related transcriptional changes (Table 3F), with the number of transcripts affected by CDDP also being very limited compared with APAP and CPA. However, on further consideration, the reason for the clustering of these three drugs is considered to be that they have similar mechanisms of causing cytotoxicity. CDDP forms adducts with DNA (Chu, 1994), whilst the reactive metabolites of APAP and CPA also form adducts with DNA, and this adduct formation is a critical part of the mechanism for their cytotoxicity. This common mechanism appears to separate these three drugs from the other drugs in hierarchical cluster analysis. In terms of the number of affected transcripts, on the other hand, CDDP-induced changes were not significant (Table 2). Also, there was no commonality with APAP or CPA for genes identified as changed and toxicity related change was not observed, in the same setting as APAP and CPA. There were similar tendencies of transcriptional changes between CDDP and APAP or CPA, i.e. increases or decreases of some particular genes, whereas the degree of the effects from CDDP was too slight to be detected as changes. Cholestatic hepatotoxicants CPZ and LCA, and the idiosyncratic hepatotoxicants DF and DSF, formed a cluster at 24 h. As already described, CDDP which rarely causes hepatotoxicity, did not belong to this cluster. These four drugs also did not induce transcriptional changes related to toxicity in the same setting as APAP and CPA (Table 3) and additionally, the number of transcripts which were affected by these four drugs was very limited (Table 2). CF induced a variety of PPAR-inducible genes (Table 3B) and in the hierarchical cluster analysis, CF was located next to the APAP/CPA/CDDP-cluster, and was clearly apart from the cluster of the cholestatic/idiosyncratic drugs (Fig. 1).

Acetaminophen (APAP)

There was some consistency with previous *in vivo* studies. Stress associated changes were observed, i.e. increased Gadd153, Gadd45α, Hsp70, a Hsp 40-like EST and Jun (Table 3A). These changes were mainly detected at 24 h. This type of change was consistent with the previously reported *in vivo* effects of APAP in rats at a single dose (Morishita *et al.*, 2006; Huang *et al.*, 2004). Slightly increased p53 targets such as Gadd45α, Gadd153, Pcna (1.7-fold, data not shown) and Hsp27 (1.4-fold, data not shown) were observed at 24 h. On the other hand, other p53 targets such as Bax, cdkn1a and Ccng1 were not affected. Although APAP-induced increases of p53 mRNA in the liver in mice have been reported (Ray *et al.*, 2001), expression change of p53 itself was not affected. This type of change appeared to be consistent with the mechanism of APAP-induced hepatotoxicity, which includes impairment of macromolecules such as proteins and DNA. Similar changes were also induced by CPA concomitant with decreased Cyp3a3, LOC191547, Calr and Arg1 (Table 3C). Increased PPAR inducible genes were observed, i.e. Cyp2c13, Cyp4a10/4a22 and Ste, whereas expression levels of PPARs themselves were not affected. This might be a part of an adaptation response of the hepatocyte against APAP because induction of PPAR has been reported to protect the liver against APAP toxicity in mice (Chen *et al.*, 2002; Manautou *et al.*, 1994; Nicholls-Grzemski *et al.*, 1992, 2000). For the metabolizing enzymes, decreased Cyp3a and increased Cyp4a were observed at 24 h. This kind of change is consistent with the previously reported *in vivo* effects of APAP in rats (Huang *et al.*, 2004). Decreases of steroid synthesis-associated genes such as LOC191574, Sult1a1, cyp51 and Hmgcr at 24 h were observed. Decreased steroid synthesis-associated genes have also been reported in an *in vivo* study (Heinloth *et al.*, 2004) but the only commonly affected gene was Sult1a1. These kinds of changes in Cyps and steroid synthesis-associated genes were not observed in the *in vitro* study at a low concentration of APAP (de Longueville *et al.*, 2003).

Although not previously reported, decreased glucocorticoid-inducible genes at 24 h were observed such as Arg1,

Table 2. The number of affected transcripts by either 6 h or 24 h exposure in collagen I-gel culture

Drug	Category	Mode of hepatotoxicity		Affected transcripts	Test conc.[a] (μM)
Directly toxic to the hepatocytes *in vivo*					
APAP	Analgesic	Hepatocyte cell death	(Klaassen, 2004)	36	4671.3
CF	Lipid-lowering	Peroxisome proliferator	(Klaassen, 2004)	14	1079.0
Indirectly toxic to the hepatocytes *in vivo*					
CPA	Anticancer, immunosuppressant	Sinusoidal epithelium disruption	(Klaassen, 2004)	26	1219.0
CPZ	Tranquilizer	Cholestatic	(Klaassen, 2004)	8	15.2
LCA	Secondary bile acid	Cholestatic	(Klaassen, 2004)	2	31.7
Rarely toxic to the liver *in vivo*					
CDDP	Anticancer	Hepatocyte cell death	(Liu *et al.*, 1998)	8	17.7
DF	NSAID[b]	Idiosyncratic	(Boelsterli, 2003)	2	87.7
DSF	Alcoholism	Idiosyncratic	(Forns *et al.*, 1994)	0	11.7

[a] Set at 1/3 the TC_{50} values for each drug, and the TC_{50} values were cited from a previous report where hepatocytes were maintained in conventional monolayer culture (Wang *et al.*, 2002).
[b] Nonsteroidal anti-inflammatory drugs.

Table 3. Transcriptional alteration (log 2 changes compared with corresponding negative controls)

Probe ID	Symbol	Description	6 h	24 h
(A) APAP				
AA892248	—[a]	—	−1.1	0.3
AI230406	—	—	−1.1	−0.1
AI229291	—	—	−1.0	−0.2
M13506	Udpgtr2	Liver UDP-glucuronosyltransferase, PB-inducible	−1.0	−0.3
AI102868	Psat1	Phosphoserine aminotransferase 1	−1.4	−0.7
E00717	Cyp1a1	CYP1A1	−1.5	−0.8
L48209	Cox8a	Cytochrome c oxidase 8a	1.0	−0.3
U30186	Ddit3	GADD153 (DNA-damage inducible transcript 3)	1.1	2.0
Z75029	Hspa1a	HSP70 1A	0.6	2.3
L16764	Hspa1a and 1b	HSP70 1A/1B	−0.1	2.0
AI070295	Gadd45a	GADD45 α	−0.5	2.2
AI070295	Gadd45a	GADD45 α	0.5	1.5
L32591	Gadd45a	GADD45 α	0.3	1.5
L32591	Gadd45a	GADD45 α	0.4	1.1
M82855	Cyp2c13	CYP2C13	−0.2	1.2
M57718	Cyp4a22/4a10	CYP4A10, 4A22	0.0	1.1
M86758	Ste	STE (STase, estrogen preferring)	−0.4	1.1
AA859648	—	Similar to HSP40	0.8	1.1
AI175959	Jun	Jun (v-jun sarcoma virus 17 oncogene homolog, avian)	−0.1	1.0
L22339	Sult1a2	SULT1A2 (STase family 1A, member 2)	0.2	1.0
AA945704	—	—	0.6	1.0
D89070	Cbr1	Carbonyl reductase 1	0.1	−1.0
X64401	Cyp3a3	CYP3A3	−0.1	−1.0
D17310	LOC191574	3α-HSD (3-α-hydroxysteroid dehydrogenase)	−0.1	−1.1
L19998	Sult1a1	SULT1A1 (STase family 1A, phenol-preferring, member 1)	−0.2	−1.0
L19998	Sult1a1	SULT1A1 (STase family 1A, phenol-preferring, member 1)	0.0	−1.1
X07467	G6pdx	G6PD (glucose-6-phosphate dehydrogenase)	0.2	−1.1
U17697	Cyp51	CYP51 (Sterol 14 α-demethylase)	0.2	−1.1
AI228738	Fkbp2, Fkbp1a	FK506 binding protein 2	0.6	−1.2
M60753	Comt	COMT (catechol-O-methyltransferase)	−0.5	−1.4
X55286	Hmgcr	3-Hydroxy-3-methylglutaryl-CoA reductase	−0.7	−1.5
X53363	Calr	Calreticulin	−0.1	−1.5
J02720	Arg1	Arginase 1	−0.3	−1.9
(B) CF				
AB010428	Cte1	Acyl-CoA thioesterase 1 (cytosolic)	4.1	4.3
Y09333	Mte1	Acyl-CoA thioesterase 1 (mitochondrial)	1.3	2.9
Y09333	Mte1, Cte1	Acyl-CoA thioesterase 1 (mitochondrial/cytosolic)	1.0	2.8
K03249	Ehhadh	Enoyl-CoA, hydratase/3-hydroxyacyl CoA dehydrogenase	1.9	3.6
J02752	Acox1	Acyl-CoA oxidase 1, palmitoyl	1.5	2.7
AJ224120	Pex11a	Peroxisomal biogenesis factor 11A	1.0	1.7
M57718	Cyp4a22/4a10	CYP4A10, 4A22	0.9	2.2
M33936	Cyp4a14	CYP4A14	0.9	1.8
D00569	Decr1	2,4-dienoyl CoA reductase 1, Mt	0.7	1.4
AI013834	Hsd17b4	17β-HSD4 (17β-hydroxysteroid dehydrogenase 4)	0.4	1.3
S83279	Hsd17b4	17β-HSD4 (17β-hydroxysteroid dehydrogenase 4)	0.6	1.1
S83279	Hsd17b4	17β-HSD4 (17β-hydroxysteroid dehydrogenase 4)	0.8	1.1
AI176422	Etfdh	Electron-transferring-flavoprotein dehydrogenase	0.3	1.1
AI014091	Cited2	CITED2 (Cbp/p300-interacting transactivator with ED-rich tail2)	−0.6	−1.1
(C) CPA				
J02722	Hmox1	Heme oxygenase (decycling) 1	1.3	1.0
D49785	Map3k12	MAP3K12 (MAP kinase kinase kinase 12)	1.3	−0.6
AA944397	Hspca	HSP 1α	−1.4	0.4
X53363	Calr	Calreticulin	−1.2	−0.2
AA963674	Eef2	Eukaryotic translation elongation factor 2	−1.0	−0.7
AI070295	Gadd45a	GADD45 α	0.0	2.0
AI070295	Gadd45a	GADD45 α	0.0	1.4
L32591	Gadd45a	GADD45 α	0.2	1.1
L32591	Gadd45a	GADD45 α	0.0	1.1
U30186	Ddit3	GADD153 (DNA-damage inducible transcript 3)	−0.2	1.7
L16764	Hspa1a & 1b	HSP70 1A/1B	−0.1	1.6
M24604	Pcna	PCNA	−0.4	1.2
AA998683	Hspb1	HSP27	0.0	1.0
K00136	—	—	0.4	1.2
M26125	—	—	0.6	1.1
AA945704	—	—	−0.1	1.0
AA997806	—	—	−0.1	−1.0
D17310	LOC191574	3α-HSD (3-α-hydroxysteroid dehydrogenase)	0.1	−1.0
AI171630	Mapk14	p38 MAPK (MAP kinase 14)	0.4	−1.1

