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High levels of radiation-induced excess acentric fragments in cells deficient in DNA-PK, p53, and p21

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Purpose: DNA-damage activates complex regulatory response pathways and several of the proteins involved have already been characterized. In the smooth muscle cell line SMCM IX, originally derived from the vein of a healthy individual, we analysed radiation-induced chromosomal aberrations, the expression of p53 and p21, and the activity of the DNA-dependent protein kinase DNA-PK.

Material and Methods: SMCM-cells in a later passage were grown in SMCM-specific medium, 10% FCS in a 5% CO2-atmosphere at 37°C. Plateau-phase cells were irradiated with graded doses of 1 Gy to 5 Gy of 200 kV X-rays. Chromosome aberrations were determined as genomic yields of dicentric chromosomes and excess acentric fragments, scored in Giemsa-stained metaphases. Protein expression of p53 and p21 was determined by Western blot analysis. DNA-PK activity was determined using the SigmaTECT(TM) DNA-Dependent Protein Kinase Assay System.

Results: We observed neither a DNA-PK-activity nor a radiation-induced expression of p53 or p21. In irradiated as well as in control cultures, a surprisingly high proportion of metaphase cells, 18± 3%, were aneuploid. Only diploid cells were analysed with respect to radiation-induced chromosomal aberrations. Here, a high proportion of excess acentric fragments (yac(ex)) was observed. The ratios of (yac(ex))/ydic were 11.5, 19.0, 19.3 and 30.0 for radiation doses of 2 Gy, 3 Gy, 4 Gy, and 5 Gy, respectively. This was a marked increase compared to yac(ex)/ydic of other normal tissues, like 0.98 for lymphocytes, 1.52 for dermal fibroblasts, and 3.64 for endothelial cells from umbilical cord veins, after a radiation dose of 4 Gy.

Conclusion: Deficiencies in proteins involved in the control of genomic integrity and DNA-dsb repair were observed in a cell line together with remarkably high levels of unrepaired DNA-dsb's manifested as excess acentric fragments. Moreover, our observation of the high proportion of aneuploid cells in the deficient cell line indicates the participation of p53/p21 in control of chromosome segregation.

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Radiation enhancement and modulations of cell cycle distribution induced by Gemcitabine (dFdC)

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Purpose: Among various mechanisms responsible for the radiation enhancing effect of low-dose Gemcitabine (2',2'difluoro desoxycytidine), cell cycle modulations may play a role. It was our aim to evaluate this effect and the underlying changes in cell cycle distribution in #4197-cells (human oropharyngeal squamous cell carcinoma).

Methods: In 96-well-plates, cells were exposed to dFdC (0-3,0 μM) for 24 hours (h). After drug removal, immediate irradiation (0-10 Gy), and incubation (120 h), viability was determined fluorometrically. For cell cycle analysis by flow cytometry (FACS), cells were irradiated (0-40 Gy) or treated with dFdC (0,012-1,0 μM) (6-well-plates). Additionally, cells were exposed to dFdC (2,0 μM) for 0-6 h. FACS-analysis of propidium iodide stained cells was performed immediately or 16-63 h later. Cell cycle kinetics were evaluated using BrdU (10 μM) S-phase labeling, given either 30 minutes prior to or in the last hour of dFdC treatment (0-6 h; 2,0 μM).

Results: The fluorometric assay revealed that dFdC enhances radiation induced cytotoxicity at marginally toxic or untoxic concentrations (<37 nM). Radiation resulted in the anticipated G2/M-arrest. DFdC induced concentration and exposure time-dependent cell cycle changes that were better resolved using BrdU demonstrating a pronounced S-phase arrest already at 12 nM. BrdU-puls labeling revealed that the cell cycle block occurred at the G1/S-boundary.

