Background: Chemokines and their receptors have been shown to play a critical role in cancer growth and metastasis. In particular, recent data have suggested that the chemokine CXCL12 and its receptor CXCR7 (also known as DRC1), which has been recently identified as a chemokine receptor, have key functions in promoting tumor development and progression. However, there is little information regarding their expression and clinical relevance in gastric cancer. Here we investigated for the first time the effects of combined CXCR7 and CXCL12 expression on the prognosis of patients with gastric cancer.

Methods: We studied CXCL12 and CXCR7 protein expression in 221 specimens of primary gastric cancer using immunohistochemistry, and investigated the relationship between CXCL12/CXCR7 expression and clinicopathological features and clinical outcomes.

Results: Patients were categorized into four groups according to CXCR7 and CXCL12 expression: low CXCR7/low CXCL12, high CXCR7/low CXCL12, low CXCR7/high CXCL12, and high CXCR7/high CXCL12. No significant differences existed in age, gender, histology, tumor location, lymphovascular invasion among the four groups. However, high CXCR7/high CXCL12 expression in tumor cells was significantly associated with invasion depth of the tumor (T status; P < 0.001), lymph node involvement (N status; P = 0.002), and proportion of tumor size >5 cm (P = 0.006) compared to tumors with low CXCR7/low CXCL12 expression or high CXCR7/low CXCL12-low CXCL12/high CXCL12 expression. Furthermore, patients with high CXCR7/high CXCL12 expression had the worst prognosis (5-year survival rate 30.6%; median, 2.3 years; range, 0.1 - 5.7 years) compared to those of other patient groups (5-year survival rate 52.4%; median, not reached; log-rank test, P = 0.008).

Conclusions: CXCR7 and CXCL12 are useful prognostic factors in gastric cancer, and the combination of high CXCR7 protein expression with high CXCL12 expression suggests a dismal prognosis.

Disclosure: All authors have declared no conflicts of interest.

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The epidermal growth factor receptor (EGFR) pathway is a regulator of cellular proliferation and tumour progression. EGFR overexpression occurs with gene amplification and activating EGFR kinase mutations (EGFRmt). Monoclonal antibody 806 (mAb806) is an anti-EGFR antibody which targets tumours with EGFR mutation variant III and overexpression but not cells with low EGFR expression (wild type EGFR, wtEGFR) and normal tissues. To characterise mAb806 binding, we correlated mAb806 expression with EGFRmt, EGFR immunohistochemical (IHC) expression and overall survival (OS). Formalin fixed paraffin embedded tissues were stained using the mAb806 (Ludwig Institute for Cancer Research) and EGFR (Dako, PharmDx kit). A H-score was obtained based on staining intensity and percentage of cells stained. mAb806 H-scores 0-50 were classified as negative (mAb806-) and ≥50 as positive expression (mAb806+). EGFR H-scores were calculated to stratify patients into low (Dako score <200) and high (≥200, EGFR≥200) expressors. DNA was isolated and assayed using Sequenom's OncosCarta panel. Clinicopathological features were correlated with mAb806 status using Fisher's exact test and the log-rank test. Harzard ratios were calculated using the Mantel-Haenszel method. Of 107 patients with non-small cell lung carcinomas (NSCLC), 94 (88%) expressed EGFR (EGFR+), of which 24 (26%) patients were EGFR≥200 expressors. Forty of 107 (37%) were EGFR+ and mAb806+, while 19 (18%) were EGFR≥200 and mAb806+. Squamous cell carcinomas (SqCC) were more likely to express mAb806+ than adenocarcinomas [16 of 29 (55%) vs 18 of 60 (30%); p = 0.0353]. Of the 15 patients with activating EGFRmt, 14 (93%) were mAb806+ and 1 was mAb806- (p = 0.0001). Six of 15 (40%) EGFRmt and 15 of 81 (19%) wtEGFR tumours were EGFR≥200 expressors (p = 0.1895). Of 107 patients, OS was similar in both mAb806+ and mAb806- cases. In patients with SqCC, mAb806+ was associated with significantly poorer survival than mAb806- tumours (HR 2.82, 95%CI 1.13-7.04; p = 0.02). mAb806+ was associated with EGFRmt but was not prognostic in adenocarcinomas. mAb806+ in SqCC was associated with a poorer prognosis. Given its use as a therapeutic target and its potential role as a prognostic biomarker, further studies exploring the treatment efficacy of mAb806 in NSCLC are warranted.

