established in BEL7402 and HLE using a lentiviral-based short-hairpin knock-down approach. For each cell line, two stable ILK knock-down (shILK) clones and one stable non-target control (shCTL) were established. To functionally characterize ILK in HCC, the knock-down stable clones were subjected to various functional assays including cell proliferation assay, soft agar colony formation assay, cell migration assay, wound-healing assay and cell invasion assay. *In vivo* tumourigenicity of BEL7402 ILK knock-down stable clones was assessed by subcutaneous injection of the cells into nude mice.
Results: Western blotting revealed a higher ILK protein expression in HCC cell lines than in normal liver cell line. In the physiological context, qPCR analysis showed that ILK was over-expressed in 36.9% (21/57) of HCC tissues when compared to the corresponding non-tumourous livers. The overall ILK expression level was significantly higher in tumourous tissues ($P = 0.005$), with a stepwise increase of expression along tumour stage. Functional characterization of ILK in HCC using the two ILK stable knock-down cell lines showed a reduction in the rate of cell proliferation, migration, invasion and anchorage-independent growth. Knock-down of ILK in BEL7402 also suppressed tumour formation in nude mice, thus decreasing the *in vivo* tumourigenicity of HCC cells. To probe the underlying mechanism, AKT activity was evaluated in the shILK clones. Western blotting analysis showed a decrease in phospho-AKT(Ser473) level upon ILK silencing.
Conclusion: Our study suggests that ILK plays a role in the progression of HCC via the activation of the PKB/AKT pathway.

728 The TGFbeta co-receptor endoglin modulates the expression and transforming potential of H-Ras

E. Perez-Gomez[1], J.F. Santibanez[2], A. Fernandez-L[3], A. Carnero[4], M. Malumbres[5], C.P.H. Vary[6], M. Quintanilla[7], C. Bernabeu[3]. [1]*Universidad Complutense de Madrid, Biochemistry and Molecular Biology, Madrid, Spain,* [2]*Universidad de Chile, Instituto de Nutricion y Tecnología de los Alimentos (INTA), Santiago, Chile,* [3]*Centro de Investigaciones Biologicas, Molecular and Cellular Medicine, Madrid, Spain,* [4]*Spanish National Cancer Research Centre, Assay Development, Madrid, Spain,* [5]*Spanish National Cancer Research Centre, Molecular Oncology, Madrid, Spain,* [6]*Maine Medical Center Research Institute, Center for Molecular Medicine, Maine, USA,* [7]*Instituto de Investigaciones Biomédicas, Cancer Biology, Madrid, Spain*

Background: Endoglin is a transforming growth factor-β (TGF-β) co-receptor that acts as a suppressor of malignancy during mouse skin carcinogenesis. H-Ras plays a crucial role in this model, by modulating tumour initiation and progression. We have studied the effects of endoglin on the expression of H-Ras in transformed keratinocytes.
Material and Methods: Epidermal mouse cell lines derived from chemically-induced skin carcinomas were used. H-Ras expression and promoter regulation was determined by RT-PCR, Western-blot and reporter assays. MAPK pathway was studied using specific antibodies and phospho-antibodies in western-blot assays. Foci formation assays were performed in mouse NIH3T3 fibroblasts transfected with $H\text{-}Ras^{Q61K}$ or $H\text{-}Ras^{G12V}$.
Results: TGF-β_1 increases the expression of H-Ras. The TGF-β_1-induced *H-Ras* promoter transactivation was Smad-independent, however it is necessary the activation of the TGF-b type I receptor ALK5 and the Ras-mitogen-activated protein kinase (MAPK) pathway. Endoglin attenuated stimulation by TGF-β_1 of both MAPK signalling activity and H-Ras gene expression. Furthermore, endoglin inhibited basal MAPK activity in transformed epidermal cells containing an H-Ras oncogene, as found by analyzing the levels of phospho-ERK1/2. Endoglin inhibited ERK phosphorylation without affecting MEK or Ras activity by an unknown mechanism strongly dependent on the endoglin extracellular domain. Finally, endoglin was able to inhibit the transforming capacity of $H\text{-}Ras^{Q61K}$ and $H\text{-}Ras^{G12V}$ oncogenes in a NIH3T3 focus formation assay.
Conclusions: The ability to interfere with the expression and oncogenic potential of H-Ras provides a new face of the suppressor role exhibited by endoglin in H-Ras-driven carcinogenesis.

