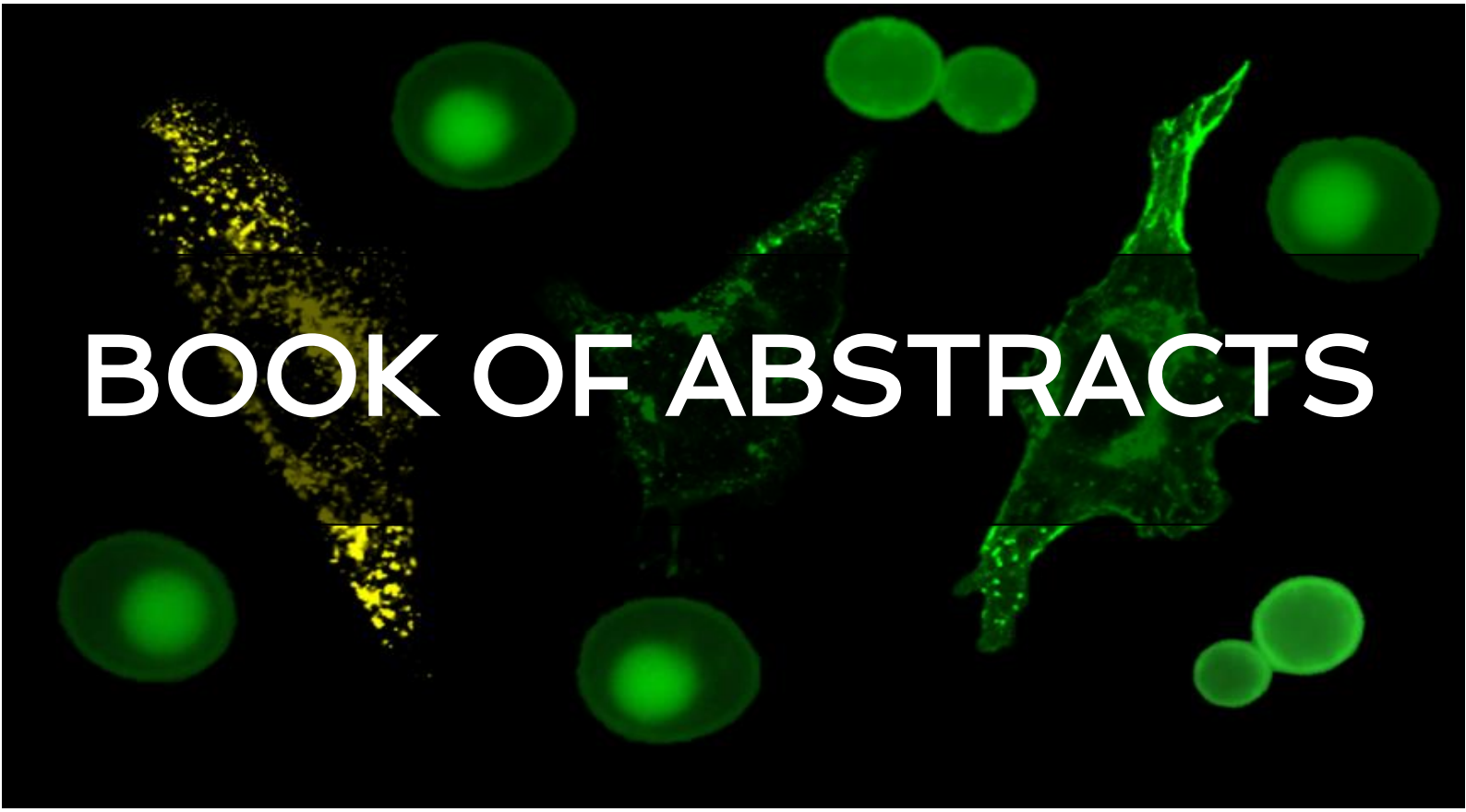
SMETS2



BOOK OF ABSTRACTS

2nd Small Meeting on Endocytic Trafficking and Signaling

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P1

A New Therapeutic Approach to Pancreatic Cancer

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal cancers with late diagnosis and lack of efficient therapeutic options. PDAC lesions are unique among solid tumors due to their extensive desmoplastic reaction and sparse cancer cells, highlighting the potential role of cell communication in the initiation and progression of this neoplasia. Despite the compelling evidence that cell communication is intrinsically involved in tumor progression, this process of tumorigenesis is still off the cancer therapy landscape. Exosomes, extracellular vesicles derived from the endocytic pathway, have emerged as mediators of intercellular communication crucially involved in different steps of tumor progression. Rab GTPases are involved in all steps of exosomes biogenesis. Therefore, we hypothesized that modulation of Rab proteins would interfere with tumor progression and its response to therapy. Using PDAC genetically engineered mouse models we show that during tumor progression there is an increase in the number of exosomes in circulation. We also show that Rab27a expression is increased in metastatic lesions when compared with the primary tumor. Rab27a expression is the one that best correlates with increased number of exosomes. We have developed an inducible Rab27a knockout mouse model conditional to the pancreas, and demonstrate that knockout of Rab27a in the healthy pancreas does not affect mouse development or pancreas functions. In human PDAC samples, high Rab27a protein levels correlate with poor prognosis. At this moment our data suggests that Rab27a is a good candidate target in pancreatic cancer to modulate cancer exosomes production and impair tumor growth.