Table 3. (*Continued*)

Probe ID	Symbol	Description	6 h	24 h
J02720	Arg1	Arginase 1	0.3	−1.1
X64401	Cyp3a3	CYP3A3	0.3	−1.1
AI014091	Cited2	CITED2 (Cbp/p300-interacting transactivator with ED-rich tail2)	−0.6	−1.0
AA900476	Cited2	CITED2 (Cbp/p300-interacting transactivator with ED-rich tail2)	−0.6	−1.1
AA900476	Cited2	CITED2 (Cbp/p300-interacting transactivator with ED-rich tail2)	−0.6	−1.3
M27440	Apob	Apolipoprotein B	0.0	−1.5
X02741	Tat	Tyrosine aminotransferase	0.2	−1.8
(D) CPZ				
E00778	Cyp1a1	CYP1A1	4.7	2.1
E00717	Cyp1a1	CYP1A1	3.8	1.9
K03241	Cyp1a2	CYP1A2	1.4	−0.2
X53363	Calr	Calreticulin	−1.2	−0.5
X79081	Cyp2c	CYP2C (mephenytoin 4-hydroxylase)	−1.1	−0.5
L48209	Cox8a	Cytochrome c oxidase 8a	0.3	1.0
U15211	Rara	RARα (retinoic acid receptor α)	−0.3	−1.1
AI014091	Cited2	CITED2 (Cbp/p300-interacting transactivator with ED-rich tail2)	−0.1	−1.2
(E) LCA				
X53363	Calr	Calreticulin	−0.4	1.2
L17127	Psmb4	PSMB4 (proteasome (prosome, macropain) subunit β4)	−1.0	0.5
(F) CDDP				
AA685903	—	Similar to Endoplasmin precursor (ER protein 99) (GRP94) (ERP99) (Tumor rejection antigen gp96)	−1.1	−0.3
L18889	Canx	Calnexin	−1.1	−0.6
X53363	Calr	Calreticulin	−2.4	−0.6
AF045464	Akr7a3	Aflatoxin aldehyde reductase	0.0	1.4
M26125	—	—	0.2	1.2
M24604	Pcna	PCNA	−0.2	1.0
E03229	Cdo1	Cytosolic cysteine dioxygenase 1	−0.6	−1.0
X02741	Tat	Tyrosine aminotransferase	−0.4	−1.5
(G) DF				
X53363	Calr	Calreticulin	−1.4	0.3
AA944397	Hspca	HSP1α	−1.6	−0.1
(H) DSF				
No change				

* No symbol or no annotation.

LOC191547, G6pd, Cyp1a1, Cyp3a, Sult1a1 and tyrosine aminotransferase (TAT), even though the decrease of TAT was slight (approx. 0.5 fold, data not shown). The expression level of the glucocorticoid receptor itself was not confirmed in this study because the microarray used did not include the relevant gene probes. This type of change has not been reported previously.

There were some discrepancies between previously reported *in vivo* studies and the present results. No effects were observed on proliferation-associated genes or anti-oxidants, unlike CPA (Table 3C). A previous *in vivo* study showed that oxidative stress in the rat liver represented by an increased Hmox1 was induced by a high dose of APAP but not by low doses (Heinloth *et al.*, 2004) and so it was considered that the lack of oxidative stress in the present study could possibly be from the low concentration. On the contrary, a previous study in rat hepatocytes exposed to a lower concentration (1000 μM) of APAP for 24 h showed induced liver Gst-Ya (de Longueville *et al.*, 2003), which appeared to be consistent with the above-mentioned *in vivo* study in rats at an intensive dose by Huang and colleagues. Therefore, the reason for the lack of the increased oxidative stress related gene was not clear. No decreased genes related to gluconeogenesis or fatty acid synthesis were observed, unlike an *in vivo* study of APAP (Heinloth *et al.*, 2004). These changes were also not observed in the *in vitro* study at a low concentration (de Longueville *et al.*, 2003).

Clofibrate (CF)

The PPARα agonist CF induced PPARα-inducible genes (Table 3B), which were consistent with previous *in vivo* studies: acyl-CoA thioesterase, enoyl-CoA hydratase/3-hydroxyacyl CoA dehydrogenase and acyl-CoA oxidase 1, Cyp4a10/4a14, Hsd17b4 and peroxisomal biogenesis factor 1. Increases of other β-oxidation enzymes, such as 2,4-dienoyl CoA reductase 1 and flavoprotein dehydrogenase, were also observed. These changes continued from 6 h to 24 h, and the degree was time dependent. Similar tendencies were shown in a previous report with rat hepatocytes exposed to CF for 24 h (de Longueville *et al.*, 2003). A transcription factor cofactor CITED2 decreased under the condition of ligand-dependent

activation of PPAR in the present experiment. This could be a negative feedback from PPARα activation, because CITED2 has been reported to be involved in the activation of PPARα in a ligand independent manner (Tien *et al.*, 2004).

In contrast to a report in rat liver (Baker *et al.*, 2004), no increases of Cyp4a1 or epoxide hydrase, a decrease of PPAR, or alterations of proliferation-associated genes were observed. Similarly, effects of CF on proliferation-associated genes in rat hepatocytes were not observed.

Drugs Not Directly Toxic to Hepatocytes *In Vivo* or Rarely Toxic to the Liver

The principal target of CPA-induced hepatotoxicity *in vivo* is the SEC because of a greater susceptibility to CPA than the hepatocytes (DeLeve, 1996). Direct effects on the hepatocyte transcription are not well characterized except for some effects on some metabolic enzymes. In this study, increased p53 targets, such as Gadd45α, Gadd153, Pcna and Hsp27, and decreased glucocorticoid inducible genes, such as Arg1, LOC191547, Cyp3a, Tat, Cyp1A1 and Sult1a1, at 24 h were observed, although the decreases of Cyp1A1 and Sult1a1 were slight (0.6-fold each, data not shown) (Table 3C). These changes and the timing were similar to APAP. It appeared reasonable that there was some commonality between CPA and APAP, because these drugs damage cells in a similar manner; reactive metabolites of these drugs covalently bind to DNA or proteins. Decreased Cyp3a3 (Table 3C) was consistent with a previous *in vivo* study in rats, whereas no increases of Cyp2b or Cyp2c, which have been reported in the *in vivo* study (Xie *et al.*, 2005), were observed. Increased Hmox1 may be consistent with a previous report that showed increased heme oxygenase content in the liver in rats *in vivo* (Rizzardini *et al.*, 1984).

CPZ did not induce transcriptional changes related to toxicity (Table 3D), which appeared consistent with the mechanism of CPZ-induced hepatotoxicity being cholestasis (Klaassen, 2004). Although the effects of CPZ on liver transcription are not well characterized, a previous study showed increased activities of CYP1A1/2 and CYP2B1 and a decreased activity of CYP2C11 in rats *in vivo* (Murray, 1992). Increases of Cyp1a1 and Cyp1a2 and a decrease of Cyp2c at 6 and/or 24 h were consistently observed.

LCA did not induce transcriptional changes related to toxicity (Table 3E), which appeared consistent with the mechanism of LCA-induced hepatotoxicity being cholestasis (Leuschner *et al.*, 1977). Only increased Calr and decreased Psmb4 were observed. Although effects from LCA on liver transcription are not well characterized, a previous study showed induction of UGT in primary rat hepatocytes exposed to LCA at a high concentration 100 mM for 48 h (Li *et al.*, 2000). This type of change was not observed in this study.

CDDP did not induce transcriptional changes indicative of toxicity (Table 3F), although increased Pcna suggested possible cell cycle effects. This lack of toxicologically significant changes appeared consistent with the fact that CDDP rarely induces hepatotoxicity *in vivo*. This is also consistent with a previous *in vivo* expression analysis which showed CDDP at a nephrotoxic dose, a maximum tolerated dose, did not cause expression changes in the rat liver after 24 h and 7 days of administration (Huang *et al.*, 2001). On the other hand, in the rat hepatocyte cell line, clone-9, CDDP has been shown to cause transcriptional changes including increases in genes related to stress and cell cycle after 24 h exposure to a low concentration of 5 $\mu g\ ml^{-1}$ (Huang *et al.*, 2001).

As for DF, only two genes were identified as being affected, i.e. decreases of two chaperons at 6 h (Table 3G). It appeared reasonable that the number of affected genes was very limited because DF is an idiosyncratic hepatotoxicant. Adduct formation of protein with reactive metabolites (Miyamoto *et al.*, 1997; Pumford *et al.*, 1993) and hypersensitivity (Romano *et al.*, 1994; Salama *et al.*, 1991; Kretz-Rommel and Boelsterli, 1993) have been proposed as mechanisms for DF-induced idiosyncratic hepatotoxicity which is not well understood. Although transcriptional effects of DF on hepatocytes are not well characterized, a previous study showed that DF induced Hmox1 mRNA in rat liver and in rat primary hepatocytes at intensive doses: 300 μM for 3 h *in vitro* (Cantoni *et al.*, 2003). This type of change was not observed in this study.

In the case of DSF, no changes were observed (Table 3H), which appeared consistent with the fact that DSF is an idiosyncratic hepatotoxicant where involvement of the immunological process has been proposed (Eliasson *et al.*, 1998).

