Resistance of SKW6 cell to apoptosis induction with anti-Fas antibody upon transduction of a reverse fragment to a cDNA encoding human 6A8 α-mannosidase

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Abstract The effect of transduction with a reverse fragment to a cDNA encoding human 6A8 α-mannosidase on apoptosis induction of human B cell line SKW6 by anti-Fas antibody was tested. Apoptosis-inducer of anti-Fas monoclonal antibody was used to induce apoptosis in SKW6 cells. Giemsa’s staining, Annexin-V-FLUOS staining and DNA ladder test were used to determine the events of apoptosis. Indirect immunofluorescent staining with anti-Fas antibody was performed to detect the surface Fas expression. In a time-course test of 12, 24 and 36 h for apoptosis induction by anti-Fas antibody, DNA ladder was observed in the wild-type SKW6 cells in a time-dependent fashion. Mock transduction had no effect on DNA ladder production. However, no DNA ladder was detected in the rAAV-antisense 6A8 cDNA-transduced SKW6. Results from Annexin-V-FLUOS staining on anti-Fas antibody-treated cells revealed that the staining-positive rate in the rAAV-antisense 6A8 cDNA-transduced SKW6 cells was decreased in comparison to that in the wild-type and the mock-transduced cells. Giemsa’s staining observation showed that the number of dying (with apoptotic bodies) and dead cells was reduced in the rAAV-antisense 6A8 cDNA-transduced SKW6 cells in comparison with that in the wild-type and the mock-transduced cells upon anti-Fas antibody induction. The transduction did not affect the expression of Fas molecular on cell surface. 100% cells in all the groups showed Fas expression. The SKW6 cells became resistant to apoptosis induction by anti-Fas antibody upon transduction with a reverse fragment to a cDNA encoding human 6A8 α-mannosidase. The transduction did not affect the expression of Fas molecule on cells.

Keywords: apoptosis, antisense 6A8 cDNA, 6A8 α-mannosidase, anti-Fas antibody, B cell line SKW6.

Protein glycosylation plays an important role in the biological activities of organisms[1]. Glycosylation is closely related to various functions of cells. Since death is one of the bases in cell functioning, researchers have studied the relationship between protein glycosylation and cell death in recent years. It has been observed that cells could be induced to apoptosis by treatment with tunicamycin (TM), an inhibitor of N-glycosylation. For example, Larsson et al. reported that treatment with a low dose of TM blocked the cell cycle progression of both normal Balb/c 3T3 cells (A31) and their SV 40-transformed derivatives (SV A31) specifically in early G1 and that
upon release after an 8-h treatment the postmitotic SVA31 cells did not divide after the removal of TM, instead came to death\textsuperscript{[2]}. Carlberg et al. observed the similar phenomena on fibroblasts\textsuperscript{[3]}. Effect of glycosylation on the sensitivity of cells to apoptosis induction was also observed. For example, Walker et al. found an enhancement of the sensitivity of cells with less N-linked glycosylation after TM treatment to temperature-induced apoptosis\textsuperscript{[4]}. Keppler et al. found that differential sialylation of cell surface glycoconjugates in human B lymphoma cell line BJAB could regulate susceptibility for CD95-mediated apoptosis\textsuperscript{[5]}. In addition, glycosylation alteration of cells was detected in late phase apoptosis from colon carcinomas\textsuperscript{[6]}.

Apoptosis is a very complicated process, in which a number of proteins play a role orchestrally\textsuperscript{[7]}. It could be supposed that glycosylation of the protein(s) in the process would influence the activity of the protein(s) in apoptosis. Based on the isolation of a cDNA encoding a new human α-mannosidase (6A8)\textsuperscript{[8]} and the high expression of 6A8 α-mannosidase in human B cell line SKW6 cells (a manuscript in publication), the effect of transduction with an antisense 6A8 cDNA on apoptosis induction by anti-Fas antibody was examined on SKW6 cells. It was observed that the transduction causing inhibition of 6A8 α-mannosidase activity resulted in resistance of SKW6 cells to the apoptosis induction.

1 Materials and methods

1.1 Cell line

EB virus-transformed human B cell line SKW6 (CD19\textsuperscript{+}CD3\textsuperscript{−}CD4\textsuperscript{−}CD8\textsuperscript{−}) was obtained from ATCC. The cells were incubated in RPMI1640 (GIBCO/BRL) containing 10% newborn calf serum (NCS, TBD Co. Tianjin), 100 U/mL penicillin and 100 μg/mL streptomycin.

1.2 Transduction with rAAV-antisense 6A8 cDNA

rAAV-antisense 6A8 cDNA was constructed by means of inserting a 6A8 cDNA fragment into the adeno-associated virus vector pAGX(+) in a reverse direction which was then packaged (a manuscript in publication). The fragment is the 3′ end 1358 bp of the 3300 bp 6A8 cDNA. 1×10\textsuperscript{6} SKW6 cells were washed with RPMI1640. After the supernatant was discarded, 100 μL rAAV-antisense 6A8 cDNA or 100 μL rAAV was applied to the cell pellet. The cells were resuspended and incubated in 5% CO\textsubscript{2} at 37°C for 1 h. The cell culture was supplemented with flesh complete RPMI1640 medium to 3 mL and incubated for another 24 h. Then the medium was replaced by flesh complete RPMI1640 medium. After 24 h incubation, G418 at a final concentration of 600 μg/mL was added to the cell culture. Wild type cells were used as a control. 7 days after 100% of the wild type cells came to death, the medium in the cell cultures was replaced by flesh complete RPMI1640 medium. The cells were cloned by means of limiting dilution.

1.3 Northern blotting

Total RNA was extracted from 5×10\textsuperscript{6} flesh cells using TRI\textsubscript{ZOL} kit (GIBCO/BRL). The RNA was transferred onto a nylon membrane (Amersham). The 6A8 mRNA was detected by \textit{in situ}