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Organ-specific Responses of Total Body Irradiated Doxycycline-inducible Manganese Superoxide Dismutase Tet/Tet Mice

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Abstract. *Background/Aim: We evaluated doxycycline-inducible manganese superoxide dismutase (MnSOD$^{tet/tet}$) mice after 9.25 Gy total-body irradiation (TBI) or 20 Gy thoracic irradiation. Materials and Methods: Six-week-old MnSOD$^{tet/tet}$ or control C57BL/6NHsd mice on or off doxycycline (doxy) in food received 9.25 Gy TBI, were sacrificed at day 19 and bone marrow, brain, esophagus, heart, intestine, kidney, liver, lung, spleen and tongue harvested, total RNAs extracted and transcripts for irradiation response genes quantitated by real time-polymerase chain reaction (RT-PCR). Results: MnSOD$^{tet/tet}$ mice only survived with daily injections of doxy beginning 5 days after birth until weaning, at which time they were placed on food containing doxy. Manganese superoxide dismutase (MnSOD) transcript levels were reduced in all tissues except the lung. Adult mice survived with low MnSOD levels, but induced by doxy or TBI. Thoracic-irradiated MnSOD$^{tet/tet}$ mice survived past day 120. Conclusion: MnSOD$^{tet/tet}$ mice should be valuable for elucidating the role of MnSOD in growth and irradiation response.*

Reactive oxygen species (ROS) have been implicated in pathological processes, including diabetes, atherosclerosis, heart failure, neurodegenerative disease, carcinogenesis and chemical- and radiation-induced tissue damage (1-3). Furthermore, the stability of intracellular antioxidant levels has been shown to be critical for embryogenesis, organogenesis, growth and development (4-6).

Ionizing irradiation damage is associated with depletion of cellular antioxidants (7-9) including: glutathione, vitamins such as ascorbate, catalases, glutathione peroxidases and superoxide dismutase. In particular, manganese superoxide dismutase (MnSOD) has been shown to be critical for cell, tissue and organ protection from ionizing irradiation damage (10, 11). Deletion recombinant-negative MnSOD$^{-/-}$ mice are neonatal lethal and die with cardiomyopathy, liver steatosis, neurodegeneration, accumulation of oxidative DNA damage and defects in mitochondrial respiration (6, 12-14). Embryo fibroblast cell lines from MnSOD$^{-/-}$ mice are radiosensitive (15). Tissue specific deletion of the MnSOD gene product using the Cre-loxP system has shown that MnSOD is critical for normal organ development and function (16). Mice with heart-specific or brain-specific MnSOD gene deletion showed progressive dilated cardiomyopathy and spongiform encephalopathy, respectively (16). Transient tissue-specific overexpression of MnSOD has been shown to protect against both acute and late irradiation damage to the lung, esophagus, intestine, urinary bladder and oral cavity in mouse models (17-21).

We recently developed a strain of MnSOD$^{tet/tet}$ C57BL/6NHsd mice (22) to study the organ-specific effects of regulated levels of MnSOD on mitochondrial ROS during growth and development. Cell lines from doxycycline (doxy)-inducible MnSOD$^{tet/tet}$ mice display MnSOD expression that is regulated by a tetracycline responsive element in the gene promoter. Bone marrow stromal cell lines derived from MnSOD$^{tet/tet}$ mice showed that the doxy-induced higher levels of MnSOD correlated with radioresistance, improved cell viability, rapid cell doubling, faster DNA strand-break repair and increased antioxidant stores (22).

In the present study, we quantitated the effects of doxy-induced MnSOD levels in MnSOD$^{tet/tet}$ mice on organ-specific gene transcripts *in vivo*. Doxy administration was required continuously for survival, after birth, and until weaning. MnSOD$^{tet/tet}$ adult mice that were taken off doxy to reduce levels of MnSOD, were subjected to total-body irradiation (TBI), sacrificed 19 days later and showed a range of different organ specific RNA transcript responses that were distinct from those of wild-type mice.

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Materials and Methods

MnSOD$^{tet/tet}$ mice. The generation of MnSOD$^{tet/tet}$ mice has been previously described (22). Briefly, to derive MnSOD$^{tet/+}$ mice all pups were genotyped on days 12 to 15 after birth using polymerase chain reaction (PCR) as previously described using the following primers: MnSODwtR 5' CAT GAT CTG CGG GTT AAT GT 3', MnSODwtF 5' AAT TTG GCA CAG GGG AGA C 3' and MnSODTetR 5' CAA ATC CTC CTC GTT TTTGG 3' (22). For the MnSOD$^{tet/tet}$ pups to reach weaning, doxy was administered using multiple methods. Initially, breeding pairs were placed on drinking water containing 2.5 mg of doxycycline/ml and water changed weekly. Some of the pups at 5 days after birth had implantation of 21-day release pellets containing 2.5 mg of doxycycline placed subcutaneously. Slow release doxy pellets were used (Innovative Research of America, Sarasota, Florida, USA). For insertion, a small incision was made in the skin and pellets were placed under the skin, skin glued together using opthalmalic glue. To maintain doxycycline levels in breast milk, mothers were injected intraperitonealy (I.P.) with 100 μl containing 2.5 mg doxycycline/ml in water daily. Sub-groups of pups in some experiments beginning on day 5 after birth were injected daily with 50 or 100 μl of water containing 2.5 mg of doxycycline/ml. To increase the number of MnSOD$^{tet/tet}$ pups, nursing mothers received food containing doxycycline (Harlan Spraque Dawley, Indianapolis, IN, USA), also supplied to MnSOD$^{tet/tet}$ mice after weaning. Food contained 625 mg/kg doxycycline for a daily doxy dose of 1.6 to 2.7 mg of doxy per 3 to 5 gm diet.

Effect of TBI on induction of RNA transcripts in MnSOD$^{tet/tet}$ mouse organs. Some six-week-old male and female MnSOD$^{tet/tet}$ C57Bl/6 mice continuously receiving doxy in chow were switched to normal chow without doxy and remained off doxy for four days. Mice were then either irradiated to the lethal dose $LD_{50/30}$ dose of 9.25 Gy TBI or nonirradiated. Subgroups of mice in each group were kept on doxy, and others taken off doxy. Mice were sacrificed at day 19 after TBI and eleven tissues (bone marrow, brain, esophagus, heart, intestine, kidney, liver, lung, muscle, spleen and tongue) were harvested from each mouse for total RNA extraction.

