The project focuses on the analyses of cell cycle regulating enzyme activities at the G2-phase/mitosis transition in relation to DNA damage, DNA repair, and the development of chromosomal damage. The working hypothesis is that the higher the endogenous cdk1/cyclin B activity is in a cell, the higher will be the non-repaired damage in DNA at the time when the cell enters mitosis. To test this hypothesis, different human cell lines and embryonic cells from different mouse strains with known differences in radiation sensitivity were analysed for cell cycle activity, chromosomal damage, and DNA repair capacity.

The project was separated into two major parts, one on adult somatic cells and a second on embryonic cells. The two major parts were further subdivided in 4 work packages each. The work package aims were, (I) to analyse changes in cdk/cyclin enzyme complexes in different human cell lines in response to irradiation, (II) to study the evolution of chromosomal damage after premature chromosome condensation in these cell lines, (III) to measure the repair capacity in cells by using the comet assay, and (IV) to analyse intracellular signal transduction pathways in irradiated cells.

Evolution of genetic damage in relation to cell cycle control: a molecular analysis of mechanisms relevant for low dose effects

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<th>Kinase Activity</th>
<th>MCF7</th>
<th>HeLa</th>
<th>SCL2</th>
<th>TK6</th>
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Cdk1/cyclin B kinase activity in four different cell lines (MCF7, HeLa, SCL2, TK6) after irradiation (1) and in non-irradiated controls (0).
to detect this strong correlation between sensitivity and DNA repair, we conclude that cell cycle regulation is a key player in determining human radiosensitivity.

To study cell cycle regulation in early mouse embryos and effects of irradiation on this regulation, the activity of the cdk1/cyclin B protein kinase was determined in single oocytes or embryos. After fertilisation, cdk1/cyclin B protein kinase activity in mouse embryos is low during interphase of the first cell cycle, increases during the first mitosis, and decreases again during the next interphase.

Embryos irradiated in the first cell cycle were arrested during G2. However, the dynamic of this radiation-induced G2-block in the first embryonic cell cycle is totally different between the mouse lines tested. This differential dynamic in the G2-block in one-cell embryos of the different strains is strictly paralleled by a similar dynamic in the cdk1/cyclin B activity.

Using the comet assay the amount of initial radiation induced DNA damage in embryos for a particular dose was comparable to the amount in the cell lines mentioned above. The repair in embryos of the mouse strains tested is extremely fast and almost complete after 30 minutes. This is significantly faster than in all cell lines and primary cells tested so far.

The results of these studies will allow a better understanding and assessment of risks from those effects which are characteristic consequences of exposure to small radiation doses, i.e. cancer, genetic damage and disturbance of development in utero. All results obtained so far are consistent with the proposed hypothesis that following exposure to ionising radiation, the onset and the efficiency of chromatin condensation-decondensation which is dependent on cdk1/cyclin B activity levels is the important determinant of the process that converts initial radiation-induced DNA damage into chromosomal breaks. This may, therefore, explain the differential radiosensitivity observed at the various stages of the cell cycle as well as among mutant cells and cells of different origin. More important we were able to show differences in in vitro chromosomal radiosensitivity of peripheral lymphocytes of cancer patients compared to lymphocytes from healthy control individuals.

**Partnership**

The project integrated the expertise of five laboratories all of which have made important contributions to progress of the project and the development of new methods needed. Their co-operation allowed the concerted approach to correlate key mechanisms in cell cycle control with damage to the DNA molecules and chromosomes at different stages of the cell cycle.

**Selected references**

