Intraperitoneal Mechanobiology in Ovarian Cancer: A Biomimetic Approach

Leigh Campbell
University of Notre Dame
Glynn Family Honors Program

April 2019

Advised by:

_____

Dr. M Sharon Stack

Table of Contents

Abstract

Ovarian Cancer is the fifth leading cause of cancer-related mortalities in women. Ovarian cancer is typically detected and diagnosed after extensive intraperitoneal (IP) metastases, contributing to 5-year survival rates that have not substantially improved in over 30 years. Unlike other cancers, ovarian cancer metastasis occurs when tumor cells detach from the primary tumor, permeate throughout the peritoneal cavity in the peritoneal fluid, adhere to the mesothelial layer of peritoneal tissues and invade the collagen-rich submesothelial matrix. The peritoneum is a tissue membrane under longitudinal tensile stress which fluctuates in response to changes in IP fluid volume. Normal intraperitoneal pressure (IPP) is ~5 mmHg, but averages 22.1 mmHg in women with ovarian cancer and tense ascites. Previous research indicates that high IPP is associated with increased abdominal metastasis in a murine ovarian cancer model. Our lab previously found that increased IPP results in increased tissue stiffening. Moreover, nano-indentation, a technique used to test the mechanical properties of materials, also demonstrated an increase in tissue stiffness in peritoneum tissues from aged versus young mice. Aged tissue exhibits an elevated melting point for collagen, indicating increased collagen crosslinking, resulting in stiffer tissue. Epidemiologic data suggests that age is a significant risk factor for ovarian cancer incidence. Nevertheless, there have been few investigations of age-related changes in peritoneal tissues and their impact on ovarian cancer metastasis. The purpose of this study is to further examine how age-induced changes in peritoneum physical properties, specifically tissue rigidity, affect ovarian cancer cell adhesion, spreading, and gene expression using an innovative biomimetic stiffened gel model. We report that two human ovarian cell lines have increased adhesion and spreading on the stiffened gels, which are both key indicators of metastatic process. Taken together, these findings suggest that aged peritoneum stiffening may play a role in metastatic susceptibility.

Introduction

1. Ovarian Cancer

Although ovarian cancer is rare, it is the fifth leading cause of cancer-related deaths in women with the highest case-fatality ratio of all gynecologic malignancies, more than three times than breast cancer's case-fatality ratio [1, 2, 3]. Ovarian cancer's disproportionately high mortality rate is due to its late presentation. Symptoms are typically non-specific and can include bloating, appetite-changes, indigestion, and nausea due to extension or encasement of the peritoneum, reproductive organs, sigmoid colon, and omentum [4]. There are also no current screening tests that have proven to reduce mortality or improve diagnosis rates [5, 6]. Moreover, five-year survival rates have not substantially improved in over thirty years, remaining steady at approximately 25% [4]. Thus, ovarian cancer often goes undiagnosed until advanced metastasis within the peritoneal cavity occurs, stage III to IV, and prognosis is poor.

Generally, there are three types of ovarian cancer: epithelial, germ cell, and specialized stromal cell tumors. Epithelial ovarian cancer, the focus of this thesis, is the most common type. There are two subtypes of epithelial ovarian cancer tumors, type 1 and type 2 [7]. Type 1 tumors develop insidiously from normal ovarian tissue into low-grade serous, mucinous, endometrial, and clear cell carcinomas. Somatic mutations in genes KRAS, BRAF, PI3KCA, ERRB2, and PTEN are characteristic of type 1 epithelial ovarian cancer tumors. In comparison, type 2 tumors are typically more aggressive malignancies like high-grade carcinoma, malignant mixed mesodermal tumors, and undifferentiated carcinomas [8]. Additionally, type 2 tumors are usually associated with mutations in the tumor suppressor gene TP53 [9]. The cells used in this study are type 2 epithelial ovarian cancer cells, OVCAR8 (RRID:CVCL_1629) and OVCAR5 (RRID:CVCL_1628) cell lines. OVCAR8 cells derive from a human

patient with high grade ovarian serous adenocarcinoma. OVCAR5 cells are also high grade serous adenocarcinoma cells and derived from a metastatic site, the ascites fluid.

2. Method of Metastasis

In most cancers, like breast and colon cancer for instance, cancer cells transgress through intra- and extravasation before metastasizing to other organs such as bone, liver, and the brain [10]. However, ovarian cancer metastasis occurs uniquely through the intra-abdominal cavity. First, tumor cells detach from the primary tumor. Cancer cells then permeate throughout the peritoneal cavity in the peritoneal fluid. The passive movement in the peritoneal fluid allows cancer cells to adhere to the mesothelial layer of peritoneal tissues and invade the collagen-rich submesothelial matrix as seen in Figure 1 [11].

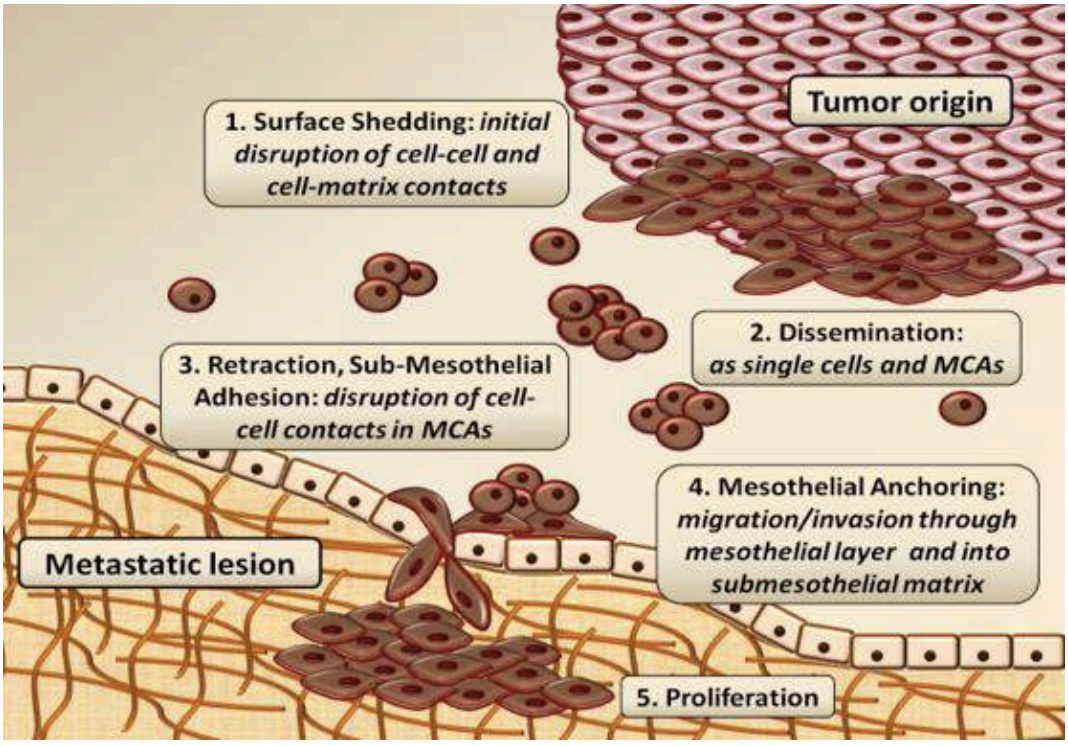


Figure 1. Epithelial ovarian cancer model.

In their first step of metastasis, exfoliation from the primary tumor, epithelial ovarian cancer cells undergo an epithelial-to-mesenchymal transition (EMT). EMT permits biochemical changes that loosen both the attachment to the basement membrane and the intercellular adhesions between cancer cells by lowering E-cadherin expression [12]. EMT also enables epithelial ovarian cancer cells to assume a mesenchymal cell phenotype characterized by enhanced migratory capacity, invasiveness, increased resistance to apoptosis, and increased production of extracellular matrix components [13]. Collagen-binding integrins on the cancer cell induce matrix metalloproteinase (MMP)-9 which cleave E-cadherin ectodomain allowing the more invasive cells to shed into the ascites fluid [14]. Free floating single ovarian cancer cells and multiple cellular aggregates next adhere to a secondary site, most commonly the peritoneum and the omentum. Adherence to mesothelial cells is mediated by integrins and surface cell receptors. Following adherence, ovarian cancer cells invade the mesothelial layer and the submesothelial matrix. The submesothelial basement membrane is composed of a variety of extracellular matrix proteins, including collagen I, collagen IV, fibronectin, and laminin. Finally, after implanting on the omentum and peritoneum, primary ovarian cancer cells proliferate to form secondary tumors [7].

3. Collagen and the Extracellular Matrix

Host mesothelial and peritoneal tissue play an important role in ovarian cancer's unique method of metastasis. The adhesion and invasion of host tissues is a paramount precursor in the transgression from a primary tumor to secondary lesions. Therefore, a careful, parallel examination of these processes and the tumor microenvironment may provide greater insight into metastasis.

