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.
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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.