In summary, the types of transcriptional changes observed in this study were consistent with previously reported *in vivo* data, although there were some differences. In the hierarchical cluster analysis, drugs formed clusters depending on their mode of toxicity against cells. The number of transcripts affected by the cholestatic hepatotoxicants, or drugs which rarely cause hepatotoxicity, were limited compared with the other drugs, where they did not induce transcriptional changes apparently related to toxicity. It is concluded that *in vitro* gene expression analysis of hepatocytes using microarray is a useful tool in evaluating the toxicological profile of drugs and in screening for direct toxicity of drugs against hepatocytes.

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p53 (TRP53) Deficiency-Mediated Antiapoptosis Escape after 5 Gy X Irradiation Still Induces Stem Cell Leukemia in C3H/He Mice: Comparison between Whole-Body Assay and Bone Marrow Transplantation (BMT) Assay

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Mice exposed to a lethal dose of radiation were repopulated with heterozygous p53$^{+/-}$ (TRP53$^{+/-}$) bone marrow cells and then exposed to doses of 1, 3 and 5 Gy 1 month later. This resulted in the transplanted bone marrow-specific diseases other than competitively induced nonhematopoietic neoplasms. Interestingly, the present study showed a high frequency of stem cell leukemia, i.e., leukemias characterized by a lack of differentiation due also to p53 deficiency, even after 5 Gy irradiation. The frequencies of stem cell leukemias (and those of total hematopoietic malignancies) were 16% (24%) at 1 Gy and 45% (75%) at 3 Gy. Furthermore, markedly high incidences of stem cell leukemias were observed at 5 Gy in p53$^{+/-}$ mice, i.e., 87% (100%) in the transplantation assay and 60% (83.3%) in the whole-body assay, whereas a conventional whole-body assay induced only 14% in wild-type mice. The high incidence of stem cell leukemias observed in this study using heterozygous p53-deficient mice agrees with results of a previous study of homozygous p53-deficient mice and is consistent with the high frequency of loss of heterozygosity in the p53 wild-type allele observed in leukemias. This suggests that the target cells for radiation-induced stem cell leukemias may be p53-deficient hematopoietic stem cells.

INTRODUCTION

In general, the incidence of radiation-induced leukemias after radiation exposure may be a function of the surviving fraction of hematopoietic stem cells[2] and the minimum number of presumptive hematopoietic stem cells required for the induction of one leukemia case (*1*). Because caloric restriction decreases the number of hematopoietic progenitor cells, presumed to be targets of leukemogenesis, our recent results were in good agreement with the decrease in the incidence of myeloid leukemia by 14% (8% compared to 22%) after 3 Gy X irradiation (*2, 3*). Classic studies of radiation-induced leukemia also showed a marked decrease in the incidence of leukemias after splenectomy (*4–6*), which was also interpreted to be a consequence of the decrease in the number of hematopoietic stem cells and/or progenitor cells. The incidence of radiation-induced leukemia increases with the dose of radiation up to 3 Gy but then starts to decrease at doses over 3 Gy and is only 2% or less at more than 6 Gy (*7, 8*) due to the decrease in the number of hematopoietic progenitor cells; thus this may also be the case at the stem cell level.

Previously, we examined the radiation-induced leukemias in homozygous p53 (TRP53)-deficient C3H/He mice. In that study, we found a high frequency of unusual stem cell leukemias with a trace of myeloblastic differentiation in the nonirradiated control group, the onset of which became significantly earlier when mice were irradiated with 3 Gy (*9*). Because the p53-homozygous deficiency generally caused an early onset of spontaneous and/or induced neoplasms even in the nonirradiated group, neither the relationship between radiation dose and the incidence of hematopoietic neoplasm nor the possible association with the induction of stem cell leukemias in terms of radiation effects was observed previously.

A heterozygous deficiency in the p53 tumor suppressor gene (*Trp53*) in mice, which provide a model of human Li-

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[2] An assay should be made by counting the number of hematopoietic progenitor cells, i.e., descendants of hematopoietic stem cells, which can be assayed only with a fraction of the long-term sustainability of lethally irradiated mice.

Fraumeni syndrome, greatly enhances predisposition to various malignancies characterized by reduced differentiation (*9–14*). Because of the slightly later onset of spontaneous and induced neoplasms in heterozygous p53-deficient mice than in homozygous p53-deficient mice, the former have been used for a more rapid carcinogenicity bioassay than the standard assay that uses wild-type mice. In this study, heterozygous p53-deficient C3H/He mice were used to compare the whole-body assay and the bone marrow transplantation (BMT) assay using mice repopulated with bone marrow cells from heterozygous p53-deficient mice. The BMT assay is an experimental tool for observing bone marrow-specific hematopoietic malignancies with the minimum competitive risk of developing neoplasms other than those arising from nonhematopoietic tissues. Three possibilities were hypothesized in this study: (1) a possible radiation dose-associated increase in the incidence of stem cell leukemias, (2) a possible relationship between stem cell leukemias and the loss of heterozygosity (LOH) for the remaining p53 allele, and (3) a possible induction of stem cell leukemias at a higher dose, 5 Gy, due to radioresistance, i.e., the p53 deficiency-mediated antiapoptosis escape of hematopoietic progenitor cells.

Wild-type mice were repopulated by transfusion of wild-type or heterozygous p53-deficient bone marrow cells after exposure to a lethal dose of radiation, followed by a whole-body exposure to increasing doses of radiation, to examine the incidence of stem cell leukemias throughout the lifetime of the mice.

MATERIALS AND METHODS

Mice

Mice lacking one allele of the p53 gene, which were derived by gene targeting in the embryonic stem cell line TT2 (*15*) and backcrossing with C57BL/6 mice, were kindly provided by Tsukada and Aizawa. These hybrid mice with heterozygous p53 deficiency were bred with normal C3H/HeNirsMs females in the Experimental Animal Care and Welfare Board-approved laboratory animal facility of the National Institute of Radiological Sciences (NIRS), Chiba, Japan.

All experimental protocols involving the laboratory mice in this study were reviewed by the externally established peer review panel, the Committee of the Ethics of the Research and Welfare of the Experimental Animals (ERWEA) of the NIRS, and were approved by the Animal Care and Use Committee at the NIRS. The experiments were performed according to the NIRS Guidelines for the Care and Use of Laboratory Animals. After 13 generations of backcrossing between heterozygous p53-deficient mice and C3H/HeNirsMs mice, heterozygous p53-deficient males and females were crossed to generate wild-type ($p53^{+/+}$) and p53-deficient ($p53^{+/-}$, $p53^{-/-}$) mice. The neonates were genotyped by targeted DNA screening by polymerase chain reaction (PCR) analysis using tissue obtained from the tail. The PCR primers used were the same as those used to detect the loss of heterozygosity (LOH) for leukemias induced in spleen tissues. Therefore, the band for the wild allele might be equivocal due to partial coverage of PCR primers for full-length p53; LOH might be detectable if the full-length p53 gene were examined. (See Table 1 in the Results.)

Eight-week-old C3H/HeNirsMs males bred in the NIRS were used as the recipient mice in the BMT assay and the assay of colony-forming units in the spleen.

Irradiation

Cells or mice were irradiated with an X irradiator (Shimadzu, Tokyo) operated at 200 kV and 20 A with 1.0-mm aluminum and 0.6-mm copper filters at a dose rate of 0.614 Gy/min and a 56 cm focal surface distance. During the irradiation, the mice were irradiated whole body, were not anesthetized, and were allowed to move freely in individual circular chambers.

Preparation of Bone Marrow Cells

The femoral bone marrow was harvested from $p53^{+/+}$ and $p53^{+/-}$ mice, and $p53^{-/-}$ mice if applicable, suspended in α-MEM, and then processed to obtain a single-cell suspension by repeated aspiration through a 27-gauge hypodermic needle.

Assay of Colony-Forming Units in Spleen (CFU-S)

The method of Till and McCulloch (*16*) was used to determine the number of CFU-S. Aliquots of bone marrow cell suspensions were used to evaluate the number of CFU-S. The number of bone marrow cells with or without irradiation was adjusted to that appropriate for producing nonconfluent spleen colonies, and the cells were then transplanted into lethally irradiated mice by injection through the tail vein. For the assay of spleen colonies, increasing doses of X radiation were given *in vitro* (irradiation conditions were similar to those for mice described above). Spleens were harvested 8 and 12 days after the injection and fixed in Bouin's solution. Macroscopic spleen colonies were counted under an inverted microscope at a magnification of 5.6×.

Irradiation for Induction of Leukemias

Twenty-four to 30 mice per group for the whole-body assay and 20 to 25 mice per group for the BMT assay were subjected to whole-body irradiation at doses of 0 (control), 1, 3 and 5 Gy and were observed throughout their lifetime. For the whole-body assay, mice were irradiated at 10 weeks of age. For the BMT assay, recipient mice grafted with heterozygous p53-deficient cells or those grafted with wild-type cells were subjected to a second irradiation 4 weeks after transplantation (see the next paragraph for the assay method).

Bone Marrow Transplantation (BMT) Assay

Eight-week-old male C3H/HeNirsMs mice were used as recipient mice. The mice were subjected to 9.45 Gy of whole-body X irradiation, as described above. Bone marrow cells were harvested from heterozygous p53-deficient C3H/HeNirsMs male donors, and 1×10^6 cells were injected into the tail vein of lethally irradiated wild-type C3H/HeNirsMs mice. The same number of wild-type bone marrow cells was also injected into lethally irradiated recipients.

Analysis by Fluorescence-Activated Cell Sorting (FACS) to Determine Stem Cell Leukemias

The leukemia cases negative for Thy-1.2, CD3, CD4, CD8, TL-2, IL-2, B220, sIgM, Mac-1 and Gr-1, and those positive for c-kit and CD44 were designated as stem cell leukemias and were confirmed immunohistopathologically. The immunophenotypic definition of stem cell leukemias has been fully described elsewhere (*9*); the analytical method is described briefly here. A single-cell suspension was prepared from leukemic spleens. One million leukemic spleen cells were suspended in phosphate-buffered saline (PBS) containing 0.3% bovine serum albumin and 0.1% NaN_3 and then incubated for 30 min with FITC-conjugated antibodies (anti-Thy1.2, anti-CD4, anti-TL-2, anti-IL-2 , B220, Gr-1 and anti-c-Kit antibodies), biotin-conjugated antibodies [anti-CD3, anti-CD8, anti-s-IgM (Zymed Lab., Inc., San Francisco, CA)], an anti-Mac-1 antibody (Caltag Lab, San Francisco CA), an anti-CD44 (*17*) (PgP-1: Becton Dickinson) antibody, and an anti-TER-119 monoclonal antibody (Becton

Dickinson). Data were analyzed using Lysis II software (Becton Dickinson). Cells from the thymus and mesenteric lymph nodes of mice with severe leukemia were also immunostained and analyzed as described above.

