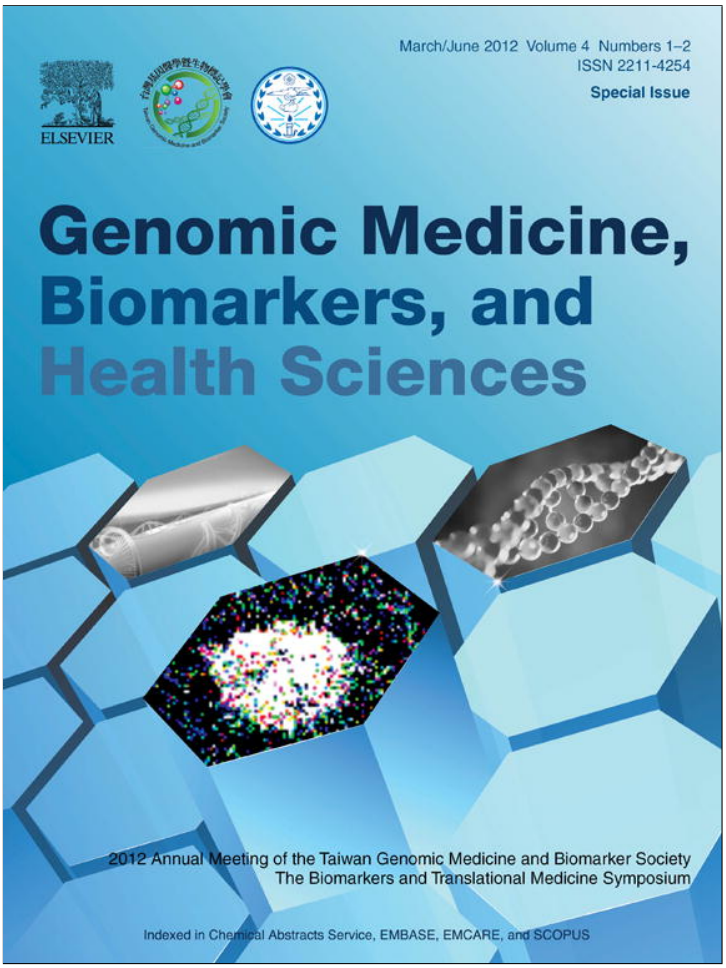

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SHORT COMMUNICATION

DNA-dependent protein kinase regulated glioblastoma survival in doxorubicin-induced cytotoxicity

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Abstract Generally, chemotherapy is effective when the cancer cell is dividing; most drugs trigger cancer cells to undergo apoptosis by attacking the cell's DNA. In the process of cancer cell apoptosis, cancer cells become more resistant to chemotherapy treatments over time. Since DNA-dependent protein kinase (DNA-PK) plays an important role in DNA repairing, it is interesting to investigate the relationship between this particular enzyme and the development of multidrug resistance. In this study, we chose the commonly used chemotherapy drug doxorubicin to treat glioblastoma cells (M059k and M059j), and performed 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenykterazolium bromide (MTT) assay and immunofluorescence staining to assess the presence of DNA-PK. The result of MTT assay showed that the concentration of an inhibitor/drug required to reduce the cell viability by half of M059j is 1.75 μm while that of M059k is 0.71 μm after doxorubicin treatment. Comparing the staining result of M059j and M059k, DNA-PK was more detectable in M059k than in M059j. It suggested that further experiments need to be performed to identify and characterize the proteins that are important for signal transduction pathways that actually link DNA-PK with doxorubicin-induced cytotoxicity as well as those that are drug resistant.