P2

Lactoferrin perturbs intracellular trafficking in yeast and highly metastatic cancer cells

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Lactoferrin (Lf) is an iron-binding glycoprotein present in several biological fluids. It exhibits a broad range of interesting biological activities, from which its anticancer and antifungal activities stand out. Our group has been studying the mechanisms and targets underlying Lf anticancer and antifungal activities to improve its therapeutic efficacy and rational application. Indeed, we previously demonstrated that Lf triggers a mitochondrial and caspase-dependent regulated cell death in *Saccharomyces cerevisiae* (1). Moreover, we found that Lf selectively induces apoptosis in highly metastatic cell lines displaying the proton pump V-ATPase at the plasma membrane (2). Herein, we show how studies with yeast unveiled a novel effect of Lf on intracellular trafficking, which was then validated in highly metastatic cancer cell lines. Results will be discussed in an integrated manner regarding their contribution towards a better understanding of the molecular basis of Lf anticancer activity and its impact on a potential expanded clinical application.

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P3

LRP1B endocytic activity: possible implications in the uptake of liposomal anticancer drugs

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The low-density Lipoprotein Receptor-related Protein 1B (LRP1B) belongs to the Low-Density Lipoprotein Receptor superfamily, scavengers for multiple ligands and major functional mediators of endocytosis [1]. Although LRP1B has been described has a putative tumour suppressor, being amongst the 10 most significantly deleted genes in human cancers [2], the exact role of LRP1B in cancer is still not fully disclosed. LRP1B binds to several ligands, activating extracellular proteolytic cascades and regulating adhesion, motility and invasion. LRP1B re-expression in cancer reduce cell proliferation, colony formation and tumourigenicity in vitro and in vivo. We have previously presented an alternative/complementary role for LRP1B endocytic activity, as modulator of cancer secretome, through depletion of soluble factors critical for tumour invasion/progression [3]. LRP1B endocytic activity may have impact in the uptake of liposomal drugs [4]. LRP1B deletion/downregulation was shown to significantly correlate with resistance to liposomal doxorubicin in ovarian cancer patients [5]. In this work we developed human tumour cell lines (derived from thyroid, melanoma, urothelial and ovarian cancer) with LRP1B over-expression and present preliminary results concerning their response to liposomal doxorubicin. Future studies will be carried out to validate the role of LRP1B's endocytic activity in the response of tumour cells to liposomal therapy and its use as predictive marker of response to liposomal anticancer drugs.

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P4

A novel iRhom interactor, controls TNF secretion by policing the stability of iRhom/TACE sheddase complex

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The metalloprotease TACE catalyzes the release of multiple important signaling proteins, including epidermal growth factor receptor ligands and the principal inflammatory cytokine, TNF. Often, it participates in intramembrane proteolysis of membrane receptors, regulating signalling further. Membrane proteins called iRhoms are essential for the trafficking of TACE within the secretory pathway and play a crucial role in the stimulation of TACE's proteolytic activity on the cell surface. To delineate further how the TACE/iRhom axis is regulated, we performed an immunoprecipitation / mass spec screen to identify proteins that interact with the two mammalian iRhom paralogs. We identified a novel protein, that we name iTAP (iRhom Tail-Associated Protein). iTAP contains a FERM domain and binds to both iRhoms, enhancing the cell surface stability of iRhoms and TACE, preventing their degradation in lysosomes. Consequently, cells null for iTAP exhibit depleted levels of iRhom2, lack mature TACE and hence are devoid of proteolytic activity. Depleting iTAP in primary human macrophages profoundly impaired TNF production while tissues from iTAP KO mice exhibit a pronounced depletion in active TACE levels. Our work reveals the important role of trafficking control of iRhom / TACE in the late secretory pathway and identifies iTAP as a physiological regulator of TNF and growth factor signaling making it a promising novel target for the control of disease.

