

Ultrastructural Analysis of Inflammatory Breast Cancer Cell Clusters in an Ex Vivo Environment Mechanically Mimicking the Lymph Vascular System

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ABSTRACT

BACKGROUND: Inflammatory breast cancer (IBC) is a rare form of breast cancer with a poor prognosis. IBC is characterized by florid lymphovascular tumor emboli in the skin and the parenchyma of the breast. We hypothesized that the formation of these emboli/clusters plays a pivotal role in IBC metastasis and its rapid progression, and that their structure and function may be a key to identifying molecular biological differences between IBC and non IBC.

METHODS: Mechanical methods were used to mimic the lymph fluid viscosity by adding 2.25% of PEG8000 to the media. Clusters were obtained for IBC tumor cell lines (SUM149 and IBC-3), non IBC tumor cell lines (MDA-MB-231, MDA-MB-468, and MCF7), and a non-tumorigenic human mammary epithelial cell line (MCF10A). Clusters were analyzed by light microscopy, and then prepared for and observed by transmission electron microscopy (TEM).

RESULTS: Significant differences were seen between IBC and non IBC clusters. The TEM analysis revealed that IBC cells harbored numerous microvilli and microvesicles, both on the free outer surface and inside the cluster. Microvilli from IBC cell clusters were noted at higher density and were longer than those of non IBC cell clusters.

CONCLUSIONS: IBC tumor cell clusters exhibited distinct ultrastructural features characterized by the presence of long, crowded microvilli and numerous microvesicles. These microvilli may play an important role in the biology and aggressiveness of IBC.

KEYWORDS: Cell clusters, inflammatory breast cancer, electron microscopy, lymph vascular environment

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Background

Inflammatory breast cancer (IBC) is a rare form of breast cancer, which accounts for 1% to 5% of all breast cancers in the United States and about 6% to 11% in North African countries.¹ IBC is highly aggressive breast cancer with a five-year survival rate of less than 40%. IBC patients typically do not present with a tumor mass, instead, the affected breast often becomes swollen, red, and tender, hence the name “inflammatory” breast cancer. However, these symptoms are not caused by inflammation but rather by lymphovascular dermal tumor emboli which result from cancer cells blocking lymphovascular spaces in the skin and soft tissue leading to the characteristic dimpled “peau d’orange” or orange skin appearance. IBC progresses rapidly and has a poor overall outcome which is mainly attributed to the systemic dissemination of tumor emboli in the body.²

The diagnosis of IBC is based on clinical and pathologic features and to date, no major molecular distinctive profile has been identified to distinguish between IBC and non IBC

tumors. It has been shown that circulating tumor cells (CTCs) can be detected in a large proportion of patients with newly diagnosed IBC.³ The presence of CTC clusters as compared to single CTCs are reported to be associated with a 23-50-fold increased metastatic potential.⁴

Therefore, an in-depth analysis of the structure and function of such clusters may identify molecular differences between IBC and non IBC tumors and may lead to a better understanding of the underlying mechanisms associated with the rapid disease progression and metastasis in IBC.

To date, little is known about the ultrastructural differences between IBC and non IBC tumor clusters. Recently, Lehman reported that a concentration of 2.25% PEG8000 provides a dynamic viscosity of 18 cSt at 37 degree Celsius; comparable to the average dynamic viscosity of the dermal lymphatic fluid and the pH of the PEG8000 containing medium is pH 7.54, also within the average range of dermal lymphatic fluid.² In the current study, we used a modified version of this method to generate IBC and non IBC tumor clusters to recapitulate



the lymphovascular spaces and to characterize the morphology of these clusters using transition electron microscopy. Our data revealed significant differences in the morphology between the IBC and non IBC clusters with IBC having denser, longer microvilli and microvesicles both on the surface and within the clusters. These morphological differences may contribute to the rapid progression and high metastatic potential of IBC.

Methods

Cell culture

All cell lines were verified for authenticity by the University of Texas MD Anderson Cancer Center Cell line characterization core and maintained according to the suppliers' recommendations. The IBC cell lines are not readily available as the non IBC cell lines and the IBC cell lines used in the current study were a generous gift courtesy of Dr. Wendy Woodward in the Morgan Welch Inflammatory Breast Cancer Research Program at the University of Texas MD Anderson Cancer Center, and non-IBC cell lines were matched by subtype for comparison. HER2 overexpressed breast cancer cell line SK-BR-3 (ATCC® HTB-30) was tested for subtype comparison to IBC-3, however, SK-BR-3 did not form the clusters in this emboli-formation system and was therefore excluded from the study. The SUM149 IBC cell line and the IBC-3 cell line were grown in Gibco™ Ham's F-12 Nutrient Mix (Fisher Scientific, Hampton, NH) supplemented with 7.5% Fetal Bovine Serum (FBS) (Corning Inc., Corning, NY), 1% penicillin/streptomycin insulin (Fisher Scientific, Hampton, NH), 1 µg/mL hydrocortisone (Sigma Chemical Co., St. Louis, MO) and 5 µg/mL insulin (Fisher Scientific, Hampton, NH). The MCF10A (ATCC® CRL-10317) cell line was grown in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F12 medium) (Fisher Scientific, Hampton, NH) with 10% FBS, 20 ng/mL epidermal growth factor (EGF) (Sigma Chemical Co., St. Louis, MO), 0.5 mg/mL Hydrocortisone, 10 µg/mL insulin, 10 mM HEPES (4-[2-hydroxyethyl]-1-piperazineethanesulfonic acid) (Fisher Scientific, Hampton, NH) and 1% penicillin/streptomycin. The MDA-MB-468 (ATCC® HTB-132) non IBC cell line was grown in DMEM/F12 medium supplemented with 5% FBS, 1% penicillin/streptomycin. The MDA-MB-231 (ATCC® CRM-HTB-26) non IBC cell line was grown in DMEM medium (Fisher Scientific, Hampton, NH) supplemented with 10% FBS, 1% penicillin/streptomycin. The MCF7 (ATCC® HTB-22) non IBC cell line was grown in Eagle's minimum essential medium (EMEM). All cell lines were grown at 37°C in 5% CO₂.

