ATRA alone. The diminution of leukemic clone was achieved by a complementary mechanism of chemotherapeutic killing and ATRA-induced differentiation. Meanwhile, combination therapy may alleviate the side-effects of long-term chemotherapy and reduce leukemic cell resistance to ATRA[5,8].

The PML-RARα fuse gene is a specific molecular marker of APL. Several investigators[8-10] have adopted the term molecular remission, which is defined to be the negative PML-RARα fusion gene in

the bone marrow, as a more advanced therapeutic goal in this disease. On the other hand, molecular relapse after treatment is defined as the reappearance of the positive PML-RARα fusion gene in 2 successive bone marrow samples collected after consolidation treatment and no evidence of disease at the morphologic analysis[11,12]. Diverio et al[13] recently reported the results of a prospective study in 163 patients who were investigated for the PML-RARα fusion gene at regular intervals after the end of treatment. Twenty of 21 patients whose negative PML-RARα fusion gene converted to positive relapsed within a median follow-up time of 3 months, whereas only 8 of 142 patients with at least two successive negative PML-RARα fusion genes relapsed within a median follow-up time of 18 months. In the 4 relapses reported in this study, the PML-RARα fusion gene was persistently positive in 2 cases and converted to positive in another 2 cases through treatment duration. However, relapse can potentially be averted through intensive consolidation with hematopoietic stem cell transplantation, arsenic trioxide, etc. Lo Coco et al[14,15] recently reported that 14 patients were given salvage therapy consisting of ATRA and chemotherapy at the first molecular relapse and 12 of them achieved the second molecular remission. In this study, 3 patients with a positive PML-RARα fusion gene were given salvage therapy with combined chemotherapy or arsenic trioxide and obtained molecular remission and long-term survival. It is suggested that the kinetic detection of the PML-RARα fusion gene is important for modifying chemotherapy regimes and averting relapses in the treatment of APL.

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