Microvesicles released from tumor cells disrupt epithelial cell morphology and contractility

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Abstract

During tumor progression, cancer cells interact and communicate with non-malignant cells within their local microenvironment. Microvesicles (MV) derived from human cancer cells play an important role in mediating this communication. Another critical aspect of cancer progression involves widespread ECM remodeling, which occur both at the primary and metastatic sites. ECM remodeling and reorganization within the tumor microenvironment is generally attributed to fibroblasts. Here, using MCF10a cells, a well-characterized breast epithelial cell line that exhibits a non-malignant epithelial phenotype, and MVs shed by aggressive MDA-MB-231 carcinoma cells, we show that non-malignant epithelial cells can participate in ECM reorganization of 3D collagen matrices following their treatment with cancer cell-derived MVs. In addition, MVs trigger several changes in epithelial cells under 3D culture conditions. Furthermore, we show that this ECM reorganization is associated with an increase in cellular traction force following MV treatment, higher acto-myosin contractility, and higher FAK activity. Overall, our findings suggests that MVs derived from tumor cells can contribute to ECM reorganization occurring within the tumor microenvironment by enhancing the contractility of non-malignant epithelial cells.

Introduction

Early in tumor progression, extensive extracellular matrix (ECM) remodeling occurs (Butcher et al., 2009; Levental et al., 2009; Provenzano et al., 2008). Reorganization of the ECM is dependent on cell-mediated collagen bundling as well as deposition of new ECM components (Even-Ram and Yamada, 2005; Friedl and Wolf, 2010). Additionally, cells can reorganize collagen into parallel fibers radiating perpendicularly to the tumor to facilitate invasion (Provenzano et al., 2006). Interestingly, ECM remodeling and reorganization is observed in the early stages of tumor progression, and even adjacent non-tumoral sites show signs of ECM remodeling (Theret et al., 2001; Zhu et al., 1995). While fibroblasts are often described as mediating these changes (Cukierman and Bassi, 2010; Dumont et al., 2013; Mao et al., 2013), healthy epithelial and myoepithelial cells residing near the premalignant lesion could also contribute to these changes and respond to signals from cancer cells.

There is now a large body of evidence that shows that cancer cells can interact and communicate with healthy cells present within the local microenvironment (Al-Nedawi et al., 2009; Al-Nedawi et al., 2008; Brucher and Jamall, 2014). Interestingly, cells have the ability to release extracellular vesicles that appear to play an important role in the transfer of signaling proteins and cargo to other cells (Al-Nedawi et al., 2009; Al-Nedawi et al., 2008). There are two known types of extracellular vesicles, namely the smaller exosomes (<100nm) and larger microvesicles (MVs) (Santana et al., 2014). While exosomes are produced by a wide variety of cell types, production of significant quantities of MVs appears to be limited to tumor cells (Santana et al., 2014). MVs shed by tumor cells can contain ECM components, growth factor receptors, cytoskeletal

proteins and signaling molecules as cargo (Antonyak et al., 2011; Grange et al., 2011). When these MVs interact with non-tumorigenic cells, they can promote transformation-like characteristics such as increased cell growth, survival, and migration (Antonyak et al., 2011; Webber et al., 2010). Of note, recent evidence suggests that tumor-shed extracellular vesicles can prime the pre-metastatic niche (Costa-Silva et al., 2015; Grange et al., 2011; Peinado et al., 2012), suggesting a role for extracellular vesicles in modifying the local microenvironment (Costa-Silva et al., 2015). However, it remains unclear whether MVs can contribute to the local ECM remodeling that occurs during the establishment of the pre-metastatic niche.

