Involvement of p53-Dependent Apoptosis in Radiation Teratogenesis and in the Radioadaptive Response in the Late Organogenesis of Mice

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The irradiation of fetuses at the late period of organogenesis has been known to induce a dramatic increase in malformations. The mechanisms involved, however, have remained unclear for a long time. Using the mouse limb bud system, we first found that radiation-induced apoptosis is involved in the malformation, namely, radiation-induced apoptosis in the predigital regions of embryonic limb buds is responsible for digital defects in mice. An examination of embryonic C57BL/6J mice with different p53 (trp53) status enabled us to further find that susceptibility to radiation-induced apoptosis in the predigital regions and digital defects depend on both the p53 status and the radiation dose; p53 wild-type mice appeared to be the most sensitive, while p53 knockout mice were the most resistant. These results indicate that p53-dependent apoptosis mediates radiation-induced digital defects in the later organogenesis period. The existence of a radioadaptive response in embryonic mice, which has not been reported so far, was found by irradiating embryos with either 5 cGy or 30 cGy on embryonic day 11 prior to a challenging irradiation at 3 Gy on embryonic day 12. p53-heterozygous embryos did not show the radioadaptive response, indicating the involvement of p53 in the radioadaptive response in embryogenesis.

INTRODUCTION

The effects of radiation on the embryo are of great concern both from the viewpoint of public health and also for academic investigation. Irradiation in utero at the pre-implantation period induces a high incidence of prenatal death, but a low incidence of abnormalities, while exposure at the organogenesis period induces high incidences of both abnormalities and neonatal death. Although cell death has been believed to be responsible for radiation-induced malformations at the late period of organogenesis, no direct evidence has so far been
reported.

Apoptosis is centrally involved in morphogenesis and in the regulation of cell populations in embryos. Its significance is highlighted by the facts that an altered location and degree of apoptosis could result in developmental abnormalities. Although radiation-induced apoptosis has been intensively studied in organs and established cell lines, the function of apoptosis in radiation-induced malformation remains obscure. During the late period of gestation, apoptosis plays a critical role in shaping the organs and structuring their architecture. The developing limb is an excellent model for studying the role of apoptosis, which is particularly prominent in the development of digits. There is a definite relationship between spontaneous apoptosis and the pattern of limb bud formation. The limb bud staging system and fate maps enable us to correlate the initial pathological changes in early limb buds with final limb defects.

Radiation-induced apoptosis plays an important role in the responses to radiation exposure. Radiation-induced apoptosis is mostly p53-dependent. Recently, Norimura et al. reported using mice with different p53 status that apoptosis induced by radiation at the early period of organogenesis aborted damaged embryos from further development, resulting in a low incidence of congenital malformations. Those embryonic cells bearing non-reparable radiation-induced teratogenic damage died via apoptosis. They were subsequently replaced by cell-replacement repair or cellular-proofreading mechanisms, which led to a low incidence of malformation. Thus, the p53 gene plays a role of guardian against radiation-induced teratogenesis during the early organogenesis period. In the late period of organogenesis, however, the role of the p53 gene in radiation-induced apoptosis, and the relation between radiation-induced apoptosis and teratogenesis, remains to be elucidated. The p53 gene sometimes has a negative effect on the development of embryos. When the blastomers of Xenopus embryos were flooded with plastids, which express a wild-type p53 gene, the germ-layer formation was severely disrupted, and the embryos finally died of neurulation.

Radio-adaptation is characterized as a reduction of the effects of relatively large doses by a preceding small priming dose. The induction of radioprotection by low doses has important implications for understanding the effects of radiation on the biodefense mechanisms. Since the first discovery by Olivieri et al., the adaptive response to ionizing radiation, or radioadaptation, has been found in a variety of cells and organisms. However, studies using either mouse preimplantation embryos or rat fetal brain have failed to show any adaptive response. There has so far been no report concerning the radio-adaptive response in mammalian embryos.

Fetuses at the organogenesis period are highly radiosensitive. A dose lower than 50 cGy given in the late period of organogenesis usually increases the incidence of malformations in certain organs, and a dose higher than 2 Gy increases the prenatal mortality. Given in the late period of organogenesis, a dose as low as 5–10 cGy resulted in postnatal behavioral changes and a dose lower than 50 cGy usually increases the incidence of malformations in certain organs. Because apoptosis is centrally involved in morphogenesis and in regulating cell populations in the embryo and because the radiation-induced apoptosis in the embryo is
mostly p53-dependent, the possible involvement of apoptosis and the role of the p53 gene in
the induction of radioadaptation are of great interest. In a series of the present studies, we
investigated the correlation between both the p53 gene status and the radiation-induced
apoptosis with the induction of radioadaptation in the late period of embryogenesis.

