Endothelial Cell Ca\(^{2+}\) Increases upon Tumor Cell Contact and Modulates Cell–Cell Adhesion

Rapid Publication

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Abstract

The signal transduction mechanisms involved in tumor cell adhesion to endothelial cells are still largely undefined. The effect of metastatic murine melanoma cell and human prostate carcinoma cell contact on cytosolic [Ca\(^{2+}\)] of bovine artery endothelial cells was examined in indo-1–loaded endothelial cell monolayers. A rapid increase in endothelial cell [Ca\(^{2+}\)] occurred on contact with tumor cells, but not on contact with 8-μm inert beads. A similar increase in endothelial cell [Ca\(^{2+}\)] was observed with human neutrophils or monocyte-like lymphoma cells, but not with endothelial cells, red blood cells, and melanoma cell–conditioned medium. The increase in endothelial cell [Ca\(^{2+}\)] was not inhibited by extracellular Ca\(^{2+}\) removal. In contrast, endothelial cell pretreatment with thapsigargin, which releases endoplasmic reticulum Ca\(^{2+}\) into the cytosol and depletes this Ca\(^{2+}\) store site, abolished the cytosolic [Ca\(^{2+}\)] rise upon melanoma cell contact. Endothelial cell pretreatment with the membrane-permeant form of the Ca\(^{2+}\) chelator bis-(O-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid blocked the increase in cytosolic [Ca\(^{2+}\)]. Under static and dynamic flow conditions (0.46 dyn/cm\(^2\)) bis-(O-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid pretreatment of bovine pulmonary artery endothelial cell monolayers inhibited melanoma cell adhesion to the endothelial cells. Thus, tumor cell contact with endothelial cells induces a rapid Ca\(^{2+}\) release from endothelial intracellular stores, which has a functional role in enhancing cell-cell adhesion. (J. Clin. Invest. 1993. 92:3017–3022.) Key words: melanoma • endothelium • adhesion • calcium

Introduction

The interaction of tumor cells with postcapillary venule endothelium and subendothelial basement membrane is crucial in modulating the frequency of organ-specific metastasis (1). Specific adhesion molecules on endothelial cells (EC) and tumor cells (TC) are involved in this process. Melanoma cell adhesion occurs to unactivated EC in vitro and may be modulated by the interaction between glycosphingolipids (2). EC activation with inflammatory cytokines, such as interleukin 1, tumor necrosis factor-α and interleukin 4, increases TC adhesion through the expression of newly synthesized endothelial adhesion molecules (3, 4), such as the endothelial-leukocyte adhesion molecule-1 and the vascular cell adhesion molecule-1. The same adhesion molecules are also involved in leukocyte adhesion to EC (5, 6). It is noteworthy that these adhesion molecules contain an extracellular Ca\(^{2+}\) binding site and require Ca\(^{2+}\) to be functionally active (7, 8).

Alterations of cytosolic [Ca\(^{2+}\)] ([Ca\(^{2+}\)]) and extracellular [Ca\(^{2+}\)] ([Ca\(^{2+}\)]\(_{EC}\)) may influence endothelial cell–leukocyte interaction. Exposure of EC to the calcium ionophore A23187 stimulates secretion of von Willebrand factor and translocation of a selectin adhesion molecule, the intracellular granule membrane protein (GMP-140), to the cell surface (9). GMP-140 is involved in neutrophil early adhesion to EC and can also be recognized by the tumor-associated antigens sialosyl Le\(^a\) and sialosyl Le\(^a\) (10). Furthermore, the calcium ionophore A23187 acts synergistically with suboptimal concentrations of phorbol ester to augment the expression on EC of the intercellular adhesion molecule-1 (ICAM-1), involved in strengthening neutrophil adhesion (11). Interestingly, in a recent study on heterotypic cell–cell adhesion, neutrophils were found to respond with a transient rise in Ca\(^{2+}\) upon binding to interleukin-1-activated EC (12). However, the functional role of Ca\(^{2+}\) in the signal transduction mechanisms that modulate cell–cell and, more specifically, TC–EC interactions is poorly understood. In the present study, we examined the effect of tumor cell–EC contact on Ca\(^{2+}\) homeostasis of the endothelium and the role of endothelial Ca\(^{2+}\) in melanoma cell–EC adhesion.