729 The impact of hypoxia on differential expression of neurotensin receptors (NTR) in colorectal and prostate carcinoma cells

K. Schlottig[1], R. Bergmann[1], J. Steinbach[1], C. Haase-Kohn[1], J. Pietzsch[1]. [1]*Forschungszentrum Dresden-Rossendorf, Institute of Radiopharmacy, Department of Radiopharmaceutical Biology, Dresden, Germany*

Background: Recent studies showed increased expression of neurotensin receptors (NTR), particularly, NTR1 and NTR3, in various tumours, thus NTR is assumed a potential target for tumour imaging and therapy. However, the knowledge about the quantitative expression of NTR on mRNA and protein level, e.g., under hypoxic conditions is limited. The aim of this study was to develop a quantitative method for determination of absolute NTR mRNA amount in tumour and non-tumour cells and tissues. For method evaluation the NTR mRNA amounts in human colorectal (HT-29) and prostate (PC3) carcinoma cell lines under normoxic and hypoxic conditions *in vitro* were compared.
Material and Methods: A novel real-time RT-PCR method using an external standard was established. The elongation factor 1 alpha (EF1α) gene served as housekeeping gene and glucose transporter protein type 1 gene (GLUT1) was used as indicator for cellular hypoxic regulation effects. The derived standard curves allow for calculation of the number of specific mRNA molecules normalized to 1000 molecules of EF1α. Acute and chronic experimental hypoxia was induced by cultivation of cells at an oxygen concentration of 0.6% for 4 to 72 hours.
Results: Both HT-29 cells and PC3 cells show high mRNA expression of NTR1 in normoxia. In acute hypoxia (till 24 hours) the expression level of NTR1 did not change. However, under conditions of chronic hypoxia in HT-29 cells, at the latest after 48 hours, the NTR1 mRNA expression was significantly decreased. In contrast, the NTR1 mRNA in PC3 cells remained at a high level also in hypoxia. The mRNA level of NTR3 was about 5 orders of magnitude lower than NTR1 in both cell lines. Expression of NTR3 in both cell lines showed no significant differences during hypoxia, with a tendency to increase.
Conclusion: A novel standardizable and reproducible quantitative method for measurement of NTR mRNA in cancer cells was established. The use of NTR1 as a target for imaging or therapy strongly depends on tumour cell type and tumour hypoxia. Ongoing investigations will compare quantitative mRNA expression with data on functional expression of NTR, e.g., protein synthesis and radioligand interaction, in human samples and rodent tumour (xenograft) models.

730 MicroRNA-based, p53 dependent post-transcriptional circuits: mechanisms, targets and inter-individual variation

A. Bisio[1], Y. Ciribilli[2], V. Del Vescovo[2], V. De Sanctis[2], C. Tonelli[2], A.G. Jegga[3], M.A. Denti[2], A. Inga[2]. [1]*National Institute for Cancer Research, Unit of Molecular Mutagenesis and DNA Repair, Genova, Italy,* [2]*University of Trento, Center for Integrative Biology, Trento, Italy,* [3]*Cincinnati Children's Hospital Medical Center, Biomedical Informatics, Cincinnati, USA*

