mesenchymal transition resulting in enhanced tumour aggressiveness. However, aside from its RAS-GAP function, it is not clear how NF1 mitigate cell invasion and mesenchymal transition. Herein, we show that the leucine-rich domain (LRD) of NF1 plays a role in cell invasion.

**Material and methods**

Patient-derived glioma cells was used in this study. Immunohistochemistry staining was used for assessing proneural and mesenchymal markers expression.

**Results and discussions**

Exogenous expression of LRD in NF1-knockdown glioma cells inhibited cell invasion in vitro and in vivo. Using immunohistochemistry staining for cancer stem cell markers associated with proneural (Sox2) and mesenchymal (CD44 and vimentin) subtype of glioblastoma, we found high Sox2 but low vimentin expression in LRD-expressing tumour when compared with the NF1-knockdown tumour.

**Conclusion**

Our data suggests that LRD may play a role in reverting mesenchymal GBM to a less invasive and therapy-sensitive subtype.

---

**PO-204 THE CONTRIBUTION OF EPITHELIAL TO MESENCHYMAL TRANSITION (EMT) TO THE METASTASES OF PANCREATIC CANCER**

1Q Müller*, 1K Schuck, 1A Kroemer, 2D Saux. 1Klinikum rechts der Isar Technische Universität München, Department of Internal Medicine II, München, Germany; 2Klinikum rechts der Isar Technische Universität München, Chair of Translational Cancer Research and Department of Internal Medicine II, München, Germany

10.1136/esmoopen-2018-EACR25.239

**Introduction**

The role of epithelial to mesenchymal transition in metastatic processes remains a controversial field. While EMT is easily accessible in vitro, tracking EMT cells in vivo has been difficult for a long time. Therefore we created a dual recombinase, dual fluorescence reporter system which can monitor EMT and non-EMT related processes in vitro.

**Material and methods**

Pdx1-Flp; FSF-KrasG12D; Fsp1-Cre; genotypes with two different fluorescence reporter systems (R26mTmG or R26Ai-65-tdTom) were used to specifically induce pancreatic cancer and label EMT events in vivo.

**Results and discussions**

R26mTmG labels unrecombined cells with a red fluorescence (tomato) while cre-recombined cells express a green fluorescence protein (eGFP). The R26Ai-65-tdTom reporter only labels Flp and Cre recombined cells with tomato. To monitor EMT events Fsp1 (S100A4) was administered to control the activation of Cre. Fsp1 plays a major role in the early stages of EMT and is normally non present in pancreatic tumour cells. An activation of Fsp1 – and therefore EMT induction – leads to a change in the fluorescence properties of the reporters as mentioned above. In addition, this change is irreversible, so a backswitch of the cell via mesenchymal to epithelial transition (MET) won’t influence the signal.

---

**PO-203 PEROXIREDOXIN 5 IN COLON CANCER PROMOTES EPITHELIAL-MESENCHYMAL TRANSITION**

DH Kim1,2, J.H. Lee1, D.S. Lee1,2, 1School of Life Sciences and Biotechnology- BK21 Plus KNU Creative BioResearch Group, Kyungpook National University, Daegu, South Korea; 2College of Natural Sciences, Kyungpook National University, Daegu, South Korea

10.1136/esmoopen-2018-EACR25.238

**Introduction**

Globally, colorectal cancer (CRC) is common cause of cancer-related deaths. The high mortality rate of patients with colon cancer is due to cancer cell invasion and metastasis. Initiation of the epithelial-to-mesenchymal transition (EMT) is essential for the tumorigenesis. Peroxiredoxins (PRX1-6) have been reported to be overexpressed in various tumour tissues, and involved to be responsible for tumour progression. However, the exact role of PRX5 in colon cancer remains to be investigated enhancing proliferation and promoting EMT properties.

**Material and methods**

In this study, we constructed stably overexpressing PRX5 and suppressed PRX5 expression in CRC cells.

**Results and discussions**

Our results revealed that PRX5 overexpression significantly enhanced CRC cell proliferation, migration, and invasion. On the other hand, PRX5 suppression markedly inhibited these EMT properties.

Our data suggests that LRD may play a role in reverting mesenchymal GBM to a less invasive and therapy-sensitive subtype.

**Conclusion**

Therefore, PRX5 can be used as a predictive biomarker and serves as a putative therapeutic target for the development of clinical treatments for human CRC.
Orthotopic transplantation of primary pancreatic tumour (PPT) cells into immunocompetent (wildtype C57BL/6) and immunodeficience (NOD/SCID IL2Rnull (NSG)) mice were performed to further investigate metastatic patterns and the influence of the immune system.

Analyses of tumour, disseminated and metastatic cells via immunofluorescence staining and FACS were performed.

**Results and discussions** We observed recombined (R) and non recombined (NR) metastases in all our experimental settings (endogenous; implanted - wildtype and immunodeficient - mice). Interestingly the ratio of R to NR metastases changed according to the organ (e.g. 10:2 in lungs; 1:10 in lymphnodes). Surprisingly, the amount of NR metastases (non-EMT) in total were significantly higher than recombined (75% NR). On a side note we found that the recombined (EMT) tumour cells were much more likely to become circulating tumour cells (CCTs). A 5- to 50-fold increase of the recombined cell percentage was observed in the blood compared to the PPT.