Wild-type (MnSOD$^{+/+}$) littermate control C57Bl/6 mice underwent a similar protocol. Doxy-treated wild-type mice received doxy in food for two weeks prior to TBI.

RNA was extracted from harvested tissues using the Trizol reagent (Life Technologies, Grand Island, NY, USA) and cDNA synthesized for testing the control (*Gusb*) for quantitation of levels of expression using primers specific for the promoter regions of: manganese superoxide dismutase (*Sod2*), glutathione peroxidase 1 (*Gpx1*), nuclear factor (erythroid-derived2)-like 2 (*Nrf2*), nuclear factor kappa beta (*Nfkβ*), transforming growth factor beta (*TGFβ*), Fas ligand (*Fas*), transcription factor Sp1 (*Sp1*), glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) and peptidylprolyl isomerase A (*Ppia*) using RT-PCR as described previously (23). Results were presented as fold increases in RNA above baseline levels of each tissue from either non-irradiated MnSOD$^{+/+}$ littermate control or MnSOD$^{tet/tet}$ mice respectively. Baseline levels allowed determination of the magnitude of detectable elevation in RNA by robot RT-PCR attributable to doxy treatment and/or irradiation in each organ of each mouse genotype.

Thoracic irradiation of MnSOD$^{tet/tet}$ mice. MnSOD$^{tet/tet}$ or control MnSOD$^{+/+}$ mice were taken off doxy for five days and then were irradiated to 20 Gy single fraction to thoracic cavity. Some MnSOD$^{tet/tet}$ and control were taken off and others maintained on doxy containing food. All thoracic irradiated mice were to be sacrificed at time of moribund condition expected from radiation pulmonary fibrosis in C57BL/6NHsd mice (17).

Statistical methods. For each removed organ (marrow, brain, esophagus, heart, intestine, kidney, liver, muscle, spleen and tongue) and each gene (*Sod2, Gpx1, Nrf2, Nfkβ, TGFβ, Fas, Sp1, Gapdh* and *Ppia*), the RNA transcript levels were measured by RT-PCR. These data were normalized by calculating the differences (ΔCt) between the Ct-*Gapdh* and Ct-Target genes isolated from bone marrow, esophagus, muscle, spleen and tongue. The Ct-*Pipa* was used to normalize the target genes for RNA isolated form brain, heart, intestine, kidney, liver and lung. The relative increase or decrease in expression was calculated by comparing the reference gene with the target gene (ΔΔCt) and using the formula for relative expression ($=2^{\Delta\Delta Ct}$). Groups were summarized and compared in terms of the relative expression. In each group, these levels of expression were summarized by mean±standard deviation (SD). Expression levels were compared to baseline levels (which were assumed to be constant) with the two-sided one sample *t*-test. Levels were compared to each mouse group on doxy "for 2 weeks, total body irradiated to 9.25 Gy, then on doxy" to the group "never on doxy, total body irradiated to 9.25 Gy, then remaining off doxy". Levels were also compared between groups "always on doxy, then total-body irradiated to 9.25 Gy, then remaining on doxy" with groups "non-irradiated always on doxy". All comparisons were carried out with the two-sided two sample *t*-test. In all these exploratory studies we did not adjust *p*-values for multiple tests.

Results

Neonatal lethality of MnSOD$^{tet/tet}$ mice in the absence of maternal and newborn doxy administration. MnSOD$^{tet/tet}$ mice were bred by mating MnSOD$^{tet/+}$ mice genotyped by RT-PCR using primers specific for the mouse MnSOD gene or MnSODtet transgene using PCR conditions previously published (22). Pups from the first four litters were genotyped at 12 to 15 days after birth and there were no MnSOD$^{tet/tet}$ mice identified. The data support previous publications which demonstrated that MnSOD$^{-/-}$ mice died *in utero* or within five days after birth. Breeding pairs were next placed on water containing 2.5 mg doxycycline (5 mM) to determine if increased levels would activate the MnSOD$^{tet/tet}$ gene in pups *in utero* and during nursing. Six litters were born from females on doxy, but no MnSOD$^{tet/tet}$ pups were identified (all were MnSOD$^{+/+}$ or MnSOD$^{tet/+}$ by genotype) (Table I). In four litters from mothers receiving I.P. doxy, the mothers died of dehydration and produced no tet/tet newborns (Table I). Next, on day 5 after birth, we implanted 21 day release doxy-pellets containing 2.5 mg doxycycline to all newborns in each of 3 litters. Newborns from 3 litters died after implantation of pellets or within 24 h, and no MnSOD$^{tet/tet}$ mice survived to time of weaning (Table I).

We next tested intraperitoneal injection of 250 μg of doxycycline into new born pups, but waiting to start until 5 days after birth, then daily until time of weaning, when doxy-containing food was supplied. This method was

Table I. *Effect of doxy administration to mother and newborns on survival of MnSOD$^{tet/tet}$ mice.*

Mouse strain	Number of litters	Number of mice genotyped	Number of mice reaching weaning	Number of mice reaching maturity
MnSOD$^{+/+}$	128	204	204	204
MnSOD$^{tet/+}$	128	459	459	459
MnSOD$^{tet/tet}$	128	187	62	30
	Subgroups			
MnSOD$^{tet/tet}$ Mothers on doxy H_2O	6	0	0	0
MnSOD$^{tet/tet}$ Mothers injected I.P. with doxy	4	0	0	0
MnSOD$^{tet/tet}$ Pups with doxy pellets	4	0	0	0
Mothers on doxy food and/or water MnSOD$^{tet/tet}$ Pups injected I.P. with doxy Pups on doxy food after weaning	80	113	33	12
MnSOD$^{tet/tet}$ Mothers on doxy food, no doxy H_2O Pups injected I.P. with doxy Pups on doxy food after weaning	34	74	29	18

To obtain MnSOD$^{tet/tet}$ mice, MnSOD$^{tet/+}$ mice were bred. Different methods were used to administer doxy to the MnSOD$^{tet/tet}$ mice to keep mice alive until weaning. The number of litters and the number of MnSOD$^{tet/tet}$, MnSOD$^{tet/+}$ and MnSOD$^{+/+}$ mice genotyped, weaned and reaching maturity is shown. The different sub-groups indicate the different methods used to administer doxy, and the number of litters and number of MnSOD$^{tet/tet}$ mice that were genotyped, weaned and reached maturity.

successful and MnSOD$^{tet/tet}$ mice survived to weaning. Most survived to genotyping at days 12 to 15 (Table I).