In the work reported here, we investigated the effect of MVs on non-malignant epithelial cell-mediated matrix reorganization within *in vitro* 3D collagen scaffolds. MCF10a epithelial cells were cultured in the presence of MVs collected from highly aggressive MDA-MB-231 carcinoma cells. Notably, the MCF10a cells cultured in 3D collagen scaffolds showed altered cell morphology and increased ECM reorganization following their treatment with MVs. In addition, 2D traction force microscopy measurements reveal that MCF10a cells generate more traction when they are cultured in the presence of MVs. Correspondingly, we observe a MV-mediated increase in both focal adhesion kinase (FAK) and myosin light chain phosphorylation. Overall, our results indicate that MVs shed by tumor cells can induce phenotypic changes in non-malignant epithelial cells, resulting in increased contractility and modifications to the ECM in the local microenvironment.

Materials and methods

Cell culture and reagents

MCF10A mammary epithelial cells (American Type Culture Collection (ATCC), Rockville, MD) were maintained in Dulbecco's Modified Eagle's Media supplemented with 5% horse serum, 20 ng/mL EGF (Invitrogen, Carlsbad, CA), 10 mg/mL insulin, 0.5 mg/mL hydrocortisone, 100 ng/mL cholera toxin (Sigma-Aldrich, St. Louis, MO), and 1% penicillin-streptomycin (Invitrogen). All cells were cultured at 37°C and 5% CO₂. Primary antibodies used were rabbit anti-phospho Y397 FAK (p-FAK, #3283) and anti-phospho threonine-18 and serine-19 myosin light chain (p-MLC, #2101; Cell Signaling Technology, Danvers, MA, USA), anti-vinculin (Sigma) and mouse monoclonal anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH, MAB374, Millipore). Secondary antibodies used were Alexa 594-goat anti-mouse IgG, Alexa 488-goat anti-rabbit IgG and Alexa-594-goat anti-rabbit IgG (Invitrogen). Phalloidin-Alexa Fluor® 488, Goat serum and phosphate buffered saline (PBS) were purchased from Invitrogen; Triton X-100 was from JT Baker (Phillipsburg, NJ, USA). All other chemicals were from Sigma-Aldrich (St. Louis, MO, USA).

MV isolation and characterization

Two 150 mm dishes of MDA MB 231 cells (~35 million cells) were rinsed with phosphate-buffered saline (PBS) several times and incubated in serum free RPMI medium for between 8-12 hours. The conditioned medium was removed from the cells and initially centrifuged at 300 x g for 10 minutes to pellet intact cells, and then again at 1000 x g for 10 minutes to pellet debris. The partially clarified medium was filtered

through a 0.22 μ m SteriFlip filter unit (Millipore), and rinsed with 15 ml of PBS. The MVs retained by the filter were resuspended in 1.5 ml DMEM/F12 medium.

3D cell culture

Three-dimensional collagen matrices for cell migration experiments were prepared as previously described (Bordeleau et al., 2013). Briefly, acid-extracted collagen I from rat tail tendon (Rockland Immunochemicals, Gilbertsville, PA) was diluted to 1 mg/mL from a 10 mg/mL stock collagen solution by gently mixing with 0.1% acetic acid on ice and neutralized to pH 7.0 with 1 M HEPES and 1 N NaOH. 50 μ l of DMEM/F12 containing 20,000 MCF10a cells was then gently mixed with the collagen. 500 μ l of the collagen solution was then allowed to polymerize for 60 minutes on at 37°C in 24 well plates. The collagen matrices were overlaid with culture medium following polymerization.

Time-lapse imaging

Cellular behavior in response to MVs treatment was observed with a wide-field digital imaging system (Zeiss Axio Observer Z1, Hamamatsu ORCA-ER camera and Axiovision software v. 4.8.1.0) equipped with an environmental chamber. Phase-contrast images were captured at 30 min intervals over a 72-h period, using a 20X/NA0.5 ph2 dry objective. After each 24-h period, the cells were fed fresh MVs. The cell morphology was quantified using ImageJ software (v. 1.46, National Institutes of Health, Bethesda, MD, USA). Quantification of cell morphology was obtained from at least three independent experiments.

