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

As the most lethal gynecologic cancer, ovarian cancer leads to significant human suffering. The vast majority of patients are diagnosed with advanced stage disease involving metastasis to the omentum. After debulking surgery, most patients initially respond to platinum-based chemotherapeutics, however, their tumors inevitably develop resistance and recur. This combination of late diagnosis and chemoresistance explains the dismal 5-year survival rate of ovarian cancer and emphasizes the need for novel therapeutics to improve survival and quality of life.

Developing new therapeutic interventions and biomarkers for ovarian cancer will require a thorough and multidisciplinary understanding of the biology underlying ovarian cancer initiation and progression. We believe that unless we achieve a better understanding of the biology of ovarian cancer we will not be able to identify novel treatments. Recent evidence has emerged that the fallopian tube may in fact be the tissue of origin for "ovarian cancer". *In situ* lesions classified as serous tubal intraepithelial carcinomas (STIC) are observed in the fallopian tubes of patients with ovarian cancer or at high risk of developing ovarian cancer (e.g., BRCA mutation carriers).

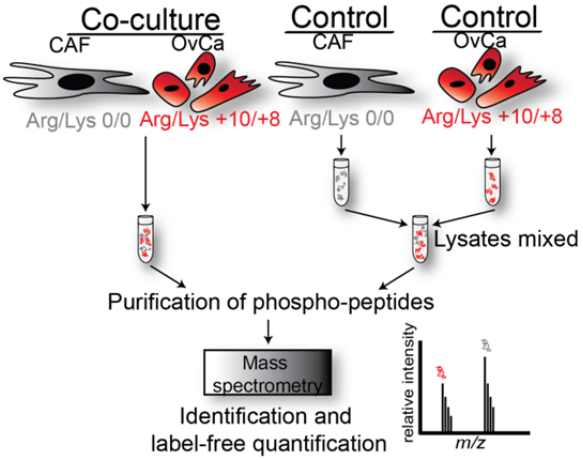

***Figure 1**. Experimental approach. Cells were co-cultured for 4hrs prior to collection for phoshoproteomic analysis. Ovarian cancer cells (red) were labeled with heavy isotopes of arginine and lysine while CAFs (grey) were light labeled to distinguish the peptides from each cell type.*

As is the case with many different types of solid tumors, more than half of the cells that compose the tumor microenvironment at the ovarian cancer metastatic site are cancer-associated fibroblasts (CAFS). CAFs promote cancer cell invasion and tumor growth by supplying growth factors, cytokines, and extra-cellular matrix. Although a few studies have begun to address the role of CAFs in the metastatic ovarian tumor microenvironment (TME), these studies have been limited by their focus on a single pre-selected signaling pathway in ovarian cancer cells and do not address the large network of signaling events that occur in both CAFs and cancer cells. We hypothesized that when ovarian cancer cells and CAFs come into contact with one another, reciprocal and bi-directional signaling pathways are activated in both cell types which fundamentally alter them and result in the promotion of tumor progression and metastasis.

Using an unbiased strategy of mass spectrometry (MS)-based quantitative phosphoproteomics combined with SILAC labeling (**Figure 1**), we have identified and quantified over 400 unique phospho-tyrosine sites in both ovarian cancer cells and CAFs which are phosphorylated upon their interaction at an FDR <0.05. SILAC labeling of the ovarian cancer cells allowed us to clearly distinguish the cell type of origin for each identified protein. Pathway analysis of the proteomic data revealed a dominant role for integrin and FAK signaling in both cell types upon co-culture (**Figure 2**). In addition, many previously described signaling nodes involved in cancer cell invasion and survival were activated in the ovarian cancer cells, including ERK, paxillin, Erbb2, and MIRK/DYRK1B. Notably, several proteins formerly thought to only play a role in tumor cells were found to be dramatically phosphorylated in CAFs upon co-culture. These included the well-known adaptor proteins, NEDD9 and BCAR1, which are classic signaling adaptors activated down-stream of receptor tyrosine kinase (RTK) and integrin

signaling. Unexpectedly, we also observed activation of the receptor tyrosine kinase EphA2 in CAFs, which has not been previously found to play a role in CAFs and represents a novel potential drug target in the TME.

