The Role of p38 Mitogen–Activated Protein Kinase in the Pathogenesis of Chronic Myelogenous Leukemia

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Abstract **Objective** To study the the role of p38 mitogen–activated protein kinase(p38MAPK) in the pathogenesis of chronic myelogenous leukemia(CML). **Methods** After bcr3/abl2 antisense oligodeoxynucleotide(ASO) was introduced into K562 cell line by liposomal transfection, the effect was evaluated by apoptotic rate of K562 cell, p38MAPK activity, p38MAPK expression and p38MAPK content. **Results** The rate of apoptotic cells, the activity, expression and content of p38MAPK were 3.9, 4.4, 3.2 and 3.8 times higher than that in control groups respectively (P<0.05) after bcr3/abl2 ASO was introduced into K562 cell 48 hours. **Conclusion** p38MAPK plays an important role in the pathogenesis of chronic myelogenous leukemia by directing apoptotic signal transduction.

Key words Leukemia; Antisense oligodeoxynucleotide; Mitogen–activated protein kinase; Signal transduction

Mitogen–activated protein kinase (MAPK) signal pathway has been more studied in recent years. In the eukaryotic cells, extracelluar singal–regulated kinase pathway, JNK/SAPK(JunN–terninal kinase/stress–activated protein kinase)pathway, ERK5 pathway and p38MAPK pathway were identified. They were involved in physiological processes, such as cell growth, development, division, intercellular synchronization functions, as well as pathologic processes of cells malignant transformation [1]. p38MAPK plays an important role both in cell stress, inflammatory reaction and development, differentiation, apoptosis [2]. Chronic myelogenous leukemia (CML) is the result of the oncogene bcr/abl encoding P210 by inhibiting apoptosis [3]. Related reports of p38MAPK in the pathogenesis of chronic myelogenous leukemia are different at present, the aim of this experiment was to study the role of p38MAPK in the pathogenesis of chronic myelogenous leukemia in order to approach a more effective way of research and therapy of CML.

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MATERIALS AND METHODS

Materials

Fully phosphorothioated antisense oligodeoxynucleotide (ASO) sequence complementary to nine bases both sides of the bcr abl junction (b3/a2) is 5′–GAAG GGCTTTTGAACTCT–3′; Unrelated oligodeoxynucleotide sequence having no homology with human gene including bcr/abl after computered is 5′–CATTT CTTGCTCTCCAC–3′. The above sequences were synthesized by Takara Corporation. RPMI1640, liposomes, rabbit anti–human MAPK monoclonal antibodies, peroxidase conjugated goat anti–rabbit secondary antibody(IgG) were produced by Sigma Corporation. Trizol regent, p38MAPK activity assay kit., [γ–32P]ATP (111TBq/mmol/l), TritonX–100 were produced by American GIBCO Corporation. K562 cells were cultured by RPMI1640 culture medium containing 5% calf serum.

Methods

ODN liposomal transfection Liposome–encapsulated ODN solution was prepared by 4μmol/L ODN and 80μg/ml liposomal(Lipo) solution of equal volume. Cultured 10 hours with 1μmol/l ODN and 20μg/ml Lipo in the culture medium, then abandoned the supernatant. Added the same ODN culture medium and

experimented after 48 hours.

Experiment Groups This experiment includes four groups: control group (only with cell suspension, Con); mutant decoy ODN group (Non); liposomal group (Lipo)and treated group (ASO) randomly. Three wells were designed in each group and took the average value.

Detection of cell apoptosis With the DNA fragment content detection, 3×10^6 cells in each group were collected, then washed them three times with PBS, filtered cells by adding 0.2%Triton X-100. Supermatant and precipitation contained fragment DNA and completed DNA respectively after high speed centrifugation. Both required the additon of 12.5%trichloroacetic acid to denature, then disolved in 80μl 5% trichloroacetic acid, 90℃ pyrolyzed 10min. The DNA was colour quantitative analyzed with the diphenylamine reagent method (590nm). Fragment DNA(%)= fragment DNA/(fragment DNA +completed DNA) × 100%.

Detection of p38MAPK activity With the method of γ-32P incorporation, measured the radioactivity with β liquid scintillation counter according to p38MAPK activity assay kit and the method introduced in references 4.

Detection of p38MAPK expression 3×10^6 cells were collected in each group, abandoned the supernatant after centrifuge(1500rpm, 5min) and washed once with PBS. Then fixed with 80% precooling ethanol for 2 hours and washed three times with PBS containing 1% bovine serum albumin. Suspension cultured in PBS with 0.25% TritonX-100 dyeing application liquid. Centrifuged and discarded the supernatant after ice bath 5min, then added rabbit anti-human MAPK monoclonal antibodies (first antibody)diluted by PBS(1:2000) containing 1% bovine serum albumin, incubated overnight at 4℃. Finally, washed twice with PBS, then added goat anti-rabbit second fluorescent antibody by diluting 1:50, incubated for 30min at 37℃, centrifuged and discarded the supernatant, suspension cultured in 200μl PBS. The expression of p38MAPK was detected with FCM.

