adenomas (NFPA) carries the increased risks of recurrence and requires adjuvant radiotherapy and surgeries. It is necessary to clarify the molecular mechanisms and markers of invasiveness to guide the management of NFPA patients. Here we describe a comprehensive phosphorproteomic evaluation of 20 NFPA followed by validation in a larger set of samples.

**Material and methods** Peptides from 20 tumours were enriched with TiO$_2$ beads, fractionated using bRPLC and subjected to high-throughput LC-MS/MS-Orbitrap Fusion Trivid Mass Spectrometer. Up to 5 precursor ions were chosen for MS/MS analysis. Following Mascot and SEQUEST analysis our bioinformatics pipeline (PhosphositePlus, Gene Ontology, DAVID, and KEGG) identified 1345 phosphoprotein groups and 2233 unique phosphopeptides. Eight candidate phosphoproteins involved in cell proliferation and growth were selected for validation using immunohistochemistry (tissue microarray containing 200 NFPA samples) and immunoblotting (n=18).

**Results and discussions** Hsp27 phospho-Ser82 was found 2.1 fold upregulated in invasive and 7.8 fold hyperphosphorylated in recurrent group while Hsp phospho-Ser15 was found 2.1 fold upregulated in invasive and 7.8 fold hyperphosphorylated in recurrent group in our mass spectrometry experiment. Immunohistochemistry for Ser82 revealed 2.1-fold upregulation (p<0.0001) in the recurrent and 1.6 (p<0.01) fold upregulation in InvasiveNFPA. There was no significant upregulation in Ser15 was observed in invasive and recurrent groups compared to non-invasive and non-recurrent. In agreement with the mass spectrometry and IHC data, immunoblotting also revealed significantly upregulated Hsp27 phospho-Ser 82 in invasive and recurrent group. HSPB1 serine 82 phosphorylation by VEGF activation of PKC-mediated PKD induces endothelial migration and tubulogenesis, indicating the potential importance of HSPB1 in VEGF-dependent angiogenesis and hence pituitary tumorigenesis.

**Conclusion** Hsp27 phosphorylated at Ser82 is significantly associated with NFPA invasion and recurrence. It Provides a roadmap for patient stratification, and prognostication for recurrence and trials for targeted therapy.

**Material and methods** Fluorescent Recovery After Photobleaching (FRAP) was used to quantify actin dynamics at invadopodia from cancer cells expressing GFP-fused actin and plated on HDFC-coated slides. In addition, the role of two invadopodial actin-bundling proteins associated with metastatic breast cancer (Fascin and CRP2) was evaluated using a RNAi strategy, and their biochemical activities were compared in *in vitro* reconstituted assays using TIRF microscopy.

**Results and discussions** Our FRAP analyses revealed that a gelatin matrix induces the formation of invadopodia with identical actin dynamics. In contrast, HDFC induced the formation of an additional population of invadopodia with significantly decreased actin dynamics. The proportion of this stable invadopodia population increased over time and was associated with MT1-MMP accumulation. Knocking down actin bundling proteins, such as fascin or CRP2, induced an increase in the mobile actin fraction and a shift toward the dynamic subpopulation. Such effects were associated with a decrease in MT1-MMP levels at invadopodia, and a reduction in the invasive potential of breast cancer cells. TIRF microscopy analyses revealed that only CRP2 protects actin bundles against cofilin-mediated severing, suggesting only partial redundancy between the two actin regulators.

**Conclusion** In this study we found that HDFC decreases actin dynamics at invadopodia, which in turn increases tumour cell invasive capacity. In addition, our data suggest that such decrease in actin dynamics involves functionally similar, but not identical, actin bundling factors.
all animal works has been obtained from the Institutional Animal Care and Use Committee, Academia Sinica.

**Results and discussions** Galecin-4 was upregulated during the development of castration resistance and metastasis. High expression of galecin-4 in PCa cells exhibited castration resistance, tumor-regenerating stem-like cells, expressed SOX9 and ALDH1A1, and ability to colonize distant metastases. We identify a feed-forward signalling pathway by which galecin-4 signalling through RTK-Myc axis upregulated gene transcription of enzymes in a specific O-glycosylation pathway and the biosynthesis of their substrates, thus altered the cellular modification of O-glycosylation, and bolstered castration resistance and metastasis. These coordinated effects conferred more and more binding sites for galecin-4 and amplified the RTKs signalling, implicating crosstalk mechanisms to activate androgen receptor signalling and drive the PCa progression.

