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Identification and functional analysis of radiation-induced microRNA changes in endothelial cells

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Due to the damage incurred by normal tissue, especially the endothelium, radiation doses used in tumour therapy are limited. A better understanding of the processes governing the radiosensitivity of endothelial cells is therefore desirable for improved radiation therapy. We have previously demonstrated that the sensitivity of endothelial cells to ionizing radiation depends on microRNA-regulated gene expression (Kraemer et al.). We now report the analysis of individual microRNAs (miRNAs), this reveals involvement of both indirect and direct protein targets.
By siRNA-mediated knockdown of two miRNA-processing enzymes (Ago2 and Dicer) we showed that miRNA-regulated gene expression has a prosurvival function in endothelial cells after radiation exposure. Specific miRNAs responding to ionizing radiation were identified. The effects of individually upregulated miRNAs on survival and cell cycle regulation after radiation were measured by transfection with either specific precursor- or anti-miRs. Out of 7 analyzed miRNAs, 3 miRNAs (miR-216a, miR-518d-5p and miR-525-3p) had an impact on the cellular response to irradiation. All three enhanced apoptosis and reduced survival, as predicted from the inhibtion of miRNA processing. We were able to identify proteins targeted of these miRNAs through proteome analysis of anti-miR transfected cells using two-dimensional difference gel electrophoresis and mass spectrometry (2D-DIGE-MS). Gene Ontology (GO Term) analysis assigned the majority of the changed proteins to cell cycle regulation, apoptosis and DNA repair. Ingenuity pathway analysis categorized the deregulated proteins to biological networks of cell death, lipid metabolism and biochemistry of small molecules. Using sequence analysis to identify miRNA-target sequences in the 3´-UTRs, and luciferase reporter assays of the miRNA-UTR target interaction we validated the detected target proteins as direct (presence of miRNA binding site and regulation of lucifease stability by the miRNA) or indirect targets of the investigated miRNAs.

Our data suggest that miRNA-mediated gene silencing has an essential function in the radiation response. In the future, targeting these miRNAs or their target proteins could be an important tool to sensitize tumors during radiation therapy and to protect normal tissue.

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Kraemer et al. Radiat Res. 2011 Nov; 176(5):575-86

Primary author: Dr KRAEMER, Anne (Institute of Radiation Biology, Helmholtz Center Munich, Germany)

Co-authors: Dr SARIOGLU, Hakan (Department of Protein Science, Proteomics Core Facility, Helmholtz Center Munich, Germany); Prof. ATKINSON, Michael John (Institute of Radiation Biology, Helmholtz Center Munich, Germany); Dr MOERTL, Simone (Institute of Radiation Biology, Helmholtz Center Munich, Germany); Dr TAPIO, Soile (Institute of Radiation Biology, Helmholtz Center Munich, Germany); Dr BARJAKTAROVIC, Zarko (Institute of Radiation Biology, Helmholtz Center Munich, Germany)

Presenter: Dr KRAEMER, Anne (Institute of Radiation Biology, Helmholtz Center Munich, Germany)

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