Methods

Cell cultures. Murine melanoma cells B16/G3.26 (a generous gift from Dr. Christopher Stackpole), which are highly metastatic to the lung (13), metastatic human prostate carcinoma cells (TSU-prl and LNCap) (American Type Culture Collection, Rockville, MD), bovine pulmonary artery endothelial cells (BPAEC) (American Type Culture Collection), and bovine aorta endothelial cells (BAEC) (National Institute on Aging Cell Repository) were cultured in Dulbecco's modified Eagle's medium (National Institutes of Health Medium Center, Be-

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1. Abbreviations used in this paper: BAEC, bovine aorta endothelial cells; BAPTA-AM, bis-(O-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid; BPAEC, bovine pulmonary artery endothelial cells; Ca\(_{i}\), cytosolic [Ca\(^{2+}\)]; Ca\(_{EC}\), extracellular [Ca\(^{2+}\)]; EC, endothelial cells; ER, endoplasmic reticulum; GMP-140, intercellular granule membrane protein; ICAM, intercellular adhesion molecule; InsP\(_3\), inositol-1,4,5-triphosphate PAF, platelet-activating factor; RBC, red blood cells; TC, tumor cells.
bicarbonate buffer of cells
For some mM): washed collected were corresponding 

bicarbonate-buffered 1 38. Indo-1 fluorescence was measured using a xenon strobe lamp (model 236 stroboscope and model 35S lamp; Chadwick-Helmuth Electronics, Inc., El Monte, CA). Band-pass interference filters (Andover, Lawrence, MA) were used to select wavelength bands of emitted fluorescence at 391–434 nm (“410-nm channel”) and 457–507 nm (“490-nm channel”), corresponding to the Ca\(^{2+}\)-bound and Ca\(^{2+}\)-free forms of the indicator, respectively. The 410:490 nm ratio was used as an index of Ca\(_2\). For these studies, we used a >63x objective and emitted indo-1 fluorescence was collected from an optical field of view with ~10–15 confluent endothelial cells. Autofluorescence of a comparable field of unloaded cells was <5% of indo-1-loaded endothelial cells. EC were bathed in a bicarbonate buffer of the following composition (in mM): 116.4 NaCl, 5.4 KCl, 1.6 MgSO\(_4\), 26.2 NaHCO\(_3\), 1.0 NaHPO\(_4\), 5.6 d-glucose, and 1.5 CaCl\(_2\), continuously gassed with 5% CO\(_2\) (pH = 7.38±0.01; 23°C). For some experiments, we used a bicarbonate-free solution containing (in mM): 137.0 NaCl, 4.9 KCl, 1.2 MgSO\(_4\), 1.2 NaHPO\(_4\), 20 Hepes, 15 d-glucose, and 1.5 CaCl\(_2\) (pH 7.38±0.01; 23°C). No difference in EC Ca\(_2\) response was detected between bicarbonate and bicarbonate-free solutions. The same bicarbonate-free solution without added Ca\(^{2+}\) and with 1 mM EGTA was used to achieve Ca\(_2\)-free conditions, while in other experiments, extracellular Na\(^+\) was completely removed by substituting NaCl with LiCl and NaHPO\(_4\) with KHPO\(_4\). We have previously shown that in indo-1–loaded rat aorta endothelial cells there is no significant mitochondrial compartmentalization of the Ca\(^{2+}\) indicator (15). However, a technically satisfactory intracellular indo-1 calibration using deenergized endothelial cells and the calcium ionophores ionomycin and 4-bromo A23187 could not be obtained. Such a calibration is dependent on the ability to randomly expose a given endothelial monolayer to solutions of varying [Ca\(^{2+}\)], making repeated measurements of emitted indo-1 fluorescence ratio at a given [Ca\(^{2+}\)]. We were unable to reproducibly equilibrate monolayers to changes in Ca\(^{2+}\) concentration to permit a full range of measurements. The difficulties stemmed, apparently, from the low efficacy of the calcium ionophores on the plasma membrane of this cell type. In addition, after endothelial cell loading with indo-1, spectrophotometric analysis of digitonin-releasable fluorescence demonstrated the presence of Mn\(^{2+}\)-insensitive fluorescence, which may represent a partially desensitized form of the indicator. Subtraction of this Mn\(^{2+}\)-insensitive component from total indo-1 fluorescence yielded a spectral scan similar to that obtained from indo-1–free acid solution (not shown). The presence of this Mn\(^{2+}\)-insensitive fluorescence would also invalidate calibration of this indicator. The results are, therefore, reported as an emitted indo-1 fluorescence ratio and not as [Ca\(^{2+}\)]. To minimize loss of the indicator from the cells, experiments were performed at 23°C, as a more rapid indo-1 loss from cells has been shown to occur at 37°C (14). The cells were resuspended either in bicarbonate- or Hepes-buffered solution to match the buffer bathing the endothelial cell monolayer and were added to the chamber (50 μl; 1 x 10^6 cells/ml). Contact between the endothelial cell monolayer and the cells added to the chamber was established when both cell types were in the same plane of focus, and after each addition, 5–15 cells appeared in the optical field. Alternatively, biologically inert beads (Medical/Surgical Division 3M, St. Paul, MN) of comparable diameter (8 μm) to cells in suspension, or tumor cell–conditioned medium were added in place of cells. In some experiments, EC intracellular Ca\(^{2+}\) stores were depleted by exposing the cells to 0.5 μM thapsigargin (Calbiochem-Novabiochem Corp., La Jolla, CA). In other studies, EC Ca\(_2\) was buffered by preincubating the cells with the Ca\(^{2+}\) chelator bis-(O-aminophenoxy)ethane-N,N',N'-tetraacetic acid (BAPTA-AM) (Molecular Probes). BPAEC monolayers were incubated with 15 μM BAPTA-AM for 45 min or DMSO alone (0.1%). At the end of the incubation period, 30 min were allowed for deestherification of the Ca\(^{2+}\) chelator before the beginning of the experiment.

