Methods: SiRNA mediated silencing technique was adopted to ensure the depletion of myoferlin to reveal the biological function of myoferlin during tumor progression in vivo and in vitro. Scoring of immunohistochemically stained patients' cohort sections was taken up to investigate correlation between myoferlin expression and micro-vessel density in patients.
Results: SiRNA-mediated myoferlin-silencing significantly reduced the volume of BxPC-3 tumors developed onto the chorioallantoic membrane of fertilized chicken eggs. Intriguingly, aside their reduced volume, myoferlin-silenced tumors appeared whitish and exhibited a significant decrease of blood vessel density as shown by FITC-conjugated Sambuccus nigra agglutinin staining. This observation suggested that, in addition to an inhibition of BxPC-3 cell growth after myoferlin silencing, this protein may exhibit a pro-angiogenic activity. Accordingly, we next showed that myoferlin-silencing significantly inhibited VEGF-A secretion without decreasing VEGF-A gene expression. Immunofluorescence revealed that VEGF-A seemed to accumulate in the cytosol at the vicinity of the plasma membrane concomitantly together with vesicle like structures, seen by the electron microscopy, in myoferlin depleted cells. Furthermore, immunofluorescence techniques showed a colocalization of myoferlin with Sec5/Exoc2, a component essential for exocytosis, raising the hypothesis that myoferlin plays a role in VEGF-A secretion.
Conclusions: Our work highlight a new function of myoferlin in pancreatic cancer progression. We show that myoferlin is essential for pancreatic cancer cell lines proliferation and tumor growth. We also report for the first time myoferlin as a key regulator in VEGF-A secretion by controlling the exocytosis of VEGF-A secretory granules in the tumor stroma.

O11

EXPEL: A Novel Non-Destructive Method for Mining Soluble Tumor Biomarkers

B. COSTANZA (1), A. BLOMME (1), A. BELLAHCENE (1), O. PEULEN (1), E. MUTIJIMA (2), P. DELVENNE (3), E. DE PAUW (4), O. DETRY (5), C. COIMBRA (5), V. CASTRONOVO (1), A. TURTOI (1) / [1] University of Liege, Liège, Belgium, Metastasis Research Laboratory, [2] CHU Liege, Liège, Belgium, Department of Pathology , [3] CHU Liege, Liège, Belgium, Department of Pathology, [4] University of Liege, Liège, Belgium, Laboratory of Mass Spectrometry, Dept. of Chemistry, [5] CHU Liege, Liège, Belgium, Department of Abdominal Surgery

Introduction: The search for biomarkers able to detect and evaluate disease such as cancer at an early stage, or to predict resistance and response to therapies, has been and remains a major challenge. Despite very important progresses in all fields of omics technologies, the success of discovery of clinically valuable biomarkers is surprisingly disappointing. Difficult mining of secreted proteins in biological fluids poses the first major hurdle, mainly because the concentration of interesting proteins in serum or urine is generally very low. The second key limitation in the field is the inaccessibility of tissue specimens from early lesions. Those are routinely required in their integrity for the complete histological evaluation in the clinical routine, leaving no residual material for research.
Aim: Aim of the study is the discovery of new soluble biomarkers for non invasive diagnosis of colorectal cancer and its liver metastasis.
Methods: Here we present an innovative procedure that we have named EXPEL, which entirely overcomes the mentioned limitations. It makes any tissue, regardless of its size, available for both omics research and histological investigation.

Results: Our original device and approach extracts soluble tumor biomarkers and small metabolites within few minutes and without altering the tissue morphology. For this purpose a small tissue biopsy is incubated in a slightly hypertonic extraction buffer while subjected to alternating pressure. Upon extraction the tissue is fixed in formalin and can be used for histological analysis. The soluble extract is further prepared for proteomic and metabolomic analysis. In a proof of concept study we have extracted and analyzed soluble biomarkers from human colorectal carcinoma liver metastases (N=10) as well as primary colorectal tumors (N=10). Pathology validation demonstrates that EXPEL procedure does not alter tissue morphology or subsequent molecular and clinical tests. The comparison of proteins and metabolites identified in tumor lesions with those found in adjacent normal tissues revealed a promising group of novel and differentially expressed targets. Their potential usefulness as diagnostic or predictive markers is currently being explored.
Conclusions: The Expel method provides clinicians with a new tool enabling them to non-destructively discover new biomarkers and preserve precious tissues (like colon polyps) for pathology evaluation

O12

Quality Control of pancreatic tissue samples in Tumorbank@UZA

S. GOETHALS (1), A. DE WILDE (1), K. LESAGE (2), G. VANHOUTTE (1), E. SMITS (3), M. PEETERS (1), P. PAUWELS (4) / [1] University Hospital Antwerp, , Belgium, Multidisciplinary Oncological Centre Antwerp, [2] University Hospital Antwerp, , Belgium, ICT, [3] University Hospital Antwerp, , Belgium, Medical Director, [4] University Hospital Antwerp, , Belgium, Pathology

Introduction: Since 2008, snap frozen tumor tissue and FFPE material is being collected for translational and clinical research in Tumorbank@UZA. In this study a total of 87 frozen (-80°C) tissue samples from 52 patients with pancreatic cancer and their corresponding uninvolved tissue samples (if available) were selected for quality control.
Aim: We checked if there was a correlation between the RNA quality based on the RIN value and pre-analytical variables such as cold ischaemia time and/or sample age. We also evaluated if there was a difference in RNA quality between tumour tissue versus reference pancreatic tissue.
Methods: A trained pathologist evaluated the hematoxilin eosin stainings for the presence of tumour and estimation of tumour cell percentage. From the samples that passed this first quality control, DNA and RNA was simultaneously isolated using the Qiagen Allprep Micro kit. Concentration of nucleic acids was measured by spectophotometry and RNA quality, expressed as RNA integrity number (RIN) was measured with the Agilent Bioanalyzer.
Results: From all selected samples, 70% were acceptable for downstream research after evaluation of the HE stainings. Rejection of samples was based on the presence of tumour cells in samples that were annotated as reference material and vice versa (16%) or on difficulties in assessment of the slide due to presence of artefacts (14%). An overall mean RIN value of 4,2 reflects that pancreatic tissue is among the most difficult tissues to isolate RNA due to the abundant presence of nucleases. In this sample set there was no significant correlation between cold ischaemia time or storage time and RNA quality. However, RNA quality was significantly higher for tumour tissue as compared to reference tissue (mean RIN respectively 5,1 and 3,2).
Conclusions: In conclusion, our findings underline the necessity of quality control of banked tissue samples in order to find “fit-for-purpose” applications.