P5

Lysosomal dysfunction in the retinal pigmented epithelium and the pathogenesis of age-related macular degeneration

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The retinal pigment epithelium (RPE) plays a critical role in retinal homeostasis. A major RPE function is the daily ingestion and degradation of photoreceptor outer segments (POS), which places a significant burden on the lysosomes of the RPE cells. RPE degeneration underlies one of the common blinding disorders, age-related macular degeneration (AMD). We hypothesise that chronic lysosome dysfunction in RPE cells is a key event in the early stages of AMD, as in other age-related conditions such as Parkinson's disease. We are investigating the effects of chronic partial lysosomal inhibition on RPE cells using inhibitors of lysosomal acidification or incubation with UV-irradiated POS. Using three different differentiated RPE culture systems, we utilise a combination of flow cytometry, fluorescence-based and electron microscopy techniques to characterise the in vitro models. We have demonstrated the presence of lysosomal sub-populations in RPE cells that differ significantly in both their cathepsin D content and morphology and may represent different stages of the lysosome life cycle. Chloroquine treatment of RPE cells leads to a feedback stimulation of lysosome activity with up-regulation of cathepsin B catalytic activity and induces nuclear translocation of the lysosomal transcription factor TFEB. Conversely, RPE cells accumulate lipofuscin-like material in lysosomes upon incubation with UV-irradiated POS. Identification and characterisation of defective pathways responsible for the regulation of lysosomal biogenesis and activity will contribute to a better understanding of AMD pathogenesis and could be relevant to other age-related diseases.

P6

Investigation of human copper transporter proteins pore formation through number and brightness analysis

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In this study we present the investigation of pore formation of human copper transporter (hCtr) proteins in live cancer cells using the number and brightness (N&B) analysis. Copper is one of the most important metals for cell metabolism, considered a crucial cofactor for several processes in eukaryotic cells such as respiration, detoxification and secretion[1]. Any imbalance on copper homeostasis can lead to severe diseases. In this homeostasis process, the hCtrs are the proteins responsible for copper uptake by the cell. It is known that hCtrs can exists as monomers, dimers or trimers and that the trimers seem to be responsible for the functional pore formation[2]. The N&B analysis is a potent statistical method based on fluorescent intensity fluctuations of single pixels recorded using a photon-counting confocal microscopy[3]. Our results indicate that after stimulation with a cooper chelator the hCtrs aggregate. We were able to map the aggregation of hCtr monomers by using N&B. In order to understand the pore formation in cancer cells, we will treat the cells with different concentrations of copper.

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P7

Unraveling the intracellular trafficking mechanism of monocarboxylate transporter 1 in mammalian cells

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Plasma membrane (PM) proteins, such as nutrient transporters and receptors, have a determinant role in the regulation of the overall cellular metabolism. They contribute to sensing, adhesion, signaling and nutrient uptake, allowing the cell to adapt and respond to distinct environmental cues. Rapid and dynamic regulation of PM proteins is achieved by means of selective endocytosis, where target proteins are internalized into endosomes and then they are either degraded or recycled back to the PM.

This study focuses on monocarboxylate transporters (MCTs), which play essential metabolic roles in most tissues. These proteins are found to be upregulated in several cancer cell lines, displaying an enhanced glycolytic activity. MCTs contribute to fuel the metabolism of tumor cells, so reducing their expression can somehow starve cancer cells and make them more vulnerable to chemotherapy, opening new pathways for future therapies.

We have previously generated several gene-edited cancer cell lines, known to express differently MCT transporters, using the CRISPR-Cas9 system (1). These cells will be applied to a combined microscopy platform (2) in the attempt to characterize the internalization dynamics of MCT transporters upon distinct environmental stimuli. This will be achieved by utilizing fluorescence optical sectioning microscopy obtained through aperture correlation microscopy with a Differential Spinning Disk (DSD) and nanomechanical mapping with an Atomic Force Microscope (AFM). An overview of the most significant results will be presented.

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P8

Functional analysis of the Human Copper Transporters using yeast as a host

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Copper (Cu) is a crucial ion for both eukaryotic and prokaryotic organisms. It serves as a co-factor of metalloenzymes that participate in important cellular processes involved in growth, development and physiology of the organisms. Although its importance in maintaining cell health, high level of this ion is extremely toxic (Wang et al., 2011). Therefore, cells possess tight regulated systems to preserve copper homeostasis. Indeed, at high Cu levels, the Copper Transporter 1 (Ctr1) is endocytosed, a process already verified in yeast and human cells (Liu et al., 2007; Maryon et al., 2013). Besides these new advances, the molecular mechanisms that are behind the intracellular trafficking of the hCtr1 protein are still poor understood. So, to better understand this process, an heterologous expression system was created using the yeast *Saccharomyces cerevisiae* as host (Pereira et al., 2016). Human *CTR1* and *CTR2* optimized genes tagged with GFP were cloned into pYPKpw plasmid and transformed into a *S. cerevisiae* strain disrupted for copper transporters. Importantly, phenotypic assays demonstrated that human Ctr1 complemented the yeast *ctr*-mutant strain for the ability to grow in a medium containing non-fermentable carbon sources. Moreover, hCtr1 and hCtr2 were localized at the plasma membrane and intracellularly. Data will be presented regarding the expression of hCTRs in different conditions.

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