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The impact of ionizing radiation on placental trophoblasts

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Abstract

Introduction—Exposure to low-dose radiation is widespread and attributable to natural sources. However, occupational, medical, accidental, and terrorist-related exposures remain a significant threat. Information on radiation injury to the feto-placental unit is scant and largely observational. We hypothesized that radiation causes trophoblast injury, and alters the expression of injury-related transcripts *in vitro* or *in vivo*, thus affecting fetal growth.

Methods—Primary human trophoblasts (PHTs), BeWo or NCCIT cells were irradiated *in vitro*, and cell number and viability were determined. Pregnant C57Bl/6HNsd mice were externally irradiated on E13.5, and placentas examined on E17.5. RNA expression was analyzed using microarrays and RT-qPCR. The experiments were repeated in the presence of the gramicidin S (GS)-derived nitroxide JP4-039, used to mitigate radiation-induced cell injury.

Results—We found that survival of *in vitro*–irradiated PHT cell was better than that of irradiated BeWo trophoblast cell line or the radiosensitive NCCIT mixed germ cell tumor line. Radiation altered the expression of several trophoblast genes, with a most dramatic effect on CDKN1A (p21, CIP1). Mice exposed to radiation at E13.5 exhibited a 25% reduction in mean weight by E17.5, and a 9% reduction in placental weight, which was associated with relatively small changes in placental gene expression. JP4-039 had a minimal effect on feto-placental growth or on gene expression in irradiated PHT cells or mouse placenta.

Discussion and conclusion—While radiation affects placental trophoblasts, the established placenta is fairly resistant to radiation, and changes in this tissue may not fully account for fetal growth restriction induced by ionizing radiation.

Keywords

Placenta; Trophoblast; Ionizing radiation; Microarray; JP4-039

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1. Introduction

Exposure to ionizing radiation remains a reality in today's world. Worldwide, the average annual exposure to natural radiation is about 2.4 milli Sievert (mSv) [1]. Occupational exposures are most relevant to people working with nuclear fuel and medical devices, in defense-related functions, and in occupations associated with enhanced exposure to natural sources of radiation. For example, aircrew members are exposed to 5–8 mSv per hour while flying [1]. Medical sources of radiation include diagnostic procedures that expose individuals to low doses (commonly 0.1–10 mSv) and therapeutic exposures, typically 20–60 Gray (Gy), to a targeted tissue [1]. Accidental exposures in nuclear fuel processing plants typically expose workers to 1–20 Gy [1]. These risks may be greatly amplified if “dirty bombs” are deployed by terrorists against civilians [2].

Research into diagnosis, treatment, and prevention of radiation injury in pregnancy is limited by appropriate ethical concerns and by the scarcity of information on mechanisms underlying the effect of ionizing radiation on the developing feto-placental unit. Anecdotal reports or observational studies have generated some information pertaining to gestational age and radiation dose. During the pre-implantation period, as little as 0.3 Gy is lethal to the mouse embryo [3]. In the post-implantation period, the main risks from radiation include embryonic death, congenital anomalies, growth restriction, and neurologic maldevelopment [4]. Exposing mice at E14 to 0.3–1.5 Gy of whole body irradiation caused decreased neonatal body length and body weight [5]. Minimal effects on litter size or fetal growth were observed when mice at E7-16 were exposed to low dose radiation, 10–13 mSv per day over 10 days [6]. In humans, data from children exposed to *in utero* radiation after catastrophic events in Hiroshima, Nagasaki, and Chernobyl revealed lower height and weight in adolescence [4,7].

Ionizing radiation damages tissues through diverse mechanisms [8]. A major consequence of radiation is direct and indirect DNA damage. Direct effects include the transfer of kinetic energy from radioactive particles to the DNA backbone, which breaks phosphodiester bonds. Indirect effects include the generation of reactive oxygen species, which cause DNA double-strand breaks and cell-cycle arrest. Other types of injury include p53-dependent and -independent apoptosis [9], mitochondrial damage, loss of regenerative capacity, and premature senescence [8]. NFκB mediates several radiation-stimulated signal transduction pathways, which may explain the degree of radiation-sensitivity of differing cells types [10]. These pathways implicate CDKN1A (also known as p21, CIP1), epidermal growth factor receptors, and the apoptosis-related proteins BAX and BCL2 in radiation injury [11]. Whereas radiation-induced pathways have been interrogated in non-placental cell types, there are no studies of radiation injury to cultured primary human trophoblast (PHT) cells; there has been a single study that included the choriocarcinoma line JEG3 and showed no effect on gene expression of gap junction protein alpha 1 [12].