Confocal cell imaging

Confocal reflectance microscopy was used to image collagen matrix organization mediated by the cells. A Zeiss LSM700 confocal microscope equipped with a long

working distance water-immersion C-Apochromat 40×/1.1 NA Zeiss objective was used as previously described (Carey et al., 2012). A solid state 405 nm laser illuminated the gel and backscattered light from collagen fibers was captured through a 32-mm pinhole, giving 1-mm thick confocal slices. Pixel dwell time was 1.57 ms and two scans were averaged per image. For imaging MVs inside the collagen gels, MVs were pre-stained after centrifugation with the membrane dye FM 1-43X (Invitrogen). MVs were washed one additional time before proceeding with the isolation step as described above. Stained MVs were added to acellular collagen gels and 3D time lapse images were immediately acquired using a 20X/NA0.5 ph2 dry objective at the fastest scanning setting. Z-oriented cross sections were reconstructed with ImageJ and used to quantify MV penetration within the collagen gels.

For 2D immunofluorescence imaging, MCF10a cells were seeded on coverslip pre-coated with 0.1 mg/ml rat tail collagen type I (BD Biosciences, San Jose, CA) as described before (Huynh et al., 2013). Cells were fixed 10 min at 37 °C with 3.7% formaldehyde in PBS, followed by 1% Triton X-100 (JT Baker) in PBS for 5 min. Fluorescent images were acquired with a Zeiss 710 confocal microscope and Zen software (v. 2009, Carl Zeiss, Germany). Z-stacks were reconstructed with ImageJ.

Western blotting

MCF10a cells were cultured on tissue culture plastic dishes for 24 h and then treated with MVs or with media alone for 2 h. The cells were rinsed with PBS and lysed. Western blots were performed as described previously (Bordeleau et al., 2010). Transferred membranes were blocked with 5% milk in TBS-polyoxyethylene (20) sorbitan monolaurate (Tween; JT Baker) or 5% BSA. Membranes were incubated overnight 1:500

in TBS-Tween with anti p-FAK, p-MLC or 1:2000 anti-GAPDH at 4°C, and 1:2000 in TBS-Tween with 5% milk with a horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature. Samples were imaged with a Las-4000 imaging system (Fujifilm Life Science, Japan) after the addition of either SuperSignal West Pico or West Femto Chemiluminescent Substrate (Thermo-Fisher, USA).

Traction Force Microscopy

Traction force microscopy was performed as previously described (Kraning-Rush et al., 2011). Briefly, polyacrylamide (PA) gels embedded with 0.5 μm diameter fluorescent beads (Life Technologies) and a Young's modulus of 5 kPa were made with a bisacrylamide:acrylamide ratio of 7.5:0.175 (Bio-Rad, Hercules, CA). The PA gels were coated with 0.1 mg/ml rat tail type I collagen (BD Biosciences, San Jose, CA) and cells were allowed to adhere for 24 hours followed by a 2 h MV treatment. Phase contrast images of single cells were then taken immediately followed by fluorescent images of the bead field at the gel surface. Cells were then removed with 0.05% trypsin/EDTA (Life Technologies) and a second fluorescent image of the bead field was captured. Bead displacements between the stressed and null state were calculated using the LIBTRC analysis library developed by M. Dembo (Dept. of Biomedical Engineering, Boston University) and used with the PA gel properties to calculate the most likely cellular traction vectors, T as well as the traction vector T_S and T_L along the cell short and long axis respectively (Dembo and Wang, 1999). The overall cellular force is the integral of the traction vector magnitudes over the total cell area (Reinhart-King et al., 2005).

