Materials and Methods: Retroviral mediated expression of wild type and mutant Trib proteins and in vivo bone marrow cell transduction and transplantation used to assay AML. Protein expression analysis of C/EBPalpha following knockdown of COP1 E3 ligase. Binding interaction assays performed using immunofluorescence, subcellular fractionation, GST pulldown and peptide array technology.
Results: In a murine bone marrow transplant model, mice reconstituted with hematopoietic stem cells (HSC) retrovirally expressing Trib1 or Trib2 but not Trib3, uniformly developed fatal transplantable AML. Investigation of the structural domains of Trib2 showed that the C-terminal COP1 E3 ligase binding site and the kinase domain are required for its oncogenic activity. Trib2 contains variant catalytic loop sequences compared to conventional kinases that we show are necessary for Trib2 function. Trib2 (and Trib1) associated with and led to the proteasomal-dependent degradation of C/EBPalpha, a critical nuclear transcription factor frequently dysregulated in AML. Trib2 localizes to both the cytoplasm and the nucleus, but interaction with C/EBPalpha is exclusively nuclear. Trib2 binding to C/EBPalpha results in COP1 E3 ligase mediated degradation of C/EBPalpha and is essential for Trib2-induced AML.
Conclusion: This works highlights Trib proteins as potent AML oncogenes, and identify the structural domains that are required for oncogenic function in AML. These data strengthen the correlation between Trib2 mediated C/EBPalpha degradation and leukaemia which may have prognostic and therapeutic implications.

644 A screen for cellular senescence reveals candidate tumour suppressor genes

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Background: Of interest to cancer researchers, cellular senescence upon prolonged oncogene activation or loss of tumour suppression is an emerging paradigm, and is detected in early stage human tumours. Since *in vivo* senescence thus acts as a tumour suppression mechanism, we reasoned that a senescence screen should reveal novel cancer genes.
Materials and Methods: We developed a high-content screening assay applying cell morphology (increased nuclear size) and proliferation (decreased Ki67) parameters to identify senescence-like siRNAs in a human kinome library. The screen was performed in hTERT-immortalised human Retinal Pigment Epithelial (RPE) cells expressing an inducible p53shRNA. Data mining was readily achieved using the Acuity Express software package. Actual senescence was quantitated by staining for senescence-associated β-galactosidase marker (SA-βGal), followed by measurement of typical senescence gene expression signatures and genomic profiling analysis.
Results: A high-content screen for senescence-like kinome siRNAs yielded 17 candidate genes. Of these, 14 genes were confirmed to be senescence regulators by SA-βGal staining. Senescence was predominantly p53-dependent and often correlated with DNA damage and p16INK4A induction. Interestingly, genomic profiling of tumour samples revealed that a significant proportion of the genes showed copy number alterations, and suggests that we may have identified novel tumour suppressor genes. To link senescence gene functions back to replication stress and/or DNA damage, we are characterising molecular components of novel senescence pathways through proteomic approaches. Special focus lies on the EPHA3 receptor tyrosine kinase, a gene found to be frequently mutated in human lung and colorectal cancers in recent genome sequencing efforts.
Conclusion: We successfully applied a high-content cellular senescence siRNA screen to identify a defined set of candidate tumour suppressor genes.