Newborn MnSOD$^{tet/tet}$ mice were smaller with less than one out of ten surviving to time of weaning (day 21) (Table I, Figure 1). Finally, we optimized the breeding. We decreased doxy levels at injection to 125 μg per day and placed nursing mothers on food containing doxy. There was an increase to 20% in the number of MnSOD$^{tet/tet}$ newborns surviving until weaning (Table I). Using these measures, we derived 30 MnSOD$^{tet/tet}$ mice surviving to 6-8 weeks of age. Thus, 30 out of 187 genotyped mice were MnSOD$^{tet/tet}$ derived from experiments with 128 litters (Table I). The data establish a clear requirement that MnSOD$^{tet/tet}$ mice receive doxy during both gestation and the neonatal period.

Surviving MnSOD$^{tet/tet}$ mice were smaller at the time of weaning, but once on solid food containing doxy they rapidly gained weight and grew to match the size of littermate MnSOD$^{+/+}$ and MnSOD$^{tet/+}$ mice (Figure 1). Some MnSOD$^{tet/tet}$ mice that survived to reach 6-8 weeks age (adulthood) were taken-off doxycycline. There was an initial weight loss, but after 5 days weight stabilized and mice again appeared healthy and active (Figure 1) despite the absence of doxycycline.

Fertility of MnSOD$^{tet/tet}$ mice. We evaluated whether MnSOD$^{tet/tet}$ mice were fertile. Two female and two male MnSOD$^{tet/tet}$ mice were paired, one male and one female per cage, and allowed to mate. Animals were kept on doxy in the food, as described in the optimal maintenance paradigm in Table I. Both female mice became pregnant and delivered litters. The litters were small containing 2 or 3 pups, which did not survive to time of genotyping. Since, the breeding of heterozygous MnSOD$^{tet/+}$ mice resulted in surviving MnSOD$^{tet/tet}$ mice, we no longer attempted to breed MnSOD$^{tet/tet}$ mice.

Effect of doxy on levels of gene expression in organs of total-body irradiated wild-type mice. We first examined whether doxy administration altered levels of expression of MnSOD and other irradiation response genes in wild type mice. Wild-type mice showed little effect of doxy on baseline gene expression (Figure 2).

There were no statistically significant differences in levels of *Sod2, Gpx1, Nrf2, Nfkβ, TGFβ, Fas* and *Sp1* in MnSOD$^{+/+}$ wild-type mice on doxy-treated compared to non-treated wild-type mice.

A. Appearance of 2 week old MnSOD$^{tet/tet}$ mouse (left) compared to wild type littermate (right).

Mouse strain	Weight at day 14 after birth
MnSOD$^{tet/tet}$	3.8 ± 0.2 gm
MnSOD$^{+/+}$	10.3 ± 0.3 gm

B. Weight of individual tet/tet mice. Mice over 10 days after 9.25 Gy TBI

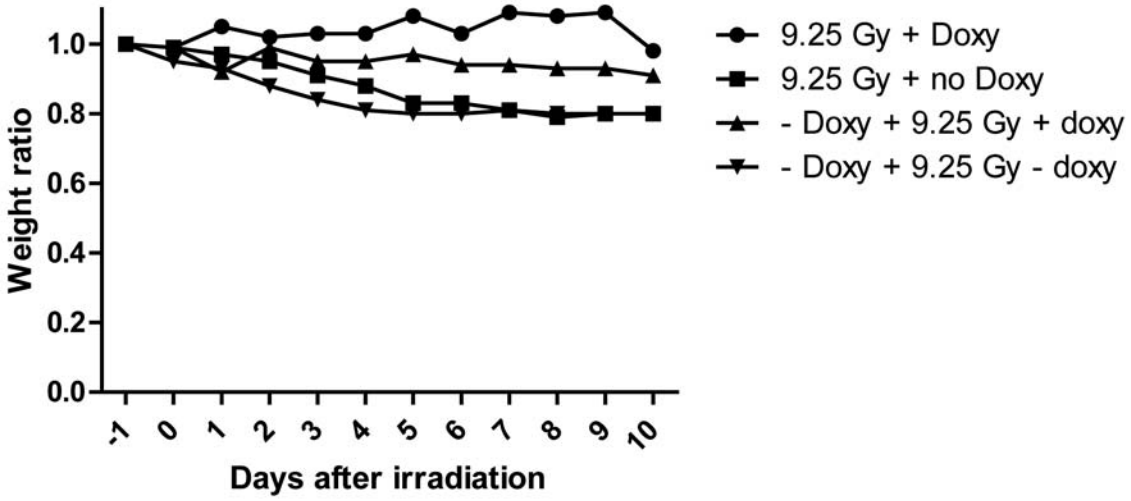

Figure 1. *Appearance and growth of MnSOD$^{tet/tet}$ mice. A) decreased size and body weight of MnSOD$^{tet/tet}$ mouse compared to the MnSOD$^{+/+}$ mouse at day 14 after birth. B) Change in body weight of MnSOD$^{tet/tet}$ mice following 9.25 Gy total body irradiation on Day 0. Mice in the two groups were taken off doxycycline on day 1 and weighed daily. Mice taken off doxycycline-containing food showed a decrease in body weight initially. If the mice were placed back on doxycycline, they returned to normal weight. If the mice were off doxycycline, then loss of weight stabilized after 10 days.*