**Static adhesion assay.** BPAEC were grown in a 24-well plate to confluence; melanoma cells were harvested with brief trypsinization (0.125% trypsin and 2 mM EGTA), resuspended in adhesion medium (DME with 0.1% bovine serum albumin; Gibco Laboratories) and added (1 x 10^5 cells/well) to the EC monolayer. At different time points the wells were washed three times with adhesion medium and melanoma cells adherent to the endothelium were counted under the microscope (x100; five fields in triplicates).

**Dynamic adhesion assay.** EC were grown to confluence on a 75 x 38-mm glass slide (Corning Inc.). Before the beginning of the adhesion assay, the glass slide was assembled in a parallel plate chamber where hydrodynamic shear stress forces could be calculated as previously described (16) and placed on an inverted microscope (Diaphot; Nikon, Japan). Melanoma cells harvested and resuspended in adhesion medium with 25 mM Hepes (5 x 10^5 cells/ml) were perfused into the chamber for 25 min with continuous laminar flow, with a syringe pump (Harvard Apparatus, Millis, MA) at 0.46 dyn/cm^2. The shear stress was increased for 1 min to achieve shear-stress forces of 12 dyn/cm^2 to remove nonadherent cells. Subsequently, flow was lowered again and TC adherent to the endothelium were counted under the microscope (x40; 15 fields/slide).

**Statistical analysis.** Results are reported as the mean±SD. Differences between means of unpaired comparisons were evaluated by Student’s t test. P < 0.05 was taken to indicate statistical significance.

