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