645 Array comparative genomic hybridization (aCGH) differentiates the major intrinsic subgroups of breast carcinomas

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Introduction: Breast Cancer is a heterogeneous disease as reflected by histopathology, molecular alterations and clinical behavior. Gene expression based classification as proposed by Perou et al in 2000 has proven to give important biological insight into the diversity of breast cancer. The intrinsic subtypes includes Luminal A, Luminal B, Normal-like, Basal-like and ERBB2-enriched subtype and have been shown to have clinical relevance and to be robust in several datasets. Due to strict tissue requirements and technical complexity, whole genome gene expression profiling is not likely to be applicable to a routine use in the clinical setting. Immunohistochemistry (IHC) for selected markers has been proposed as a surrogate for expression data, but a consistent system for scoring of intrinsic subtypes has been challenging. A limitation of IHC is the lack of a consistent scoring system and the subjectivity in the scoring. It has been shown that the intrinsic subtypes harbor major differences in copy number change, but a classifier based on genomic changes alone is yet to be defined.
Material and Methods: Tumour material from two clinical cohorts (MicMa and Ull) was included in this study. (1) MicMa: Fresh frozen biopsies were collected from 130 of the 920 patients included in the "Oslo Micrometastasis Project" from 1995 to 1998, data previously reported. Tumour DNA from 49 of these samples was available for aCGH-analysis. (2) Ull: Fresh frozen tumour specimens from 212 patients with primary breast cancer were collected at Oslo University Hospital from 1990 to 1994, and aCGH-analysis was performed on the total cohort. Expression data from 73 of these samples were available.
DNA was isolated and analyzed using the Agilent Human-Genome-CGH Microarray 244k platform. A piecewise constant regression function were fitted to the log transformed aCGH data, using the algorithm multi-PCF (Piecewise Constant Fit) on each subgroup separately. This resulted in a combined matrix with 1303 segments. Both ANOVA and multiclass SAM analyses were performed with the intrinsic subgroups as response variable.
Results: The ANOVA analysis gave 89 significant segments after Bonferroni correction. This included major regions on 1p, 1q, 2p, 3p, 4p, 5q, 6p, 7q, 10p, 16p and 16q, and smaller segments on other chromosomal arms. Multiclass SAM gave similar significant aberrations in the same regions. A clustering analysis using the significant regions for the aCGH-data showed clearly a separation between a luminal and a non-luminal group of tumours. In the non-luminal branch of the dendrogram, an erbB2 and a basal cluster were evident.
Conclusion: Our analyses show that the aCGH-data alone may separate between the major intrinsic subclasses of breast cancer. A classifier for the intrinsic subtypes based on genomic changes alone is yet to be defined. Such a DNA-based scoring system for breast cancer would be a valuable clinical tool and will add to our biological understanding of breast cancer subtypes.

646 MeCP2 has no role in intestinal tumourigenesis but is required for normal homeostasis in the murine intestine

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Mutations of the methyl binding domain protein MeCP2 underlie RETT syndrome (RTT), a human neurodevelopmental disorder. Other methyl binding proteins like MBD2 and Kaiso, have been shown, when deficient, to delay onset of Apc ^{Min} tumourigenesis. Given the observation that spatial gene expression in the gut is regulated by MBD2, and the role of MBD2 and KAiso in tumourigenesis, we have addressed the hypothesis that mutation of *Mecp2* will directly impact upon intestinal physiology and intestinal tumourigenesis.
To investigate the consequences of MeCP2 deficiency in the intestine, we used a Cre-Lox strategy to delete *Mecp2* from the adult mouse small intestinal epithelium. Male mice bearing the cytochrome p450 inducible AhCre transgene and the LoxP flanked *Mecp2* allele were induced with b-naphthoflavone. Small intestine homeostasis, proliferation, cell death, differentiation and transcriptome were analysed. MeCP2 role in intestinal tumourigenesis was studied using 2 Apc^{Min} model: $Apc^{Min/+}$ where Apc loss is occurring after LOH; and $Rb9Apc^{Min/+}$ where the loss of Apc is favoured towards epigenetic silencing.
Although $Mecp2^{-/y}$ mice did not develop intestinal symptoms of disease, loss of MeCP2 altered intestinal homeostasis, increasing the proliferative compartment within the crypt, decreasing cell death in the crypt-villus axis, increasing cell migration rates onto the villus and leading to longer villi. Cell differentiation was not altered. Transcriptome analysis identified upregulation of the MeCP2 target *Igfbp3*. This provides a ready mechanism for our observations, as Igfbp3 is implicated in the control of villus length by altering cell proliferation and death rates in the crypt-villus. The Survival and tumour burden was unchanged in the two $Apc^{Min/+}$ models in presence or absence of Mecp2 despite the increase of the tumour size in absence of Mecp2.
We show here that *Mecp2* is required for normal intestinal homeostasis but not tumourigenesis. RTT is caused by *MECP2* mutation, a neurological disorder however, our results extend the role of *Mecp2* beyond its known neurological role to the intestinal epithelium.

647 Withdrawn