Histopathological Examination

Mice were observed three times daily with gentle care throughout their lifetime. Mice exhibiting anemia with pale appearance and/or hypothermia of extremities were isolated and euthanized humanely as soon as they showed signs of distress.

Blood samples collected from the circulating blood were examined immunohematologically and histopathologically for evidence of immunohematological and histopathological abnormalities. For histopathological examination, all the visceral organs including the thymus, spleen, sternum and femoral bone marrow were fixed in 4% neutral-buffered formalin for 24 h. The sternum and femoral bone marrow were decalcified in 7.5% formic acid for 72 h. After routine processing, paraffin-embedded sections were stained with hematoxylin and eosin (H&E) and then examined under a light microscope. The histopathological classification of murine hematopoietic malignancies including stem cell leukemias was based on the authors' previous report (*9*) and the monograph (*18*) published by one of the present authors, along with the results of the FACS analysis.

Examination of Loss of Heterozygosity (LOH) for p53 Wild-Type Hemi-allele (PCR)

During the course of radiation-induced leukemogenesis, the wild-type allele of the p53 gene remaining in the heterozygous p53-deficient mice may be inactivated. The frequency of such LOH was evaluated in mice with radiation-induced leukemias. The band corresponding to the wild-type allele indicated that there was no complete loss of the allele in most of the leukemia cases with LOH, but the band showed a weak signal due to stromal tissue contamination by leukemic cells whose wild-type allele signals were noticeable weaker than those of the positive control band or paired mutated p53. Whenever possible, tissues were obtained immediately after death and the p53 hemi-allele derived from the heterozygous p53-deficient mice with leukemia was determined using the same PCR primers used in the genotyping of the mice for breeding. The PCR primers used to detect the p53 gene in this study cover exon 1 and its vicinity.

Statistical Analysis

The data were analyzed using the generalized Wilcoxon test for determining the statistical significance of differences.

RESULTS

Survival Curves after Exposure to Graded Increasing Doses of Radiation: Whole-Body Assay and BMT Assay

Figure 1A shows the survival curves for groups of 10-week-old mice subjected to whole-body irradiation. The mean survival time for the control group (0 Gy) was 380 ± 19 days. With increasing doses of radiation (1, 3 and 5 Gy), the mean survival time decreased to 323 ± 24, 212 ± 13 and 152 ± 9 days, respectively ($P = 0.1$, $P < 0.001$ and $P < 0.0001$, respectively, compared to the 0-Gy control group by the generalized Wilcoxon test). Because p53-deficient mice have higher incidences of various nonepithelial tumors other than hematopoietic neoplasms, a BMT assay focusing on the induction of hematopoietic neoplasms was designed to examine the incidence of C3H/He-specific myeloid leukemias after exposure to increasing doses of radiation. The experimental protocol for the BMT assay in this study using heterozygous p53 deficiency is similar to that used in a previous study of homozygous p53-deficient mice (*9*). One million bone marrow cells were transfused into 6-week-old lethally irradiated C3H/He mice followed by another whole-body irradiation with increasing doses to induce leukemia when they were 10 weeks old.

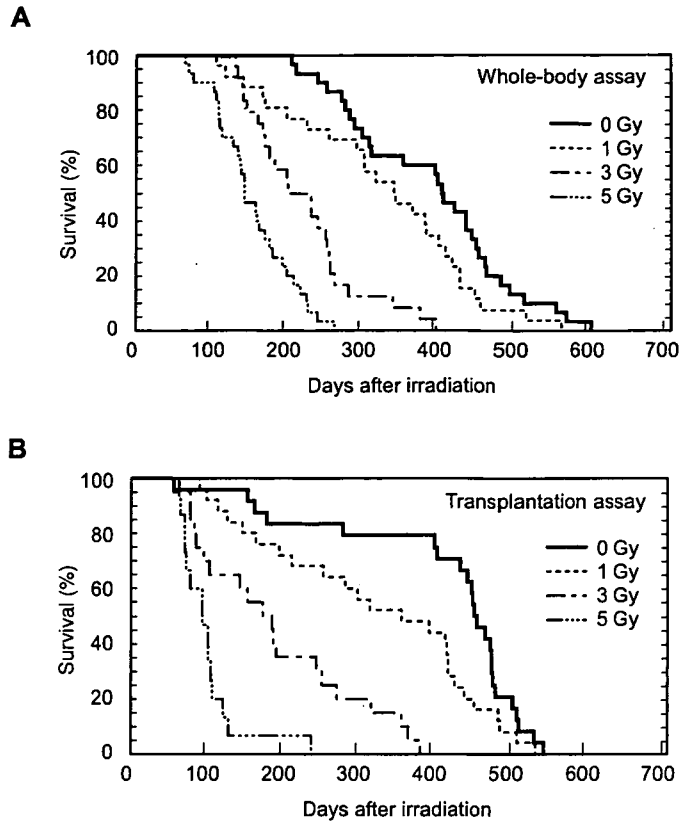

FIG. 1. Survival curves for mice irradiated with 1, 3 and 5 Gy and for nonirradiated mice. Panel A: Whole-body assay; 10-week-old mice were irradiated and observed throughout their lifetime. Panel B: Transplantation (BMT) assay (*9*): Recipient mice that had been lethally irradiated with 9.45 Gy were repopulated with 1×10^6 bone marrow cells from heterozygous p53-deficient mice. The mice were allowed to recuperate for 4 weeks before X irradiation. After irradiation, they were observed throughout their lifetime.

Figure 1B shows the survival curves for the groups in the BMT assays that were exposed to increasing doses of radiation and for the 0-Gy control group. The mean survival time for the 0-Gy control group was 402 ± 27 days. With increasing doses of radiation (1, 3 and 5 Gy), the mean survival time decreased to 318 ± 28, 188 ± 23 and 99 ± 11 days, respectively ($P = 0.0168$, $P < 0.001$, and $P < 0.0001$, respectively, compared to the 0-Gy control group). The survival curve for the 5-Gy-irradiated group in the BMT assay (Fig. 1B) shows earlier deaths than in the corresponding group in the whole-body assay shown in Fig. 1A ($P < 0.0001$). However, the survival curves for other groups exposed to less than 3 Gy had slightly lower death rates than the corresponding groups in the whole-body as-

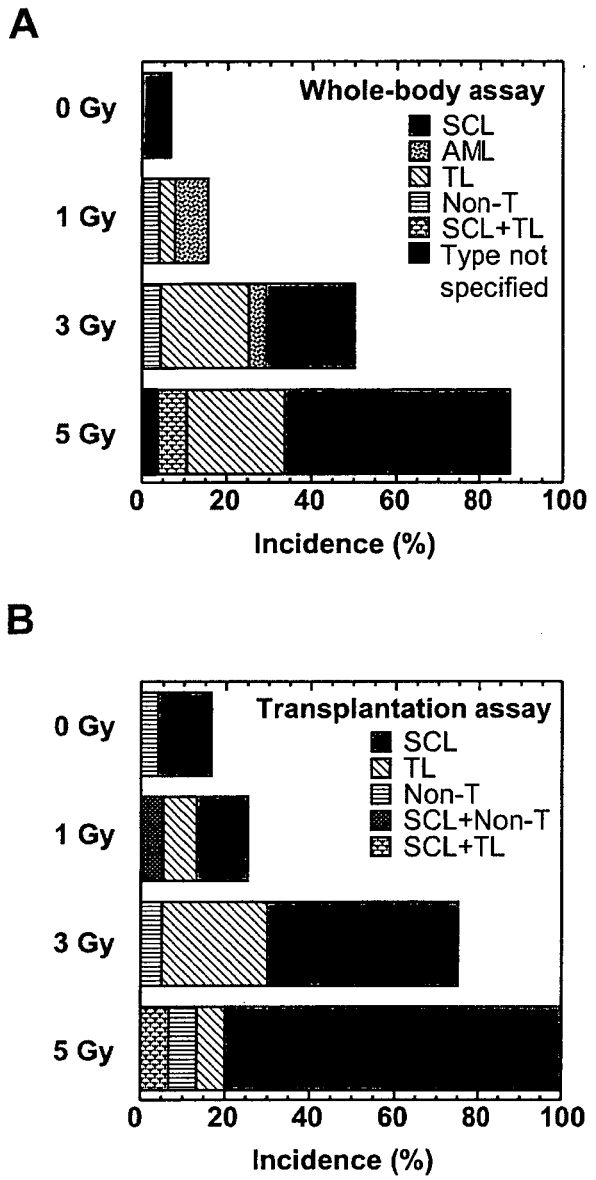

FIG. 2. Incidences of hematopoietic neoplasms in heterozygous p53-deficient mice irradiated with 1, 3 and 5 Gy X rays and in nonirradiated mice. The diagnosis for each hematopoietic malignancy was made on the bases of immunocytochemical analysis, fluorescence-activated cell sorting, and histopathological analysis. Panel A: Whole-body assay; panel B: transplantation (BMT) assay. SCL, stem cell leukemia; AML, acute myeloid leukemia; TL, thymic lymphoma; Non-T, nonthymic lymphoma.

say (statistically insignificant). The differences in the fatal disease spectra in the BMT and whole-body assay groups may have been the result of different p53 environments of the host tissue as well as factors attributable to a lethal whole-body irradiation prior to the BMT.

p53 Deficiency Induces Stem Cell Leukemias: Immunocytological and Histopathological Examinations

The incidences of hematopoietic neoplasms in each experimental group are shown in Fig. 2A for the whole-body assay and in Fig. 2B for the BMT assay. As observed previously, stem cell leukemias that developed with a trace of myeloid differentiation were examined immunocytologically to determine their cytological origin (*9*). The leukemia cases negative for Thy-1.2, CD3, CD4, CD8, TL-2, IL-2, B220, sIgM, Mac-1 and Gr-1 and positive for c-kit and CD44 were designated as stem cell leukemias. The incidences of stem cell leukemias increase with the dose of radiation as shown in Fig. 2A and B; however, the dose–incidence relationship was much clearer in the BMT assay (Fig. 2B).

