assessors (1 surgery, 1 pathology). Survival was analyzed using Kaplan–Meier curves and the Log-rank test.
Results: There is increased tumour epithelial cytoplasmic staining for NR4A2, and an altered nuclear-cytoplasmic ratio with more cytoplasmic NR4A2 in tumour compared to matched normal tissue ($p = 0.001$). High tumour cytoplasmic NR4A2 is associated with a significantly worse overall and disease specific survival in colorectal cancer. (5-year DSS 64% high cytoplasmic NR4A2 versus 80% low cytoplasmic NR4A2, log-rank test $p=0.049$, 5-year OS $p=0.003$)
Conclusions: There is marked cytoplasmic mislocalisation of NR4A2 in colon cancer. High tumour cytoplasmic NR4A2 is associated with an adverse prognosis.

0921: LOCALISED INHIBITION OF MICRORNA-15 AND -16 RESULTS IN AN IMPROVED ANGIOGENIC RECOVERY FOLLOWING LIMB ISCHAEMIA

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Introduction: MicroRNAs (miRNA; miR) are small non-coding RNAs which negatively regulate mRNA translation. miR-15 & miR-16 are upregulated in response to myocardial ischaemia, whilst inhibition protects against cardiac ischaemic injury in a mouse model. We investigated the effect of adenovirus-mediated local miR-15/16 inhibition in a mouse model of hindlimb ischaemia.
Method: We generated an adenovirus capable of overexpressing a miRNA inhibitor (Ad.Decoy-15/16), containing multiple, tandem complementary binding sites for miR-15 and miR-16, and thus allowing specific and efficient depression of miR-15/16 expression. Unilateral hindlimb ischaemia was surgically induced in anaesthetised CD1 mice ($n=12$ per group) and Ad.Decoy-15/16 or an Ad.Null control (109 virus particles) were delivered to the ipsilateral adductor muscle.
Results: Blood flow to the ischaemic limb was significantly improved by Ad.Decoy-15/16 at 7 ($p<0.04$), 14 ($p<0.01$) and 21 ($p<0.03$) days post-surgery, with an average increase of ~50% compared to control mice. Subsequent histopathological analyses at day 21 demonstrated an increase in both capillary density (by ~30%, $p<0.041$) and arteriolar density (by ~40%, $p<0.03$) in the ischaemic adductor muscles of Ad.Decoy-15/16-treated mice compared with control.
Conclusions: These data indicate a pro-angiogenic effect of localised miR-15/16 inhibition in the setting of peripheral limb ischaemia in mice.

0942: THE ROLE OF MICRORNA-30C-2* AS AN ANTIANGIOGENIC MEDIATOR

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Introduction: MicroRNAs (miRs) negatively regulate the expression of targeted mRNAs. Here, we aim to elucidate the contribution of miR-30c2* in endothelial cell (EC) dysfunction in diabetes.
Methods: miR30c2* functional studies were performed in human umbilical vein endothelial cells (HUVECs) under conditions of high glucose (HG, mimics diabetes) and low growth factors (LGF, mimics ischaemia). Expressional analyses were performed in mice, with and without diabetes, using whole limb muscle and the ECs derived from these tissues.
Results: MicroRNA-30c-2* expression was upregulated under LGF, HG and a LGF/HG combination ($p<0.01$). In addition, miR-30c-2* was three-fold upregulated in muscular ECs of diabetic mice ($p<0.01$). miR-30c-2* overexpression induced HUVEC apoptosis and impaired angiogenesis. Conversely, miR-30c-2* inhibition prevented LGF- and LGF/HG-induced apoptosis and impaired angiogenesis.
The cell cycle regulator minichromosome maintenance complex component 7 (MCM7) is a putative miR-30c-2* target. MCM-7 mRNA levels were decreased in p75NTR-HUVECs ($p<0.05$ vs. Null) and in HUVECs cultured in HG/LGF ($p<0.05$). miR-30c-2* overexpression reduced MCM7 mRNA ($p<0.01$).
Conclusions: In conclusion, the p75NTR-induced miRNA miR-30c-2* represses EC survival and angiogenesis, and may prove to be a novel therapeutic target for diabetes-induced endothelial damage in limbs.

