Characterization of Radioresistant Cell Clones from Human U251 Glioblastoma Cell

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Background Malignant gliomas comprise more than 40% of central nervous system malignanacies. Radiation therapy is used extensively in the primary management of patients with glioblastoma multiforme (GBM). But GBM is one of the most resistant of human tumors to radiation whether used alone or in combination with surgery and/or chemotherapy. The way to investigate how cells respond to ionizing radiation is observation of response of mutant cell lines that differ from their parent lines in their radiosensitivity.

Methods To investigate the responses to ionizing radiation, we established radioresistant clones (RRC) from human U251 glioblastoma cell line through intermittently exposed to 3 Gy γ-radiation for six months. Then, we compared the radiation responses and characteristics between the two cell lines. Radioresistance was performed by colony forming assay on monolayer cultures in exponential growth. Cell cycle analysis was examined by FACS after radiation exposure. The expression of signal molecules involved in the cell cycle and MAPKs was detected by immunoblot assay. The differential gene expression profile was obtained by DD-PCR and cDNA microarray.

Results RRC showed the increased resistance to radiation induced apoptosis than U251. RRC grows more slowly than U251, and has a delayed cell cycle and G2-phase accumulation after radiation. The MAPK cascade was increased after radiation in RRC. Activities of antioxidant enzymes (catalase, SOD, glutathione peroxidase, glutathione transferase) are higher than U251 after radiation exposure. To screen and search for genes involved in the radioresistance, we performed the DD-PCR analysis and cDNA microarray. In DD-PCR analysis, vimentin was up-regulated genes and hRAD17 was down-regulated in RRC. In cDNA microarray, YB-1, p21/WAF1, heat shock factor1, replication protein P1, and STAT3 were decreased in RRC.

Conclusion Radioresistance of RRC is caused by increased cellular protection activity including changes of cell cycle distribution, high antioxidant activities and MAPK cascade. This approach contributes to the elucidation of radioresistance mechanism and development of its suppression/inhibition.