

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),