radiation with chemotherapy and/or surgery. The interaction between tumour and inflammatory cells, e.g., via S100A4 (metastasin) and the receptor for advanced glycation endproducts (RAGE), is hypothesized to play a key role in metastasis of melanoma. In this study the contribution of para- and autocrine S100A4-RAGE activation to growth, motility and migration of metastatic melanoma and inflammatory cells before and post irradiation was investigated.
Materials and Methods: Mouse melanoma cells (B16), macrophages (RAW; a model for tumour associated macrophages (TAM)) and B16/RAW cocultures (ratio 1 to 5) were exposed to single dose irradiation (5, 10, and 20 Gy, compared to sham-irradiated controls) for 0, 3 and 6 days. S100A4 and RAGE expression in these cells was quantified via real-time RT-PCR, Western-blot analysis and immunochemistry. Cell growth and cellular viability was detected by MTT assay. Migration assays of non- and irradiated cells were performed with and without chemoattractants (supernatants of irradiated cocultures after 6 days). Additionally, the actin cross-linker L-plastin was investigated as a migratory marker.
Results: Post irradiation, S100A4 and RAGE mRNA expression was significantly increased in B16 and RAW cells but not in cocultivated cells. S100A4 protein expression was only detected in irradiated B16 cells whereas RAW cells always showed high levels in non- and irradiated cells. Interestingly, cocultures showed only minor S100A4 expression levels with a further reduction of S100A4 after irradiation. In contrast, RAGE protein showed only slight differences. A significant reduction of cell viability was observed after irradiation as supposed and the remaining cells seem to be in an exceptionally aggressive shape. On the other hand, migratory activity was significantly increased in B16 and cocultures after irradiation whereas RAW cells showed a significant decrease. Furthermore, chemoattractants significantly induced the migration in non-irradiated B16 cells.
Conclusion: Irradiation of both melanoma cells and macrophages alters their migratory and invasive activity. Under conditions of cocultivation these effects were more pronounced. We suppose an involvement of para- and autocrine regulation of extracellular S100A4 and its receptor RAGE in melanoma cells and TAM, thereby changing functional properties of melanoma cells towards a promigratory phenotype.

841 PARP-1 dependent DNA damage repair and formation of reactive oxygen species in response to ionizing radiation

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Background: Poly(ADP-ribose) polymerase (PARP) is an enzyme responsible for synthesis of poly(ADP-ribose) polymers in cell nuclei in response to DNA strand interruptions. Due to PARP's ability to recognize DNA strand breaks, poly (ADP-ribose) plays an important role in repair of DNA. Modulation of its activity by inhibition is considered as a potentially important strategy in clinical practice, especially to sensitize tumour cells to chemo- and radio-therapy. This study examines the influence of PARP inhibition on DNA repair and formation of reactive oxygen species (ROS) in cells exposed to ionizing radiation (IR), and also tries to answer the question whether the addition of a PARP inhibitor to irradiated cells is reflected in an altered level of apoptosis and necrosis.
Materials and Methods: Human K562 (myelogenous leukaemia) cells pre-treated or not with the PARP inhibitor Nu1025 (100 mM) were exposed to 4 Gy of ionizing radiation. The kinetics of DNA strand break rejoining were measured by alkaline single cell gel electrophoresis (Comet) assays. Intracellular reactive oxygen species were assayed using the probe 2,7-dichlorofluorescein diacetate (DCF-DA) with detection by flow cytometry. To measure PARP activity, its product poly(ADP-ribose) was detected immunocytochemically. Apoptotic and necrotic cells were quantitated using AnnexinV-PI staining and measured by flow cytometry.
Results: The data showed a ~100-fold increase in poly(ADP-ribose) formation during the first 10 min of recovery from IR, followed by a gradual decrease up to 30 min. Addition of the PARP inhibitor almost completely stops the production of poly(ADP-ribose) and significantly decreases the rate of DNA single strand rejoining. Three hours after IR we did not observe any changes in the amount of DNA single strand breaks (ssb) between groups but ROS concentration continued to grow up to 10 h. In this time point PARP inhibitor treated cells showed 250% higher ROS level than control and irradiated cells. We didn't find any significant changes in the apoptosis and necrosis level after 48 and 72 h.
Conclusions: Poly(ADP-ribosylation) and inhibition of PARP show a critical influence on DNA repair, and appear to be responsible for keeping the concentration of ROS at a high level after exposure of cells to genotoxic stress. Significant increase of ROS level in PARP inhibited cells seems not to have any influence on apotosis and necrosis. To determine the role of PARP in formation of reactive oxygen species, further studies have to be done.
Supported by Polish Ministry of Science and Higher Education grant N N514 411936; supported by the European Community from the European Social Fund within the RFSD 2 project.