Detection of P38MAPK Content Used western blot method. Transferred 25μl cell lysis buffer to nitrocellulose membrane by. Marker the standard protein by the ponceau G stain. Sealed 1h at 37℃ in PBST containing 5% (Wt/Vol) BSA. Added first antibody (rabbit anti-human MAPK monoclonal antibodies, 1:2000) and second antibody (peroxidase conjugated goat anti-rabbit IgG, 1:1000), then incubated for 1h at 37℃. Membrane and chemiluminescence reacted for 5 min, then exposed X-ray. Detect light density in western blot band by laser light density scanner after development and fixation.

Statistical analysis The result was analyzed with $x\pm s$, Comparison between groups was performed by *t*-test while rate variation was performed by χ^2 test, when $P<0.05$ was considered as statistically significant.

RESULTS

Cell Apoptosis

After corresponding treatment, fragment DNA content(%): Con groups is 7.5±1.1, Non groups is 9.0±1.4, Lipo group is 7.8±1.3, ASO group is 36.7±1.6 respectively (compared with other three groups, $P<0.05$).

Table 1 Effects of ASO on MAPK of K562 cells($x\pm s$)

Group	MAPK activity	MAPK expression	MAPK content
Con	10.1±1.1	0.45±0.3	0. 76±0.1
Non	11.3±1.8	0.46±0.4	0.62±0.6
Lipo	10.7±2.9	0.43±0.6	0.78±0.5
ASO	54.5±1.4★	1.90±0.1★	3.66±0.6★

Note: Compared with other three groups, ★$P<0.05$

Activity of p38MAPK

After 48 hours treatment by ASO, each group MAPK activity is different(as table 1). ASO group is 4.4 times higher than Con group.

Expression of p38MAPK

After 48 hours treatment by ASO, cell absorbance value in each group is different. ASO group is 3.2 times higher than Con group (as table 1).

Content of P38MAPK

The luminous intensity in each zone is different with western blot method, ASO group is 3.8 times higher than Con group (as table 1).

DISCUSSION

It is generally accepted that bcr/abl gene inhibiting apoptosis is fundamental pathogenesis of chronic myelogenous leukemia(CML). It has become the ideal model of anti−apoptosis mechanism study that antisense bcr/abl oligonucleotide introduced into CML cell can inhibit the expression of bcr/abl protein and induce apoptosis. The content of the specific fragment DNA in apoptosis cells significantly increased showed that apoptosis happened during this experiment.

p38MAPK pathway is one kind MAPK pathway found in 1993. It showed that the specific activator−PMA for lipopolysaccharide, paclitaxel and PKC, can induce strongly the activation of p38 MAPK[5]. In 1994, Han *et al*[6] firstly cloned p38MAPK gene in mouse liver cells and found it expressed in both macrophagocytes and lymphocytes. p38MAPK was usually activated by ultraviolet, high osmotic environment, arsenic salt, heat shock, H_2O_2, cytokine and physiological stress and so on, then started transcription by displacing to corresponding transcription factor. p38MAPK played an important role both in inflammatory reaction and cell development, differentiation and survival. Sen *et al*[7] found that IL−1 and TNF−α can activated p38MAPK in thymocyte and it proved that p38MAPK participated in the thymocyte development and differentiation. p38MAPK also played an important role in regulating apoptosis. Xia *et al* [4] found that p38MAPK pathway action can lead to nerve cell apoptosis while EPK pathway action can inhibit the apoptosis. Some studies showed that p38MAPK activity increase in tumor cells and can induce apoptosis. p38MAPK can be activated in one hour and may be participate in starting the apoptosis during human colon cancer cell SW480 apoptosis which xenograft to athymic mice induced by Pc4 light treatment [5]. p38MAPK can be activated and may be directing apoptosis during colon adenocarcinoma cell apoptosis by withdrawal serum [8]. Studies on glioma showed that dopamine, high osmotic environment and LPS can act p38MAPK. Hernandez *et al*[9] found that p38MAPK can make TNF−α induce cPLA2 phosphorylation and participated in retinoic acid metabolism in astrocytoma lines, which suggested that p38MAPK expressed and acted in astrocytoma. Ozaki *et al* [10] detected the p38MAPK activity increased in the study of human glioma apoptosis induced by PKC specific inhibitor−calphostin C, but it was not the necessary for calphostin C to induce apoptosis.

In this study, we introduced the bcr3/abl2 ASO into K562 cell line by liposomal transfection method and built CML cell apoptosis model successfully to further elucidate the role of p38 MAPK in the pathogenesis of chronic myelogenous leukemia. We found that p38MAPK has a certain low−level amount in normal K562 cells. Its activity, expression and content were 10.1±1.1, 0.45±0.3 and 0. 76±0.1 with γ−32P incorporation, FCM and western blot detection, and they increased to 54.5±1.4, 1.90±0.1 and 3.66±0.6 respectively after transfected bcr/abl anti −sense oligodeoxynucleotide, which showed that sealed bcr/abl expression can induce p38MAPK action. p38MAPK plays an important role in CML cell apoptosis induced by bar/abl antisense oligodeoxynucleotide. The apoptotic signal may be transported by p38MAPK pathway. In other hand, bcr/abl cancer gene to inhibit CML cell apoptosis may be realized by inhibiting p38MAPK activity. The result also suggested that p38MAPK may be become the new target in CML treatment, that is to realize treating CML by regulating p38MAPK activity.

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