Results and discussions The relative mRNA expression of VCAN and its isoforms were found significantly higher in MM patients in both BMMNCs and BMMSCs with higher expression in BMMSCs than in BMMNCs. VCAN being produced in stroma found at lower levels in MM cell lines. Furthermore, BMMSCs CM showed the presence of VCAN whose effect was evaluated in MM cell lines in vitro. Upon treatment with BMMSCs CM, proliferation and angiogenesis increase while apoptosis decrease in cell lines, however, effect of CM neutralised in presence of VCAN antibody. The downstream signalling of VCAN was observed to entail phosphorylation of FAK and STAT3 which subsides by treatment with VCAN antibody.

Conclusion Augmented levels of VCAN and its isoforms in BM of patients especially in BMMSCs imply their involvement in BM microenvironment of MM. The neutralisation of the oncogenic effect of BMMSCs CM upon treatment with VCAN antibody affirms the plausible role of VCAN in pathogenesis of MM. These findings open up new avenues for exploration of VCAN as a therapeutic target for treatment of MM in future.

PO-286 EVALUATING THE INFLUENCE OF MESENCHYMAL STROMAL CELLS DERIVATIVES ON EARLY STAGE OF BREAST CANCER

Introduction Tumour microenvironment plays a decisive role in cancer development and metastasis, and affects the therapeutic effectiveness of anticancer drugs. Mesenchymal stromal cells (MSCs) are an important elements of tumour stroma. MSCs can both stimulate and inhibit tumour progression, depending on the components of microenvironment, genesis and stage of cell differentiation. Special attention focused on the paracrine effect of products secreted by MSCs. The aim of this study was to characterise the influence of derivatives from human bone marrow MSCs on proliferation, survival, receptor profile of MCF-7 in 2D and 3D cell cultures in vitro.

Material and methods The monolayer MCF-7 cell culture was cultured in standard conditions in DMEM nutrient medium (Sigma, USA), with 2 mM l-glutamine (Sigma, USA), 40 mg/mL Gentamicin (Biopharma, Ukraine). The initial density of MCF-7 cells was 2 × 10^4 cells/cm². Human bone marrow multipotent mesenchymal stromal cells (MSCs) were used. MCF-7 cells were incubated in fully conditioned media from MSCs in the ratio 1:1. For the initial generation of spheroids the DMEM nutrient medium with 2% carboxy-methyl cellulose (Bio-Rad, USA) was used. Plates with spheroids were being incubated on an orbital shaker (PSU-10i, Biosan, Latvia) at 80 rpm for 3–5 hours. The spheroid culture was maintained for 7 days. Cell viability was evaluated by MIT assay. The Stemis2000 software Axio Vision Red 4.7 (Zeiss, Germany) was used for processing the images. The

PO-285 IMMUNOMODULATORY EFFECTS OF LACTOBACILLUS STRAINS: EMPHASIS ON IDENTIFICATION OF PROBIOTIC CANDIDATES WITH ANTI-TUMOUR RESPONSES

Introduction Mucosal macrophages are essential for driving immune responses in mucosal membranes. In homeostatic environments, regulatory responses are predominated by the M2 subset, whereas inflammatory responses are mediated via their ability to switch to a pro-inflammatory M1 subset. Tumour-associated macrophages (TAMs) have been found to play a fundamental role in tumour development. Therefore, the aim of this study was to identify macrophage subset-specific responses to a panel of Lactobacilli with the objective of determining candidates that elicit anti-tumour responses for future in vitro management of colorectal cancer.

Material and methods A panel of candidate strains; L. plantarum (C28, VD23, MS18) and L. salivarius (MS3, MS6, MS16) were grown in MRS broth and harvested at the end of the exponential phase. The human monocytic cell line THP-1 was differentiated into M1 and M2 macrophages. Pro-inflammatory cytokine profile (IL1β, IL18, IL23 and TNFα) was investigated upon macrophage culture with Lactobacilli and quantified by sandwich ELISA. Phagocytic properties were measured by culturing macrophage subsets with CFSE-loaded bacteria and analysed by flow cytometry.

