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The Role of p53 Gene in Benzene-Induced Leukemogenesis

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Introduction

Benzene is an environmental pollutant that is present in mineral oil, natural gas, coal tar, gasoline, motor vehicle emissions, and tobacco smoke. The importance of benzene resides in the fact that it can induce hematotoxicity and leukemia in human and mice. However, the underlying mechanism of benzene hematotoxicity and leukemogenicity is still not fully understood.

To date, studies on benzene have focused on its metabolic pathways to determine the metabolites responsible for its hematotoxicity and leukemogenicity, for benzene and its major metabolites are not mutagenic in the Ames *Salmonella* test. As postulated by several investigators, the metabolism to reactive metabolites by hepatic enzyme, mainly cytochrome P450-2E1 (CYP2E1), is a prerequisite to the cyto- and genotoxicities associated with benzene exposure. Benzene metabolites subsequently undergo secondary activation by myeloperoxidase (MPO) that is present at high level in the bone marrow tissue. Also, it was demonstrated that synergistic interactions between phenolic metabolites such as phenol, hydroquinone, catechol and *trans-trans* muconic acid exacerbate benzene toxicity.

Exposure duration and dose are also important factors in determining benzene-induced hematotoxicity and leukemogenicity, which may be related to the limited capacity of enzymes for benzene metabolism and to the dynamic responses of hemopoietic microenvironmental conditions against the adverse effects of benzene. It was shown in the previous and our studies that 300 ppm benzene is a critical inhalation dose for hematotoxicity and leukemogenicity of benzene.

Recently, we have begun the studies to elucidate the underlying mechanism of benzene hematotoxicity and leukemogenicity at the molecular level, which included the cell cycle kinetic studies using bromodeoxyuridine UV cytocidal (BUUV) assay, the leukemogenicity studies and the microarray analyses during and after benzene exposure. Based on the data from those studies, we disclosed the important roles of p53 gene in the benzene-induced hematotoxic and leukemogenic mechanism.

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p53 mediates benzene-induced cell cycle suppression by inducing p21 overexpre- ssion.

There had been a controversy as to whether benzene accelerates or suppresses cell cycle of hemopoietic stem cells, although all of the investigators consistently reported severe decreases in the cellularity of peripheral blood and bone marrow following benzene exposure in mice. To elucidate the effect of benzene on the cell cycle of hemopoietic stem cells, in wild-type (WT) and p53 knockout (KO) mice, we performed cell cycle kinetic studies using BUUV assay that would show the cell cycle fraction and the cell cycling speed in the resultant graph (Fig. 1).

The result of cell cycle kinetics demonstrated that, when the WT mice were inhaled by 300 ppm of benzene for 6 hours/day, 5 days/week for 2 weeks, benzene does suppress but not accelerate the cell cycling of hemopoietic progenitor cells during the 2 week exposure (Fig. 2A). However, it should be noted here that such cell cycle suppression was not noted in the p53 KO mice exposed to benzene under the same exposure condition (Fig. 2B). With regard to the mechanism, our results clearly indicated that p53 gene mediates the cell cycle suppression of hemopoietic stem cells induced by benzene. In western blot analyses for cyclin dependent kinase inhibitors, including p21, p27 and INK4 family, it was shown that p21 was highly expressed by benzene exposure in the benzene-exposed wild-type mice, but not in the p53 KO mice, demonstrating the molecular mechanism of benzene-induced cell cycle suppression, that is by the p53 mediated

