EXOSOMES FROM PLASMA OF HNSCC PATIENTS TREATED WITH PHOTODYNAMIC THERAPY ARE BIOMARKERS FOR EPITHELIAL-MESENCHYMAL TRANSITION

Introduction Photodynamic therapy (PDT) is a palliative treatment option for patients with head and neck squamous cell carcinoma (HNSCC). We and others have shown that PDT induces a local inflammatory reaction with the potential to initiate antitumor immune responses. Recent studies indicate that cancer cells can change their morphology after PDT due to cytoskeleton alterations and decreased cell adhesion. To see whether the cargo of exosomes released by these cancer cells reflects cellular alterations after PDT, plasma was collected from PDT-treated patients prior to and at time points after therapy for exosome isolation and molecular characterisation.

Material and methods HNSCC patients (n=9) were treated with PDT in a palliative setting. All patients had previously undergone several oncologic treatment regimens. Blood samples were taken before and after PDT. Exosomes were isolated from plasma by mini-size exclusion chromatography and were co-incubated with cancer cells. Following co-incubation, tumour cell migration, proliferation and chemotaxis were measured. Expression on tumour cells of the EMT markers: Vimentin, EpCAM (by flow cytometry, PCR, immunofluorescence), Snail, Twist, ZEB1, Slug (by PCR), E-Cadherin, N-Cadherin (by flow and PCR) was determined. Total plasma exosomes were separated by immune capture on beads into CD3 + and CD3 neg fractions. The CD3 neg exosomes enriched in tumor-derived vesicles were tested by on-bead flow cytometry for the presence of E-Cadherin- and N-Cadherin.

Results and discussions Exosomes harvested pre- and 24 hour after PDT contained high levels on N-Cadherin. In contrast exosomes isolated on day 7 and 4–6 weeks after PDT contained high levels of E-Cadherin. Exosomes collected before and 24 hour post PDT and co-incubated with tumour cells altered cell morphology and induced mesenchymal features, including co-expression of Vimentin, N-Cadherin, snail, slug, twist and ZEB1. In functional experiments, exosomes collected pre- and 24 hour after PDT significantly enhanced migration, proliferation and chemotaxis of tumour cells relative to exosomes harvested at two later time points.

Conclusion We have shown for the first time that PDT can change the mesenchymal character of tumour cells converting it into an epithelial phenotype, and that exosomes in plasma of the PDT–treated patients were responsible for mediating the conversion. In addition, plasma-derived exosomes served as biomarkers of response to PDT, as their molecular cargo reflected the phenotypic changes occurring in the tumour cells during therapy.
EMT and cell surface vimentin was shown in CSC. The present study aimed to evaluate vimentin expression in prostate cancer (PCa).

**Material and methods** 36 cases of PCa were immunohistochemically stained with rabbit polyclonal antibodies to vimentin (ThermoScientific). Membranous and cytoplasmic expression was evaluated separately semi-quantitatively in PCa as well as in the adjacent non-cancerous glands (NCG) with calculation of weighed staining index (WSI).

**Results and discussions** In the present study vimentin exhibited membranous and cytoplasmic expression. The pattern of staining varied in NCG and PCa. In the former membranous staining prevailed and was especially evident in benign prostatic hyperplasia, mainly in basolateral membranes. In NCG cytoplasmic vimentin expression was generally weaker than membranous and seen mostly in basal part of the cell. Conversely, in PCa vimentin was much more often expressed in cytoplasm, but staining was usually weak or moderate, and it was seen mostly in apical part of cytoplasm, while membranous staining was seen on the whole cell membrane. There was a patchy distribution of cell groups with membranous vimentin.

We propose that such membranous expression may correspond to cell-surface vimentin. Membranous staining in PCa was absent in 32.3% of cases, present in <5% of cells in 45.2%, 5%-20% in 16.1%, 20%-50% in 3.2% and in >50% of cells in 3.2%. For cytoplasmic staining the corresponding numbers were 0%, 6.5%, 32.3%, 16.1% and 32.3%. When WSI was counted, it was significantly higher for cytoplasmic staining in PCa and for membranous in NCG (p<0.05). In PCa, cytoplasmic vimentin was higher and membranous lower than in NCG (p<0.05). No significant associations were found of tumour EMT, and membranous vimentin seen in 50% in 16.1%, 20%-50% in 3.2% and in >50% of cases in 3.2%. For cytoplasmic staining the corresponding numbers were 0%, 6.5%, 32.3%, 16.1% and 32.3%. When WSI was counted, it was significantly higher for cytoplasmic staining in PCa and for membranous in NCG (p<0.05). No significant associations were found of tumour EMT, and membranous vimentin seen in 32.3% of cases as a sign of tumour EMT, and membranous vimentin seen in 67.7% of cases may also mark CSCs. We are planning to confirm the results of the present study in a larger series and the specificity of membranous staining with another antibody.

