7 Phosphorylation of p53 caused by radiation and telomere-shortening

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p53 protein is phosphorylated both in irradiated cells and in senescent cells. In this report, we examined whether the same signal transduction pathway is involved in this process. We have established a system, which the wtp53 gene can be induced in p53 lung carcinoma cells. p53 was induced by administration of Ponasterone A(PA). The induced p53 protein was phosphorylated at Ser15, and its phosphorylation level was significantly by X-ray irradiation. The radiation-induced phosphorylation was inhibited by Wortmannin treatment, and it also decreased Ser15 phosphorylation in unirradiated cells. These results indicate the possibility that ATM function may be stimulated by telomere-shortening. However SV40-immortalized Ataxia telangiectasia(AT) cells also showed p53 phosphorylation at Ser15. Therefore signal transduction pathway activated by telomere-shortening can be distinctive from that by ionizing radiation.

8 Accumulation and activation of p53 protein through its phosphorylation and acetylation after heat shock

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p53 protein is accumulated and activated following exposure to various stresses and prevents growth of damaged cells. The accumulation and activation of p53 are regulated by its amino acid modifications such as phosphorylation and acetylation. After X-irradiation, p53 is phosphorylated at Ser15 and Ser20 by ATM and ATR, which results in an accumulation of p53. The acetylation at Lys382 by p300/CBP and phosphorylation at Ser392 by CKII cause p53 activation. We examined here the mechanism of p53 accumulation and activation after heat shock.

Normal human fibroblast (HE49) and ATM-deficient cells (AT2KY, AT5BI) were heated at 43°C for 2 hour and recovered at 37°C subsequently. p53 was phosphorylated at Ser15 and accumulated in HE49. In AT cells, p53 was not phosphorylated at Ser15 but accumulation was observed. These results suggest that p53 was phosphorylated at Ser15 by ATM and that p53 accumulation was independent of phosphorylation at Ser15. We also found that p53 is not acetylated at Lys382 and not phosphorylated at Ser392 while p21 was induced following p53 accumulation. In summary, the results show that ATM activated by heat shock phosphorylates p53 at Ser15 and that p53 modification other than phosphorylation at Ser15, acetylation at Lys382 and phosphorylation at Ser392 may be involved in heat shock-induced p53 accumulation and activation.

9 p53 protein contributed to repair of double strand break

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Human lymphoblastoid cell line WIL2-NS has mutation in p53 gene at codon 237. This cell is closely related to p53 wild type TK6 cell. However, WI-L2-NS shows more resistant to killing and higher mutation frequency after ionizing radiation than TK6. Moreover recombination activity is reported to be greater in WIL2-NS than in TK6. Since, homologous recombination repair system is known to be less mutable than non-homologous end-joining repair system, error- prone repair mechanism might activate in WIL2-NS because of mutant p53gene. Now we hypotheses that many errors might occur in repair at G1 phase in WIL2-NS due to lack of p53-dependent p21 protein. To examine this hypothesis, we try to study cell cycle regulation and protein expression after X-ray irradiation. In TK6, DNA synthesis was inhibited immediately after the irradiation and started to recover DNA synthesis within 3h post-irradiation. Little number of cells to start replication from G1 phase to S phase and remarkable G2 arrest was observed after 9 hour post-irradiation. In contrast, such decrease in number of cells to start replication might be hardly expected in WI-L2NS because of the p21 deficiency. We now test this possibility. Besides, the population of cells at late S to G2 phase is increased by p53-independent regulation of cell cycle, and this increase might provide higher frequency of homologous recombination repair in WI-L2NS. This possibility in also now under test.