Quantitative characterization of collagen 3D microarchitecture

Quantification of collagen radial compaction in 3D gels was performed as described before (Kraning-Rush et al., 2011) with the following modification: briefly, after 3 days of treatment, cells embedded within collagen gels were fixed and imaged in 3D with confocal reflectance microscopy. Further image quantification steps were performed on confocal reflectance sections corresponding to the middle plane of the imaged cell cluster. The radial profile of the collagen fibers surrounding the cells was obtained from linear intensity profile scanned in 2° increments using a custom ImageJ script. The cell membrane surface contour was used as the origin, and reflectance intensities were averaged as a function of the radius. The intensity profile was then normalized by subtracting a baseline average intensity value measured 70-80 μm away from the cells. The resulting collagen intensity profiles were then fit to an exponential decay model using Matlab:

$$I = I_0 e^{\frac{-r}{\tau}} \quad (1)$$

Where I is the collagen signal intensity, I_0 is the collagen intensity at the cell membrane and τ is the rate of exponential decay.

Collagen fiber alignment and orientation was quantified using the OrientationJ plugin for ImageJ (Rezakhaniha et al., 2012). To obtain the coherency metric, which is a measure of local fiber alignment (Fonck et al., 2009), confocal reflectance images were segmented into 6 triangular sections spanning 60° each around the centroid of the cells. The area occupied by the cells was subtracted from each triangular region. The OrientationJ measure function was then used to extract the coherency values and obtain an average for each image. A color-coded orientation map of the collagen fibers [HSB color mode; orientation, coherency; intensity] was first obtained by analyzing each image with the

OrientationJ Analysis function. Matlab was then used to transform the reference Cartesian coordinate to an elliptic-like coordinate system in order to express the measured fiber orientation relative to the cells' surface. Briefly, the cells were fit with an ellipse to approximate the membrane surface orientation. The ellipse parameters were then used to compute the new fiber orientation and setting the 0 radian origin as the direction tangent to the ellipse surface. The possible angle values lies within an interval that span $-\pi/2$ to $\pi/2$. The resulting converted angle distribution was then computed by weighting the angle orientations to their matching coherency to give priority to fibrous regions (Rezakhaniha et al., 2012).

Statistical analysis

Statistical analyses were performed using the Matlab Parametric one-way ANOVA and linear regression models with post-hoc Tukey's honest significance test. A p-value below 0.05 was considered statistically significant.

Results

MVs can diffuse inside a collagen gel

Considering that a 3D collagen gel can be a barrier for efficient diffusion of macromolecules (Kihara et al., 2013), we investigated the extent to which MVs can penetrate the gels. MVs generated by MDA-MB-231 breast cancer cells were isolated as described in the Materials and Methods section. Dynamic light scattering (DLS) was used to determine the size of the isolated MVs. DLS measurements yielded an average MV diameter of ~300 nm (Fig. 1A). Of note, the filtration step was sufficient to eliminate the smaller exosomes (Santana et al., 2014). Fluorescently labelled MVs were added on top of the collagen gels and time lapse confocal imaging was performed to monitor how efficiently the MVs entered the gel (Fig. 1B and C). Notably, we observed a gradual increase of the average signal as a function of depth over time (Fig. 1C). This data indicates that MVs can uniformly diffuse throughout the collagen gel.

Cancer cell MVs influence the morphology of non-malignant epithelial cells cultured in 3D collagen scaffolds

We have shown previously that MVs from cancer cells can confer transformed characteristics to fibroblast such as promoting anchorage independent growth (Antonyak et al., 2011). In that context, we first asked whether MVs isolated from MDA-MB-231 cells could induce morphological changes in MCF10a epithelial cells cultured within 3D collagen scaffolds. Notably, after 24 h of MV treatment, the MCF10a cells displayed a more elongated and spindle-like morphology compared to the controls (Fig. 2A). Measurements of the cell aspect ratio indicated that the MV-treated cells were

significantly more elongated after 24 h, and that this difference continued to increase through the 48 h of observation (Fig 2B). The number of protrusions, as indicated by measures of circularity, was only significantly different after 72 h of MV treatment (Fig. 2C). When comparing the cell population as a function of the aspect ratio versus circularity distribution, the cells exhibited characteristic shape factors. Cells treated with MVs were far more likely to fall into a high aspect ratio/low circularity population (Fig. 2D). In addition, the low aspect ratio/high circularity population was mostly absent in the MV treated cells at 72 h. These results indicate that MVs can alter the morphology of non-malignant epithelial cells in the 3D setting.

