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Investigating the E-Cadherin/β-catenin interaction in colon cancer: a proximity ligation approach

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Background

Colon cancer, a leading cause of cancer-related deaths worldwide, is driven by a complex interplay of molecular interactions. Among these, the interaction between beta-catenin and E-cadherin stands out due to its pivotal role in cell-cell adhesion and the Wnt signaling. Disruption of this interaction can lead to aberrant Wnt signaling, promoting uncontrolled cell growth, altered cell differentiation, and increased metastatic potential. Studying this interaction in colon cancer tissue is of great importance. The tissue-specific context provides insights into the in vivo dynamics of the disease, capturing the intricate cellular environment and the influence of surrounding stromal cells. Understanding the beta-catenin and E-cadherin interaction in colon cancer tissue deepens our knowledge of disease progression but also offers a foundation for developing targeted therapeutic strategies. By focusing on tissue-specific interactions, researchers can pave the way for more effective treatments and improved patient outcomes.

In this study we have utilized an advanced technique, called proximity ligation, optimized on the Leica Bond platform, to delve deeper into these molecular interactions. This approach offers high precision and reproducibility, enabling researchers to elucidate the dynamics of the beta-catenin and E-cadherin complex in colon cancer cells (Fig.1).

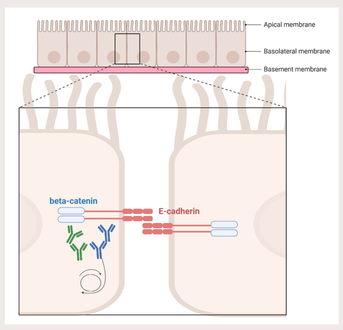


Fig. 1. Schematic representation of proximity ligation assay for the detection of beta-catenin-E-Cadherin interaction.

Materials and Methods

In our research, we utilized tissue microarrays (TMA) containing various types of colon cancer samples. The first TMA included 56 cases of colon adenocarcinoma, each matched with adjacent normal tissue sections, covering pathology grades 1 to 3 and clinical stages I to IV. The second TMA contained 86 cases of colon adenocarcinoma, featuring various subtypes, such as papillary adenocarcinoma, mucinous adenocarcinoma, adenoma, and congenital megacolon. These TMA sections were subjected to antigen retrieval using citrate buffer and heat. Primary antibodies targeting β-Catenin and E-Cadherin were applied, followed by detection using the NaveniFlex™ Tissue system for fluorescence-based analysis and the NaveniBright™ system for chromogenic analysis. Staining procedures were automated using the Bond™ platform. This approach allowed us to comprehensively investigate β-Catenin-E-Cadherin interactions within a diverse set of colon cancer samples, providing valuable insights into the cellular context of these interactions alongside traditional histological evaluation.

