and -49(G > T) from the transcription start site. We could demonstrate that the variants -186(GGGC)5 ($p < 0.05$), and -49T ($p = 0.05$) were significantly or strongly associated with colorectal cancer as compared to the healthy controls. We found the highest promoter activity to be associated with -186(GGGC)3 and -49T.
Conclusion: These results suggest that the two pKi-67 promoter polymorphisms 186(GGGC)3 > (GGGC)5 and -49G > T located in the basic promoter may play a crucial rule in the development of colorectal cancer.

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P25. MATRIX METALLOPROTEINASE 9 SINGLE NUCLEOTIDE POLYMORPHISM ANALYSIS IN BLOOD OF UROLOGICAL CANCER PATIENTS

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Introduction: Ample evidence indicates that MMPs contribute in multiple ways to all stages of malignant progression, including tumor invasion, blood vessel penetration, metastases and tumor angiogenesis. MMP-9 has been found to be specifically associated with prostate cancer metastasis. A number of DNA polymorphisms in the MMP genes are associated with differences in MMP activity. However, the relationship between the polymorphism and susceptibility of cancer remains ambiguous. A cytosine (C) – thymidine (T) single nucleotide polymorphism (SNP) at position -1562 in the MMP-9 promoter is reported to affect expression of this gene.
Aim: To determine the prevalence of a single nucleotide polymorphism (-1562 C/T) in the MMP-9 gene promoter in cancer patients and evaluate it's correlation with tumor type and stage.
Methods and Materials: DNA from the cancer patients' blood was extracted and amplified with PCR. PCR-RFLP method was used to determine MMP-9 polymorphism in 18 prostate cancer cases, 4 benign prostate hyperplasia cases, 14 invasive bladder cancer cases, 5 non-invasive bladder cancer cases, 4 renal cancer cases and 1 adrenal gland cancer case.
Results: Prevalence of C/C, C/T, T/T genotypes was similar among bladder and renal cancer patients. In prostate cancer patients a significant association ($P = 0.0052$) between clinical stage and MMP-9 polymorphism was found.
Conclusion: Our data demonstrate that MMP-9 (-1562 C/T) polymorphism may modify susceptibility to prostate cancer. We hypothesized that this polymorphism might act as a genetic modifier in the development and progression of prostate cancer. Additional studies with larger population are warranted.

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P26. ANTAGONISTIC FUNCTION OF S100 PROTEINS DURING TUMOR DEVELOPMENT

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Background: Despite compelling data demonstrating a direct link between altered expression of S100 proteins located on human chromosome 1q21 and epithelial malignancies, the knowledge of their function and mode of action in epithelial cells and in tumor promotion or progression is largely unknown.
Methods: Gene expression profiling of the in vivo model of chemically induced skin carcinogenesis revealed differential regulation of genes coding for S100 proteins. Subsequent studies using in vitro models and tissue microarrays are currently extended by to suitable in vivo models.
Results: Here, we have identified a novel signaling pathway in epithelial cells initiated by extracellular S100A8/A9 resulting in the activation of AP-1-dependent gene expression. Importantly, co-expression of S100A3 inhibits S100A8/A9 mediated AP-1 activation, which is in line with repression of this gene during skin carcinogenesis suggesting a negative role for S100A3 in epithelial malignancy. We found elevated levels of MMP2 and MMP9, two well-known AP-1 regulated genes, and identified S100A6 as an additional target gene of S100A8/A9 signaling. Moreover, significant co-expression of S100A8 and S100A9 together with phosphorylation of c-Jun and elevated S100A6 protein levels were evident in eSCC. The in vivo relevance of S100A8/A9 interaction with RAGE is discussed.
Conclusion: Our data suggest that targeting the net activity of S100 induced signaling represents an auspicious strategy for cancer prevention and/or therapy.

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P27. DEFICIENT MITOTIC CHROMATIN CONDENSATION IN RESPONSE TO Chk1 INHIBITION IS MEDIATED BY DEREGULATED Cdc25B

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Background: One of the most common properties of cancer is genomic instability, a leading cause of which are defects of the DNA damage response (DDR). DDR defects in tumors and germline have been linked to clinical outcome and cancer susceptibility, respectively. Among DDR regulators, the nuclear checkpoint kinase Chk1 is an established transducer of ATR- and ATM-dependent signalling in response to DNA damage. Additional functions of Chk1 include regulation of unperturbed cell cycle progression. Recently, we have shown that Chk1 localizes to inter-

phase centrosomes and thereby negatively regulates entry into mitosis by preventing premature activation of cyclin B-Cdk1 [*Nat Cell Biol* 2004;**6**:884–91].