LOH in Leukemia Tissue of Heterozygous p53-Deficient Mice

The normal allele of the p53 gene remaining in heterozygous p53-deficient mice was examined in tissues from those mice that developed leukemias. Figure 3 shows an example of a PCR analysis of a leukemic mouse with the wild-type p53 allele. Except for lanes 7, 9 and 10, the wild-type p53 allele, which corresponds to the lower band with a weaker signal than the band corresponding to the p53 mutated allele for knockout strategy (p53 mut-signal), was regarded as essentially deleted in all leukemic tissues (M, molecular marker; A-C, positive control for PCR; D, negative control). Results for LOH showed good agreement with the characteristics of stem cell leukemias determined by immunocytochemical and histopathological examinations. Lanes 7 and 10 correspond to samples from the same mouse with angiosarcoma originating from the spleen, and lane 9 corresponds to the sample from a mouse with myelogenous leukemias other than stem cell leukemias. Table 1 shows the data from all the stem cell leukemia cases in which LOH for the p53 hemi-allele could be examined. Despite the fact that nearly half of the cases could not be examined randomly, 86% of the leukemia cases in the whole-body assay groups and 92% of the mice in BMT assay groups showed LOH for the remaining p53 allele. (Refer to an unpaired staining intensity comparison between the mutated and wild-type p53 alleles at the same mobilities for the positive control, lane C in Fig. 3.)

Cumulative Incidence of Leukemias and Dose Response

When one compares the incidences of stem cell leukemias in the whole-body assay and BMT assays (Fig. 4A and B, respectively), the curves for the BMT assay show a higher incidence of stem cell leukemias than those for the whole-body assay, owing in part to possible exclusion of the competitive induction of neoplasms other than hematopoietic neoplasms. The incidences of ordinary myeloid leukemia in wild-type mice at each dose are shown in Fig. 5 as closed circles with a solid line. In contrast, a high incidence (87%) of stem cell leukemias was seen in the heterozygous p53-deficcient 5-Gy-irradiated group in the BMT assay, whereas a low incidence of leukemias was observed in the wild-type 5-Gy-irradiated group in the whole-body assay (14%). No leukemias developed as shown by

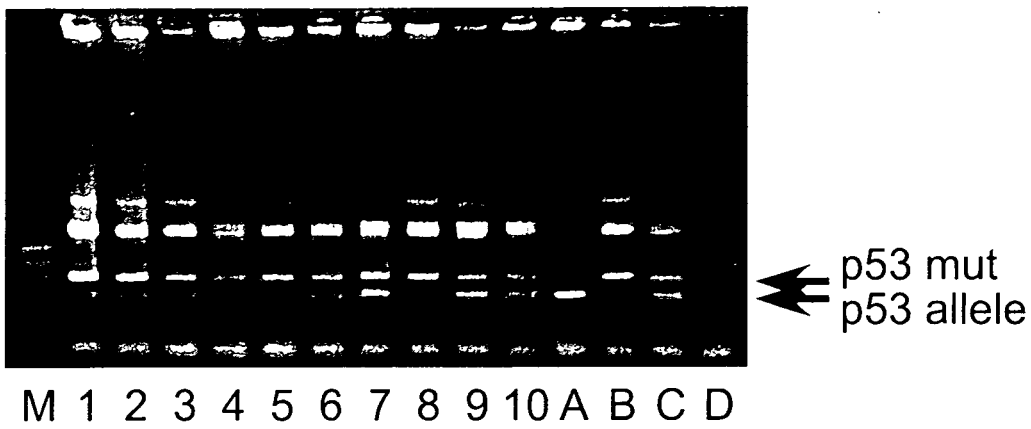

FIG. 3. An example of PCR data for wild-type p53 allele in each leukemia case. M indicates molecular marker; A–C, positive controls for PCR; D, negative control. Lanes 7, 9 and 10 show two bands corresponding to a p53 mutated allele for knockout strategy (p53 mut) and a p53 wild-type allele (p53 allele). Lanes 1–6 and 8, corresponding to samples from stem cell leukemia cases, show weak signals of the lower bands. Lanes 7 and 10 correspond to two samples from the same mouse with an angiosarcoma originating from the spleen, and lane 9 corresponds to a sample from a mouse with myelocytic leukemia other than stem cell leukemia.

the BMT assay of wild-type mice and as indicated by the open circles on the horizontal baseline. In this BMT assay, mice repopulated with wild-type bone marrow cells did not show any leukemia and died mostly of nephrosclerosis induced by a lethal dose of radiation.

Surviving Fractions of Hematopoietic Progenitor Cells after Exposure to Increasing Doses of Radiation

The survival of hematopoietic progenitor cells after exposure to radiation was found to be higher for p53-deficient cells than for wild-type cells on the basis of spleen colony-forming units [assay of CFU-S, both day 12 immature colonies (Fig. 6A: CFU-S late) and day 8 mature colonies (Fig. 6B: CFU-S early)]. As shown in Fig. 6, the survival curves for both day 12 immature colonies and day 8 mature colonies derived from the bone marrow of p53-deficient mice ($p53^{+/-}$ and $p53^{-/-}$) are flatter than those colonies derived from the bone marrow of wild-type mice ($p53^{+/+}$). This increased survival of the p53-deficient progenitor cells is assumed to be due in part to prevention of apoptosis as well as cell cycle arrest. This increased survival due to p53 deficiency might be responsible for the induction of stem cell leukemias at a higher incidence in a dose-dependent manner up to 5 Gy.

TABLE 1
Frequency of Loss of Heterozygosity in p53 Hemi-allele[a]

Assay	Number of cases[b]	Number examined (A)[c]	Number showing loss of wild allele[c] (B)	Ratio[c] (%)
Whole body	26	14	12	86
BMT	30	12	11	92

[a] Whenever tissues were obtained immediately after death, LOH was examined in mice with radiation-induced leukemia (See Materials and Methods).

[b] All leukemia cases observed in present study (from 0 to 5 Gy radiation exposure).

[c] Loss of wild allele = LOH. Frequency of LOH, (B/A) × 100 (%).

DISCUSSION

p53-deficient mice develop undifferentiated immature hematopoietic neoplasms (*9*), and in C3H/He mice, we previously observed a high frequency of stem cell leukemias with a trace of myelogenous differentiation in homozygous p53-deficient mice with or without radiation exposure (*9*). In the present study, as shown in Figs. 2A and B, regardless of the assay used, whole-body irradiation at increasing doses resulted in a high, dose-dependent frequency of stem cell leukemias. The incidence of stem cell leukemias was higher in the BMT assay, possibly owing to the deletion of competitive risks other than hematopoietic malignancies. Thymic lymphomas were also observed in all the irradiated groups. A few cases of double hematopoietic neoplasms (stem cell leukemias and thymic lymphomas) were observed in the 5-Gy-irradiated groups, implying that the loss of dose dependence at high doses may be because of competition between stem cell leukemias and thymic lymphomas. The mechanism underlying the loss of differentiation in neoplasms deficient in the p53 gene has been discussed elsewhere (*9, 19–22*).

Because most of the leukemias that developed in a previous study using homozygous p53-deficient mice were stem cell leukemias, the high incidence of stem cell leukemias observed in heterozygous p53-deficient mice in the present study suggests a high frequency of loss of the remaining heterozygous p53 allele in mice that developed leukemias. This possibility was supported by the observation of 86% LOH in leukemias in the whole-body assay and 92% LOH in leukemias in the BMT assay. In other words, among the cases of hematopoietic neoplasms that were examined for LOH, the cases showing LOH were all diagnosed as stem cell leukemias. In the present study, al-

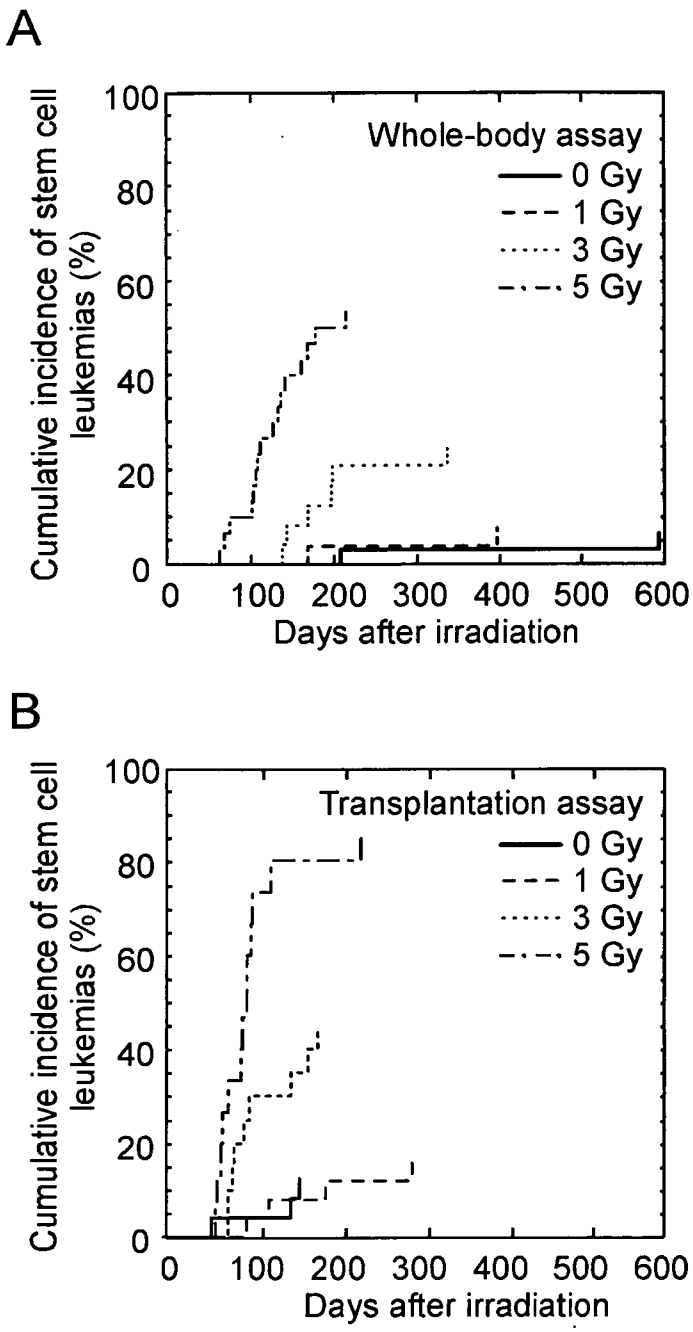

FIG. 4. Cumulative incidences of stem cell leukemias in heterozygous p53-deficient mice exposed to 1, 3 and 5 Gy X rays and in nonirradiated mice. Panel A: Whole-body assay; panel B: transplantation (BMT) assay.

though not all the stem cell leukemia cases showed LOH, the frequency might be higher, because the PCR primers used in the present study cover a limited part of exon 1 (*23*). Together with the other evidence observed, these results suggest that the stem cell leukemia may require complete inactivation of p53 and that LOH is readily achieved by radiation exposure in a dose-dependent manner in our C3H/He mice.