Emboli culture system

We used the in vitro emboli cultures system described in detail by Lehman with slight modifications.² Briefly, a 10 cm

bacterial dish was used instead of the low adherent flask to generate the clusters. Monolayer cells were trypsinized with TrypLE™ Express Enzyme (Gibco Cat#12605036), followed by centrifugation at 1200 rpm for 5 min and cells were counted using Countess™ II Automated Cell Counter (Thermo Fisher Scientific). A total of 10 ml of suspension liquid containing 100 000 cells, was placed in each 10 cm dish. Polyethylene Glycol 8000 (PEG8000) (2.25%) was added to the media to create a viscous medium simulating the dermal lymphatic fluid. Dishes were then placed on a belly button dancer shaker at 37°C, with shaking at 40 rpm for 72 hours. Clusters were then harvested using 40 µm cell strainers (Sigma CLS431750-50EA), washed twice with Phosphate buffered saline (PBS), and fixed following the TEM fixation protocol (described in detail below).

Trypan blue staining assay

We used trypan blue staining to determine the percentage of dead cells in each of the cell lines. Clusters were collected manually by pipetting using a light microscope, washed twice in PBS, and trypsinized with TrypLE™ Express Enzyme. After 10 min incubation, clusters were dissociated into single cells by pipetting. 10 µL of 0.4% trypan blue was added to 10 µL of cell suspension. After 3 min of incubation at room temperature, the unstained (viable) and stained (nonviable) cells were counted using a hemocytometer. Counting was repeated twice, and the average dead cells / total cell in each cell line was calculated.

Transition electron microscopy (TEM)

TEM was conducted at the University of Texas MD Anderson Cancer Center Electron Microscopy Core facility. Clusters were fixed with a solution containing 3% glutaraldehyde plus 2% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.3. Clusters were then washed in 0.1 M sodium cacodylate buffer and treated with 0.1% Millipore-filtered cacodylate buffered tannic acid. Clusters were post fixed with 1% buffered osmium, and stained en bloc with 1% Millipore-filtered uranyl acetate for 30 min. The samples were dehydrated (5 min each of 30%, 50%, 70%, 80%, 90%, 95%, 100%, 100%, 100% ethanol), infiltrated, and embedded in LX-112 medium. The samples were polymerized in a 60°C oven for approximately 3 days. Ultrathin sections were cut in a Leica Ultracut microtome (Leica, Deerfield, IL), stained with uranyl acetate and lead citrate in a Leica EM Stainer, and examined in a JEM 1010 transmission electron microscope (JEOL, USA, Inc., Peabody, MA) at an accelerating voltage of 80 kV. Digital images were obtained using AMT Imaging System (Advanced Microscopy Techniques Corp, Danvers, MA). The length of microvilli was quantified with TEM images at 5000x magnification. The presence of lipid droplets within the clusters of each cell line were also identified with TEM images.

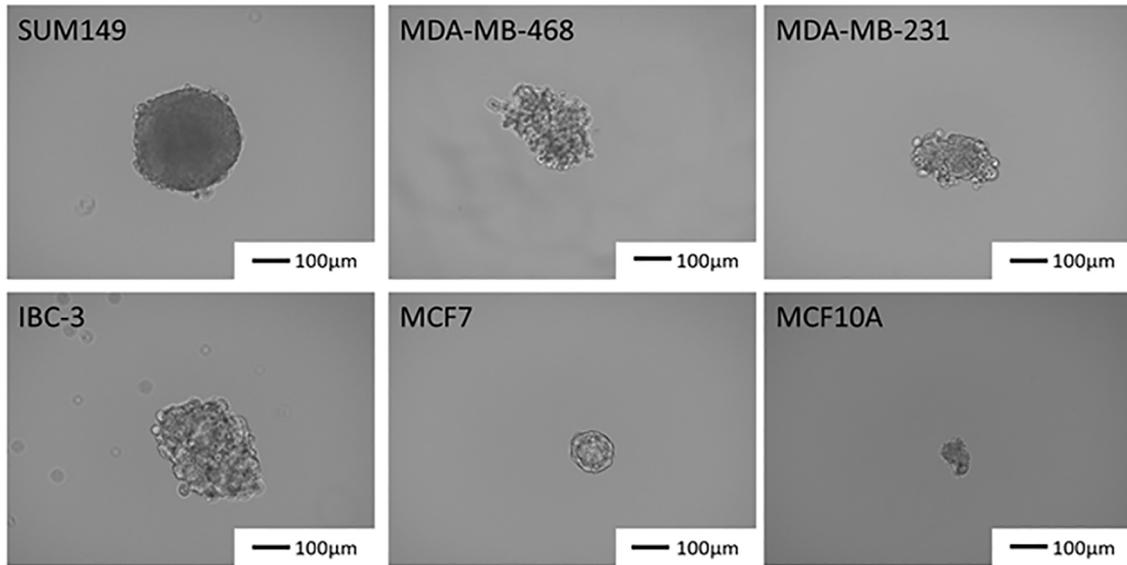


Figure 1. A phase contrast light microscopy revealed bigger and more compact IBC clusters compared to non IBC clusters. IBC cell line (SUM149 and IBC-3) clusters appear more packed compared to non IBC cell line (MDA-MB-231, MDA-MB-468, MCF7 and MCF10A) clusters. IBC indicates inflammatory breast cancer.

