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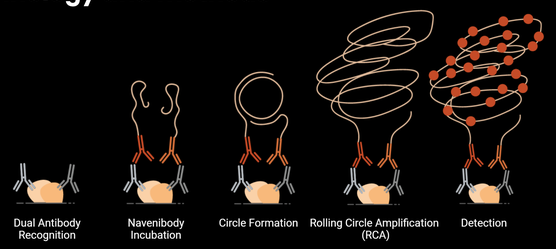
Exploring the role of the MSLN/MUC16 interaction in cancer progression via the *in situ* proximity ligation technique

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Introduction

Mesothelin (MSLN) is a glycoprotein present on the surface of mesothelial cells, sometimes used as a tumor marker. Its overexpression in various cancers, including mesothelioma, ovarian, pancreatic, and lung, is linked to increased tumor aggressiveness, evasion of apoptosis, metastasis, and resistance to treatments. Mucin16 (MUC16) belongs to a family of proteins that play a crucial role in cell adhesion, signaling, and immune responses. In cancer, alterations in mucin expression and structure may contribute to tumor progression and metastasis by enhancing cell survival and immune evasion. Mesothelin is known to interact with mucins, influencing tumor cell behavior and metastatic potential. More specifically, the MSLN/MUC16 interaction facilitates tumor cell invasion and peritoneal spread in ovarian cancer. However, reliable molecular tools to study protein interactions have been scarce. Here, we applied the Naveni® *in situ* proximity ligation technology to identify MSLN/MUC16 in various cancer samples. The method detects proteins located within interaction range (<40 nm) by means of antibodies conjugated to oligonucleotides that create amplified fluorescent signal. Since the technique does not compromise the structural integrity of tissues, communication between proteins can be studied in their native environment.

Technology and methods



Human FFPE tissue microarrays (TMA) with ovarian primary carcinoma and matched metastatic cancer (80 cores) and ovarian cancer tissue with normal tissue (100 cores) were deparaffinized and subjected to standard heat-induced antigen retrieval and blocking. Staining against the MSLN/MUC16 interaction was performed with the NaveniFlex™ Tissue MR Atto647N kit (Navinci Diagnostics). Co-staining antibodies against pan-Cytokeratin, Ki67 or MMP1 were included in the detection mix as indicated. Immunofluorescence (IF), using the same MSLN and MUC16 primary antibodies, was completed on an additional slide of each TMA, and then secondary antibodies conjugated to Alexa Fluor™ 488 and 594 were used for detection. Imaging was performed on an Olympus scanner VS200.

Conclusion

In this study, we showed that the interaction is abundant in ovarian cancer, with a distinguishable difference in the signal presence between cancerous and normal adjacent tissue. Furthermore, the additional tumor marker co-staining provide insights to the tumor invasion and growth. When comparing the Naveni® signal of MSLN/MUC16 against IF staining of each, the Naveni® signal shows true interactions, as opposed to individual expression patterns. Understanding the roles of mesothelin and mucin in cancer, both individually and in interaction, is crucial for developing targeted therapies and diagnostic tools. Further research on their interaction and specific molecular pathways triggered by it is essential for devising effective strategies to combat cancer progression.

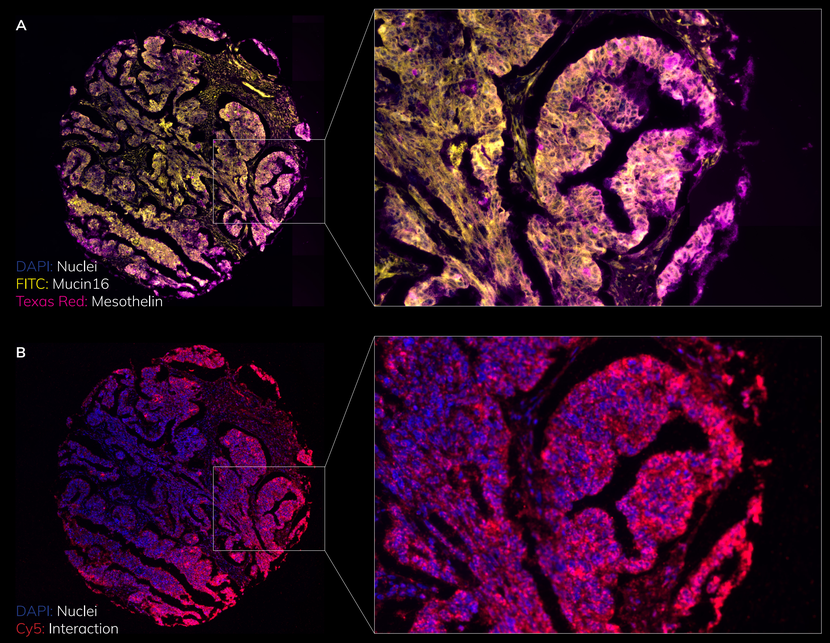


Fig. 1. Comparison between expression of MUC16 and MSLN and their interaction levels in ovarian cancer TMA cores. (A) Immunofluorescent staining against Mucin16 (yellow) and Mesothelin (magenta). The zoomed-in section shows an area of high overlap of fluorescent signals (white) suggesting but not proving interaction between the two proteins. (B) Mucin16/Mesothelin interaction (red) detected with NaveniFlex™ Tissue. Although the individual expression patterns of the two proteins largely overlap with the isPLA staining, IF co-expression is not a synonym for interaction. The zoomed-in area shows the same region as in (A) with a lot of interactions visible.