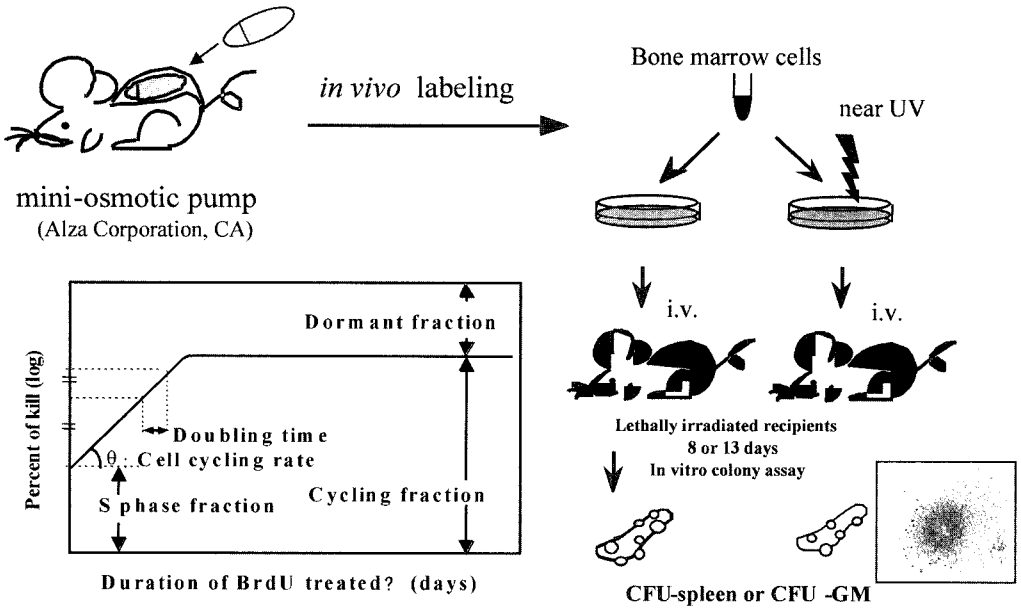

Fig. 1. BUUV assay is composed of three major steps. First is to label bromodeoxyuridine (BrdU) *in vivo* using mini-osmotic pumps, second is to kill the BrdU labeled cells that have undergone cell cycling during the labeling period by UV radiation, and then followed by colony assay for CFU-spleen or granulocyte-macrophage (CFU-GM) as the last step.

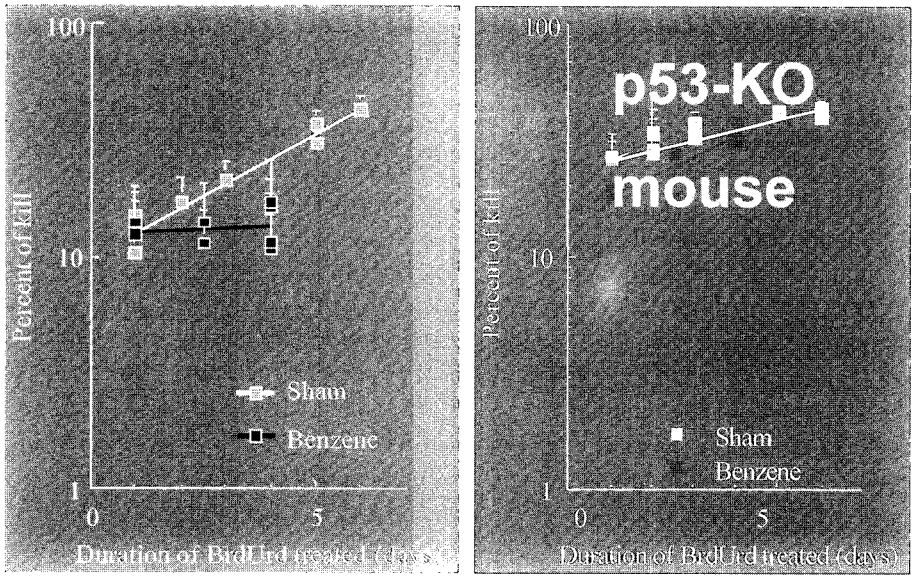

Fig. 2. Cell cycle kinetics during benzene exposure in wild-type (A) and p53 knockout mice (B). Note the severely suppressed cell cycle of CFU-GM in benzene-exposed wild-type mice but not in p53 KO mice. Benzene inhalation : 300 ppm, 6 hours/day, 5 days/week.

overexpression of p21 (Fig. 3).

What we are worthy to note here associated with benzene leukemogenesis is the dynamically responsive hemopoiesis characterized by the repeated cell cycle suppression and rapid recovery (Fig. 4) that was absolutely dependent on benzene exposure, which made the dramatic oscillatory changes of bone marrow cellularity during and after benzene exposure (Fig. 5). It is strongly possible that this phenomenon is associated with the genetic instability followed by the consequent

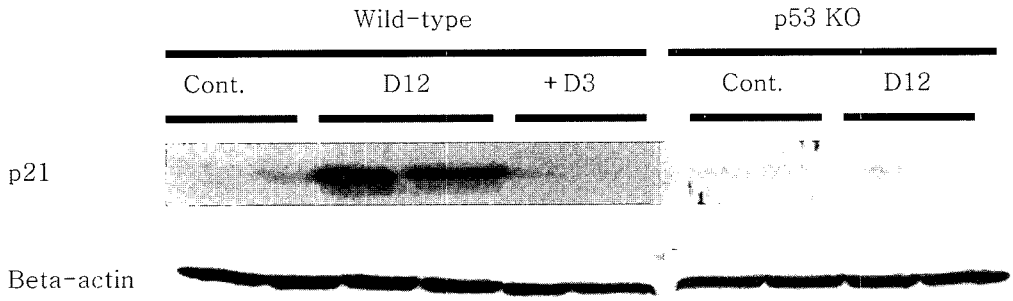

Fig. 3. Western blot analyses for p21 cyclin dependent kinase inhibitor in the benzene-exposed wild-type and p53 knockout mice. Note highly expressed p21 in wild-type mice but not in p53 knockout mice.

