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Subject	Ionizing radiation induced transcriptional changes in the developing mouse brain
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Abstract

Brain damage induced by prenatal irradiation is of major concern in radioprotection. The brain is the final result of a series of well timed consecutive waves of cellular proliferation, migration, and differentiation. Acute irradiation during pregnancy could selectively disturb these events to result in various forms of malformations such as microencephaly, reduced cortical thickness, glioblastoma tumours and/or mental retardation. In this work we concentrated on the transcriptional alterations induced by ionising radiation in the mouse developing brain and its different cell-types.

Using cDNA-microarrays and real-time PCR, we analysed the modulated gene expression profile after 50 cGy X-ray exposure in embryonic mouse total brains at three developmental stages. Functional grouping of the modulated mRNA transcripts revealed that the main activated pathways in irradiated wild type embryos are involved in the induction of *Trp53* dependent programmed cell death and intracellular signalling cascades. The strong upregulation of *Ccng1*, *Trp53inp1* and *Cdkn1a* suggested that the tumour suppressor P53 protein is an essential regulator of the radiation induced stress response. Moreover, a decreasing expression profile could be identified at later development, suggesting a reducing sensitivity to radiation.

The information obtained lead to a subsequent experiment in which the ionising radiation response in P53 deficient embryonic brains at the same developmental stages was determined. Since both genotypes showed the strongest gene expression modulation at developmental stage E13, we concentrated our initial analysis on this developmental stage. In one hand, wild type embryos show a strong upregulation for *Trp53inp1* and *Ccng1* in the irradiated E13 mouse brain was observed. Considering the fact that they are involved in similar functions, and that *Trp53inp1* is less strongly induced then *Ccng1*, let us suggest that P53 is tightly regulated through different mechanisms after radiation exposure. The *Trp53* null mutants at this stage exhibited a decreased expression profile for various Cyclins and Cyclin-dependent kinases, suggesting the induction of a *Trp53* independent cell cycle arrest. The observed absence of high levels of apoptosis is in concordance with the described phenotype of *Trp53* null mutant mice that show an increased risk for tumour formation. Next, we questioned if the developing brain is characterized by a regional dependent differences on radiation sensitivity. Therefore, additional transcriptional analyses of the different regions (hippocampus, pallidal neuroepithelium and cortex of control and irradiated brain embryos) of the ventral wild type brain at E15 were performed. In these three regions of the developing brain, the differential expression of *Trp53inp1* and *Ccng1* by *in situ* hybridization suggested that the ventral brain shows a specific regional dependent expression pattern. This was further evidenced by real time qPCR, by which it also became clear that the strongest radiation induced effect can be observed in the cerebral cortex. However, this experiment didn't allow making a distinction between neural cells in this experiment, and different cell types contribute to correct neural network formation. Detailed analysis of cultured and irradiated neural cell types indicated that the expression of the *Trp53inp1/Hipk2* and *Ccng1/Mdm2/P19Arf* signalling pathways are activated in a cell-type dependent matter. From this data it appears that, depending on the cell type and the stage of differentiation, two possible mechanisms for the induction of apoptosis are activated. In one hand cell cycle arrest occurs via P21 (*Cdkn1a*) induction and to certain extends via P19ARF in the mitotic astrocytes, while in short-term neurons this mechanism seems to be preferentially induced by Cyclin G1. This arrest is then followed by the induction of the mitochondrial proapoptotic pathway. Interestingly, long term cultured neurons show also an induction of apoptosis after 50 cGy ionising radiation, but they were not characterized by an increased expression of *Cdkn1a*. The elimination of neurons or their supporting cells can thus result in an impaired brain functioning, with the risk of causing cognitive dysfunctions, even in the more mature brain.