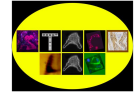




Do Ultrastructural Changes in the Aged Peritoneum Contribute to Ovarian Cancer Metastasis?

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Background

Epithelial ovarian cancer (EOC) will affect 1 in 69 women born in the United States today. Currently, 80% of women newly diagnosed with EOC already have metastatic disease, thus early intervention during the metastatic process will improve the long-term survival rates of women with EOC. Metastasis in EOC occurs through a unique process where cells are shed from a primary tumor and form multicellular aggregates (MCA) that disseminate intraperitoneally in the ascites fluid. Once the MCA reach the mesothelium, the MCA implant and disseminate [Figure 1]. EOC displays an age-specific incidence that increases and peaks in the eighth decade of life. Epithelial tumors, unlike stromal or germ cell tumors, are uncommon before the age of 40. These epidemiologic factors form the basis of the current hypothesis, that **the aging of the mesothelium alters the receptivity to implantation of metastatic cells.**

Figure 1

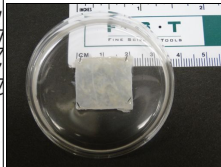
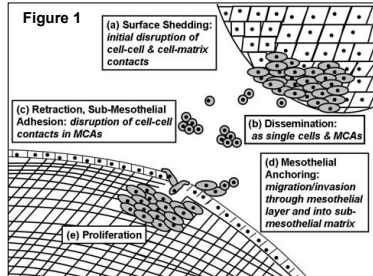


Figure 2: Ex vivo experimental set-up with pinned murine peritoneum in a dish coated with silicon elastomer.

Methods

- Young (5 months) and aged (18 months) mice were anesthetized, then euthanized with a subsequent cervical dislocation. The peritoneum was removed and placed in phosphate buffered saline (PBS) to wash and prevent desiccation.
- OvCa429 cells were then "seeded" to the tissue for 2 hours at 37°C (Figure 2), then the tissue was washed with PBS.
- The tissue was processed for scanning electron microscopy (SEM) using 2% glutaraldehyde and 2% paraformaldehyde as a primary fixative, and 2% OsO₄ in 0.1 M Na-cacodylate buffer, pH 7.35 as a secondary fixative.
- A Hitachi S-4700 FESEM was used to image the samples ($V_{acc}=4.0kV$; $I_e=10.0\mu A$).

Results

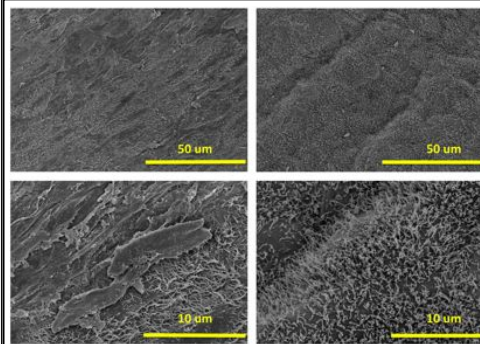


Figure 3: Scanning Electron Microscopy (SEM) images of old (left column) and young (right column) murine peritoneum. Note the areas of fibrosis visible in the old specimen.

Figure 4: SEM images of microvilli on old (left column) and young (right column) murine peritoneum. The microvilli in the old specimen appear elongated and areas of the peritoneum are devoid of microvilli. The microvilli in the young tissue display round projections distally and the peritoneal surface appears "freckled."

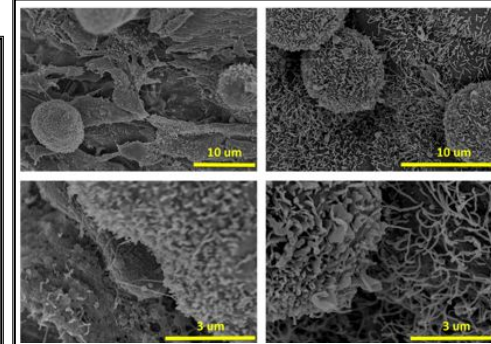
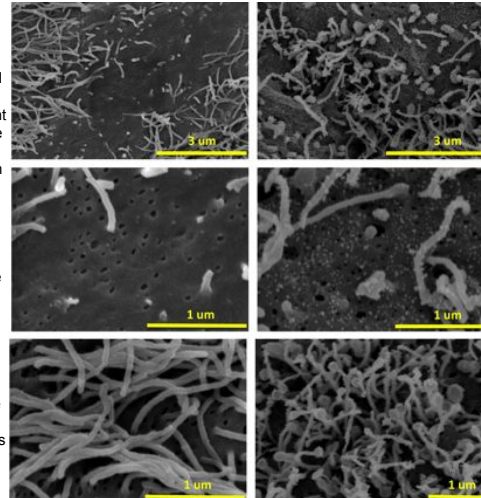


Figure 5: SEM images of old (left column) and young (right column) murine peritoneum with OvCa 429 cells attached (2hr). The cells adhered to areas of fibrosis in the old tissue.

Conclusions and Future Directions

- The overall topography of the old and young tissues was drastically different; the old peritoneum displayed signs of fibrosis (Figure 3).
- The structure of the microvilli differs between the old and the young tissues. The old peritoneum has areas devoid of microvilli, whereas the young peritoneal tissue possesses numerous microvilli throughout. In addition, the structure of the microvilli varies between the tissues; the old microvilli are elongated, whereas the young microvilli have round projections distally. Finally, the peritoneal surface is different between the two ages; young peritoneum displays "freckles" on the surface which may also be observed on the surface of the microvilli in the young samples (Figure 4).
- When OvCa429 cells were seeded to tissues for 2 hours, the cells adhered more readily to areas of fibrosis in the old tissues. The microvilli appear to play a role in the attachment of the ovarian cancer cells, and fibrosis may enhance this attachment (Figure 5).
- A method is being developed to quantitate the number of cells that attach to the tissue. This method could help determine whether or not more cells attach to old tissue.

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