Sphingosine-1-phosphate reduces adhesion of malignant mammary tumor cells MDA-MB-231 to microvessel walls by protecting endothelial surface glycocalyx

L. Zhang, M. Zeng, B. M. Fu*

Department of Biomedical Engineering, The City College of the City University of New York, 160 Convent Ave, New York, NY 10031

Correspondence to: fu@ccny.cuny.edu

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Abstract: Sphingosine-1-phosphate (S1P) is a sphingolipid in plasma that plays a critical role in cardiovascular and immune systems. Endothelial surface glycocalyx (ESG) decorating the inner wall of blood vessels is a regulator of multiple vascular functions. To test the hypothesis that S1P can reduce tumor cell adhesion to microvessel walls by protecting the ESG, we quantified the ESG and MDA-MB-231 tumor cell adhesion in the presence and absence of 1μM S1P, and in the presence of the matrix metalloproteinase (MMP) inhibitor in post-capillary venules of rat mesentery. We also measured the microvessel permeability to albumin as an indicator for the microvessel wall integrity. In the absence of S1P, ESG was ~10% of that in the presence of S1P, whereas adherent tumor cells and the permeability to albumin were ~3.5-fold (after 30 min adhesion) and ~7.7-fold that in the presence of S1P, respectively. In the presence of the MMP inhibitor, the results are similar to those in the presence of S1P. Our results conform to the hypothesis that protecting ESG by S1P inhibits MDA-MB-231 tumor cell adhesion to the microvessel wall.

Key words: Post-capillary venule in rat mesentery; Microvessel permeability to albumin; In situ immunostaining; Heparan sulfate; Matrix metalloproteinase.

Introduction

Tumor metastasis is the leading cause of cancer-related death among cancer patients (1, 2). To search for effective anti-metastatic therapies, many in vivo, ex vivo and in vitro studies have been conducted to understand underlying mechanism by which tumor cells interact with endothelial cells lining the microvessel wall for the adhesion and extravasation. The luminal surfaces of endothelial cells (ECs) that line our vasculature are covered by an endothelial surface glycocalyx layer (ESG), composed of proteoglycans, glycosaminoglycans (GAGs) and glycoproteins (3-6). The GAGs in the ESG are heparan sulfate (HS), hyaluronic acid (HA), chondroitin sulfate (CS) and siaic acid (SA), of which, the most abundant one is HS, accounting for 50–90% of the GAGs (4). This layer serves many vascular functions, including being a mechano-sensor and transducer (6, 7), maintaining vessel wall permeability (8-11) and modulating circulating blood cell-vessel wall interaction (3, 12-15). Recent study by Cai et al (2012) showed that tumor cell adhesion to the microvessel wall degrades ESG and increases microvessel permeability to a large molecule, albumin (16).

Sphingosine-1-phosphate (S1P), is a sphingolipid in plasma that plays a critical role in the cardiovascular and immune systems. Red blood cells (RBCs) are a major source of S1P in plasma, which acts continuously to maintain normal vascular permeability under physiological conditions (17-20). Serum albumin and high-density lipoprotein (HDL) carry ~90% of the S1P and both elicit the release of S1P from RBCs (20-22). Recent studies investigated S1P effects on hydraulic conductivity (Lp) and solute permeability (P) of intact post-capillary venules in rat mesentery (20, 23-28). These results showed that via receptor S1P1, S1P can attenuate acute microvascular permeability increases by inflammatory agents such as platelet-activating factor (PAF) (23-25) and bradykinin (24, 26). In addition to attenuating increased microvessel permeability by inflammatory mediators, 1μM S1P decreased basal Lp by 63% for a group of vessels with slightly higher Lp (23) and by 30% for those with normal Lp (24). 1μM S1P also maintained normal albumin permeability in the absence of BSA in the perfusate (27).

To investigate the underlying structural mechanisms by which S1P maintains microvessel permeability, in an in vitro study using HMVEC (human dermal microvascular endothelial cells) monolayers, as well as in intact microvessels of rat mesentery, Adamson et al.(2010) found that S1P pretreatment inhibited rearrangements of VE-cadherin and occludin induced by PAF or bradykinin and preserved peripheral cortactin (24). In addition to endothelial junction proteins, endothelial surface glycocalyx (ESG) forms a barrier between blood circulation and the surrounding tissues and plays an important role in controlling microvessel permeability, especially microvessel permeability to large molecules. A recent study by Zeng et al (2014) using an in vitro cultured cell monolayer (rat fat-pad endothelial cells) has shown that S1P plays a critical role in protecting the ESG via the S1P1 receptor and inhibits the matrix metalloproteinase (MMP) activity-dependent shedding of heparan sulfate (HS), chondroitin sulfate (CS) and the ectodomain of syndecan-1 (29). Figure 1 demonstrates this molecular mechanism. More recent study in Zhang et al. (28)
confirmed the same role of S1P in protecting the ESG in vivo.

