W-II-3 Regulation mechanism of the activity of MDM2, ubiquitin ligase toward p53

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We previously showed that oncoprotein MDM2 has ubiquitin ligase activity toward tumor suppressor p53. When we mutated the cysteine residues of RING finger domain of MDM2 in the carboxyl terminus, the disruption of each residue in the RING finger completely diminished the ubiquitin ligase activity of MDM2 toward MDM2 itself and toward tumor suppressor p53. These data suggest that the RING finger domain in MDM2 is the catalytic site of the ligase as suggested in the ROC1 in SCF and in APC11 in the anaphase- promoting complex/cyclosome (APC/C). Furthermore, a serine residue in the amino-terminus is essential for the activity. Also we show that MDM2 is modified by SUMO-1, ubiquitin like protein and that this modification affects the activity of MDM2 ubiquitin ligase.

W-II-4 Regulation of p53 by p38 MAP kinase cascade during the cellular response to ionizing radiation

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p38 MAP kinase (p38 MAPK) is a member of MAP kinase superfamily and known to be activated in response to a variety of stresses such as ionizing radiation (IR). We have reported that p38 MAPK plays a key role in radioadaptive response. Furthermore, we demonstrated that p38 MAPK phosphorylates p53, a tumor supressor which plays an important role in cellular response to IR *in vitro*, and up-regulates p53's transactivation activity. Our recent *in vitro* study revealed that p38 MAPK phosphorylates p53 at Ser33 residue. Now we have identified an another protein kinase which cooperatively phophorylates p53 with p38 MAPK and regulates its transactivaion function. This kinase can phosphorylate p53 at Ser392 residue, at least, *in vitro*. How p38 MAPK cascade can work in concert with p53 function in response to IR will be discussed.

W-II-5 **Glycerol-induced restoration of mutant p53 to normal p53 activity after radiation/heat treatment**

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We previously reported that glycerol was effective in restoring mutant p53 (mp53) to normal p53 function leading to normal *WAF1* expression after heating in m*p53*-transfected human glioblastoma cells (A-172/m*p53*). We report here whether radiation/heat-induced cellular signal transduction contributes to restoring mutant p53 (mp53) to normal p53 function. Gel mobility-shift assay showed DNA binding activity of mp53 increased in *in vivo* assay of cultured cells treated with radiation/heat and glycerol, but not in *in vitro* assay of nuclear or cytoplasmic proteins extracted from intact cells. Wortmannin suppressed the phosphorylation of serine 15 of p53, the DNA binding of p53 and WAF1 accumulation after combined treatments of glycerol and radiation/heat. No DNA binding of mp53 was observed in the mixture of whole cell proteins of A-172/m*p53* cells and nuclear or cytoplasmic proteins of p53-defective Saos-2 cells. These results suggested that radiation/heat-induced cellular signal transduction pathway is required for the restoration of mp53 to normal p53 by glycerol. Furthermore, the subsequent X-ray irradiation after glycerol pre-treatment for 48 h induced DNA fragmentation and apoptotic bodies in human squamous cell carcinoma transfected with mutant *p53* (SAS/m*p53*), but not in SAS/m*p53* cells non-treated with glycerol. In contrast, DNA fragmentation or apoptotic bodies was clearly observed in SAS/neo cells with or without glycerol. The results of apoptotic cell responses were closely correlated with cell surviving rate. It is strongly suggested that glycerol may function as a chemical chaperone that restores mp53 to wtp53 function.