**PO-179** BREAST CANCER CELLS AND MACROPHAGES IN A PARACRINE-JUXTACRINE LOOP

1S Oral, 2M Turk, 3G Bati Ayas, 4D Pesen Okur*, 1Izmir Institute of Technology, Biotechnology, Izmir, Turkey; 2Izmir Institute of Technology, Molecular Biology and Genetics, Izmir, Turkey; 3Izmir Institute of Technology, Biomedical Engineering, Izmir, Turkey

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**Introduction** Metastasis is the leading cause of death for cancer patients. As cancer cells metastasize, they interact with various extracellular molecules and stromal cells such as macrophages. Macrophages have been shown to promote invasion and change multicellular organisation of cancer cells. Breast cancer cells (BCC) and macrophages are known to interact via epidermal growth factor (EGF) produced by macrophages and colony stimulating factor-1 (CSF-1) produced by BCC. Despite contradictory findings, this interaction is perceived as a paracrine loop. Yet, an in-depth understanding of the mechanistic basis of this interaction is lacking: It is not known whether the interactions between breast cancer cells and macrophages are based on chemotaxis or haptotaxis or direct contact.

**Material and methods** We used a multidisciplinary approach including classical and up-to-date techniques such as cell-on-a-chip devices. MDA-MB-231 and RAW264.7 cell lines were used to represent BCC and macrophages, respectively. Secretion of EGF and CSF-1 was determined using ELISA. Cell adhesion and motility were assessed via live cell imaging in the presence and absence of iressa. Classical 3D cell culture, cell-on-a-chip 3D cocultures and custom designed 3D cell-on-a-chip cultures were performed to determine migration of cells in matrigel and collagen. Immunofluorescence and live cell imaging were used to determine endocytosis of EGF receptors.

**Results and discussions** BCC did not show chemotaxis to macrophages in custom designed 3D cell-on-a-chip devices, which was in agreement with ELISA results showing that macrophage-derived-EGF was not secreted into macrophage-conditioned-medium. Live cell imaging of BCC in the presence and absence of iressa showed that macrophages but not macrophage-derived-matrix mediated adhesion and motility of BCC in 2D. 3D co-culture experiments in matrigel and collagen showed that BCC changed their multicellular organisation in the presence of macrophages. In custom designed 3D co-culture cell-on-a-chip devices, macrophages reduced and promoted migration of BCC in matrigel and collagen, respectively. Furthermore, adherent but not suspended BCC endocytosed their EGF receptors when in contact with macrophages.

**Conclusion** Collectively, our data revealed that macrophages showed chemotaxis towards BCC-derived-CSF-1 whereas BCC required direct contact to interact with macrophage-derived-EGF. We propose that the interaction between cancer cells and macrophages is a paracrine-juxtacrine loop of CSF-1 and EGF, respectively.

**PO-180** TRANSFORMING GROWTH FACTOR BETA 1 ACTIVATES CELL MIGRATION THROUGH THE DOWN-REGULATION OF PRH/HHEX EXPRESSION

1E. Marcolino de Assis Junior*, 2PS Jayaraman, 3K Gaston, 1University of Bristol, School of Biochemistry, Bristol, UK; 2University of Birmingham, College of Medical and Dental Sciences- Institute for Cancer and Genomic Sciences, Birmingham, UK; 3University of Nottingham, Division of Cancer and Stem Cells- School of Medicine, Nottingham, UK

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**Introduction** The transcription factor PRH/HHEX (Proline-Rich Homeodomain/Haematopoietically Expressed Homeobox) controls cell proliferation, cell differentiation and cell migration/invasion in a diverse range of cell types. Our recent work showed that PRH expression is down-regulated in breast and prostate cancer cells and that this results in increased cell proliferation and cancer cell invasion. We also showed that PRH inhibits breast and prostate cancer cell migration and invasion in part at least by activating the transcription of Endoglin, a Transforming Growth Factor beta1 (TGFβ1) co-receptor. Here we show that TGFβ1 down-regulates PRH expression in prostate cells and thereby up-regulates cell migration.

**Material and methods** Normal immortalised prostate epithelial cells (PNT2-C2 cells) and prostate adenocarcinoma cells (PC3 cells) were treated with TGFβ1 (5 ng/ml) or vehicle for 48 hours. mRNA expression was then assayed using quantitative PCR. Protein expression was assayed using western blotting and immunofluorescence microscopy. Cell migration was analysed using chemotaxis assays. pSMAD3 binding was examined using quantitative chromatin immunoprecipitation assays.