Quantification of microvilli length on TEM images

The length of microvilli was quantified using ImageJ software. Five TEM images of the clusters per cell line were used for this quantification. We used a one-way analysis of variance (ANOVA) followed by the Tukey Test for cell line comparison. All statistical calculations were performed by using GraphPad Prism version 8.4.0. Graphs were made by using GraphPad Prism version 8.4.0. A *P* value of $< .01$ was considered statistically significant.

Results

A phase contrast light microscopy

Figure 1 shows the representative images of the clusters formed by the different cell lines for size and shape comparisons (Figure 1). IBC clusters, SUM149 and IBC-3, were more packed than the non IBC cell clusters from MDA-MB-231, MCF7, and human mammary epithelial cell cluster MCF10A. We observed that the MDA-MB-468 formed sheet-like aggregates that easily dissociated. In addition, we observed that tumor clusters formed by SUM149 were more rounded and compact than those formed by IBC-3.

Transition electron microscopy

We describe below the ultrastructural morphological differences observed between tumor clusters generated from the different cell lines with TEM.

Inflammatory breast cancer cell line clusters

SUM149 (Figure 2A and B). SUM149 is a highly aggressive triple negative IBC tumor cell line. Figure 2 shows

the characteristics of the clusters formed by SUM149. The clusters contained loosely packed cells, many of which were dividing as evident by the alignment of the genetic material at the metaphase plate (Figure 2A). The clusters also contained very few dead cells. We observed numerous microvilli and microvesicles between cells as well as on the surface of clusters (Figure 2B). By definition, microvilli are formed as cell extensions from the plasma membrane surface (look homogeneous and have the same density), and microvesicles are vesicular structures (0.1-1.0 μm , that look round and faint) shed by outward blebbing of the plasma membrane.^{5,6} Microvilli and microvesicles in SUM149 clusters were dense and interlaced both on the cell surface and between the cells in the cluster. The gaps between cells were also filled with numerous long interlacing microvilli. In addition, multiple lipid droplets were observed in the cytoplasm of almost all SUM149 clusters (Figure 2B, arrow).

IBC-3 (Figure 2C to F). IBC 3 is a HER2 + IBC tumor cell line; clusters from IBC-3 were slightly different from SUM149. We observed two different types of clusters, tightly packed (Figure 2C) and loosely packed ones (Figure 2D). Both had numerous microvilli on the surface. In both loosely and tightly packed IBC-3 cluster, numerous microvilli (Figure 2E and F, arrow) and microvesicles were found both on the free outer surface of the cluster as well as on the surface of cells inside the cluster (Figure 2E and F). Unlike SUM149 clusters only a few lipid droplets in the cytoplasm were found in IBC-3 clusters.

Non inflammatory breast cancer cell line clusters and human mammary gland cell line clusters (Figure 3)

MDA-MB-231 (Figure 3A to D). MDA-MB-231 is a highly aggressive triple negative non IBC cell line. Similar to