A few cases of nonhematopoietic soft tissue tumors and leukemias other than stem cell leukemias were observed,

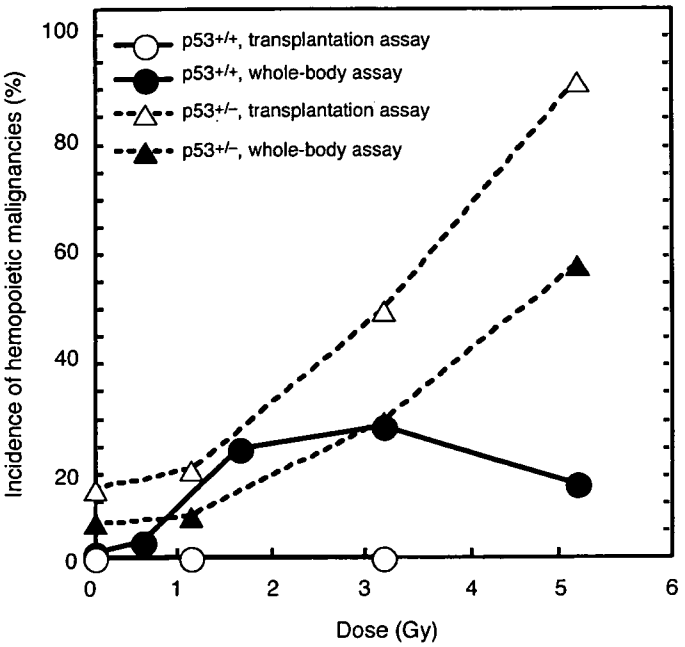

FIG. 5. Dose–response relationship of hematopoietic malignancies for different assays and different p53 genotypes. Data for wild-type mice are from Seki *et al.* (*8*). No hematopoietic malignancies were observed in the wild-type group subjected to the BMT assay.

neither of which showed any deletion or mutation of the wild-type p53 allele in heterozygous p53-deficient bone marrow. The mechanisms underlying these cases were not examined in this study.

Myeloid leukemia cases in C3H/He mice lack a part of chromosome 2 in 49 out of the 52 cases examined (94%) regardless of radiation exposure (*24*). However, interestingly, none of the leukemia tissues examined showed evidence of the deletion in chromosome 2 (*25*). In the stem cell leukemia cases that developed in the p53-deficient mice in this study, the deletion of a part of chromosome 2 might be skipped because of the rapid development of leukemias, but this possibility has not been fully clarified in this study.

Because an increase in the dose of radiation exponentially decreases the number of hematopoietic progenitor cells, exposure to a dose of more than 5 Gy will not yield a high frequency of leukemias, but instead it will induce a significant decrease in the incidence of leukemias; this is in agreement with the loss of hematopoietic progenitor cells (Fig. 6). Conversely, damaged p53-deficient progenitor cells escaped from the pathway leading to apoptosis after exposure to increasing doses of radiation, and their survival curves indicate resistance to radiation (Fig. 6). Therefore, such surviving damaged hematopoietic progenitor cells are to be targets of leukemogenesis at an even higher radiation dose, for example, 5 Gy. The high incidences of stem cell leukemias observed in both the whole-body assay and BMT assay in heterozygous p53-deficient mice at 5 Gy are possibly attributable to p53-deficient bone marrow cells escaping radiation-induced DNA damage (*26, 27*) and apoptosis (*28–31*). This was observed for the first time in the present

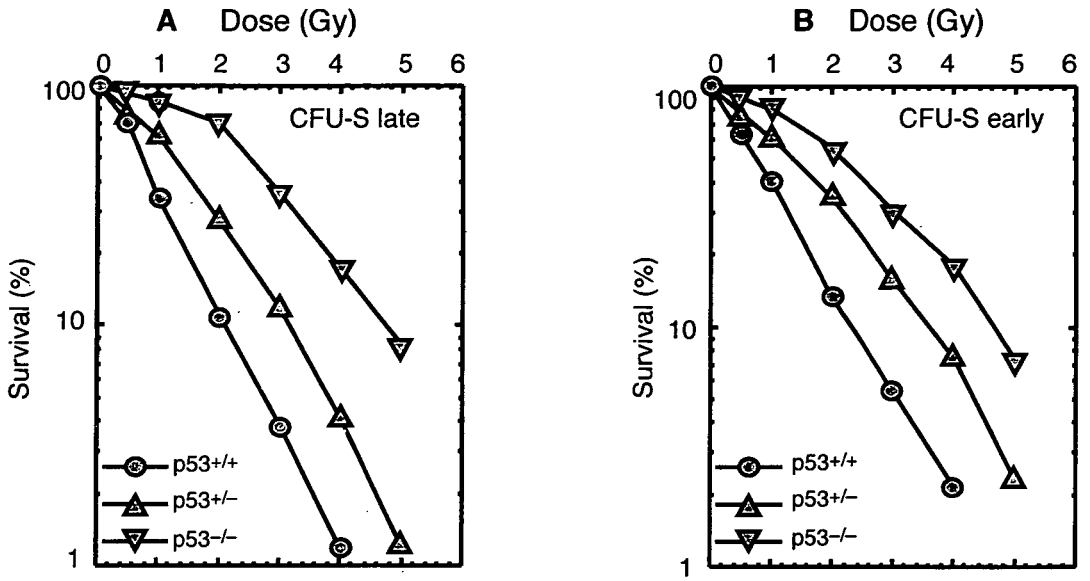

FIG. 6. Radiation survival curves for hematopoietic progenitor cells, colony-forming units in spleen (CFU-S) with p53 deficiency. Bone marrow suspensions were exposed to increasing doses of radiation *in vitro*. Panel A: CFU-S late, counted on day 12; panel B: CFU-S early, counted on day 8.

study. It is of interest that the incidences of stem cell leukemias at 5 Gy were extremely high in both the whole-body and BMT assays. As shown in Fig. 2B, because of the nullification of competitive risk, the incidence of hematopoietic neoplasms at 5 Gy is 100%. This presumably is due to an increase in the incidence of targeted hematopoietic progenitor cells that lack differentiation due to p53 deficiency (*9, 19–22*), resulting in an increase in the incidence of stem cell leukemias. In contrast, with increasing radiation dose in the p53-deficient mice, hematopoietic target stem cells and/or progenitor cells may undergo p53-independent apoptosis. Although have no data for irradiation with 6 Gy or higher, the incidence of leukemias in heterozygous p53-deficient mice may peak at 5 Gy.

The present leukemogenicity studies using p53-deficient mice have elucidated much more clearly the nonthreshold incidence of leukemogenicity than those using incidence curves for wild-type mice (*32*). Conversely, leukemogenesis in wild-type mice is presumably and potentially prevented by p53 gene expression not only at high doses of radiation but also at low doses, as supported by a conventional radiation-induced leukemogenicity curve showing a linear-quadratic relationship.

Assaying leukemogenicity by BMT eliminates the competitive induction of neoplasms other than hematopoietic neoplasms, which occur at an increased frequency in heterozygous p53-deficient mice. Thus the increase in the risk of radiation-induced leukemogenesis with increasing dose was clearly visible in our model in which the "first hit" was genetically engineered. therefore, our model may be a good one for studying for the promotion stage of leukemogenesis. Sporadic nullification of the heterologous p53 allele in somatic cells is also observed in senescent animals and humans. In this regard, the radiation-induced risk of hematopoietic leukemogenesis in p53-deficient mice may be not only a theoretical model but also a practical tool for studying the possible mechanism(s) of senescent tumorigenesis in animals and humans, although p53 alteration generally is not involved in experimental models. Moreover, the present results suggest that using ex.p53ER^{TAM} for tamoxifen-derived p53 gene alteration (*33*) may be useful for developing other experimental models to examine an earlier part of leukemogenesis and/or leukemogenesis of differentiated leukemias. Furthermore, because p53 deficiency tends to induce spontaneous neoplasms other than leukemias, the BMT assay is a useful experimental tool for high-risk neoplastic modeling analogous to the p53-deficient system modeling used in the leukemogenicity bioassay.