The tumour suppressor p53 is a sequence specific transcription factor that regulates the expression of many target genes linked, among others, to the control of cell cycle, apoptosis, angiogenesis and DNA repair. Recent studies identified direct p53 regulation of miRNAs and related regulatory circuits.
Using bioinformatics approaches, we identified an additional group of candidate miRNAs for direct p53 transcriptional control. Furthermore, some of those miRNAs can be predicted to target mRNAs in genes relevant to p53-mediated responses. Notably, we found examples of miRNA seed binding sequences at target 3′UTRs that contain SNPs predicted to modulate miRNA binding. Our work aims at the validation of p53-mediated control of the newly predicted miRNA genes and related circuitries that would provide additional negative and/or positive feedback loops for p53 regulation. To validate p53-responsiveness of 13 miRNA promoters not previously described to be under control of this family of transcription factors, we initially evaluated the potential for wild type p53, p63 and p73 to transactivate the predicted p53 response elements (REs) in those miRNA promoters. For these experiments we developed in the model system *S. cerevisiae* a panel of isogenic reporter strains harboring the chosen p53 REs upstream of the firefly luciferase reporter gene. 9 REs (including miR10b, 23b, 106a, 151, 191, 198, 202, 221, 320) were responsive to p53 of which 7 were also inducible by p63 or p73, even though to a lower extent. Moreover, we developed RT-qPCR and ChIP assays in human cell lines where p53 proteins could be ectopically expressed or induced by genotoxic stress. In general, results confirmed p53-dependent transcriptional regulation of the studied miRNAs, although cell line differences were observed. To establish miRNA targeting of selected mRNAs and the functional impact of SNPs at the miRNA binding sites we developed 3′UTRs reporter constructs differing for the SNP status or with mutagenized miR binding sites. We also measured allele imbalance at the endogenous gene level by quantitative RT-PCR analysis in cell lines heterozygous for the SNPs and relative protein levels by western blot to evaluate the impact of the SNP allele as well as of p53-dependent or -independent miR modulation. Specific examples of p53-directed post-transcriptional circuits will be presented.

731 Chemical induction of mitotic slippage by proteolytic degradation of spindle assembly checkpoint proteins

J.L. Riffell[1], M. Roberge[1]. [1]*University of British Columbia, Biochemistry and Molecular Biology, Vancouver, Canada*

Background: Chemicals were recently identified that induce cells arrested at mitosis by antimitotic agents to undergo mitotic slippage and enter interphase without chromosome segregation (Riffell *et al.*, Cell Cycle 8(18): 3025−38 (2009)), resulting in DNA endoreduplication and cell death through apoptosis; this project examines the mechanism whereby the mitotic slippage inducers SU6656 and geraldol force cells to escape mitotic arrest.
Materials and Methods: T98G glioblastoma cells were arrested in mitosis by exposure to paclitaxel or vinblastine and induced to undergo mitotic slippage by incubation with SU6656 or geraldol in the absence or presence of protease

inhibitors. DNA content was monitored by flow cytometry and the levels of mitotic regulators were measured by immunoblotting.
Results: SU6656 and geraldol induced the degradation of the spindle assembly checkpoint protein BubR1, the anaphase promoting complex activator Cdc20, and the CDK1 regulatory subunit cyclin B1. This degradation was not observed during completion of mitosis or early G1 in T98G cells. The effects of SU6656 and geraldol were observed in cells arrested at mitosis but not in cycling cells. Simultaneous exposure of cells to a proteasome inhibitor or a caspase inhibitor and SU6656 or geraldol prevented protein degradation and mitotic slippage. Further, MCF-7 cells, which are deficient in caspase-3, were resistant to induction of mitotic slippage by SU6656 or geraldol. Stable transfection of MCF-7 cells was used to examine the requirement for caspase-3 activity for mitotic slippage.
Conclusions: The mitotic slippage inducers SU6656 and geraldol act via proteasome- and caspase-dependent degradation of proteins required to maintain mitotic arrest.

732 Role of microRNAs and microRNA machinery in the pathogenesis of diffuse large B-cell lymphomas

S. Caramuta[1], M. Berglund[2], D. Özata[1], P. Akçakaya[1], P. Georgii-Hemming[2], H. Xie[1], G. Enblad[2], C. Larsson[1], W.O. Lui[1]. [1]*Karolinska Universitetssjukhuset, Molecular Medicine and Surgery, Solna, Stockholm, Sweden,* [2]*Uppsala University, Oncology Radiology and Clinical Immunology, Uppsala, Sweden*