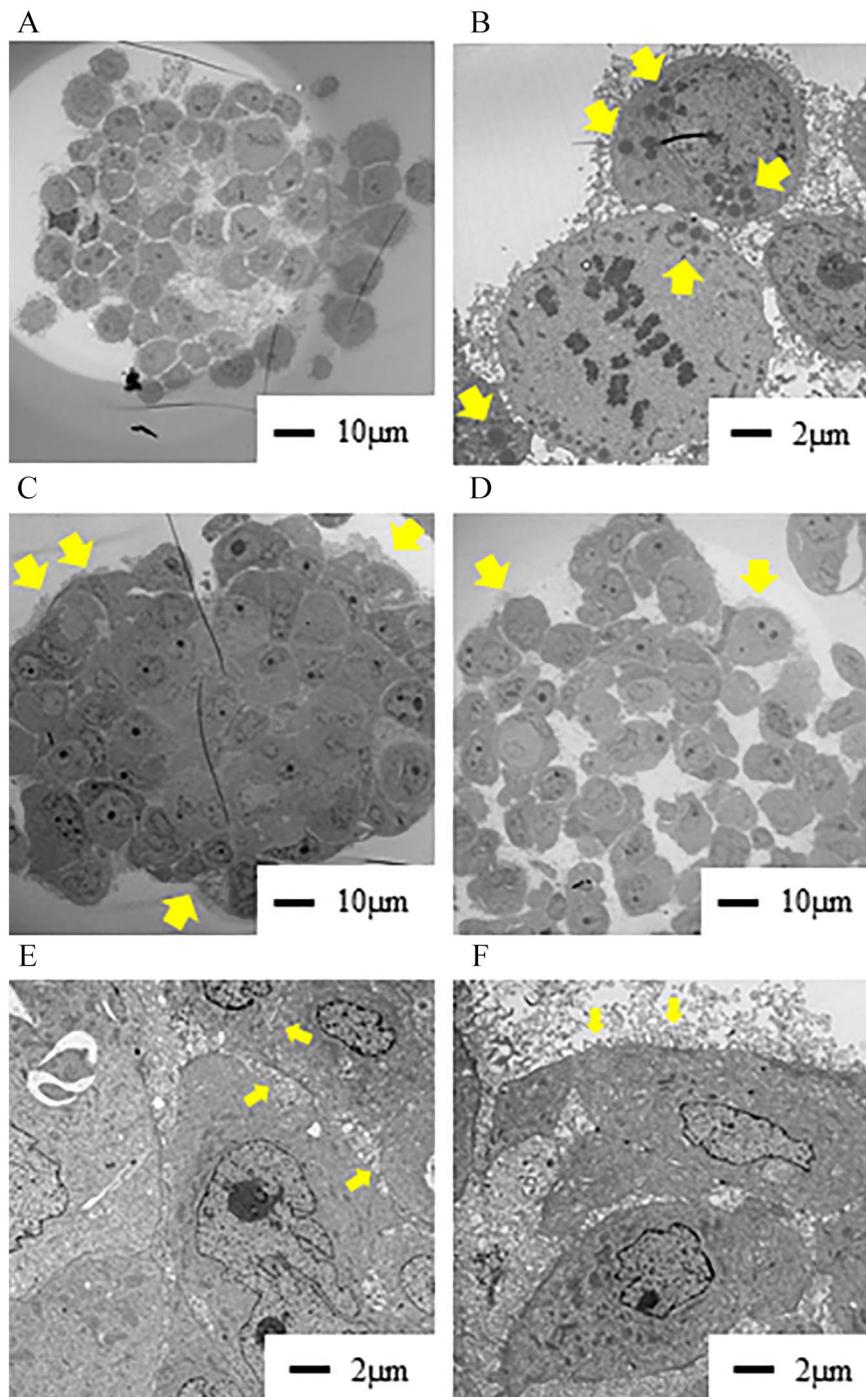


Figure 2. TEM images of IBC cell line clusters (SUM149 and IBC-3). SUM149 clusters show many dividing cells (A arrowhead) and few dead cells (A arrow). Long, dense microvilli and numerous microvesicles were found both on the free outer surface of the cluster as well as on the surface of cells inside the cluster (B). SUM149 cells in the cluster have multiple lipid droplets in the cytoplasm (B arrowhead). IBC-3 clusters have two different types: tightly packed clusters (C) and loosely packed clusters (D). Both had numerous microvilli and vesicles between cells inside the cluster as well as the outer surface of the clusters (E and F, arrow indicates microvilli). IBC indicates inflammatory breast cancer; TEM, transmission electron microscopy.

IBC-3 clusters, two types of clusters were observed, loosely packed (Figure 3A) and tightly packed clusters (Figure 3B). Very few microvilli on the outer surface of the cluster were seen in both types of clusters (Figure 3C and D). We observed a larger number of dead cells that clustered together within the MDA-MB-231 clusters (Figure 3A and B, arrow). Higher

magnification images of loosely packed clusters revealed short and sparse microvilli as well as microvesicles between cells (Figure 3C). Only a few cells contained lipid droplets in the cytoplasm (Figure 3C and D arrowhead). Within the tightly packed clusters, the cell borders were poorly defined due to the tight packing of the cells (Figure 3D).

