identify potential mechanisms resulting in LOH around the *p53* locus in its carcinogenesis.

Materials and Methods: We investigated ten esophageal cancer cell lines and 91 surgically-resected specimens, examining them for LOH at the *p53* locus on chromosome 17. LOH was screened using microsatellite markers by DNA sequencing. To test whether copy number loss was seen at the *p53* locus, we examined the *p53* gene using comparative genomic hybridization (CGH) and fluorescence in situ hybridization (FISH). Finally, we performed single-nucleotide polymorphism (SNP) − CGH analysis to clarify potential mechanisms of disruption of the intact allele in *p53* mutant ESCCs.

Results: In an analysis of specimens using microsatellite markers, a close positive correlation was found between *p53* mutations and LOH at the *p53* locus ($p < 0.01$). Although four cell lines were found to be homozygous for *p53* mutations, LOH at the *p53* locus was not detected by CGH. Among two *p53* mutant cancer cell lines and five *p53* mutant/LOH cancer specimens analyzed by FISH, both of the cell lines and four of the specimens exhibited no obvious copy number loss at the *p53* locus. SNP-CGH analysis, which allows both determination of DNA copy number and detection of copy-neutral LOH, demonstrated no chromosomal alterations in the *p53* wild type/retention of heterozygosity ESCC specimen and all normal samples. On the other hand, data from the *p53* mutant/LOH cancer specimens indicated that LOHs without copy number change were caused by whole or large chromosomal alteration.

Conclusions: LOH without copy number change at the *p53* locus was frequently observed in *p53* mutant ESCC. Our data suggest that copy-neutral LOH occurring as a result of chromosomal instability might be the major mechanism for inactivation of the intact allele in esophageal squamous cell carcinogenesis associated with *p53* mutation.

1051 POSTER

High Level of MiR-21, MiR-10b and MiR-31 Expression in Bilateral Versus Unilateral Breast Carcinomas

A. Iyevleva[1], E. Kuligina[1], N. Mitiushkina[1], A. Togo[1], Y. Miki[1], E. Imyanitov[1]. [1]*N.Petrov Research Institute of Oncology, Laboratory of Molecular Oncology, Saint-Petersburg, Russian Federation*

Bilateral breast cancer (biBC) represents approximately 5% of total breast cancer (BC) incidence. Patients with bilateral breast cancer (biBC) develop the same disease twice therefore they are likely to accumulate women with particularly elevated genetic or non-genetic susceptibility to BC. We analyzed the expression of several microRNAs implicated in BC pathogenesis (miR-21, miR-10b, miR17−5p, mir-31, miR-155, miR-200c, miR-18a, miR-205, miR-27a) in 80 breast carcinomas obtained from biBC patients and 40 cases of unilateral BC (uBC). Unexpectedly, 3 miRs (miR-21, miR-10b and miR-31) demonstrated significantly higher level of expression in biBC *versus* uBC ($p = 0.0001$, 0.00004 and 0.0002, respectively). Increased content of miR-21, miR-10b and miR-31 was observed in all categories of biBC tumours, i.e. in synchronous biBC as well as in 1st and 2nd tumours from metachronous biBC cases. Synchronous biBC showed more similarity of miR expression profiles within pairs that the metachronous doublets. This study suggests that bilateral breast tumours have somewhat distinct pattern of molecular events as compared to the unilateral disease.

1052 POSTER

MicroRNA-mediated Restriction of Cell Proliferation and Tumour Growth

V. Halytskiy[1]. [1]*Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine, Molecular Immunology Department, Kiev, Ukraine*

Background: Tumour growth is tightly associated with regular shifts in microRNA (miRNA) expression pattern as well as with amplification, translocation and other damages in fragile chromosome regions containing more than 50% of miRNA genes. Usually, expression of miRNAs miR-15a, miR-16, miR-17−5p, miR-31, miR-125a/b, miR-143 and miR-145 is down-regulated in cancer cells whereas expression of miRNAs miR-21, miR-155, miR-206, miR-221 and miR-222 is up-regulated. This investigation aims to identify how abnormalities in miRNA network contribute to the excessive proliferation of transformed cells.

Material and Methods: miRNA targets within gene transcripts were predicted *in silico* using TargetScan software.