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予測・予防型医療安全への転換
――「重篤副作用疾患別対応マニュアル」作成事業の概要

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はじめに

厚生労働省では，2005年度から４年計画で「重篤副作用総合対策事業」の一環として，関係学会の専門家などの協力を得て，「重篤副作用疾患別対応マニュアル」（以下，本マニュアル）の作成を進めている。2006年11月に，第１弾として「スティーブンス・ジョンソン症候群」,「間質性肺炎」などの９つの重篤副作用疾患のマニュアルをとりまとめた。また，2007年３月には第３回検討会において，引き続いて10のマニュアルについて検討を行った。本稿では，事業の目的，その進め方，本マニュアルについて紹介する。

重篤副作用総合対策事業について

１．目的

従来の安全対策は，医薬品に着目し，医薬品ごとにその使用により発生した副作用を収集・評価し，添付文書の改訂などを通じて臨床現場に注意喚起する「警報発信型」,「事後対応型」が中心となっていたが，医薬品の副作用は担当医の専門分野とは異なる臓器にも発生しうること，重篤な副作用の発生頻度は一般に低く，臨床現場において遭遇する機会が少ない場合がありうることなどから，場合によっては発見が遅れ，重症化することもある。

本事業においては，従来の安全対策に加え，個々の医薬品に着目した対策から，医薬品の使用により発生する副作用疾患に着目した対策整備を行っている。さらに副作用発生機序解明研究などを推進することにより，副作用のリスクの高い患者をあらかじめ予測したり，発生した副作用を初期のうちに発見し，重症化を防ぐ「予測・予防」的な安全対策への転換を図ることを目的として，2005年度より重篤副作用総合対策事業をスタートした[1)-3)]。

２．本事業の進め方

本事業は，2005年度から以下の３つの段階を踏まえ進めることとしている（表１）。本事業の第１段階「早期発見・早期対応の整備」（４年計画）では，重篤度などから判断して必要性の高いと考えられる副作用について，患者および臨床現場の医師，薬剤師などが活用する治療法，判別法などを包括的にまとめた「重篤副作用疾患別対応マニュアル」を作成することとしている（図１）。

また，計画的にリスク因子の解明と副作用の発生機序研究を推進することにより，将来的には副作用の発生を低減した新薬開発を可能とするなど，医薬品の安全対策について，これまでの事後対応型に加え，予測・予防型の積極的な副作用対

表 1　重篤副作用総合対策事業の進め方

第 1 段階：「早期発見・早期対応の整備」
重篤度などから判断して必要性の高いと考えられる副作用について，患者および臨床現場の医師，薬剤師などが活用する判別法，治療法などを包括的にまとめたマニュアルを４年計画で作成・公表することにより，臨床現場における副作用の早期発見，早期対応を促進する
第２段階：「予測対応の整備」
副作用症例の集積・解析などから得られるハイリスク患者群に関する知見をもとにマニュアルの改訂を行う
第３段階：「予防対応の整備」
リスク因子の解明と副作用の発生機序研究を推進し，臨床現場においてはハイリスク患者群への投薬を避け，また製薬企業においては副作用の発現を低減した新薬の開発を目指す

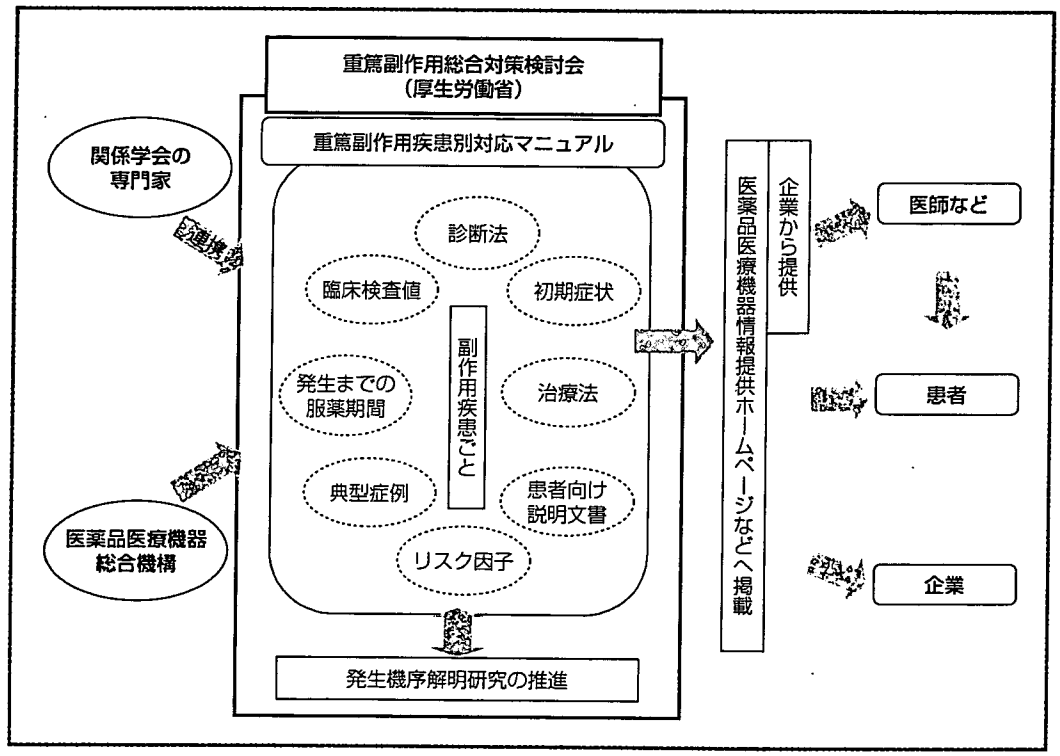

図 1　重篤副作用総合対策事業について

策を展開することとしている。

現在，遺伝子多型と薬剤応答性に関する知見が蓄積されており，今後，より有効性・安全性の高い薬物治療の実現に向けた方策の一つとしてファーマコゲノミクスを利用した，よりいっそうの取り組みが期待される。特に，副作用のリスク因子をもつ患者を事前に同定することができれば，予測・予防型の安全対策に資するものと考えられる。

重篤副作用疾患別対応マニュアルについて

2005年７月19日に開催した第１回重篤副作用総合対策検討会（座長：松本和則，国際医療福祉大学教授）において，マニュアルの作成について検

表２　重篤副作用疾患別対応マニュアルの作成対象副作用疾患と作成状況

領域	学会名	対象副作用疾患	状況
皮膚	日本皮膚科学会	スティーブンス・ジョンソン症候群	2006年11月公表
		中毒性表皮壊死症	2006年11月公表
		薬剤性過敏症症候群	2007年3月検討会
		急性汎発性発疹性膿疱症*	作業中
肝臓	日本肝臓学会	薬物性肝障害	作業中
腎臓	日本腎臓学会	急性腎不全	2007年3月検討会
		間質性腎炎	2007年3月検討会
		ネフローゼ症候群*	作業中
血液	日本臨床血液学会	再生不良性貧血	2007年3月検討会
		出血傾向	2007年3月検討会
		薬剤性貧血	2007年3月検討会
		無顆粒球症	2007年3月検討会
		血小板減少症	2007年3月検討会
		血栓症	2007年3月検討会
		播種性血管内凝固	2007年3月検討会
		血栓性血小板減少症紫斑病	作業中
		ヘパリン起因性血小板減少症	作業中
呼吸器	日本呼吸器学会	間質性肺炎	2006年11月公表
		非ステロイド性抗炎症薬による喘息発作	2006年11月公表
		急性肺損傷・急性呼吸窮迫症候群	2006年11月公表
		肺水腫*	作業中
		急性好酸球性肺炎*	作業中
		肺胞出血*	作業中
消化器	日本消化器病学会	麻痺性イレウス	作業中
		消化性潰瘍	作業中
		偽膜性大腸炎	作業中
		膵炎（急性膵炎）*	作業中
心臓・循環器	日本循環器学会	心室頻拍	作業中
		うっ血性心不全	作業中
神経・筋骨格系	日本神経学会	薬剤性パーキンソニズム	2006年11月公表
		横紋筋融解症	2006年11月公表
		白質脳症	2006年11月公表
		末梢神経障害	作業中
		無菌性髄膜炎	作業中
		急性散在性脳髄膜炎	作業中
		ギラン・バレー症候群	作業中
		ジスキネジア*	作業中
		痙攣・てんかん	作業中
精神	日本臨床精神神経薬理学会	悪性症候群	作業中
		うつ状態	作業中
代謝・内分泌	日本内分泌学会	偽アルドステロン症	2006年11月公表
		甲状腺機能亢進症*	作業中
		甲状腺機能低下症*	作業中
	日本糖尿病学会	低血糖	作業中
		高血糖*	作業中
過敏症	日本アレルギー学会	アナフィラキシー	作業中
		蕁麻疹・血管浮腫	作業中
感覚器（眼）	日本眼科学会	網膜・視路障害*	作業中
		緑内障*	作業中
口腔	日本口腔外科学会	薬物性口内炎*	作業中
骨	日本口腔外科学会	顎骨壊死*	作業中
	日本整形外科学会	骨粗鬆症*	作業中
泌尿器	日本泌尿器科学会	尿閉（排尿困難）*	作業中

＊：第２回重篤副作用総合対策検討会において追加作成することとされたもの

討された。頻度や重篤度から判断して必要性が高く，マニュアル作成に着手すべき副作用疾患として表２の作成対象副作用疾患を選定し，また，表３のマニュアルの記載項目をとりまとめた。

記載の要点としては，①患者，医療関係者（医師，歯科医師，薬剤師，看護師）向けの早期発見，早期対応のポイントを簡潔に記載すること，②医療関係者が臨床現場で遭遇する機会が少ないことを念頭に，副作用疾患の判別方法や治療方法を記載すること，③典型的な症例を紹介すること――などがあげられる。

本検討結果に基づき，学術論文，各種ガイドライン，厚生労働科学研究事業報告書，医薬品医療機器総合機構の保健福祉事業報告書などを参考に，厚生労働省の委託により，関係学会においてマニュアル作成委員会を組織し，日本病院薬剤師会とともに議論を重ねてマニュアル案の作成を進めた（図２）。2006年10月19日には，第２回重篤

表３　重篤副作用疾患別対応マニュアルの記載項目

		項目	内容
	1	副作用名	同義語などを記載
患者の皆様へ	2	副作用の概要 早期発見と早期対応のポイント	患者さんおよび患者のご家族向けの早期発見と早期対応のポイント
医療関係者の皆様へ	3	早期発見と早期対応のポイント	医療関係者が注意すべきポイント
	4	副作用の概要	対象副作用疾患の症状などの概要
	5	副作用の判別基準（判別方法）	副作用と原疾患などを判別する基準の概要
	6	判別が必要な疾患と判別方法	他の疾患などとの判別方法の概要
	7	治療方法	副作用が発現した場合の治療
	8	典型的症例概要	典型的な副作用として参考となる症例の概要
	9	引用文献・参考資料	マニュアル中の引用文献および関連資料の一覧

