Results: Seven of 12 liver metastases from colon carcinoma (58.33%) were positive for Prox1. We noticed Prox1 positive cells with nuclear expression pattern in tumour cells of liver metastasis from well (4 positive cases), moderately (2 positive cases) and poorly differentiated adenocarcinoma (1 positive case). We found a heterogenous distribution and different intensity of Prox1 expression. The highest number of Prox1 positive cells was observed at the edge between restant liver tissue and metastases. Few Prox1 positive cells were present in intratumoural area also, but with low intensity expression. Rare Prox1 positive reaction was observed into the cells lining vascular structure from tumour area.
Conclusions: To our knowledge, this is the first study which reported the presence of Prox1 positive cells in tumour cells of human liver metastases from colon carcinoma. Their distribution at the edge between liver tissue and tumour area suggests their involvement in the progression of liver metastases. Further studies will be needed to demonstrate the mechanism of Prox1 involvement in this type of liver metastasis. Our evidences concerning Prox 1 expression in vascular structures from liver metastases areas were not strong enough to support a lymphangiogenic process but it could be launch the question if such type of metastases are able to produce their own lymphathic vessels.

500 Anti-tumour effects of zoledronic acid and somatostatin analogues in murine androgen-independent neuroendocrine carcinoma as hormone-refractory prostate cancer model

K. Hashimoto[1], N. Masumori[1], T. Tanaka[1], K. Uchida[2], T. Tsukamoto[1].
[1]Sapporo Medical University School of Medicine, Urology, Sapporo Hokkaido, Japan, [2]Sanjukai Hospital, Urology, Sapporo Hokkaido, Japan

Background: The treatment strategy for patients with hormone-refractory prostate cancer (HRPC) eventually emerging during androgen deprivation therapy presents many challenges to oncologists. Neuroendocrine (NE) cells in cancerous tissue have biological and histological features that might affect progression to hormone-refractory status. Previously, we developed an NE allograft (NE-10) and its cell line (NE-CS) from the prostate of the LPB-Tag 12T-10 transgenic mouse. We demonstrated that NE carcinoma promoted the pulmonary metastasis of human prostate cancer cell line LNCaP as well as androgen-independent growth of LNCaP. It was hypothesized that NE cells could be a therapeutic target for HRPC. To clarify the treatment options for HRPC, we investigated whether NE-10 and NE-CS were controlled by several agents, including zoledronic acid (ZOL) and somatostatin analogues such as octreotide (SMS) and pasireotide (SOM), having potential anti-tumour activity.
Material and Methods: Nine-week-old male BALB/c nude mice, which were castrated and inoculated subcutaneously (s.c.) with a 50 mg tissue block of an NE-10 tumour, were treated for 6 weeks with ZOL (3 μg/body/week s.c.), SMS (2 μg/body/day s.c.), SOM (4 μg/body/day s.c.), ZOL plus SMS, ZOL plus SOM, or saline (an equal volume of solvent/day s.c.). The effects of treatment on tumour growth were determined by measuring tumour volume. *In vivo and vitro*, tumour cell apoptosis and proliferation were determined by immunohistochemistry using TdT-mediated dUTP-biotin nick-end labeling (TUNEL) and a Ki-67 antibody, respectively.
Results: Growth of NE-10 tumours in mice treated with ZOL, ZOL plus SMS, or ZOL plus SOM was significantly slowed compared to the saline control ($p = 0.003$, $p < 0.001$, $p = 0.001$, respectively). The number of TUNEL-positive cells per 1000 NE cells was significantly increased in tumours from mice treated with ZOL, ZOL plus SMS, or ZOL plus SOM compared to the saline control (means: 9.2, 11.6, and 12.7, respectively, vs. 2.4, $p < 0.001$). In contrast, the number of Ki-67 positive cells per 1000 NE cells was significantly decreased in tumours from mice treated with ZOL, ZOL plus SMS, or ZOL plus SOM compared to the saline control (means: 5.3, 8.3, and 4.2, respectively, vs. 15.9, $p < 0.05$). *In vitro*, ZOL induced time- and dose-dependent growth inhibition and apoptosis of NE cells involving Ras/MAPkinase pathway via mevalonate pathway inhibition. Neither SMS nor SOM induced growth inhibition of 50% or greater in NE cells.
Conclusions: These results suggest that ZOL induces growth inhibition and apoptosis of murine androgen-independent NE carcinoma, which supports the possibility that ZOL can be an effective therapeutic agent for HRPC.