**Results.**

The effect of the interaction between melanoma cells and the endothelium on BPAEC or BAEC Ca\(_2\) was examined. The addition of inert beads to the EC monolayer did not elicit any response (Fig. 1). In contrast, in the same monolayer immediately upon contact with melanoma cells there was a rapid and transient increase in endothelial Ca\(_2\), and after the initial peak EC Ca\(_2\) remained elevated (Fig. 1). The response appears specific for cell–cell contact and was not caused by mechanical deformation of the EC or to soluble factors, since it was never elicited by inert beads (n = 5) or tumor cell–conditioned medium (n = 4) (data not shown). In additional experiments, we examined whether the increase in EC Ca\(_2\) could be triggered by other cell types or was specific for melanoma cells. We were not able to elicit an increase in EC Ca\(_2\) when red blood cells (RBC) (Fig. 2) or endothelial cells (BAEC) in suspension (not shown) came in contact with the EC monolayer. Fig. 2 shows that the addition of RBC to BPAEC did not affect EC Ca\(_2\), while the successive TSU-pr1 cell addition to the same monolayer triggered an increase in Ca\(_2\) similar to that observed with melanoma cells. Furthermore, the EC Ca\(_2\) rise also occurred upon contact with two human prostate carcinoma cell lines, TSU-pr1 (Fig. 2) and LNCaP (not shown), human neutrophils (n
To investigate the Ca\(^{2+}\) source for the increase in C\(_a\), BPAEC in the chamber were perfused with a bicarbonate-free buffer without added Ca\(^{2+}\) and with 1 mM EGTA. Under these conditions, Ca\(^{2+}\) influx from the extracellular space does not occur. Nevertheless, melanoma cell–EC contact still elicited an increase in C\(_a\) (Fig. 3 A), suggesting that the transient C\(_a\) increase upon melanoma cell–EC contact is not caused by Ca\(^{2+}\) influx from the extracellular space. Furthermore, \(\Delta\) indo-1 fluorescence ratio from control at 1 min after the initial peak was smaller in the experiments in Ca\(^{2+}\)-free buffer than in the studies in 1.5 mM C\(_a\) (0.03±0.01, \(n=4\) vs 0.18±0.09, \(n=5; P<0.01\)). This raises the possibility that Ca\(^{2+}\) influx from the extracellular space may keep C\(_a\) elevated after the initial C\(_a\) transient. In additional experiments, we examined whether Ca\(^{2+}\) release from intracellular Ca\(^{2+}\) pools could be the mechanism for the C\(_a\) transient upon melanoma cell–EC contact. For these experiments, we used thapsigargin, a sesquiterpene lactone that in several cell types (17–19) selectively and irreversibly inhibits the endoplasmic reticulum (ER) Ca\(^{2+}\)-ATPase by blocking Ca\(^{2+}\) uptake into inositol-1,4,5-trisphosphate (InsP\(_3\))-sensitive and InsP\(_3\)-insensitive intracellular Ca\(^{2+}\) pools. The end-result of thapsigargin treatment is intracellular Ca\(^{2+}\) stores depletion via an InsP\(_3\)-independent mechanism. In response to perfusion with 0.5 \(\mu\)M thapsigargin there was a rise in C\(_a\) that slowly recovered toward control. Melanoma cells were added to the EC monolayer after 25 min exposure to thapsigargin. Under these conditions, the endothelial C\(_a\) transient was abolished (Fig. 3 B). This result shows that upon melanoma cell–BPAEC contact, there was a rapid Ca\(^{2+}\) release from the intracellular Ca\(^{2+}\) stores, presumably from the ER. However, it cannot be excluded that sustained elevation in C\(_a\) after exposure to thapsigargin may have inhibited the ER Ca\(^{2+}\)-release channel as observed in myocardial cells (20). Similar to other cell types, endothelial cells appear to have a plasmalemmal Na\(^+\)/Ca\(^{2+}\) exchange (21). Thus, by removing extracellular Na\(^+\), it is possible to enhance C\(_a\) without depleting ER Ca\(^{2+}\). After BPAEC superfusion with a Na\(^+\)-free buffer, C\(_a\) increased. Nevertheless, the C\(_a\) transient elicited by melanoma cell addition was also observed under these conditions (Fig. 3 C).

