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Tumor suppressor genes in chromosome 3p21.31

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Genetic alterations in the short arm of chromosome 3 in different epithelial tumors suggested that 3p contains several tumor suppressor genes (TSGs). It was established that 3p21.3 LUCA region (lung cancer TSG region, 600kb) is one of the most frequently rearranged site of 3p and intrinsically involved in development of major forms of human carcinomas (Lerman et al., 2000). In this region twenty candidate tumor suppressor genes were identified. Epigenetic methylation and inactivation of RASSF1A promoter was shown with high frequency in lung, ovary and renal cancer. For another candidate TSG SEMA 3B decrease or loss of expression was found. It was suggested, SEMA3B action in tumorigenesis involve inhibition of tumor angiogenesis through interference with VEGF function (Keith et al., 2000;Tomizawa et al., 2001). Here we analyzed methylation status of SEMA3B CpG-sites in promoter region by sodium bisulfite treatment with followed sequencing. For lung cell lines and primary tumors with silencing SEMA3B Hypermethylation of promoter region was shown. Detailed 3p allelotyping in four epithelial malignancies (approx. 400 T/N DNA samples) with 23 polymorphic markers (including tri- and tetramers) allowed us to describe additional important DNA segment (between D3S2409-D3S2456, 3p21.31) closely telomeric to LUCA site (Braga et al., 2002). Computational analysis of gene sequences mapped in this D3S2409 region was done using GDB, NCBI and other databases.32 UniGene clusters including 22 unique genes and 10 ESTs clusters were identified in this 600kbp DNA segment. Therefore, gene density in D3S2409 region is even higher than in LUCA site (19 genes in 600kb). Using local versions of SAGE database (tag libraries),Tag in UniGene Mapping database and comparative Count Display program from SAGEmap server expression of all these genes was compared in tumor cell and their normal counterpart for breast and ovary cell lines libraries.Four new gene-candidates (MST1,DAG1, USP4, RON) were selected for subsequent investigations.Potential high expression of DAG1 and USP4 in breast and lung cancers is consistent with allele amplifications observed in surrounding D3S2409 and D3S2456 markers.MST1 receptor (RON) and its ligand MST1 gene are located in 3p21 and their overproduction can result in autocrine stimulation and uncontrolled proliferation (Angeloni and Lerman, 2001).

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The transcription factor Egr-1 promotes prostate carcinoma and systemic treatment of TRAMP mice with antisense Egr-1 inhibits tumour formation

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The transcription factor Egr1 or immediate early growth response-1 gene, is rapidly induced by growth factors to transduce proliferation signals. Induction of Egr1 is usually transient but appears to be sustained in some prostate tumor cell lines and tumors, suggesting that Egr1 stimulates tumor growth. In contradiction, in breast, lung, and brain tumors, Egr1 expression is often absent or reduced. When re-expressed, Egr-1 causes growth suppression via induction of TGF β(1), fibronectin(2), and PTEN(3). Several groups(4-5) have observed that Egr-1 is over-expressed in human prostate cancer and likely plays an oncogenic role. To test this, we re-expressed Egr-1 in immortalized normal human 267B prostate epithelial cells and observed accelerated growth, increased focus formation, and growth in soft agar. In contrast, Egr-1 is expressed in mouse TRAMP tumor cells. We developed a high affinity (ED50 = 0.15 μM) antisense Egr-1 reagent targeted against both human and mouse Egr-1 that substantially reduces Egr-1 mRNA and protein expression but not that of other family members (Egr-2 to Egr-4 and WT1). Antisense treatment inhibits proliferation by 50% ($p < 0.001$), reduces focus-forming frequency by 40 - 60% ($p < 0.005$), and eliminates growth in soft agar. When male TRAMP mice were treated systemically by I.P. injection 3 d/week for 10 weeks from an age of 22 weeks, a marked reduction prostate carcinoma incidence (3/7) as judged by staging necropsy was observed whereas 3-bp mismatch control oligonucleotide treated mice and vehicle alone treated mice exhibited frequencies of 6/8 and 7/7 yielding a significant overall decrease ($p = 0.045$). Mechanistic studies of TRAMP C-cells indicate that Egr-1 induces TGF β1, a potential growth factor but does not regulate PTEN in contrast to the role of Egr-1 in normal cells3. Taken together, these observations strongly indicate that Egr-1 is a functional growth and transformation promoting agent in human prostate carcinoma cells and in the TRAMP model and is a potential new target of therapy. Antisense Egr-1 maybe a useful reagent in the analysis and treatment of prostate cancer.

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Molecular class prediction of acute myeloid leukemia and myelodysplastic syndromes

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Despite recent advances in the treatment of acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS), mechanisms underlying the disease are still not completely understood. In addition, relevant diagnostic markers and surrogate clinical endpoints of chemotherapeutic efficacy and survival are needed. Treatment approaches for AML and MDS are different and correct diagnosis of the patients is critical. As part of an ongoing pharmacogenomic analysis of AML and MDS patients before and after treatment with the antibody-targeted chemotherapy gemtuzumab ozogamicin (anti-CD33 calicheamicin immunoconjugate, Mylotarg®, we have undertaken a global molecular classification of bone marrow to identify diagnostic and surrogate biomarkers of AML and MDS. Bone marrow samples from normal volunteers, AML and MDS patients prior to Mylotarg treatment (n = 38) were obtained and white blood cells purified using Ficoll gradients. Hematological parameters were measured on all samples to determine variation in cell populations across individuals. Total RNA was isolated from individual samples and analyzed on oligonucleotide arrays containing over 12,000 full-length human genes. Correlation metrics determined a high degree of relationship within the similar bone marrow populations. However, nonsupervised hierarchical cluster analysis demonstrated that the gene expression signatures of AML, MDS, and normal bone marrow were distinct from each other. A supervised class distinction algorithm identified unique gene sets that could accurately distinguish AML from MDS and normal marrow. Interestingly, transcript levels that differentiated AML from MDS included an overabundance of members of the HLH transcription factor. Several of these genes have been shown to map to chromosomal breakpoints in a number of leukemia's and solid tumor types suggesting that the deregulation of these genes may play a fundamental role in the oncogenic process. These data indicate that gene expression profiling will allow diagnostic differentiation of patients with AML and MDS and will ultimately contribute to our understanding of disease development, efficacy and treatment resistance.

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Gene expression microarray and 2D proteomic profiling of human ovarian adenocarcinoma cells following treatment with 17AAG, an inhibitor of the molecular chaperone Hsp90

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The molecular chaperone Hsp90 is of interest as a therapeutic target because of its importance in maintaining the stability and function of key client proteins required for tumour cell proliferation and survival. The natural product Hsp90 inhibitors geldanamycin and radicicol exert their antitumour effect by inhibiting the intrinsic ATPase activity of Hsp90, resulting in degradation of Hsp90 client proteins. A geldanamycin derivative, 17AAG, has