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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