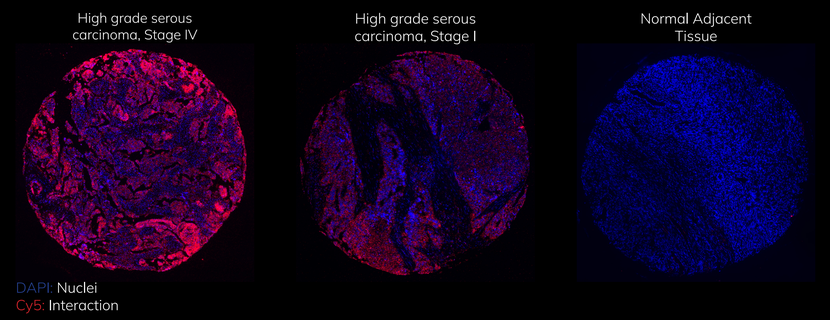


Fig. 2. Comparison of MUC16/MSLN interaction levels in different disease stages compared to normal adjacent tissue. High grade serous ovarian carcinoma cores in different stages and a normal adjacent tissue core were stained for the Mucin16/Mesothelin interaction and variable degrees were observable. Although additional studies are needed in order to make a definitive conclusion, the staining patterns suggest that the severity of disease is possitively correlated to the number of interactions detected (core on the far left vs middle core) and thus healthy tissue (core on the far right) is negative for the interaction.

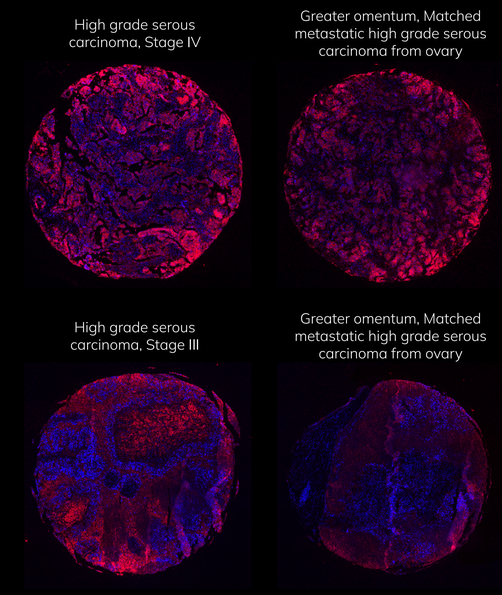


Fig. 3. MUC16/MSLN interaction levels in primary and metastatic high grade serous carcinoma of different stages. Metastasis of ovarian cancer to the peritoneal cavity can occur in an alternative manner compared to classic hematogenous metastasis seen in many solid cancers. Ovarian cancer cells detach from the main tumour and form spheroids that survive and recruit specific cell types to create a specialized TME that they can survive in. They then metastasise predominantly to the peritoneal milieu. We compared stage III (below) and stage IV (above) high grade serous ovarian carcinoma to a matched metastatic carcinoma in the greater omentum and observed similar levels of interaction between the primary and secondary site tumors.

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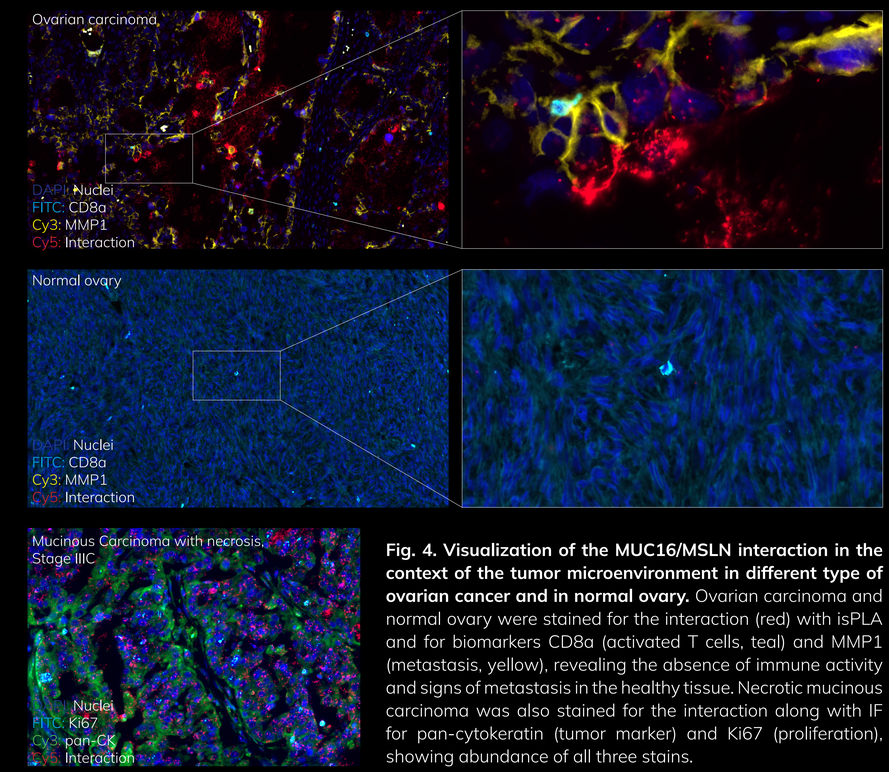


Fig. 4. Visualization of the MUC16/MSLN interaction in the context of the tumor microenvironment in different type of ovarian cancer and in normal ovary. Ovarian carcinoma and normal ovary were stained for the interaction (red) with isPLA and for biomarkers CD8a (activated T cells, teal) and MMP1 (metastasis, yellow), revealing the absence of immune activity and signs of metastasis in the healthy tissue. Necrotic mucinous carcinoma was also stained for the interaction along with IF for pan-cytokeratin (tumor marker) and Ki67 (proliferation), showing abundance of all three stains.