659 Podoplanin, a non-canonical signaling transmembrane glycoprotein that promotes tumour cell migration and invasion

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Podoplanin is a small transmembrane mucin anchored to F-actin via association with ezrin and/or moesin members of the ERM (ezrin, radixin, moesin) family of plasma membrane-cytoskeleton crosslinkers. Podoplanin expression is upregulated in a variety of cancers, including testicular germ cell tumours, malignant mesotheliomas, central nervous system tumours, and squamous cell carcinomas (SCCs). Furthermore, podoplanin has recently been proposed as a candidate cancer stem cell marker in SCCs. Regulation of podoplanin expression in tumour cells involves a complex array of mechanisms including promoter methylation, alternative polyadenylation, alternative splicing and calpain-mediated protein degradation. Podoplanin is expressed at the invasion front of SCCs where it appears to promote cell migration, invasion and metastasis. Thus, podoplanin is able to induce epithelial-mesenchymal transitions (EMTs) in MDCK cells and premalignant keratinocytes. Nevertheless, other authors postulated that podoplanin promotes collective tumour cell migration without the requirement of EMT. We have analyzed the role of different structural domains on podoplanin function. The cytoplasmic (CT) tail contains a juxtamembrane dipeptide (RK) involved in binding to ERM proteins that is necessary for podoplanin-mediated RhoA GTPase activation, EMT and cell migration. Both the CT and transmembrane (TM), but not the extracellular (EC) domain, are involved in podoplanin association with ganglioside GM1-containing membrane lipid rafts and EMT. We are now investigating the implications of podoplanin-mediated signal transduction and protein-protein interactions on the function of this glycoprotein in cell migration and cancer.

660 Withdrawn

661 SKP2 oncogene is a MYC target gene in human leukemia cells

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Background: SKP2 gene is frequently overexpressed in human cancers and has been associated with tumour progression. SKP2 is the ubiquitin ligase subunit that targets $p27^{KIP1}$ for degradation, and the levels of SKP2 and p27 inversely correlate in many tumours and cell models. MYC antagonizes p27 inhibitory activity in cell cycle.
Material and Methods: We used human leukemia K562 cells carrying zinc-inducible MYC transgenes and tamoxifen-activable MycER proteins. The expression of SKP2 was assayed at the mRNA level by qRT-PCR and at the protein level by immunoblot. The binding of MYC to SKP2 promoter was analysed by chromatin and the transcriptional activity of MYC was assessed by rerpoter luciferase assays.
Results: MYC induces SKP2 expression at the mRNA and protein levels in four different models based in K562 cells with conditional MYC expression. SKP2 up-regulation was independent from cell cycle. MYC up-regulates SKP2 mRNA expression when protein synthesis is inhibited, binds to a 5′ region of human SKP2 that includes an E-box. Silencing of MYC by siRNA results in SKP2 down-regulation. MYC also regulated SKP2 in a human lymphoid and mink epithelial cell lines with conditional MYC expression. We also found that the MYC-induced expression of SKP2 resulted in decrease in p27 protein in K562 cells. siRNA-mediated silencing of SKP2 resultked in increased p27 but had no effect on MYC levels or activity. Moreover, MYC induced the phosphorylation of p27 in Thr-187 and the association of p27 with CDK-cyclin complexes, a prerequisite for p27 being targeted by SKP2.
Conclusions: Altogether, our data shows that SKP2 is a direct target gene of MYC at least in human leukemia K562 cells, and that SKP2 regulation leads to reduced p27 levels in this model. The results suggest a new mechanism for the MYC transformation activity through the up-regulation of the SKP2 oncogene and the reduction of p27 levels in tumour cells.

662 Mitotic spindle stress elicited by TACC3 depletion as a major trigger of premature senescence

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Background: Mitotic spindle stress caused by microtubule-interfering (MT) drugs like paclitaxel® is a potent trigger of mitotic cell death or p53-dependent post-mitotic cell cycle arrest in the case of aberrant cell division. Paclitaxel® elicits these cellular effects in a highly dose-dependent manner. Here we tested the role of TACC3 (transforming acidic coiled coil 3), a mitotically expressed centrosomal protein and major regulator of mitotic MT dynamics, as a potentially novel molecular target in the induction of premature senescence.
Material and Methods: To elucidate the molecular and cellular effects triggered upon TACC3 depletion we established an inducible, lentiviral-based shRNA system against human TACC3. To investigate cellular senescence we examined cell cycle status, cell morphology, and β-galactosidase activity at pH 6.0 (SA-bgal). Furthermore we established the SAHF marker HP1g (pS83) in combination with nuclear $p21^{WAF}$ as a potent marker for premature senescence in breast carcinoma and epithelial cells. Lastly, genearray analysis was employed to characterize expression changes upon mitotic spindle stress and consecutive senescence.
Results: Knock-down of TACC3, in contrast to treatment with paclitaxel®, failed to induce a cell death response in immortalized MCF10a epithelial cells or MCF7 breast carcinoma cells. Consistent with this unexpected result, down-regulation of TACC3, but not paclitaxel treatment, led to a progressive loss of the pro-apoptotic Bcl-2 family member Bim that links microtubule integrity and mitotic cell death induction. Rather, TACC3-depleted cells arrested in G_1 through a cellular senescence program characterized by the upregulation of p53 and nuclear $p21^{WAF}$, downregulation of pRb levels, formation of HP1g (pSer83) positive senescence-associated heterochromatic foci (SAHF), and increased senescence-associated b-galactosidase activity. Using gene expression profiling we observed an upregulation of various senescence-associated transcripts including PML and SP110 as well as downregulation of ERK signaling and c-FOS expression. Interestingly, the onset of senescence through TACC3 depletion was strongly accelerated by treatment with paclitaxel concentrations as low as 1 nM.
Conclusion: Mitotic spindle stress triggered by TACC3 knock down is an efficient inducer of premature senescence of human breast carcinoma cells.

663 Age dependence of T-cell lymphoma induction by radiation exposure in Mlh1-deficient mice

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Background: The carcinogenic effects of ionizing radiation vary markedly with age and genetic background of exposed individuals, as revealed by epidemiological studies such as the survivors of atomic bomb exposure, Chernobyl accidents and medically irradiated patients. We studied effects of age at exposure on radiation carcinogenesis using mouse model for T-cell lymphomas in mismatch repair (MMR)-deficient mice. Homozygous germline mutations of MMR genes in humans are manifested by an early onset of childhood T- or B-cell leukemias.
Materials and Methods: *Mlh1*-deficient mice were irradiated with 2 Gy of X-rays at late fetal (17 dpc), 2-weeks and 10-weeks of age, and underwent autopsy at the time of spontaneous death. Collected T-cell lymphomas were examined for the expression and mutation status of Ikaros, which is a potent tumour suppressor for this tumour type in human and mouse.
Results: *Mlh1*-deficient mice develop T-cell lymphomas spontaneously at the incidence of 71%. Ionizing radiation exposure further accelerated lymphoma development; 2-week-old mice were more susceptible to radiation than 10-week-old mice. Radiation-induced lymphoma was characterized by high rate of invasion into other tissues. Unexpectedly, the 17-dpc mice were resistant. When lymphomas were molecularly analyzed, the frame shift mutations at mononucleotide repeat sequences in *Ikaros* gene were frequently observed, which resulted in loss of the protein. The point mutations in either DNA binding domain or dimerization domain was found in 2-week-old (15%) and 10-week-old (46%) irradiated groups.
Conclusion: These results suggest that the age at exposure to ionizing radiation affects not only tumour incidence but also the mechanism of lymphomagenesis.