**Results and discussions** The treatment of immortalised prostate epithelial cells and prostate cancer cells with TGFβ1 increases cell migration. In immortalised prostate epithelial cells this is accompanied by morphological changes suggestive of an epithelial to mesenchymal transition (EMT). In both immortalised prostate epithelial cells and prostate cancer cells TGFβ1 treatment results in the down-regulation of E-cadherin expression and the up-regulation of SNAIL expression, two established hallmarks of EMT. In both cell lines TGFβ1 treatment results in increased levels of the TGFβ1 effector pSMAD3, increased binding of pSMAD3 to the PRH/HHEX gene, and down-regulation of PRH expression at both the protein and mRNA level. Interestingly, PRH over-expression increases E-cadherin levels and represses cell migration.

**Conclusion** TGFβ1 treatment increases prostate cell migration in part at least by the down-regulation of PRH expression. PRH up-regulates E-cadherin expression and down-regulates cell migration suggesting that TGFβ1 alters E-cadherin levels and increases cell migration via its effects on PRH. Since PRH also regulates TGFβ1 signalling this creates a feedback loop that enables a more precise control of cell behaviour. Changes in PRH levels or activity during tumourigenesis disrupt this control mechanism.

**PO-181** PREDICTING HOMING ABILITY OF HEPATOCELLULAR CARCINOMA CELLS BY USING A LAB-ON-A-CHIP SYSTEM

12,4J Topel*, 12,4Y Yilmaz, 1A Gunes, 1,4E Bagirsakci, 1BA Gizem, 1,4D Celenci, 1,4G Bagci, 1E Kahraman, 4D Pesen Okur, 4N Atayeb, 1Izmir Biomedicine and Genome Center IBG, Cancer Signalling, Izmir, Turkey; 3These authors contributed equally, to this study, Izmir, Turkey; 2Institute of Health Sciences, Medical Biology and Genetics, Izmir, Turkey; 2Izmir Faculty of Medicine, Department of Biotechnology, Izmir, Turkey; 3Izmir Institute of Technology, Department of Molecular Biology and Genetics, Izmir, Turkey

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**Introduction** Lab-on-a-chip (LOC) systems can present cells multiple cues that cells are subjected to, including gradients of cytokines and secreted proteins from neighbouring cells, biochemical and mechanical interactions with the extracellular matrix, and direct cell-to-cell contacts in a controllable and
The upregulation of EPDR1 is related to identification of differentially methylated genes associated with colorectal cancer patients

**Introduction**

Colorectal cancer (CRC) is an important public health problem worldwide. In Chile, CRC is the fourth most frequent cancer and its incidence is rising. Sporadic CRC results from the accumulation of both acquired genetic and epigenetic changes that transform normal glandular epithelium into invasive adenocarcinoma. DNA methylation has an important role in colon carcinogenesis and also has been found involved in metastasis pathways of CRC. However, there are no studies that analysed whole genome in the search of specific methylated genes in metastasis of CRC, despite the next-generation sequencing platforms and methylation arrays available. Therefore, in this project it is proposed to find novel methylation markers of metastasis by comparing primary tumours and their corresponding lymph node metastasis, using a next-generation sequencing platform. The aim of this study was to identify differentially methylated genes associated with metastasis tumour behaviour in colorectal cancer.

**Material and methods**

Five paired FFPE samples of CRC primary tumour and its corresponding lymph node metastasis were used as homing targets to simulate bone, lung and liver, respectively. Image analysis was performed with fluorescent and confocal microscopy.

**Results and discussions**

Knockdown of the gene results in a decrease of cell proliferation, adhesion to collagen-coated plates, invasion and migration, while it was possible to observe an increase in necrosis among CRC cell lines. EPDR1 is more expressed in tumour than normal tissue among patients diagnosed with CRC. Interestingly, EPDR1 expression is directly related to T parameter, being higher among patients diagnosed with T3 and T4 CRC, independently from nodal involvement.

**Conclusion**

EPDR1 seems to be a new marker of tumour invasiveness in CRC patients and its detection could predict tumour infiltration.