A shared feature among different types of ovarian cancer is the ability of some cancer cell populations to form papillary structures that detach and seed peritoneal metastases causing mesothelial cell layer disruption. We have developed a combination of organoid and spheroid culture approaches with live cell imaging to study, in time and space, the formation of polarized papillary outgrowths. We previously documented that a mutant p53/integrin/myosin axis was required for ovarian cancer cells to intercalate into mesothelium. We provide new evidence that phosphorylated myosin light chain (pMLCII) is enriched in cancer cells that form papillary outgrowths. Remarkably, in an ovarian cancer patient-derived xenograft (PDX) model, we detected a population of cells with high phosphorylation of MLC. Inhibition of ROCK decreased MLCII phosphorylation and blocked papillary formation in organoid cultures. Puzzlingly, however, ROCK activation and MLC phosphorylation and blocked papillary formation in organoid cultures. Puzzlingly, however, ROCK activation and MLC phosphorylation. Inhibition of ROCK decreased MLCII phosphorylation and blocked papillary formation in organoid cultures. Puzzlingly, however, ROCK activation and MLC phosphorylation.

**Muscle Myosin II Activity Defines Cell Populations that Initiate Dissemination of Ovarian Cancer**

**Modulation of Non-Muscle Myosin II Activity Defines Cell Populations that Initiate Dissemination of Ovarian Cancer**

**1. Dissemination of Papillary Serous Ovarian Cancer**

**2. Engineering Fallopian Tube Carcinogenesis**

**3. Mutant p53 promotes papillary outgrowth in Fallopian Tube Cell Organotypic Cultures**

**4. GEM Mouse Fallopian Tube Cells and Human Tumor Cells Make Papillary Structures Similar to Engineered Human Fallopian Tube Cells**

**5. Loosely Attached Cells Initiate Papillary Outgrowth**

**6. Loosely Attached Cells are Enriched with pMLCII**

**7. Blebbistatin Treatment Promotes Appearance of Loosely Attached Cells**

**8. Defining pMLCII State in PDX Tumor Populations**

**9. Increased Affinity for ATP Treatment Promotes Appearance of Loosely Attached Cells**

**10. Defining pMLCII State in PDX Tumor Populations**

**11. Model and Conclusion**

**Activation of ROCK Contributes to Papillary Formation**