The peritoneum, the primary microenvironment for metastatic lesions, covers the visceral organs and abdominal and pelvic cavities. There are five layers of the

peritoneum. The first layer is composed of endothelial cells lining the intravascular space of capillaries, and the second layer is the basement membrane of these cells. The third layer contains fibroblasts, collagen, and hyaluronic acid. The fourth layer is the submesothelial basement membrane of the fifth layer, mesothelial cells [16].

Numerous studies have implicated the role of extracellular matrix restructuring in tumor progression. Shed ovarian cancer cells adhere preferentially to type 1 collagen, the most abundant fibrous proteins of the extracellular matrix [16]. Collagen 1 has been shown to enhance migration of multiple epithelial ovarian cancer cell lines as well [17, 18]. The alignment of collagen 1 is correlated with cell invasion and poor prognosis in cancers [19, 20, 21]. Physiologically healthy collagen alignment is typically random, isotropic wavy fibrils parallel to the epithelial boundary [20]. However, tumorigenesis is associated with collagen characterized by decreased fiber density and sinusiodosity, increased fiber alignment, and collagen tracts perpendicular to the epithelial boundary [22]. This pattern is known as the "tumor-associated collagen signature" (TACS) [6, 11]. Remodeling of the extracellular matrix is associated with increased stiffness and increased invasion and migration of cancer cells. The collagen-rich tumor microenvironment is thus an important mediator in epithelial ovarian cancer progression and an alluring avenue for understanding ovarian cancer.

4. Intraperitoneal Pressure and Ascites Fluid

The peritoneum is a tissue membrane under longitudinal tensile stress which fluctuates in response to changes in IP fluid volume [23-24]. Normal intraperitoneal pressure (IPP) is ~5 mmHg [25], but averages 22.1 mmHg in women with ovarian cancer and tense ascites [26].

Normally, oncontic pressure across the peritoneal membrane is high at the endothelial layer and low at the mesothelial layer thereby limiting capillary fluid from filtering into the peritoneal cavity. However, secondary ovarian cancer tumors lining the peritoneal membrane increase the surface area of microvessels, allowing for increased filtration surface of fluid [17]. Vascular endothelial growth factor (VGEF) is also implicated in the altercation of peritoneal permeability and subsequent accumulation of ascites fluid [27, 28]. VGEF is a component of normal ovarian function, but overexpression in epithelial ovarian cancer patients is correlated with increased ascites [29, 30]. VGEF is believed to increase permeability through downregulation of claudin 5 in the peritoneal endothelium [31,32]. The inducer of the upregulation of VGEF is not well-defined, but includes lysophosphatidic acid, tumor necrosis factor, epidermal growth factor and transforming growth factor-B (among other factors) [33]. The increased concentration of proteins within the ascites creates a flow of fluid into the peritoneal cavity as well. These alterations combine to alter the flow of fluid within the peritoneal cavity and change the oncotic pressure across the peritoneal membrane [17]. As noted previously, normal IPP is ~5 mmHg [25], but averages 22.1 mmHg in women with ovarian cancer and tense ascites [26]. Previous research indicates that high IPP is associated with increased abdominal metastasis in a murine ovarian cancer model [34].

Our lab has investigated the effect of compressive force associated with accumulated ovarian cancer ascites by subjecting peritoneal tissue explants to mechanical strain and measuring the stiffness by atomic force microscopy (AFM). AFM determined unstretched peritoneal tissue had a stiffness of 650 Pascal (Pa) and stretched tissues measure 3 kiloPascal (kPa), enabling us to model tissue stiffness changes present in ovarian cancer patients with tense ascites. Therefore, the accumulation of ascites,

combined with extracellular matrix remodeling, suggest a stiffer peritoneum microenvironment for ovarian cancer cells to adhere.

5. Tissue stiffness and Ovarian Cancer

Various studies have demonstrated the effect of substrate and extracellular matrix stiffness on cancer progression, particularly in breast cancer [35, 36, 37]. Nevertheless, two previous studies reached conflicting results on the effect of stiff versus soft matrices on ovarian cancer cells EMT-transition phenotype changes, spreading, and adhesion [37, 38]. However, in addition to the AFM experiment previously conducted, our lab observed increased adhesion on stiffer peritoneum stretched to model ascites induced mechanical forces. In this experiment, peritoneal tissue was dissected from mice and subjected to mechanical strain. Fluorescently labeled Skov3-ip cells were applied to the tissue and allowed to incubate for 2 hours. The ex-vivo peritoneal explant adhesion assay demonstrated a significant increase (p <0.05) of human ovarian cancer cells (Skov3-ip-RFP) adhesion on stretched (stiffened) peritoneum compared to non-stretched tissue as seen in Figure 2.

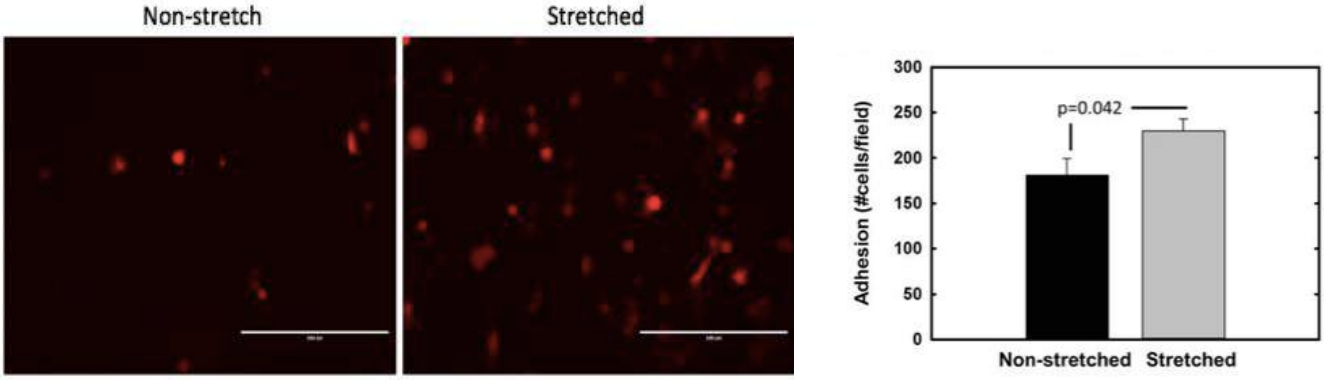


Figure 2. Non-stretched vs. stretched mouse peritoneum. A Skov3-ip-RFP ex-vivo assay demonstrated increased adhesion on the stiffer, stretched peritoneum. Cells were incubated for 2 hours on strained and unstrained

peritoneum. Adherent cells were enumerated in 11 fields and a significant increase in adhesion to stretched tissue was observed.

This data suggests that stretched peritoneum, due to ascites-induced forces, may indeed increase susceptibility of metastasis.

6. Aging and Ovarian Cancer

Epidemiologic data suggests that age is a significant risk factor for ovarian cancer incidence [3]. Older patients also have poorer prognosis [39]. Yet, it is unclear why aged hosts are most susceptible to metastasis. A study in our lab found that aged peritoneal adipose tissue immune composition changes contributes to the age-related differences in metastasis [40]. These findings implicate the aged-host's tumor microenvironment in disease progression. Upon further investigation of aged versus young peritoneum, nano-indentation, a technique used to test the mechanical properties of materials, demonstrated an increase in tissue stiffness in peritoneum tissues from aged versus young mice. These values were similar to those determined by AFM associated with ascites forces (650 Pa and 3 kPa). Additionally, thermal denaturation of collagen in young versus aged murine peritoneal tissues exhibited an elevated melting point for collagen in aged tissue, indicating increased collagen cross-linking, resulting in stiffer tissue. Taken together, we hypothesize that alterations in aged collagen affect the mechanical properties of host peritoneum, and thus affect susceptibility to metastasis.

7. Current study

The overall goal of this project is to model and examine how age-induced changes in peritoneal tissue affect ovarian cancer cell adhesion following the schematic in Figure 3.