Results: miRNA miR-17−5p can target transcripts of genes coding proliferative signal pathway components E2F1, E2F2, STAT3, Rb, p107, p130 and ErbB3. miR-221 and miR-222 silence genes encoding cell cycle inhibitors p27 and p57 as well as receptor c-Kit. Nevertheless, miR-17−5p targets also transcript of gene coding another cell cycle inhibitor, p21, whereas miR-155, miR-221 and miR-222 silence *E2F2* gene. miR-15a and miR-16 can suppress genes encoding transcription factors E2F3 and E2F7 as well as gene of cyclin-dependent kinase CDK6 and main antiapoptotic gene *bcl-2*. *E2F2*, *STAT3, erbB2* and *bcl-2* gene transcripts carry miR-125a/b binding sites. Also, miR-31 silences *E2F2* gene. miR-143 can suppress *abl2*, *erbB3* and *bcl-2* genes. miR-145 targets transcripts *of RASA1*, *RASA2, erbB3* and *bcl-2* genes. miR-320 can suppress *E2F1*, *E2F3, RASA1*, *CDK6* and *p57* genes. miR-21 as well as miR-155 target transcript of gene encoding TGF-β receptor that can induce cell-cycle arrest through suppression c-Myc-regulated genes. miR-205 silences *E2F1*, *erbB3*, *erbB4* genes. miR-206 targets *RARB* gene encoding retinoic acid b-receptor. miR-181, which is responsible for differentiation of some cells, e.g. lymphopoiesis, can suppress *E2F5*, *E2F7*, *bcl-6* and *bcl-2* genes. miR-150, another differentiation hallmark, targets transcript of gene encoding transcription factor Elk1.

Conclusions: Cell miRNA network is intertwined with signal transduction pathways. Cancer cells down-regulate expression of miRNAs that silence proliferative and antiapoptotic genes and thus can prevent from abnormal cell proliferation and surviving. Up-regulated miRNAs suppress genes encoding cell cycle inhibitors as well as genes responsible for cell differentiation. Therefore, shifts in miRNA expression pattern can themselves cause reactivation of cell oncogenes and antiapoptotic genes as well as repression of cell cycle inhibitor genes. Such alterations facilitate proliferation of transformed cells. Moreover, as each miRNA impairs the expression of many genes, including genes of other miRNAs, illegitimate activation or repression of some miRNA genes can be the first event in carcinogenesis, leading to the reorganization of epigenetic pattern in transforming cells through the RNAi-dependent DNA methylation.

1053 POSTER

Snail1 and Estrogen Receptor Signaling: Two Crossing Pathways in Breast Cancer

A. Scherbakov[1], O. Andreeva[2], M. Krasil'nikov[2]. [1]*N.N.Blokhin Cancer Research Center, Laboratory of Clinical Biochemistry, Moscow,* [2]*N.N.Blokhin Cancer Research Center, Laboratory of Molecular Endocrinology, Moscow, Russian Federation*

Epithelial-to-mesenchymal transition (EMT) is one of key steps in breast cancer progression. The transcription factor Snail1 helps the breast cancer cells to lose their epithelial morphology (in particular E-cadherin adhesive contacts) and to acquire a fibroblast-like phenotype and a high expression of mesenchymal markers. Estrogen receptor (ERalpha, ER) signaling plays the significant role in breast cancer development and treatment, but relations between Snail1 and ER pathways remain unclear. The goal of this study was to investigate the relationship between ER and Snail1 signaling in breast cancer cells.

Methods: MCF-7 (ER+, hormone responsive), MCF-7/LS (ER+, hormone resistant) and HBL-100 (ER-) cells was cultured in standard DMEM medium supplemented with 7% fetal calf serum. Estrogen receptors and Snail1 expression was assessed by immunoblotting. The transcriptional activity of ER and Snail1 was determined by luciferase assay.

Results: The inverse relationship between Snail1 and estrogen receptors has been demonstrated: MCF-7 and MCF-7/LS cells were characterized by high ER expression and low Snail1 activity/expression, on the contrary, HBL-100 cells were ER-negative and had high level of Snail1 activity/expression. The study of the expression of the Snail-regulated epithelial marker, E-cadherin, has shown high E-cadherin expression in ER-positive MCF-7 and MCF-7/LS cells and a loss of E-cadherin in ER-negative HBL-100 cells. The wild type Snail transfection into ER-positive cells has caused no changes in ER expression, but significantly decreased ER transcriptional activity determined by luciferase assay. Contrariwise wild type Snail transfection into ER-negative HBL-100 cells was not accompanied with the alterations in the reporter gene activity. The transfection of siRNA Snail into MCF-7 and MCF-7/LS cells has caused stimulation of ER activity, totally supporting the involvement of Snail1 in ER down-regulation. Thereby the results show the inverse relationship between ER and Snail1 content/ activity in breast cancer lines demonstrating the possible Snail involvement in the regulation of hormonal signaling.

1054 POSTER

17beta-estradiol Signaling via Estrogen Receptor Alpha-36 Enhances Survival and Invasion of ERalpha-negative Breast Cancer Cells

R.A. Chaudhri[1], N. Cuenca[2], A. Hadadi[1], Z. Schwartz[2], B.D. Boyan[2]. [1]*Georgia Institute of Technology, School of Biology, Atlanta GA,* [2]*Georgia Institute of Technology, Department of Biomedical Engineering, Atlanta GA, USA*

Background: While several factors associated with tumour metastasis are known to be upregulated in breast carcinomas, the molecular mechanisms of metastasis are not well understood. ERα36, a variant of ERα that we found in ERα-negative HCC38 breast cancer cells, is responsible for 17β-estradiol(E2)-dependent protein kinase C (PKC) activation from the