Diffuse large B-cell lymphoma (DLBCL) is one of the most common cancers among all B-cell non-Hodgkins lymphomas and is characterized by high genetical, morphological and clinical heterogeneity. Although different studies have been conducted to investigate the dysregulation of microRNAs in DLBCLs, the role of these small non-coding RNAs is still not well understood. In this study we aimed to explore the contribution of microRNA expression alteration in DLBCL carcinogenesis. Using miRNA microarray and qRT-PCR approaches, we analyzed global microRNA expression and their processing machinery in a set of 70 DLBCLs (62 *de novo* and 8 transformed) and 10 non-neoplastic lymph nodes (LN). Our results show a significant over-expression of *TRBP* gene in tumours as compared to LN suggesting its potential role in development/progression of diffuse large B-cell lymphoma. Further, a comparison between *de novo* and transformed cases revealed an up-regulation of *DROSHA*, *TRBP* and *PACT* in *de novo* DLBCLs. Deregulated microRNAs, identified by microarray analysis, were evaluated in relation to clinical and molecular characteristics of lymphoma cases. Forty-four differentially expressed microRNAs could distinctly classified DLBCL tumours from normal lymph node samples. We also identified 11 candidate microRNAs that could distinguish GCB-DLBCL subtype from non-GCB. Subsets of down-regulated microRNAs were associated with high expression of *BCL6* and *IRF4*, and higher expression of *miR-494* and *miR-638* was observed in advanced stages of DLBCLs. Furthermore we found an association between microRNA deregulation and high expression of *DROSHA*, *DICER* and *TRBP* in lymphoma tumours.
In conclusion, our findings give new insights in the understanding the role microRNA machinery and microRNAs in the diffuse large B-cell lymphomas carcinogenesis.

733 HER2 status in breast carcinomas: comparison between silver in situ hybridization, chromogenic in situ hybridization and fluorescence in situ hybridization

S.A. Md Ali[1], H.M. Nurhayati[1], M.A. Munirah[1], M.Z. Reena[1], N. Masir[1], S.F. Chandramaya[1], M. Rohaizak[2], A. Asmiati[3], N.A. Sharifah[1], M.K. Rafie[3]. [1]*Universiti Kebangsaan Malaysia, Pathology, Kuala Lumpur, Malaysia,* [2]*universiti Kebangsaan Malaysia, Surgery, Kuala Lumpur, Malaysia,* [3]*Hospital Putrajaya, Pathology, Putrajaya, Malaysia*

Introduction: Determination of HER2 amplification in breast carcinoma was regularly reported using fluorescence in situ hybridization (FISH) as a golden method. However, HER2 FISH method required specialized fluorescence microscope, high cost and their signals definitely faded over time causing it to be impractical for routine laboratories. Chromogenic in situ hybridization (CISH) and silver in situ hybridization (SISH) have been reported to be suitable option to FISH as their stability of signals were archival. The aim of this study was to evaluate HER2 and compare it using different techniques, SISH, CISH and FISH.
Methods and Materials: HER2 expression was evaluated by immunohistochemistry (IHC) in 234 breast carcinoma samples. Whole sections of HER2 of 26 cases with borderline positive (2+) IHC were further validated by using manual dual-colour FISH, manual single colour CISH and automated single colour SISH.
Results: By IHC, the samples for HER2 were negative in 70.1%, 1+ in 6.8%, 2+ in 12.2% and 3+ in 10.9%. The 26 cases that were borderline positive (2+) IHC were further analysed for FISH and 88.0% (22) of the cases showed HER2 amplification, 12.0% showed no amplification and 2 cases were non-interpretable. By using CISH, 22 cases showed low to high amplification with more than 5 dots to clusters in more than >50% of carcinoma cells and one case was equivocal. Whereas by using SISH, 19 cases were HER2 amplified and 5 cases were not assessable due to absence of adequate SISH signals. A high level of concordance between FISH and CISH, FISH and SISH, CISH and SISH were observed in 92% ($p = 0.029$, $k = 0.621$), 95% ($p = 0.095$, $k = 0.644$) and 100% ($p = 0.05$, $k = 1.00$) respectively. Technically, we experienced that SISH saved time as it could be done in a short time compared to CISH and FISH. Besides, the signals when using SISH were seen under the ordinary light microscope showed discrete metallic silver black and have a permanent result compared to CISH whose signals became less discrete over long period. FISH method was time consuming and laborious. However, CISH equipment was less expensive than FISH or SISH.
Conclusion: This study shows that CISH and SISH are practical methods that can detect HER2 amplification and may be an alternative used in routine laboratories which are not equipped to do FISH.