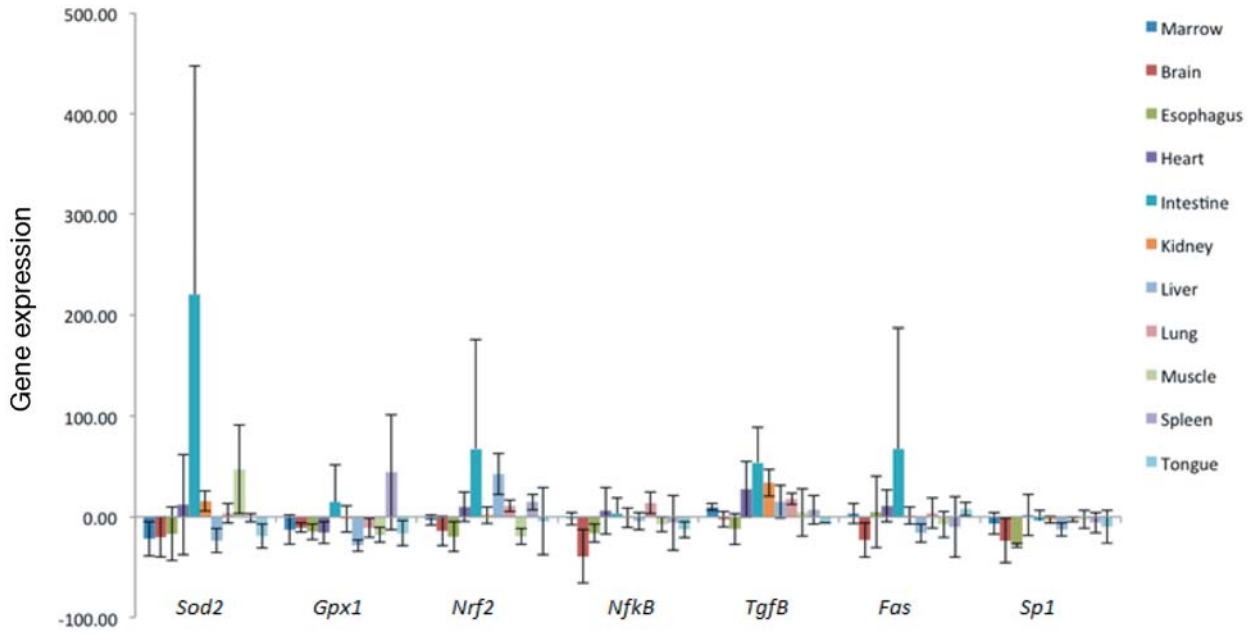

Figure 2. *Lack of effect of doxycycline on baseline gene transcript expression in MnSOD$^{+/+}$ wild type mice. The effect of doxycycline on induction of irradiation response genes in MnSOD$^{+/+}$ wild type mice organs and tissues was determined. Harvested organs and tissues were assayed two weeks after doxycycline treatment by RT-PCR for transcripts Sod2, Gpx1, Nrf2, Nfkβ, TGFβ, Fas and Sp1, as described in the Material and Methods.*

Effect of irradiation on levels of gene expression in wild-type mice. Levels of *Sod2* and other gene transcripts induced by 9.25 Gy total-body irradiation in wild-type mice were next measured (Figure 3). Previous studies demonstrated that day 19 was the time on the mouse survival curve after the LD 50/30 dose of TBI when death of the 50% destined to die was occurring at a rapid rate. At day 19 after 9.25 Gy TBI, irradiation decreased levels of Sod2 transcripts in brain and kidney of wild-type mice and increased levels in spleen. Doxycycline treatment of irradiated wild-type mice prevented the drop in *Sod2* levels in brain (Figure 3A). At this time, levels of *Gpx1* were increased in brain and intestine, while decreased in kidney, liver and lung of wild type mice (Figure 3A).

Doxy treated irradiated wild type mice showed decreased Gpx1 in brain and increased *Nrf2* in liver, lung and muscle (Figure 3). In contrast, *Nfkβ* transcripts were decreased in heart, lung and tongue at day 19 after 9.25 Gy TBI of wild-type mice (Figure 3B); *TGFβ* was increased in brain and liver and decreased in intestine, lung and tongue (Figure 3C). Doxy reduced the irradiation induced *TGFβ* in brain and increased *TGFβ* in tongue. Levels of *Fas*, a marker of apoptosis, were increased in esophagus and tongue (Figure 3C) and decreased in bone marrow. The doxy effect on irradiated wild-type mice was most prominent in the brain. The data support prior studies indicating that doxy protects neurons from ionizing radiation-induced apoptosis (24).

Effect of doxy on gene transcripts in organs of total-body irradiated MnSOD$^{tet/tet}$ mice. We first measured baseline transcript levels in MnSOD$^{tet/tet}$ mouse organs. Most organs of MnSOD$^{tet/tet}$ mice (exception esophagus, intestine and muscle) had lower levels of *Sod2* transcripts compared to wild-type despite doxy-containing food (Figure 4A). TBI irradiated MnSOD$^{tet/tet}$ mice continuously on doxy showed higher MnSOD levels in bone marrow, lung and intestine. Elevated levels of Sod2 transcripts in intestine of irradiated MnSOD$^{tet/tet}$ mice were significantly higher than that in total body irradiated MnSOD$^{+/+}$ mice (Figure 4A). Despite doxy administration, *Sod2* gene transcript levels in brain, heart, liver, spleen and tongue of total-body irradiated MnSOD$^{tet/tet}$ mice were lower than in total-body irradiated wild type mice.

MnSOD$^{tet/tet}$ mice on doxy had levels of *Gpx1* similar to those in wild-type mice (except in bone marrow, kidney and tongue) (Figure 4A). In MnSOD$^{tet/tet}$ mice continuously on doxy, irradiation increased *Gpx1* levels in intestine but decreased levels in liver and muscle. There were no doxy-specific differences in *Gpx1* levels in irradiated MnSOD$^{tet/tet}$ mice. Therefore, changes in *Gpx1* transcript levels were similar in MnSOD$^{tet/tet}$ and wild-type mice.

