EMT and cell surface vimentin was shown in CSC. The present study aimed to evaluate vimentin expression in prostate cancer (PCa).

**Material and methods** 36 cases of PCa were immunohistochemically stained with rabbit polyclonal antibodies to vimentin (ThermoScientific). Membranous and cytoplasmic expression was evaluated separately semiquantitatively in PCa as well as in the adjacent non-cancerous glands (NCG) with calculation of weighed staining index (WSI).

**Results and discussions** In the present study vimentin exhibited membranous and cytoplasmic expression. The pattern of staining varied in NCG and PCa. In the former membranous staining prevailed and was especially evident in benign prostatic hyperplasia, mainly in basolateral membranes. In NCG cytoplasmic vimentin expression was generally weaker than membranous and seen mostly in basal part of the cell. Conversely, in PCa vimentin was much more often expressed in cytoplasm, but staining was usually weak or moderate, and it was seen mostly in apical part of cytoplasm, while membranous staining was seen on the whole cell membrane. There was a patchy distribution of cell groups with membranous vimentin.

We propose that such membranous expression may correspond to cell-surface vimentin. Membranous staining in PCa was absent in 32.3% of cases, present in <5% of cells in 45.2%, 5%–20% in 16.1%, 20%–50% in 3.2% and in >50% of cells in 3.2%. For cytoplasmic staining the corresponding numbers were 0%, 6.5%, 32.3%, 16.1% and 32.3%. When WSI was counted, it was significantly higher for cytoplasmic staining in PCa and for membranous in NCG (p<0.05). In PCa, cytoplasmic vimentin was higher and membranous lower than in NCG (p<0.05). No significant associations were found between vimentin expression and Gleason score or pT category (p>0.05). Previously we studied E- and N-cadherin expression varied in NCG and PCa. In the former membranous staining with another antibody.

Results of the present study in a larger series and the specificity of membranous staining with another antibody.

**Conclusion** Vimentin neoeexpression is seen in PCa as a sign of tumour EMT, and membranous vimentin seen in 67.7% of cases may also mark CSCs. We are planning to confirm the results of the present study in a larger series and the specificity of membranous staining with another antibody.

**PO-247 MESOTHELIN REGULATES INVASION AND PERITONEAL METASTASIS OF OVARIAN CANCER**

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**Introduction** Ovarian cancer is the fifth leading cause of cancer death in women, and is characterised by widespread metastatization in the peritoneal cavity. Mesothelin (MSLN) is overexpressed in several human cancers, including ovarian cancer, but its exact function remains unclear. The aim of the present study was to evaluate the role of MSLN expression in ovarian cancer progression/metastatization using in vitro and in vivo experiments.

**Material and methods** To study the function of MSLN in ovarian cancer progression/metastatization we generated ovarian cancer cell lines (OVCAR3 and OVCAR8) with stable knockdown of MSLN expression using short-hairpin RNA. The biological effects of MSLN knockdown were evaluated on migration, invasion, proliferation and apoptosis assays. Further, to disclose the role of MSLN expression in the peritoneal dissemination and metastatization process we established peritoneal xenografts by injecting intraperitoneally OVCAR8 cells with and without knockdown of MSLN in athymic nude mice [NIH(II)s:nu/nu] (n=3).

**Results and discussions** Downregulation of MSLN decreases cell invasion, whereas in vivo animal experiments...
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 I3S 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.1 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 glycans, which lead to the activation of tyrosine kinase receptors, such as HER2, MET, and RON;2,3,4,5 (C) the identification of altered glycosylated proteins, carrying simple mucin-type carbohydrate structures, in engineered cancer cell models and in sera of cancer patients.5,6

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.5,6

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 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, HRAK, PIK3CA). To assess 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%), NRAS=3 cases (23%), KRAS=3 cases (23%), HRAS=2 cases (15.4%) and PIK3CA=2 cases (15.4%). Among the mutated cases 67% exhibited more than one 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 HRAK 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.

Conclusion The number of mutational events in PTC with DM is strikingly higher than in PTC 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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Abstracts