

Background

Various studies have shown that early embryos of several mouse strains are sensitive to radiation-induction of congenital anomalies. The presence of mutations in particular genes seems to increase the sensitivity of embryos to such effects, but studies in that field are extremely limited.

Objectives

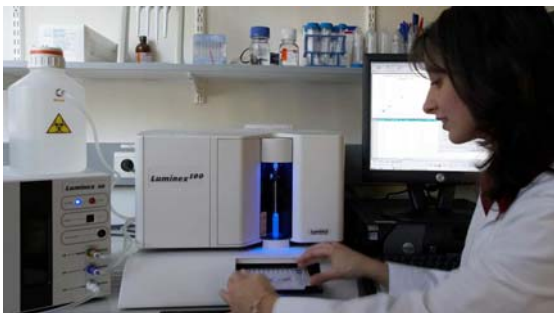
The aim of these studies is to determine 1) whether the sensitivity to radiation-induction of congenital anomalies during very early stages of gestation is transmitted to the following generation; 2) how mutations in genes involved in important cellular processes, like DNA repair, cell cycle regulation and apoptosis (cell suicide of a damaged cell), can influence the radiation sensitivity of the mammalian embryo during sensitive stages of early pregnancy. Emphasis is put on heterozygous mutations (in which only one of the two copies of the gene is mutated), which are relatively numerous in the human population and do not affect the viability and the fertility of individuals carrying them. The studies under way in that field are supported by contracts with the European Union and the Federal Agency for Nuclear Control (FANC-AFCN).

Principal results

The embryonic stages at which irradiation is delivered are the *zygote* or 1-cell stage (first day of gestation in mammals) and the *gastrula* stage (beginning of the second week in humans and mice). Both stages occur while women are, generally, not aware of pregnancy, meaning that irradiation of the embryo during a radiological examination of the mother cannot be excluded. Mouse embryos are irradiated with moderate doses of x-rays, e.g. 200 or 400 mGy. Our studies concentrate essentially on congenital anomalies, *cytokine* secretion in the amniotic fluid, *chromosome instability* and modifications of *gene expression* in the embryos. *Cytokines* are regulatory proteins which are released by cells of the immune system and act as intercellular mediators in the generation of an immune response. Measuring cytokines in the amniotic fluid will enable us to determine whether there is a correlation between specific cytokines and radiation-induced congenital anomalies. *Chromosome instability* has been reported in mouse foetuses from 2 strains (Heiligenberger and C57BL), after x-irradiation at the zygote stage. This phenomenon was characterized by an abnormally high frequency of chromosomal anomalies in the cells of the foetuses. The chromosomal anomalies had developed many cell cycles after x-irradiation of the zygotes. The long-term consequences of chromosome instability are still unclear, but, in the Heiligenberger strain, there was an apparent link between chromosome instability and the presence of external malformations in the foetuses. Early alterations of *gene expression* in the irradiated embryos could be a key component of the various developmental effects appearing later in the foetuses.

Developmental effects are assessed in pregnant animals before delivery, *i.e.* on day 19 of gestation. A sample of amniotic fluid surrounding each foetus is collected. The pre-implantation and post-implantation losses are evaluated in the pregnant mice and the living foetuses are weighed and analyzed under the stereomicroscope for the presence of congenital anomalies

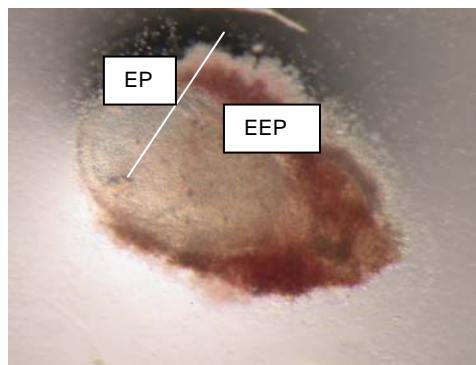
The method used for the detection of cytokines in the amniotic fluid is based on the so-called "multiplex bead assay" or Luminex technology, which has been adapted recently in our laboratory.



The Luminex technology allows the simultaneous detection and dosage of dozens of cytokines present in only 50 µl of amniotic fluid, with a sensitivity of a few pg/ml (picograms or 10⁻¹² grams/ml)

Chromosome aberrations are analyzed in the embryonic part of early gastrulas, because our studies on various mutants have shown that this part of the gastrula is much more sensitive to radiation induction of chromosome damage than the extra-embryonic part. For this purpose, total gastrulas (about 1 mm length) are

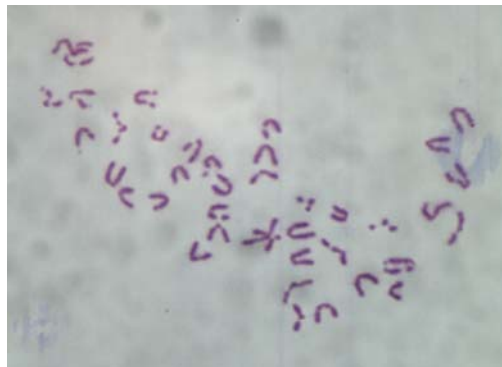
dissected under the stereomicroscope, and their embryonic parts are isolated and cultured *in vitro* for 6 h in the presence of colchicine, in order to block the cells in the metaphase of mitosis, at which stage chromosomes can be visualized.



The

picture on the left illustrates the collection of early gastrulas, before their *in vitro* culture. Gastrulas are composed of an embryonic part (EP) and an extra-embryonic part (EEP) (picture on the right). Isolation of the embryonic part is performed under the stereomicroscope, using sharpened tungsten needles with extremely fine tips

After culture, embryonic cells are fixed, spread on microscopic slides and stained for cytogenetic analysis, as illustrated on the following pictures.



Two metaphases from embryonic parts of mouse gastrulas, as they can be seen under the microscope. The first metaphase originates from a control embryo and shows 40 normal chromosomes, while the second is from an irradiated embryo and contains various aberrations (gaps and fragments)

Analysis of gene expression in irradiated embryos is performed with our genomic platform which permits rapid, large-scale mapping of almost whole genomes (about 20,000 genes in the mouse) by directly analyzing single genomic DNA fragments. Our studies have shown that, after x-irradiation with a moderate dose (0.5 Gy), a high number of genes are down-regulated in the embryonic part of the gastrula, while the same genes are up-regulated in its extra-embryonic part. This might explain the high sensitivity of the embryo at this important stage and suggests a role of the extra-embryonic part in its protection. Down-regulation of several embryonic functions at the start of the organogenesis could have drastic consequences on survival and further development.

Future developments

Studies on the heritability of the sensitivity to radiation induction of congenital anomalies are under way in the CF1 and ICR mouse strains. Likewise, experiments on the influence of heterozygous mutation in the p53 gene (the so-called "genome guardian", which exerts a key role in the apoptotic process) are being pursued. Two other genes will be investigated in the near future for their influence on embryonic radiation sensitivity. The first of them -PARP- is involved in the repair of single-strand breaks in DNA, while the second -ATM- is involved in cell cycle regulation, DNA repair and apoptosis.

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Main reference

P. Jacquet, Genetic susceptibility to radiation-induced effects in embryos, in "Effects of *in utero* exposure to ionising radiation during the early phases of pregnancy", Office for Official Publications of the European Communities, (Ed. European Communities), Luxembourg, ISBN-92 894-4536-X, pp. 17-36, 2002.