501 Analysis of the contractility of prostatic cancer-associated fibroblasts in a 3D collagen gel contraction assay

A. Henke[1], O. Brigid[1], E. Katz[2], G. Stewart[3], A. Riddick[3], A.A. Thomson[1].
[1]Medical Research Council – Human Reproductive Sciences Unit, Prostate Group, Edingburgh, United Kingdom, [2]University of Edinburgh, Breakthrough Research Unit, Edingburgh, United Kingdom, [3]University of Edinburgh, Department of Urology, Edingburgh, United Kingdom

Background: In recent years, the stroma of several cancer types has been recognized as a source of pro-tumourigenic signals mediated via e.g. paracrine signaling from cancer associated fibroblasts (CAFs) or physical stimuli such as tissue stiffness. Tissue stiffness can be regulated by e.g. lysyl-oxidase (LOX), as this enzyme cross-links proteins of the extracellular matrix, and has been shown to promote tumour progression.
Our aim was to investigate the effects of TGFβ1 protein and neutralizing antibody, and the LOX inhibitor bAPN upon the contractility of prostate CAFs and non-cancer prostate fibroblasts using a collagen contraction assay (CGC).
Methods: CAF and non-cancerous fibroblasts were isolated via tissue culture from patients undergoing transurethral resection of the prostate, and used at passages 4–7 for CGC assays and proliferation assays.
Cell proliferation and cytotoxicity of compounds were assayed in 96 well plates with the MTS assay provided by Promega's CellTiterOne Assay.
The CGC assay serves as surrogate assay for tumour stiffness and CAF activity. Cells were used in 500 μl collagen type I gels in a 24 well plate format. After gel solidification, 1ml medium was added per well, the gel mechanically released and photographed 24 and 48 hours later. The area of gels was measured with ImageJ.
Results:
1. CAF were more contractile than non-tumourigenic fibroblasts
2. Non-tumourigenic fibroblast gels were inhibited in a dose-dependent manner by bAPN but not by TGFβ1 protein or a TGFβ1-neutralizing antibody (nAB).
3. Contraction of CAF gels was not significantly inhibited or enhanced by bAPN, TGFβ1 or TGFβ1-nAB
4. Proliferation assays: no significant effects upon growth were observed following treatment with bAPN, TGFβ1 protein or a TGFβ1-nAB

Conclusions: There was a significant difference between CAFs and non-tumourigenic fibroblasts in regard to their contractility and in responsiveness towards the LOX inhibitor bAPN. This suggests that tissue stiffness is influenced not only by LOX but also by other factors. Furthermore, it appears that CAF themselves can be resistant to treatment. However, this does not exclude the possibility that other compounds might actually prevent tumour stiffness and/or pro-tumourigenic signaling pathways.

502 Mammospheres phenotype in expressing hormonal receptors and triple negative breast cancer cell lines

M. Laranjo[1], M.J. Carvalho[2], A.M. Abrantes[1], I. Torgal[3], C.F. Oliveira[4], M.F. Botelho[1]. *[1]Biophysics and Biomathematics Institute IBILI CIMAGO, Faculty of Medicine of Coimbra University, Coimbra, Portugal, [2]Gynaecology Service University Hospitals of Coimbra Biophysics and Biomathematics Institute IBILI CIMAGO, Faculty of Medicine of Coimbra University, Coimbra, Portugal, [3]Gynaecology Service University Hospitals of Coimbra, Faculty of Medicine of Coimbra University, Coimbra, Portugal, [4]CIMAGO, Faculty of Medicine of Coimbra University, Coimbra, Portugal*

Background: Clinical behavior of breast cancer is influenced by several parameters, particularly the expression of hormonal receptors (HR) and HER2 overexpression.
The application of certain growth factors (as bFGF and EGF) *in vitro* develops groups of spherical colonies in suspension with stem cell properties designated spheres. In breast cancer have been identified a subpopulation with tumourigenic capacity. The expressing of $CD44^+/CD24^{low}$ is being studies as possibly stem cell markers.
The objective of this study is to characterize the CD44 and CD24 expression in cells exposed to bFGF and EGF. We aim to analyze the differential expression profile in breast cancer cell lines expressing HR as well as triple negative.
Material and Methods: The adherent cell lines MCF7 (HR positive) and HCC1806 (triple negative) were propagated according with ATCC recommendations. Subsequently cells were rinsed 10 minutes in trypsin-EDTA 0.25%. Mammospheres protocol consisted on cell cultured in 10 mL DMEM-F12 supplemented with 10 ng/ml bFGF and 20 ng/ml EGF. The medium of each culture was renewed every 2 days during 15 days. Afterwards were analyzed in a FACS Canto II flow cytometer, with monoclonal antibodies anti-CD44 and anti-CD24. The results were interpreted in the form of mean fluorescence intensity (MFI) for the receptors studied.
Results: The breast cancer cell line HCC1806 exposed to growth factors developed a sparse population of suspense cells. Comparing with controls not exposed to mammosphere protocol, the mammospheres expressed CD44 in a higher degree than the controls. This difference was substantial considering a subpopulation of suspense cells representing 1%. In HCC1806, the expression of CD24 decreased after mammospheres protocol, despite this difference being scarce.
Focusing on MCF7, the treatment with growth factors developed dense suspense groups of spheres. In this group, the cells gained considerable CD44 expression in most of the suspense population (99%), comparing with the controls. The expression of CD24 diminished on treated cells, but not markedly.
Conclusions: The mammospheres protocol formation developed a minority of cells $CD44^+/CD24^{low}$ in triple negative cell lines. On the contrary, this phenotype was more frequent in breast cancer cells expressing HR in the same conditions. These differential phenotypes may represent a higher stem cell population in HR positive than in triple negative breast cancer cells.