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TITLE: The Role of Mesothelial Omentin in Ovarian Cancer Progression

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<b>13. SUPPLEMENTARY NOTES</b>						
<b>14. ABSTRACT</b> We hypothesize that omentin (ITLN1) suppresses ovarian cancer progression by sequestering lactoferrin from its receptor on the ovarian cancer cells and adipocytes, and that lactoferrin promotes ovarian cancer cells motility and growth. A majority of experiments proposed under Major goal 1 and 5, and subsets experiments proposed in Major goal 2, 6 have been accomplished. Our results demonstrated that lactoferrin triggers intracellular Ca <sup>2+</sup> mobilization, enhances the amplitude of cellular traction exerted on substratum, and increases expression of matrix metalloproteinase-1 (MMP1) in ovarian cancer cells. We also showed that omentin could counteract the stimulating effects of lactoferrin on ovarian cancer cells. For Major goal 8, we have been successful to boost the circulating omentin levels in C57BL/6 mice using adeno-associated virus.						
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## 1. INTRODUCTION

Advanced epithelial ovarian cancer, which accounts for the majority new diagnoses of epithelial ovarian cancer every year, occurs most frequently in postmenopausal women and metastasizes preferentially to the soft omentum. The omentum is covered by a layer of mesothelial cells. Disseminated ovarian cancer cells have to adhere to and invade through the mesothelial cell layer before developing as metastasized tumor nodules in the omentum. However, the exact role of mesothelial cells in ovarian cancer cell adhesion and growth is unclear. Alteration in gene expression in mesothelial cells by ovarian cancer cells may facilitate the adhesion and progression of ovarian cancer cells. Using RNAseq analysis on mesothelial cells co-cultured with ovarian cancer cells, we identified a differential gene signature for ovarian cancer associated mesothelial cells. One of the most significantly down regulated genes in mesothelial cells co-cultured with ovarian cancer cells is Intestinal Lactoferrin Receptor (ITLN1), also known as omentin. Omentin is a 38 kDa secreted protein and can bind and sequester lactoferrin from its receptors. Lactoferrin is known to trigger FAK, Erk1/2 and Akt dependent signaling processes through its receptor, low density lipoprotein receptor-related protein-1 (LPR-1), which might stimulate ovarian cancer cell proliferation and invasiveness. Western blot analyses confirmed omentin was down-regulated in mesothelial cells obtained from patients with ovarian cancers compared to body mass index (BMI) matched healthy individuals. Circulating omentin levels is significantly lower in patients with high-grade serous ovarian cancer (HGSOC) compared with those in BMI matched healthy individuals. Survival correlation studies demonstrated that high levels of pre-operative serum omentin (>350 ng/ml) in patients with high-grade serous ovarian cancer were associated with longer survival times. Our preliminary data showed that omentin significantly lowered the number of ovarian cancer cells adhering to the mesothelial cell monolayer. Omentin alone did not inhibit ovarian cancer growth. However, conditioned media collected from adipocytes treated omentin inhibited the growth of ovarian cancer cells, which suggested that omentin inhibited ovarian cancer growth only in the presence of adipocytes.

Based on our preliminary studies, we hypothesize that cancer or stromal cells in the omental tumor microenvironment produce tumor specific mediators or proinflammatory cytokines such as TNF- $\alpha$  and TGF- $\beta$  down-regulate omentin expression in the mesothelial cells covering the omental adipose tissue, which subsequently facilitates ovarian tumor progression. Since secreted omentin binds lactoferrin, it can sequester lactoferrin from its receptor LRP-1 on ovarian cancer cells and adipocytes. Unopposed lactoferrin due to down-regulation of omentin results in the activation of LPR-1 mediated downstream signaling events in the ovarian cancer cells, which confers a more aggressive ovarian cancer cell phenotype, and leads to poor clinical outcomes. Strategies that aim to increase omentin levels in patients should therefore suppress ovarian cancer progression and improve survival.

