Characterization of the bi-directional cross talk between cancer cells and carcinoma-associated fibroblasts

by *Ernst Lengyel*, University of Chicago, Section of Gynecologic Oncology

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Most ovarian cancer (OvCa) patients are diagnosed when they have advanced stage disease with metastasis to the omentum. In the omentum, more than half of the tumor microenvironment is composed of fibroblasts. It is widely acknowledged that cancer-associated fibroblasts (CAFs) play a key role in tumorigenesis by promoting tumor growth, invasion, and metastasis. However, the signaling networks activated by the interaction between CAFs and OvCa cells have yet to be discovered. We hypothesize that, when OvCa cells and CAFs come into contact, reciprocal signaling pathways are activated in both cell types which fundamentally alters them, resulting in the promotion of tumor progression and metastasis.

In order to identify these signaling pathways in an unbiased way, we used a strategy that combines SILAC (Stable Isotope Labeling of Amino acids in Culture) labeling and mass spectrometry (MS) based quantitative phosphoproteomics. First, an OvCa cell line, SKOV3ip1, was grown in media containing heavy isotopes of lysine and arginine which were incorporated into each protein. This SILAC labeling allows us to identify the cell line of origin for each peptide upon MS analysis of the co-culture of OvCa and CAF cells. The heavy labeled OvCa cells were then cultured either on plastic or with CAFs (light labeled) lysed and processed for MS analysis. We then used two separate methods of phosphopeptide enrichment in order to maximize the number of peptides identified. The first method was enrichment with titanium dioxide (TiO_2), which binds preferentially to peptides containing, in order of abundance, phosphorylated serine, threonine, and tyrosine. Using this method we identified 63 unique sites which were statistically different when the cancer cells were cultured alone and when they were cultured with fibroblasts. While the data produced by the TiO_2 enrichment provides some insight into the signaling pathways of interest, the low number of proteins containing phospho-tyrosine within the cell resulted in limited identifications.

Phospho-tyrosine modifications are of unique interest in cancer, since they control many oncogenic pathways. Therefore, in order to specifically enrich p-tyr peptides, we used an antibody to immunoprecipitate the p-tyr peptides prior to MS analysis. This method produced identifications of 21 unique peptides which were significantly decreased in CAFs and 37 peptides which were significantly increased. In SKOV3ip1 cells, we identified 161 peptides that were significantly reduced and 75 peptides that were significantly increased.

We currently use the unique software capabilities of our collaborators at Brown University to visualize and predict novel pathways of interest in both OvCa and CAF cells. In the second year of funding we will validate the bi-directional signaling network formed between CAFs and OvCa. Our long term goal is to use this data as a rational basis for the development of new therapeutic strategies for the treatment of metastatic ovarian cancer. Moreover, we believe that the identification of the key signaling pathways activated by the interaction between cancer cells and CAFs could provide knowledge relevant to the biology of cancers other than OvCa which also involve CAFs, such as pancreatic, breast, and colon cancers.

PUBLICATIONS:

1) Romero I.L., McCormick A., Angell K., Park S.Y., Karrison T., Pannain S., and Lengyel E. Metformin use is associated with improved ovarian cancer survival in patients with type II diabetes. **Obstetrics & Gynecology**, 2012, 119 (1): 61-67. PMID: 22183212
2) Tergas A., Buell-Gurbrod R., Gwin K., Kocherginsky M., Temkin S., Fefferman A., Lengyel E., Yamada S.D. Clinico-pathologic comparison of type II endometrial cancers based on tamoxifen exposure. **Gynecologic Oncology**, 2012, 127 (2): 316-320. PMID 22835717
3) Ko S.Y., Barengo N., Lee J., Ladanyi A., Marini F., Lengyel E., Naora H. HOXA9 promotes human ovarian cancer growth via paracrine effects on mesenchymal stem cells and peritoneal fibroblasts. **Journal of Clinical Investigation**, 2012, 122 (10): 3603-3617. PMID 22945634
4) Mitra A.K., Zillhardt M.R., Hua Y.J., Tiwari P., Murmann A.E., Peter M.E., and Lengyel E. microRNAs mediate early reprogramming of fibroblasts into cancer-associated fibroblasts. **Cancer Discovery**, 2012, 2 (12): 1100-1108. PMID: 23171795
5) Lengyel E., Fleming S., McEwen K.A., Montag A., and Temkin S. Serial sectioning of the fallopian tube allows for improved identification of primary fallopian tube carcinoma. **Gynecologic Oncology**, 2013, 129 (1): 120 – 123. PMID 23237768
6) Ohyagi-Hara C., Sawada K.,Kamiura S.,Tomita Y., Isobe A.,Hashimoto K., Kinose Y.,* Seiji Mabuchi,* Nagata S., Morishige K.I., Lengyel E., Kurachi H.,Kimura T. MiR-92a inhibits peritoneal dissemination of ovarian cancer cells by inhibiting integrin α5 expression. **American Journal of Pathology**, 2013, 182 (5): 1876-1889. PMID 23499550, *In press*
7) Nieman K.M., Romero I.L., Van Houten B., and Lengyel E. Adipose tissue and adipocytes supports tumorigenesis and metastasis. **Biochimica et Biophysica Acta** 2013, *in press*.
8) Landen C and Lengyel E. Summary of the 2013 American Association for Cancer Research (AACR) Annual Meeting. Gynecologic Oncology

TALKS:

2013

- Angiogenic genetic signatures in ovarian cancer
American Society of Clinical Oncology (ASCO) Annual Meeting – Discussant at Plenary session (6/1/2013)
- Ovarian cancer metastasis
Max Planck Institute for Biochemistry, Munich, Germany (5/5/2013)
- The origin of ovarian cancer
University of Chicago (4/17/2013)
- Metabolic changes during ovarian cancer metastasis
University of Richmond, Dept. of Biochemistry weekly seminar (2/28/2013)
- Co-organized with Drs. Bill Beck (UIC) and Skip Schink (NWU) a daylong “Ovarian Cancer Workshop”. Received funding from the Chicago Biomedical Consortium. Gave

talk: “Current state and future directions of basic and translational science in ovarian cancer” (1/12/2013)

2012

- How does adipose tissue promote tumor growth?
Mayo clinic – Oncology Society lecture – Rochester (12/7/2012)
- Interactions between Ovarian Cancer Cells and the Microenvironment during early Metastasis
Magee-Womens Research Institute's Research Seminar – University of Pittsburgh Medical Center (UPMC) (11/13/2012)
- Ovarian cancer cells and the microenvironment during early ovarian cancer metastasis
Stanford University, Grand Rounds (07/07/2012)
- The clinical behavior of ovarian cancer: What are the critical scientific questions?
Midwestern Epithelial Cancer Coalition, University of Notre Dame, South Bend (6/10/2012)
- Mechanisms of early ovarian cancer metastasis (*Plenary Session*)
American Association for Cancer Research Annual Meeting, Chicago (4/1/2012)
- Interactions between ovarian cancer cells and microenvironment during early metastasis
Grand Rounds, Northwestern University Comprehensive Cancer Center (2/10/2012)
- Cross talk between cancer cells and adipocytes
Section of Endocrinology. Research seminar series, University of Chicago (2/6/2012)