significantly increased angiogenesis in endothelial cells and growth rate of cancer cells. We found that miR-619–5p directly targeted RCAN1.4 which has the capability to inhibit endothelial cell proliferation and angiogenesis and ectopic expression of miR-619–5p markedly decreased RCAN1.4 expression.

Conclusion Our findings provide the first evidence that tumor-derived MVs can promote angiogenesis in endothelial cells and aggressive phenotype in cancer cells through the transfer of miRNAs including miR-619–5p. Clinical validation and exploring the way of therapeutic intervention should be followed.

**PO-311**

**CHARACTERISATION OF COLORECTAL TUMOUR ENDOTHELIAL CELLS**

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**Introduction** Endothelial Cells (ECs) have a substantial influence on the tumour microenvironment. This aberrant tumour microenvironment mediates cancer progression. Although the tumour vasculature is understood to be unique, few studies have directly isolated and compared tumour-associated and normal endothelial vasculature. Understanding the specific characteristics of tumour endothelial cells, and their respective influence on tumours, may be a crucial step in developing effective and comprehensive, anti-tumour therapies.

**Material and methods** We applied a fully optimised 8 colour flow cytometry marker panel to characterise and compare endothelial cells from colorectal cancer biopsies, and normal-adjacent, non-involved colonic tissue. With the advent of the tumour vasculature ‘normalisation’ hypothesis, and cancer immunotherapy, understanding the specific characteristics of tumour endothelial cells, and their respective influence on tumours, may be a crucial step in developing effective and comprehensive, anti-tumour therapies.

**Results and discussions** Tumour-associated endothelial cells (TECs) express significantly less CD144, CD34 when compared to normal ECs (NECs). Conversely, TECs demonstrate higher expression of CD105. No consistent trend was observed for CD31 expression. Using this unique approach, we were able to uncover previously unidentified subpopulations within NEC, that are significantly altered in TEC. Additionally, using various gating strategies — we analysed specific subpopulations of cells that appear to be significantly increased or reduced between colorectal tumours and normal colon.

**Conclusion** Taken together, these results accurately depict a tumour endothelium that is functionally inept, and permit new insights into the origin, and adaptation, of tumour endothelial cells, which is still debated. The remarkable consistency between patients in CD54/CD34/CD105 expression may argue that MPIO of both types were largely present in macrophages in gadolinium-enhancing tumours in mice receiving RDG-MPIO, but not with RDG-MPIO. Molecular magnetic resonance imaging (MRI) using targeted microparticles of iron oxide (MPIO) has been used to image angiogenic vessels in solid tumours by targeting integrin αvβ3 with the peptide RGD. The aim of this study was to determine whether RGD conjugated MPIO can be used to detect angiogenic vessels in a mouse model of brain metastasis.

**Material and methods** Mice (n=34) were injected intracerebrally with 4T1 murine metastatic mammary carcinoma cells. Mice underwent MRI following intravenous injection of either RGD-MPIO or control scrambled RDG-MPIO, at days 7, 14, 21, 28 or 35 using a T2* weighted sequence to detect MPIO-induced hypointensities. Following imaging, brains were perfusion-fixed for histology.

**Results and discussions** In mice receiving RGD-MPIO, postcontrast hypointensities were evident at most time-points, and were significantly increased in tumour-bearing vs. contralateral striatum at day 35 (p<0.05). At days 7 and 14, a trend towards increased hypointensities was observed in RGD-MPIO injected mice compared to both the control hemisphere and mice injected with RDG-MPIO. No significant differences were found between the tumour-bearing and contralateral striatum in RDG-MPIO injected mice. Thus, hypointensities observed in mice injected with RGD-MPIO likely reflect specific binding to endothelial αvβ3.

At later stages, however, hypointensities were also observed in gadolinium-enhancing tumours in mice receiving RDG-MPIO and unconjugated MPIO. Histological analysis indicated that MPIO of both types were largely present in macrophages associated with tumour blood vessels, suggesting that perivascular macrophages may actively phagocytose MPIO once the BBB is no longer intact. Nevertheless, endothelium-specific binding was also evident histologically in mice imaged with RDG-MPIO, but not with RDG-MPIO. The RGD-MPIO appear to detect integrin αvβ3, positive blood vessels in a mouse model of brain metastasis. However, molecularly targeted MRI using MPIO may be precluded once tumours reach later stages with overt BBB breakdown, since retention is no longer target-specific.
Introduction The neddylation, a process of Neddy8 modification, is important for the complete activity of cullin-scaffold RING-finger E3 ligases (CRLs) which promote proteins’ ubiquitination and degradation. The inhibition of neddylation is reported to interfere with the neddylation of cullins for subsequently inactivating CRLs, leading to cell cycle arrest and apoptosis of tumours. This study investigated the effects of neddylation status on angiogenesis and tumour growth using in vitro and in vivo models.

