BAALC-mediated signalling complexes involved in DNA repair were identified by immunoprecipitation.

Results and discussions BAALC, but not EV, overexpression resulted in elevated DNA fragmentation (comet assay), and sustained gH2AX and ATM activation (pSer1981) post-daunorubicin and etoposide-induced DNA damage, indicative of an inability to repair the damage. Gene expression analyses in BAALC overexpressing cells identified that the expression of components (*RAD51D*, *TP53BP1*, *BTG2*) of the high-fidelity homologous recombination double strand break repair pathway were significantly decreased. Furthermore, BAALC interacts with several proteins (RAD51, CaMKII, dermcidin) implicated in DNA repair. Further supporting a role for BAALC overexpression in inducing defective homologous recombination repair, BAALC overexpressing AML cells were hypersensitive to cisplatin treatment.

Conclusion As perturbations in DNA repair pathways in AML have been associated with increased resistance to a range of chemotherapeutics, and BAALC overexpression is associated with primary refractory AML, our data suggests that BAALC overexpression induces decreased chemosensitivity by perturbing DNA repair pathways.

PO-145 ERK5 PATHWAY INHIBITORS INHIBIT THE MAINTENANCE OF CHRONIC MYELOID LEUKAEMIA STEM CELLS

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Introduction Chronic myeloid leukaemia (CML) is a hematopoietic stem cell (HSC)-driven neoplasia characterised by the expression of the constitutively active tyrosine kinase BCR/ABL. CML therapy based on tyrosine kinase inhibitors (TKi) is highly effective in inducing remission but not in targeting leukaemia stem cells (LSC), which sustain the minimal residual disease and are responsible for CML relapse following discontinuation of treatment. Our aim was to address the effects of the inhibition of the ERK5 pathway on the maintenance of CML LSC.

Material and methods KCL22 and K562 CML cell lines, patient-derived CML cells or CD34 +peripheral blood cells from healthy donors (informed consent) were incubated in normoxic or hypoxic (0.1% O_2) primary cultures (LC1) in the presence or the absence of drugs. At the end of incubation (day 7), cells were analysed on a flow cytometer to determine the expression of stem cell markers or transferred to drug-free normoxic secondary cultures (LC2) to measure LC2 repopulation as a read-out of progenitor/stem cell potential (CRA assay). In the serial Colony Formation Ability (CFA) assay colonies were scored on day 7 of each passage (III passages). In the Long-Term Culture-Initiating Cells (LTC-IC) assay the number of colonies was scored after 14 days. Compounds: XMD8-92 (ERK5 inhibitor) and BIX02189 (MEK5 inhibitor); imatinib and dasatinib (BCR/ABL inhibitors).

Results and discussions In CML patient-derived cells and cell lines, we found that the MEK5/ERK5 pathway is active and necessary for optimal proliferation in low oxygen, a condition typical of normal hematopoietic and leukemic stem cell niches. Treatment of primary CML cells with XMD8-92 or BIX02189, but not with TKi, strikingly reduced Culture Repopulation Ability (CRA), serial Colony Formation Ability and Long-Term Culture-Initiating Cells (LTC-IC). Importantly, inhibition of MEK5/ERK5 was effective on CML cells regardless of the presence or absence of imatinib (IM), and did not reduce CRA or LTC-IC of normal CD34 +cells. Interestingly, in hypoxia, combined treatment XMD8-92/IM decreased the expression of genes relevant for stem cell maintenance such as c-MYC, SOX2 and NANOG and the expression of CD26, a CML LSC marker.

Conclusion We propose ERK5 pathway inhibitors as a novel therapeutic approach to prevent CML relapse and, in combination with TKi, enhance induction of remission.

Metastases and EMT

PO-146 MULTIPLE MYELOMA-DERIVED EXOSOMES CARRY AMPHIREGULIN AND ARE RESPONSIBLE FOR THE UNCOUPLED BONE REMODELLING

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Introduction Multiple myeloma (MM) is a hematologic malignancy associated with osteolytic bone disease caused by the perturbation of the functional balance between bone resorption and bone formation. Exosomes, nanosize lipoproteic structures, have been recently recognised as a new mechanism of cell to cell communication during tumour growth and progression. We have previously shown that MM-exosomes are involved in osteolytic lesions but the underlying mechanism is still understood. We hypothesise that the epidermal growth factor receptor ligand Amphiregulin (AREG) can be delivered by multiple myeloma-derived exosomes and participate in modulating the response of the bone microenvironment to the tumour.

Material and methods Exosomes were isolated from the conditioned medium of MM1 cell line and from BM plasma samples of patients. In order to test whether MM-exosomes could affect osteoclastogenesis through the activation of the EGFR pathway, primary CD14 +monocytes and a murine cell line (RAW264.7) were used as osteoclast (OC) models. Cells were treated with exosomes from both MM1 and plasma samples, pre-treated or not with anti-AREG neutralising antibodies and OC specific markers were measured. In addition, to further explore whether exosomes were able to promote osteoclastogenesis by affecting mesenchymal stem cells, hTERT-MSC were treated with exosomes; the conditioned medium were collected to measure the secretion of IL8 and to stimulate primary CD14 +monocytes.