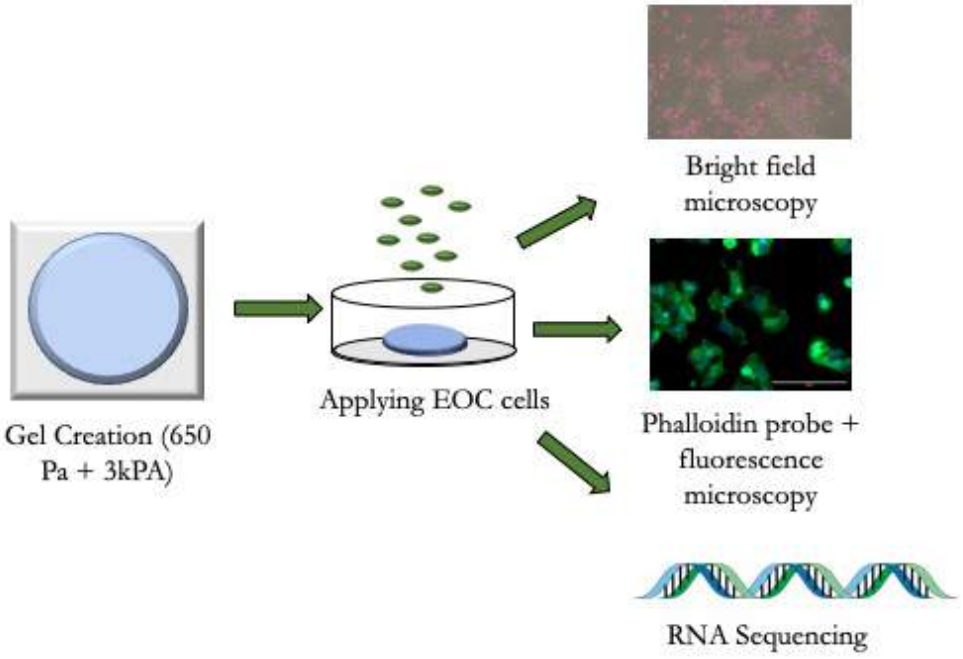


Figure 3. Study workflow. Gels of 650 Pa and 3000 Pa stiffness are generated, Epithelial ovarian cancer cells applied, and adhesion, spreading, and RNA studied.

We hope to elucidate the impact of age-induced collagen crosslinking, and subsequent stiffening of the matrix, on ovarian cancer cell metastatic anchoring with this gel model approach. Using this model, we will be able to quantify ovarian cancer cell adhesion and measure cell spreading, which is characteristic of increased ovarian cancer cell migration and invasion. Furthermore, the RNA sequencing and bioinformatic analysis from adherent ovarian cancer cells on collagen gels may identify genes that are involved in transducing mechanical signals into cell gene expression. As age-induced biophysical changes in cancer are still relatively unexamined, the RNASeq data generated in this study will aid in the identification of genes that can be used in future studies that look at abnormally-expressed proteins or oncogenic signaling pathways as biomarkers or potential therapeutic targets.

Ultimately, the disruption of identified mechano-sensitive pathways may be an approach to inhibit ovarian cancer cell adhesion, spreading, and subsequent metastasis.

Materials and Methods

1. General Cell Culture

The human epithelial ovarian cancer cell lines OVCAR5 and OVCAR8 were maintained in a humidified incubator at 37°C in Dulbecco's Modified Eagle Media (DMEM) supplemented with 10% fetal bovine serum, 1% penicillin and streptomycin, and 1% MEM non-essential amino acids solution. Cells were trypsinized and split every 3-4 days to maintain subconfluency.

2. Adhesion Assay

In order to evaluate the contribution of the host peritoneal mesothelial cell aging to age-related stiffness in the intraperitoneal microenvironment, gels with measured stiffness of 650 Pa and 3 kPa (~5 mmHg and 22.1mmHg respectively) were generated. The gels were engineered based off a protocol adapted from research in the Zorlutuna Lab, Notre Dame. Glass sides of size 12-14 mm^2 were treated with (3-Aminopropyl)triethoxysilane , washed, and treated with 400 uL of 0.5% glutaraldehyde in water. Gel solutions were made based on Table 1 below. Twenty uL of the polymerizing solution was immediately pipetted on the prepared glass and a 12 mm^2 glass cover slip placed on top. Gels were allowed to polymerize approximately 15 minutes in a moisture chamber before cover slip removed and placed in PBS in a 12-well plate.

	650 Pa	**3000 Pa**
Rat tail type 1 collagen (Corning REF#354236)	171 uL	171 uL
40 mM Hepes	689 uL	609 uL
40 % Acrylamide	125 uL	200 uL
2.0% BIS	10 uL	15 uL
10% APS	4 uL	4 uL
Temed (1:1 diluted)	1 uL	1 uL

Table 1. Gel components and volumes. Gels were made with 600 mg collagen.

OVCAR5 and OVCAR8 cells were prepared for plating using sterile cell culture techniques. Each cell line was trypsinized, resuspended in serum-medium, and diluted to a concentration of 50,000 cells/mL. The cells were seeded 2 mL per gel in duplicate. They were then incubated at 37°C for 15 minutes, 30 minutes, and 1 hour. After the given time point, gels were removed from the well, dipped in PBS, and placed in a new well with 1 mL of 4.0% paraformaldehyde and incubated at 37°C for 45 minutes. Next, gels were washed with PBS and stained with 1 mL of 0.01% crystal violet for approximately 30 minutes. Finally, gels were washed with PBS and were ready for analysis. Independent validation of gel stiffness was completed by the Zorlutuna Lab with nano-indentation.

3. Visualization of Cell Spreading

To visualize the spreading of cells adhered to the 650 Pa and 3000 Pa gels, a phalloidin (F-actin binding phallotoxin) fluorescent probe was used. Gels and adherent cells were prepared in the same manner discussed in the adhesion assay. After cells were fixed in 4.0% paraformaldehyde, they were washed with PBS and treated with 0.05% tween20 in PBS. Cells were blocked with 2.5% goat serum for 1 hour. Next, cells were treated with 1:120 phalloidin in 2.5% goat serum for 20 minutes and washed with PBS. Finally, gels were treated with TO-PRO-3 Stain (ThermoFisher REF#T3605) and mounted for confocal microscopy.

4. RNA Sequencing

RNA sequencing was used in order to assess differences in gene expression between Ovarian Cancer cells on 650 Pa and 3000 Pa gels. Gels and adherent cells were

prepared in the manner previously described in the adhesion assay. OVCAR5 and OVCAR8 cells were incubated on the gels at 37°C for four hours. Gels were dipped in PBS and a lysis buffer applied. Following lysis, the RNAeasy Plus Mini Kit was used to isolate RNA (Qiagen 74134). RNA purity was verified through nanodrop. Three independent trials were conducted and RNA sent to Novogene for sequencing. The top 10 differentially regulated genes will be validated with qRT-PCR.

Results

Cell adhesion measurement was performed through bright field microscopy and cell counting in ImageJ. Cells were plated on gels in duplicate. Once crystal violet staining was complete, four pictures at a magnification of 10X were taken of each gel. Cells were enumerated on ImageJ and averaged. OVCAR5 adhesion increased on 3000 Pa gels compared to the 650 Pa gels. Adhesion increased expectedly at each time point as well as seen in Figure 4. Quantification of adhesion showed statistically significant differences in adhesion in OVCAR5 cells at each time point as seen in Figure 5.

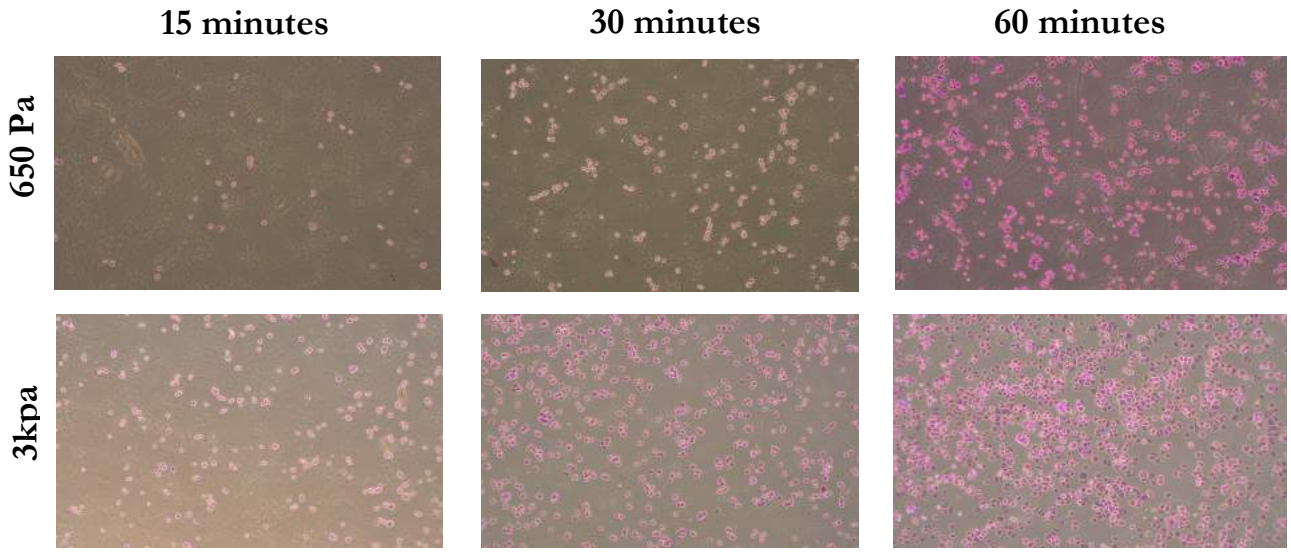


Figure 4. OVCAR5 Adhesion Imaging. OVCAR5 cells were allowed to adhere to 3000 Pa and 650 Pa gels for 15, 30, and minutes. Cells were stained with crystal violet and images were taken at 10X magnification.