Material and methods Human umbilical vein endothelial cells (HUVECs) were used to examine the angiogenic effects of neddylation, including assays in viability, trans-migration, nitric oxide (NO) production, and angiogenic Matrigel tube formation. Further, protein profiles in neddylation, apoptosis, and angiogenesis-associated signalling pathways were verified in HUVECs by Western blotting. Additionally, dominant negative constructs of cullins were applied to investigate each cullin’s neddylation on VEGF-activated VEGFR2 degradation and signalling. Moreover, Matrigel-plug and xenograft tumour models were utilised to study tumour angiogenesis and growth.

Results and discussions Neddylation inhibition delayed vascular endothelial growth factor (VEGF)-activated VEGF receptor 2 (VEGFR2) degradation for sustaining VEGF2 phosphorylations and its down-stream MAPKs/ATK-eNOS signalling, which accelerated the abnormal increment of NO, a biphasic pro- and anti-angiogenic factor, in HUVECs. Furthermore, neddylation inhibition exhibited biphasic effects on HUVECs’ viability, migration, and angiogenic tube formation. L-NAME, the NO inhibitor, notably blocked the proangiogenic activities and partly restored the anti-angiogenic and apoptotic activities of neddylation inhibition in HUVECs. Furthermore, we evidenced that the neddylation status of cullin 1 rather than other cullins was critically important in VEGF-activated VEGFR2 degradation and phosphorylations. Moreover, neddylation inhibitor displayed biphasic effects on angiogenic Matrigel-plug assay and on tumour angiogenesis and growth in xenograft tumour model.

Conclusion Neddylation inhibitor could block the neddylation of cullin 1 through delaying and sustaining VEGF-activated VEGFR2 degradation and signalling, which accelerated the abnormal amount of NO production on mediating the biphasic effects of endothelial angiogenesis and tumour growth. Therefore, exploring the neddylation status in angiogenesis may result in a new perspective for targeting tumours and angiogenesis-dependent diseases.

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PO-315 INTERTUMOR HETEROGENEITY IN VASCULARITY AND RESPONSE TO BEVACIZUMAB TREATMENT IN ARTIFICIAL MELANOMA BRAIN METASTASES

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Introduction Patients diagnosed with melanoma brain metastases have few treatment options and poor prognosis, and anti-angiogenic agents targeting the vascular endothelial growth factor A (VEGF-A) could represent a potential treatment strategy. The purpose of this preclinical investigation was to study the vascularisation pattern and the effect of the VEGF-A targeting agent bevacizumab in artificial brain metastases established from four human melanoma cell lines with different angiogenic and invasive properties.

Material and methods A-07, D-12, R-18, and U-25 cells transfected with GFP were injected intracerebrally in nude mice treated with bevacizumab (10 mg/ml) or vehicle. Treatment was initiated one day before tumour cell injection, and continued twice a week until the mice became moribund. Moribund mice were killed and autopsied, and the brain was evaluated by fluorescence imaging or by histological examination.

PO-314 LOSS OF HOST SECRETORY LEUKOCYTE PROTEASE INHIBITOR REDUCES LUNG ADENOCARCINOMA BURDEN

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Introduction Lung cancer accounts for approximately 11.8% of all cancer diagnoses within the European Union.1 One area of interest in lung cancer is the role of the secretory leukocyte protease inhibitor (SLPI) in lung tumorigenesis. SLPI has been identified to possess anti-bacterial, anti-protease and anti-inflammatory activity. However, SLPI levels are substantially increased in lung adenocarcinoma patient tumour tissue, with SLPI−/− mice presenting with significantly reduced pulmonary nodules in a urethane model.2 Zelyute et al showed a significant increase in plasma SLPI levels in lung cancer patients compared to healthy controls, and that metastatic patients exhibited increased SLPI plasma levels compared to local disease however this was not significant.3

Material and methods Human tissue microarrays were stained for SLPI. 5 × 10⁷ 3 LL murine lung adenocarcinoma cells were subcutaneously (S.C.) injected into C57BL/6 WT and SLPI−/− mice. Evan’s blue dye (45 mg/kg) was intravenously injected for vessel functionality assessment. S.C. tumours were excised and stained for H and E, CD31, Ki67, TUNEL and CD45. In an experimental metastasis (I.V) model 1 × 10⁶ 3 LL cells were intravenously injected into C57BL/6 WT and SLPI−/− mice. Flow cytometry and qPCR analysis was performed on S.C. tumours and experimental metastasis lung tissue.

Results and discussions Human lung adenocarcinoma tissue exhibited substantially increased SLPI expression. In a subcutaneous model SLPI−/− mice exhibited significantly reduced tumour growth and cellular proliferation compared to WT mice. Tumours from SLPI−/− mice also displayed significantly reduced functional vessel number and vessel leakiness compared to WT mice. Host SLPI loss altered the immune micro-environment in both the subcutaneous and experimental metastasis model upon qPCR and flow cytometry analysis.

Conclusion The findings to date highlight that SLPI may play a notable role in lung tumorigenesis. This may be due to altered neo-angiogenesis or tumour immune cell infiltration and requires further scientific investigation. These findings propose a role for SLPI as a biomarker or potential treatment target in lung cancer.