**Conclusion** The data suggest that there might be an EMT but also a non-EMT route to form metastases. Those routes seem to coexist and are to some point organ-specific. Also, there was no evidence for the amount of CCTs to predict the metastases pattern.

**PO-205** CHARACTERISATION OF THE INTEGRIN ALPHA V-DEPENDENT ADHESOME IN MDA-MB-435S MELANOMA CELLS

1 M Paraščić, 1D Humphries, 1D Nestić, 1D Majhen, 1A Dekanić, 1N Stopanović, 1D Sedda, 1I Weber, 1M Humphries, 1A Ambrović Ristov, 1Ruder Bošković Institute, Division of Molecular Biology, Zagreb, Croatia; 2Faculty of Biology Medicine and Health University of Manchester, Wellcome Trust Centre for Cell-Matrix Research, Manchester, UK

10.1136/esmoopen-2018-EACR25.240

**Introduction** Integrins are heterodimeric glycoproteins that bind cells to extracellular matrix proteins. Upon integrin clustering, multimolecular integrin adhesion complexes (IACs) form that facilitate the linkage between integrins and the actin cytoskeleton and permit bidirectional signalling. The αV integrin is expressed in most tumour cells where it regulates an array of cellular functions and plays a role in anti-tumour drug resistance. The aim of our work was to assess αV-dependent changes in IAC composition in MDA-MB-435S melanoma cells in order to better understand the increased sensitivity to paclitaxel and vincristine upon integrin αV knockdown.

**Material and methods** Integrin αV-specific shRNA was cloned into pSUPER.puro, transfected into MDA-MB-435S cells using Lipofectamine, and cell clones were selected using puromycin. The sensitivity of cells to antitumour drugs was determined using an MTT assay. Cell migration was monitored using a Transwell assay. IACs were isolated following crosslinking and their molecular composition analysed using mass spectrometry (MS)–based proteomics.

**Results and discussions** In two MDA-MB-435S-derived cell clones with decreased expression of integrin αV, expressing 15% (2αV) or 5% (3αV) of the control cells amount, increased sensitivity to paclitaxel and vincristine, decreased sensitivity to cisplatin, and decreased migration were observed in line with previous results obtained following transient transfection with integrin αV siRNA. In cell clones 2αV and 3αV, which were smaller than control cells and lacked stress fibres, the number of focal adhesions was shown to be significantly lower as observed by interference reflection microscopy and immunofluorescence detection of phospho-paxillin, phospho-FAK and phospho-Src. MS analysis of isolated IACs from control MDA-MB-435S, 2αV and 3αV cells identified 282 proteins, including 36 out of 60 consensus adhesome proteins. As expected, in clones 2αV and 3αV, integrins αV, β3 and β5 were detected at much lower levels compared with control cells. In addition, lower levels of talin-1 and –2, vinculin, alpha-actinin-4, tensin-3, filamin-A and -B, liprin β1 and plec- tin were detected.

**Conclusion** These data will enable follow-up analyses of the mechanisms of signalling by integrins αVβ3/5 and therefore represent a valuable resource to improve our understanding of the mechanisms involved in adhesion control of cell sensitivity to antitumour drugs and metastatic potential.

**PO-206** CALPONIN 3 (CNN3) PROMOTES EPITHELIAL TO MESENCHYMA TRANSITION AND DRUG RESISTANCE OF COLON CANCER CELLS

1WM Abdel-Rahman, 2V Nair, 3N Al-Khayal, 1University of Sharjah, Medical Laboratory Sciences- College of Health Sciences and and Sharjah Institute for medical Research SIMR-, Sharjah, United Arab Emirates; 1University of Sharjah, Sharjah Institute for medical Research SIMR, Sharjah, United Arab Emirates

10.1136/esmoopen-2018-EACR25.241

**Introduction** Calponin 3 (CNN3) is one of the three isoforms in the Calponin family of actin-binding proteins. It is expressed in smooth muscle cells and non-smooth muscle cells and is required for cytoskeletal rearrangement and wound healing. Epithelial to Mesenchymal Transition (EMT) is important step in the development of epithelial cancers during which the cancer cell exploit the preexisting wound healing program. Therefore, it is expected that CNN3 plays a role in EMT. The few available data on the role of CNN3 in cancer shows that CNN3 is expressed in mammary cells in response to ErbB2-overexpression and in Duke’s stage C of colon cancer, but there is no comprehensive analysis of its role in cancer which is the aim of this study.

**Material and methods** Total of 21 cell lines were included in this study (8 from breast cancer, 12 from colon cancer, and a cervical cancer cell (HeLa) as a positive control for EMT). CNN3 expression was examined by Western blotting, followed by siRNA silencing in the metastatic cell line SW620. The influence of CNN3 silencing on EMT was detected by EMT markers expression by Western blot and collagen invasion assay. Cell viability was performed after exposure to 5 Fluorouracil (5FU) using Sulforhodamine B (SRB) assay. Furthermore, CNN3 expression was examined by immunohistochemistry in 56 formalin-fixed paraffin-embedded (FFPE) colon cancer tissue samples.

**Results and discussions** CNN3 showed positive expression in 6/8 breast lines and 7/12 colon lines. The primary line SW480 was negative while its metastasis from the same patient (SW620) was positive, suggesting that CNN3 was associated with metastasis. The CNN3 silencing was >98% efficient in the metastatic cell line SW620. Silenced cells were less invasive compared to the control and showed...