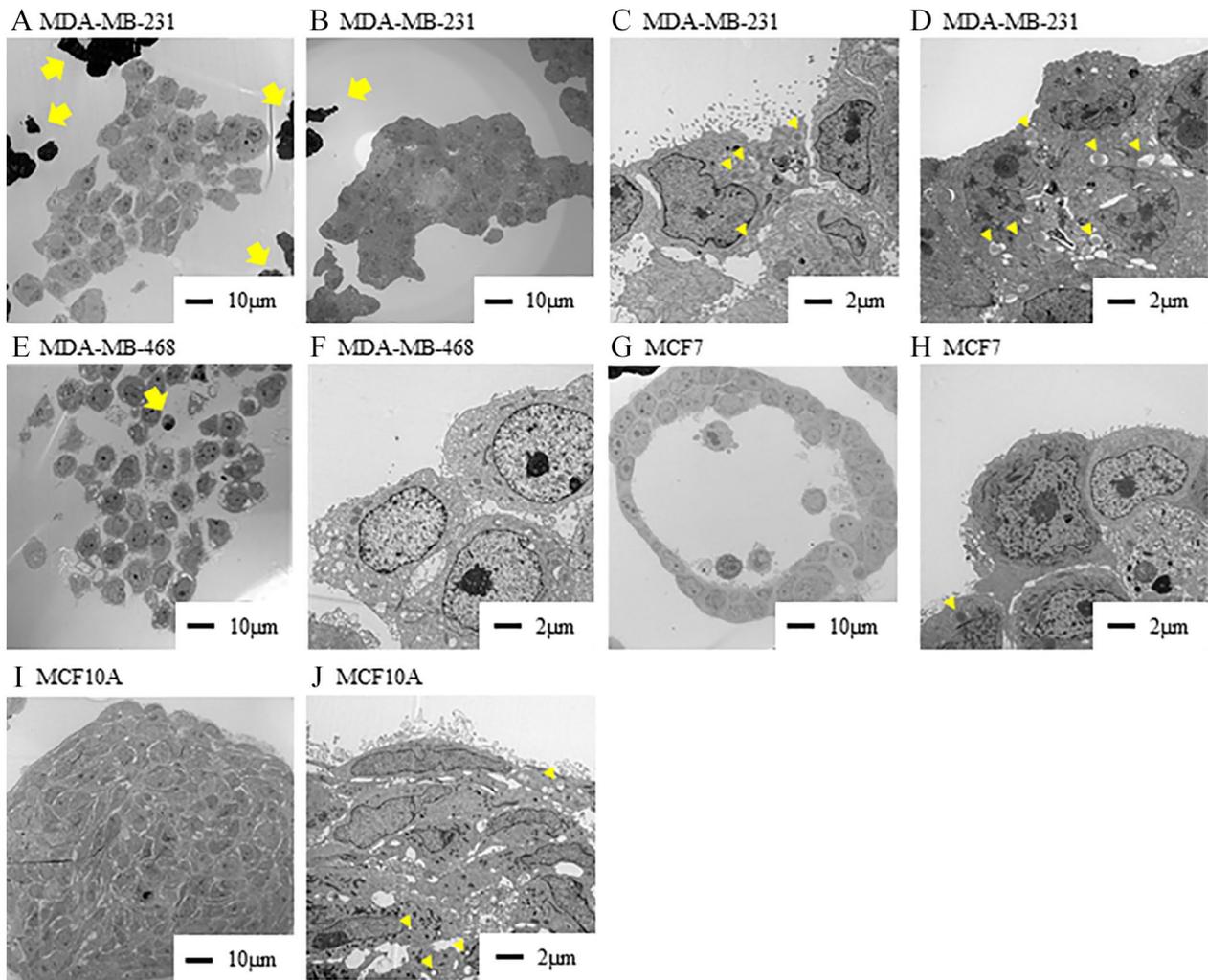


Figure 3. TEM images of non-IBC cell line and human mammary gland cell line clusters. Two types of clusters were found, loosely packed (A) and tightly packed (B) in MDA-MB-231 clusters. Clusters consist of dead cells were pointed by arrows (A and B). With higher magnification, short and few microvilli on the outer surface of the cluster were seen in both loosely packed clusters (C) and tightly packed clusters (D). The borders of each cell in the tightly packed clusters were difficult to identify (D). All MDA-MB-468 clusters were loosely packed (E). A few, short microvilli were observed on the free surface of the cluster (F). MCF7 clusters were very compact with tightly attached cells and a formation of luminal space was observed in some clusters (G). A sparse, short microvilli were seen on the free surface of the clusters but not inside the clusters (H). MCF10A clusters were tightly packed (I). Numerous microvesicles on the free surface of the cluster but no microvilli were observed (J) and some cells had lipid droplets in their cytoplasm (J arrowhead). IBC indicates inflammatory breast cancer; TEM, transmission electron microscopy.

MDA-MB-468 (Figure 3E and F). MDA-MB-468 is a highly aggressive triple negative non-IBC cell line. Cells within the MDA-MB-468 clusters were loosely packed, with large spaces between cells that were free of microvilli or microvesicles (Figure 3E). A small number of dead cells were observed in the cluster (Figure 3E arrow). In addition, a few microvilli were observed on the surface of clusters, and between cells, microvilli were short and sparse (Figure 3F). No lipid droplets were observed in the cytoplasm.

MCF7 (Figure 3G and H). MCF7 is a Hormone Receptor (HR) + weakly invasive non-IBC cell line. MCF7 clusters were very compact with tightly attached cells and some of the MCF7 clusters formed a luminal space, an observation that was previously reported (Figure 3G).⁷ Short and scarce microvilli were only seen on the surface of the cluster but not on the surface of

the cells inside the clusters (Figure 3H), and few cells had lipid droplets inside the cytoplasm (Figure 3H, arrowhead).

MCF10A (Figure 3I and J). MCF10A is a non-tumorigenic human mammary epithelial cell line. MCF10A clusters showed microvesicles on the outer surface which did not seem to be protruding from the cell but rather resting on the cell surface (Figure 3I and J). No microvilli or microvesicles were seen between cells however, some cells have lipid droplets structure that seemed different from other cell line clusters; with empty areas surrounded by dark stained areas (Figure 3J, arrowhead).

Summary of differences between IBC and non-IBC clusters on TEM images. Figure 4A to E is the representative TEM image of the clusters of each cell line to show a side-by-side comparison of lipid droplet morphology where the size, color, and