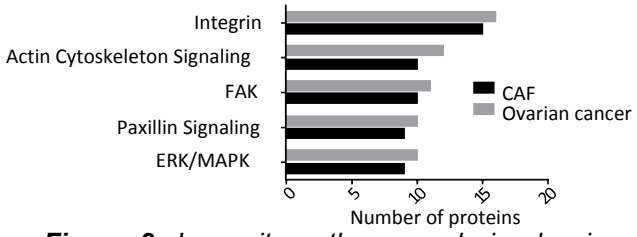

***Figure 2**. Ingenuity pathway analysis showing canonical pathways enriched in the phosphoproteomic dataset from both cell types.*

In order to further validate our findings and to demonstrate the functional relevance of the identified signaling pathways, we established an *in vitro* assay in which invasion through extra-cellular matrix could be tracked in real-time over a period of 3 days. Co-culture of GFP-labeled ovarian cancer cells with CAFs greatly enhanced invasion of the cancer cells over time (**Figure 3a**). Using siRNA to target proteins identified in the phosphoproteomics dataset, we show that transient knock down of the RTK adaptors, NEDD9 and BCAR1, in CAFs greatly reduces the invasive behavior of the cancer cells (**Figure 3b**). This confirms the important role of RTK and integrin signaling in CAFs and clearly demonstrates that, by blocking key signaling pathways specifically in CAFs, we can drastically alter the invasive ability of ovarian cancer cells in the co-culture.

In addition, we also found that PGM1 and LDHA were both phosphorylated within ovarian cancer cells in response to the co-culture, suggesting that their cellular metabolism is affected by direct interaction with CAFs. LDHA was phosphorylated at a known tyrosine residue which has been shown to increase activity of the enzyme that converts pyruvate to lactate, a key hallmark of anerobic glycolysis in cancer cells. Using glycolytic flux analysis, we demonstrated that both co-culture with CAFs and secreted factors from CAFs stimulate glycolytic flux in ovarian cancer cells. Studies are currently being conducted to determine the specific factors and direct mechanism by which CAFs promote glycolysis in ovarian cancer cells.

Ovarian cancer is a complex disease with many contributing factors and, unfortunately, there are no specific targetable mutations which occur in all ovarian cancer patients. By directing therapeutics at common mechanisms of tumor promotion in the normal cell populations of the TME, it may be possible to circumvent the widespread genomic instability of ovarian cancer cells which contributes to drug resistance. The present study provides new insight into the complex nature of the fibroblast and cancer cell interaction. Not only have we identified key signaling pathways, we have also discovered several novel mechanisms which could play important roles in the biology of metastatic cancer. We are completing the study and plan to publish the results in 2015.

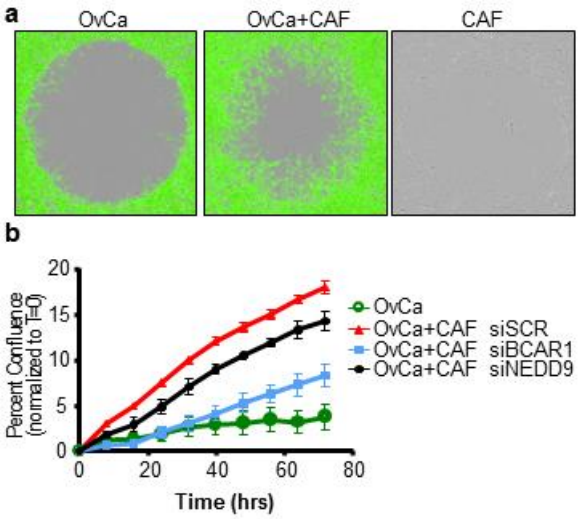

***Figure 3**. Real-time invasion assay (Incucyte). **a**, GFP-labeled OvCa cells are plated with unlabeled primary CAFs around a stationary plug. At the start of the assay, the plug is removed and invasion of the OvCa cells is quantified by fluorescence over time. **b**, CAFs were transfected with targeted siRNAs prior to invasion assay.*

I want to thank the Cancer Research Foundation, its entire board and the reviewers, for giving us a chance to investigate the fascinating topic of bidirectional signaling between normal host and tumor cells.

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