Methods to scavenge reactive oxygen species have been proposed to mitigate radiation damage. This effect has been attributed, at least in part, to the action of manganese superoxide dismutase (MnSOD, [13]). The nitroxides, which have superoxide dismutase–mimetic activity and inhibit lipid peroxidation [14], constitute one such class of radioprotectors. JP4-039 is a nitroxide linked to a short alkene isostere analog of hemigramicidin S, which allows concentration at the mitochondrial membrane, the site of radiation-induced lipid peroxidation [15]. It has been shown to protect against radiation damage *in vivo* [16,17]. In this study, we tested the hypothesis that ionizing radiation causes injury to PHT cells *in vitro* and to the mouse placenta *in vivo*. We also assessed whether the nitroxide JP4-039 mitigates that damage.

2. Materials and methods

2.1. Cell culture and irradiation in vitro

All studies involving human placental cells were approved by the Institutional Review Board at the University of Pittsburgh. For control, we used the immortalized choriocarcinoma line BeWo (ATCC, Manassas, VA), which captures aspects of trophoblast biology but maintains its undifferentiated state and proliferative capacity [18], and NCCIT cells (ATCC, Manassas, VA), a mixed germ cell tumor line that is particularly radiosensitive [19]. Term PHT cells were isolated and cultured using a modified version of the trypsin–deoxyribonuclease–dispase/Percoll method described by Kliman et al. [20,21]. After 4 h in culture, non-adherent cells and syncytial fragments were removed by washing in phosphate-buffered saline (PBS). All human placental cells were maintained, for 72 h after plating, in Dulbecco's modified Eagle's medium (DMEM, Fisher Scientific, Hampton, NH) containing 10% fetal bovine serum (Fisher Scientific), 20 mmol/l HEPES (pH 7.4, Sigma–Aldrich, St. Louis, MO), and antibiotics at 37 °C in a 21% oxygen/5% carbon dioxide atmosphere. The quality of PHT cells was routinely monitored every 24 h by cell morphology and by ELISA assay of medium human beta chorionic gonadotropin (β-hCG, DRG International, Mountainside, NJ), showing a characteristic increase in medium β-hCG as cytotrophoblasts differentiate into syncytiotrophoblasts [21,22].

The cells were irradiated 24 h after initial plating, defined as time zero. Cells were irradiated at the dosage noted in Results vs. sham, defined as 0 Gy [23], using a Clinac 600C (Varian Medical Systems, Palo Alto, CA) with a 6 MV photon beam and a dose rate of 250 cGy/min. The flasks containing the cells were placed on 1.5 cm of bolus (a tissue equivalent material) since the maximum irradiation depth was 1.5 cm, which corresponded to the plated cell layer. In some of the experiments, irradiated cultured cells were exposed to either JP4-039 (10 μM) in DMSO [24], added to the medium 1 h before radiation, or to DMSO alone. Cells were collected for microarray analysis at 4, 8, and 24 h after irradiation, and for all other analyses at 24 h after exposure to radiation.

Cell numbers (BeWo and NCCIT) were assessed by rinsing the monolayer with PBS, followed by trypsinization, resuspension in DMEM, and counting using a hemocytometer. Total cellular protein concentration was measured with the Pierce BCA Protein Assay Reagent Kit (Thermo Scientific, Rockford, IL). For DNA extraction, PHT cells were lysed in a buffer containing NaCl 0.4 M, Tris 10 mM, EDTA 2 nM, SDS 25% with proteinase K (200 μg/ml). The DNA was precipitated in saturated NaCl and isopropanol, washed, resuspended in Tris, and quantified using a NanoDrop 1000 spectrophotometer (NanoDrop, Wilmington, DE).

2.2. Irradiation of pregnant mice

All experiments using mice were approved by the Institutional Animal Care and Use Committee protocols at the University of Pittsburgh. Female C57Bl/6HNsd adult mice were fed standard laboratory chow. Mice were mated and separated the next morning (termed E0.5). Pregnancy was confirmed by weight change on E12.5. On E13.5, mice were irradiated at 0 Gy (sham) or 4 Gy using a Gamma Cell cesium irradiator (JL Shepherd, San Fernando, CA), with a dose rate of 70 cGy/min. This radiation dose was selected based on our experience with mouse irradiation [25], and because a higher dose increased the risk of fetal or early neonatal death, or severe malformation (data not shown). All mice were unanesthetized at the time of irradiation.

Ten minutes after radiation or sham, half the mice from each group received JP4-039, prepared as previously described [26,27]. JP4-039 was dissolved at a concentration of 3 mg/ml in 10% ethanol, 10% Cremophor EL (BASF SE, Limburgerhof, Germany), and 80%

water and administered intravenously at a dose of 10 mg/kg, with control mice receiving solvent alone. The mice were monitored for 4 days, and on E17.5, they were re-weighed and then sacrificed. Fetuses and placentas were procured and immediately weighed. One portion of the placenta was placed in RNAlater (Qiagen, Valencia, CA) overnight at 4 °C, then frozen at –80 °C until RNA extraction. Another portion was snap-frozen in liquid nitrogen. Tissue was also fixed in 4% paraformaldehyde, paraffin-embedded, sectioned, stained with hematoxylin and eosin, and microscopically examined using a Nikon Eclipse 90i microscope equipped with Nikon Elements software (both, Nikon Corporation, Tokyo). The image was then imported into ImageJ, version 1.44k (Rasband, W.S., ImageJ, U. S. NIH, Bethesda, MD), for stereologic analysis. Using the methods outlined by Howard [28], we processed vertical uniform random sections of mouse placenta with the Grid plugin. Systematic random vertical lines, 500,000/μm^2 with random offset, were used to measure the height of the labyrinth and junctional zones.