Results and discussions MS3 strain was the biggest inducer of pro-inflammatory cytokine secretion. This strain was even more potent inducer of IL1β, IL18 and IL23 secretion in M2-like macrophages and would represent the most appropriate strain to re-programme M2-like TAMs. VD23 and C28 exhibited a TNFαβ, IL1βα, IL18α, IL23β phenotype. The phagocytic response was generally greater for M2 macrophages, whereby the greatest phagocytosis was observed with VD23 strain (MFI ratio 4,06±0,67) and the lowest response with the MS6 strain (MFI ratio 1,38±0,08).The highest phagocytic properties in M1 macrophages were observed with MS6 (MFI ratio 3,36±0,21), whereas the lowest was with MS3 (MFI ratio 1,28±0,12).

Conclusion Selection of potential anti-tumour strains would be dependent on tumour stage, facilitating an M2-like phenotype for earlier stages and an M1-like phenotype for later stages. VD23 would appear to be the best strain to programme anti-inflammatory activity in M1/M2s whereas MS3 would represent the most appropriate strain to re-programme M2-like TAMs. The findings of this study suggest new strategies to probiotic bacteria modulating immune responses, leading to the treatment of diseases by switching to favourable macrophage subsets.

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volume of aggregates was calculated by Bjerkvig formula after 7 days of cultivation. Markers were detected by applying IHC method with primary monoclonal antibodies Ck (clone AE1/ AE3, IS053, Dako, USA), vim (Clone V9, IS630, Dako, USA), EpCAM (Sigma, HPA026761, USA).

**Results and discussions** Incubation of tumour cells with c-medium from MSCs led to decreasing of cell viability by 45% and 60% after 48 and 96 hours, respectively, compared with the control samples. MSCs derivatives reduced the volume of tumour spheroids by 45% and 60% on 2nd and 4th day of cultivation respectively compared with the control. Expression of epithelial markers was increased under influence of MSCs derivatives in both 2D and 3D cell cultures, but expression of vimentin was only in 3D cell culture.

**Conclusion** MSCs derivatives inhibited proliferative activity, reduced migration ability and increased differentiation properties of breast cancer cells in 2D and 3D cell cultures of MCF-7.

**PO-288**

**STROMA CELLS INCREASE EXPRESSION OF TUMOR-PROMOTING RAC1B IN COLORECTAL CANCER CELLS**

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**Abstract**

**Introduction** An inflammatory microenvironment is a tumor-promoting condition that provides survival signals to which cancer cells respond with changes in their gene expression. One key gene regulatory mechanism that responds to extracellular signals is alternative splicing. For example RAC1B, a RAC1 alternative splicing variant that we previously identified in a subset of BRAF-mutated colorectal tumours, was found increased in samples from inflammatory bowel disease patients or following experimentally-induced acute colitis in a mouse model. The main goal of this work is to determine the pro-inflammatory signals that lead to increased RAC1B expression in colorectal cells.

**Material and methods** Caco-2 colorectal cells were either grown as polarised cell monolayer on porous filter membranes and then co-cultured with different stromal cell lines (fibroblasts, monocytes and macrophages) or grown as cysts in 3D matrices. RAC1B expression was analysed by RT-PCR, Western blot and confocal fluorescence microscopy.

**Results and discussions** Culture conditions for polarised 2D and 3D models were established as physiologically more relevant colon cell models. Co-culture experiments with polarised cells revealed that the presence of fibroblasts and/or M1 macrophages induced a transient increase in RAC1B protein levels in the colorectal cells, accompanied by a progressive loss of epithelial organisation. The cytokines secreted by fibroblasts and macrophages are currently being identified.

**Conclusion** Our data indicate that extracellular signals from stromal cells can affect gene expression in colorectal cancer cells. The observed increase in alternatively spliced RAC1B will help to understand the tumor-promoting effect of inflammation and identify novel therapeutic strategies.

**PO-289**

**IDENTIFICATION OF MICROENVIRONMENTAL REGULATION AND THERAPEUTIC TARGETING OF ONCgenic EF-2 KINASE IN PANCREATIC CANCER**

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**Abstract**

**Introduction** Pancreatic cancer (PaCa) is one of the most aggressive and deadliest cancer with 6 months average survival