Increase in microvessel permeability is a critical step in many pathological processes including tumor cell adhesion to the microvessel wall (16, 30). The microvessel permeability or microvessel integrity is determined by the structural components of endothelial cells forming the microvessel wall (31). The structural components are ESG and endothelial junctions. Fu et al (2015) demonstrated that cAMP can enhance the endothelial junction integrity, decrease microvessel permeability and reduce tumor cell adhesion to the microvessel wall (30). To search for another approach to prevent tumor cell adhesion to microvessel walls, the aim of the present study was to test the hypothesis that S1P inhibits tumor cell adhesion to microvessel by preserving the ESG in intact microvessels. To test this hypothesis, the ESG in the post-capillary venules of rat mesentery was first quantified by immunostaining heparan sulfate (HS), the most abundant glycosaminoglycans (GAGs) which forms the base of the observation platform as previously described (33). The gut was gently pinned out against a silicon elastomer barrier to maintain the spread of the mesentery. The upper surface of the mesentery was continuously superfused by a dripper with mammalian Ringer solution at 35–37°C, which was regulated by a controlled water bath and monitored regularly by a thermometer probe. The microvessels chosen for the study were post-capillary venules, with diameters of 35–50 μm. All vessels had brisk blood flow immediately before cannulation and had no marginating white cells. At the end of experiments, the animals were euthanized with excess anesthetic. The thorax was opened to ensure death.

**Materials and Methods**

**Animal preparation**

All experiments were performed on female Sprague-Dawley rats (250–300g), supplied by Hilltop Laboratory Animals (Scottsdale, PA). All procedures were approved by the Animal Care and Use Committees at the City College of the City University of New York. The methods used to prepare rat mesenteries, perfuse solutions, and micropipettes for microperfusion experiments have been described in detail in (16, 28, 32, 33).

Rats were first anesthetized with pentobarbital sodium given subcutaneously. The initial dosage was 65 mg/kg, and an additional 3 mg/dose was given as needed. After a rat was anesthetized, a midline surgical incision (2–3 cm) was made in the abdominal wall. The rat was then transferred to a tray and kept warm on a heating pad. The mesentery was gently taken out from the abdominal cavity and spread on a glass coverslip, which formed the base of the observation platform.

**Solutions and reagents**

Mammalian Ringer solution was used for all dissections, perfusion, and superfusion. The solution compositions are (in mM) 132 NaCl, 4.6 KCl, 1.2 MgSO$_4$, 2.0 CaCl$_2$, 5.0 NaHCO$_3$, 5.5 glucose, and 20 HEPES. Its pH was balanced to 7.4 by adjusting the ratio of HEPES acid to base. In addition, the perfusate into the microvessel lumen contained fatty acid-free bovine serum albumin (BSA, A0281, Sigma, 66kDa, ~3.6 nm Stokes radius) at 10 mg/ml (1% BSA-Ringer solution). This fatty acid-free BSA has about 50 nM S1P (29), which is negligible compared to 1 μM S1P. FITC-conjugated mouse anti-human HS (anti-HS, 10e epitope) was purchased from United States Biological (Swampscott, MA). It was diluted to 1:50 (20 μg/ml) in 1% BSA-Ringer solution for labeling HS in the microvascular ESG (34). A blocking solution was made of 5% goat serum (Invitrogen, Eugene, OR) in 1% BSA-Ringer. All of the solutions described above were made at the time when the experiment was performed and were discarded at the end of the day. Sphingosine-1-phosphate (S1P) (73914, Sigma) was first dissolved in 95% methanol and prepared as a stock solution of 125 μM in 0.4% fatty acid-free BSA (29). A broad-spectrum hydroxamic acid inhibitor of MMPs, GM6001 and its negative control GM6001 NC were purchased from EMD Millipore.

To determine microvessel permeability to albumin, fatty acid-free bovine serum albumin was labeled with AlexaFluor 555 (Invitrogen) according to the manufacturer’s instructions. The details for generating AlexaFluor 555-BSA were described in (27, 28).