2.3. RNA extraction and processing for RT-qPCR and microarrays

RNA was extracted from PHT cells using both TRI Reagent (Molecular Research Center, Cincinnati, OH) and the RNeasy Mini Kit (Qiagen) and following the respective manufacturers' instructions. For extraction of mouse placental RNA, each placental specimen was homogenized and RNA extracted using TRI Reagent (Molecular Research Center) as above. RNA samples were incubated with DNase I, using a TURBO DNase Kit (Life Technologies, Grand Island, NY), and RNA quality and quantity were determined using a NanoDrop 1000 spectrophotometer (NanoDrop) as well as visualization on a denaturing agarose gel.

Reverse transcription of 1 μg of total RNA to cDNA was performed using the High Capacity cDNA Reverse Transcription Kit (Life Technologies), with amplification in a Veriti thermal cycler (Applied Biosystems/Life Technologies Corp). Synthesized cDNA was then diluted 1:5 using nuclease-free water. Quantitative PCR was performed in duplicate. For *in vivo* analysis, ribosomal protein L32 (*Rpl32*) was the internal control, while for *in vitro* analysis, tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide (YWHAZ) was the internal control. Quantitative PCR was performed using a 384-well plate with a total reaction volume of 10 μl that included 3 μl of cDNA, 1 μl of forward primer, 1 μl of reverse primer, and 5 μl of SYBR Green PCR Master Mix (Life Technologies). Quantitative PCR was performed using a 7900HT Fast Real-Time PCR System (Applied Biosystems). Because each placental preparation yielded cultured trophoblasts that were subject to radiation or control, gene expression *in vitro* was performed using the delta delta CT method [29], whereas the delta CT method was used for samples derived from *in vivo* experiments.

We used high-throughput microarray analysis to screen for radiation-induced transcriptional changes in cultured PHT cells or mouse placentas. All samples were first examined with an Agilent High-Resolution C Scanner (Agilent Technologies, Santa Clara, CA) to ensure RNA integrity and quality. For cultured PHTs, we analyzed the RNA using the Agilent SurePrint G3 Human GE 8 × 60K arrays (Agilent Technologies). Mouse placental RNA was analyzed using the MouseWG-6 Expression BeadChip arrays (Illumina, San Diego, CA). Microarray data were analyzed using a moderated t-statistic [30]. We then ranked the $\log_2$ expression ratio (radiation:sham) for each significantly changed transcript. For the PHT RNA data, which encompassed 3 time points (4 h, 8 h, and 24 h), we ranked transcripts by the maximum $\log_2$ expression ratio over the entire 24 h time course. We then selected a merged subset of the top 1% and bottom 1% of differentially expressed RNA from PHTs and from mouse placentas.

2.4. Western immunoblotting

PHT proteins were extracted with cell lysis buffer (Tris–HCl 50 mM pH 7.4, NaCl 150 mM, Triton-X100 1%) containing protease inhibitors. Protein concentrations were measured with the Pierce BCA Protein Assay Reagent Kit. Each protein lysate (75 μg) was loaded into each lane of 12% polyacrylamide gel electrophoresis, and then transferred to polyvinylidene fluoride (PVDF) membrane. Mouse anti-CDKN1A antibody (1 μg/ml, Santa Cruz Biotechnology, Santa Cruz, CA), mouse anti poly (ADP-ribose) polymerase (PARP, 0.1 μg/ml, EMD Millipore, Billerica, MA), mouse monoclonal anti-cytokeratin 18 (0.1 μg/ml, Roche Diagnostics, Indianapolis, IN), or mouse anti-MnSOD (0.1 μg/ml, R&D, Minneapolis, MN) were used to detect protein expression at 4 °C overnight, while mouse anti-actin antibody [0.08 μg/ml] (EMD Millipore) was used to measure β-actin, which served as a loading control.

2.5. Statistical analysis

We used the Q–Q plot graphical method to determine whether the data were normally distributed. Missing data were removed after informal tests to ensure they were missing at random. When there were no correlated data, we analyzed the data with linear regression. When there were correlated data, we used linear mixed-effects modeling. The clustered variables were the mouse placenta *in vivo* or PHT cultures *in vitro*. For mouse placentas, radiation and JP4-039 were used as categorical predictor variables. The likelihood ratio test was used to determine whether a fixed or random effect was a significant contributor to the model. Alpha values < 0.05 were considered significant when building multivariable models. Parameters were estimated using the restricted maximum likelihood approach. All linear mixed-effects models were examined to ensure homoscedasticity of the response variable. We used R, version 2.15.1, for all our statistical analyses [31]. For linear mixed-effects model analyses, we used the lme4 package [32,33].

