ABSTRACT

Aim: autophagy is a pivotal physiological process for survival during starvation, differentiation and normal growth control. It is defined as the process of sequestrating cytoplasmic proteins or even entire organelles into the lytic compartment (lysosome/vacuole). This study investigates the expression of autophagy in Hodgkin lymphoma cells treated with various anti-cancer drugs.

Methods: Hodgkin’s lymphoma cells (HD-My-Z cells) were cultured with various anti-cancer drugs, such as bleomycin, adriamycin, gemcitabine and paclitaxel. Autophagy was detected by fluorescent pattern of light chain 3 (LC3) proteins and the apoptotic cell death was determined by annexin V binding.

Results: autophagy was detected in HD-My-Z cells treated with gemcitabine, but not with bleomycin, adriamycin and paclitaxel. Adriamycin exhibited the strongest cytotoxic action, and the cytotoxic action of bleomycin and gemcitabine was less marked compared with adriamycin. Paclitaxel did not cause significant cell death in the cells.

Conclusion: autophagy was differentially expressed in Hodgkin lymphoma cells treated with anti-cancer drugs and the expression did not correspond to the apoptotic cell death.

Key words: hodgkin lymphoma, HD-My-Z cells, autophagy, gemcitabine, apoptosis.

INTRODUCTION

Autophagy is a pivotal physiological process for survival during starvation, differentiation and normal growth control. It is defined as the process of sequestrating cytoplasmic proteins or even entire organelles into the lytic compartment (lysosome/vacuole). On the other hand, apoptosis activates autophagy and, at least in some cases, autophagy is required for efficient apoptosis. There was a correlation between defects in autophagy and carcinogenesis. Further, autophagy is found in malignant cells treated with anti-cancer drugs, starvation and ionising radiation. However, there is no report on participation of autophagy in the cell death of hematopoietic neoplasms treated with anti-cancer drugs.

Rat microtubule-associated protein 1 light chain 3 (LC3) is a homologue of Apg8p essential for autophagy in yeast and consisting of two forms of cytosolic LC3-I and membrane bound LC3-II. The amount of LC3-II is correlated with the extent of autophagosome formation and it is used as a marker of autophagy. In the present study, we studied the participation of autophagy in various anti-cancer drugs-induced cell death of Hodgkin lymphoma HD-My-Z cells with the expression of LC3. Here, we report the participation of autophagy in the cells treated with gemcitabine.

METHODS

Cell Culture, Transfection and Autophagy Detection

HD-My-Z cells were grown in RPMI 1640 (Sigma Biosciences, St Louis, MO, USA) supplemented with antibiotic and 10% fetal bovine serum (Tracescientific, Melbourne Australia) at 37 °C under 5% CO₂. HD-My-Z cells at subconfluency and were transfected with the green fluorescence protein (GFP)-LC3 plasmid (a kind gift from Dr. T. Yoshimori) in a 24 well plastic plate by using lipofectamine 2000 CD (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instruction. GFP-LC3-transfected HD-My-Z cells were further cultured in the culture medium before use. The cells were inspected under a fluorescence microscopy. The digital images were processed by using the metaview imaging system software.
Cytotoxicity

HD-My-Z cells were plated at a cell density of 2 x 10^4 cells/100 ml/well in a 96-well microplate, and incubated at various concentrations of anti-cancer drug for 48 h. After 24 or 48 h, 10 ml of the WST-1 solution (Dojindo, Maryland, USA) was added into the cultures. After 4 h, the absorbance was measured at 450 nm with a microplate reader (Multiscan JX, Osaka, Japan). The absorbance was expressed as the mean of triplicate with standard deviation in 3 independent experiments.

Apoptosis Assay

The cells were plated in a slide chamber at a cell density of 4 x 10^4 cells/200 ml in a Lab-Tek 8 chamber slide with cover (Nalge Nunc International, Naperville, IL, USA). Various concentrations of anti-cancer drugs were added and incubated for 48 h. After the incubation, the cells were washed 2 times with phosphate-buffered saline and treated with the binding buffer (200 ml) containing annexin V (2 ml) for 30 min in the dark at room temperature. The cells were inspected under a fluorescence microscopy. The frequency of annexin V-positive cells was expressed as the mean of triplicate with standard deviation in 3 independent experiments.