**Cell culture**

Human breast carcinoma cells (MDA-MB-231) from ATCC (Manassas, VA) were cultured in Dulbecco’s Modified Eagle’s Medium/Nutrient Mixture F-12 Ham (DMEM/F-12), 2 mM L-glutamine, and 100 U/
Intravital microscopy

A Nikon Eclipse TE2000-E inverted fluorescent microscope was used to observe the mesentery. A 10× lens (NA 0.3, Nikon) gave a field of view of approximately 2 mm in diameter. The tissue was observed with either transmitted white light from a light pipe suspended above the preparation or with fluorescent light from an illumination system (the monochromator with a xenon lamp FSM150Xe, Bentham Instrument Ltd., UK). The monochromator can generate the light of wavelength from 200 to 700 nm. The observation of fluorescently labeled glycocalyx and measurements of P to albumin were done by a high-performance digital 12-bit CCD camera (SensiCam QE, Cooke Corp., Romulus, MI, USA) with a Super Fluor 20x objective lens (NA=0.75, Nikon) and recorded by InCyt Im™ imaging and analyzing system (Intracellular Imaging Inc., Cincinnati, OH, USA).

Immuno-labeling and quantification of microvessel endothelial surface glycocalyx (ESG)

To compare the ESG of the microvessel wall in the presence or absence of S1P, FITC-conjugated heparan sulphate (HS) antibody was used to label HS, one of the most abundant glycosaminoglycans (GAGs) forming the ESG (6, 36). The detailed method was described in (26, 34). Briefly, a post-capillary venule was first cannulated with a single lumen micropipette with or without 1 μM S1P in the perfusate and perfused for 20 min. The upper surface of the mesentery was continuously superfused by a dropper with mammalian Ringer solution at 37°C, which was regulated by a controlled water bath and monitored by a thermometer probe. Then the cannulation was changed to a glass theta micropipette filled with 1% BSA-Ringer solution containing ~4 million/ml cells. The perfusate was perfused with a driving pressure of ~10cmH2O to maintain a flow velocity of ~1 mm/s, which is a typical blood flow velocity in the post-capillary venules of rat mesentery. The perfusion flow velocity was determined by the driving pressure and was calculated from the movement of a marker tumor cell (33). The rate of cells out of the micropipette was also measured, which was ~1 cell/s at the perfusion velocity of~1 mm/s. The adhesion process was recorded at ~2 frames/s in a ~1-min interval for ~30 min in each experiment. A single experiment was carried out in one microvessel per animal. Since 20x/numerical aperture (NA) 0.75 objective lens was used to observe the cell adhesion, which has a depth of light collection ~100 μm (37), the cells adhering at the top and bottom of the vessel can also be observed when focusing at the midplane of the vessel. To calculate the distance of the two dimensional (2D) images, the midplane area was used as the projection of the surface area of the vessel where the cells adhere.

Measurement of microvessel solute permeability P

To further investigate if S1P maintains normal microvessel permeability, we measured apparent microvessel permeability (P) to a large solute, albumin (AlexFluor 555-BSA, ~3.6 nm Stokes radius) in the presence of absence of 1 μM S1P, and in the presence of a generic MMP inhibitor, GM6001 (10μM), or its negative control GM6001NC (10μM). Before P measurement, the microvessels were pretreated with these solutions for 20 min. These concentrations were the optimized concentrations used in (20, 23, 25, 29) for the S1P and inhibitor effects on the endothelial permeability in vivo and in vitro. The detailed method for P measurement has been previously described in (32, 33, 38). Briefly, a post-capillary venule was cannulated with a theta pipette. One lumen was filled with 1% BSA-Ringer (washout) and another lumen with the same solution additionally containing fluorescently labeled solutes (dye). When the dye solution was perfused into the vessel lumen, the vessel was exposed to 555nm wavelength light, the images were recorded simultaneously by a high-performance digital 12-bit charge-coupled device (CCD) camera (SensiCam QE, Cooke, Romulus, MI) with a Super Fluor x20 objective lens (NA =
0.75, Nikon). Then the P was determined offline. The total fluorescence intensity (I) in the lumen of a straight vessel and surrounding tissue was determined by image analysis software (Intracellular Imaging, Cincinnati, OH). The measuring window was 200-500 μm long and 100-200 μm wide and was set at least 100 μm from the cannulation site and from the base of the bifurcation to avoid solute contamination from the cannulation site and from the side arms. P was calculated by \( P = \left( \frac{1}{\Delta I_n} \right) \left( \frac{dI}{dt} \right) \left( \frac{r}{2} \right) \), where \( \Delta I_n \) was the step increase in fluorescence intensity in the measuring window when the perfused dye just filled up the vessel lumen, \( \left( \frac{dI}{dt} \right) \) was the initial rate of increase in fluorescence intensity after the dye filled the lumen and began to accumulate in the tissue, and \( r \) was the vessel radius. The assumption for using the above equation for determining the \( P \) was that the fluorescence intensity is linearly related to the fluorescence concentration. AlexFluor 555-BSA was at the concentration of 0.75 mg/ml, which was in the linear range under the imaging settings according to in vitro calibrations (28, 32, 38).