3. Results

3.1. The effect of cell irradiation on PHT cells in vitro

We first examined the effect of *in vitro* irradiation on PHT cell number. We measured total protein as a surrogate for cell number, because PHT cells do not divide *in vitro*, but differentiate into syncytia. As shown in Fig. 1A, radiation had no effect on protein content in PHT cells, determined 24 h after exposure. In contrast, radiation markedly reduced protein content in the BeWo placental line, as well as in the radiosensitive NCCIT cells, both capable of proliferation *in vitro*. We obtained similar results by measuring cellular DNA content (not shown). Exposure to radiation also reduced cell number in the radiosensitive NCCIT line, as expected, but not in BeWo cells (Fig. 1B). As shown in Fig. 1C, hCG production was diminished in irradiated PHT cells, compared to control, and was accompanied by a 73% reduction in hCG mRNA ($p < 0.0001$) This was accompanied by enhanced apoptosis, determined by the increased expression of PARP and cytokeratin 18 (Fig 1D).

To comprehensively interrogate the impact of cell irradiation on gene expression in PHT cells, we used high-throughput microarray screening to measure gene expression at 4, 8, and 24 h after exposure to radiation, at a dose of 10 Gy (NCBI-GEO reference number GSE49924). Using a mixed-effects model with adjustment for false discovery, our microarray screen identified 1200 genes that were upregulated in irradiated PHT cells and 1050 genes that were downregulated by irradiation ($q < 0.05$). We ranked the $\log_2$ expression ratio of these significantly affected genes and chose a subset of the top 1% and bottom 1% for RT-qPCR validation, including the upregulated genes *CDKN1A*, *DDB2*, *EGR2*, *FAS*, *SULF2*, and *MAP4K4* and the downregulated genes *CCNA2* and *CTSC*. As seen

in Fig. 2A, most expression changes observed in the selected genes by microarrays were verified using RT-qPCR, with the greatest effect commonly in the first 4 h after irradiation. We further confirmed our results by determining protein expression for the most upregulated mRNA, cyclin-dependent kinase inhibitor 1A (*CDKN1A*). As expected, the expression of the CDKN1A protein was upregulated by PHT cell irradiation (Fig. 2B). In contrast, the expression of the MnSOD, which is known to be radioprotective [34], was unchanged (Fig. 2C). To assess whether the nitroxide JP4-039 mitigates irradiation injury in PHT cells, we added the drug to the cell culture 1 h before radiation or sham exposure. We found that a single dose of 10 μM JP4-039 had no effect on medium hCG levels (not shown) or on the genes presented in Fig. 2 (including the largest effect, on *CDKN1A*), except for a weak attenuation of MAP4K4 expression change at 8 h (Fig. 3).

3.2. The effect of whole body irradiation on the mouse placenta in vivo

To determine the impact of radiation injury on placental function and gene expression, we exposed pregnant mice at E13.5 to whole body irradiation as describe in Methods and analyzed physiologically relevant outcomes at E17.5. As shown in Fig. 4, radiation reduced fetal weight, with an insignificant effect on litter size and a small, yet significant, effect on placental weight. No gross fetal or placental anomalies were noted. Interestingly, maternal weight gain was also reduced after exposure to ionizing radiation. None of these parameters was significantly affected by treatment with a single dose of 10 mg/kg of JP4-039, 10 min post irradiation (Fig. 4).

To gain further insight into the effect of total body irradiation on the placenta, we used stereology, as described in Methods, to analyze irradiated placental morphology. Focusing on the labyrinthine and junctional zones, we found no significant differences between irradiated or sham-exposed placenta. Specifically, using vertical uniform random sections, the mean height of the junctional zone was 365 ± 24 μm for the sham-exposed dams, which decreased by 56 ± 43 μm in irradiated placentas ($p = 0.2$). The mean height of the labyrinth was 935 ± 28 μm for the sham-exposed dams, which was unchanged in the radiation-exposed dams (934 ± 45 μm, $p = 0.99$).

We employed high-throughput microarray screening to examine the impact of whole body irradiation on gene expression in the mouse placenta ($n = 6$ for sham vs. radiation, NCBI-GEO reference number GSE49924). We found that the $\log_2$ expression ratio ranged between –2.01 and 1.96, and none of the approximately 30,000 tested mRNAs was significantly different after adjustment for false discovery. We used RT-qPCR analysis of gene products exhibiting the greatest change from the 1% most up- or downregulated genes, and included genes that are relevant to mouse placental biology or to radiation injury. The expression of a subset of these mRNA was also tested in mice exposed to JP4-039. As seen in Fig. 5A–B, radiation weakly affected the expression of *Vldlr* (1.6-fold increase), *Gcgr* (1.6-fold decrease), and *Ndufs7* (1.5-fold decrease). We also found that JP4-039 modified the effect of radiation on three genes: *Apoe*, *Ctgf*, and *Ndufs7*. Most gene products, however, were resistant to the effect of radiation or JP4-039 treatment.

