• Invasive capacity was evaluated using Matrigel-coated transwells.

Results and discussions Through RT-qPCR, we could observe that ATRA (1 μM) and Lapatinib (1 μM) treatments, separately or in combination, were able to increase retinoic acid receptors RARα and RARγ and decrease RARβ receptor levels. Moreover, the same treatments induced an increment in E-Cadherin and reduced the expression of main pluripotential genes: NANOG, OCT4 and SOX2. Combination treatment induced growth inhibition in 4 T1 mammospheres and in their clonogenic capacity. Regarding parameters associated with malignant progression using Matrigel-coated transwells, we detect that combined treatment increase CSC invasive capacity, however in experimental metastases assays with pretreated-CSC, treatments separately or in combination significantly decreases lung colonisation.

Conclusion Treatments with both ATRA and Lapatinib were able to induce CSC differentiation and reduced their lung nesting ability, leading to a less malignant phenotype.

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NOVEL ANALYSIS OF CANCER CELL INVASION USING A THREE-DIMENSIONAL CULTURED TISSUE MODEL MIMICS HUMAN TISSUE ORGANISATION.

1S Iwai*, 2S Kishimoto, 1K Nishiyama, 3M Matsusaki, 2T Akagi, 3M Akashi. 1Osaka University- Graduate School of Dentistry, 2Nd Department of Oral and Maxillofacial Surgery, Suita- Osaka, Japan; 2Osaka University- Graduate School of Engineering, Department of Applied Chemistry, Suita- Osaka, Japan; 3Osaka University, Graduate School of Frontier Biosciences, Suita- Osaka, Japan

Introduction Preventing cancer metastasis requires a through understanding of cancer cell invasion. Two-dimensional (2D) culture systems are typically used to analyse these processes; for instance, wound healing and transwell assays are used to evaluate cell migration, whereas the Matrigel chamber assay is used to assess cell invasion. However, these may not reflect actual events that occur in human tissue. We developed a human cell-based three-dimensional (3D) cultured tissue model that mimics human tissue and used it evaluate cell invasion in human oral squamous cell carcinoma (OSCC).

Material and methods The 3D tissue structure consisted of five layers of normal human dermal fibroblasts along with human oral squamous cell carcinoma (OSCC). Material and methods The 3D tissue structure consisted of five layers of normal human dermal fibroblasts along with human oral squamous cell carcinoma (OSCC).

The results of invasion capacity using the 3D cultured tissue model were comparable to those obtained using conventional 2D culture-based transwell and Matrigel chamber assays. In addition, our findings using the 3D cultured tissue model are consistent with those of our previous study reporting that SAS-Venus cell migration and invasion were increased by stimulation with Wnt5b. Importantly, we clearly observed invasion of these cancer cells into the 3D tissue structure and HDLEC cells were established from the SAS-Venus and HSC-Venus cell lines through in vivo selection individually. Conventional methods were transwell chamber assay and Matrigel chamber assay also used for comparison.

Results and discussions The results of invasion capacity using the 3D cultured tissue model were comparable to those obtained using conventional 2D culture-based transwell and Matrigel chamber assays. In addition, our findings using the 3D cultured tissue model are consistent with those of our previous study reporting that SAS-Venus cell migration and invasion were increased by stimulation with Wnt5b. Importantly, we clearly observed invasion of these cancer cells into the 3D tissue structure and HDLEC cells were established from the SAS-Venus and HSC-Venus cell lines through in vivo selection individually. Conventional methods were transwell chamber assay and Matrigel chamber assay also used for comparison.

Conclusion Our 3D cultured tissue system allows detailed analysis of cancer cell migration and invasion in an...
environment that mimics actual human tissue. It is expected that the expression of genes and proteins involved in these processes. It is a very useful tool for analysing a process of cancer cell invasion through a human living tissue.

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**PO-243 EXOSOMES FROM PLASMA OF HNSCC PATIENTS TREATED WITH PHOTODYNAMIC THERAPY ARE BIOMARKERS FOR EPITHELIAL-MESENCHYME TRANSITION**

1MN Theodorakis, 2S Yenemi, 3K Lorenz, 4L Saban, 5P Schuler, 6C Brunner, 7T Hoffmann, 8A Theodorakis, 9L Whitehouse. 1University of Ulm, ENT Head and Neck Surgery, Ulm, Germany; 2Carnegie Mellon University, Department of Biomedical Engineering, Pittsburgh, USA; 3Hospital of German Armed Forces, Department of Oto-Rhino-Laryngology, Ulm, Germany; 4Geniki Klinik, Urology, Thessaloniki, Greece; 5University of Pittsburgh, Pathology, Pittsburgh, Germany

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**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− fractions. The CD3− enriched exosomes were isolated from tumour-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. Exosomes collected before and 24 hour post PDT and co-incubated with tumour cells underwent 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− fractions. The CD3− enriched exosomes were isolated from tumour-derived vesicles were tested by on-bead flow cytometry for the presence of E-Cadherin and N-Cadherin.

**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 reflects the phenotypic changes occurring in the tumour cells during therapy.