The functional role of the increase in endothelial C\(_a\) on melanoma–BPAEC interaction was examined in BPAEC treated with BAPTA-AM, an intracellular Ca\(^{2+}\) chelator. After incubation with 15 \(\mu\)M BAPTA-AM for 45 min, BPAEC did not exhibit an increase in C\(_a\) upon contact with melanoma cells (Fig. 4). Thus, in additional experiments, we examined melanoma cell adhesion to BPAEC monolayers under control conditions and to BPAEC monolayers pretreated with BAPTA-AM for 45 min. Both static and dynamic adhesion assays were used to examine the effect of BAPTA-AM pretreatment on melanoma cell adhesion to BPAEC monolayer. Under static conditions, EC pretreatment with BAPTA-AM inhibited TC adhesion only at 3 and 5 min (47.3±15.4% and 49.4±9.7% inhibition respectively), while it was ineffective at 15 min (Fig. 5 A). In contrast, under dynamic conditions, which more closely mimic in vivo adhesion, there was a significant inhibition of adhesion (70.3±18.5% inhibition) even after 25 min at 0.46 dyn/cm\(^2\) (Fig. 5 B). Thus, EC treatment with BAPTA-AM abolished the increase in C\(_a\) upon melanoma cell contact and significantly reduced subsequent melanoma cell adhesion to BPAEC monolayer. Inhibition of TC adhesion to BAPTA-AM–treated EC appeared more effective in a dynamic flow system, possibly because of additional adhesion enhancement occurring after the initial contact under dynamic conditions. In addition, it is noteworthy that in the dynamic adhesion assay we observed a rolling phenomenon similar to that previously described by Lawrence and Springer during neutrophil addition to artificial bilayers containing GMP-140 (22). Melanoma cell rolling was confirmed by observing (×200) rotation of TC; it was characterized by transient melanoma cell attachment to the EC monolayer with a decrease in cell velocity followed by an increase in velocity during which the cells appeared to detach from the EC and then to reattach downstream.

Figure 1. Effect of inert beads and melanoma cells on Ca\(_a\) of the same BPAEC monolayer. Inert beads and melanoma cells (50 \(\mu\)l, \(1 \times 10^6\) cells/ml; arrows) were added to the same EC monolayer during indo-1 fluorescence collection. Similar results with the beads and with the tumor cells were observed in 5 out of 5 and 11 out of 11 experiments, respectively.

Figure 2. Effect of red blood cells and prostate carcinoma cells on Ca\(_a\) of the same BPAEC monolayer. RBC and TSU-prl cells (TC) (50 \(\mu\)l, \(1 \times 10^6\) cells/ml; arrows) were added to the same EC monolayer. Similar results were observed in five out of five experiments.
Discussion