All experiments were performed at the Department of Microbiology and Immunology of Aichi Medical University School of Medicine, Nagakute, Aichi Japan.

Statistical Analysis

Statistical comparison of variables was performed by student’s T-test. Statistical significance was defined as p < 0.05.

RESULTS

Cells Viability

The cell viability in HD-My-Z cells treated with adriamycin, bleomycin, gemcitabine and paclitaxel for 48 h was examined with a WST assay. As shown in figure 1A, adriamycin exhibited the strongest cytotoxic action on HD-My-Z cells among anti-cancer drugs tested whereas bleomycin and gemcitabine exhibited the weaker action than adriamycin. Paclitaxel caused slight inhibition of the cell viability. The viability of the cells treated with them for 48 h was lower than that for 24 h.

Cells Apoptosis

In order to clarify the participation of apoptosis in their cytotoxicity, the cells treated with anti-cancer drugs were stained by fluorescent-annexin V. A large number of annexin V-positive cells were detected in the cells treated with adriamycin contained. (Figure 1B)
Autophagy

We tried to examine the relationship between apoptosis and autophagy. GFP-LC3-transfected HD-My-Z cells were treated with adriamycin, bleomycin, gemcitabine or paclitaxel for 48 h. The appearance of autophagy characterized by punctuated pattern of green fluorescence was inspected under a fluorescence microscope. (Figure 2) The typical punctuated pattern of GFP-LC3 dots was detected in approximately 15% and 20% of the cells treated with gemcitabine for 24 h and 48 h, respectively. On the other hand, such punctuated pattern was not detected in the cells treated with adriamycin, bleomycin and paclitaxel.

DISCUSSION

Programmed cell death comprises several subtypes. Apoptosis or type I programmed cell death is characterized by condensation of cytoplasm and preservation of organelles, essentially without autophagic degradation. Autophagic cell death or type II programmed cell death exhibits extensive autophagic degradation of Golgi apparatus, polyribosomes and endoplasmic reticulum, which precedes nuclear destruction. During autophagy-literally “selfeating”-cells deliver cytoplasmic constituents, including whole organelles, to the lysosome for degradation. Autophagy often gets overlooked as “just housekeeping”. In fact, failures in keeping house likely to diseases such as cancer and neurodegeneration.

In a previous study, autophagic cell death was demonstrated in the human mammary carcinoma cell line MCF-7 treated with tamoxifen. Apoptosis plays little or no role on the killing of epithelial neoplastic cells by radiation. Paglin et al demonstrated a novel response to radiation manifested by autophagy.

High dose gemcitabine (1g/m²) alternating with dexamethasone/paclitaxel (135 mg/m²) has been given in clinical trial of autotransplantation for advanced Hodgkin’s disease followed by post-transplant rituximab/GM-CSF and consolidation therapy. Based on that study, we treated Hodgkin lymphoma cell line by various anti-cancer drugs, including gemcitabine and paclitaxel.

The present study for the first time demonstrates that autophagy is differentially expressed in the cell death of Hodgkin lymphoma cells treated with various anti-cancer drugs. Although the correlation between apoptosis and autophagy has been suggested, we found that adriamycin and bleomycin-treated HD-My-Z cells undergo apoptosis but do not express autophagy. On the other hand, gemcitabine-treated HD-My-Z cells demonstrated both apoptosis and autophagy. The present study suggests that apoptosis and autophagy occur independently in anti-cancer drugs-treated Hodgkin lymphoma cells. It is consistent with the previous report that autophagy, but not apoptosis, is induced in malignant glioma cells by the treatment with temozolamide.

Autophagy might be a survival mechanism for escape from cytotoxic actions of anti-cancer drugs.

CONCLUSION

There was differential expression of autophagy in Hodgkin lymphoma cells treated with various anti-cancer drugs. The expression did not correspond to apoptosis-dependent cytotoxicity.
ACKNOWLEDGEMENT

We would like to thank Dr. T. Yoshimori for providing GFP-LC3 plasmid. This work was partially supported by a Grant for Foreign Researcher from Aichi Medical University School of Medicine.

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