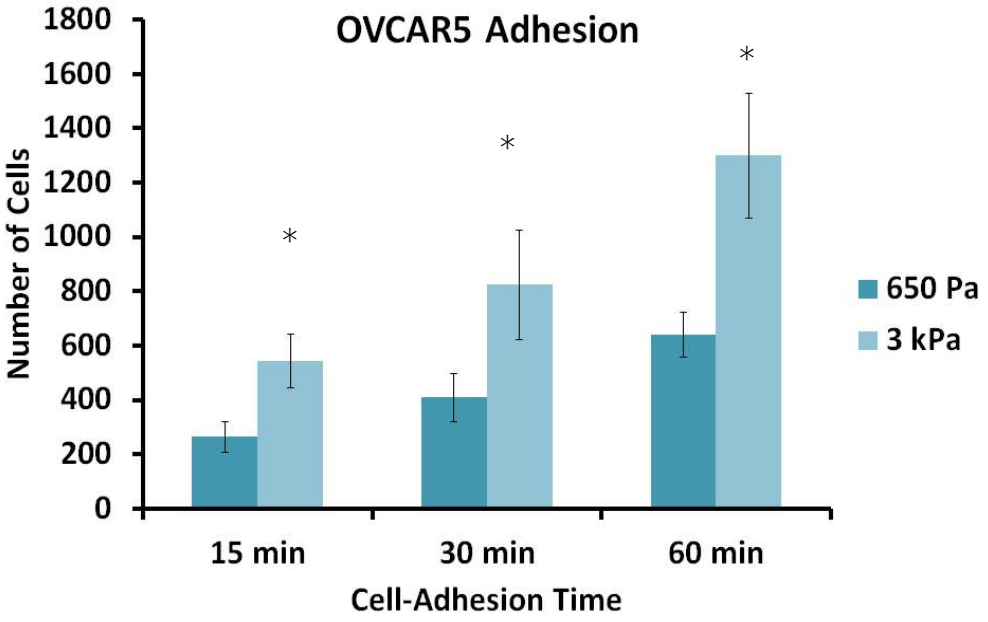


Figure 5. Quantification of OVCAR5 Adhesion. Cell Adhesion was quantified using ImageJ software. Significant data was determined with a Student's t-test. *(p<0.05).

The OVCAR8 cell line adhesion was imaged and quantified in the same manner as OVCAR5 cell line. Adhesion also increased on the 3000 Pa gels compared to the 650 Pa gels as seen in Figure 6. Quantification of adhesion showed statistically significant differences in adhesion in OVCAR8 cells at each time point as seen in Figure 7.

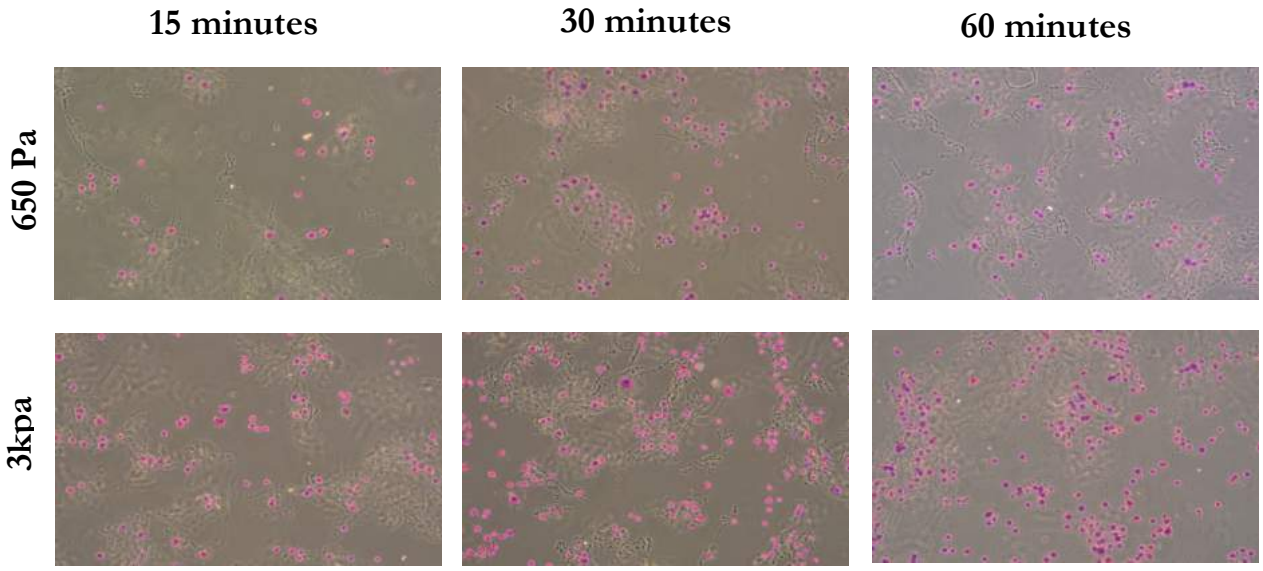


Figure 6. OVCAR8 Adhesion Imaging. OVCAR8 cells were allowed to adhere to 3000 Pa and 650 Pa gels for 30 minutes, 1 hour, and 4 hours. Cells were stained with crystal violet and images were taken at 10X magnification.

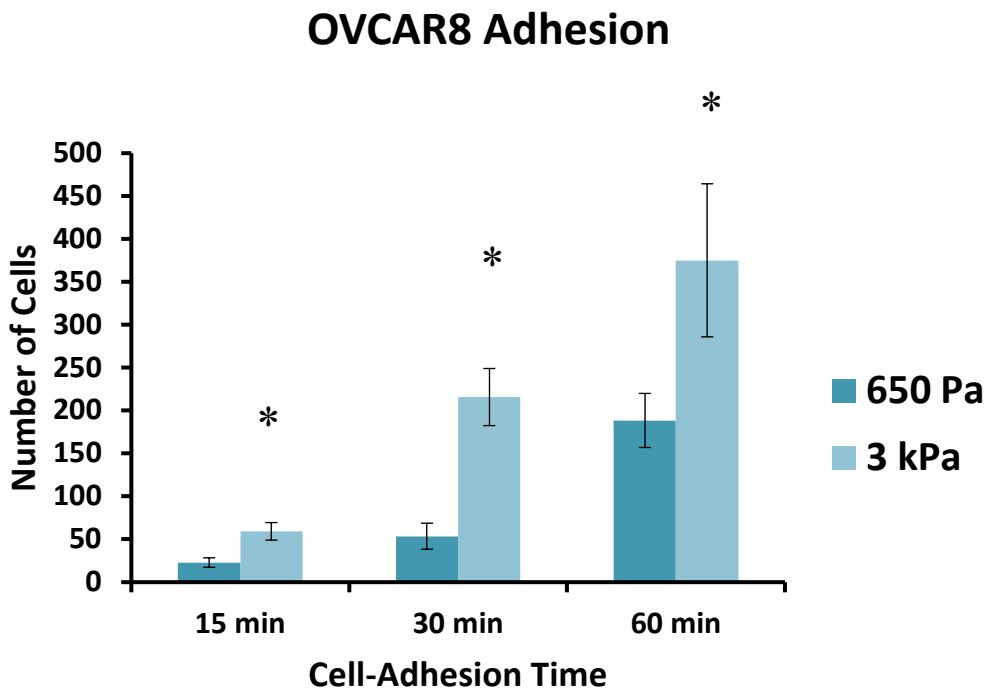


Figure 7. Quantification of OVCAR8 Adhesion. Cell Adhesion was quantified using ImageJ software. Significant data was determined with a Student's t-test. *(p<0.05).

Cytoskeleton spreading on gels was visualized using confocal microscopy. Gels were imaged at 20X and 60X. Confocal imaging was compressed and compiled as composite images in ImageJ. OVCAR5 and OVCAR8 cells showed increased spreading on the 3000 gels compared to 650 gels as seen in Figure 8 and Figure 9.

