The expression of immediate early gene in leukemia

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Abstract

The aim of this study was to analyse the expression of immediate Early Genes (IEG) c-myc, c-myc and c-fos on leukemia cells. The relation of overexpression to survival and progression (prognosis) of the patients and also the possible mechanism of the overexpression were also studied. The expression of acute lymphoblastic leukemia (ALL), acute myeloblastic leukemia (AML) and chronic myeloblastic leukemia (CML) were examined in total RNA by Northern hybridization. The results showed that overexpression of c-myc related to poor prognosis of the ALL, but no difference of a rate of good prognosis between c-myc mRNA alone and c-myc plus c-myc mRNA. C-myc overexpression in ALL, AML, and CML showed poor prognosis and c-fos leukemia patients who did not express c-fos mRNA survived for more than 2 years. However, c-fos expression did not related to the prognosis of leukemia. The mRNA turnover study using Actinomycin D blockade showed that there were prolongation of mRNA turnover of c-myc, c-myc and c-fos more than 75% (normally < 50%).

This study concluded that overexpression of c-myc increased the aggressiveness of the disease (poor prognosis) and the mechanism of this overexpression partly caused by prolongation of mRNA turnover.

Keywords: c-myc, c-myc and c-fos - overexpression - prognosis - leukemia

Introduction

Acute leukemia is a bone marrow based malignancy characterized by maturation arrest of immature myeloid and lymphoid cells and inhibition of normal hematopoiesis (Cavathys et al., 1990). The control of normal growth and differentiation involves a number of signal transduction pathways that transmit extracellular signal to the cell nucleus. These signals can alter gene expression and regulate cell growth by balancing growth promoting and growth arresting factors, which can result in uncontrolled cell proliferation (Bishop, 1991). Growth promoting molecular defects include the presence of mutant protein, the abnormal persistence of certain normal growth promoting factors or a decrease in the activity of growth suppressing proteins. Many of the molecules alteration involve growth promoting cancer genes or oncogenes (Weinberg, 1991).

Oncogenes of c-myc, c-myc and c-fos are members of a class of genes, the immediate early gene (IEG), whose transcription is transiently activated by growth factor stimulation and whose mRNA are specifically targeted for rapid degradation (Zobel et al., 1991). The products of c-myc, c-myc and c-fos genes are nuclear transcription factors which regulate cell growth and differentiation (Strassburg et al., 1992). The c-myc proto-oncogene encodes a helix loop helix DNA-binding protein associated with the control of proliferation (Marcus et al., 1992). This gene is expressed in immature myeloid cells and is down regulated during myeloid differentiation (Greil et al., 1992). Constitutive expression of c-myc inhibits myeloid maturation and inhibition of c-myc expression by c-myc antisense oligomers induces maturation of leukemia cell lines (Selvanayagam et al., 1988). The c-myc proto-oncogene is another nuclear proto-oncogene encoding a DNA-binding protein, which regulates myeloid maturation (Machinski et al., 1991). c-myc down regulation has been shown to be necessary to induce maturation of leukemia (Selvakumaran et al., 1992). Overexpression of those protooncogenes has been reported in many leukemia cells that may affect prognosis of the disease. However, the role of overexpression in the pathogenesis and its relation with prognosis of leukemia cells is not clearly resolved.

This study is aimed to analyse the expression of IEG, the possible mechanism of the overexpression and prognosis of the disease.

Materials and Methods

Subjects. Subjects for this study consisted of 52 patients, clinically diagnosed as leukemia based on classification of French American British criteria. All patients gave informed consent before acquisition of peripheral blood and bone marrow samples for study. All studies were approved by the Institutional Board Review. The patients were treated using standard therapy.

Preparation of cell. Peripheral blood and bone marrow cells were aspirated using syringes containing sodium citrate, which were immediately placed on ice. Mononuclear cell fraction were isolated by centrifugation over 0.77 density Ficoll Hypaque. Differential cell counts were performed on Wright-Giemsa stained of the mononuclear cells.

mRNA turnover analysis. mRNA turnover was analysed using actinomycin C transcription blockades. Peripheral blood mononuclear cells were cultured in RPMI 1640 medium with 10% FCS and 100 µg/ml actinomycin D at 37°C in 5% CO2 incubator. RNA was prepared from 5x10^6 cells, and harvested at 15', 30', 60', and 75' after cultured (Iwa et al., 1991).

