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

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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

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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

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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

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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

plasma membrane. We have shown that PKC activity is associated with increased breast cancer tumorigenicity and recurrence, and in patients with higher PKC activity, metastasis is also more prevalent. The aim of this study was to examine the role of ERα36 in promoting survival and invasiveness of breast cancer cells.
Materials and Methods: HCC38 cells were treated with increasing concentrations of E2 and ERα36 antibody and PKC activity was measured. Cells were treated with chelerythrine, and MTT incorporation and DNA fragmentation were determined after 24 hours. Cells were treated with E2 with or without ERα36 antibody for 24 hours, and DNA synthesis was measured by [3H]-thymidine incorporation. Cells were treated with taxol with or without E2-BSA, and TUNEL and caspase-3 activity were measured. Control and ERα36-blocked cells treated with E2 were analyzed for effects on metastatic(CXCR4, snail1, e-cadherin) and osteotropic(RANKL, IL6, OPG) factors by qRT-PCR and ELISA.
Results: E2 increased PKC activity in a membrane-associated manner through ERα36. Chelerythrine, a specific PKC inhibitor, caused a dose-dependent decrease in MTT and an increase in DNA fragmentation, indicating the role of PKC in cell survival. E2-BSA increased proliferation and ERα36 antibody blocked this effect, indicating that E2 signaling through ERα36 enhances proliferation. Taxol caused apoptosis as indicated by TUNEL and caspase-3 activity, and this was blocked by E2-BSA. Membrane E2 signaling caused increased expression of CXCR4 and Snail1 with downregulation of E-cadherin, suggesting a role for ERα36 in epithelial to mesenchymal transition. RANKL expression also increased without any changes in OPG production, indicating that E2 signaling through ERα36 enhances osteoclastogenesis and osteoclast activation.
Conclusions: Our results indicate that E2 can enhance survival of ERα-negative breast cancer cells *in vitro* via ERα36 and signaling through this receptor at the membrane enhances proliferation and anti-apoptotic activity of breast cancer cells. ERα36-dependent E2 signaling also activates expression of metastatic and osteolytic factors, suggesting its role in metastasis of ERα-negative breast cancer. Further analysis of the role of ERα36 in breast cancer metastasis *in vivo* may reveal its role in membrane-targeted therapies against breast cancer progression.

1055 POSTER

Signalling Mechanisms Mediating EGF- and HGF-induced Migration in Carcinoma Cells in Vitro

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Background: Cell migration is an integrated and necessary part of cancer cell invasion. We have studied mechanisms mediating the effects of epidermal growth factor (EGF) and hepatocyte growth factor/scatter factor (HGF/SF) on cell migration in oral squamous carcinoma cells and other carcinoma cells *in vitro*.
Methods: A wound scratch assay was performed in a confluent layer of carcinoma cells. Wound closure was measured after 24 hours. Phosphorylation was assessed by the use of western blot.
Results: In the hepatoma cell line MH_1C_1 and in the oral squamous carcinoma cell line E10 both EGF and HGF activated the ERK 1/2 and PI-3 kinase pathways. In the E10 cell line both EGF and HGF induced cell migration, while in the MH_1C_1, only HGF induced significant cell migration. A more detailed investigation of the mechanisms in the E10 cells showed that both EGF and HGF dose-dependently induced wound closure within 24 hours and led to phosphorylation of EGF receptor (EGFR) and Met respectively. The addition of the EGFR-specific inhibitors cetuximab (antibody) and gefitinib (tyrosine kinase inhibitor) abolished cell migration and receptor activation induced by EGF. Similarly, addition of a Met kinase inhibitor (SU11274) prior to HGF stimulation abolished cell migration and receptor activation. To examine the contribution to cell migration from different downstream pathways, we added inhibitors of the MEK/ERK 1/2 and p38 MAP kinase pathways, or PI-3 kinase before growth factor stimulation. We found that all three pathways examined were contributors to both EGF- and HGF-induced cell migration, as their individual inhibition decreased cell migration in the scratch assays. However, compared to EGF-stimulation, the effect of HGF was more sensitive to inhibition of ERK and particularly PI3K.
Conclusion: The results show that EGF and HGF can exert motogenic effects through main signalling pathways that also mediate other responses such as proliferation and apoptosis. Cell migration is a necessary part of invasive growth, and further studies of the quantitative differences observed here may contribute to a better understanding of mechanisms involved.