## 2. KEYWORDS

Ovarian cancer, omentin, lactoferrin, mesothelium, tumor microenvironment

### **3. ACCOMPLISHMENTS**

#### **a. What were the major goals of the project?**

Major Goal 1: Measure changes in cytosolic  $\text{Ca}^{2+}$  and cell traction force in different cancer cells induced by lactoferrin (Months 1-9).

Major Goal 2: Evaluate the effects of lactoferrin and omentin on MMP1 expression in ovarian cancer cells. (Months 10-18).

Major Goal 3: Evaluate the role of store-operated  $\text{Ca}^{2+}$  entry (SOCE) in ovarian cancer cell motility and invasion potential. (Months 19-25).

Major Goal 4: Evaluate the effects of lactoferrin and omentin on the invasion of ovarian cancer cells into mesothelial cells (Months 26-34).

Major Goal 5: Evaluate the effects of omentin on insulin dependent glucose uptake in adipocytes, and test whether omentin suppress ovarian cancer growth by glucose deprivation. (Months 1-6).

Major Goal 6: Evaluate whether glucose deprivation plays a role in suppressing tumor growth in the omental tumor microenvironment using Induced Metabolic Bioluminescence Imaging (Months 7-14).

Major Goal 7: Evaluate the effects of lactoferrin and omentin on GLUT4 expression of (Months 15-21).

Major Goal 8: Boost circulating omentin levels in C57BL/6 mice using adeno-associate virus (AAV). (Months 1-24).

Major Goal 9: To evaluate the therapeutic potential of recombinant omentin. (Months 25-34).

#### **b. What was accomplished under these goals?**

A majority of experiments proposed under Major goal 1 and 5, and subsets experiments proposed in Major goal 2, 6 and 7 have been accomplished. Firstly, we confirmed our hypothesis that proinflammatory cytokines such as  $\text{TNF-}\alpha$  and  $\text{TGF-}\beta$  down-regulate omentin expression in the mesothelial cells that covering the omental adipose tissue (Fig.1).

Since an increase in cytosolic  $\text{Ca}^{2+}$  level and stress fiber formation will contribute to the increase in cell traction force required for cancer migration. To test whether lactoferrin can trigger  $\text{Ca}^{2+}$  dependent signal cascade in ovarian cancer cells and increase of cell traction force, we monitored the effects of lactoferrin in cytosolic  $\text{Ca}^{2+}$  mobilization and the amplitude of cell traction force in ovarian cancer cell lines (A224, OVA433, and SKOV3) using confocal fluorescence microscopy and traction force microscopy. The results demonstrated that lactoferrin can trigger cytosolic  $\text{Ca}^{2+}$  mobilizations and increase of cell traction in all three cell lines tested. Data extracted from ovarian cancer cell line A224 are shown in Fig.2. and 3.

Cancer cells migrate using cell-matrix mechanocoupling mechanisms through paths generated by the degradation (mediated by matrix metalloproteinases) and remodeling of the extracellular matrix, and by force-mediated deformation (powered by cell traction force from cancer cells). We next tested whether lactoferrin induces the expression of matrix metalloproteinase-1 (MMP1) in ovarian cancer cells, and whether omentin can suppress MMP1 expression. The results demonstrated that lactoferrin increases the expression of MMP1 (Fig.4), and omentin decreases expression of MMP1 (Fig.5) in A224 and SKOV3 cancer cells.

We also demonstrated that omentin reduced both glucose uptake and lactate secretion in ovarian cancer cells when they were co-cultured with adipocytes but not with other stromal cell types (Fig.6). These observation indicated that omentin-induced glucose uptake in adipocytes may deplete the surrounding glucose that fuels the glucose-addicted ovarian cancer cells in the omental microenvironment and thus drive metabolic shift in ovarian cancer cells. To delineate the underlying mechanism by which omentin induced glucose uptake in adipocytes, we have initiated the studies as described in Major Goal 6 and 7.

