We investigated changes in the sub-cellular distribution of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) after X-ray irradiation in HeLa cells. Twenty-four h after irradiation at 5 Gy, nuclear GAPDH levels increased 2.6-fold, whereas total GAPDH levels increased only 1.2-fold. Knockdown of GAPDH using specific small interfering RNA (siRNA) led to sensitization to X-ray-induced cell death. These results suggest that GAPDH plays a role in the radioresponse.

Key words: glyceraldehyde-3-phosphate dehydrogenase (GAPDH); X-ray radiation resistance; cell survival.

Exposure to ionizing radiation including that from space and terrestrial sources is a regular occurrence for human beings. In addition, although radiation has the ability to provide humans with life-saving technologies, it causes DNA damage, stimulates a plethora of signal transduction cascades, and alters expression of a number of genes responsible for maintaining cellular homeostasis and triggering apoptosis.1)

To identify proteins that are differentially expressed in response to irradiation, a proteomic approach using two-dimensional gel electrophoresis and mass spectrometry is useful. Candidates for biological marker proteins for detecting radiation-induced cell damage and for proteins involved in radiation sensitivity have been identified.2,3) In our previous study, we identified six candidate proteins in the nuclear fraction by proteomic analysis, and found that aldolase A, a glycolytic enzyme, is involved in the X-ray resistance of human HeLa and UV²¹ cells.4)

For over four decades, glycolytic enzymes have been considered to be distributed in the cytosol, and they are mainly involved in energy production. On the other hand, numerous studies have demonstrated the presence of at least four glycolytic enzymes, lactate dehydrogenase, phosphoglycerate kinase, aldolase A, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), in the nuclear compartment of several cell systems, suggesting functional diversity of the glycolytic enzymes.5) Among these, GAPDH has been well investigated for its physiological functions in the nuclei.6) In this study, we investigated to determine whether the sub-cellular distribution of GAPDH protein is altered after X-ray irradiation. In addition, the involvement of GAPDH in X-ray resistance was studied by colony survival assay.

First we performed cell fractionation of 5 Gy-irradiated and unirradiated HeLa cells and determined GAPDH protein levels in the samples by Western blotting analysis (Fig. 1) to determine whether the expression or distribution of GAPDH would be altered after X-ray irradiation. In the nuclear sample, the GAPDH protein level increased 2.6-fold after irradiation. In the total cell lysate, the level increased only 1.2-fold, and it did not increase in the post-nuclear samples. This alteration by irradiation was similar to that of aldolase A protein, which was demonstrated previously (Fig. 1, second row from top).4) The level of nuclear-distributed GAPDH in the non-irradiated cells was approximately 5–10% of the total amount as estimated by Western blot analysis. This result suggests the possibility that newly synthesized GAPDH protein after irradiation is preferentially imported to the nuclei, or that some of the cytosolic GAPDH protein is redistributed to the nuclei after irradiation, as with aldolase A.

Next we performed knockdown experiments using siRNA targeted to GAPDH mRNA to determine the possible involvement of GAPDH protein in the cellular response to X-ray irradiation. Transfection of two siRNAs, GAPDH-#1 and GAPDH-#2 siRNA, led to suppression of GAPDH protein by more than 50% at 48 h after transfection (Fig. 2A). GAPDH protein levels in the nuclei were also suppressed by the specific siRNA.
The cell-cycle distribution 48 h after transfection was also determined by flow cytometric analysis. The average \( \mu/C_6 \) SD percentages of the cell population in the \( G_1 \), S, and \( G_2/M \) phases were 70/\( \mu/C_6 \)1:0%, 23/\( \mu/C_6 \)0:6%, and 10/\( \mu/C_6 \)0:4% in GAPDH-\#2 siRNA-transfected cells, and 74/\( \mu/C_6 \)1:1%, 20/\( \mu/C_6 \)2:1%, and 6:3/\( \mu/C_6 \)1:0% in non-specific negative control (NC) siRNA-transfected cells respectively. This result indicates that reductions in GAPDH protein levels did not result in a great change in cell-cycle distribution, at least at 48 h after transfection. Then we performed a colony survival assay of siRNA-transfected cells to determine whether knockdown of GAPDH protein sensitizes HeLa cells to X-ray irradiation (Fig. 2B). GAPDH-\#1 and GAPDH-\#2 siRNA-transfected cells exhibited substantially increased sensitivity to X-ray irradiation as compared with NC siRNA-transfected cells; the \( D_0 \) value decreased from 1.9 to 1.1 Gy, suggesting that GAPDH protein is involved in sensitivity to X-rays, as well as aldolase A protein. The average plating efficiencies of unirradiated cells transfected with GAPDH-\#1 and GAPDH-\#2 siRNA (26.5% and 28.3% respectively) were about half of that transfected with NC siRNA (54.2%).