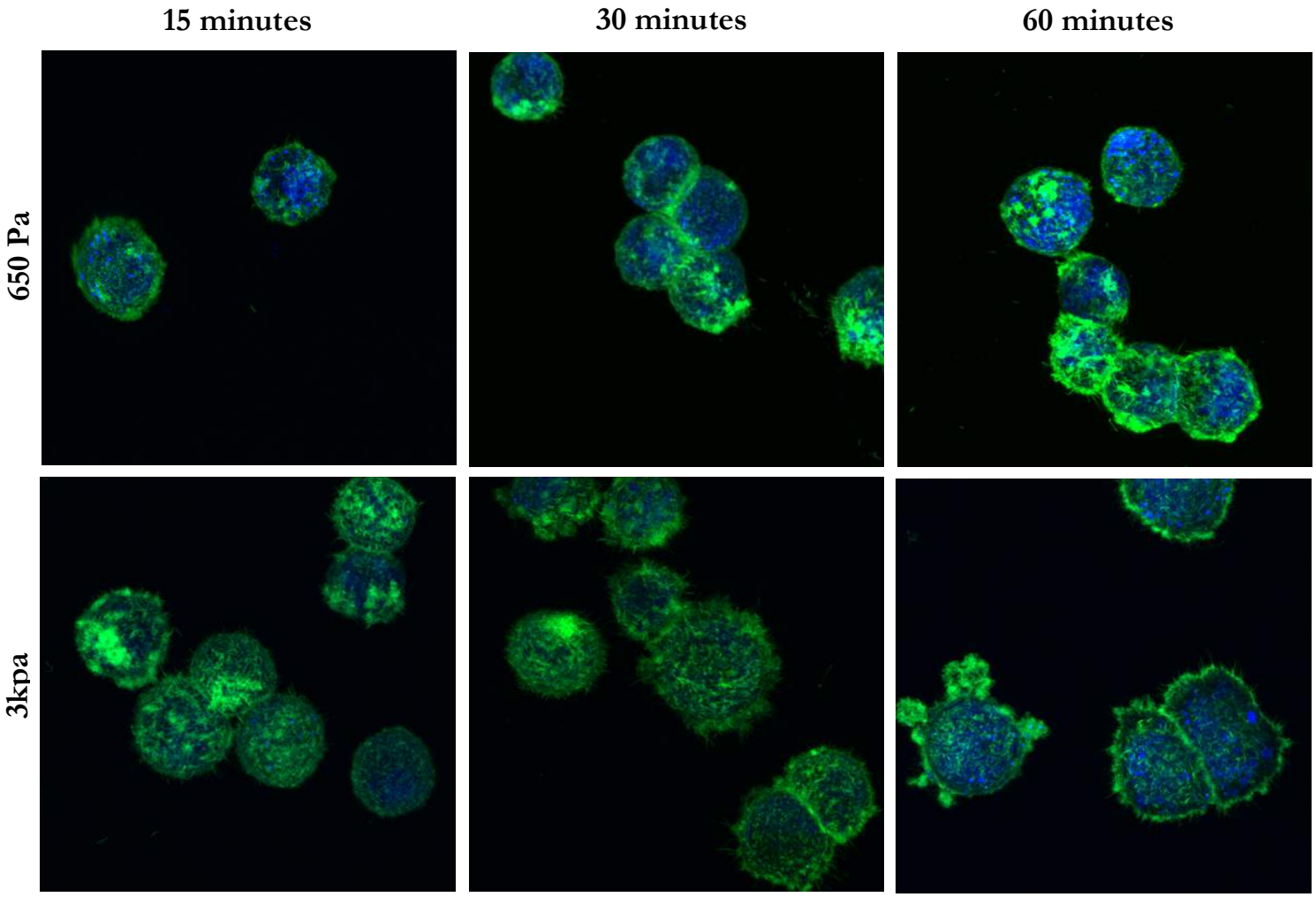


Figure 8. Confocal Images of OVCAR5 cells. Green fluorescence is the phalloidin probe illuminating OVCAR5 f-actin filaments. Pseudopodia and cells disaggregation

increased at each time point and on the 3000 Pa gels compared to the 650 Pa gels. All images are at 60X magnification.

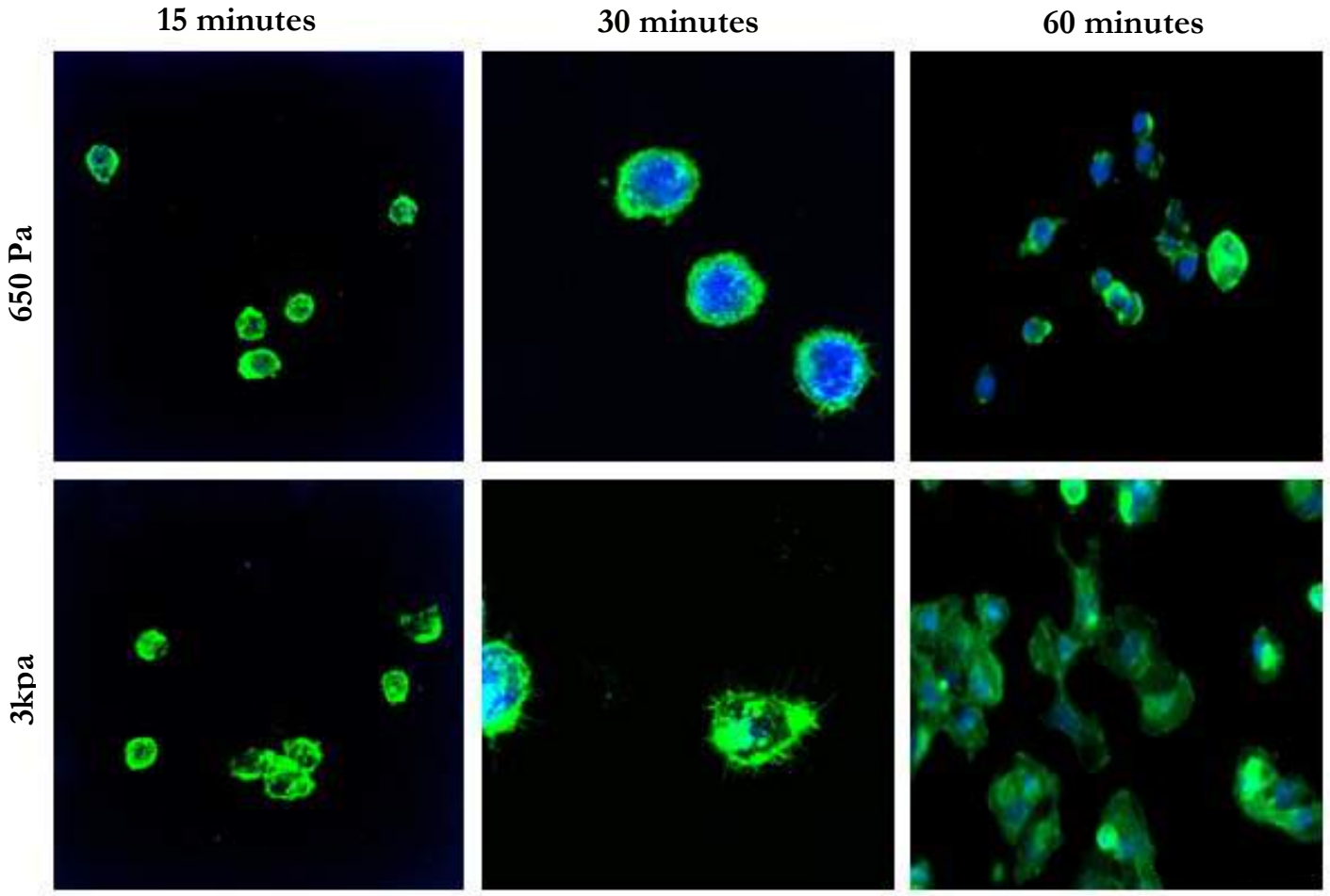
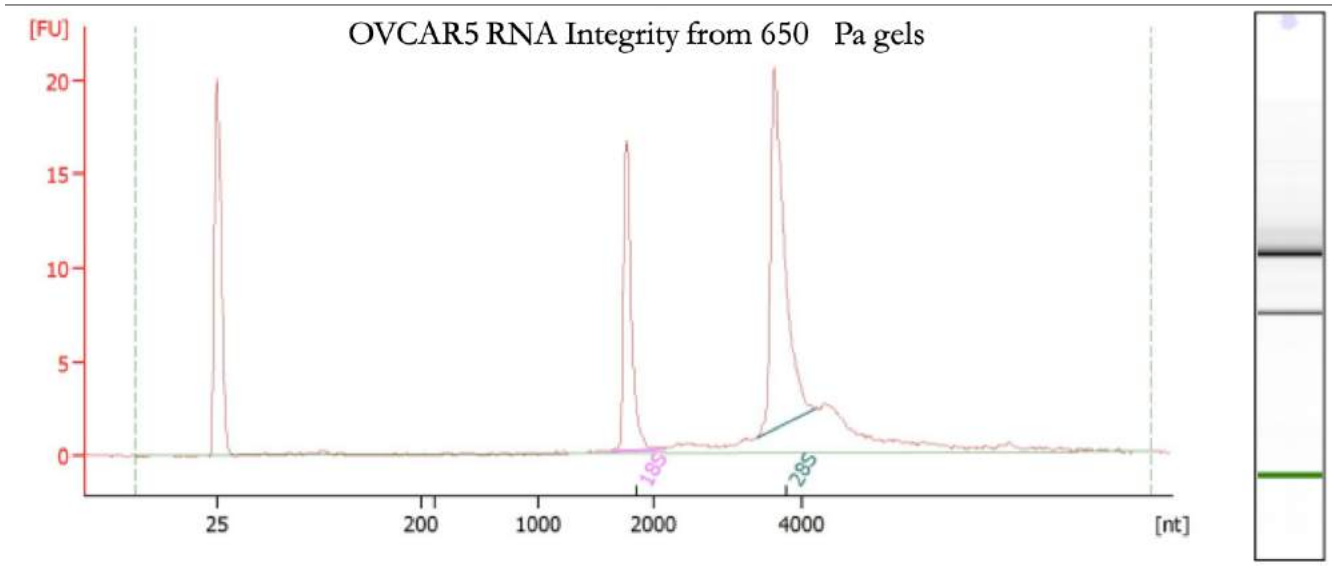


Figure 9. Confocal Images of OVCAR8 cells. Green fluorescence is the phalloidin probe illuminating OVCAR8 f-actin filaments. Pseudopodia and cells disaggregation increased at each time point and on the 3000 Pa gels compared to the 650 Pa gels. All images are at 40X magnification.