In order to test the direct effects of circulating omentin levels on tumor growth and overall survival, we employed a gene therapy approach to boost the circulating omentin levels in C57BL/6 mice using a custom-made adeno-associated virus (AAV-mITLN1), which transfers a constitutively expressed omentin gene to organs particularly the liver in the peritoneal cavity. Our preliminary indicated that a single injection of our custom-made AAV-mITLN1 particles increased the serum level of ITLN1in C57BL/6 mice for up to 2 weeks (Fig.7)

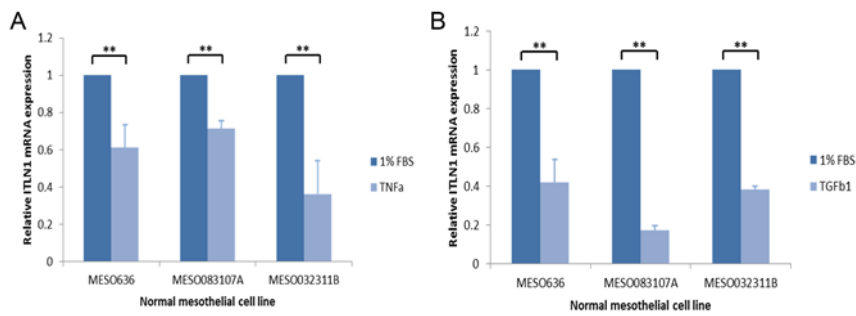


Figure 1. Bar charts showing the effect of TNF- $\alpha$  (10ng/ml) (A) and TGF- $\beta$  (5 ng/ml) (B) on omentin (ITLN1) mRNA expression in three primary mesothelial cell cultures, , respectively. Experiments were performed in triplicate. \*\*P<0.01

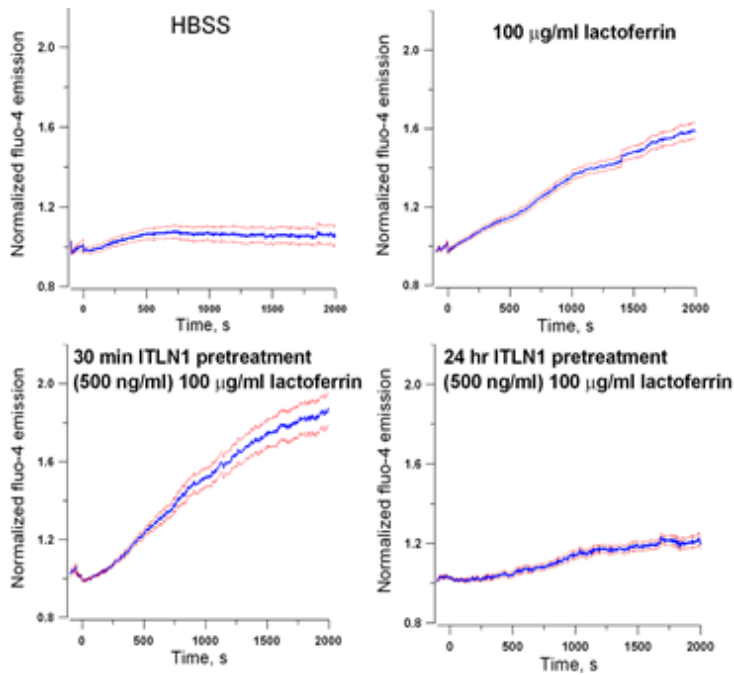


Figure 2. Graphs showing the mean normalized time course of cytosolic Ca<sup>2+</sup> mobilization in A224 cells induced by lactoferrin. Cells were pretreated with omentin (ITLN1) or Hanks' Balanced Salt Solution (HBSS) for 30 min or 24 hours before lactoferrin was added to the bath of cells at t = 0 (mean  $\pm$  SEM of four independent experiments for each treatment group).