Methods: In synchronized U2OS cells, we inhibited Chk1 kinase by the specific inhibitor CEP-3891 and manipulated this system using different constructs and siRNAs.

Results: Chk1 inhibition induced premature mitotic entry displaying regular spindles but deficient chromatin condensation – which we have termed the 'paraspindle' phenotype –, apparently resulting in mitotic nuclear fragmentation. The paraspindle phenotype was reverted by inhibition of the Cdc25B phosphatase using siRNA, which restored normal mitoses with regular chromosome condensation.

Conclusion: Cdc25B is an important downstream target of Chk1 in the regulation of mitotic entry. Our data may help elucidate the mechanism of genomic instability as an early step in carcinogenesis.

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P28. PROMOTER ANALYSIS OF HUMAN SMALL REGULATORY SUBUNIT CALPAIN, CSS1

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Background: Calpains are a family of 14 known intracellular calcium dependent cysteine proteases that share a similar catalytic structure. Calpains are involved in several key aspects of migration, including: adhesion and spreading; detachment of the rear; and membrane protrusion. The best described calpains are μ and M calpain which are ubiquitous heterodimeric proteins composed of a distinct catalytic subunit and a common regulatory subunit called regulatory calpain (CSS1). Studies have shown the importance of CSS1 for the function of both μ and M calpains. So far there is not much known about the promoter and transcriptional regulation of this important gene, therefore we conducted the following study to characterize the promoter and major *cis* acting elements in the 5′ prime region of CSS1 gene.

Methods: Different CSS1 promoter regions spanning ∼2.0 kb 5′ upstream and ∼1.0 kb downstream region from the transcription start site were PCR amplified using Hela cell genomic DNA, cloned into pGEMT-Easy cloning vector, and sequenced. Luciferase reporter constructs were made by subcloning these fragments into pGL3-Basic vector. Luciferase assay was carried out in Hela, MCF7 and HCT116 cell lines. EMSA and supershift with specific antibodies was done using the standard protocol. TRANSFAC database was used to scan the sequence for putative transcription factor binding sites.

Results: All the three cell lines tested showed high constitute expression of CSS1 mRNA and showed more or less similar pattern of promoter activity for different deletion constructs of CSS1, with construct −121 to +274 showing the highest activity. Region −55 to −25 seems to be important for the core promoter activity, this region contains a putative NRF1 biding site. NRF1 was found to bind to this region in EMSA. Interestingly, deletion construct −55 to −2 which has lost the first noncoding exonic region showed complete lack of promoter activity, suggesting that the first noncoding exon containing putative AP-1-and Ets-1-binding sites is required for basal promoter activity. Binding of AP-1-transcription factors to this exonic region has already been confirmed in gelshift analysis.

Conclusion: This study describes for the first time the minimal region required for the basal activity of CSS1 promoter and defines *cis*-elements and transcription factors regulating this vital gene.

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P29. MULTIPLEX Rt-Pcr DETECTION OF PROSTATIC GENES EXPRESSING CELLS IN THE BLOOD OF PROSTATE CANCER PATIENTS

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Background: Circulating cell detection using RT-PCR techniques has been studied as new prognostic factor in prostatic cancer patients. To enhance detection sensitivity of these techniques, we have developed a new multiplex RT-PCR assay for prostate circulating cell detection based on the combined expression of KLK3 (formerly PSA), FOLH1 (formerly PSAM), PSCA, KLK2 and KLK15 (formerly prostinogen).

Methods: Our approach associate classical RT-PCR technics, fluorescent labelling and a very sensitive capillary electrophoresis detection. After RT-PCR, PCR products are visualized using capillary electrophoresis and fluorescent detection which is very reliable to indicate the precise size of the detected fluorescent products. We first test our new detection technique using prostate cancer cell lines known to express all of these genes and improved its sensibility using serial dilution of these cells in healthy blood samples: the limit of detection was found to be as few as 1 expressing cell in 10^6 nucleated blood cells. Between July 2005 and January 2006, 41 prostate cancer patients, 12 non prostate cancer patients and 11 healthy individuals were included and this multiplex RT-PCR assay was used to detect prostate circulating cells.

Results: Multiplex RT-PCR assay was positive in 13/41 (32%) of the prostate cancer patients for 3 of the 5 markers, whereas 4/41 (10%) were found positive for 5 markers. The test was negative for the 23 non prostate cancer patients or healthy individuals.

Conclusion: This multiplex RT-PCR assay with five markers proved to be more sensitive than a single one in detecting prostate circulating tumor cells. The discrepant expression of KLK3, FOLH1, PSCA, KLK2 or KLK15 may label circulating tumor cells with different levels of differentiation and subsequent aggressive behaviour, and increases the capability to detect prostate circulating cells.

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