Non-malignant epithelial cell mediated collagen reorganization is augmented after MV treatment

It has been suggested that the exosome counterpart of the MVs could contribute to the formation of a pre-metastatic niche (Grange et al., 2011; Peinado et al., 2012). In that context, and in light of the above data showing that MVs can trigger morphological changes in cells embedded within collagen, we proceeded to characterize the extent to which the MVs can influence local collagen reorganization by MFC10a cells. To that end, the angular orientation of the collagen fibers surrounding MCF10a cells was measured from confocal sections (Fig 3a). Computation of the average local coherency, which is a measure of the local alignment of fibrillar components (Fonck et al., 2009), indicates that the collagen surrounding the MV-treated cells is significantly more radially aligned relative to the cells than the collagen surrounding the control cells (Fig 3b). The collagen in both the control and the MVs treated samples presented a preferential

orientation perpendicular to the cellular membrane (Fig. 3c). However, the angle distributions in the MV treated sample showed a far greater number of collagen radially-oriented components than the control sample. Acellular collagen gels showed overall poor coherency and no preferential radial orientation (Fig. 3b and c).

To further analyze collagen reorganization, we measured the collagen signal intensity as a function of the distance from the cellular membrane (Fig. 3d). The resulting data points were then fit to an exponential decay function (Fig. 3d) to extract the τ parameter representative of collagen compaction (equation 1, (Kraning-Rush et al., 2011)). The resulting decay rate τ was significantly higher for cells treated with the MVs (Fig. 3e). Taken together, our results indicate that MVs increase the ECM remodeling ability of epithelial cells.

MVs modulate non-malignant epithelial cell contractility and focal adhesions

The ability of a cell to reorganize the surrounding ECM is dependent on its contractile ability to generate the required force to rearrange matrix fibers (Kraning-Rush et al., 2011). Therefore, we assessed the extent to which MVs could regulate non-malignant epithelial cell contractility using TFM. Graphical representation of traction stress magnitudes due to the cell provides a visual representation of the increase in force generation mediated by the MVs (Fig. 4a). Indeed, the MV treatment resulted in significantly higher net traction force (Fig. 4b). In addition, the increase in traction force as function of cell area was greater following the MVs treatment than in the control (Fig. 4c). Interestingly, the traction force ratio between the short and long axis of the cell

indicate a significantly higher traction force polarization with MVs treatment compared with the control (Fig. 4D).

To better understand the increased traction force mediated by cancer cell-derived MVs, we extended our analysis to both MLC and FAK activation. Notably, MLC is required for cellular contractility while FAK activation is known to be dependent on cell contractility (Bordeleau et al., 2012; Huynh et al., 2013; Pasapera et al., 2010; Wei et al., 2008). Immunostaining of the active phosphorylated form of MLC reveals increased staining in MV-treated MCF10a cells compared to the untreated control (Fig. 5a). Similar to what was observed for MLC, the MV treatment resulted in an increase in the FAK phosphorylation staining (Fig. 5b). Of note, there was no significant difference in the actin microfilament co-staining or in FAs size (Fig. 5b and c). Quantification of the fluorescence signal further showed that MVs mediate an increase in both MLC phosphorylation and FAK phosphorylation in vinculin tagged FA (Fig. 5d and e). Western blotting was then used to confirm these observations showing phosphorylation increases in both MLC and FAK due to the MV treatment (Fig. 5f and g). Overall, these data confirm that MVs regulate cellular contractility, which in turn enables ECM reorganization.