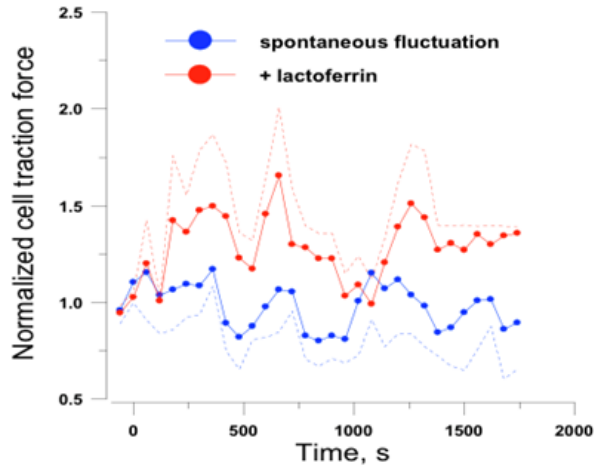


Figure 3. Mean normalized time course of cell traction force induced by lactoferrin (100  $\mu\text{g/ml}$ ) in A224 cells. Dotted lines are SEM ( $n = 6$ ). Lactoferrin was applied to the cells at  $t = 0$ .

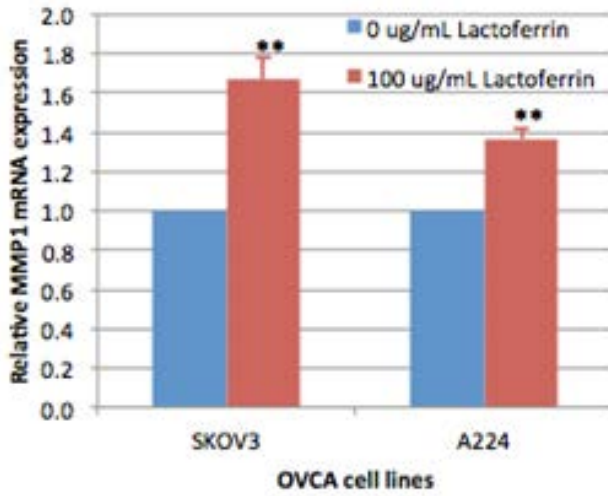


Figure 4. qRT-PCR analysis showing significantly higher levels of MMP1 expression in cells treated with 100  $\mu\text{g/ml}$  lactoferrin.  $P < 0.01$ .

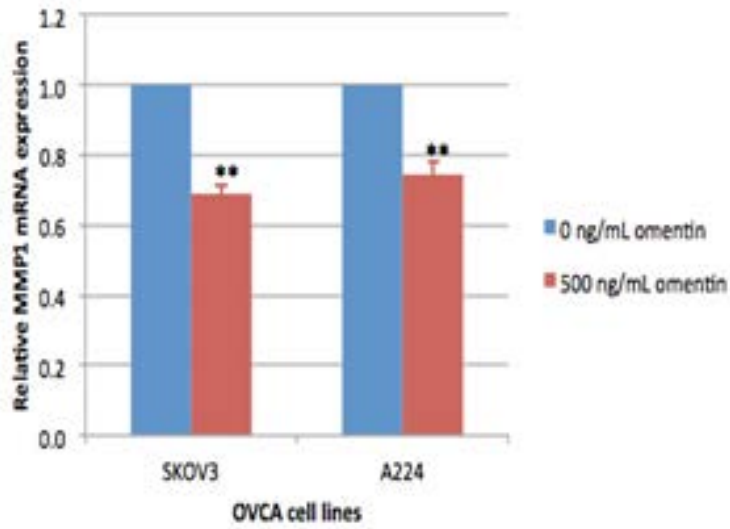


Figure 5. qRT-PCR analysis showing significantly lower levels of MMP1 mRNA in A224 and SKOV3 cancer cells treated with omentin.  $<0.01$ .