Disclosure: A. Scott: A.S is an inventor of a patent for mAb806, and a consultant to Life Science Pharmaceuticals which has the license for mAb806. All other authors have declared no conflicts of interest.

Background: Ephitelial-to-mesenchymal transition (EMT) is originally proposed as a process of organogenesis. In recent years, the association between EMT and cancer invasion and metastasis has been advocated and actively investigated in various cancers. In addition, chemoresistance and cancer stemness could be involved in EMT, and the elucidation of this association might contribute to improved outcomes of hepatocellular carcinoma (HCC). We utilized surgical specimens of HCC from our department to examine the clinical implications of EMT.

Methods: One hundred and one patients with hepatocellular carcinoma, who underwent resection in our department between 1994 and 2003 were analyzed. The mRNA expression of E-cadherin and Vimentin were measured by quantitative real-time PCR and EMT status of each patient was determined as follows: Vimentin/ E-cadherin < 2 = Epithelial (E), Vimentin/E-cadherin ≥ 2 = Mesenchymal (M). Moreover, transcription factors which are involved in EMT (Twist, Snail, Slug, Zeb-1, and Zeb-2) and also IL-6 and its receptor IL-6R were measured. The correlation between these values and clinicopathological factors and prognosis were analyzed statistically.

Results: 1) AFP values were significantly higher in the epithelial group than in the mesenchymal group (P = 0.0229). There was no difference in overall survival, but a significant difference was found in disease-free survival (P = 0.0042), which showed that patients with a mesenchymal tumor were more prone to have earlier recurrence than those with an epithelial tumor. 2) All transcription factors were more highly expressed in mesenchymal than in epithelial tumors, and in particular, Twist and Zeb-2 were significantly overexpressed (P = 0.0012, P = 0.0017, respectively). 3) IL-6 expression was also significantly higher in mesenchymal than in epithelial tumors, but there was no difference in IL-6R expression.

Discussion: Our study using resected surgical specimens suggest that EMT could be involved in cancer invasion and metastasis at the clinical level. In particular, Twist and Zeb-2 might be important for inducing EMT in HCC and patients with mesenchymal tumors are more prone to have early recurrence or metastases after resection. In addition, IL-6 might be an important factor for HCC EMT and could be a potential therapeutic target.

Disclosure: All authors have declared no conflicts of interest.
expression, but not in Gli3-undetectable HCT116 or DLD-1 cells. Silencing of endogenous Gli3 down-regulated colony formation and proliferation in HT29 and SW480 cells. However, truncated Gli3 (Gli3-R; repressor isomer) transduction had no effect on these parameters although Gli1 expression was inhibited. After implantation of Gli3- or mock-transfected DLD-1 cells into immunodeficient SCID mice, tumor formation was observed in only Gli3-transfected DLD-1 group but not in mock control. In surgically resected colorectal cancer specimen, Gli3 expression was heterogeneously detected and Shh expression was highly observed.

Conclusions: Gli3-FL and Shh signals induce tumorigenicity in Gli3 independent manner, and Gli3-FL may be molecular targets for refractory colorectal cancer.

Disclosure: All authors have declared no conflicts of interest.

SEMULOPARIN EFFICIENTLY INHIBITS THROMBIN GENERATION TRIGGERED BY PANCREAS ADENOCARCINOMA CELLS BXPC3. DISTINCT ROLES OF ANTI-XA AND ANTI-IIA ACTIVITY

G. Gerotziafas1, M.P. Roman1, E. Mdemsba1, M. Halt1, J. Fareed2, J. F. Bernaudin3 and I. Elaïamy3
1EP2UCMC, Faculty of Medicine, Université Pierre et Marie Curie (Paris VII, Paris, FRANCE, 2Pathology, Loyola University Medical Center, Maywood, IL, UNITED STATES OF AMERICA

Background: Venous thromboembolism (VTE) is a common complication in patients with cancer receiving chemotherapy. Currently no anticoagulant is approved for VTE prophylaxis in this setting. Semuloparin is an ultra-LMWH generated through a highly selective dephosphorylation of heparin which protects the antithrombin (AT) binding site in order to improve the benefit/risk ratio compared to existing anticoagulants.