**PO-246** AXL RECEPTOR TYROSINE KINASE EXPRESSION AS A PROGNOSTIC MARKER AND THERAPEUTIC TARGET IN NEUROENDOCRINE TUMOURS.

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**Introduction** Neuroendocrine tumours (NETs) present a clinical challenge due to their late presentation, limited treatment options and a lack of accurate biomarkers to guide their management. Increasing evidence has confirmed the oncogenic potential of the receptor tyrosine kinase Axl, which is implicated in several hallmarks of cancer progression. This study aimed to evaluate the prevalence, prognostic role and therapeutic potential of Axl expression and its ligand Gas6 in NETs.

**Material and methods** Tissue microarray blocks were constructed from a consecutive cohort of 54 patients with surgically resected NETs. The prevalence of the expression of Axl and Gas6 as well as markers of hypoxia were identified and correlated with clinicopathologic features and overall survival. To test the hypothesis of whether Axl is involved in the metastatic progression of NETs, immunostaining for Axl and Gas6 was evaluated in paired primary and metastatic tumour specimens in a group of post mortem cases (n=7) with adequately preserved tissues.

**Results and discussions** In the 54 consecutive patients, n=46 (85%) presented with a pancreatic primary, n=24 (44%) were well-differentiated tumours and 35% were metastatic. Axl/Gas6 overexpression was identified in n=28 (52%) of the resection specimens.

In isogenic primary/metastatic NETs, Axl was overexpressed in 29% of primary tumour specimens and 61% of metastatic deposits and interestingly, Gas6 was also expressed in 29% of primaries and 61% of metastases. Axl and Gas6 expression did not correlate with VEGF-A or Calix, nor was it predictive of overall survival in univariate analyses. However, Axl and Gas6 overexpression correlated significantly with decreased HIF-1α expression levels.

**Conclusion** Overexpression of Axl and Gas6 was found in >50% of NETs and correlated with decreased HIF-1α expression. However, it did not influence patient’s overall survival.

**PO-247** MESOTHELIN REGULATES INVASION AND PERITONEAL METASTIZATION OF OVARIAN CANCER

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**Introduction** Ovarian cancer is the fifth leading cause of cancer death in women, and is characterised by widespread metastatization in the peritoneal cavity. Mesothelin (MSLN) is overexpressed in several human cancers, including ovarian cancer, but its exact function remains unclear. The aim of the present study was to evaluate the role of MSLN expression in ovarian cancer progression/metastatization using in vitro and in vivo experiments.

**Material and methods** To study the function of MSLN in ovarian cancer progression/metastatization we generated ovarian cancer cell lines (OVCAR3 and OVCAR8) with stable downregulation of MSLN in isogeneic primary/metastatic NETs. Axl was overexpressed in 29% of primary tumour specimens and 61% of metastatic deposits and interestingly, Gas6 was also expressed in 29% of primaries and 61% of metastases. Axl and Gas6 expression did not correlate with VEGF-A or Calix, nor was it predictive of overall survival in univariate analyses. However, Axl and Gas6 overexpression correlated significantly with decreased HIF-1α expression levels.

**Conclusion** Overexpression of Axl and Gas6 was found in >50% of NETs and correlated with decreased HIF-1α expression. However, it did not influence patient’s overall survival.

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**Material and methods** To study the function of MSLN in ovarian cancer progression/metastatization we generated ovarian cancer cell lines (OVCAR3 and OVCAR8) with stable downregulation of MSLN expression using short-hairpin RNA's. The biological effects of MSLN downregulation were evaluated on migration, invasion, proliferation and apoptosis assays. Further, to disclose the role of MSLN expression in the peritoneal dissemination and metastatization we established peritoneal xenografts by injecting intraperitoneally OVCAR8 cells with and without downregulation of MSLN in athymic nude mice [NIH(II)s:nu/nu] (n=3).

**Results and discussions** Downregulation of MSLN decreases in vitro cell invasion, whereas in vivo animal experiments