Northern analysis. Pellets of mononuclear cells were dissolved in guanidium isothiocyanate (GTC). Total 10 µg of RNA was electrophoresed in 1% agarose gel with formaldehyde. The RNA in the gel was blotted into a Hybond N-membrane (Amersham) by capillary blotting. A baked filter was prehybridized and then hybridized overnight with Digoxigenin (Dig) labelled cDNA probe of c-myc, c-myc and c-fos (Boehringer Mannheim). After washing the filter, the remaining probes were detected by chemiluminescent detection system using anti-Dig antibody. The filter was exposed to KODAK X-Omat AR film at room temperature using an intensifying screen (Chomczynski et al., 1987).

The Dig labelled cDNA probes. These probes for human c-myc, c-myc and c-fos were prepared by polymerase chain reaction (PCR). Fragments of 250 bp of PstI/Pvu from the exon 2 of human genomic c-myc 532 bp of EcoRI/BamHI fragment of the human c-myc cDNA and 800 bp of Apal fragment from the exon 3 of the human genomic c-fos were amplified with specific primers in the presence of Dig labelled dCTP by PCR (Chomczynski et al., 1987).
Results and Discussion

Leukemia samples. Mononuclear cells were isolated from peripheral blood of leukemia patients before treatments. More than 30% of these cells were leukemia blast cells. Type of leukemia cell was diagnosed by morphological examination based on the FAB classification. Leukemia cells used in this study were summarized in Table 1.

Table 1. A list of leukemia patients. Leukemia was classified according to the FAB classification.

<table>
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<th>Case</th>
<th>ALL</th>
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The expression of c-myc, c-myc, and c-fos proto-oncogenes were analyzed in total RNA isolated from leukemia cells by Northern blot. Figure 1 shows the 2.3 Kb band for c-myc RNA, 3.8 Kb band for c-myc mRNA and 2.2 Kb band for c-fos mRNA were detected in samples on the filter. Those strong expressions on the samples were determined as positive expression although those three mRNA were not detected in samples from healthy persons (data not shown). In summary shown in Table 2, expression of c-myc mRNA was detected in about 50% of leukemia cells (40% in ALL, 60% in AML and 45% in CML). Almost all of the leukemia cells which expressed c-myc or c-fos mRNA also expressed c-myc mRNA which is predominantly expressed in acute type of leukemia.

![Figure 1. Oncogene expression in seven leukemia cell lines by Northern analysis.](image)

Figure 1. Oncogene expression in seven leukemia cell lines by Northern analysis. c-myc (a), c-myc (b), and c-fos (c) mRNA expression were detected in 10 of total RNA. Lane 14 were ALL and lane 5-7 were AML. Eshamidrome binding assay of the cell was shown at the bottom of the figure.

A relation between the proto-oncogene expression and prognosis of the disease. Since expression of the c-myc, c-myc and c-fos genes involved in the proliferation and differentiation of hematopoietic cells (Cattovsky et al., 1991; Tsa et al., 1991; Yang et al., 1992) overexpression of those genes might affect growth and differentiation of leukemia cells and prognosis of the disease. To examine whether expression profile of those genes affected the prognosis, the proto-oncogene expression-profile shown in Table 2 was compared with prognosis of the patients. All of the patients were given standard regimen therapy after they were diagnosed. The patients who survived for more than 2 years after the diseases was diagnosed were described as good prognosis in this study. The results were summarized in Table 3. The patients with good prognosis were 37% of total leukemia patients examined. In ALL, expression of c-myc mRNA made prognosis very poor (13%). Expression of c-myc (29%) or c-fos mRNA (40%) did not affect prognosis. Negative expression of c-myc mRNA in ALL was detected only in one patient who showed good prognosis. In AML, expression of c-myc mRNA in ALL was detected only in one patient who showed good prognosis. In CML, percentage of the good prognosis was rather high (57%). Expression of c-myc or c-fos mRNA did not change the prognosis. No-expression of c-myc mRNA demonstrated high percentage (10%) of the good prognosis. In total, all of the 4 patients with leukemia cells which did not express c-myc mRNA survived for more than 2 years. Logistic regression analysis indicated that the probability of death in patients who expressed c-myc was about 400 times more than that in patients who showed negative c-myc expression. These findings suggest that c-myc expression indeed increase the level of aggressiveness.