4. Discussion

Our data indicate that, while ionizing radiation affects human trophoblasts *in vitro* and mouse fetal growth *in vivo*, the impact of radiation on placental gene expression is relatively modest and might not account for the full effect of radiation on fetal growth.

In vitro, irradiation of PHT cells led to reduced medium β-hCG levels and gene expression, and enhanced apoptosis. The extent of global gene expression was overall small. Among gene products that exhibited a significant change *in vitro*, the greatest effect was an increase

in CDKN1A mRNA and protein expression. Indeed, CDKN1A, commonly regulated by p53, is known to be upregulated by radiation injury, resulting in G1-phase cell cycle arrest and probable protection against apoptosis [35–37].

Other gene products that we found to be regulated *in vitro* or *in vivo* might reflect radiation injury, or its consequences. FAS plays a key role in trophoblast apoptosis [38]. FAS and MAP4K4 both activate MAPK8, and are required for TNFα-induced apoptosis [35]. SULF2, a member of the heparan sulfate 6-O-endosulfatases family, regulates signaling cascades through release of growth factors from storage sites and is possibly involved in tissue regeneration [39]. Interestingly, SULF2 is a target of p53 during doxorubicin-induced DNA damage in HepG2 cells [40]. DDB2, a damage-related protein and part of a heterodimeric complex that participates in nucleotide excision repair, is increased in irradiated human fibroblasts [41]. EGR2 is a member of the early growth response family of transcription factors and is known to be upregulated after wound injury in the mouse [42]. The VLDL receptor (VLDLR) probably plays a role in placental cholesterol metabolism, which may be disrupted as a consequence of cell injury [43]. The glucagon receptor (GCGR) is implicated in maintaining adequate carbohydrate supply to the fetus. *Gcgr−/−* fetuses exhibit placental vascular damage, vacuolization of syncytiotrophoblasts, and diffuse interstitial trophoblast hyperplasia [44]. NDUFS7 is a complex 1 mitochondrial respiratory chain protein. In addition to a role in Leigh Syndrome, with severe lactic acidosis in infants or muscle weakness in adults, reduced levels of NDUFS7 have been associated with oxidative damage [45].

Although the changes in gene expression identified in our study may not account for the 25% reduction in mean fetal weight, we emphasize that we exposed mice to radiation injury at E13.5, a time when placental growth has nearly plateaued, yet fetal growth remains rapid [46]. This may also explain the relatively small effect of radiation on placental phenotype. Because we used whole body irradiation, the observed fetal growth restriction may reflect a direct radiation effect on the fetus, affecting neurons, soft tissue and epithelial surfaces, and thus impacting growth. Such a direct injury was not probed in the present study. It is likely that radiation at an earlier point in pregnancy might have exerted a greater impact on the developing placenta. Another confounding factor that we could not control was for the significant reduction in maternal weight gain, reflecting reduced fetal weight and a small reduction in litter size. In addition, it likely reflects reduced food intake by irradiated dams, with resultant reduction in fetal growth. It is intriguing that, despite irradiation and reduced maternal food intake, placental histology and gene expression patterns were relatively intact, further supporting the notion that the established placenta, in the last third of pregnancy, is fairly resistant to these injuries.

The dose of radiation used in our study was intended to affect fetal growth, with minimal effect on survival. Indeed, the use of 5 Gy in preliminary experiments led to a high rate of fetal death, that was prohibitive for our studies on fetal growth. A previous study showed a 5% reduction in fetal growth when Swiss albino mice were irradiated at a similar gestational age, but at ≤1.5 Gy [5]. We used C57BL/6HNsd. It is known that different mouse strains exhibit a range of susceptibility to total body irradiation [47].

Our attempt to mitigate radiation injury with the GS-nitroxide JP4-039 led to a weak and sporadic effect on several gene products. JP4-039 did not alter *in vitro* differentiation of control or irradiated cells (not shown). A prior study showed that JP4-039 given 1 h before radiation improved survival of 32Dcl3 cells [24], probably reflecting cell sensitivity to radiation injury. Importantly, JP4-039 did not affect fetal weight, placenta weight, or mouse weight gain. The impact of JP4-039 or other targeted reactive oxygen species scavengers [48] at different gestational ages, any beneficial or deleterious effects, the timing of

administration relative to the exposure, trans-placental transport, and the optimal dose remain to be established.

Although exposure of pregnant women to less than 0.05 Gy has not been associated with an increased rate of fetal anomalies or spontaneous abortion [49], a safe radiation dose for trophoblasts has not been defined. Our data suggest that even though radiation affects placental trophoblasts, the established placenta is fairly resistant to radiation. Our study underscores the need to interrogate the impact of radiation and radiation-mitigating drugs on the feto-placental unit. Additional doses, used at early and late gestational ages, might be instrumental in guiding safety parameters in humans.