**Conclusion** MYC regulates oncofetal O-glycosylation in RTKs thus primes the cells for binding to galecin-4 and downstream signalling, which promotes PCa castration resistance, metastasis, and clinically poor survival.

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**PO-254**

**Merkel cell polyomavirus T-antigen regulate microRNAs post-transcriptionally through Dicer in merkel cell carcinoma**

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**Introduction** Background and aim Merkel cell carcinoma (MCC) is an aggressive type of skin cancer. About 80% of MCCs harbour integrated Merkel cell polyomavirus (MCC) genome with a mutation in the large T (LT) gene. MCC T-antigens are required for neoplastic transformation and maintenance of cell growth; however the molecular mechanism by which the virus induces tumorigenesis remains unclear. In this study we aimed to identify and characterise functional role of MCC-regulated microRNAs in MCC.

**Material and methods** We did silencing or ectopic expression of MCC T-antigens to identify specific miRNAs regulated by MCC T-antigens by RT-qPCR. The involvement of MCC T-antigens and miRNAs in autophagy was evaluated using LC3-II conversion, mRFP-EGFP-LC3 reporter and/or transmission electron microscopy. The targets of miRNAs were verified by western blot analysis and luciferase reporter assays. We also look for the DICER expression by western blot.

**Results and discussions** We identified specific miRNAs regulated by MCC T-antigens. Using both gain- and loss-of-function experiments, we showed that MCC T-antigens and MCC-regulated miRNAs (miR-375, miR-30a-5p and miR-30a-3p) could regulate autophagy. We further demonstrated that miR-375 could directly regulate ATG7 and SQSTM1, while both miR-30a-3p and miR-30a-5p could target BECN1. We also identified the MCC T-antigens regulates these miRNAs through post-transcriptionally by regulating DICER expression in MCC positive MCC.

**Conclusion** We provide evidence that MCC T-antigens regulate miRNAs and autophagy in MCC. MCC T-antigens increase miRNA expressions through pots-transcriptional regulation, and several of these miRNAs can directly target multiple autophagy genes that lead to suppression of autophagy in MCC.

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**Tumour Microenvironment**

**PO-255**

**The role of solid stress in tumour-stromal interactions**

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**Introduction** Solid tumours are characterised by an abnormal microenvironment that drives tumour progression. Tumour abnormalities include matrix stiffening and solid stress accumulation in the tumour interior. Several studies have dealt with the effect of solid stress on cancer cells using different experimental setups including the application of a predefined mechanical compression on a cell monolayer that transmits intracellular stress. To date, results indicated that solid stress guides cancer cell migration, however there are no pertinent studies taking into account the effect of solid stress on other cellular components of the tumour microenvironment, such as fibroblasts, and whether is implicated in tumor-stromal interactions.

**Material and methods** In order to investigate the effect of solid stress on fibroblasts we employed a transmembrane pressure device to simulate the compressive solid stress encountered in the tumour microenvironment. Briefly, fibroblasts were cultured overnight in the inner chamber of a 24 mm diameter transwell insert with 0.4 μm pores. An agarose cushion was placed on top of the cells and a 24 mm diameter piston of adjustable weight was placed on the top of the agarose gel applying a predefined compressive stress. Next, we set up a novel co-culture system consisting of compressed fibroblasts seeded in the upper chamber of the transwell insert and pancreatic cancer cells (CFPAC-1 or Mia PaCa-2) seeded in the lower chamber of the transwell insert. Cells were allowed to interact for 48 hours. At the end of the co-culture period, a scratch wound assay was performed on cancer cells to study their migratory ability in response to compressed fibroblasts. Total RNA and protein extracts were isolated in order to be subjected in Real Time PCR and Western Blotting.

**Results and discussions** Fibroblasts were exposed to 4.0 mmHg of compressive stress for 48 hours and the expression of a-SMA, one of the most established markers for fibroblast activation was found to be upregulated as revealed by qPCR and Western Blotting. Moreover, we observed that two distinct pancreatic cancer cell lines exhibited higher migratory ability when co-cultured with compressed compared to uncompressed fibroblasts suggesting that fibroblasts-derived factors may act in a paracrine fashion to stimulate the migration of adjacent cancer cells.

**Conclusion** Our results highlight the involvement of biophysical factors, such as solid stress in the activation of normal pancreatic fibroblasts and the migration of pancreatic cancer cells.