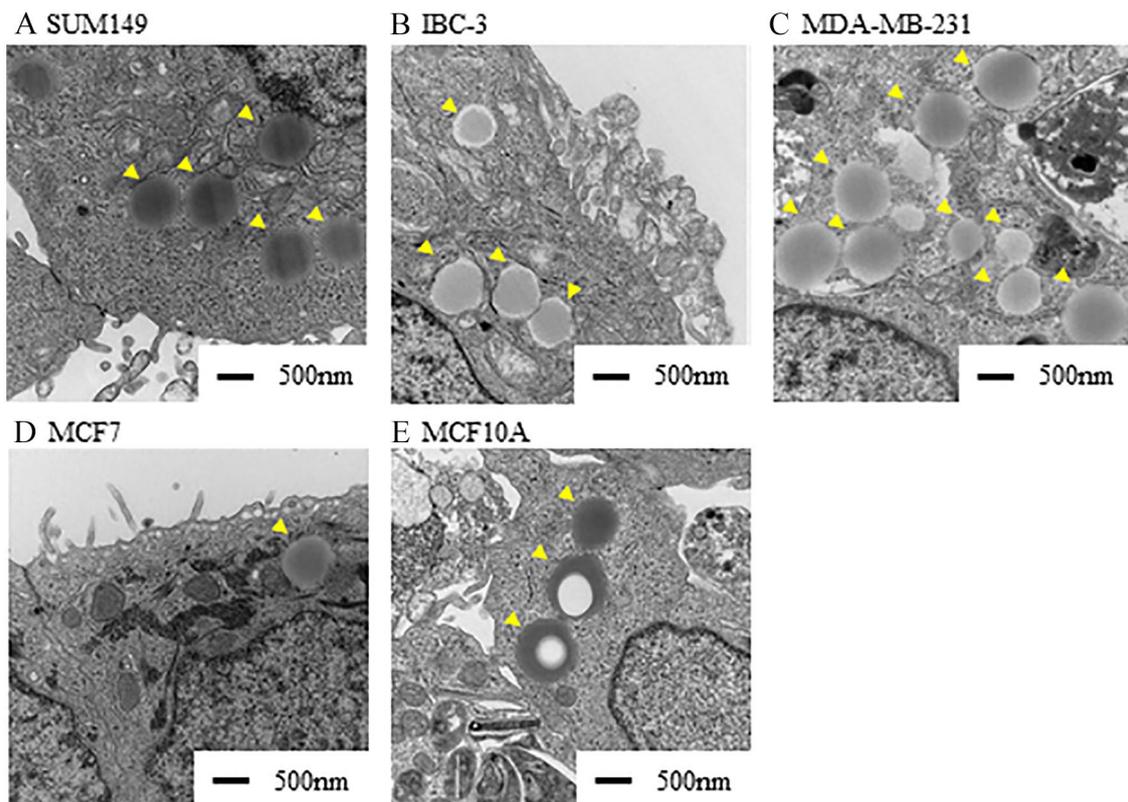


Figure 4. Comparison of lipid droplets in the clusters. All cell line clusters used in this study except MDA-MB-468 showed lipid droplets in their cytoplasm. SUM149 (A) showed multiple darker lipid droplets compared to IBC-3 (B), MDA-MB-231 (C) and MCF7 (D), MCF10A (E) lipid droplets had a vacuolated appearance.

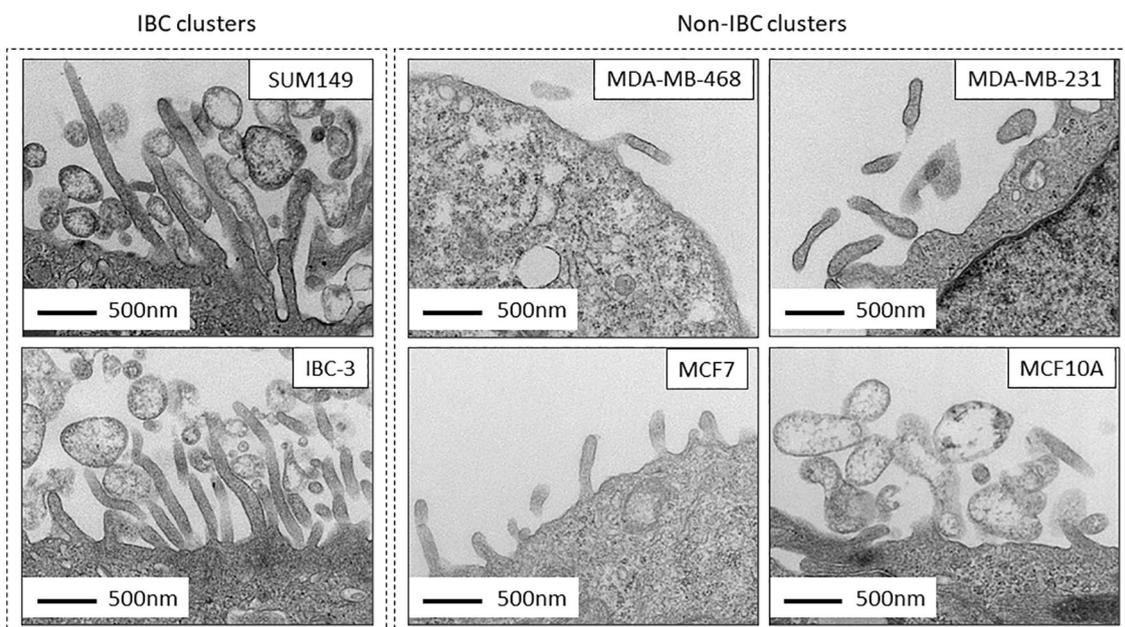


Figure 5. Comparison of free outer surface of the clusters. IBC clusters (SUM149 and IBC-3) have longer, and denser microvilli compared to non IBC clusters (MDA-MB-231, MDA-MB-468, MCF7 and MCF10A). IBC clusters (SUM149 and IBC-3) have numerous microvesicles. IBC indicates inflammatory breast cancer.

structures were different. No lipid droplet was identified in MDA-MB-468 in our TEM images. Lipid droplets in SUM149 were darker compared to those in IBC-3, MDA-MB-231, and MCF7 while lipid droplets in MCF10A had a

vacuolated appearance. Figure 5 shows a side-by-side comparison between the length and shape of the outside surface microvilli in all the tested cell lines; both SUM149 and IBC-3 cell line clusters showed longer microvilli as compared to non IBC