Aims: We studied in vitro the mechanism of action of semuloparin on the inhibition of thrombin generation (TG) of human platelet poor plasma (PPP) triggered by human pancreatic cancer cells BXPC3. We compared the antithrombotic efficiency of semuloparin to that of enoxaparin and the specific AT-dependent factor Xa inhibitor fondaparinux.

Materials and methods: BXPC3 cells were suspended in PPP spiked with clinically relevant concentrations of semuloparin, enoxaparin and fondaparinux. The endogenous thrombin potential (ETP) and the mean rate index (MRI) of the propagation phase of TG were monitored with the CAT assay (Stago France) as described previously (Gerotziafas et al Thromb Res 2011). Anti-Xa and anti-IIa specific activities were assessed assays obtained from Diagnostica Stago.

Results: Both semuloparin and enoxaparin, at the concentration of 0.4 anti-Xa IU/ml, completely abrogated TG. Total inhibition of TG occurred in the presence of 0.002 anti-IIa IU/ml of semuloparin and 0.05 anti-IIa IU/ml of enoxaparin. Fondaparinux, even at concentrations higher than 2 μg/ml, reduced the MRI but did not completely affect the ETP and did not completely inhibited TG. The IC50 for the ETP of the anti-IIa activity of enoxaparin was 37-fold higher as compared to that of semuloparin.

Conclusion: In a cancer cell model of hypercoagulability, semuloparin reduced efficiently thrombin generation. The unique anticoagulant profile of semuloparin based on its high AT-affinity differentiates it from enoxaparin and fondaparinux. The residual anti-IIa activity of semuloparin amplifies its antithrombotic efficacy. This profile is expected to translate into an improved benefit/risk ratio.

Disclosure: All authors have declared no conflicts of interest.

DO THE WELL-KNO own RISK Factors OF BREAST CANCER HAVE THE SAME IMPACT ON DEVELOPMENT OF MOLECULAR SUBTYPES?  

F. Paksos Türköz1, M. Solak1, O. Keskin2, Z. Ark1, F.S. Sanci2, İ.H. Petelekaya1, T. Babacan2 and K.M. Altundag3
1Medical Oncology, Abdurrahman Yurtaslan Ankara Oncology Research and Education Hospital, Ankara, TURKEY, 2Medical Oncology, Hacettepe University Oncology Institute, Ankara, TURKEY

Background: Although clinical differences between breast cancer (BC) subtypes have been well-described, etiologic heterogeneity have not been fully studied. The aim of this study was to assess the associations between risk factors and molecular subtypes of BC.

Methods: 1884 invasive BC cases were retrospectively analyzed. The odds ratios (OR) and 95% confidence intervals (CI) were estimated using multiple logistic regression analysis.

Results: 1249 patients had luminal A, 234 had luminal B, 169 had HER-2
overexpressing and 232 had triple negative BC. The age of ≥40 years was found to be a risk factor for luminal A (OR 1.41 95% CI 1.15-1.74; p = 0.001) and HER-2 overexpressing subtype (OR 1.51, 95% CI 1.01-2.25; p = 0.04). Women who were nulliparous (OR 1.60 95% CI 1.03-2.21; p = 0.03) and who had their first full-term pregnancy ≥30 years (OR 1.25 95% CI 0.83-1.86; p = 0.04) were at increased risk of luminal BC, whereas women with ≥2 children had a decreased risk (OR 0.68, 95% CI 0.47-0.97; p = 0.03). Breast-feeding was a protective factor for luminal BC (OR 0.74, 95% CI 0.53-1.04; p = 0.04). We found increased risks for postmenopausal women with HER-2 overexpressing (OR 2.20, 95% CI 0.93-5.17; p = 0.04) and luminal A (OR 1.87, 95% CI 0.93-3.90; p = 0.02) BC, who used hormone replacement therapy for ≥5 years. Overweight and obesity increased the risk of triple negative BC (OR 1.89 95% CI 1.06-3.37; p = 0.04 and OR 1.90 95% CI 1.30-3.61; p = 0.03), on the contrary, decreased the risk of luminal BC (OR 0.63 95% CI 0.43-0.95; p = 0.02 and OR 0.50 95% CI 0.32-0.76; p = 0.002, respectively) in premenopausal women. There were no significant differences between risk of BC subtypes and early menarche, late menopause, family history, postmenopausal obesity, oral contraceptive use, smoking, in vitro fertilization and blood groups.