Baseline RNA transcript levels of *Nrf2* in brain, heart, lung and tongue of MnSOD$^{tet/tet}$ mice on doxy were lower compared to wild-type mice (Figure 4B). Irradiation increased

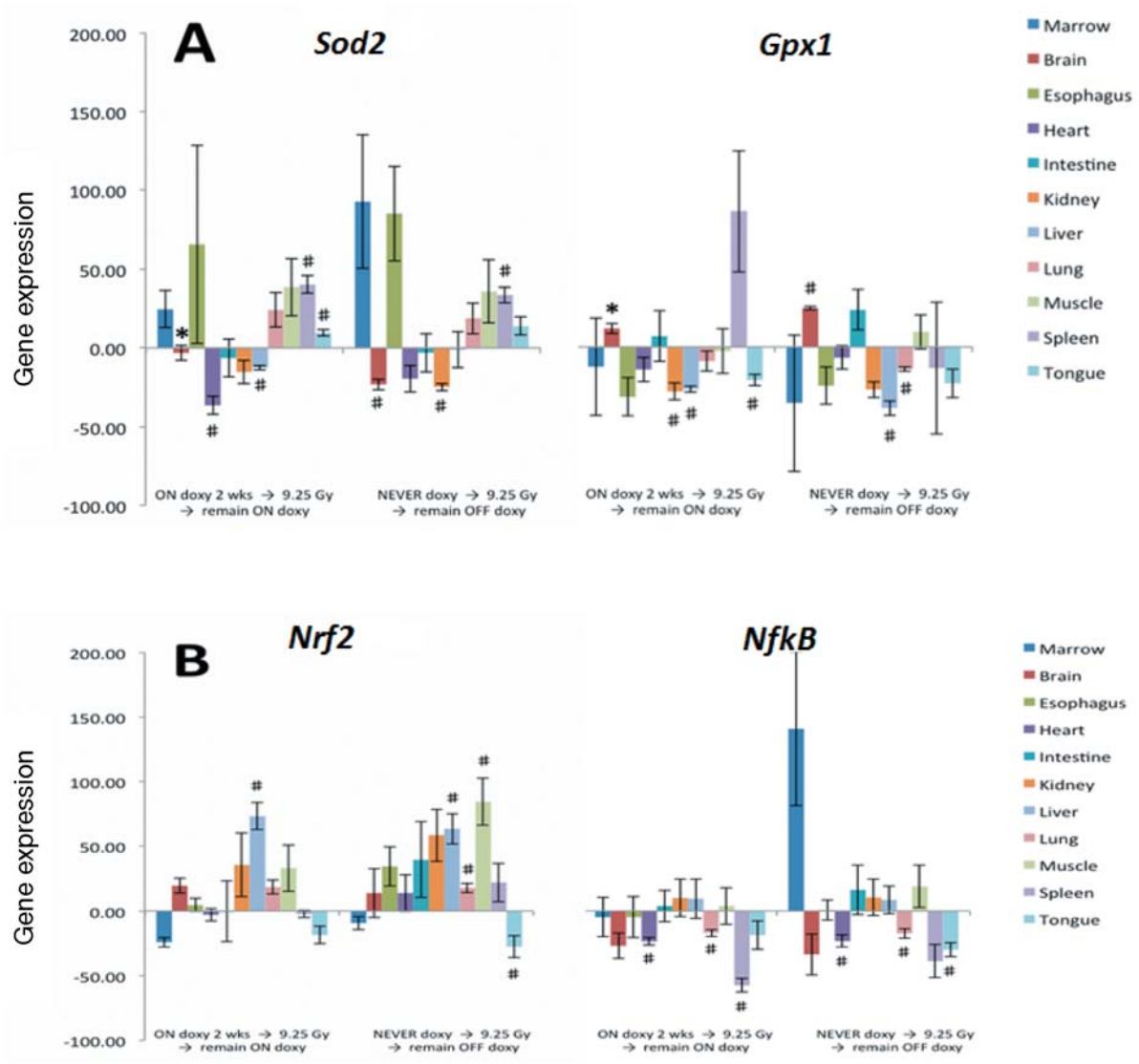

Figure 3. *continued*

Nrf2 levels in brain, esophagus and lung of MnSOD$^{tet/tet}$ mice on doxy. Irradiated MnSOD$^{tet/tet}$ mice continuously on doxy had significantly higher esophagus levels of *Nrf2* than MnSOD$^{tet/tet}$ mice off doxy (Figure 4B).

Nfkβ levels were also lower in brain and tongue but higher in intestine and muscle of MnSOD$^{tet/tet}$ mice on doxy compared to wild-type mice (Figure 4B). Irradiation of MnSOD$^{tet/tet}$ mice significantly decreased *Nfkβ* transcript levels in muscle and spleen. There were no differences in levels of *Nfkβ* between organs and tissues of doxy-treated or untreated irradiated MnSOD$^{tet/tet}$ mice.

TGFβ levels were higher in muscle of MnSOD$^{tet/tet}$ mice on doxy compared to wild type mice (Figure 4). TBI significantly increased *TGFβ* in heart of MnSOD$^{tet/tet}$ mice on doxy but not in MnSOD$^{tet/tet}$ mice off doxy, while levels were higher in the heart than that observed in wild-type mice (Figure 4C).

Fas levels in MnSOD$^{tet/tet}$ mice were lower in brain, kidney, lung and tongue when on doxy compared to that in wild-type mice (Figure 4C). Irradiation increased *Fas* levels in the brain and lung but reduced levels in bone marrow and heart of doxy-treated MnSOD$^{tet/tet}$ mice. The bone marrow of irradiated MnSOD$^{tet/tet}$ mice on doxy showed a significant decrease in Fas compared to mice off doxy (Figure 4C).