0967: CHARACTERIZATION OF INITIATING PHYSIOLOGICAL EVENTS OF MAMMARY GLAND INVOLUTION: EVIDENCE FOR CATHEPSIN B INVOLVEMENT

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Introduction: The mechanism of one of the most extensive physiological cell death in mammals, postlactational regression (involution) of the mammary gland, was recently characterized as a non-classical, lysosomal-mediated and caspase-independent pathway of cell death. Expression of cysteine proteinases cathepsins B and L was shown to be highly increased via Stat3 pathway during this process. The present study aimed to investigate the intracellular trafficking and potential involvement of cathepsins B and L in the physiological events leading to involution mediated by lysosomal membrane permeability.
Methods: Fluorescent immunohistochemical staining of mammary gland sections from wild type and Stat3 KO mice was performed for cathepsins B, L and α–lactalbumin, alongside co-staining of the lysosome associated membrane protein-2 (LAMP2). Intracellular (co-) localization was analysed by deconvolution fluorescence microscopy.
Results: Both cathepsins B and L, but not α–lactalbumin, showed strong co-localization with LAMP2, indicating lysosomal translocation, in the presence of functional wild-type Stat3. The co-localization with the lysosomal membrane was significantly reduced, most severely in the case of cathepsin B, in Stat3 KO mice compared with wild-type.
Conclusions: The strong Stat3-dependent lysosomal membrane localization of cathepsin B suggests a role for this proteinase in triggering the lysosomal membrane permeability associated with the mammary gland involution.

0988: M1 POLARISED MACROPHAGES DEVELOP AN ENDOTOXIN TOLERANCE-LIKE PHENOMENON IN RESPONSE TO BACTERIAL STIMULATION

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Introduction: To establish a predominant M1and M2 macrophage polarisation pattern in vitro. To examine the inflammatory cytokine response to bacterial stimulation in polarised cells. To determine the phagocytic activity in polarised cells after bacterial stimulation.
Methods: Peritoneal and bone marrow derived macrophages were harvested from C57BL/6 mice. Cells were exposed to polarising stimuli for 18-24 hours. (M1 - LPS and IFN-γ, M2 - IL-4). Polarised cells were further stimulated with heat-killed Staphylococcus aureus and Salmonella Typhi or FITC-labeled E coli. Inflammatory cytokine production and phagocytosis were assessed by ELISA and FACScan analysis.
Results: M1 macrophages were characterised by high levels of TNF-α and IL-12p70 and M2 macrophages by high levels of TGF-β and low levels of IL12p70. M1 polarized macrophages, when exposed to gram-positive bacteria, had lower levels of TNF-α than M2 macrophages. Phagocytosis assays revealed similar results for both macrophage subpopulations.
Conclusions: M1 macrophages are expected to produce higher levels of TNF-α, however we found that M1 macrophages, when stimulated with bacteria, had lower levels of TNF-α compared with their M2 counterparts. This unexpected result indicates a tolerisation effect developed during the M1 polarisation and further work is required to clarify the underlying mechanism(s).

1094: ASSOCIATION BETWEEN LEVELS OF MATRIX METALLOPROTEINASE-3 AND JOINT REPLACEMENT OF THE HIP AND KNEE IN RHEUMATOID ARTHRITIS

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Introduction: Within 20 years 25% of rheumatoid arthritis (RA) patients will undergo total joint replacement. Matrix metalloproteinase-3 (MMP-3) is released during the disease process. Previous studies suggest that MMP-3 levels decrease following joint replacement. The objective of this study was to investigate if serum levels of MMP-3 at baseline are associated with future joint replacement of the hip and knee.