

- 168 **Role of Ser15, Thr18, and Ser20 phosphorylation in p53 stabilization after X-irradiation**
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- p53 tumor suppressor protein has been shown to be degraded through ubiquitin-proteasome pathway, and the level in unirradiated cells is maintained at low level. In response to X-irradiation, p53 protein is stabilized and accumulated. Previous studies have indicated that phosphorylation at Ser15, Thr18, and Ser20 is involved in p53 stabilization after IR, but there is no detailed examination of the physiological role of phosphorylation of these three sites. In the present study, we constructed the expression vectors for the p53 gene, whose Ser15, Thr18, and Ser20 are replaced with Alanine (Ala). The vectors were transfected into HT1080 cells, which have the normal p53 response. We found that Ala substitution alone changed the stability of p53 in unirradiated cells. The level of p53 was examined 2 h after 4 Gy X-irradiation, and accumulation of p53 was observed in every single Ala mutants. Similar results were obtained using double or triple Ala mutants. These results suggest that phosphorylation of p53 at Ser15, Thr18, or Ser20 may not be indispensable for p53 accumulation after X-irradiation.
- 169 **Expression Profiling of cells irradiated with ionizing radiation**
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- A novel technique exhibiting the whole transcriptional events in a cell would provide us a great tool to understand such important mechanisms in life as differentiation and apoptosis. We have developed an expression profile system that can detect approximately 80% of the entire transcripts from a minimal size of cells or tissue samples (HiCEP). We investigated the sensitivity and the reproducibility of this novel strategy. (1) Different amount of synthesized polyA+RNA was added to total RNA from budding yeast and tested for detection by HiCEP. The result showed that HiCEP is so sensitive as detects one copy per cell of the contaminated polyA+RNA. (2) We performed HiCEP analyses for mouse embryonic fibroblast (MEF) cell samples, which were harvested at 0, 3, 6 and 24 hr after X-ray irradiation (7 Gy). More than 200 transcripts exhibited altered level of expression among approx. 20,000 transcriptoms detected in the HiCEP analysis. Those include p21 gene, of which expression increased to 2-folds in the first 3 hrs after irradiation. Quantitative RT-PCR targeting the each individual transcript verified that alteration of expression level by 1.3-fold is reliable in the HiCEP analyses. We are investigating the expression profile of cells after ionizing irradiation with different dose rate.
- 170 **Accumulation and phosphorylation of p53 by hypergravity**
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- We investigated the p53 signal pathway by hypergravity in human glioblastoma cell line, A172. Hypergravity (20 g) accumulated p53 at 3h and induced Waf-1 slightly. Hypergravity induced the phosphorylation of p53 at Ser15. The induction of p53 phosphorylation at Ser20, Ser37 or Ser46 was not observed under hypergravity condition. The accumulation of p53 and the phosphorylation at Ser15 by hypergravity were inhibited by wortmannin. We considered that PI3-kinase, such as ATM, might be concerned with the signal pathway of p53 by hypergravity.