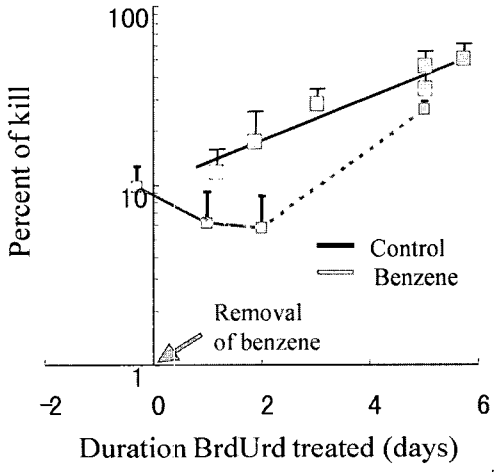

Fig. 4. Cell cycle kinetics after cessation of benzene exposure in wild-type mice. Note rapid recovery of cell cycle 3 days after benzene exposure

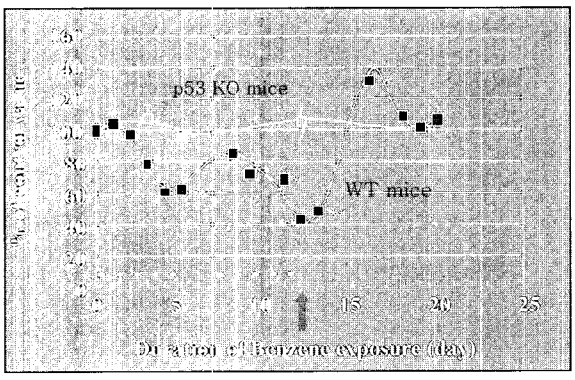

Fig. 5. The oscillatory changes of bone marrow cellularity depending on benzene exposure.

epigenetic leukemogenicity in wild-type mice.

p53 deficient mice produce leukemia by different mechanism

In the leukemogenicity study using WT, p53 hetero- and homozygous p53 deficient mice, the p53 deficient mice showed not only earlier onset of hemopoietic neoplasia but also much higher frequency of leukemia development than did the WT mice (Fig. 6). When we consider the leukemogenicity of benzene in p53 deficient mice together with the results of cell cycle kinetic studies described above, we could postulate that leukemogenicity induced in the p53 KO mice is, because of lack with p53, resulting in non-expression of p21 even during benzene exposure, is possibly due to insufficient DNA repairs and retained DNA damages. This is, hence, much more likely a genotoxic leukemogenesis, where reactive oxygen species, dysfunction of topoisomerase, and covalent binding of adducts formation of DNA actively participate in further leukemogenic development without repairing system. Thus, leukemogenesis between the mice carrying wild-type p53 and mice lacking of p53 gene seems to differ clearly from one another; the epigenetic in WT mice v.s. genetic in p53 knockout mice.

Advanced insight into the action mechanism of benzene and its leukemogenesis, based on microarray analyses in WT and p53 KO mouse bone-marrow tissues.

As postulated in the previous studies and ours, the mechanisms underlying benzene-induced toxicity and leukemogenicity are likely to be complicated by various pathways, including those of metabolism, growth factor regulation, oxidative stress, DNA damage, cell cycle regulation, and programmed cell death. To clarify the

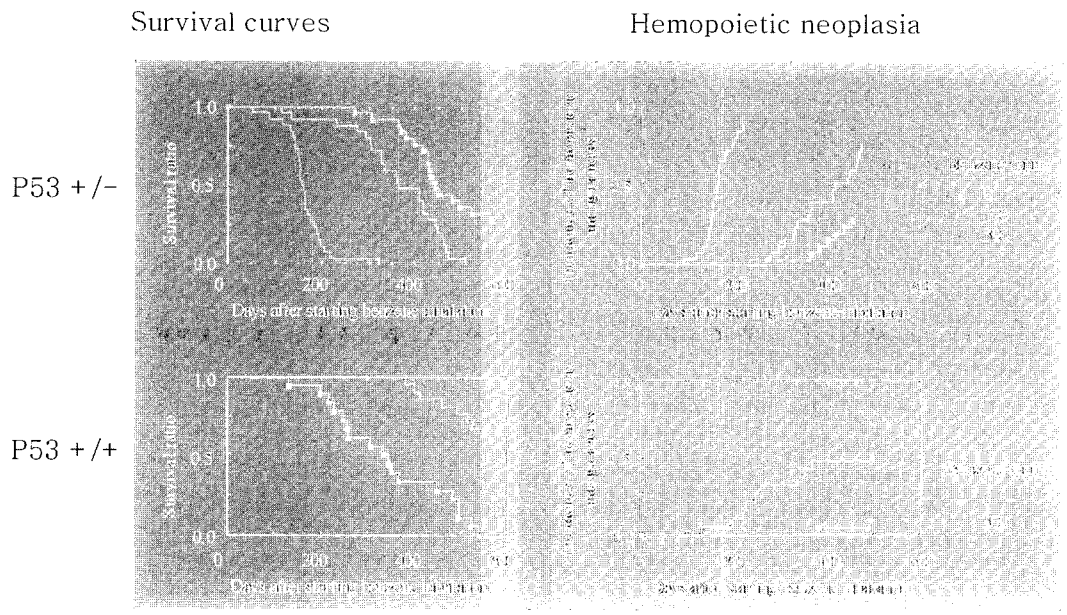

Fig. 6. Leukemogenicity of benzene in wild-type and p53 knockout mice when the mice were inhaled with 300 ppm benzene for 26 weeks.