**Determination of diffusive solute permeability \( P_d \) from measured apparent permeability \( P \)**

The albumin \( P \) measured in our experiments (apparent \( P \)) tends to overestimate the true diffusive \( P (P_e) \) of albumin because solute flux can be coupled to water flow (solute drag). Using the hydraulic conductivity \( L_p \) and reflection coefficient \( \sigma \) to albumin, which were measured under control and tumor cell adhesions (16), we calculated the \( P_e \) to albumin by employing the following formula in (32, 39, 40).

\[
P = P_d \frac{P_e}{\exp(P_e) - 1} + L_p (1 - \sigma) \Delta p_{eff} \tag{1}
\]

\[
P_e = \frac{L_p (1 - \sigma) \Delta p_{eff}}{P_d} \tag{2}
\]

\[
\Delta p_{eff} = \Delta p - \sigma \Delta \pi_{albumin} \min - \sigma \Delta \pi_{AlexFlur-albumin} \min - \sigma \Delta \pi_{AlexFlur-albumin} \min \tag{3}
\]

Here \( \Delta p_{eff} \) is the effective filtration pressure across the microvessel wall; \( \Delta p \) and \( \Delta \pi \) are the hydrostatic and osmotic pressure drops across the microvessel wall, respectively, and \( Pe \) is the Peclet number. We assumed \( \sigma_{albumin} = \sigma_{AlexFlur-albumin} = \sigma \) in our study.

**Data analysis**

Data are presented as means ± SE, unless indicated otherwise. Statistical analysis was performed by a T-test or two-way ANOVA using Sigma Plot 11.2 from Systat Software Inc. (San Jose, CA). A level of \( p < 0.05 \) was considered significant difference in all experiments.

**Results**

**S1P protects ESG of the microvessel**

To show that S1P can protect the ESG in intact microvessels as in cultured endothelial cells (29), the post-capillary venule was perfused in the presence or absence of 1 μM S1P in 1% BSA-Ringer for 20 min at 37˚C degree. Then the microvessel ESG was quantified by immunolabeling HS, one of the most abundant GAGs in the ESG. Figure 2 shows the fluorescence images of the FITC-anti-HS labeled ESG in a post-capillary venule in the presence and absence of S1P and in the presence of a broad-spectrum MMP inhibitor. Figure 2A shows the mid-plane views of the FITC-anti-HS labeled ESG in the microvessels taken by our highly light-sensitive CCD camera. Since the 20x/NA0.75 objective lens was used to observe the ESG, which has a depth of light collection ~100 μm (37), the intensity of the FITC-anti-HS labeled ESG at the top and the bottom of the vessel can both be detected when we focus at the mid-plane of the vessel. The left image in Fig. 2A shows the FITC-anti-HS labeled ESG in the microvessel in the presence of S1P; the middle image is for that with the MMP inhibitor and the right image is for that in the absence of S1P. Figure 2B compares the intensity of the FITC-anti-HS labeled ESG under these three conditions. In the absence of S1P, the intensity of the FITC-anti-HS labeled ESG was only ~10% of that in the presence of S1P. In the absence of S1P but in the presence of the MMP inhibitor, the intensity of the FITC-anti-HS labeled ESG was 0.88 ± 0.11 of that in the presence of S1P (\( p = 0.56 \)). S1P does protect the ESG in intact microvessels by inhibiting MMP activity.

**Effect of S1P on MDA-MB-231 cell adhesion to post-capillary venules**

To investigate whether S1P inhibits tumor cell adhesion to the microvessel wall via protecting the ESG, the MDA-MB-231 adhesion on post-capillary venules was reduced with S1P (left), with a generic MMP inhibitor (middle) and in the absence of S1P (right). (A) Comparison of the intensity of the fluorescently labeled heparan sulfate in 4 vessels with S1P, in 3 vessels with the generic MMP inhibitor and that in 3 vessels without S1P. Values are mean±SE. * \( p < 0.001 \).
S1P reduces tumor cell adhesion.