Conclusion: Our data reconfirm the already known radiation enhancement and S-phase specific activities of dFdC. In literature data, it is discussed that the progression of cells through the S-phase seems to be important for the radiosensitizing properties of dFdC. However, we could demonstrate that before progressing into the S-phase, cells were blocked at the G1/S-boundary which is a more radiation sensitive cell cycle stage. We conclude that dFdC enhances the radiation effect by accumulating cells in this stage. Furthermore, cells progressing past the block might accumulate pro-apoptotic signals caused by both, radiation and dFdC which will also results in cell death.

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The relationship between Fludarabine-induced radiosensitisation and apoptosis in six human squamous cell carcinoma lines

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Purpose: To determine the relationship between Fludarabine-induced radiosensitisation, and apoptosis in six human squamous cell carcinoma lines.

Methods: Head and neck cancer (ZMK-1, OH-65, GR-145, BW-225-l); lung cancer (A-549), or cervical cancer (CaSki) cells were treated with escalating doses of Co-60-g-irradiation, different doses of Fludarabine (0.5 - 0.005μg/ml), or a combination of both. Cell survival was measured by a standard colony-forming assay; apoptosis was evaluated morphologically in acridin-orange-stained cells.

Results: Radiosensitisation was only observed for the more fludarabine-resistant ZMK-1, CaSki, and A-549 cells. The corresponding SER values at the 37% survival level were 1.5, 1.3, and 2.5, respectively. For, ZMK-1 and CaSki cells a fludarabine-induced doubling of the amount apoptotsis was observed, whereas A-549 cells showed a marked radiosensitisation and no apoptosis. For the OH-65, GR-145, and BW-225-l cell lines, neither radiosensitising effects, nor an increase of the amount of apoptosis was found.

Conclusions: We could demonstrate a fludarabine-induced radiosensitisation in 3 out of 6 cell lines tested, which for two cell lines seems to be related to the degree of apoptosis-induction of fludarabine alone.

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Identification of target cells for CNS radiation injury: Is apoptosis of neural stem cells a pathogenesis of radiation-induced leukoencephalopathy?

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Purpose: Pathophysiological mechanisms of radiation-induced leukoencephalopathy are yet to be clarified. Subependymal zone of the lateral ventricles and dentate gyrus in the hippocampus are unique regions of the CNS where majority of neural stem cells exist. We demonstrate that ionizing radiation induces apoptosis of the stem cells and then accelerates differentiation of stem cells into oligodendrocyte lineage leading to myelin synthesis disorder.

Materials and Methods: Adult C57BL/6J female mice were irradiated at 10 Gy and was sacrificed 4 h, 8 h, 24 h, 7 days, 14 days, and 28 days after irradiation. Whole brain samples were dissected into three portions (the ventricle-hippocampal region, the rest of forebrain, and the cerebellum). Apoptosis was assessed by the in situ immunohistochemical analysis (Tunel assay). An anti-nestin antibody (a marker for neural stem cells), an anti-O4 antibody (a marker of premature oligodendrocytes), an anti-NeuN antibody (a maker for neurons), and an anti-GFAP antibody (a marker for astrocytes) were used. The O4 protein and myelin basic protein (MBP) (a marker of mature oligodendrocytes) were consecutively assessed by western blotting. The brain sections of each time point were evaluated using Luxol Fast Blue (a method for myelin staining).

Results: (1) In both the subependymal zone and the dentate gyrus, the number of apoptotic cells increased at 2 h and reached its maximum (20%) at 6-8 h. The apoptotic cells were also detected by the anti-nestin antibody suggesting neural stem cells. (2) Characteristics of the apoptotic stem cells at subependymal zone and those in dentate gyrus were different (subependymal:GFAP-positive, dentate gyrus:O4positive). (3) The O4 protein and MBP syntheses in the ventricle-hippocampal region showed different style consecutive changes. The changes of the O4 protein and MBP synthesis were not observed from the samples of the rest of forebrain and those of cerebellum. (4) Myelin synthesis decreased sporadically 28 days after receiving 10 Gy irradiation.