Redox regulation

139 Proteome analysis of protein expression caused by hydroperoxide or radiation

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Reactive oxygen species (ROS) give rise to various types of oxidative damage and some responses such as activation of stress responsive transcription and/or repair factors in cells. Proteome analysis using 2D-PAGE and peptide mass fingerprinting is suitable for the study on the post-translational modifications of proteins. When glial cells were exposed to hydroperoxide, the relative abundance of 9 spots changed on 2D gels, as compared with control gels. MALDI-TOF MS analysis after in-gel digestion revealed that these spots corresponded to at least 3 pairs of proteins. These pairs of protein spots had different isoelectric points each other and were identified as peroxiredoxin II, peroxiredoxin III and calpactin. It was suggested that peroxiredoxin II, peroxiredoxin III and calpactin were changed to their modified forms of a different isoelectric point by hydroperoxide. In the present work, we studied also the variant expressions of proteins caused by irradiation, and will discuss the effects of oxidative stress on proteins in the presentation.

140 Inhibition of the MEK/ERK Pathway Induces Radioresistance in Rat 3Y1 Cells

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Activation of MEK/ERK pathway generally results in stimulation of cell growth and confers a survival adavantage. The potential involvement of this pathway in cellular radiosensitivity, however, still remains unclear. The purpose of this study was to examine whether ERK pathway affects intrinsic radiosensitivity in mammalian cells. In order to inhibit MEK/ERK pathway, a MEK inhibitor, PD98059 was employed. When rat 3Y1 cells were treated with PD98059, at a concentration of 25 uM, ionizing radiation-induced ERK activation at 6 Gy was almost completely inhibited. Cell growth was significantly suppressed even at 1 uM. PD98059 treatment unexpectedly induced clonogenic radioresistance in a dose-dependent manner. Similar effect was observed by expression of kinase-deficient MEK, confirming the effect induced by PD98059 treatment. Radiation-induced apoptotic activity was significantly reduced in cells PD98059-treated cells as evaluated by PARP cleavage. The present study serves as a reminder that therapeutic interventions established based upon a general view could conversely work in certain conditions; characterization of individual tumors and a careful consideration are required.

141 The role of site-specific phosphorylation on X-ray-induced p53 accumulation

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p53 protein is a stress-responsive molecule that is accumulated and activated by X-irradiation and induces a cell cycle arrest or apoptosis. It has been thought that p53 is accumulated by the inhibition of the interaction between p53 and its specific ubiquitin ligase MDM2 caused by the phosphorylation of p53 at Ser15, Thr18, or Ser20. However, the physiological significance of the phosphorylation of each site remains elusive. Therefore, in the present study, we aimed at elucidateing a role of the phosphorylation on X-ray-induced p53 accumulation. First, we constructed ecdyson-inducible vectors containing the mutant *p53* gene in which Ser15, Thr18, and Ser20 were substituted by alanine. Then, these vectors were stably transfected into HT1080. The expression of Ala mutant p53 was induced by ponasterone A (PA). In response to 4 Gy of X-rays, every PA-induced mutant p53 protein was accumulated, although the basal levels and the degree of accumulation were slightly different among the clones. These results indicate that other major mechanism than the inhibition of p53-MDM2 binding can be involved in p53 accumulation.

142 *In situ* observation of DNA base radical using an EPR spectrometer combined with a synchrotron soft X-ray beamline in SPring-8

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To understand the molecular mechanism of base damages by Auger effect on oxygen and nitrogen atom in DNA molecule, guanine radicals have been studied using a newly developed an EPR spectrometer installed in a soft X-ray beamline in SPring-8. This system enables us to irradiate monochromatic ultrasoft X-rays to a sample and observe radicals induced simultaneously. The unique character of this apparatus revealed the induction of unstable short-lived radicals in guanine pellet sample irradiated in a vacuum at 77 K. The EPR spectrum was clearly distinguished from that of stable one which still exists after exposing to ultrasoft X-rays. Thus, we conclude that 1) short-lived species are mainly induced by as a result of the final state of the resonant Auger