be novel therapeutic or diagnostic targets for lymphatic metastasis. Recently, we have established "antibody proteomics technology" which accelerates the discovery of proteins useful for diagnosis and therapies of cancer. In this study, we applied this technology for seeking proteins specifically expressed on tumour LECs and related to lymph node metastasis.

Material and Methods: Tumour LEC model; Human primary LECs were cultured in conditioned media (CM) prepared from the cultures of low-metastatic human lung cancer cell line (RERF-LC-MS), high-metastatic cell line (RERF-LC-KJ) or normal bronchial epithelial cells (NHBE) and used as the lymphatic vessel model in tumour tissues or in normal tissues.

In vitro invasion assay; LECs of each model were seeded to the upper chamber of an invasion assay plate and incubated for 72 hr. The invasive cells were stained with calcein-AM and measured by fluorescence.

2D-DIGE analysis; Lysates of each model LECs were labelled with Cy-dyes and analyzed by 2D-DIGE according to the manufacture's protocol. Proteins of interest were extracted from the gel and subjected to protein identification by mass spectrometry analysis.

Tumour tissue microarray (TMA) analysis; Expression profiling of candidate proteins was performed by immunostaining of tumour TMAs.

Results and Conclusions: Results from the invasion assay show that RERF-LC-KJ-CM treated LECs possess a more invasive character than RERF-LC-MS-CM. Therefore, CM-treated LECs were considered to gain one of the metastasis related characters. The differential proteomes were then analyzed by 2D-DIGE in order to identify metastasis-related proteins on LECs. MS analysis identified fourteen proteins over-expressed in the RERF-LC-KJ-treated LECs. These candidate proteins were then validated by TMA analysis. Thus, proteins expressed in lung tumour LECs and tumour cells but not in LECs or normal lung were identified. Although further analysis is required, the identified proteins are potentially useful drug targets for lymphatic metastasis.

246 Utilisation of the DNA repair enzyme, N-Methylpurine DNA glycosylase, as a biomarker of oesophageal Cancer

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Background: Barrett's Oesophagus (BO) is an inflammatory disease where continuous exposure of the distal oesophagus to stomach acids can lead to uncontrolled cellular division and ultimately oesophageal adenocarcinoma (OAC). Such uncontrolled cellular division is multifaceted, including the loss of DNA fidelity and genomic miss-repair. Altered expression of the DNA repair enzyme *N*-Methylpurine DNA Glycosylase (MPG), for instance, has previously been associated with breast and colon cancer. Where disruption of DNA repair may well prove deleterious in a normal cell, execution of DNA repair in a tumour cell conversely becomes counterproductive during chemotherapy. Use of a biomarker that will both allow for detection of early stage oesophageal carcinoma as well as predict a patient's response to chemotherapy would thus be most invaluable.

Aim: To explore the role of MPG as a predictive and therapeutic marker of oesophageal cancer.

Material and Methods: Levels of MPG mRNA expression was determined from control and cancer tissues derived from a cDNA panel (Origene, USA). qRT-PCR was carried out using TaqMan probes for MPG (Applied Biosystems, UK) and data normalised to the expression of the endogenous genes B-actin and RPLO. MPG mouse monoclonal antibody (Sigma-Aldrich) was used for protein detection and immunohistochemistry was carried out using the Ventana automated system. H-score was used for semi-quantification of MPG protein levels.

Results: Gene expression studies revealed high levels of MPG mRNA in OAC tissue relative to healthy tissue. Further analysis showed a tumour grade dependent increase in MPG expression at both mRNA and protein levels, signifying MPG as a promising diagnostic marker for the detection of MPG. Relative to cancer tissues derived from the enterohepatic system, altered expression of MPG mRNA was specific to the oesophagus. Indeed with amenable access to the oesophagus, focussed targeting of cancer cell DNA repair, via MPG modulation, could be used as a means to enhanced therapeutic treatment.

Conclusion: Production of a clinically useful biomarker that is capable of detecting oesophageal carcinoma at the earlier stages of tumour development could allow for early intervention of the disease resulting in increased survival. This study highlights the potential use of the DNA repair enzyme, MPG, as a diagnostic marker for the early detection of oesophageal cancer and a therapeutic marker for enhanced chemotherapy.