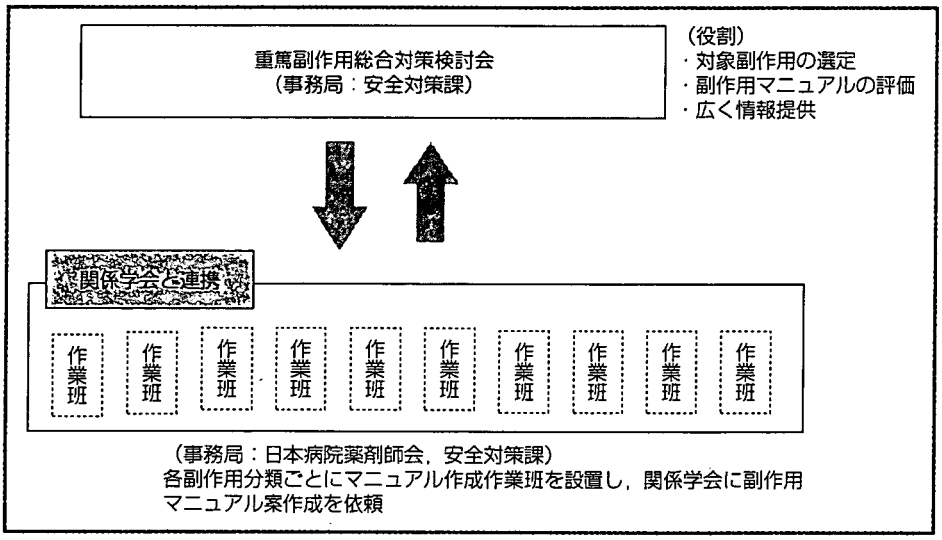

図２　重篤副作用疾患別対応マニュアルの作成体制

副作用総合対策検討会において，スティーブンス・ジョンソン症候群，中毒性表皮壊死症，間質性肺炎，急性肺損傷・急性呼吸窮迫症候群，非ステロイド性抗炎症薬による喘息発作，薬剤性パーキンソニズム，横紋筋融解症，白質脳症および偽アルドステロン症について，評価・検討され取りまとめた。また，この第２回の検討会では，ネフローゼ症候群，肺水腫などの副作用について追加選定されるとともに，感覚器（緑内障など），口腔（薬物性口内炎），骨（骨粗鬆症など），泌尿器（尿閉）の各領域が新たに選定された。

さらに，2007年３月22日の第３回重篤副作用総合対策検討会では，薬剤性過敏症症候群，再生不良性貧血，出血傾向，薬剤性貧血，無顆粒球症，血小板減少症，血栓症，播種性血管内凝固，間質性腎炎，急性腎不全についての検討を行った。

重篤副作用疾患別対応マニュアルの活用について

これまでに完成したマニュアルについては，厚生労働省ホームページ（http://www.mhlw.go.jp/）

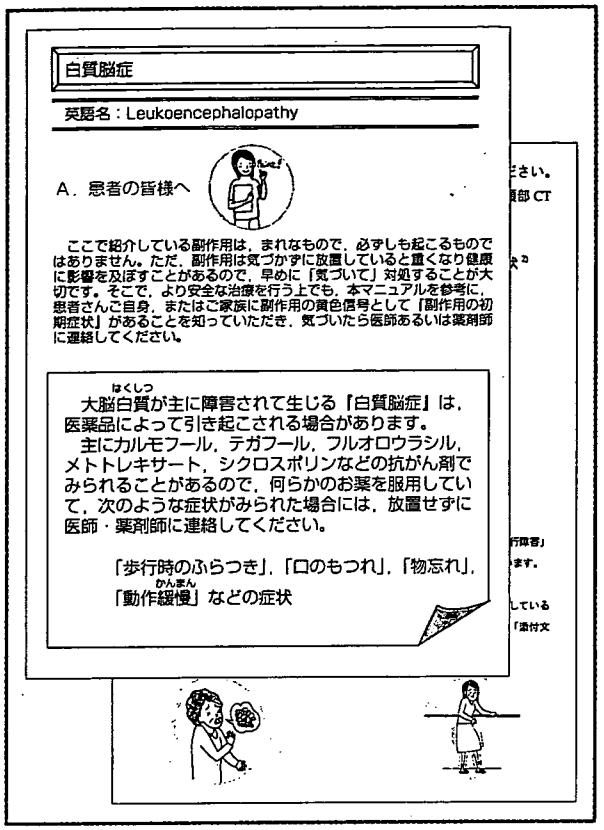

図３　重篤副作用疾患別対応マニュアルのイメージ（患者向け説明部分）

および医薬品医療機器総合機構の医薬品医療機器情報提供ホームページ（http://www.info.pmda.go.jp/）にて公表するとともに，都道府県，日本医師会，日本薬剤師会，日本病院薬剤師会などにお知らせしているところである。

今後とも，関係学会および日本病院薬剤師会の協力を得て，順次マニュアル案を作成し，重篤副作用総合対策検討会における評価・検討を経たうえで公表する予定である。

本マニュアルは，一般の国民，患者やその家族の方が直接インターネットを介して情報を入手すること，また，医療従事者の方が患者への説明に使用することを想定している。このため，患者向けの説明部分については，思わぬ副作用が生じた際の「気づき」を促すため，注意すべき初期症状について，イラストなどを用いてできるだけ平易に説明し，緊急度に応じて医療機関への受診などを促している（図３）。これまでにマニュアル（案）を作成済みの副作用の代表的な症状を表４に示す。

また，医療従事者向けの部分についても，当該副作用を専門としない医療従事者が早期の兆候を見逃すことのないよう，図表などを用いて副作用の概要をわかりやすく記載するとともに，必要な処置についても，包括的に示している。

おわりに

医師，歯科医師，薬剤師などの医療関係者におかれては，副作用の発生時のみならず，日頃の院内情報活動や患者への服薬指導などで本マニュアルをご活用いただき，重篤な副作用の早期発見・早期対応に努めていただきたい。また，製造販売業者の方におかれても，製品の安全性確保の資料としてご活用いただきたい。

この他，医薬品医療機器情報提供ホームページからは，添付文書情報に加え，医療用医薬品の適正使用と患者自らが自覚症状を確認し，重大な副作用の早期発見ができるよう，添付文書情報をもとに特に知っておくべき事項をわかりやすくまとめた「患者向け医薬品ガイド」などの情報も入手できるので，あわせてご活用いただきたい[4), 5)]。

最後に，本マニュアルの作成にご尽力いただいている関連学会および日本病院薬剤師会に感謝する。

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表 4　副作用名と主な症状

副作用名	症状
スティーブンス・ジョンソン症候群	「高熱（38℃以上）」，「目の充血」，「めやに（眼分泌物）」，「まぶたの腫れ」，「目が開けづらい」，「くちびるや陰部のただれ」，「排尿・排便時の痛み」，「のどの痛み」，「皮ふの広い範囲が赤くなる」
中毒性表皮壊死症	「高熱（38℃以上）」，「目の充血」，「くちびるのただれ」，「のどの痛み」，「皮ふの広い範囲が赤くなる」
薬剤性過敏症症候群	「皮膚の広い範囲が赤くなる」，「高熱（38℃以上）」，「のどの痛み」，「全身がだるい」，「食欲が出ない」，「リンパ節がはれる」
急性腎不全	「尿量の減少」，「ほとんど尿が出ない」，「一時的に尿量が多くなる」，「発疹」，「むくみ」，「体がだるい」
間質性腎炎	「発熱」，「発疹」，「関節の痛み」，「はき気，嘔吐，下痢，腹痛などの消化器症状」，「むくみ」，「尿量の減少」，「体重増加」
再生不良性貧血	「あおあざができやすい」，「歯ぐきや鼻の粘膜からの出血」，「発熱」，「のどの痛み」，「皮膚や粘膜があおじろくみえる」，「疲労感」，「どうき」，「息切れ」，「気分が悪くなりくらっとする」，「血尿」
出血傾向	「手足に点状出血」，「あおあざができやすい」，「鼻血」，「歯ぐきの出血」，「便が黒くなる（タール便）」
薬剤性貧血	「顔色が悪い」，「疲れやすい」，「だるい」，「頭が重い」，「どうき」，「息切れ」，「認知症または狭心症の症状」
無顆粒球症	「突然の高熱」，「さむけ」，「のどの痛み」
血小板減少症	「手足に点状出血」，「あおあざができやすい」，「出血しやすい（歯ぐきの出血・鼻血・生理が止まりにくい）」
血栓症	「手足のまひやしびれ」，「しゃべりにくい」「胸の痛み」，「呼吸困難」，「片方の足の急激な痛みや腫れ」
播種性血管内凝固	「あおあざができやすい」，「鼻血」，「歯ぐきの出血」，「血尿」，「鮮血便」，「目（結膜）の出血」，「意識障害」，「呼吸困難」，「どうき」，「息切れ」，「尿が出なくなる」，「黄疸」
間質性肺炎	「階段を登ったり，少し無理をしたりすると息切れがする・息苦しくなる」，「空咳（からせき）が出る」，「発熱する」
非ステロイド性抗炎症薬による喘息発作	「息をするときゼーゼー，ヒューヒュー鳴る」，「息苦しい」
急性肺損傷・急性呼吸窮迫症候群	「息が苦しい」，「咳・痰が出る」，「呼吸がはやくなる」，「脈がはやくなる」
薬剤性パーキンソニズム	「動作が遅くなった」，「声が小さくなった」，「表情が少なくなった」，「歩き方がふらふらする」，「歩幅がせまくなった（小刻み歩行）」，「一歩目が出ない」，「手が震える」，「止まれず走り出すことがある」，「手足が固い」
横紋筋融解症	「手足・肩・腰・その他の筋肉が痛む」，「手足がしびれる」，「手足に力がはいらない」，「こわばる」，「全身がだるい」，「尿の色が赤褐色になる」
白質脳症	「歩行時のふらつき」，「口のもつれ」，「物忘れ」，「動作緩慢」
偽アルドステロン症	「手足のだるさ」，「しびれ」，「つっぱり感」，「こわばり」がみられ，これらに加えて，「力が抜ける感じ」，「こむら返り」，「筋肉痛」が現れて，だんだんきつくなる」

4）奥山　清，寺澤路子，高橋利幸，他：インターネットサイトと他の情報経路における添付文書改訂情報に関する比較検討．薬学雑誌，125：639-642，2005

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