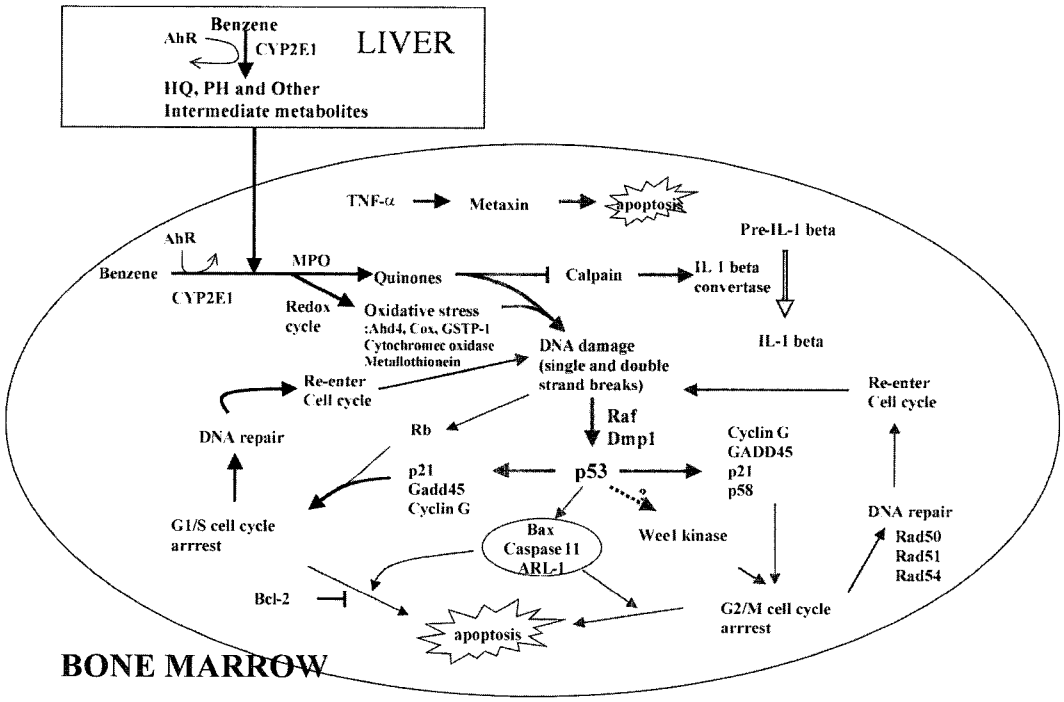

Fig. 7. Mechanism of benzene toxicity at the molecular level based on thealtered multigene expression profiles after benzene exposure.

mechanisms at the level of altered gene expression, we, therefore, performed microarray analyses on the mouse bone marrow tissue of WT and p53 KO mice during and after a 2-week benzene exposure by inhalation. Our microarray analyses not only corroborated the past data indicating the important roles of CYP2E1 and

myeloperoxidase for benzene metabolism and of p53, cyclin G, Gadd 45, and bax genes in the cell cycle regulation and apoptosis following benzene exposure, but also provided advanced insight into the whole scheme of action mechanism of benzene. On the basis of our microarray results, we could summarize the effects of benzene on the bone marrow cells of p53 KO mice as follows: a) cellular damage due to benzene metabolites and oxidative stress, b) dysfunction of the machinery of cell cycle arrest for repairing damaged DNA, resulting in continuous cycling of damaged cells even without undergoing repair, c) inhibition of apoptosis by both disruption of p53-dependent pro-apoptotic signaling and activation of survival genes, and d) failure of activating DNA repair genes. Such phenomena may lead to the increase in cell mutation frequencies at the candidate DNA locus, for instance, the *hprt* locus, responsible for benzene carcinogenesis, resulting in the development of hemopoietic malig-nancies. A possible signaling pathway induced by benzene exposure is shown by a schematic in Fig. 7, illustrating the role of p53 gene not only in during benzene exposure, but also in the recovery state, and the gene expression profiling from p53 KO mice visualize such oscillatory changes hidden behind the homeostatic balance organized by the p53 gene in WT mice.

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