247 The medicinal and antitumour mushroom, agaricus blazei murill, activates NF-KB via TLR2 in monocytic cells and induces expression of cell surface markers and production of cytokines in human monocyte-derived dendritic cells (MDDC) in vitro

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The edible *Basidiomycetes* mushroom *Agaricus* bM (AbM) of Brazilian rain forest origin is used in traditional medicine against cancer and various diseases. It is rich in immunomodulating substances like beta-glucans, and is previously shown to induce apoptosis in cancer cells and inhibit tumours in mouse models. Activation of transcription factor NF-kB, which is linked to cytokine production and cell cyclus regulation in cancer defence, was determined by a luciferase assay system as translocation of NF-kB from cytosol to nucleus of cells stimulated with AbM extract. Surface markers on the cells were studied by flow cytometry, and cytokine release from them to the cell culture supernatnants, were measured in by a 17-plex Luminex system. Activation of NF-kB was examined in promonocytic THP-1 cells and in HEK 293 cells, transfected with CD14/MD2 and TLR2 or TLR4, after the AbM stimulation. The mushroom extract induced NF-kB activation via TLR2, but not TLR4, in both cell lines. Then we studied effects on MDDC cultures of the AbM-based (82%) extract AndoSan^{TM}, also containing *Basidiomycetes* mushrooms *Hericium erinaceum* and *Grifola frondosa*. After 24 h there was down-regulated CD11c, *de novo* CD69 and enhanced CD86 expression on the cells, as well as dose-dependent increased levels of IL-1beta, IL-6, IL-8, MIP-1beta, G-SCF, and TNFalfa in the cell culture supernatants. Whereas synthesis of IL-2, IL-8 and IFNgamma was similar for 10% of AndoSan^{TM} and 0.5 mg/ml of *E. coli* LPS, the AbM-based extract induced a 2- to 10-fold higher production of IL-1beta, TNFalfa and G-SCF, respectively, than did LPS. We conclude that AbM activates transcription factor NF-kB via TLR2 in monocytes and most probably also in MDDC. In MDDC an AbM-based extract induced differential expression of cell surface markers for cell adhesion, activation and antigen presentation, and increased production of proinflammatory, chemotactic and some Th1-type cytokines *in vitro*. This may explain some of the known antitumour properties of AbM. The antitumour effect of the AbM extract is currently being tested in a tumour stem cell model in PVG rats.

248 Histone deacetylase inhibitor vorinostat suppresses the growth of uterine sarcomas in vitro and in vivo

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Background: Uterine sarcomas are very rare malignancies with no approved chemotherapy protocols. Histone deacetylase (HDAC) inhibitors belong to the most promising groups of compounds for molecular targeting therapy. Here, we described the antitumour effects of suberoylanilide hydroxamic acid (SAHA; vorinostat) on MES-SA uterine sarcoma cells *in vitro* and *in vivo*.

Material and Methods: We investigated effects of vorinostat on growth and colony forming ability by using uterine sarcoma MES-SA cells. We analyzed the influence of vorinostat on expression of different HDACs, p21^{WAF1} and activation of apoptosis. Finally, we examined the antitumour effects of vorinostat on uterine sarcoma *in vivo*.

Results: Vorinostat efficiently suppressed MES-SA cell growth at a low dosage (3 μM) already after 24 hours treatment. Decrease of cell survival was even more pronounced after prolonged treatment and reached 9% and 2% after 48 and 72 hours of treatment, respectively. Colony forming capability of MES-SA cells treated with 3 μM vorinostat for 24 and 48 hours was significantly diminished and blocked after 72 hours. HDACs class I (HDAC2 and 3) as well as class II (HDAC7) were preferentially affected by this treatment. Vorinostat significantly increased p21^{WAF1} expression and apoptosis. Nude mice injected with 5×10^6 MES-SA cells were treated for 21 days with vorinostat (50 mg/kg/day) and, in comparison to placebo group, a tumour growth reduction of more than 50% was observed. Results obtained by light- and electron-microscopy suggested pronounced activation of apoptosis in tumours isolated from vorinostat-treated mice.

Conclusions: Our data strongly indicate the high therapeutic potential of vorinostat in uterine sarcomas.