842 Transcripts from genes located in different isochores are differently regulated in cells exposed to ionizing radiation

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Background: Studies on eukaryotic genome organization have revealed the existence of long (~300 kb average) chromosomal regions that differ significantly in GC base pair content. Vertebrate genomes contain five distinct families of DNA regions termed isochores L1, L2, H1, H2 and H3, with increasing GC content (Constantini et al., 2006). Here we compared the change of level of transcripts from genes lying in different isochores in the human genome induced by ionizing radiation. The presence of repetitive and transposon-like sequences in up- and down-regulated transcripts was also analyzed.
Materials and Methods: Transcript levels were measured with Affymetrix HG-U133A microarrays after exposing K562, Me45 and HCT116 p53+/− cells to 4 Gy of ionizing radiation. The average values of hybridization signals for multiple microarray probes corresponding to the same transcript were calculated, and sequence analysis of up- and down-regulated transcripts was performed after extraction of sequences from the EMBL Reference Sequence transcript database using mostly our custom-made computer applications.
Results: Genes whose transcripts were up-regulated after irradiation differed significantly in nucleotide composition from those down-regulated. The largest differences were observed in Me45 melanoma cells where the median GC content was 58.8% in up-regulated genes and 42% in those down-regulated. These differences were seen in the GC content of both the 3′ untranslated region and the coding sequences, which also revealed a very high codon bias. The nucleotide compositional differences between up- and down-regulated transcripts were highly correlated with the isochore location of their genes; about 78% of genes from the down-regulated group were in the GC-rich H2 and H3 isochores compared to less than 17% of up-regulated genes. The largest differences in regulation of genes lying in different isochores were observed 1 h after irradiation, with a decrease over the next 12−24 h. Up- and down-regulated transcripts also showed cell type-specific differences in the distribution of repetitive transposon sequences.
Conclusions: These observations suggest that responses of the transcriptome to radiation are related to the isochore organization of the genome.
This work was supported by grant: N N514 411936.

843 Rapamycin sensitizes glioblastoma cells to radiation by inhibiting survivin

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Background: Rapamycin, a specific inhibitor of mTOR, has a cytotoxic effect and sensitizes to radiation therapy in various types of cancer. Survivin is elevated and attributes to radiation resistance in many tumours. We investigated the radiosensitizing mechanism of rapamycin in terms of mTOR signalling, survivin and cell cycle change using glioblastoma cells which is one of the most radioresistant.
Material and Methods: Three different glioblatoma cell lines, A172, T87 and U87 were treated with rapamycin and/or radiation. We did Westernblot for the study of protein expression and analyzed the cell cycle change using flowcytometry.
Results: Rapamycin resulted in a significant reduction in the phosphorylation (ser 473) of AKT and mTOR (Ser 2448 and Ser 2481). Rapamycin also reduced XIAP and survivin. Rapamycin along with radiation more significantly reduced phosphorylation of AKT and mTOR, and suvivin level than rapamycin alone. The cell cycle assay showed that cotreatment of rapamycin and radiation increased sub-G1 population, compared with rapamycin and radiation alone. Rapamycin enhanced radiation induced $p21^{cip1}$ and $p27^{kip1}$ expression and downregulated cyclin D1 and D3. In clonogenic assay, rapamycin sensitized glioblastoma cells to radiation.
Conclusions: Rapamycin enhances apoptosis by inhibiting the pro-survival protein survivin in glioblastoma cells. Rapamycin in combination with radiation may be efficacious in the treatment of glioblastoma.