Discussion

While data exists regarding how extracellular vesicles are generated (Antonyak et al., 2012; Hanson and Cashikar, 2012) and the function of exosomes (Katsuda et al., 2014; Minciacchi et al., 2015), there is little information regarding the function of MVs. It is known that MVs shed by tumor cells can confer to healthy cells characteristics reminiscent of transformed cells (Antonyak et al., 2011; Webber et al., 2010). Here, we show that MVs also induce morphological changes in non-malignant epithelial cells within a 3D culture. These morphological changes are associated with increased ECM reorganization. Furthermore, we demonstrate that non-malignant epithelial cells exposed to MVs shed by tumor cells generate significantly higher polarized traction force as well as have increased myosin activity and FAK signaling at FAs.

Changes in the tumor microenvironment are an integral part of cancer progression. Recent evidence suggests that extracellular vesicles may be one of the components capable of initiating these changes (Costa-Silva et al., 2015; Grange et al., 2011; Peinado et al., 2012). Our results provide new insights into how tumor cell-shed MVs can influence the microenvironment by promoting ECM reorganization. Specifically, we showed that MVs from cancer cells increased epithelial cell contractility, which is known to be required for ECM remodeling and reorganization (Alcoser et al., 2014; Yamaguchi et al., 2014). Interestingly, tumor shed MVs can also mediate the transformation of fibroblasts into cancer associated fibroblasts (Webber et al., 2010). Notably, cancer associated fibroblasts are highly contractile cells that play a major role in ECM remodeling (Alcoser et al., 2014; Cukierman and Bassi, 2010; Dumont et al., 2013; Mao et al., 2013; Webber et al., 2010). In our case, the effect observed with non-

malignant epithelial cells indicates that MVs can promote ECM reorganization *in vitro*. . This effect also correlates with more directed traction forces generation after MVs treatment in 2D. In this context, further assessment of traction forces both in 3D and with additional cell models representative of the metastatic niche could be very informative. Overall, MV-mediated ECM remodeling could explain how MVs can prime the microenvironment, either at the primary or metastatic sites, to promote tumor progression. In such a context, future work should focus on exploring MV-mediated ECM remodeling *in vivo*.

MVs can contains a wide variety of cargo, and their effects are still poorly understood (Antonyak and Cerione, 2014; Antonyak et al., 2011). Some of the cargo includes cytoskeletal proteins such as actin, moesin, vimentin and actinin (Antonyak et al., 2011). Both moesin and actinin are actin scaffolding proteins involved in regulating the overall actin cytoskeleton organization (Arpin et al., 2011; Ehrlicher et al., 2015; Niggli and Rossy, 2008; Zaidel-Bar et al., 2003). The intermediate filament vimentin, which is not typically expressed in healthy epithelial cells, is also a known regulator of actin dynamics (Helfand et al., 2011). Moreover, MV surfaces are coated with ECM molecules, including fibronectin (Antonyak et al., 2011) which is known to play a major role in tissue morphogenesis of epithelial cells (Sakai et al., 2003). Another major cargo is tissue transglutaminase 2 (Antonyak et al., 2011), which is capable of Ca^{2+} -dependent acyl transferase activity able to mediate crosslinking of ECM molecules such as collagen and fibronectin (Antonyak et al., 2011; Forsprecher et al., 2009; Fortunati et al., 2014). In addition, MVs have also been reported to contain various metabolic proteins, signaling proteins, mRNA and miRNA (Grange et al., 2011). Therefore, a number of type of MVs

cargo could be responsible for the morphological changes as well as the increased cell contractility and focal adhesion signaling we describe here. Much work remains to be done to fully understand MV-mediated cell-cell communication.

Interestingly, increased cell contractility is often associated with enhanced metastatic potential (Kraning-Rush et al., 2012; Paszek et al., 2005). It is still unclear whether the MV-mediated contractility changes that we describe in the present study are permanent or if they ultimately contribute to ECM remodeling in vivo. Nevertheless, a body of evidence suggests that long term changes in cell contractility can alter cell phenotype (Engler et al., 2006; Provenzano et al., 2009; Provenzano et al., 2008). For instance, stem cell differentiation can be regulated by contractility (Engler et al., 2006). In addition, epithelial cell morphogenesis is also dependent on cell contractility (Provenzano et al., 2009). Finally, we have recently shown that cell contractility can alter protein splicing (Bordeleau et al., 2015), which could in turn induce important phenotypical changes (Ghigna et al., 2008). Overall, the current findings further highlight the important roles played by tumor-derived MVs in priming the early tumor microenvironment.