IMPLICATIONS OF RADIATION-INDUCED APOPTOSIS IN LIMB MALFORMATION

We first conducted a study to elucidate the role of radiation-induced apoptosis and to
correlate the apoptosis with the final digital teratogenesis of mice. Mice were exposed to
X-rays in utero on day 11 of gestation. We observed an increase in typical apoptotic changes,
such as chromatin condensation detected by light and electron microscopic observations and
DNA fragmentation on agarose gel electrophoresis. A marked increase in the number of
apoptotic cells in the predigital regions in the forelimb buds was observed 4 h after irradiation.
The preinterdigital regions of the forelimb buds did not show an increase of apoptosis at the
same time. Prenatal irradiation-induced apoptosis in the predigital regions (Fig. 1) led to limb
digital defects dependent on the radiation dose. Thus, radiation selectively kills the cells in
the predigital regions via apoptosis. An examination of fetuses 1 day before birth revealed
severe defects in limbs in 100% of the living fetuses exposed to 5 Gy. Excessive cell death
caused a failure of limb bud development, which finally led to digital defects. The amount and
distribution of apoptosis and the regenerative capacity of the surviving cells play a role in
determining the degree and pattern of defects of the surviving fetuses.

![Fig. 1](https://example.com/image.png)

Fig. 1. Whole mount nile blue staining of limb buds from embryonic ICR mouse on E12 (day 12
of gestation). The darkly stained areas of limb buds indicate the regions of apoptosis.
A. Control. In the preinterdigital regions, cells are undergoing physiologically spontaneous
apoptosis. B. 4 h after irradiation with 5 Gy. Radiation-induced apoptotic cells appear
mainly in the predigital regions.
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We next investigated the possible involvement of the p53 gene using embryonic C57BL/6J with different p53 status (p53+/–). As shown in Fig. 2, after giving the same radiation dose, the susceptibility to radiation-induced apoptosis and digital defects becomes p53 dependent. p53+/+ animals were reported to be more sensitive to radiation-induced death than the p53+/– and p53−/– mice both during the middle of organogenesis and during the grown-up period. In our results concerning the late period of organogenesis, however, the p53−/– embryonic

Control 1 Gy 3 Gy 4 Gy

Fig. 2. Malformations of limbs due to prenatal X-irradiation. The effects of prenatal irradiation on E12 on the digital number of forelimb in all living fetuses of C57BL mice were investigated on E18. The whole mount skeletons of limbs were prepared using alizarin red S staining. The photographs showed normal forelimbs of the controls and malformed forelimbs from aphlangy to ectrodactyly of the irradiated fetuses. % indicates the percentage of malformations in the living fetuses in each group.
mice were more sensitive to the killing effect than were the $p53^{+/−}$ and $p53^{+/+}$ mice. The functions of p53, therefore, appeared to be restricted not only to specific tissues, but also to specific stages in the development of tissues, and also, the sensitivity to prenatal death varied with the timing of radiation given during the preimplantation period. Taken together, our findings suggest that the lethal effects of radiation on embryos or fetuses vary considerably during the whole process of gestation. The involved mechanisms are more than the balance of excessive cell death and p53-dependent cellular proofreading in embryos or fetuses.

**RADIOADAPTIVE RESPONSE IN EMBRYOGENESIS AND ITS RELATIONSHIP TO APOPTOSIS AND p53 GENE STATUS**

Radioadaptation is characterized by the reduction of certain deleterious effects of a challenging high dose. An adaptive response was confirmed in chicken embryos; however, studies using either mouse preimplantation embryos or rat fetal brain failed to show any positive results. We first investigated a possible existence of adaptive response by changing the dose and timing of the priming dose given before 5 Gy on day 12 of gestation of C57BL mice.