Table 2. Oncogene expression in leukemia cells. C-myc positive expression was abnormal in 95% (21 cases in ALL, 96% (22 cases) in AML and 71% (5 cases) in CML. C-myc positive expression was observed in 36% (8 cases) of ALL, 39% (9 cases) of AML and 14% (1 cases) of CML. C-myc positive expression was observed in 45% (10 cases) of ALL, 61% (14 cases) of AML and 43% (5 cases) of CML.

![Table 2](image)

The relation in the patients of leukemia cells with c-o expression of more than two of those proto-oncogenes was shown in Table 4. In general, there was no significant difference of the prognosis in leukemia cells between expression of c-myc mRNA only and coexpression of c-myc mRNA and the others. More data were needed to confirm these results.

Table 3. Relation between oncogene expression and good prognosis. Those patients with negative expression 100% of c-myc (ALL, AML and CML) showed good prognosis, achieved remission and survived for more than 2 years.

![Table 3](image)

Prolongation of mRNA turnover. Overexpression of c-myc mRNA was found in many leukemia cells, suggesting that c-myc played a critical role in multistage of leukemogenesis (Marcu et al., 1992; Selvakumar...
et al., 1992). Although constitutive activation of the c-myc gene disrupts the growth regulation of cell and has been proposed as an essential factor for carcinogenesis, mechanisms of leukemogenesis by the c-myc overexpression are still unknown (Marcu et al., 1992). Evidence showed that expression of c-myc mRNA in leukemia cells correlated with poor responsiveness to therapy and with shorter duration of remission. This phenomenon was also observed in the level of c-myc protein in bone marrow cells from AML and CML patients. Our study also support the notion since a rate of the good prognosis of the patients who suffered from c-myc overexpressed leukemia cells was poor and all four leukemia patients who did not express c-myc mRNA survived for more than two years. Therefore, expression of c-myc mRNA affect the level of aggressiveness of the disease. Enan et al (1992) revealed that constitutive c-myc expression disrupted the regulation of cell cycle progression.

Elevated c-myc expression had been observed in human multiple myeloid by the c-myc gene translocation (Selvakumaran et al., 1992; Yang et al., 1992; Baer et al., 1992). However, there was no translocation of Fc c-myc gene (chromosome 14) in leukemia cells examined in this study by karyotype analysis (Haryana et al., 1994; Baer et al., 1992). Abnormal proliferation of the c-myc mRNA turnover in some of the AML since there was a truncation of the 3' end of the mRNA which enhanced stability of the mRNA (Cavovsky et al., 1991). They also reported that enhanced c-myc stability was due to cytogenetic abnormality. Brewer (1991) reported that destabilizing element was a protein that bind to the AU rich element in 3' untranslated c-myc RNA. Our study showed that mRNA turnover of c-myc and c-myb is prolonged more than 75 minutes (Figure 2).

Oncogenic potential of the deregulated c-fos in leukemia cells is still controversial (Tsai et al., 1991). Overexpression of c-fos transforms fibroblasts in vitro (Curran et al., 1983). Many c-fos transgenic mice were generated to examine oncogenic activity of the deregulated c-fos in various organs and cell lineages in vivo (Johnson et al., 1992). Several lines of those mice developed bone hyperplasia and bone tumors. However, no malignant transformation of lymphocytes was induced in the mouse. In our study two out of two patients with leukemia cells that expressed c-fos mRNA alone survived well, suggesting that c-fos expression did not affect the aggressiveness of the disease.

In conclusion, this study showed that c-myc overexpression at the onset of the disease in leukemia increased the aggressiveness of the disease and therefore might be used as an indicator of prognosis and the overexpression was partly caused by prolongation of mRNA turnover.

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