Introduction

Cancer has been the most common cause of death in Taiwan for 29 years. Traditional treatments of cancer

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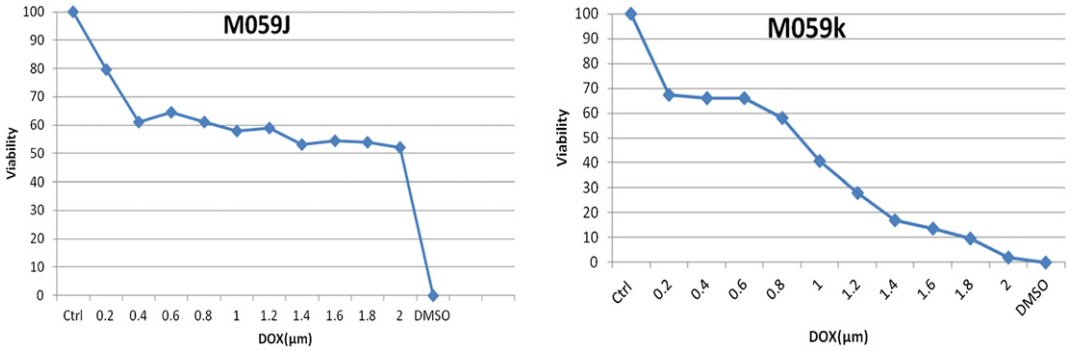

Figure 1 Cytotoxic effects of doxorubicin in M059j and M059k cells.

include surgery, radiation, and chemotherapy. Generally, chemotherapy is effective when the cancer cell is dividing. Doxorubicin is a widely used drug in cancer chemotherapy. It can directly attack DNA and cause DNA double strand to break. Accordingly, DNA-dependent protein kinase (DNA-PK) plays a role in the development of drug resistance in cancer cells,[1,2] suggesting the antagonistic interaction between DNA-PK and certain caner drugs. Therefore, we would like to explore what role DNA-PK plays after doxorubicin treatment. Apparently, glioblastoma cells M059k and M059j are good cell models in the study, as M059k has normal DNA-PK activity while M059j is a radiosensitive cell line that fails to express the catalytic subunit of DNA-PK. Further proteomics analysis and functional validation of individual proteins are ongoing and might provide new insights in doxorubicin-induced drug resistance, potentially leading to the design of novel diagnostic and therapeutic strategies.

Materials and methods

Cell culture

M059k and M059j were grown in DMEM-F12 medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin in a 5% CO_2/37°C incubator. Cells were subcultured by trypsin every 3—4 days.