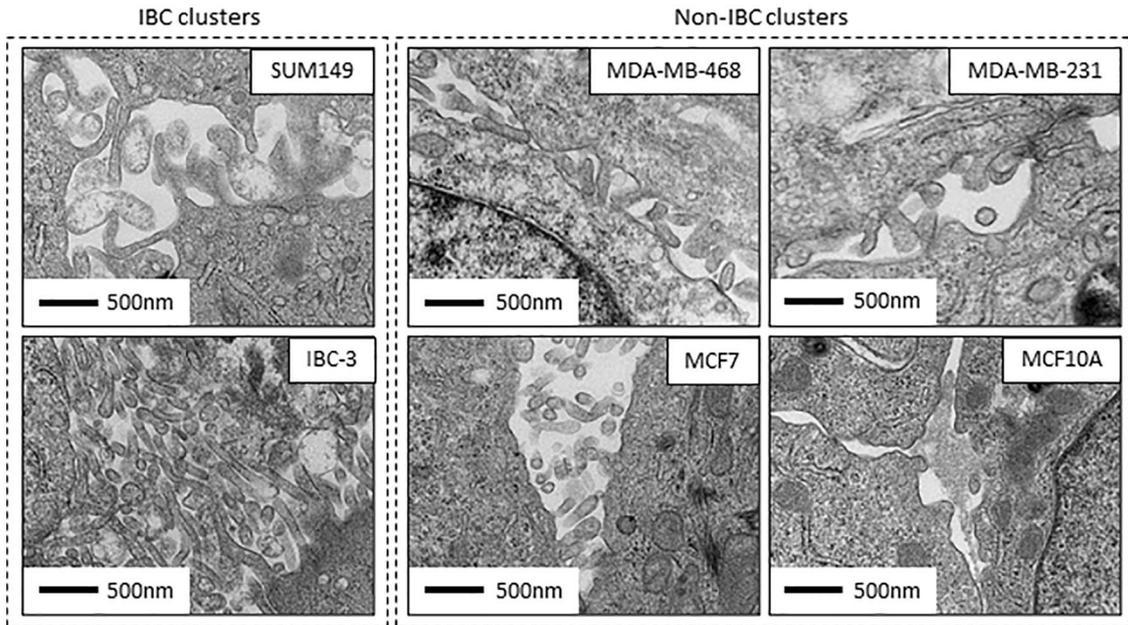
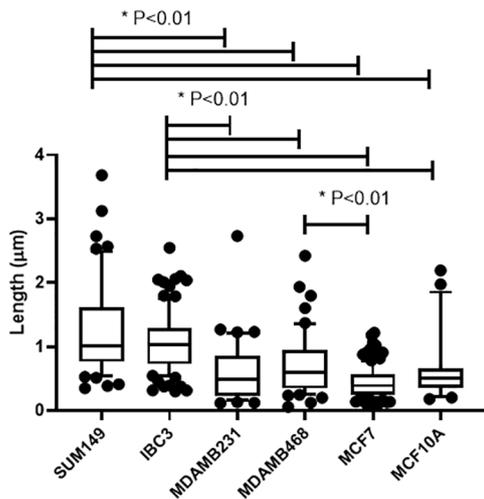


Figure 6. Comparison of the surface of cells inside the clusters. IBC clusters (SUM149 and IBC-3) have longer, and denser microvilli compared to non IBC clusters (MDA-MB-231, MDA-MB-468, MCF7 and MCF10A). IBC clusters (SUM149 and IBC-3) have numerous microvesicles. IBC indicates inflammatory breast cancer.

A Length of microvilli on the surface of the clusters



B Length of microvilli inside the clusters

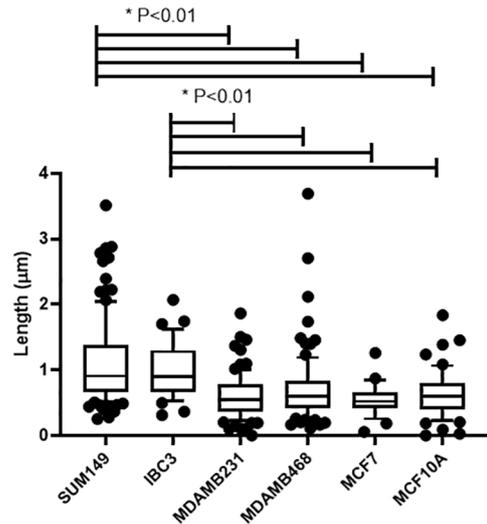


Figure 7. Quantification of microvilli length on the surface of clusters (A), and inside the clusters (B). The length of microvilli was measured using ImageJ software from 5 TEM images of each cell line clusters which is connected to the surface of the cell membrane. In the box plots, the boundary of the box closest to zero indicates the 25th percentile, a black line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 10th and 90th percentiles. Points above and below the whiskers indicate outliers outside the 10th and 90th percentiles. The IBC cell line clusters (SUM149 and IBC-3) had significantly longer microvilli both on the surface of the clusters (A) as well as the surface of cells inside the clusters compared to non IBC cell line clusters (MDA-MB-231, MDA-MB-468 and MCF7) and human mammary gland cell line clusters (MCF10A) ($P < .01$). IBC indicates inflammatory breast cancer; TEM, transmission electron microscopy.

cell line clusters. Figure 6 shows the length and shape of the microvilli between the cells inside the clusters; both SUM149 and IBC-3 cell lines had longer microvilli than non IBC cell lines. Quantification of the length of microvilli showed that the microvilli were predominantly longer (500-1000 nm) and denser in IBC tumor cell line clusters compared to non IBC

clusters (200-400 nm). The length of microvilli both on the surface and inside of IBC cell line clusters (SUM149 and IBC-3) were significantly longer than non IBC cell clusters ($P < .01$), (Figure 7). The major differences observed between the IBC and non IBC cell line clusters in TEM images are summarized in Table 1.

Table 1. Summary of the characteristics of IBC and non IBC cell line clusters on TEM images.

	IBC CELL LINES		NON IBC CELL LINES			BENIGN CELL LINE
	SUM149	IBC-3	MDA-MB-231	MDA-MB-468	MCF7	MCF10A
Cluster types	Loose	Tight and loose	Tight and loose	Loose	Tight	Tight
Surface	Long microvilli Microvesicles	Long microvilli Microvesicles	Short microvilli	Short microvilli	Short microvilli	Microvesicles
Microvilli between cells	Dense, Long	Dense, Long	Sparse, Short	Sparse, Short	Tightly attached	Tightly attached
Dead cells (%)	8.1	18.9	44.6	14.6	36.6	9.1
Lipid droplets	+	+	+	-	+	+

Abbreviations: IBC, inflammatory breast cancer; TEM, transmission electron microscopy.