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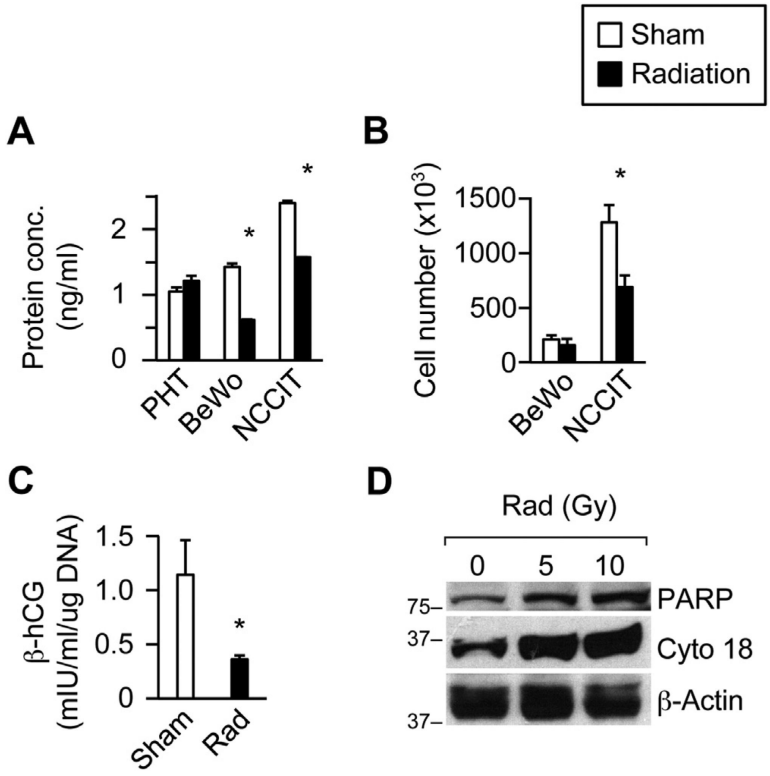

Fig. 1.

The effect of ionizing radiation *in vitro* on cultured human trophoblasts. Radiation was delivered as described in Methods. (A) Protein concentration, (B) Cell number, (C) Medium β-hCG levels normalized to total cellular DNA, (D) Expression of PARP and cytokeratin 18. Note that cell number was not recorded for PHT cells, which do not proliferate *in vitro*. Radiation dose was 10 Gy in PHT cells (5 and 10 GY in Panel D), but only 5 Gy in the placental line BeWo or in the radiosensitive mixed germ cell tumor cells NCCIT. Sham was an identical procedure without radiation. The data are for a representative experiment, which was repeated at least three times using cells from three different placentas. *Denotes $p < 0.05$.