1056 POSTER

Sorafenib Sensitizes Hepatocellular Carcinoma Cells to Radiation-induced Apoptosis Through the Inhibition of Signal Transducers and Activators of Transcription 3

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Background: Hepatocellular carcinoma (HCC) is one of the most common and lethal human malignancies. Radiation therapy is one of the major treatment modalities for cancer. However, many HCC cells show resistance to radiation therapy. Sorafenib, a tyrosine kinase inhibitor, is the first and only approved molecular targeted agent in HCC. In this study, we showed that sorafenib sensitizes resistant HCC cells to radiation-induced apoptosis.
Material and Methods: HCC cell lines (PLC5, Huh-7, Sk-Hep1, and Hep3B) were treated with sorafenib and/or radiation and analyzed in terms of apoptosis, signal transduction.
Results: HCC cells, including PLC5, Huh-7, Hep3B and Sk-Hep1, showed significant resistance to radiation-induced apoptosis (up to 6 cGy). The combination of sorafenib (starting at 5 μM) and radiation enhanced the sensitivity of HCC cells to radiation-induced apoptosis. Thorough comparisons of the molecular change before and after treatment with these agents, we found signal transducers and activators of transcription 3 (STAT3) played a significant role in mediating TRAIL sensitization of sorafenib. Our data showed that sorafenib down-regulated phospho-Stat3 (Tyr 705) and subsequently reduced the expression levels of two Stat3-related proteins, Mcl-1, cylcin D1, and survivin in radiation-treated HCC cells. Knocking down STAT3 by RNA-interference overcame apoptotic resistance to radiation in HCC cells, and ectopic expression of STAT3 in HCC cells abolished the radiation-sensitizing effect of sorafenib, indicating STAT3 inactivation plays a key role in mediating the combination effect.
Conclusions: Sorafenib sensitizes resistant HCC cells to radiation-induced apoptosis at clinical achievable concentrations, and this effect is mediated via the inhibition of STAT3.
Supported by NTUH100P04, NSC98–3112-B-002–037, NSC98–2314-B-002–067-MY3, NSC99–2314-B-002–017-MY2

1057 POSTER

The Expression of Insuline-like Growth Factors, Insuline-like Growth Factor Binding Proteins and PTEN in Receptor-negative Endometrial Carcinomas

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Background and aims: The aim of the study was to evaluate the expression of insulin-like growth factors (IGFs), insulin-like growth factor binding protein-3 and -4 (IGFBP-3 and IGFBP-4), their protease PAPP-A and PTEN in receptor-positive endometrial carcinomas.
Methods: The concentrations of IGF-I, IGF-II, IGFBP-3, -4 and PAPP-A in tumours were determined by ELISA kits (R&D Systems, DSL, USA). Results were analyzed in relation to estrogen and progesterone receptors (ER and PR) and PTEN expressions (immunohistochemistry). Tumour was considered receptor-positive if more than 5% of tumour cells expressed ER or(and) PR. The expression levels of markers were assessed by semi-quantitative method using three-point score system. A total of 54 endometrial cancer patients with I-II Stage were enrolled.
Results: The IGFBP-3 level was found to be significantly higher in ER-negative tumours than in ER-positive tumours. The IGFBP-3 level was higher in tumours with high level of PTEN expression (3 points) than in tumours with moderate and low levels irrespective of ER expression. The IGFBP-4 level was correlated with intensity of ER expression and it was maximal in tumours with low ER expression. The IGF-II level correlated with PR expression and it was significantly higher in PR-negative carcinomas.
Conclusion: Thus, the correlation between the IGF-II, IGFBP-3 and IGFBP-4 levels and ER, PR and PTEN expressions was found in endometrial adenocarcinomas. Receptor-negative tumours were characterized by high levels of IGFBP-3, IGFBP-4 and IGF-II, while high level of IGFBP-3 correlated with high level of PTEN expression.