734 Identification of gene expression alterations associated with fibrosis in breast cancer survivors

H. Landmark-Hoyvik[1], V. Dumeaux[2], K. Reinertsen[3], H. Edvardsen[1], S. Fossâ[3], A.L. Børresen-Dale[1]. [1]*Oslo University Hospital Radiumhospitalet and University of Oslo, Department of Genetics, Oslo, Norway,* [2]*University of Tromsø, Institute of Community Medicine, Tromsø, Norway,* [3]*Oslo University Hospital and University of Oslo, National Resource Center for Late Effects Department of Oncology, Oslo, Norway*

Advances in detection and treatment of breast cancer have lead to an increasing number of cancer survivors. In consequence, late effects and optimal quality of life have become new important end points in cancer care. Radiotherapy (RT) is an established treatment for breast cancer, however irradiation of normal tissue can induce side effects. Fibrosis is a frequent late side effect among breast cancer survivors treated with RT. Considered to be a consequence of complex biological processes, fibrosis gradually develops over several years. Although fibrosis has been extensively studied, high throughput assays have opened new research avenues which can be used as target discovery tools to develop clinically useful late-effects signatures.
This study included 253 survivors derived from a cohort of survivors, treated for breast cancer stage II/III with adjuvant RT at the Norwegian Radium Hospital between 1998 and 2002. The women were in 2004 invited to participate in a study assessing late side effects. A clinical examination evaluating fibrosis in the breast, axilla and supraclavicular area was performed and blood samples for RNA analyses were drawn in PAX tubes. Gene expression analysis was conducted using Illumina Human-6 version 2 expression beadchips and the data were analyzed using R version 2.8.0 with tools from the Bioconductor project.
Our analyses showed a correlation between age and fibrosis and chemotherapy and fibrosis, thus all the analyses were adjusted for these confounders. Of note, the occurrence of telangiectasia significantly correlated with fibrosis, suggesting that the patients experiencing both effects might constitute a subgroup of survivors, ultra-sensitive to radiation. Preliminary analyses show fibrosis to be significantly associated with global gene expression (p-value = 0.03). Multivariate gene-wise linear analysis identified 87 genes significantly associated with fibrosis (fdr < 0.15). Functional annotation enrichment analyses reveal that these genes are involved in cellular processes, gene expression, cellular component organization and biogenesis, intracellular transport, establishment of cellular localization and chromatin modification.
Breast cancer survivors with fibrosis show a different blood gene expression compared to non-fibrotic survivors. Finally, by analyzing our dataset using gene lists from several related studies in the literature, we aim to identify relevant biological pathways involved in fibrosis.

735 PTEN gene promoter methylation in endometrial and ovarian tumours

T. Kovalenko[1], L.I. Patrushev[1], L.A. Ozolinya[2]. [1]*Shemyakin-Ovchinnikov Institute of Bioorganic Chem, Department of Biotechnology, Moscow, Russian Federation,* [2]*Russian State Medical University, Obstetrics and Gynaecology, Moscow, Russian Federation*

Background: Tumour suppressor gene *PTEN* is implicated in the pathogenesis of several familial and sporadic cancers. The aim of our study was to analyze the possible existence of alterations in *PTEN* gene promoter in endometrial and ovarian tumours.
Material and Methods: The study included 34 patients with endometrial cancer, 26 – with endometrial hyperplasia, 24 – with ovarian cancer and 17 – with benign ovarian tumours. Normal endometrium tissue samples were obtained from 20 healthy women during therapeutic abortion. Genomic DNA was isolated and the −1148 to −727 promoter region called *PN-1* was amplified by PCR. The genomic DNA was treated with sodium bisulfite and used as a template for the amplification of the 618 bp fragment (*PN-2*) including the