The data establish that MnSOD$^{tet/tet}$ mice demonstrate three phases of growth and development: (i) Neonatal lethality in the absence of doxy induction of MnSOD and requirement for

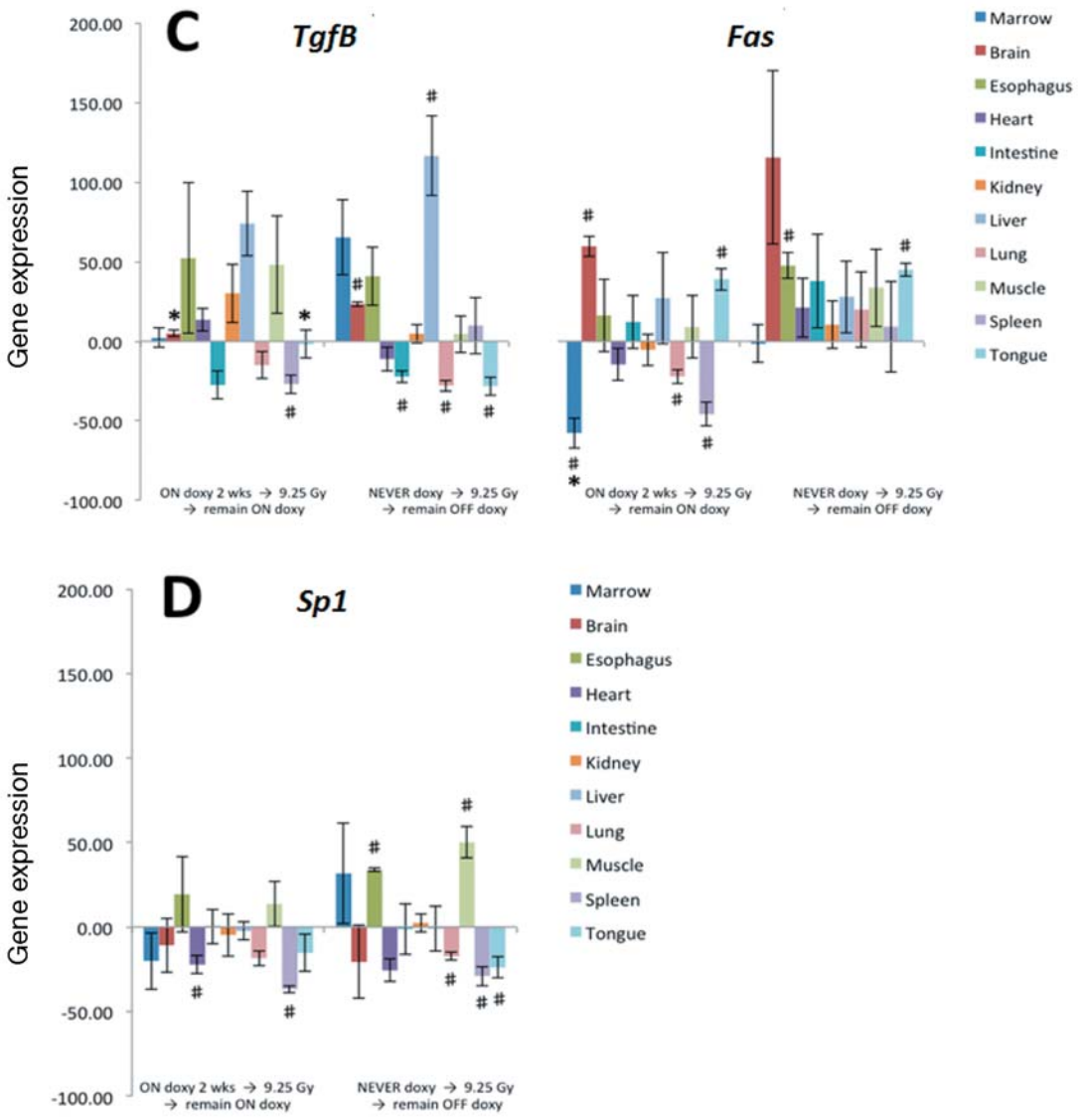

Figure 3. *Effect of irradiation on induction of gene transcripts in MnSOD$^{+/+}$ wild type mice. The baseline and radiation-induced changes in expression of irradiation response genes in doxycycline-inducible MnSOD$^{tet/tet}$ mice organs and tissues was used to quantitate irradiation effect. Expression levels were standardized to that in non-irradiated MnSOD$^{+/+}$ wild-type mice. Harvested organs and tissues were assayed 19 days after 9.25 Gy total body irradiation by RT-PCR, as described in the Material and Methods. Results are shown in Panel A: Sod2 and Gpx1; panel B: Nrf2 and Nfkβ; panel C: TGFβ and Fas; and panel D: Sp1. (# represents p-value <0.05 compared to non-irradiated non-doxy baseline wild type, while *shows where the p-value <0.05 compared to MnSOD$^{+/+}$ mice never on doxy plus 9.25 Gy and remaining OFF doxy).*

doxy by IP injection until weaning, (ii) requirement for doxy in food for survival past weaning and (iii) an adaptation phase at 6-8 weeks of age (adulthood) in which doxy was not required for survival.

Survival of thoracic-irradiated MnSOD$^{tet/tet}$ mice. At 200 days all 20-Gy thoracic-irradiated MnSOD$^{tet/tet}$, as well as control MnSOD$^{+/+}$ mice on or off doxy remained alive and healthy.

Discussion

The absence of MnSOD results in neonatal lethality in mice, characterized by multi-organ dysfunction and pathologic changes associated with oxidative damage (6, 12-14). MnSOD$^{tet/tet}$ mice in our study confirmed the neonatal lethality observed in knockout mice, in that absence of doxy induction of MnSOD was associated with *in utero* or neonatal death. High MnSOD levels were required during gestation and