RNA isolation from adherent OVCAR8 and OVCAR5 cells on 3 kPa and 650 Pa collagen gels is complete. High quality RNA was verified by BioAnalyzer (Agilent) for

quality control as seen in Figures 10-13. RNA sequencing is currently underway, but results will not be available for some time.



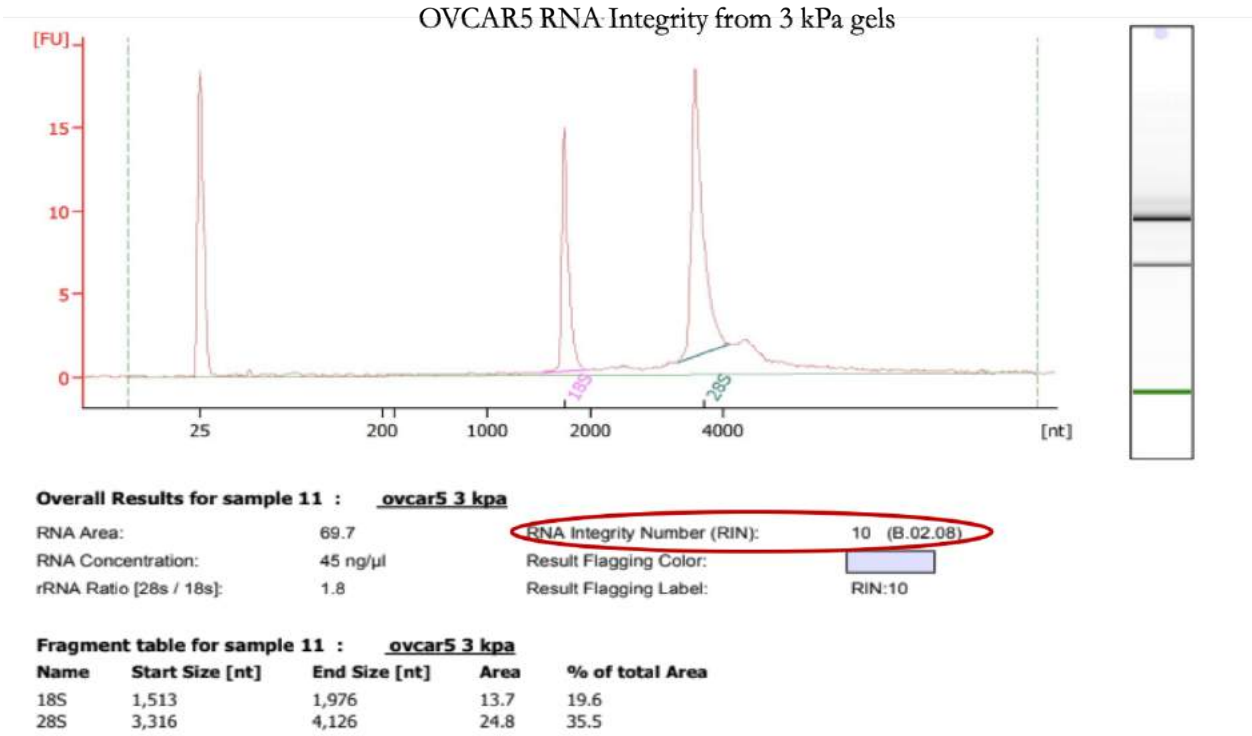
Overall Results for sample 9 : ovcar5 650pa

RNA Area:	74.0	RNA Integrity Number (RIN):	10 (B.02.08)
RNA Concentration:	48 ng/µl	Result Flagging Color:	
rRNA Ratio [28s / 18s]:	1.9	Result Flagging Label:	RIN:10

Fragment table for sample 9 : ovcar5 650pa

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,621	2,145	15.7	21.2
28S	3,398	4,178	29.8	40.3

Figure 10. Quality control results for RNA isolated from adherent OVCAR5 cells on collagen gels with 650 Pa Young's Modulus. BioAnalyzer's RNA Integrity Number for this sample was 10, indicating high quality RNA.



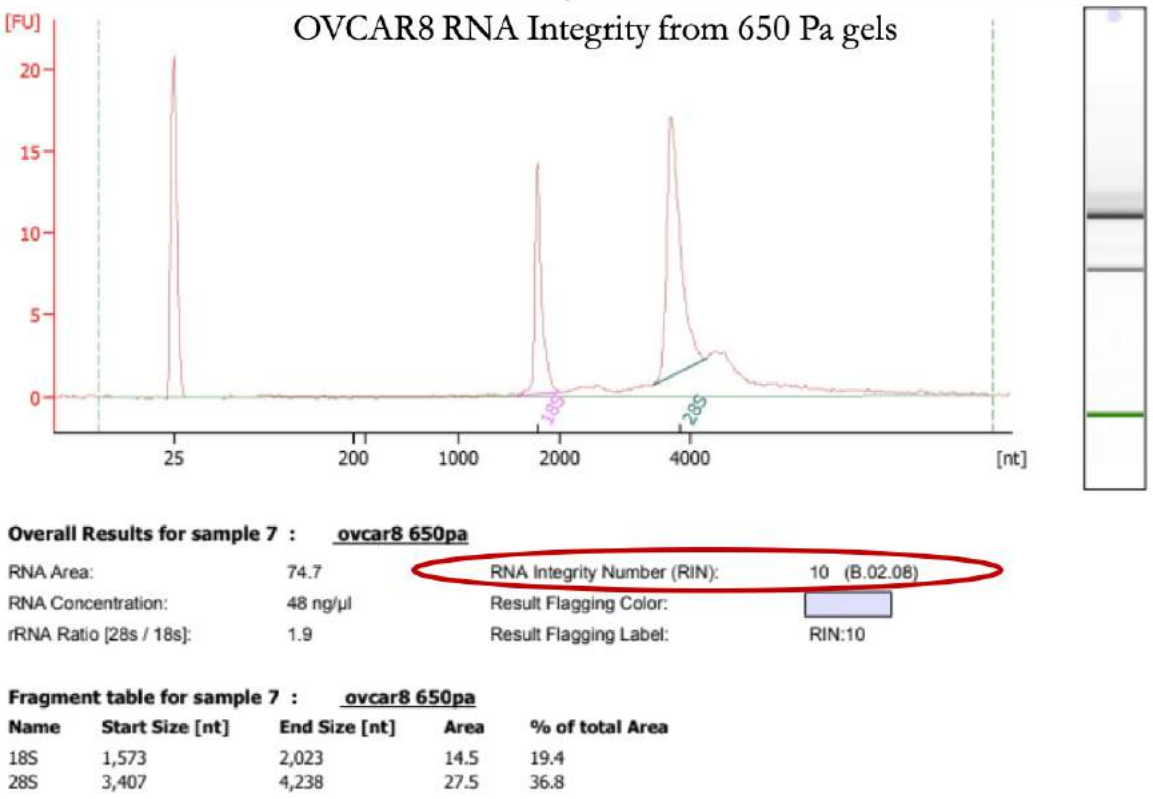
Overall Results for sample 11 : ovcar5 3 kpa

RNA Area:	69.7	RNA Integrity Number (RIN):	10 (B.02.08)
RNA Concentration:	45 ng/µl	Result Flagging Color:	
rRNA Ratio [28s / 18s]:	1.8	Result Flagging Label:	RIN:10

Fragment table for sample 11 : ovcar5 3 kpa

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,513	1,976	13.7	19.6
28S	3,316	4,126	24.8	35.5

Figure 11. Quality control results for RNA isolated from adherent OVCAR5 cells on collagen gels with 3 kPa Young's Modulus. BioAnalyzer's RNA Integrity Number for this sample was 10, indicating high quality RNA.



Overall Results for sample 7 : ovcar8 650pa

RNA Area:	74.7	RNA Integrity Number (RIN):	10 (B.02.08)
RNA Concentration:	48 ng/µl	Result Flagging Color:	
rRNA Ratio [28s / 18s]:	1.9	Result Flagging Label:	RIN:10

Fragment table for sample 7 : ovcar8 650pa

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,573	2,023	14.5	19.4
28S	3,407	4,238	27.5	36.8

Figure 12. Quality control results for RNA isolated from adherent OVCAR8 cells on collagen gels with 650 Pa Young's Modulus. BioAnalyzer's RNA Integrity Number for this sample was 10, indicating high quality RNA.