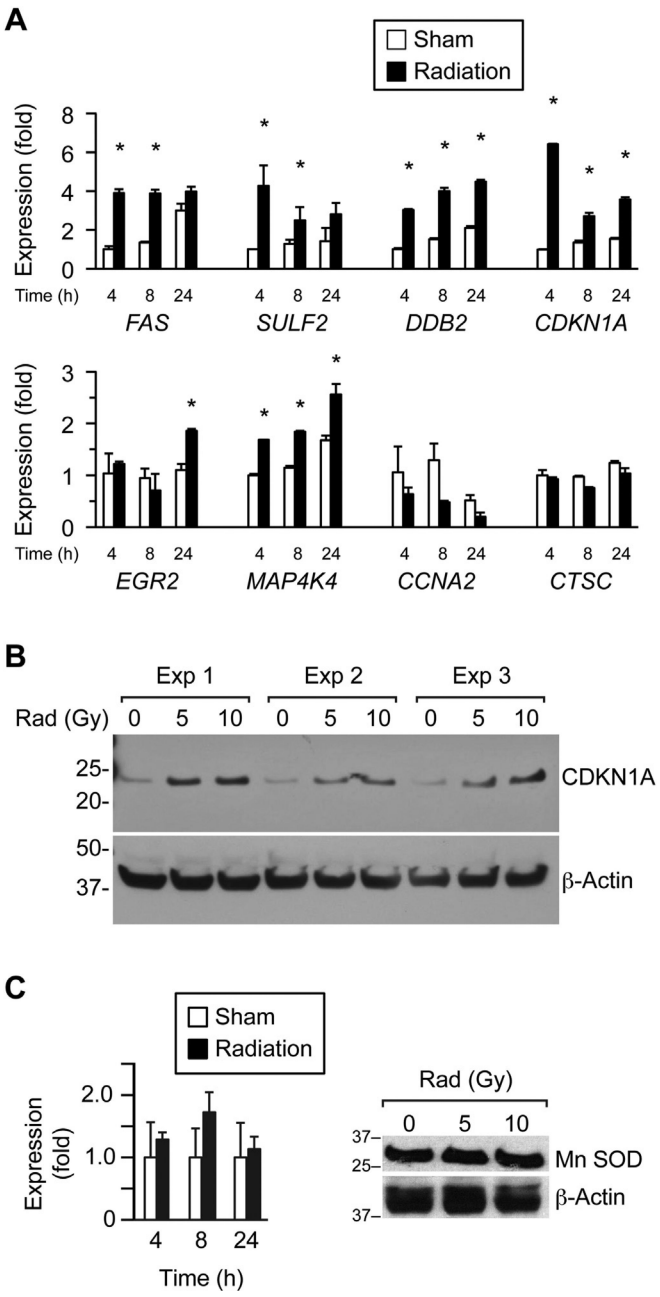

Fig. 2.

The effect of ionizing radiation *in vitro* on gene expression in cultured human trophoblasts. (A) Transcripts were selected for RT-qPCR assay ($n = 6$) based on mRNA array assays, performed as described in Methods. Radiation dose was 10 Gy, and sham was an identical procedure without radiation. *Denotes $p < 0.05$. (B) A western analysis of the expression change in CDKN1A at several radiation doses ranging from 0 to 10 Gy and repeated three times using three PHT cell cultures, each prepared from a different placenta. The data were normalized to actin and scanned, confirming ($p < 0.05$) a dose-dependent increase in CDKN1A expression. (C) The expression of MnSOD by RT-qPCR (left panel) and western analysis (right panel). None of the differences were significant.

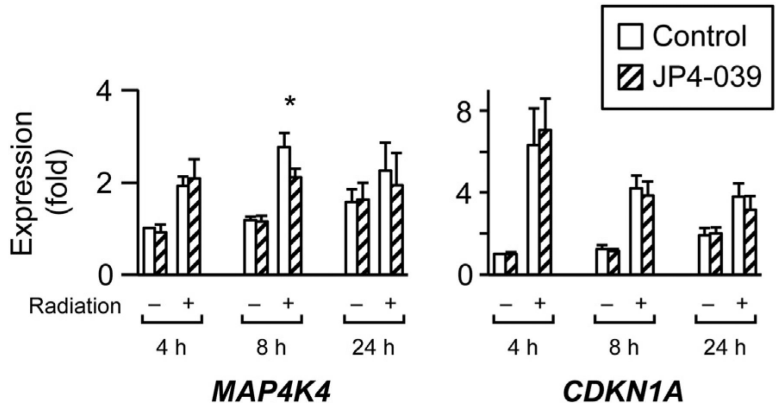

Fig. 3.

The effect of JP4-039 on the expression of selected genes in PHTs. All transcripts shown in Fig. 2 were tested using RT-qPCR. Only MAP4K4 at the 8 h time point was affected by JP4-039. CDKN1A was the most affected transcript in Fig. 2 and is shown for comparison. DMSO was used as control for JP4-039. The data are from a representative experiment which was repeated three times. *Denotes $p < 0.05$.

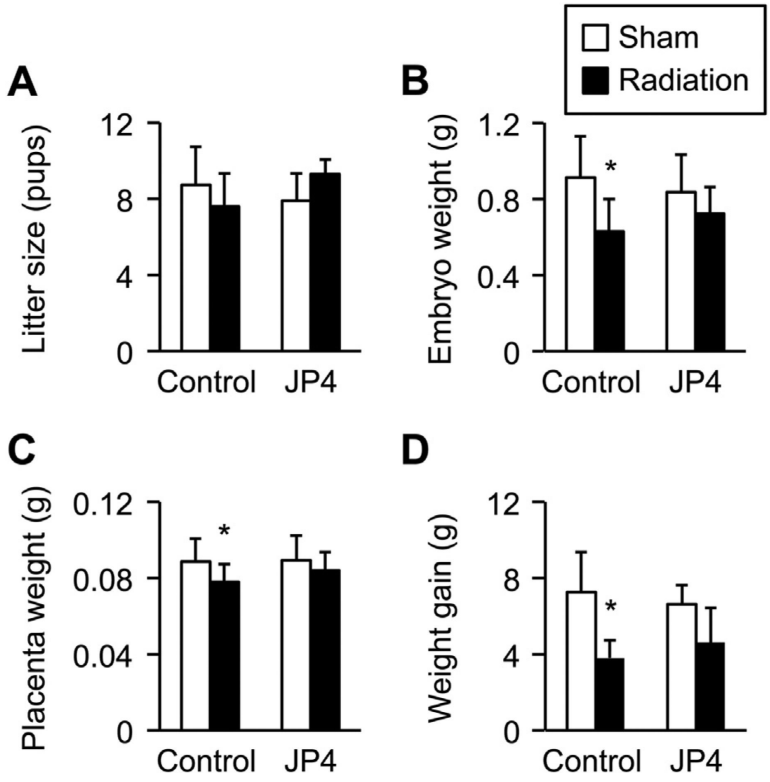

Fig. 4.