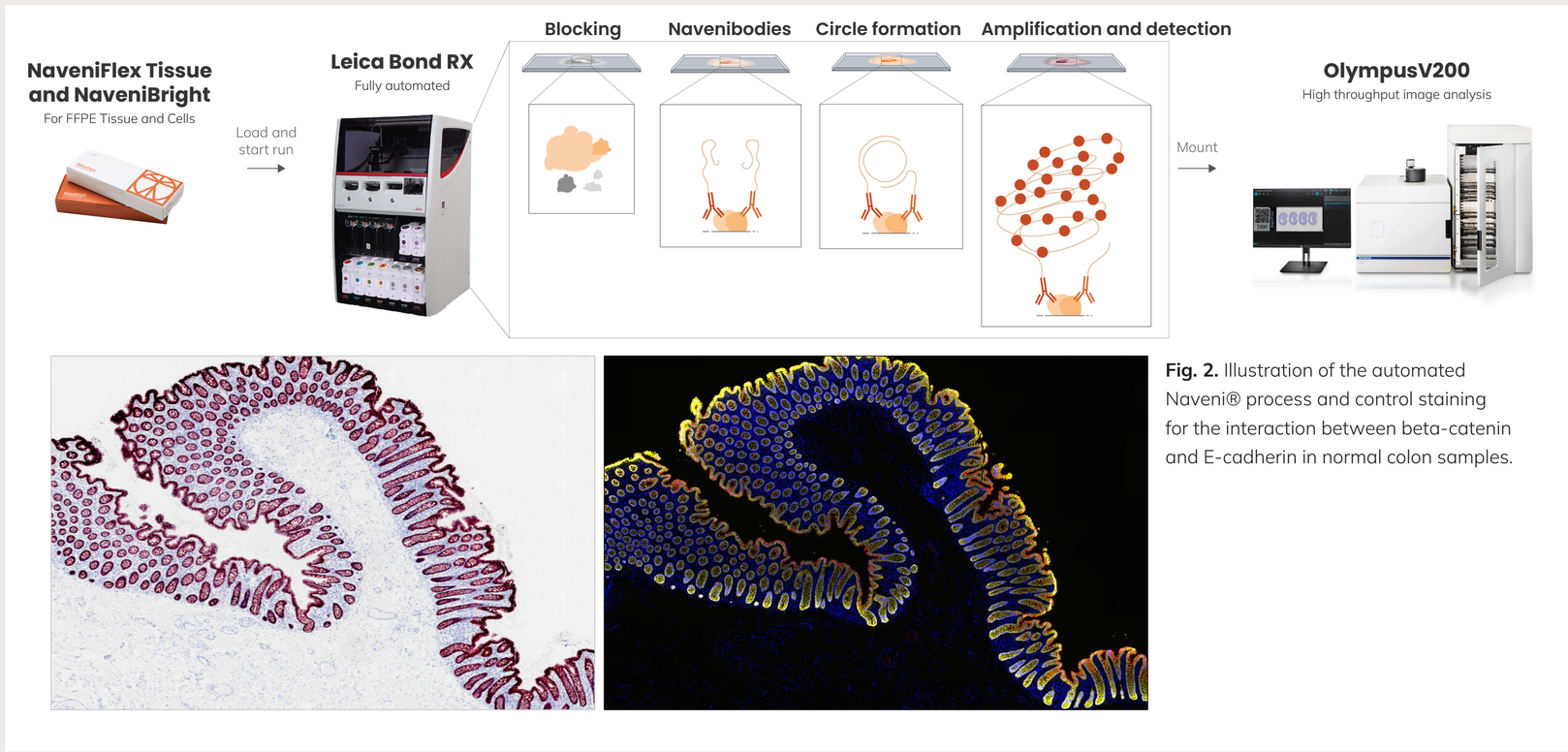


Fig. 2. Illustration of the automated Naveni® process and control staining for the interaction between beta-catenin and E-cadherin in normal colon samples.

