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Subject	Congenital malformations induced by ionizing radiation in mouse embryos: investigating molecular changes
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Abstract

Irradiation of the mammalian embryo during development results in diverse effects depending on the dose and the specific gestational phase at irradiation. In this work cellular and molecular changes associated with X-irradiation of embryos were therefore investigated at both early and late gestational stages at the moment of radiation exposure. Our goal was to find biological markers indicative of teratogenic effects of radiation, and provide a holistic model of the impact of irradiation during early and late development.

In the first part of this doctoral thesis, we investigated telomere length in the irradiated and non-irradiated embryos bearing different p53 genotypes and malformation status as telomere shortening was associated with neural tube defects in mTR^{-/-} embryos. Moreover, the loss of telomere function has been shown to elicit DNA damage checkpoints and p53-dependent apoptosis *in vitro*. We conclude that telomere shortening is associated with the malformation status as well with the p53 genotype. These data assign telomere length as a potential predictor of a malformed phenotype, a feature that is modulated according to the p53 genotype and the developmental stage at the moment of irradiation.

In the second part of this work, we focused on a specific malformation phenotype, namely: forelimb defect. To identify potential genes involved in the radiation-induced forelimb teratogenesis, we investigated differential gene expression between irradiated and non-irradiated fetuses using RT-q-PCR. The results indicate that forelimb defects observed in p53 wild type fetuses irradiated at the organogenesis period was due to excessive cellular death as shown by the high expression of the pro-apoptotic factors caspase-3 and Bax. This suggestion was supported by the positive TUNEL assay performed on forelimb tissue sections of malformed irradiated fetuses. Moreover, overexpression in malformed fetuses of MKK3 and MKK7, both members of the stress-activated MAP kinase family, could be involved in radiation-induced apoptosis through activation of the p38 and JNK pathways, respectively.

To further evaluate the biomarker value of telomere length in this forelimb defect phenotype, we assessed telomere length in normal fetuses versus abnormal ones with forelimb defects. We found that irradiated fetuses exhibiting forelimb defects showed a marked telomere shortening confirming our first findings, which showed an association between various malformations and telomere shortening. Knowing that oxidative stress and inflammation are potential accelerators of telomere attrition, and taking into account that amniotic fluid is the most accessible fetal material, we decided to explore cytokine secretion in the amniotic fluid of normal and malformed fetuses. Our results showed a considerable inflammatory reaction among the irradiated fetuses, as revealed by the high presence of pro-inflammatory cytokines.

Finally, all these results indicate that insufficiency or excess of apoptosis is probably the central process behind radiation-induced malformations. Moreover, apoptosis is strongly related to p53, which upon signaling modulates cell death response according to the developmental stage at which exposure to radiation has occurred. Furthermore, in the context of this work, telomere shortening as well as the differential gene expression described and the high pro-inflammatory cytokines measured may constitute potential indicators of the teratogenic status of the embryos after radiation exposure.