Conflict of Interest Statement

The authors declare there are no conflicts of interest.

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Figure legends

Figure 1. Characterization of MV penetration into 3D collagen gels. (A) Dynamic light scattering plot of purified MVs collected from MDA-MB-231 cells. (B) Orthogonal projections of the z-x plane reconstructed from confocal fluorescent sections showing a representative time lapse of MV diffusion inside a collagen gel. (C) Quantification of the MV fluorescence signal as a function of depth and time.

Figure 2. MVs induce morphological changes in epithelial cells cultured in 3D (A) Phase contrast images at 0 h and 24 h of MCF10a cells supplemented with MVs (+MVs) or culture media alone (Ctrl). (B) Corresponding quantification of aspect ratio and (C) circularity shape parameters measured from at least 60 cells from 3 independent experiments. Mean $\pm$ SE (D) Cell shape population distributions plotted as a function of the aspect ratio to circularity shape parameters. * $p < 0.05$.

Figure 3. Cell mediated ECM reorganization is increased by MV treatment. (A) Confocal reflectance image of acellular collagen gels as well as cell clusters after 72 h of culture with (+MVs) or without (Ctrl) MV addition. The pseudo color represents the collagen fiber angular orientation relative to a radial coordinates system centered to the cell cluster centroid (or image center for acellular collagen gel). (B) Average local coherency of collagen fiber alignment measured in acellular gels (aCell), 3d cell culture with media alone (Ctrl) or 3D culture supplemented with MVs (+MVs). Mean $\pm$ SE (C) Corresponding weighted histogram of the collagen fiber radial orientation tangent to a matching ellipse fit of the cell clusters. (D) Quantification of the average collagen intensity surrounding the cell clusters as a function of the radial distance from the cellular membrane. Both the control and MVs treatment were fit to an exponential decay (solid

line). (E) Matching decay parameter τ obtained from the exponential fit. Boxplots show 1st to 4th quartiles. ** $p < 0.01$, *** $p < 0.001$.

Figure 4. MVs increase non-malignant epithelial cell traction force generation. (A) Representative traction force maps of MCF10a cells treated with media alone (Ctrl) or with MVs (+MVs). (B) Quantification of the total traction force exerted by cells on collagen-coated polyacrylamide substrates with a Young's Modulus (E) = 5 kPa. Mean $\pm$ SE. (C) Corresponding scatter plot of the total traction force as a function of the cell area. Both control and MVs treatment were fit with a linear regression using a logarithmic transformation for cell area (solid line) with significant effect of the MVs treatment on the slope of the linear regression ** $p < 0.01$. (D) Ratio between the traction along the cell short (T_S) and long axis (T_L). Boxplots show 1st to 4th quartiles and data scatter. * $p < 0.05$.

Figure 5. MVs increase FAs signaling and cell contractility. (A) Representative images of phalloidin stained actin and phosphorylated MLC (p-MLC) or (B) of vinculin labelled FAs and phosphorylated FAK (p-FAK) in MCF10a cells supplemented with (+MVs) or without (Ctrl) MVs. Corresponding quantification of the average (C) FA size, (D) p-FAK fluorescence intensity and (E) p-MLC fluorescence intensity. (F) Representative immunoblots of p-FAK and p-MLC images. GAPDH was used as a loading control. (H) Corresponding densitometric quantification showing both p-FAK and p-MLC normalized against the total GAPDH content. Mean $\pm$ SE. * $p < 0.05$, *** $p < 0.001$.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5