Assessment of ovarian cancer spheroid attachment and invasion of mesothelial cells in real time to profile the molecular signature of the invasive interface.

Marlee Bilandzic, Adam Rainczuk, Andrew Stephens, Nicole Fairweather1, Thomas Jobling1 and Kaye L. Stenvers

MIMR-PHI Institute of Medical Research, Australia; 1-OCR, Epworth Research, Australia.

Introduction
Ovarian cancer is the most common cause of death from gynaecological cancer worldwide with over 75% of initial diagnoses made at a late stage when the cancer has metastasized (1, 2). Once this insidious disease has disseminated, treatment methods are almost never curative (3). Thus, insights into the unique biology of ovarian cancer metastasis are urgently required to derive novel strategies to block their spread.

The formation of ovarian cancer spheroids within the non-adherent peritoneal environment is a barrier to effective treatment, as spheroids have an enhanced ability to survive chemotherapies and seed distal metastases by disseminating within the peritoneal fluid (3, 4).

Invading ovarian cancer spheroids interact with mesothelial cells lining the peritoneal surface, attach to, and invade the underlying basement membrane to establish early invasive lesions (5). To date, the molecular events occurring at the interface between invading ovarian cancer cells and peritoneal cells at the onset of invasion have been rarely studied, as traditional assays generally fail to capture these early events. As such, the critical regulatory molecules which govern the initiation of a metastatic lesion within the peritoneal cavity are poorly understood.

Hypothesis: The interface between ovarian cancer spheroids and the peritoneal surface exhibits a unique protein signature as the cancer cells invade, comprising bidirectional signals governing the early events in the establishment of secondary tumors.

To address this hypothesis, we have developed an in vitro co-culture model which recapitulates key aspects of the peritoneal microenvironment to quantitatively study spheroid invasion through components of the peritoneal cavity in real time. We then identify the proteins expressed at the ovarian cancer spheroid-peritoneal interface during the earliest stages of invasion using a novel proteomics approach.

Methods

Real Time Cell Analysis (RTCA) technology (xCELLigence, ACEA) was adapted to establish a three-dimensional co-culture model of the tumor microenvironment of the peritoneum (6).

RTCA wells were coated with Matrigel (basement membrane matrix) and a confluent monolayer of human mesothelial cells (C).

Non adherent ovarian cancer cells were derived from fresh malignant ascites comprising high-grade serous carcinoma or benign specimens (B). Multicellular spheroids were generated from cells cultured under non-adherent conditions (C) and the earliest stages of spheroid invasion were established in our peritoneal microenvironment model by RTCA.

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Conclusions

Our novel methods capture and characterize the poorly understood molecular events which occur during the initiation of invasion, focusing specifically on the border between invading ovarian cancer cells and peritoneal tissue - the “invasive interface”.

RTCA methodology enables a high-throughput quantitative analysis of primary ascites-derived ovarian cancer spheroid invasion of mesothelial and ECM barriers and pinpoint the moments of early invasion in a peritoneal microenvironment model.

MALDI imaging of the “invasive interface” has provided an unprecedented insight into the molecules involved in the initiation of ovarian cancer invasion of the mesothelium.

Subsequent studies will determine the significance of identified proteins to the metastatic process, establishing their potential as therapeutic or diagnostic/prognostic agents for clinical translation, providing new and effective therapeutic options for ovarian cancer patients with advanced disease.