Conclusions: Reproductive and hormonal characteristics were associated with luminal BC. Obesity and overweight increased the risk of triple negative subtype, particularly in premenopausal women. Older age and use of hormone replacement therapy were related to the risk of HER-2 overexpressing BC. Our data suggest a significant heterogeneity in association of traditional BC risk factors and tumor subtypes.

Disclosure: All authors have declared no conflicts of interest.

DATA FROM SCREENING OF WNT-1, WNT-2, R- and B-catenin, E-cadherin and cyclin D1 in melanoma, breast, lung, prostate cancer in comparison to adjacent normal tissue and to further understand WNT ligands significance as a potential biomarker. Formalin-fixed, paraffin-embedded samples were sent from twenty women, and two from 26 breast cancer patients, 24 melanomas patients in 1-III stage of the disease. Expression of WNT-1, WNT-2, β-catenin, E-cadherin and cyclin D1 was assessed by immunohistochemistry and analyzed by two independent histopathologists. Changes of studied proteins expression profiles between normal and malignant tissues were measured with Wilcoxon test, while comparison of expression of analyzed proteins between tumors was performed with Kruskall-Wallis and post hoc test. A value of p < 0.05 was considered significant. The study received approval by the Local Bioethics Committee. In our study, WNT-1 cytoplasmic expression was increased in breast cancer (p = 0.003) and melanoma (p = 0.0001), while in lung cancer it was decreased (p = 0.002). WNT-2 cytoplasmic expression was increased in breast (p = 0.001), prostate cancer (p = 0.0002), melanoma (p = 0.0007), while in lung cancer WNT-2 increased expression showed a trend towards significance (p = 0.06). Moreover WNT-2 ligand was found in cell nuclei in all tumors and its expression level was increased in breast cancer (p = 0.007) and decreased in melanoma (p = 0.042). Our study revealed that WNT-2, but not WNT-1 expression was increased in all analyzed tumors. Moreover WNT-2 ligand was detected in cell nuclei, what could implicated its yet undiscovered role in gene expression regulation.

Disclosure: M. Wieszczek: Maciej Wieszczek is Chief Executive Officer of Celon Pharma Ltd. All other authors have declared no conflicts of interest.

WNT-2, BUT NOT WNT-1 EXPRESSION INCREASES DURING TUMOURIGENESIS IN BREAST, PROSTATE, LUNG CANCER AND MELANOMA

A. Stanczuk1, A. Brozyna1, H. Wasilewskia2, W. Jozwicki2, L. Bodnar2, M. Lamparska-Kryspin3, M. Bielefeldt-Willms1, L. Biedrzycka2, M. Wieszczek1
1Research and Development, Celon Pharma, Lomianki, POLAND, 2Department of Tumor Pathology and Pathomorphology of The Franciszek Lukaszkow Oncology Center, The Ludwik Rydygier Collegium Medicum, Nicolaus Copernicus University, Bydgoszcz, POLAND, 3Department of Oncology, Military Institute of Medicine, Warsaw, POLAND, 4Celon Pharma, Lomianki, POLAND

WNT-β-catenin pathway regulates cell cycle and proliferation. It is triggered by WNT ligands, and drives β-catenin regulated expression of cyclin D1, c-Myc, MMP7. Moreover β-catenin, along with E-cadherin, forms adherent junctions mediating cell adhesion. WNT-1 is a known oncogene and its expression occurs in several malignancies such as breast, prostate and lung cancers, while WNT-2 is less known member of WNT ligands family. The aim of our study was to investigate the expression of WNT-1, WNT-2, β-catenin, E-cadherin and cyclin D1 in melanoma, breast, lung, prostate cancer in comparison to adjacent normal tissue and to further understand WNT ligands significance as a potential biomarker.

Annals of Oncology

PROTEOMIC ANALYSIS OF HUMAN LUNG CANCER CELL LINES

M. Kormu1, J. Mokleyb, D. Chenb, T. Ohrab, N. Gotchb, A.F. Gazdarc and N. Ikedaa
11st Department of Surgery, Tokyo Medical University Hospital, Tokyo, JAPAN, 2Division of Urology, University of Alabama at Birmingham, Birmingham, AL, UNITED STATES OF AMERICA, 3Division of Preventive Medicine, University of Alabama at Birmingham, Birmingham, AL, UNITED STATES OF AMERICA, 4Division of Systems Biomedical and Genomics, The University of Tokyo, The Institute of Medical Science, Tokyo, JAPAN, 5Hamon Center for Therapeutic Cancer Research, UT Southwestern Medical Center, Dallas, TX, UNITED STATES OF AMERICA