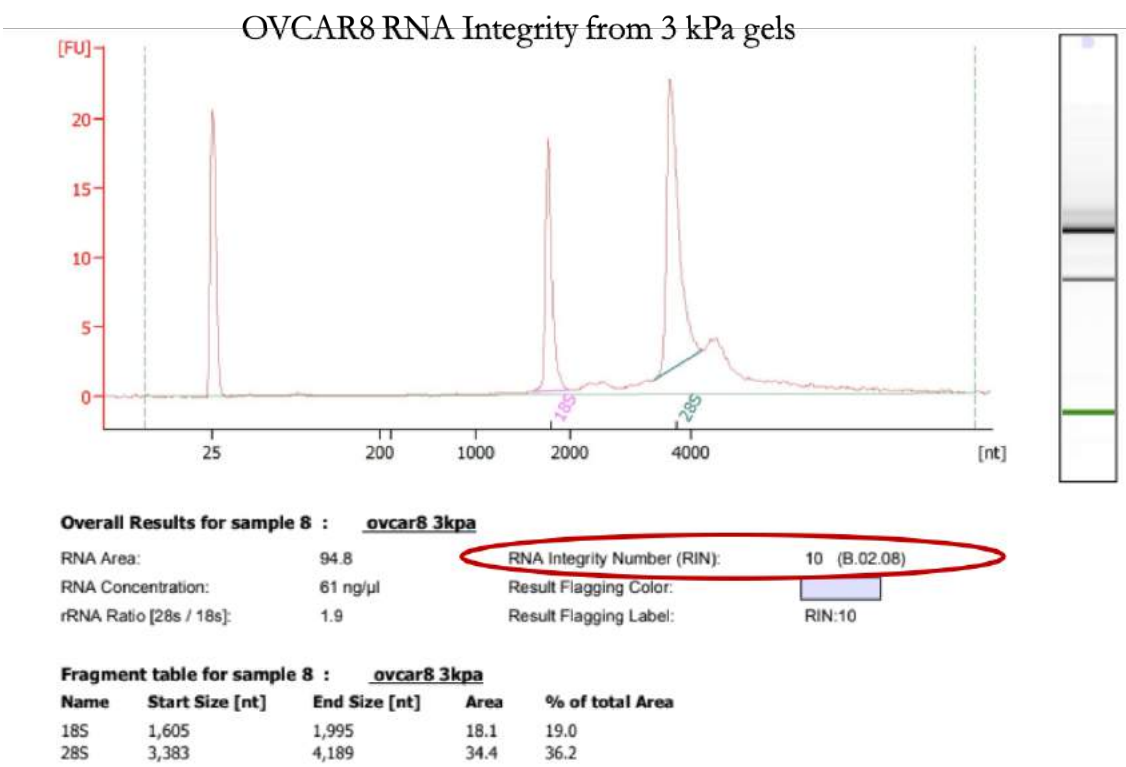


Figure 13. Quality control results for RNA isolated from adherent OVCAR8 cells on collagen gels with 3 kPa Young's Modulus. BioAnalyzer's RNA Integrity Number for this sample was 10, indicating high quality RNA.

Discussion

Aging is associated with increased incidence and poorer prognosis of ovarian cancer. Ovarian cancer metastasizes by shedding away from the primary ovarian tumor and floating freely in the abdominal cavity in ascites fluid before adhering to secondary sites. The peritoneum is the most common site of ovarian cancer metastasis. To investigate the ability of ovarian cancer cells to adhere and spread on aged versus young peritoneum, adhesion assays were performed on collagen gels that mimic the Young's modulus of young and aged murine peritoneum (650 Pa and 3000 Pa). Results suggest that the increasing substrate rigidity associated with aged tissue enhances epithelial ovarian cancer cell metastatic potential (adhesion and disaggregation). The results of the current work also concur with McKenzie et. al's conclusion that epithelial ovarian cancer is a "mechano-responsive malignancy" [37]. It should be noted that there are no current studies that identify the Young's modulus of aged human peritoneum versus young human peritoneum. Nevertheless, this study underscores the importance of the role of host mechanobiology in modulating the progression of epithelial ovarian cancer. This area of study in epithelial ovarian cancer has been previously neglected, and may provide more insights into why aging is an influential epidemiologic factor in ovarian cancer.

Although RNA-sequencing results are forthcoming, we hypothesize that adhesion molecules such as AMIGO-2 (an adhesion molecule currently being investigated in our lab) and E-Cadherin may be upregulated in cells adhered to the stiffer (aged) collagen gels. ECM remodeling proteins such as MMP2, MMP9, and LOX may also be upregulated in cells adhered to the stiffer collagen gels. McKenzie et. al. observed that FAK may be involved in a mechanotransduction pathway that regulates spreading and thus may differentially expressed on our stiffer collagen gels [37]. We also anticipate that genes involved in disaggregation such as ROCK1 and myosin may

be differentially regulated. The top ten differentially regulated genes will be verified by qt-PCR. Following validation, these genes may be further investigated by repeating the methods described in this study and knocking out genes or inhibiting their protein products in the OVCAR-5 and OVCAR8 cell lines.

It should also be noted that the findings in this study contradict McGrail et.al. who reported that epithelial ovarian cancer cells preferentially adhered, migrated, and had increased polarity of traction forces on softer matrices [38]. This study used collagen 1 but had stiffer matrices (3 kPa and 30 kPa) as well as a different cell line (SKOV3). However, our lab has previously observed that SKOV3 adhesion increases on stiffer peritoneum. McKenzie et. al. noted that the major difference in McGrail's study was culture conditions. This study and McKenzie et. al. used serum-containing medium while McGrail et. al. did not. Serum-media more closely mimics the biochemically rich ascites fluid. Moreover, bioactive molecules in serum like lysophophatidic acid (LPA) reversed the rigidity-dependence end-points of migration and traction experiments [38].

The aging process is an important modulator for the tumor microenvironment for ovarian cancer [40]. The results of this study implicate the extracellular matrix remodeling of collagen in aged peritoneum as a promoter of the metastasize of ovarian cancer cells. Following RNA-sequencing results, future studies focusing on these differentially regulated genes may further elucidate how the mechanobiology of aged host peritoneum affects mechanotransduction pathways and metastasis.

Conclusion

This study manipulated the Young's Modulus, or stiffness, of collagen gels to mimic the differentiated biological stiffness of aged and young murine peritoneal tissue (650 Pa and 3000 Pa). The human epithelial ovarian cancer cell lines OVCAR5 and OVCAR8 displayed increased metastatic behavior on stiffer collagen gels including adhesion and disaggregation. The RNA-sequencing results in this study are forthcoming, but may elucidate differentially regulated adhesion, spreading, and extracellular matrix remodeling proteins that enable this increased metastatic behavior. Ultimately, these results underline the role of intraperitoneal mechanobiology in ovarian cancer progression and a focus for the development of new therapeutics or biomarkers.

Acknowledgements

Thank you to Dr. Stack for allowing me to join the lab three years ago and for advising my thesis. I am grateful to the Research Like A Champion grant program and the Harper Cancer Research Institute for supporting this project. I am also grateful to Dr. Yuliya Klymenko for first mentoring me in lab and for her patience in teaching me research fundamentals. Thank you to Dr. Yueying Liu for spearheading this project and the many hours spent guiding and helping me to refine methods, complete assays, and work the confocal microscope. Thank you also to Stack lab members Dr. Tyvette Hilliard, Gifty Marfowaa, Brooke Kolwaskii, and Dr. Jing Yang for all their help culturing cells, completing assays, and data analysis. I would also like to acknowledge Dr. John Thyfault and Dr. Tim Downing for their mentorship in my other research endeavors at the University of Kansas and Dublin City University. Finally, thank you to the research programs K-INBRE, the Glynn Family, and Naughton Fellowship for their support.

References

[1] Jemal A, Siegel R, Xu J, Ward E (2010) Cancer statistics, 2010. CA: a cancer journal for clinicians. 60(5):277-300.

[2] Siegel R, Ward E, Brawley O, Jemal A. Cancer statistics (2011) The impact of eliminating socioeconomic and racial disparities on premature cancer deaths. CA: a cancer journal for clinicians. 2011;61(4):212-36.

[3] Siegel RL, Miller KD, Jemal A. Cancer statistics. (2016) CA Cancer J Clin; 66:7–30.

[4] Howlader N NA, Krapcho M, Carshell J, Miller D, Altekruse SF, Kosary CL, Yu M, Ruhl J, Tatalovich Z, Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KA. (2014) SEER Cancer Statistic Review, 1975-2011. SEER Cancer Statistics Review.

[5] Lengyel E, Burdette J, Kenny J, Matei D, Pilrose J, Haluska P, Newphew KP, Hales DB, Stack MS (2014) Epithelial ovarian cancer experimental models. Oncogene 33:3619-33. PMC3990646; PMIS 23934194.