Figure 3A shows the representative fluorescence images of the adherent cell tracker red labeled MDA-MB-231 breast cancer cells in post-capillary venules after 30 min perfusion under various conditions. The top image is for that in the presence of S1P, the middle one for that in the presence of the generic MMP inhibitor and the bottom one for that in the absence of S1P. Figure 3B compares the temporal tumor cell adhesion profiles under these 3 conditions. Compared to that in the absence of S1P, in the presence of S1P or the generic MMP inhibitor the adherent tumor cells decreased as early as 5 min; at 30 min, the adherent tumor cells were ~33% and ~39% of that in the absence of S1P, respectively. These results conform to our hypothesis that S1P can inhibit TC adhesion to the microvessel wall by protecting the ESG via inhibition of MMP activity.

S1P maintains normal microvessel permeability

To further test whether S1P maintains normal microvessel permeability through protecting the ESG, the microvessel permeability $P$ to albumin was measured in the presence and absence of S1P, and in the presence of the MMP inhibitor. The results were summarized in Table 1, which shows that after 20 min pretreatment with 1 μM S1P, $P$ to albumin was $8.09 \pm 2.2 \times 10^{-7}$ cm/s ($n=7$), while in the absence of S1P, $P$ increased by ~8.5-fold, to $68.8 \pm 7.6 \times 10^{-7}$ cm/s ($n=7$) ($p < 0.001$). Inhibition of MMPs reduced the $P$ to $12.2 \pm 1.9 \times 10^{-7}$ cm/s ($n=7$), which has no significant difference from that in the presence of S1P ($p = 0.34$). However, the negative control of the MMP inhibitor did not reduce the $P$. Table 1 also shows the effect of solvent drag on permeability to albumin by using $P$ from the measured values and $L_p$, $\sigma$ to albumin from Cai et al, 2012 (16). We used the averaged fluorescence intensity of adherent TCs was obtained from a vessel segment of 250-500 μm in length and vessel diameter in width. Value is mean±SE. * $p < 0.05$ compare tumor cell adhesion at the specific time with that at the initial adhesion ($t = 5$ min); # $p < 0.01$, compare tumor cell adhesion under BSA only and that in the presence of S1P or a MMP inhibitor at the same time.

![Figure 3](image)

![Figure 4](image)

Table 1. Effect of solvent drag on $P_{BSA}$ in the presence of S1P, in the presence of the MMP inhibitor, in the presence of the negative control of the MMP inhibitor, and BSA only in the perfusate.

<table>
<thead>
<tr>
<th></th>
<th>$\Delta p_{m}$ (cm H$_2$O)</th>
<th>$\sigma_{m}$</th>
<th>$L_p$ (cm/s/cm H$_2$O)</th>
<th>$P_{BSA}$ (cm/s)</th>
<th>$P_{m}$</th>
<th>$P_d$ (cm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1P</td>
<td>10</td>
<td>0.940</td>
<td>$1.1 \times 10^{-7}$</td>
<td>$8.09 \times 10^{-7}$</td>
<td>0.09</td>
<td>$7.75 \times 10^{-7}$</td>
</tr>
<tr>
<td>MMP inhibitor</td>
<td>10</td>
<td>0.940</td>
<td>$1.1 \times 10^{-7}$</td>
<td>$12.2 \times 10^{-7}$</td>
<td>0.06</td>
<td>$11.87 \times 10^{-7}$</td>
</tr>
<tr>
<td>BSA only</td>
<td>10</td>
<td>0.423</td>
<td>$2.8 \times 10^{-7}$</td>
<td>$68.8 \times 10^{-7}$</td>
<td>0.27</td>
<td>$60.04 \times 10^{-7}$</td>
</tr>
<tr>
<td>MMP NC</td>
<td>10</td>
<td>0.423</td>
<td>$2.8 \times 10^{-7}$</td>
<td>$64.9 \times 10^{-7}$</td>
<td>0.29</td>
<td>$56.44 \times 10^{-7}$</td>
</tr>
</tbody>
</table>

# The value of $\sigma_{m}$ and $L_p$ are from Cai et al. (2012).
Eqs. (1)-(3) to calculate the $P$ to albumin, which was $7.75 \times 10^{-7}$ cm/s in the presence of S1P, $11.87 \times 10^{-7}$ cm/s in the presence of the MMP inhibitor, $60.04 \times 10^{-7}$ cm/s in the absence of S1P and $56.44 \times 10^{-7}$ cm/s in the presence of the negative control of the MMP inhibitor. Figure 4 plots the relative diffusive permeability $P$ to that in the presence of S1P under these conditions. Our results demonstrate that S1P maintains normal microvessel permeability by inhibiting MMP activity, the consequence of protecting the ESG.