![Induction of radioadaptive response in late organogenesis](image)

**Fig. 3.** Induction of radioadaptive response in late organogenesis. The effects of priming doses on E11 prior to a challenging dose at 3 Gy on E12 on the digital number of forelimbs in all living fetuses of C57BL mice were investigated on E18. The whole mount skeletons of the limbs were stained with alizarin red S. % indicates the percentage of malformations among the living fetuses in each group.
ICR mice. We then succeeded in demonstrating the radioadaptive response in embryogenesis caused by giving a priming dose exclusively of 30 cGy 1 day before the challenging dose. We subsequently examined the adaptive response using C57BL mice with different p53 status. Fig. 3 shows the existence of radioadaptation in embryogenesis in p53\(^{+/+}\) mice, but not in p53\(^{+/-}\) mice. Conditioning doses of both 5 cGy and 30 cGy were applied to embryos on gestation day 11 prior to irradiation with a challenging dose of 3 Gy on day 12. The pre-exposure resulted in a significant increase in the number of living fetuses, and a decrease in the incidence of apoptosis in predigital regions of limb buds and digital defects in living fetuses. These results indicate that the induction of a radioadaptive response in embryogenesis is related to the occurrence of apoptosis, which is dependent on the p53 gene status in mice.

We further found that, though the priming dose could result in some living births, a high postnatal mortality existed in the prenatal adapted mice; all survivors suffered from various detrimental effects, such as growth retardation and behavioral alterations. These results indicate that increased survival does not always mean that the priming dose is beneficial, because the survivors are not necessarily healthy.

**PERSPECTIVES**

Since the classical work of Russell et al. in 1957, the response of mammalian fetuses during the organogenesis period to radiation has been characterized by a high incidence of malformations. An application of modern molecular biological techniques to developmental radiation biology has further revealed an involvement of p53-dependent apoptosis in malformations. Our studies on radiation teratogenesis clearly indicate that radiation-induced apoptosis is a p53-dependent event in the late period of organogenesis in mice. p53-dependent apoptosis is responsible for excessive cell loss in the predigital regions, which results in digital defects. However, p53-dependent apoptosis in preimplantation as well as in early organogenesis period has been found to prevent mice from undergoing teratogenesis. This apparent paradoxical effect of radiation-induced p53-dependent apoptosis could be explained in terms of a difference in the timing of irradiation. In the early organogenesis period, proximal and critical organs for the survival of the whole animal, such as the heart and central nervous system, are being generated, the radiation-induced defects of which eventually lead to fetal death, rather than to a living fetus with malformations. On the other hand, in the late organogenesis period, distal organs, such as limbs, are being made, the defects of which are not fatal to the fetus, but result in the birth of a malformed animal.

Thus, regarding the role of radiation-induced p53-dependent apoptosis, we propose a possible mechanism, including p53 gene status, for the embryonic response to radiation. p53-dependent apoptosis can prevent mice from malformations at the preimplantation period or at the early period of organogenesis, while apoptosis in distal organs at the late period of organogenesis causes malformations. These findings suggest that the wild-type p53 gene may be an intrinsic susceptibility factor responsible for certain congenital defects induced by prenatal
radiation. The expression of some genes other than the p53 gene has been associated with radiation-induced apoptosis \(^{11,35}\). With the recognition of p53 homologues and their functions \(^{36–38}\), it will be helpful to further determine the complexity of the p53 gene and its role in radiation teratogenesis.

Radioadaptive response is a complicated phenomenon. Changes in the expression of some genes in response to the conditioning dose may occur within a few hours after radiation, although the specific molecular components have not been identified \(^{39,40}\). Many genes may be involved, some of which may be critical. Just as the p53 gene is essential in the adaptive response in the present system, an in vitro study showed that a radioadaptive response could be induced in normal cells, but not in neoplastic cells with mutations in the p53 gene or the Ras gene \(^{41}\). At the cellular level, the adaptive response may be due either directly to an increase in DNA repair, or indirectly to a modulation of the cell-cycle progression or apoptosis. The induction of an increasing amount and the rate of DNA repair seem to dominate these mechanisms \(^{42}\). Further, sensitization for apoptosis may represent a novel adaptive response mechanism in certain cases \(^{43}\), while a reduction of the apoptosis also has a significant role in this process \(^{41}\).

The reduction of apoptosis in our studies has suggested that an anti-apoptotic pathway might be involved in the adaptive response. Other factors, such as strain differences and immunological conditions, may also be involved in the process of radioadaptation \(^{44}\). The existence of two conditioning doses in radioadaptation may be due to biological differences in the systems examined. This finding further suggests the existence of multiple pathways of signal transduction activated by different conditioning doses or/and the involvement of multiple cell populations with various susceptibilities to radiation-induced apoptosis in the present system. The findings that a decrease in radiation-induced apoptosis accompanies radioadaptation and that the p53 gene plays a role in the radioadaptation process will be of use for future investigations aimed at understanding radioadaptation in embryogenesis.

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