Results

Colon cancer tissue array with matched adjacent normal tissue

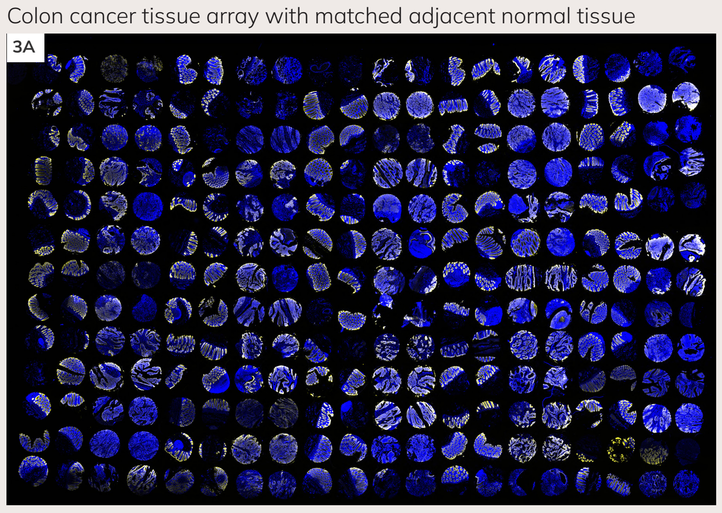
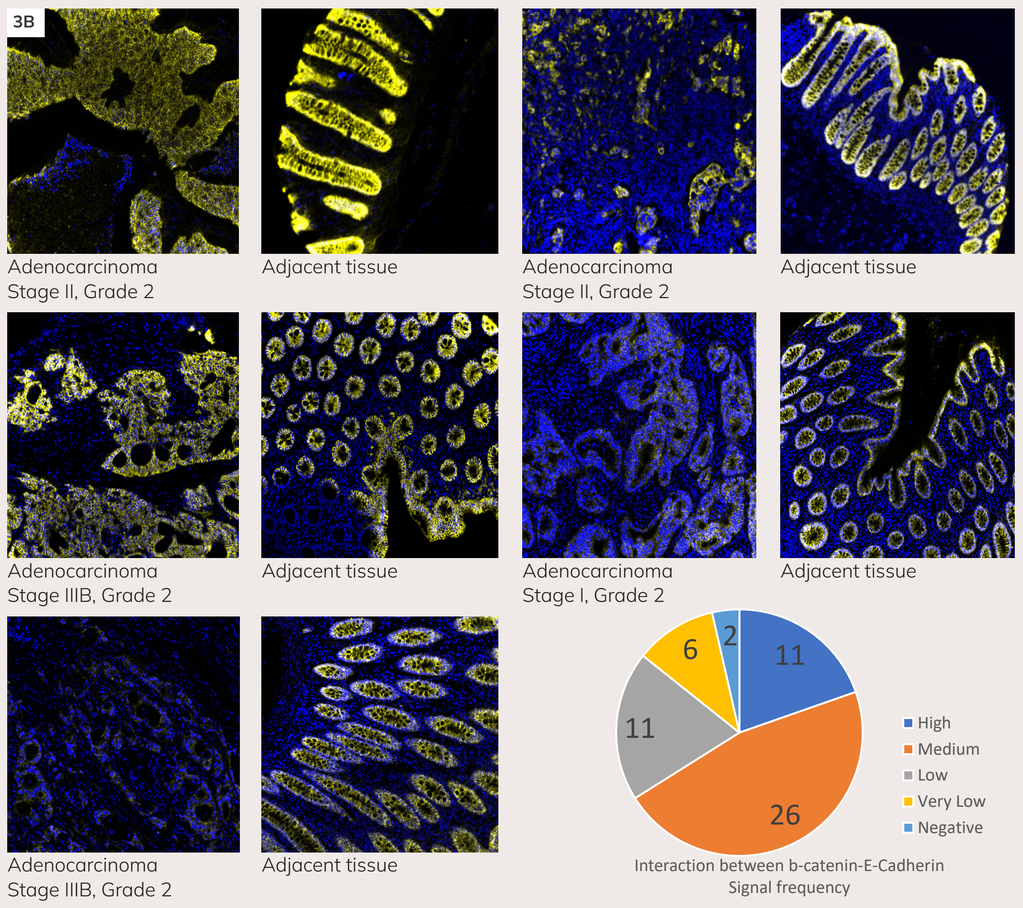
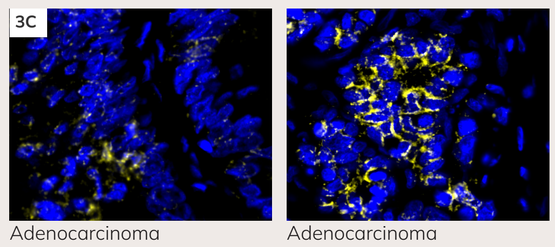


Fig. 3. Beta-catenin and E-cadherin interaction was identified in a colon TMA comprising 65 adenocarcinoma samples paired with their adjacent normal tissue **(A)**, with a detailed examination across various tumor stages/grades **(B)** and staining variability **(C)**. Staining was achieved using DAPI (blue) and isPLA (yellow).



A tissue microarray (TMA) of 56 colon adenocarcinoma samples paired with matched adjacent normal tissue, we investigated the interaction between beta-catenin and E-cadherin using the proximity ligation assay **(A)**. The results indicated a predominant localization of this interaction at cell membranes in the majority of samples **(B)**. However, a variable pattern and abundance of interaction was observed in certain tumors **(C)**. The healthy adjacent tissue to each tumor sample consistently demonstrated high levels of interaction, always localized at cell membranes. This suggests a potential baseline or normative state for these tissues. Furthermore, while some high-grade, advanced-stage cancer samples also exhibited pronounced and membrane-confined interaction, it was intriguing to note that the interaction was disrupted in some low-grade, early-stage samples. This disruption in seemingly less aggressive tumors underscores the complexity of the molecular dynamics at play and suggests that the beta-catenin and E-cadherin interaction might be influenced by factors beyond just tumor grade and stage.



In a colon TMA consisting of 86 diverse colon cancer samples (A), the interaction between beta-catenin and E-cadherin was detected using the proximity ligation assay, with concurrent staining for cytokeratin. The data revealed fluctuating levels of both targets across different tissues. Interestingly, while some samples exhibited a direct correlation between the levels of the two targets, others displayed an inverse relationship, with one being elevated while the other was reduced, and vice versa (B). Notably, a subset of samples demonstrated minimal beta-catenin and E-cadherin interaction but had pronounced cytokeratin expression.

