demonstrated that downregulation of MSLN decreases peritoneal dissemination and ascites formation in three of three animals tested. No significant effects were observed on migration, proliferation and apoptosis.

Conclusion The preliminary data suggest that MSLN plays a crucial role in the invasion process and peritoneal metastasis in an ovarian cancer model. Current studies are being conducted using CRISPR Cas9-mediated MSLN knockout ovarian cancer cell lines to expand the findings observed with the downregulation experiments.

All the procedures in animals were approved by the IJS animal ethical committee and the national regulator entity (DGAV) ref. n° 020015/2017-09-06.

**PO-248**

**NOVEL INSIGHTS ON THE ROLE OF GLYCOSYLATION IN CANCER: MOLECULAR FUNCTIONS AND CLINICAL APPLICATIONS**

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**Introduction** Glycosylation alterations are frequently found in cancer and specific aberrant glycan structures are associated with tumour development and progression. The characterisation of glycosylation modifications occurring in cancer is of high interest and represents a source of putative new biomarkers for cancer detection, therapeutic intervention and patient stratification.

**Material and methods** Glycomics, glycoproteomics, glycoprofiling, and in situ Proximity Ligation Assays of glycoengineered cancer cell models and tissue samples from gastrointestinal cancer patients were performed.

**Results and discussions** This presentation reports the recent discoveries applying several novel approaches for: (A) the characterisation of glycosylation changes in the cancer cells; (B) the identification of the aberrant expression of specific glycan structures in cancer, like terminal sialylated glycan, which lead to the activation of tyrosine kinase receptors, such as HER2, MET, and RON; (C) the identification of altered glycosylated proteins, carrying simple mucin-type carbohydrate structures, in engineered cancer cell models and in sera of cancer patients.

**Conclusion** These results demonstrate aberrant glycan structures as key functional players in tumour biology and highlight their potential as novel biomarkers and as therapeutic targets in the clinical management of cancer patients.

**PO-249**

**PHYLOGENETIC RELATIONSHIPS BETWEEN PAIRED PRIMARY PAPILLARY THYROID CARCINOMAS AND DISTANT METASTASIS: INTRA-TUMOUR MOLECULAR HETEROGENEITY AND CLONAL EVOLUTION**

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**Introduction** Though the vast majority of Papillary Thyroid carcinomas (PTC) are indolent tumours, around 5%-15% behave aggressively, developing distant metastases (DM), which cause patient’s death. The molecular events underlying metastatic spread are poorly understood. Little it is known about the contribution of intratumor molecular heterogeneity to DM.

**Material and methods** In this study, by genotyping 13 cases of matched primary tumours (PrT) and DMs, we sought to determine the prevalence of mutations in genes that have been associated with progression and aggressiveness in thyroid cancer (TERTp, BRAF, NRAS, KRAS, HRS, PIK3CA). To asses the contribution of intratumor heterogeneity and clonal evolution to DMs, 54 tumour areas, including different areas across space and time within the PrTs and DMs, were characterised. Mutational analysis was done by means of PCR and SSCP or direct sequencing.

**Results and discussions** Twelve cases (92%) were mutated in at least one of the genes screened [TERTp=9 cases (69%), BRAF=7 cases (54%), KRAS=3 cases (23%), NRAS=3 cases (23%), HRAS=2 cases (15.4%) and PIK3CA=2 cases (15.4%). Among the mutated cases 67% exhibited more than 1 gene activated. Three mutated genes coexisted in 62.5% of the cases bearing several mutations [3 cases (60%) TERTp +RAS + BRAF and 2 cases (40%) TERTp +BRAF +PIK3CA]. Concurrent activation of TERTp +RAS or TERTp +BRAF was seen in 5 cases each combination (62.5%). Parallel activation of BRAF and RAS was found in 4 of the cases (50%) with genetic heterogeneity. In all of the cases in which more than 1 area of DM, across space and time, was analysed, the mutations at TERTp, KRAS and HRAS resulted clonal. ’De novo’ mutations at DM, not present in the PrT, were seen in 3 cases mutated at TERTp, 2 cases mutated at NRAS and 1 case mutated at KRAS. Among the mutated cases, in which more than one area of PrT was analysed, TERTp mutations were clonal in 80% of the cases and BRAF mutations subclonal in 80% of the cases. The activation of RAS within the PrT tended to be a clonal event.

**Conclusion** The number of mutational events in PTC with DM is strikingly higher than in PT without DM. While TERTp and RAS mutations tend to be clonal within the PrT and the DMs, BRAF mutations tend to be subclonal. TERTp and RAS mutations may appear the novo at DM. PTC with DM display a much higher rate of genetic heterogeneity (67%). The coexistence of mutations in different genes is in agreement with the hypothesis that tumour progression relies on progressive accumulation of genetic alterations.

**PO-250**

**PHOSPHO S82 HSP27: A MARKER OF INVASIVE AND RECURRENT NON-FUNCTIONING PITUITARY ADENOMA.**

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**Introduction** Introduction: Non-functioning pituitary adenomas (NFPA) usually present as macroadenomas and 42% shows invasion of the adjacent structures, such as cavernous sinus, sphenoid bone or nasopharynx and some recur locally. The incomplete surgery section of invasive non-functional pituitary
adenomas (NFPAs) 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 phosphoproteomic evaluation of 20 NFPAs 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 Tribrid Mass Spectrometer. Upto 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 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 coflin-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.

**Introduction** Thomsen-Friedenreich (TF or T)-related antigens are short O-glycans highly expressed in over 90% of all human cancers including prostate, breast, ovary, bladder, colon, liver and gastric cancers. Especially in prostatic cancer (PCa), a higher level of T antigen was found correlated with the histological grade and bone metastasis; however, its functional impact in PCa and the underlying mechanism remain perplexing. We showed that spontaneous metastasis of human PCa xenografts expresses high levels of galectin-4, along with gene signatures for ERRB receptors signalling and sialylated Thomsen-Friedenreich (TF or T)-related antigens. Galectin-4 expression in clinical PCa is positively correlated with tumour grade, PSA recurrent rate, and poor survival. Galectin-4 binding to multiple receptor tyrosine kinases (RTKs) in PCa cells stimulates their autophosphorylation and downstream signalling pathways, therefore promoting epithelial-mesenchymal transition and invasive tumour growth.

**Material and methods** Orthotopic xenografts of LNCaP PCa cell line were implanted in athymic nude mice to derive a series of castration-resistant prostate cancer (CRPC) cell populations. Gene expression was detected using qRT-PCR, western blotting, and IHC. Manipulation of gene expression was conducted by overexpression and shRNA knockdown approaches with lentivectors in relevance cell populations. Permission for