At present, the mechanism of increased GAPDH protein in the nuclei after irradiation and that of X-ray resistance remain unknown. Recently, Sundararaj \textit{et al.}\textsuperscript{7} reported that the nuclear localization of GAPDH is regulated in a cell cycle-dependent manner, which appears to be maximum at the S phase, and can no longer be detected in the nuclei at the \( G_0/G_1 \) phase. They also reported that nuclear GAPDH was characterized by a basic pI of 8.3–8.7 while its cytoplasmic counterpart displayed a more acidic pI of 7.0–7.5. On the other hand, Brown \textit{et al.}\textsuperscript{8} identified a chromosome region maintenance 1/exportin 1 (CRM1)-dependent nuclear export signal in the C-terminal domain of GAPDH, truncation or mutation of which abrogated CRM1 binding and caused nuclear accumulation of GAPDH. From these observations, post-translational modifications that alter the pI value and/or the CRM1-dependent nuclear export system might be involved in the increase in nuclear GAPDH after X-ray irradiation. In addition, GAPDH is perhaps involved in the replication and transcription of DNA, the regulation of telomere structure, nuclear membrane fusion, and the recognition of fraudulently incorporated nucleotides in DNA with its binding partners.\textsuperscript{5,6} Thus it is possible that nuclear GAPDH makes a protein complex for DNA strand-break repair to enhance/stabilize the activity.

There is another unresolved but interesting issue, as to whether GAPDH and aldolase A are involved in radioresistance cooperatively or independently. Further studies concerning the mechanism of X-ray resistance of cells with increased GAPDH expression in the nuclei and the relationship between GAPDH and aldolase A in the radiation response should be pursued.

These results suggest a potential novel role for GAPDH in cells in resistance to X-ray irradiation and its possible involvement in the repair of X-ray-induced DNA damage.
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Fig. 2. Knockdown of GAPDH Leads to Sensitization to X-Ray Lethality in HeLa Cells.

Two siRNA duplexes with Stealth™ modification against human GAPDH mRNA (GAPDH-#1 siRNA, GAPD Validated Stealth™ Duplex #1, No. 45-1556; GAPDH-#2 siRNA, GAPD Validated Stealth™ Duplex #2, No. 45-1557) were purchased from Invitrogen (Carlsbad, CA). As a negative control (NC), Stealth™ RNAi negative control medium GC duplex (Invitrogen) was used. Cells were transfected with the above described siRNA using Lipofectamine 2000 (Invitrogen). A, Forty-eight h after transfection, total cell lysate, nuclear extract, and post nuclear lysate were prepared, and suppression of GAPDH expression by specific siRNA was determined by Western blotting analysis, as described in Fig. 1. B, Colony survival assay. Forty-eight h after transfection, cells were irradiated at the indicated doses, and then seeded in 90-mm dishes at a density of 1,000–1,500 cells per dish after X-ray irradiation, as described previously. Colony numbers were counted after staining with 0.1% (w/v) methylene blue tetrahydrate. The survival fraction was calculated as the ratio of colony numbers after X-ray irradiation to those without irradiation. Values are the mean ± SD of three independent experiments.
References