[6] Conklin MW, Eickhoff JC, Riching KM, Pehlke CA, Elicerir KW, Provenzano PP, Firedl A, Keely PJ (2011) Aligned collagen is a prognostic signature for survival in human breast carcinoma. Am J Path 178: 12212-1232.

[7] Kroeger PT Jr, Drapkin R (2017) Pathogenesis and heterogeneity of ovarian cancer. Curr Opin Obstet Gynecol 29(1): 26-34.

[8] Shih IM, Kurman RJ (2004) Ovarian tumorigenesis: a proposed model based on morphological and molecular genetic analysis. Am J Pathol. 164(5): 1511–1518.

[9] Kurman RJ, Shih IM (2011) Molecular pathogenesis and extraovarian origin of epithelial ovarian cancer—shifting the paradigm. Hum Pathol 42(7): 918-31.

[10] Gupta GP, Massague J (2006) Cancer metastasis: building a framework. Cell. 127(4):679-95.

[11] Malik R, Lelkes PI, Cukierman E (2015) Biomechanical and biochemical remodeling of stromal extracellular matrix in cancer. Trends Biotechnol 33:230-236.

[12] Huber MA, Kraut N, Beug H (2005) Molecular requirements for epithelial-mesenchymal transition during tumor progression. Curr Opin. Cell Biol. 17(5):548-58.

[13] Kalluri R, Neilson EG. Epithelial-mesenchymal transition and its implications for fibrosis. J Clin Invest. (12):1776-84.

[14] Symowicz J, Adley BP, Gleason KJ, Johnson JJ, Gosh S, Fishman DA, Hudson LG, Stack MS (2007). Engagement of collagen-binding integrins promotes matrix metalloproteinase-9-dependent E-cadherin ectodomain shedding in ovarian carcinoma cells. Cancer Res. 67(5):2030-9.

[15] Kipps E, Tan DS, Kaye SB (2013) Meeting the challenge of ascites in ovarian cancer: new avenues for therapy and research. Nat Rev Cancer. 13(4):273-82.

[16] Moser TL, Pizzo SV, Bafetti L, Fishman DA, Stack MS. Evidence for preferential

adhesion of ovarian epithelial carcinoma cells to type I collagen mediated by the $\alpha_2\beta_1$

[17] Moser TL, Pizzo SV, Bafetti LM, Fishman DA, Stack MS (1996) Evidence for preferential adhesion of ovarian epithelial carcinoma cells to type I collagen mediated by the alpha2beta1 integrin. Int J Cancer. 67(5):695–70

[18] Burleson KM, Casey RC, Skubitz KM, Pambuccian SE, Oegema TR, Jr, Skubitz AP (2004) Ovarian carcinoma ascites spheroids adhere to extracellular matrix components and mesothelial cell monolayers. Gynecol Oncol. 93(1):170–81

[19] Goetz JG, Minguet S, Navarro-Lerida I, Lazcano JJ, Samaniego R, Calvo E, Del Pozo MA (2011) Biomechanical remodeling of the microenvironment by stromal caveolin-1 favors tumor invasion and metastasis. Cell. 146:148–163.

[20] Provenzano PP, Eliceiri KW, Campbell JM, Inman DR, White JG, Keely PJ (2006) Collagen reorganization at the tumor-stromal interface facilitates local invasion. BMC medicine. 4:38.

[21] Conklin MW, Eickhoff JC, Riching KM, Pehlke CA, Eliceiri KW, Provenzano PP, Keely PJ (2011) Aligned collagen is a prognostic signature for survival in human breast carcinoma. The American journal of pathology. 178:1221–1232.

[22] Adur J, Pelegati VB, de Thomaz AA, Baratti MO, Andrade LA, Carvalho HF, Bottcher-Luiz F, Cesar CL (2014). Second harmonic generation microscopy as a powerful diagnostic imaging modality for human ovarian cancer. J Biophotonics. 7(1-2):37-48.

[23] Breton E, Choquet P, Bergua L, Barthelmebs M, Haraldson B, Helwig JJ, Constantinesco A, Fischbach M. (2008) In vivo perioneal surface area measurement in rats by micro-computed tomography study. Per Dial Intl 28: 188-194.

[24] Bergua L, Breton E, Choquet P, Barthelembs M, Haraldson B, Helwig JJ, Constantinesco A, Fischback M. (2008) Peritoneal membrane recruitment in rats: a micro-computerized tomography study. Pediatr Nephrol 23: 2179-2184.

[25] Henriksen JH, Stage JG, Schlichting P and Winkler K. (1980) Intraperitoneal pressure: ascetic fluid and splanchinic vascular pressures, and their role in prevention and formation of ascites. Scand J Clin Lab Invest 40: 493-502.

[26] Gotlieb WH, Feldman B, Feldman-Moran O, Zmira N, Kreizer D, Segal Y, Elran E, Ben-Baruch G. (1998) Intraperitoneal pressures and clinical parameters of total paracentesis for palliation of symptomatic ascites in ovarian cancer. Gyn Oncol 71: 381-5.

[27] Zebrowski BK, Liu W, Ramirez K, Akagi Y, Mills GB, Ellis LM (1999) Markedly elevated levels of vascular endothelial growth factor in malignant ascites. Ann Surg Oncol. 6(4):373-8.

[28] Barton DP, Cai A, Wendt K, Young M, Gamero A, De Cesare S (1997) Angiogenic protein expression in advanced epithelial ovarian cancer. Clin Cancer

Res. 3(9):1579-86.

[29] Kassim SK, El-Salahy EM, Fayed ST, Helal SA, Helal T, Azzam Eel-D, Khalifa A (2004) Vascular endothelial growth factor and interleukin-8 are associated with poor prognosis in epithelial ovarian cancer patients. Clin Biochem. 37(5):363-9.

[30] Paley PJ, Staskus KA, Gebhard K, Mohanraj D, Twiggs LB, Carson LF, Ramakrishnan S (1997) Vascular endothelial growth factor expression in early stage ovarian carcinoma. Cancer. 80(1):98-106.

[31] Barton DP, Cai A, Wendt K, Young M, Gamero A, De Cesare S (1997) Angiogenic protein expression in advanced epithelial ovarian cancer. Clin Cancer Res. 3(9):1579 86.

[32] Herr D, Sallmann A, Bekes I, Konrad R, Holzheu I, Kreienberg R, Wulff C (2012) VEGF induces ascites in ovarian cancer patients via increasing peritoneal permeability by downregulation of Claudin 5. Gynecol Oncol. 127(1):210-6.

[33] Rodewald M, Herr D, Fraser HM, Hack G, Kreienberg R, Wulff C (2007) Regulation of tight junction proteins occludin and claudin 5 in the primate ovary during the ovulatory cycle and after inhibition of vascular endothelial growth factor. Mol Hum Reprod. 13(11):781-9.

[34] Matsuzaki S, Bourdel N, Darcha Cl, Cechelotte PJ, Bazin JE, Pouly JL, Mage G, Canis M. (2009) Molecular mechanisms underlying postoperative peritoneal tumor dissemination may differ between a laparotomy and carbon dioxide pnumoperitoneum: a syngeneic mouse model with controlled respiratory support. Surg Endosc 23: 705-15.

[35] Yue X, Nguyen TD, Zellmer V, Zhang S, Zorlutuna P (2018) Stromal cell-laden 3D hydrogel microwell arrays as tumor microenvironment model for studying stiffness dependent stromal cell-cancer interactions. Biomaterials. 170:37-48.

[36] Piao J, You K, Guo Y, Zhang Y, Li Z, Geng L (2017) Substrate stiffness affects epithelial-mesenchymal transition of cervical cancer cells through miR-106b and its target protein DAB2. Int J Oncol. 50(6):2033-2042.

[37] McKenzie AJ, Hicks SR, Svec KV, Naughton H, Edmunds ZL, Howe AK (2018). The mechanical microenvironment regulates ovarian cancer cell morphology, migration, and spheroid disaggregation. Sci Rep. 8: 7228.

[38] McGrail DJ, Kieu QMN, Dawson MR (2014) The malignancy of metastatic ovarian cancer cells is increased on soft matrices through a mechanosensitives Rho-ROCK pathway. J Cell Sci. 127(12): 2621-2626.

[39] T Thigpen, MF Brady, GA Omura, WT Creasman, WP McGuire, WJ Hoskins, et al. (1993) Age as a prognostic factor in ovarian carcinoma. The Gynecologic Oncology Group experience. Cancer. 71: 606-614.

[40] 40. Loughran EA, Leonard AK, Hilliard TS, Phan RC, Yemc MG, Harper E, Sheedy E, Klymenko Y, Asem M, Liu Y, Yang J, Johnson J, Tarwater L, Shi Z, Leevy M, Ravosa MJ, Stack MS (2018) Aging increases susceptibility to ovarian

cancer cell metastasis in murine allograft models and alters immune composition of peritoneal adipose tissue. Neoplasia. 20(6): 621-631.