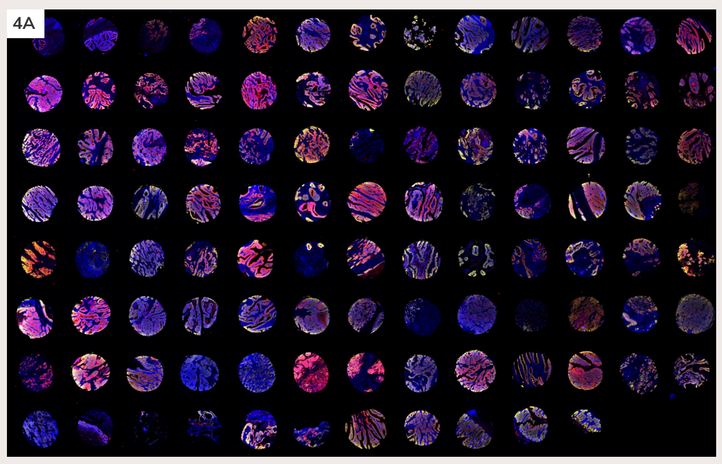
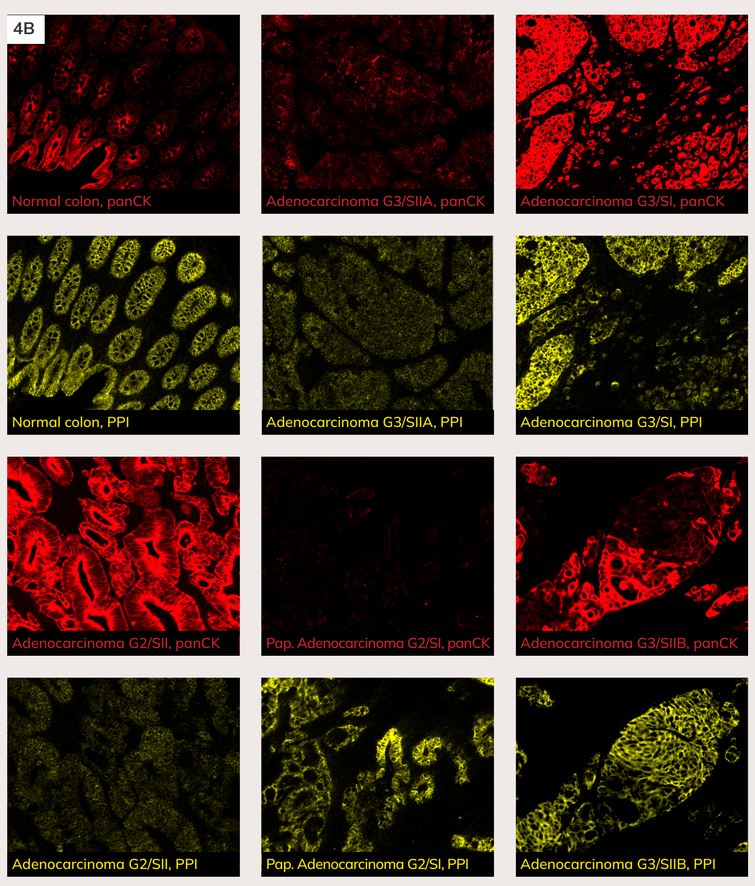


Fig. 4. The interaction between beta-catenin and E-cadherin was examined in a colon TMA containing 86 diverse colon cancer specimens **(A)**. This analysis also explored the co-expression of cytokeratin across different tumor stages and grades and assessed staining variability **(B)**. The staining process utilized a panCK antibody (red) and isPLA (yellow).



Conclusions

Our study using tissue microarrays (TMAs) of colon adenocarcinoma revealed variable interaction patterns between beta-catenin and E-cadherin across tumor samples. While healthy adjacent tissues consistently showed strong interactions at cell membranes, tumor samples displayed variability, with disruptions even in some low-grade tumors. Concurrent cytokeratin staining showed fluctuating expression levels, with some samples indicating an inverse relationship with beta-catenin/E-cadherin interaction. This aligns with studies, which highlighted cytokeratin variability in colon cancer. The observed molecular dynamics underscore the need for deeper insights to guide potential therapeutic strategies in colon cancer.

Come find us at booth 919 for further discussions



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