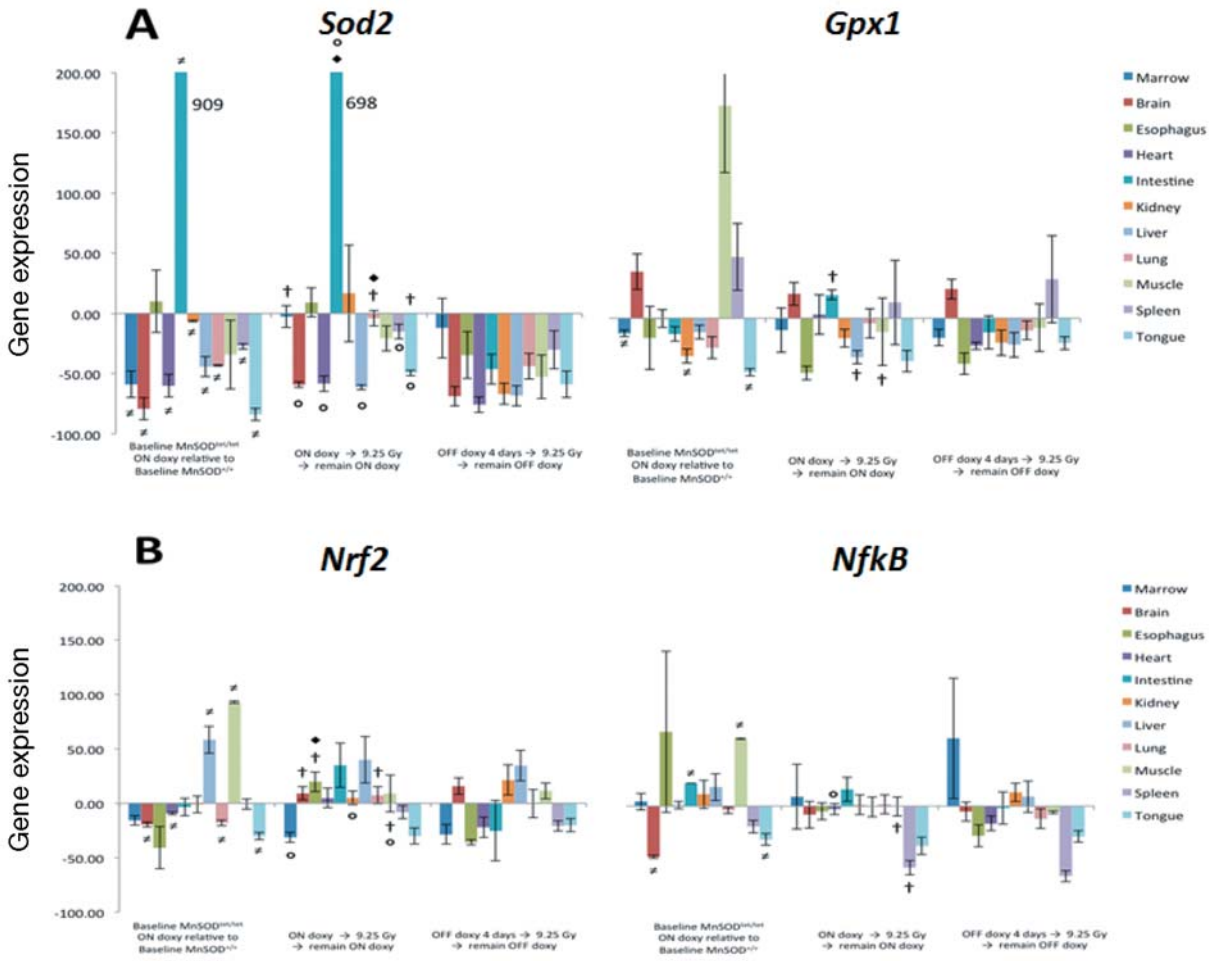

Figure 4. *continued*

in the neonatal period. MnSOD was clearly required for developing tissues and organs (25). The organs tested in 6-8-week-old MnSOD$^{tet/tet}$ mice (except intestine, esophagus and muscle) showed significantly lower baseline levels of MnSOD RNA transcripts compared to wild-type mice. Thus, there were persistent organ-specific differences in MnSOD gene transcript levels in MnSOD$^{tet/tet}$ mice that survived on doxy.

Age-related differences were observed in levels of MnSOD in MnSOD$^{tet/tet}$ mice. If MnSOD$^{tet/tet}$ mice on doxy survived past weaning, despite constitutively low levels of MnSOD in multiple organs, they continued to survive when taken off doxy. These results establish an adaptation phase in adulthood for survival despite a low level of MnSOD. The data may reflect up-regulation of other antioxidant pathways during growth and maturation. Low, but detectable levels of MnSOD have been reported in bone marrow stromal cells derived from MnSOD$^{tet/tet}$ mice (22). The low basal level of MnSOD was enough to allow survival of 6-8 week old MnSOD$^{tet/tet}$ mice even without doxy and for 19 days after 9.25 Gy TBI. Other tet-based inducible transgenic models have also shown the phenomenon of transgene leak *in vitro* and *in vivo* (26-33). Mechanisms such as protein stabilization may have contributed to survival of MnSOD$^{tet/tet}$ mice during adulthood. We also cannot rule out the possibility that MnSOD$^{tet/tet}$ mice may have displayed greater rates of death after 19 days.

MnSOD$^{tet/tet}$ mice had significantly lower body weight compared to wild type mice during the neonatal period. MnSOD levels correlated with weight of MnSOD$^{tet/tet}$ mice. MnSOD is known to be required for signal transduction pathways involved in cell proliferation and growth, including the growth stimulatory function of the mTOR signaling and growth inhibitory function of GSK-3β signaling (34). If doxy was discontinued in neonatal mice, we observed an immediate weight reduction. Skeletal muscle-specific MnSOD-knockout mice have exercise intolerance and a more rapid fatigue (35). The immediate weight loss after cessation of doxy may have