SUM149 and IBC-3 clusters showed numerous and dense microvilli with multiple microvesicles on their surface as well as inside the clusters. Dead cells in the cluster of each cell line were quantified using trypan blue staining. SUM149 had the lowest percentage of dead cells within clusters. The quantification of dead/apoptotic cells, as well as dividers in the clusters, were shown in Supplemental Table 1. Lipid droplets were present in all cell lines except MDA-MB-468 on TEM images.

Discussion

To our knowledge, this study is the first comprehensive descriptive analysis that compares IBC and non IBC clusters and showed distinctive differences in microvilli length and density in the clusters. We found that IBC tumor clusters harbor numerous microvilli and microvesicles on the surface. The microvilli in IBC tumor clusters were denser and longer compared to those observed in the non IBC tumor clusters. Numerous microvilli and microvesicles were also seen in the gaps between the cells within the IBC cluster. These findings are consistent with the previous report by Morales describing the human IBC xenograft model, MARY-X.⁸ The investigators reported that MARY-X spheroid shows extensive microvilli on the surface and many structures that were termed “canalis” or canals using scanning electron microscopy. These canals extend deep within the interior of the spheroid and were coated by long extensive microvilli lining. In our study, we observed many gaps within the IBC clusters which may be the cross-sectional images of these “canalis.” Another unique finding of the SUM149 clusters in our study was the presence of many dividing cells which reflects rapid cell proliferation (Supplemental Table 1). This observation is interesting since many dividing cells within the clusters together with the presence of gaps filled with numerous long microvilli may be a potential mechanism by which the rapidly growing cells within the cluster receive oxygen and nutrients through “canalis” formation. This also supports the hypothesis that “canalis” formation may play an important role in supporting IBC’s rapid proliferation.

The association between microvilli and the invasiveness in mesothelioma,⁹ and the growth and the metastatic potential in other tumor cells^{10,11} have been reported. Recently, Tanaka reported that TEM analysis of squamous cell carcinoma cells in the tongue revealed that the primary tumor from patients with lymph node metastasis had numerous microvilli compared to non-metastatic patient samples.¹² These findings suggest that microvilli may play an important role in the invasiveness and the aggressiveness of IBC which showed denser and longer microvilli compared to non IBC clusters in our study.

Microvesicles, a subset of extracellular vesicles released from the plasma membrane of cancer cells, contains biologically active biomolecules, including DNA, mRNA, and miRNA. Microvesicles shed from various tumor-cell lines have been thought to facilitate extracellular matrix (ECM) invasion and metastasis.¹³ They are also reported to support the establishment of a favorable tumor niche by influencing the surrounding stroma cells. The amount of microvesicles shed by tumor cells has been shown to correlate with their invasiveness both in vitro¹⁴ and in vivo.¹³ It was recently reported that microvesicles released from cancer cells changed cancer-associated fibroblast (CAF)-like fibroblast phenotypes on stiff matrices and may regulate the physical properties of the microenvironment.¹⁵ In our study, we observed the presence of numerous microvesicles between cells inside the clusters, and on the surface of the clusters, which was prominent in IBC cell lines. IBC often does not form a lump in the primary site, and this is one of the distinctive features of IBC. Microvesicles produced by IBC may change the physical properties of the microenvironment which may be the reason for the lack of primary lump. Further investigation into the bio-molecular constituents of the microvilli and microvesicles is warranted as it may provide a better understanding of the biology of IBC.

One of the most important metabolic hallmarks of cancer cells is the metabolic alteration to adapt to adverse environmental conditions. Enhanced lipogenesis is observed in many different cancer types but studies have shown that cancer cells

can also acquire exogenous fatty acids by upregulating various fatty acid uptake mechanisms.^{16–18} Different studies including ours, have reported that obesity is a risk factor for IBC regardless of the menopausal status.¹⁹ Since adipocytes are a major component of the stroma of breast cancers, obesity associated dysfunctional adipose tissue releasing increased amounts of fatty acids in addition to dietary lipids can be involved in promoting tumor growth and progression.²⁰ In this study, we observed a greater number of lipid droplets in SUM149 cytoplasm as compared to other cell lines on TEM images. We used BODIPY 493/503 staining to confirm the presence of lipid within the clusters (Supplemental Figures 1 and 2). Supplemental Figures 1 and 2 are representative images of the lipid staining from each cell line cluster which shows significant lipid droplets accumulation in SUM149 cells suggesting the enhanced lipogenesis and/or enhanced uptake of the lipid from the surroundings in SUM149 cells. In addition, lipid droplets are known to be prominent in mammary epithelial cells of lactating mammary glands, where they essentially serve as a secreted organelle, transferring lipid nutrients and energy from mother to child. In this process, lipid droplets are secreted from the apical side of epithelial cells. It has been reported that lack of breastfeeding in parous women with inflammatory breast cancer was a predictor of poor outcome²¹ underscoring a potential role of lipid droplet accumulation in IBC's aggressiveness.

Conclusions

To our knowledge, this is the first study to describe the differences in IBC and non IBC clusters formed in a mechanically simulated lymph vascular environment. Using TEM, we show that the IBC clusters have denser, longer microvilli and microvesicles on the surface and within the clusters compared to non IBC clusters. These findings suggest that IBC clusters have a distinct structural difference from non IBC clusters that may contribute to the rapid progression and high metastatic potential. Further studies are needed to clarify correlations between these cell surface features, underlying bio molecular composition and potential influence on aggressive behavior of the tumor.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Yuka Fujii, Savitri Krishnamurthy and Randa El-Zein. The first draft of the manuscript was written by Yuka Fujii and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Supplemental Material

Supplemental material for this article is available online.

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