Results and discussions We found that AREG was specifically enriched in exosome samples, leading to the activation of EGFR in pre-OC. In addition we showed a significant increase of the expression of the OC markers Cathepsin K, Matrix Metalloproteinases 9 and Tartrate-resistant Acid Phosphatase in

RAW 264.7 and CD14 +cells after treatment with MM-derived exosomes as compared to the control. The effects of MM-derived exosomes on OC activation were significantly abrogated by exosome pre-treatment with anti-AREG neutralising Ab directly on pre-osteoclast cells and indirectly by inhibiting IL8 release in MSC.

Conclusion Taken together our data indicate that MM-derived exosomes are responsible for the uncoupled bone remodelling, affecting directly osteoclast function and promoting IL8 release from mesenchymal stromal cells. In this context, AREG packed into MM-derived exosomes, represents a potential new player in MM-induced bone resorption.

PO-147 PINOSTILBENE HYDRATE SUPPRESSES HUMAN ORAL CANCER CELL METASTASIS VIA DOWNREGULATION MATRIX METALLOPROTEINASE-2 THROUGH THE MITOGEN-ACTIVATED PROTEIN KINASE SIGNALLING PATHWAY

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Introduction Oral squamous cell carcinoma (OSCC), the most common primary malignancy occurring in the head and neck, the 5 year survival rate of OSCC patients is below 50% after treatment, owing to cancer cells are highly metastatic and relapse. Despite advances in diagnostic techniques and improvements in treatment modalities, the prognosis of OSCC remains poor. Therefore, an effective chemotherapy regimen that enhances tumour sensitivity to chemotherapeutics is urgently required. Pinostilbene hydrate (PSH), a methylated derivatives of resveratrol, has been reported to possess antioxidative, cardioprotective and anticancer properties.

Material and methods Cell viability was examined by MTT assay, whereas cell motility was measured by migration, invasion and would healing assays. Zymography assay and Western blot was used to analyse matrix metalloproteinase-2 (MMP-2) protein activity, protein level and mitogen-activated protein kinase (MAPK) pathway protein level in oral cancer cells.

Results and discussions In this study, we demonstrate that PSH attenuated SCC-9 cells migration and invasion in a dose-dependent manner. The anti-metastatic activity of PSH was downregulated by MMP-2 protein activity and protein expression in SCC-9 cells. Also, PSH did inhibit the effects of MMP-2 by reducing the activation of mitogen-activated protein kinases (MAPKs) in SCC-9 cells.

Conclusion These results demonstrate that Pinostilbene hydrate may be a potent adjuvant therapeutic agent in the prevention of oral squamous cell carcinoma.

PO-148 SUPPRESSION OF CARNOSINE ON ADHESION AND EXTRAVASATION IN HUMAN COLORECTAL CELLS

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Introduction Colorectal cancer is the third most commonly diagnosed cancer in the word. Carnosine is an endogenous dipeptide found in muscle and brain tissues. The aims of this study are to investigate the regulation of carnosine on adhesion and extravasation of metastasis in human colorectal cancer.

Material and methods The human colorectal cancer cells (HCT-116) and human umbilical vein cell line (EA. hy926) cultured were as experimental models. Those adhesive and extravasate abilities and regulate molecular expression were analysed in this study.

Results and discussions In adhesion ability assay results shown carnosine were significantly decreased HCT-116 cells adhesion to endothelial cells (EA. hy926 cells). From adhesion-regulating proteins expression analysis result show that carnosine was decreased the levels of integrin in HCT-116 cells and ICAM-1 and E-selectin in EA hy926 cells after 24 hour incubation ($p<0.05$). The transepithelial electrical resistance (TEER) of the monolayer EA. hy926 endothelial cell was significantly increased after carnosine treatment for 24 hour. The phosphorylation of VE-cadherin was significantly reduced after EA. hy926 cells treated with carnosine ($p<005$). And, when EA. hy926 cells treated with carnosine, the protein levels of ROCK and RhoA, and levels of ROS were significantly increased in EA. hy926 cells ($p<0.05$). Furthermore, the DNA-binding activity of NF-κB was increased after carnosine treatment.

Conclusion The results indicate that carnosine can inhibit the adhesion and extravasation through suppressing of carnosine on NF-κB transcript activation and its regulator adhesion and extravasation related molecular expression in HCT-116 cells and EA hy926 cells.

PO-149 VIMENTIN IN EPITHELIAL CANCER CELLS DURING ADAPTATION TO THE GROWTH *IN VITRO*

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Introduction Epithelial-mesenchymal transition (EMT) plays key roles in all stages of cancer progression from primary tumour growth, invasion, and metastasis to resistance to chemotherapy. Earlier we have shown that EMT phenotype in ovarian cancer tumours associated with the increase in the metastatic potential and resistance to anticancer therapy; EMT markers were more often discovered in ascites as compared to solid cancers. The main goal of the present work was the analysis of EMT phenotype during cancer cell adaptation to the growth *in vitro*.

Material and methods Cell lines were purchased from ATCC and were cultured *in vitro* according to the manufacturer's recommendations. Level, intensity and integral index (product of level and intensity) of *de novo* expression vimentin (EMT marker) was estimated in cell cultures originated from solid tumours and extracellular fluids by immunofluorescence assay associated with flow cytometry. Primary anti-vimentin