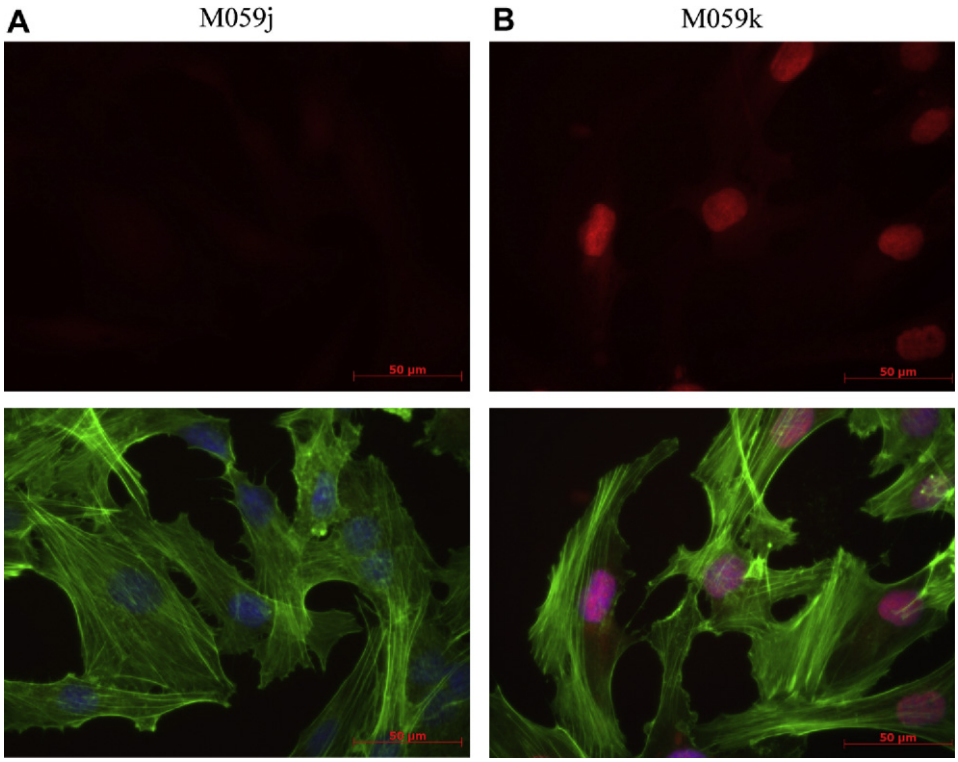

Figure 2 Immunofluorescence staining of DNA-PK in M059j and M059k cells. Cells on coverslips were fixed and stained for DNA-PK (red), F-actin (green), and DAPI (blue). Comparing with (A) M059j and (B) M059k, DNA-PK is more detectable in M059k than in M059j. Each set of three fields was taken using the same exposure, and images were representative of six different fields. DAPI = 4′,6-diamidino-2-phenylindole; DNA-PK = DNA-dependent protein kinase.(A) M059j (B) M059k.

MTT assay

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenykterazolium bromide (MTT) assay is a colorimetric assay that can assess the viability of cell. MTT could be deduced to purple formazan in living cells. The purple formazan could be dissolved with dimethyl sulfoxide (DMSO). Finally, the density was measured with a spectrophotometer. A total of 10,000 cells per well were seeded in the 96-well plate. After cells had been attached to plate button, various concentrations of doxorubicin (0.2, 0.4, 0.6, ..., 2.0 μm) were added in the wells. After 24 hours, we removed doxorubicin and added MTT. After 4 hours, DMSO was used to dissolve formazan. Finally, the spectrophotometer was used to measure absorbance at 545 nm.

Immunofluorescence staining

We seeded 5000 cells per well in a 12-well plate with 13 mm diameter glasses. After cells had been attached to the glasses, we removed the medium and washed the glasses with phosphate-buffered saline (PBS), paraformaldehyde (PFA), and bovine serum albumin (BSA). Then the cells were fixed with paraformaldehyde for 20 minutes. 0.1% Triton X-100 was used to permeabilize cell membranes, to help antibodies enter the cells more easily. After that, we used 5% bovine serum albumin to block for 1 hour, and then first antibody and secondary antibody were added subsequently and incubated for 1 hour, respectively. Finally, mounting oil was added before these stained cells could be observed under the immunofluorescence microscope.

Results

MTT-based viability assays were performed in which 10,000 glioblastoma cells were treated with various concentrations of doxorubicin for 24 hours. The result showed that half maximal inhibitory concentration of M059j is 1.75 μm while that of M059k is 0.71 μm after doxorubicin treatment (Fig. 1). Immunofluorescence analysis of morphological and DNA-PK localization in both M059j and M059k cells is shown in Fig. 2. Comparing with M059j and M059k, DNA-PK was more detectable in M059k than in M059j.

Discussion and conclusion

In theory, M059j (lacks DNA-PK activity) is supposed to be more sensitive to the cancer drugs. Instead, our result showed that the viability of M059k is lower than that of M059j after the doxorubicin treatment. Therefore, it is interesting to confirm this phenomenon by using DNA-PK inhibitors. In addition to assessing whether the presence of DNA-PK will affect doxorubicin-induced cytotoxicity in glioblastoma cells, we will utilize Western blot and flow-cytometry to investigate if doxorubicin will lead to cell apoptosis. In addition, we will perform further experiment with 2D-DIGE and MALDITOF-MS to find out the complexity in the signal transduction regulated by DNA-PK in the presence of doxorubicin-induced DNA double-strand breaks.

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