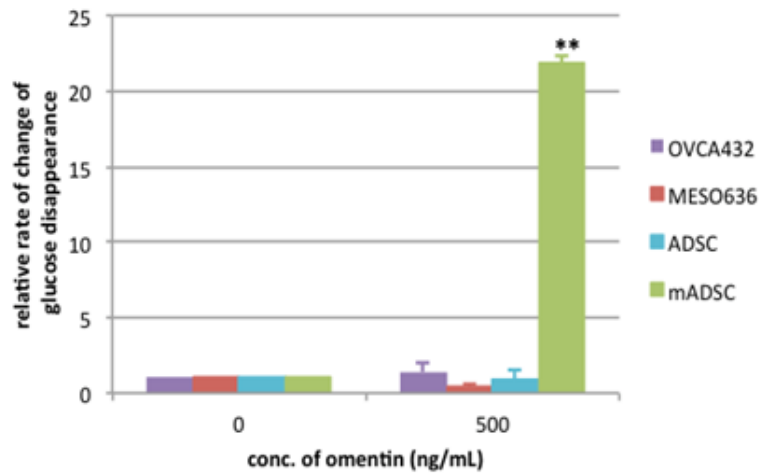


Figure 6. Effect of exogenous omentin on glucose uptake rates in pre-adipocytes (ADSC), mature adipocytes (mADSC), mesothelial cells (MESO636), and ovarian cancer cells (OVCA432).  $**P<0.01$

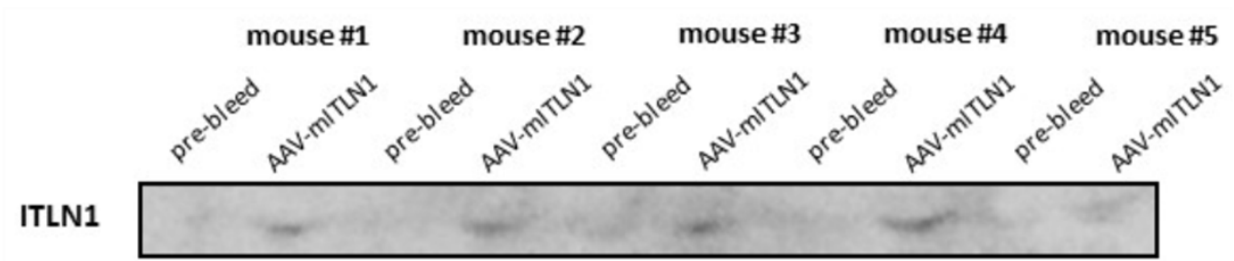


Figure 7. Western blots showing increase of serum omentin (ITLN1) level in mice two weeks after receiving a single injection of AAV-mLITN1 particles. Pre-bleed is the baseline.

- c. What opportunities for training and professional development has the project provided?**

Nothing to report

- d. How were the results disseminated to communities of interest?**

Nothing to report

- e. What do you plan to do during the next reporting period to accomplish the goals?**

In the next reporting period (months 13-24), we will perform experiments according to those outlines in the proposal to further delineate the role and mechanisms of omentin in suppressing the progression ovarian tumor.

#### **4. IMPACT**

- a. What was the impact on the development of the principal disciplines of the project?**

Nothing to report

- b. What was the impact on other disciplines?**

Nothing to report

- c. What was the impact on technology transfer?**

Nothing to report

- d. What was the impact on society beyond science and technology?**

Nothing to report

#### **5. CHANGES/PROBLEMS**

Nothing to report

#### **6. PRODUCTS**

Nothing to report

#### **7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

- a. What individuals have worked on the project?**

Kay-Pong Yip: no change  
Samuel C. Mok: no change

- b. Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Nothing to report

- c. What other organizations were involved as partners?**

Nothing to report

**8. SPECIAL REPORTING REQUIREMENTS**

No applicable

**9. APPENDICES**

None