The causes and morphological appearances of human lung cancers are variable. We analyzed thirty seven lung cancer cell lines including thirty adenocarcinoma (ADC), three small cell carcinoma (SCLC), one large cell carcinoma (LCC) and one adenosquamous carcinoma (AdCv) with LC/MS and identified less than 500 proteins of each cell line. We compared proteomic profiles between EGFR mutations and K-Ras mutations, smokers and non/less-smokers, and non-small cell carcinoma (NSCLC) and small cell carcinoma (SCLC). Eleven proteins, CALR, KNYU, SLC32A2, ALDH2, P2G, LAP3, DCL2, KIAA0664, ILKAP, ABCA13 and PTGR1 are related to the relationship between cell lines in from non/less-smokers and those from smokers and all proteins are associated to the signal network of cell morphology. Fifteen proteins, ANXA2, P4HB, ACTN4, MN, ANXA5, IDH2, DYSPS2, DYSPS3, DYSPS5, CKB, IPO8, MAP1B, EFTA, TRIM28 and USP5, are related to the relationship between cell lines in from non/less-smokers and those from smokers and all proteins are associated to the signal network of cell morphology. Fifteen proteins, ANXA2, P4HB, ACTN4, MN, ANXA5, IDH2, DYSPS2, DYSPS3, DYSPS5, CKB, IPO8, MAP1B, EFTA, TRIM28 and USP5, are related to the relationship between cell lines in from non/less-smokers and those from smokers and all proteins are associated to the signal network of cell morphology. Fifteen proteins, ANXA2, P4HB, ACTN4, MN, ANXA5, IDH2, DYSPS2, DYSPS3, DYSPS5, CKB, IPO8, MAP1B, EFTA, TRIM28 and USP5, are related to the relationship between cell lines in from non/less-smokers and those from smokers and all proteins are associated to the signal network of cell morphology. Fifteen proteins, ANXA2, P4HB, ACTN4, MN, ANXA5, IDH2, DYSPS2, DYSPS3, DYSPS5, CKB, IPO8, MAP1B, EFTA, TRIM28 and USP5, are related to the relationship between cell lines in from non/less-smokers and those from smokers and all proteins are associated to the signal network of cell morphology.
method A cohort of 113 CBC patients, without distant metastases, has been prospectively registered during 28 months. Patients are divided in 2 groups according to the time of CBC diagnosis: 1. Synchronous, if the CBC (S-CBC) was diagnosed either simultaneously or within 6 months after PBC; 2. Metachronous (M-CBC) if CBC was diagnosed > 6 months after PBC. (Table 1)

<table>
<thead>
<tr>
<th></th>
<th>Synchronous CBC N = 49</th>
<th>Metachronous CBC N = 64</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>Pts N = 113</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median age at CBC diagnose (yrs)</td>
<td>57 (33-74)</td>
<td>50 (25-75)</td>
<td>0.007</td>
</tr>
<tr>
<td>Median time to CBC (months)</td>
<td>0 (0-&lt;6)</td>
<td>60.5 (0-408)</td>
<td>/</td>
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<tr>
<td>PBC-CBC Histology identical (%)</td>
<td>76%</td>
<td>52%</td>
<td>0.006</td>
</tr>
<tr>
<td>Lobular/Ductal (%)</td>
<td>41%/59%</td>
<td>41%/59%</td>
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</table>

Results: Patient with S-CBC are median 7 years older than patients with M-CBC (p-0.007). S-CBC is more likely to be of the same histological type (76%) than M-CBC (56%) (P- 0.006). In the whole analyzed group, and each subgroup separately, lobular carcinoma is registered in higher percentage (41%) than expected. For all other characteristics (tumor grade, estrogen/progesterone receptors and HER2 status) there was no statistical difference.

Conclusion: According to these results, it seems that patients destined to develop S-CBC or M-CBC, as a first recurrence site, have a greater susceptibility to lobular carcinoma, because this histology type was confirmed in significantly higher percentage than expected. Whether this reflects different genetic susceptibility for S-CBC and M-CBC, and what could be implications on further prognosis, is yet to be analyzed.

Disclosure: All authors have declared no conflicts of interest.