Discussion

Tumor cell adhesion to the microvessel wall is one of the critical steps in tumor metastasis (2). Exploring new approaches to prevent tumor cell adhesion to endothelium lining is important in developing anti-metastatic strategies. Thus the aim of this study was to determine the effect of S1P on microvessel integrity in intact microvessels and its role in tumor cell adhesion.

It is well established that the ESG contains a wide variety of membrane-bound carbohydrate-rich macromolecules including sulfated proteoglycans and glycosaminoglycans (GAGs). Due to its composition and unique location at the interface of circulating blood and the vessel wall, this ESG plays an important role in maintaining vascular permeability, attenuating interactions between circulating blood cells and the endothelial cells forming the vessel wall, as well as sensing the hydrodynamic changes in the blood flow (4, 6, 9, 12-14, 41, 42). As shown in Figure 1, syndecans are one of the major proteoglycans found in ESG, which anchor GAGs to the endothelial cell cytoskeleton, membrane and cytoplasm. GAGs are further categorized as heparan sulfate (HS), chondroitin sulfate (CS), dermatan sulfate, keratin sulfate and hyaluronic acid or hyaluronan (HA) (4, 41, 43). HS is the most abundant one, which is $\approx 50$-$90\%$ of the total GAGs (4, 43). Our newly developed technique of in situ immunolabeling of HS, CS and HA in intact post-capillary venules of rat mesentery (16, 34) revealed that compared to HS, the amount of CS and HA can be neglected in these microvessels (7). By employing this newly developed technique for in situ immunostaining of the ESG in an individual microvessel and the measurement of microvessel solute permeability in the same type of intact microvessels in the presence and absence of S1P, we have confirmed that S1P contributes to the maintenance of normal vascular permeability by protecting the ESG in intact microvessels (28).

The cellular mechanisms by which S1P acts to enhance endothelial barrier functions both in vivo and in vitro were thought to be via S1P1 ligation(20, 23, 25, 29, 44-46). Ligation of S1P1 receptor activates the Rho family small GTPase Rac 1, leading to peripheral localization of cytoskeletal effectors such as cortactin. This localization promotes adherens junction and tight junction formation (24), which promotes cell-cell adhesion and cell-substrate adhesion, resulting in enhancing endothelial barrier function. Via S1P1 receptor, another mechanism of S1P action is to stabilize the endothelial surface glycocalyx (ESG) by reducing matrix metalloproteinase (MMP) activation and attenuating the loss of ESG (29). Our in situ immunolabeling of the ESG directly showed that S1P can preserve the ESG in intact microvessels (Fig. 2), the same as that observed in cultured endothelial cell monolayers (29).

To test the effect of S1P on preventing tumor adhesion to microvessel walls, the MDA-MB-231 breast cell adhesion rate was measured in the absence and presence of S1P, and in the presence of a MMP inhibitor. These experiments have shown that S1P does inhibit tumor cell MDA-MB-231 adhesion by over 60\% in 30 min. Preserving the ESG of the microvessel wall by S1P not only reduces the interaction between cell adhesion molecules (CAMs) at tumor cells MDA-MB-231 and those at endothelial cells lining the microvessel wall, but also prevents tumor cell MDA-MB-231 adhesion to the CAMs in the ECM of endothelium (33, 47), resulting in reduction of tumor cell MDA-MB-231 adhesion to the microvessel walls.

In summary, we have shown in this study that S1P can reduce breast carcinoma MDA-MB-231 cells to microvessel walls by protecting the ESG. Our results thus suggest a new anti-tumor metastatic therapy via ESG protection.

References

9. Curry FR and Adamson RH. Vascular permeability modulation at the cell, microvessel, or whole organ level: towards closing gaps in our knowledge. Cardiovascular research 2010; 87: 218-229.
13. Mulivor AW and Lipowsky HH. Inhibition of glycan shedding and leukocyte-endothelial adhesion in postcapillary venules by sup-


