P19. GENE THERAPEUTIC OVEREXPRESSION OF MDR1 RESULTS IN UPREGULATION OF FURTHER GENES INVOLVED IN DETOXIFICATION AND DELIVERS RADIOPROTECTION

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Background: Gene therapeutic-delivered overexpression of P-glycoprotein (P-gp), the product of MDR1 (multidrug resistance 1) gene, might protect normal tissue during chemo- and radiotherapy of P-gp-expressing tumors. However, little is known about the influence of MDR1-overexpression on the expression of other genes.
Methods: Differentially gene expression in untransduced and oncoretrovirally transduced human lymphoblastoid TK6 cells were analysed by using the GeneChip Human Genome U133 Plus2.0 (Affymetrix). The expression of several genes was validated with quantitative real-time PCR (TaqMan; Applied Biosystems). Radiation-induced apoptosis was analysed by the sub-G1 DNA content using flow cytometry. Cell survival was measured by the colony formation assay.
Results: Sixty-one annotated genes showed a significant change in expression ($p < 10^{-4}$) in MDR1-overexpressing compared to untransduced and control virus-transduced cells. Several genes coding for proteins involved in detoxification and exocytosis (e.g. ALDH1A, UNC13) were up-regulated. Additionally, proapoptotic genes were down-regulated (e.g. CASP1, CASP4) with concomitant increased expression of the potential antiapoptotic gene AKT3. In functional assays overexpression of MDR1 conferred protection against radiation-induced clonogenic inactivation and apoptosis.
Conclusion: The resistant phenotype of MDR1-mediated P-gp overexpressing cells is associated with differential expression of several genes coding for metabolic as well as pro- and antiapoptotic proteins. Our results could have important implications for MDR1-gene therapy in patients receiving chemotherapy regimens in combination with radiotherapy.

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P20. TACI-SIGNALLING IN MULTIPLE MYELOMA – FROM THE IDENTIFICATION AS POTENTIAL THERAPEUTIC TARGET BY GENE EXPRESSION ANALYSIS AND FUNCTIONAL TESTING TO CLINICAL TRIALS

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Background: BAFF, APRIL and TNFR-family members are involved in various cancers including B cell malignancies. The aim of the study is to identify new therapeutic targets in multiple myeloma using gene expression analysis.
Methods: Samples of 65 patients (CD138-purified myeloma cells (MMC) and bone-marrow-microenvironment (BMME)), 7 normal bone-marrow-plasma-cell-samples (BMPC), 7 in vitro generated osteoclast-samples, and 20 human-myeloma-cell-lines were investigated. The expression of TACI, BCMA and BAFFR and the respective ligands BAFF and APRIL on MMC and BMSC was assessed by quantitative RT-PCR and Affymetrix U133 A + B DNA-microarrays. BMMAs of MM-patients were exposed to the TACI-Fc5 fusion-protein (Serono) containing the extracellular BAFF/APRIL-binding domain of TACI and the Fc-region of human Immungobulin G, to neutralize BAFF and APRIL secreted by MMC/BMME.
Results: MMC express TACI, the receptor of BAFF/APRIL. BAFF and APRIL promote MMC growth. The main site of production for BAFF and APRIL is the BMME, i.e. monocytes/neutrophils and osteoclasts, respectively.[1,2] The TACI-Fc5 fusion protein is currently under investigation in a clinical phase I/II trial at the University Hospitals of Montpellier and Heidelberg.
Conclusion: Inhibiting TACI-signaling via BAFF and APRIL by a TACI-Fc5 fusion protein is a promising new therapeutic approach in the treatment of MM.

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P21. A MOLECULAR CLASSIFICATION OF MULTIPLE MYELOMA (MM) BASED ON GENE EXPRESSION PROFILING AND FLUORESCENCE IN SITU HYBRIDISATION AS INDEPENDENT PROGNOSTIC FACTOR FOR EVENT FREE SURVIVAL (EFS)

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Background: To test the ability of our EC-classification[1,2] to (i) predict EFS in 100 MM-patients treated with high-dose-chemotherapy and autologous-stem-cell-transplantation, (ii) investigate whether the classification represents an independent prognostic factor.
Methods: 128 newly-diagnosed MM-patients (65 training-group/63 validation-group) were included. Bone-marrow aspirates were CD138-purified and RNA subjected to Affymetrix HG U133 A + B (training-group) and 2.0$^+$ (validation-group) arrays. CCND1-, CCND2- and FGFR3-expression were verified by RT-PCR and Western-blotting. iFISH was performed for chromosomes 1q21, 11q23, 11q13, 13q14, 17p13, t(4;14) and t(11;14). Expression data were gcrma-normalised and nearest-shrunken-centroids applied to cross-validate a predictor on the training-group. Log-rank test and Cox-proportional-hazard-model