p53 and phosphrylation (5-10)

5 Phosphorylation and ubiquitination of p53 protein in normal human embryonic cells irradiated with X-rays

Keiji SUZUKI, Seiji KODAMA, and Masami WATANABE
Div. Radiat. & Life Sci., Sch. Pharm. Sci., Nagasaki University, Nagasaki 852-8521.

p53 protein is a nuclear phosphoprotein, whose level is accumulated by various cellular stresses, including ionizing irradiation, UV-irradiation, and heat shock treatment. Recently, phosphorylation of p53 protein at multiple sites has been shown to inhibit interaction between p53 and MDM2 proteins, which results in stabilization and activation of p53 protein. In the present study, we examined the kinetics of p53 protein phosphorylation in X-irradiated normal human embryonic cells using antibodies against phosphorylated p53 protein. Phosphorylation of p53 protein at Ser15 and accumulation of p53 protein were detected within 1 hour after irradiation, whereas Ser20 phosphorylation was observed only 2 hours after irradiation. Accumulated p53 protein was ubiquitinated subsequently and ubiquitinated proteins were also phosphorylated at Ser15, simultaneously. In order to determine whether ubiquitinated p53 is degraded through proteasome, cells were treated with ALLN, a proteasome inhibitor, together with cycloheximide, irradiated with X-rays, and then p53 level was determined. We found that ubiquitinated p53 without phosphorylation at serine 15 rapidly degraded but phosphorylation protected p53 protein from degradation. These results indicate that sequential phosphorylation of p53 protein at multiple sites plays an essential role on stabilization of p53 protein.

6 Radiation-induced p53 phosphorylation and stabilization

Motohiro YAMAUCHI, Keiji SUZUKI, Seiji KODAMA, Masami WATANABE, Lab. Radiat. Life Sci. Sch. Pharm. Sci. Nagasaki Univ.

The tumor suppressor gene product p53 accumulates and regulates the expression of the genes involving cell cycle arrest or apoptosis in response to external stresses, such as ionizing radiation, heat shock, and UV. Because the level of p53 mRNA does not change after irradiation, increased protein stabilization has been thought to be a major mechanism of p53 accumulation. p53 protein is targeted by the oncoprotein MDM2, which is a ubiquitin ligase of p53, ubiquitinated, and degraded through ubiquitin-proteasome pathway. After irradiation, p53 is phosphorylated in its N-terminal region, by which interaction between p53 and MDM2 proteins is suppressed. Here, we established an experimental system in which p53 protein can be induced by ponasterone A (PA), an synthetic analog of ecdyson, in order to examine a role of p53 phosphorylation on its stabilization in vivo. PA-induced p53 is transcriptionally active and it induces downstream effectors, such as $p21^{WAF/CIP1}$, and MDM2. We found that X-ray irradiation promoted p53 phosphorylation, however, there was no accumulation of p53 protein. Furthermore, X-irradiation did not affect transcriptional activity of p53 protein. These results indicate the possibility that mechanism(s) other than p53 phosphorylation are involved in p53 accumulation.