INHIBITION OF APOPTOSIS BY bcr-abl FUSION GENE IN K562 CELLS

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ABSTRACT

Objective: To investigate the effect of bcr-abl fusion gene on CML cell apoptosis. Methods: Apoptosis of *ex-vivo* cultured K562 cells were observed after exposure to synthetic 18 mer antisense oligodeoxynucleotide complementary to the bcr-abl junction (b3a2). Results: Apoptosis of K562 cells was significantly increased associated with inhibition of bcr-abl expression. Conclusion: bcr-abl fusion gene formation due to chromosome translocation may be the major mechanism of CML via inhibition of apoptosis.

Key words: Chronic myeloid leukemia, bcr-abl fusion gene, Apoptosis, Antisense oligodeoxynucleotides.

Chronic myeloid leukemia (CML) is a hematological malignancy characterized by an initial chronic phase of expanded clonal hematopoiesis with continued differentiation into mature myeloid cells. Its cytogenetic hallmark is the philadelphia chromosome (Ph), or t(9; 22) (q34.1; q11.2); this balanced translocation resulted in the creation of a chimeric bcr-abl gene, which is the molecular biology feature of CML. The bcr-abl has been proved to cause human CML-like syndrome in mice. To elucidate the effect of a chimeric bcr-abl gene in CML, we observed the apoptosis of K562 cells after exposing ex-vivo cultured K562 cells to synthetic antisense oligodeoxynucleotides (ASODN), and inquired into the pathogenesis of CML.

MATERIALS AND METHODS

MATERIALS

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K562 cell lines were kindly provided by Professor JIN Bo-quan. Antisense oligodeoxynucleotide (ASODN) sequences used were as follows: b3a2ASODN 5'-GAAGGGCTTTTGAACTCT-3'; N-ASODN 5'-CAT-TTCTTGCTCTCCACG-3' (Shanghai Institute of Cell Biology).

Cell Culture

K562 cell lines were maintained in 5% CO_2 atmosphere at 37°C with RPMI 1640 5% fetal bovine serum. Exponentially cells growing were used in all experiments.

Experimental Groups

Experimental groups included control, N-ASODN and ASODN groups.

Cell Viability Assay

K562 cells (1×10^5/ml) were incubated with different concentrations of ASODN or N-ASODN (10, 20, 40 μmol/L) in 96-well flat-bottomed plates for 24, 48, 72 h. Viable cell numbers ($\bar{x}\pm s$) were determined daily by using a hemocytometer and trypan blue dye.

Cell viability = viable cells / (viable cell + dead cells) × 100%

Morphology

The cells stained with Wright-Giemsa were observed under optical microscope.

Flow Cytometric Assays

Cells (2×10^6) were washed with 1×PBS (pH 7.4), fixed in 70% ethanol and maintained at 4°C. Before quantified by flow cytometric analysis, cells were centrifuged, resuspended and stained with propidium iodide. The fraction subdiploid cells with oligo-nucleosomal DNA degradation characteristic of apoptosis was quantified by flow cytometric analysis.

Assays of Expression of bcr-able Gene

Cell (1×10^6/ml) were exposed to ASODN or N-ASODN with a concentration of 40 μmol/L for 24 h. RNA was isolated by acid guanidinium thiocyanate-phenol-chloroform-extraction and amplified by nested RT-PCR.[1] Reaction products were electrophoresed in 1.5% agarose gel.

RESULTS

Viability of K562 Cells

After being exposed to ASODN, the viability of the cells was decreased. The effect of ASODN was dose-dependent. The viability of cells exposed to N-ASODN, compared with the cells in control group was not decreased significantly.

Morphological Changes of Cells in ASODN Group

After 72 h of treatment with 40 μmol/L ASODN, a part of the cells changed into apoptotic cells, including a reduction in cell volume, cytoplasmic condensation, compaction of chromatin, fragmentation of the nucleus into discrete masses, and separation of the cell into distinct membrane-bound vesicles (apoptotic bodies). By flow cytometric assays sub-2N DNA peak (apoptotic peak) could be observed. In contrast, apoptotic peak could not be observed in the N-ASODN group.

Inhibition of the Expression of bcr-abl Gene

The bcr-abl transcript was barely detectable after 24 h of exposure of cells to 40 μmol/L ASODN in when comparison with the same concentration of N-ASODN. The result showed that ASODN inhibited the expression of bcr-abl gene. The inhibition was antisense sequence-specific.