The effect of radiation and JP4-039 on murine pregnancy at E17.5. Whole body irradiation at 0 Gy (sham) or 4 Gy (radiation) was performed on E13.5 as detailed in Methods. JP4-039 (10 mg/kg) was administered intravenously 10 min after radiation or sham. (A) Litter size, (B) fetal weight, (C) placenta weight, (D) maternal weight gain. Data were based on non-irradiated dams (n = 30, of which 8 received JP4-039), and irradiated dams (n = 17, of which 7 received JP4-039. *Denotes $p < 0.05$).

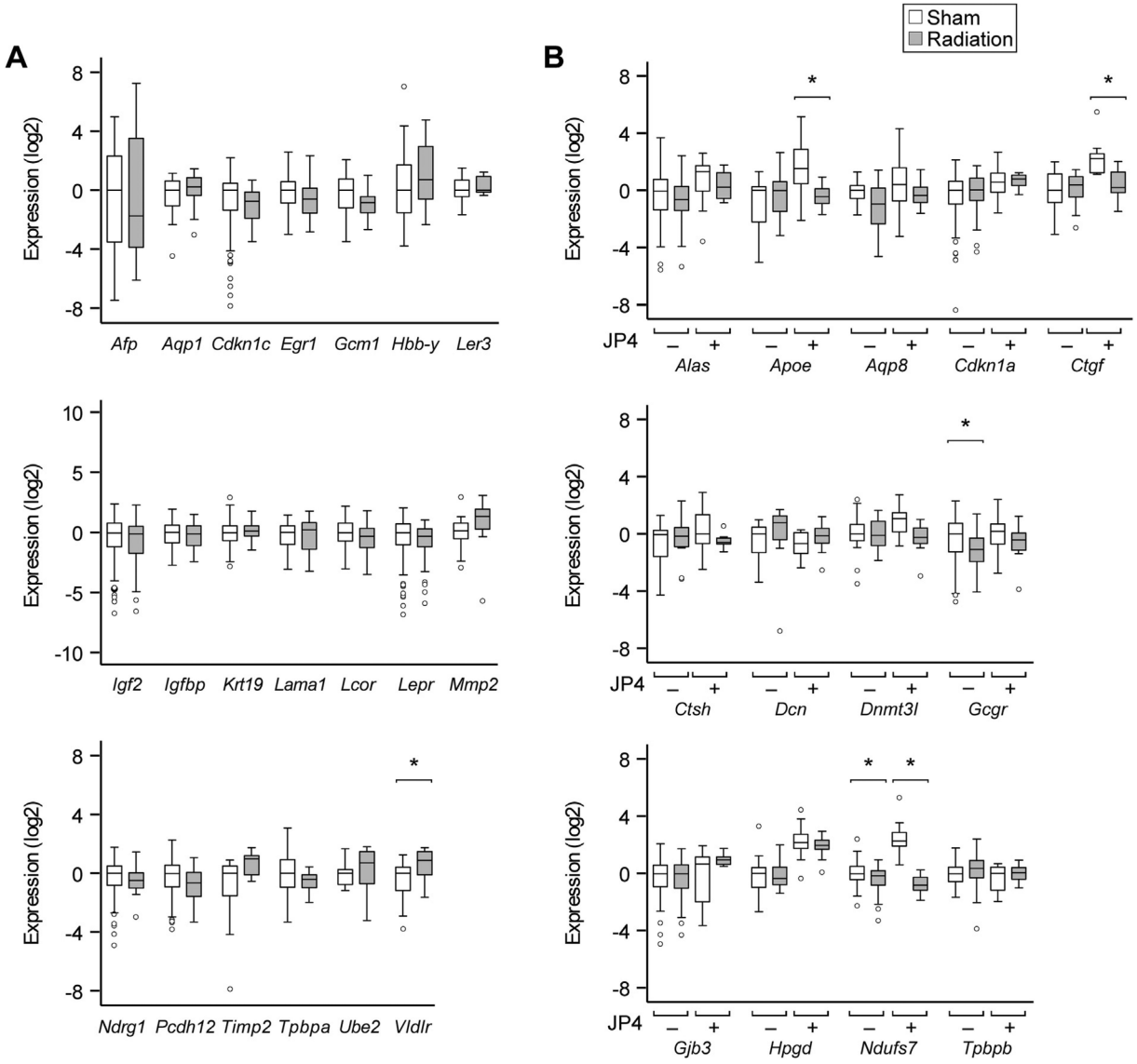

Fig. 5.

The effect of radiation, with or without JP4-039, on mouse placental gene expression. Whole body irradiation at 0 Gy (sham) or 4 Gy (radiation) was performed on E13.5 as detailed in Methods. JP4-039 (10 mg/kg) was administered intravenously 10 min after radiation or sham. (A) Gene products tested for the effect of radiation and (B) gene products tested for the effect of radiation and the interaction with JP4-039. *Denotes $p < 0.05$.