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

were used. EFS was analysed for 100 patients treated with high-dose-chemotherapy.
Results: Gene-expression and iFISH allow a molecular classification of MM (expression-cytogenetic (EC) classification): (1.1) CCND1-expression and 11q13^{+}, (1.2) CCND1-expression, translocations involving 11q13, (2.1) CCND2-overexpression without 11q13^{+}, t(11;14), t(4;14), (2.2) CCND2-overexpression, t(4;14), FGFR3-upregulation.
Conclusion: EC-groups defined by the predictor show a distinctive pattern in gene expression and significantly different EFS. EC-groups and B2M represent independent prognostic parameters.[1,2]

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P22. CLASSIFICATION OF COLORECTAL CANCER – COMPARISON BETWEEN ESTABLISHED STAGING PARAMETERS AND SIGNATURES BASED ON TRANSCRIPT PROFILING

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Background: Classification of patient samples is an important aspect of cancer diagnosis and treatment. Recent microarray studies have shown that cancer classification by gene expression profiling is feasible and provides clinicians with additional information to choose the most appropriate forms of treatment.
Patients and Methods: A "genetic algorithm", using K-Nearest Neighbour-Classification was used to identify diagnostically relevant probe set combinations (classifier) to classify patients with sporadic colorectal cancer into stages without nodal and distant metastasis (UICC I/II) and patients with advanced CRC with nodal and distant metastasis (UICC III/IV). The algorithm was feeded with the 5% top and 5% bottom probe sets after statistical ranking (Golub, Wilcoxon, foldchange).
Results: Thus 2228 probe sets have been used as a starting pool. Discriminating probe set combinations have been identified in a training data set and checked using a non-overlapping test set. Probe sets classifying correctly in more than 99% could be identified for tumor vs. normal tissue distinction and for UICC I/II vs. III/IV distinction in the training set. In non-overlapping test-set tumor/normal classifier performed well (90%), whereas UICC classifier only reached 60% performance.
Conclusion: Most likely generalization properties of the signatures are poor because data representativeness is not sufficient using this approach. Sample number is quite appropriate to identify differentially expressed genes between tumor and normal tissues but it is likely to be insufficient to reliably reveal differentially expressed genes between diverse prognostic stages based on UICC classification in colorectal cancer.

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P23. A NEW FAMILY OF KIAA1245 GENES WITH AND WITHOUT THE HERV-K LTRS IN THEIR INTRONS

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A transcript containing the long terminal repeat (LTR) and the sequence homologous to the KIAA1245 mRNA fragment were revealed among the transcribed LTRs of human endogenous viruses of the K family in normal and tumor tissues. Ten other sequences with a high level of homology to the KIAA1245 mRNA were found in the GenBank. The intron-exon structures were determined for all the sequences, and their exon sequences were compared. The comparison showed that they differ both in the extent of the exon homology and in the presence or absence of the HERV-K LTR in the second intron. The revealed sequences form a new gene family that comprises at least four subfamilies. Two of these subfamilies have the LTR, and the other two do not. We showed by PCR that the LTR was integrated into the introns after the divergence of the orangutan evolutionary branch from other hominoids but before the divergence of the gorilla branch, i.e., 8–13 million years ago. The total expression of the genes of this family was examined in a number of tissues. It was shown that LTR-containing genes of this family expressed in tumor, embryonic tissues and in transformed human cell cultures, in explored normal tissues of the mature organism the expression of genes of this family was not detected.

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P24. POLYMORPHISMS OF THE pKi-67 PROMOTER AND THEIR BIOLOGIC RELEVANCE

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Background: Monoclonal antibody Ki-67 is a well-established and widely used marker to access cell proliferation activity, particularly in tumors. Ki-67 is directed against a nuclear antigen (pKi-67) which is solely expressed in actively proliferating cells during all phases of the cell cycle, but it is absent in quiescent cells. To investigate polymorphisms or mutations of the pKi-67 promoter we sequenced the promoter region of the pKi-67 gene of 50 colorectal cancer patients and 24 healthy donors. To find differences in the biologic relevance of polymorphisms we measured the promoter-activity of all detected variants.
Methods: The pKi-67 promoter region was amplified using *Pfu*-polymerase, randomly cloned into the pCR-Blunt II-Topo-vector, and cycle-sequenced using Thermosequenase. Firefly-luciferase reporter plasmids were constructed by subcloning of the sequence-controlled inserts into the pGL3-Basic-vector. The activity of the reporter plasmids was measured using the Dual-Luciferase Reporter Assay.
Results: After comparison of the cloned promoter sequences we found four polymorphisms (three SNPs and one tetranucleotide repeat) at position -518(A > G), -351(T > C), 186((GGGC)3 > (GGGC)5),