DISCUSSION

CML is characterized by a large accumulation of relatively mature myeloid cells in the peripheral blood. The mechanism of abnormal cell growth in CML was presumed to be deregulation of cell proliferation by bcr-abl expression. However, recent studies have found that relative rates of cell proliferation are not increased in CML. CML progenitors do not manifest greater proliferative potential than normal progenitors[2] and display a normal proliferative response to growth factors.[3] The induction of P210 expression alone is unable to generate growth factor-independent proliferation of hematopoietic progenitors.[4] Deregulated proliferation does not appear to explain the myeloid expansion in CML. Therefore, it is reasonable to assume that malignant growth depends on an imbalance between the rate of cell survival and the rate of cell apoptosis.

The term of apoptosis is derived from a Greek word describing for the leaves dropping off of trees. Apoptosis is different from necrosis. It is a physiological mode of cell death where the cell actively participates in its own death process, and is a genetically regulated process. Apoptosis keeps a balance between cell proliferation and cell death. Recent reports have shown that deregulated apoptosis plays an important role in tumors. Our results indicate that apoptotic K562 cells increased obviously after inhibition of expression of bcr-abl gene by exposing K562 cells to ASODN, and this resulted in prolonged cell viability. Therefore, bcr-abl-mediated inhibition of apoptosis may be the primary mechanism in CML.

Internucleosomal DNA cleavage leading to the formation of DNA ladders in agarose gels is a hallmark of apoptosis. Here we observed that key morphological changes of apoptosis were dissociated from DNA ladder. The result was in accordance with McGabon's.[5] A number of recent reports have demonstrated that apoptosis can not occur in the absence of double-stranded breaks but only in the presence of single-stranded breaks. The lack of double-strand breaks in K562 is unclear but it is reasonable to assume that either the activity of the endonuclease is inhibited or the enyme is simply not present in these cells. Support from the former comes from a report by Wyllie[6] who demonstrated that ras-transfected cells lost their endonuclease activity, suggesting that the expression of the ras protein could interfered with the endonuclease activity.

The mechanisms of resistance of bcr-abl to apoptosis are still not completely understood. Bedi et al.[7] reported that after irradiated with ionizing radiation, Ba-F_3 and FDC-P_1 cells underwent apoptosis, while Ba-F_3^{D210} and FDC-P_1^{D210} cells (cell lines induced to express $p210^{bcr\text{-}abl}$ by retroviral infection with a full-length bcr-abl cDNA sequence) remained viable. Meanwhile, they found that the inhibition of apoptosis of irradiated Ba-F_3 and FDC-P_1 cells by enforced bcr-abl expression was associated with a prolonged arrest at the G_2/M transition. This delay of G_2/M transition induced by bcr-abl expression may grant time to repair and complete DNA replication, thereby preventing a mitotic catastrophe and inhibiting apoptosis.

In conclusion, our research has further proved that bcr-abl maintains resistance to apoptosis in CML cells and might provide an important way to investigate the treatment for chronic leukemia through switch-on of apoptosis via the effect of ASODN on bcr-abl fusion gene.

REFERENCES

[1]. Huang Wei, Wang Weicheng, Cao Qi, et al. Study on the significance of the use of "nested" polymerase

chain reaction in detection the bcr-abl fusion gene transcripts expression. Chin J Hematology 1992; 13:183.

[2]. Strife A, Clarkson B. Biology of chronic myelogenous leukemia: Is discordant maturation the primary defect? Semin Hematol 1998; 25:1.

[3]. Emanuel PD, Bates LJ, Castleberry RP, et al. Selective hypersensitivity to granulocyte-macrophage colony-stimulating factor by juvenile chronic myeloid leukemia hematopoietic progenitors. Blood 1991; 77:925.

[4]. Gishizky ML, Witte ON. Initiation of deregulated growth of multipotent progenitor cells by bcr-abl *in vitro*. Science 1992; 256:836.

[5]. McGahon A, Bissonnette R, Schmitt M, et al. bcr-abl maintains resistance of chronic myelogenous leukemia cells to apoptotic cell death. Blood 1994; 83:1179.

[6]. Wyllie AH. Apoptosis. Br J Cancer 1993; 67:205.

[7]. Bedi A, Barber JP, Bedi GC, et al. bcr-abl-mediated inhibition of apoptosis with delay of G_2/M transition after DNA damage: a mechanism of resistance to multiple anticancer agents. Blood 1995; 86:1148.