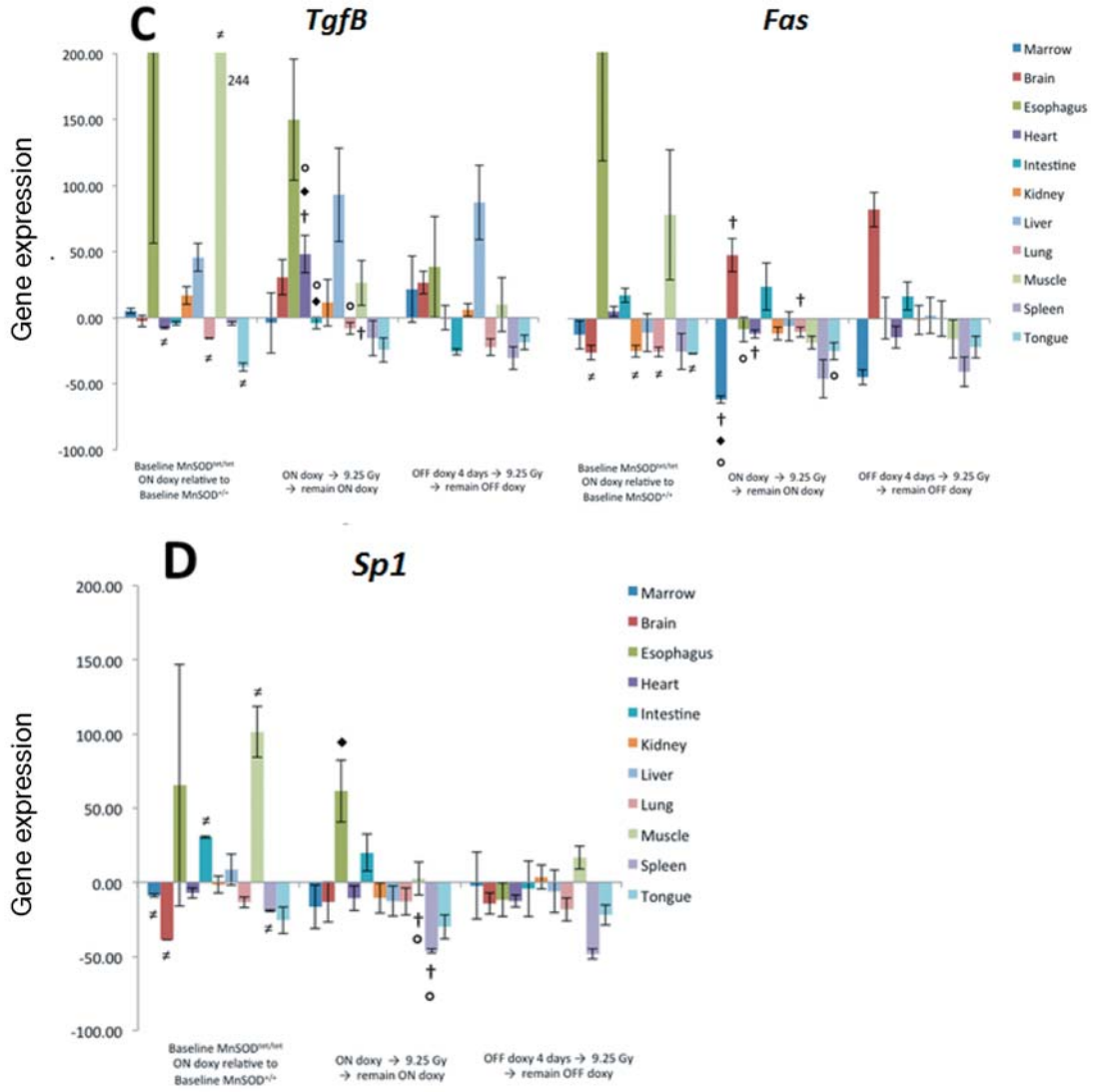

Figure 4. *Effect of irradiation on gene transcripts in doxycycline-inducible MnSOD$^{tet/tet}$ mice. The baseline and radiation-induced changes were determined for levels of expression of irradiation response genes in doxycycline-inducible MnSOD$^{tet/tet}$ mice organs. Expression levels were standardized to that in non-irradiated MnSOD$^{+/+}$ wild-type mice. Harvested organs were assayed 19 days after 9.25 Gy total body irradiation by RT-PCR as described in the Material and Methods. Results are shown in panel A: Sod2 and Gpx1; panel B: Nrf2 and Nfkβ; panel C: TGFβ and Fas; and panel D: Sp1. (The ≠ symbol represents p-value <0.05 compared to baseline non-irradiated non-doxy MnSOD$^{+/+}$ wild type. The † symbol is the p-value <0.05 compared to Baseline MnSOD$^{tet/tet}$ ON doxy. The ◆ symbol has a p-value <0.05 compared to MnSOD$^{tet/tet}$ OFF doxy 4 days then irradiated to 9.25 Gy and remaining off doxy. The ○ symbol stands for a p-value <0.05 compared to MnSOD$^{+/+}$, which were never on doxy, irradiated to 9.25 Gy and then taken off doxy for the remainder of the experiment).*

been from reduced mobility, increased fatigability and reduced food intake in neonatal MnSOD$^{tet/tet}$ mice.

Doxycycline modulated the irradiation response in MnSOD$^{tet/tet}$ mice most prominently in the brain. The brain of wild-type C57BL/6NHsd mice that received doxy had radiation-induced gene expression changes that reflected radioprotection, including: higher levels of MnSOD, lower *TGFβ* and lower *Fas*. The tetracycline family of antibiotics has been shown to display neuroprotective effects in hypoxia-ischemia animal models and protect against apoptosis from oxidative stress or inflammation (36, 37). Tetracycline is also known to be a radioprotector that inhibits irradiation-induced DNA fragmentation protecting neurons from apoptosis (24).

MnSOD$^{tet/tet}$ mice on doxy had MnSOD levels that were still significantly lower than those in wild-type mice, and transgene expression varied between organs. The intestine

had high levels of MnSOD, perhaps explained by the fact that the luminal epithelium of intestine is situated at the initial interface between the absorptive cellular environment and the highly concentrated doxycycline in the food given to the mice. Therefore, the inducible gene expression in intestine may have been higher. TBI significantly induced MnSOD transcripts in the lungs of $MnSOD^{tet/tet}$ mice on doxy, to levels higher than that in lungs of mice off doxy. That the lungs of 20 Gy thoracic irradiated $MnSOD^{tet/tet}$ mice either on or off doxy at day 120 showed no clinical signs of irradiation pulmonary fibrosis is consistent with the TBI data showing high MnSOD levels.

$MnSOD^{tet/tet}$ mice revealed several limitations as a model system. It was difficult to obtain enough viable mice for large experiments given their extreme fragility during the neonatal period. The present gene expression data in TBI mice were limited to a single 19 day time point. Expression patterns of radiation response genes at earlier or later time points may be relevant and are being evaluated in new experiments. The present $MnSOD^{tet/tet}$ total body inducible system makes difficult any study of the effects of a change on one specific organ. Other organ-specific transgenic approaches used a Tet-inducible system and Cre/lox P system, which may be of greater value to address organ-specific MnSOD levels, as well as effects on survival of mice during early development (38-41).

The high level of regulatable MnSOD in the intestine of our $MnSOD^{tet/tet}$ mice may be ideal for studying the role of MnSOD in the intestinal radiation injury model. Thus, $MnSOD^{tet/tet}$ mice should be valuable for understanding the role of MnSOD levels in organ function during gestation *in utero*, growth and development, as well as response to ionizing irradiation.

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