In this study, we have shown that the initial contact of metastatic tumor cells with unactivated endothelial cells induces a rapid and transient EC Ca\(^{2+}\) rise that is caused by Ca\(^{2+}\) release from an intracellular store rather than by Ca\(^{2+}\) influx from the extracellular space. An increase in EC Ca\(^{2+}\) was also observed with leukocytes which, similarly to tumor cells, can bind and cross the endothelium. In contrast, no increase in Ca\(^{2+}\) of EC monolayer was observed upon contact either with red blood cells or with endothelial cells in suspension. Thus, the Ca\(^{2+}\) rise may be triggered by specific adhesion molecules present on tumor cell surface, since endothelial cells and red blood cells were not able to enhance Ca\(^{2+}\). Furthermore, we excluded the possibility that soluble factors may be involved in the phenomenon, since melanoma cell conditioned medium was not able to increase EC Ca\(^{2+}\). It is also noteworthy that \(\Delta\) indo-1 fluorescence ratio from control at 1 min after the initial peak was greater in the experiments implemented in 1.5 mM Ca\(^{2+}\) than in those in Ca\(^{2+}\)-free buffer. This suggests that Ca\(^{2+}\) influx into endothelial cells may also be triggered by contact with the TC and may be responsible for the sustained increase in indo-1 fluorescence that occurs after the initial peak. However, this cannot be conclusively established with the experimental protocol used in this study, because the sustained increase in indo-1 fluorescence may also be caused by TC reaching the endothelium at different times after their addition to the chamber. It is also of interest that the first tumor cell that reaches the endothelium triggers the most rapid and marked increase in Ca\(^{2+}\); subsequent TC–EC interactions, within the ensuing 2–3 min, cause markedly smaller increases in Ca\(^{2+}\) (Fig. 1). This observation suggests that initial TC–EC contact may "prime" the surrounding endothelial cells and modulate their Ca\(^{2+}\) response to the contact with cancer cells that may occur shortly thereafter. This preliminary observation will require additional studies to establish whether the rapid Ca\(^{2+}\) rise upon initial TC–EC contact may be a mechanism for cell-to-cell signaling as described for other nonendothelial cells (23, 24). The signal transduction mechanism for the increase in Ca\(^{2+}\) reported in the present study remains to be elucidated. It is noteworthy that EC respond to interventions that cause phosphatidylinositol turnover and enhance Ins\(^{3,4,5}\)P\(_3\) and Inositol-1,3,4,5-tetrakisphosphate (Ins\(^{4,5}\)P\(_2\)) production with a biphasic Ca\(^{2+}\) response. An initial rapid and transient increase in Ca\(^{2+}\) caused by Ins\(^{3,4,5}\)P\(_3\)-mediated ER Ca\(^{2+}\) release is followed by a lower and more sustained increase in Ca\(^{2+}\) caused by Ca\(^{2+}\) influx from the extracellular space that may result from Ins\(^{4,5}\)P\(_2\) (25). In the absence of biochemical measurements, it cannot be determined whether the observations reported in this study are associated with an enhanced phosphatidylinositol turnover; nevertheless, such possibility may be considered. The results of the present study differ from those of a previous report where flow cytometry analysis (FACS®; Becton Dickinson Immunocytometry Systems, Mountain View, CA) and a suspension of leukocytes and EC were used to examine the effect of the interaction between those two cell types on EC Ca\(^{2+}\), and it was found that no increase in Ca\(^{2+}\) occurred in EC (12). However, different experimental conditions may account for the discrepancy between the results of that study and those reported in this work. We have also shown that the increase in endothelial cell Ca\(^{2+}\) upon contact with melanoma cells has a functional significance because it modulates melanoma cell adhesion.

Current knowledge on TC adhesion to endothelia is deficient as compared to leukocyte adhesion. However, findings on leukocyte adhesion may also be relevant to tumor cells, since, for example, the interaction of both melanoma cells and lymphocytes with EC appears to be mediated by the same adhesion molecule, vascular cellular adhesion molecule-1 (3). Moreover, metastatic cells, like leukocytes, adhere to and cross the
endothelium (1). Thus, it was not surprising that under our experimental conditions, neutrophils and monocytes elicited a Ca<sup>2+</sup> response similar to that of tumor cells. A general model of leukocyte-EC recognition (26) suggests an active process requiring three sequential events: the first step, described as leukocyte “rolling,” is mediated by lectin-carbohydrate interaction (GMP-140/oligosaccharides). This initial adhesion is transient and reversible unless followed by leukocyte activation caused by secretion of platelet-activating factor (PAF) or other cell contact-mediated signals (second step). PAF is not constitutively present in resting EC, it is synthesized rapidly upon agonist exposure and it requires elevation of Ca<sub>2+</sub> for its synthesis (27). PAF associated with EC is involved in the recruitment of leukocyte CD11-CD18 integrins and in enhancing the tightness of GMP-140-mediated adhesion (28). Interaction of the activation-dependent leukocyte integrin with its EC counterreceptor ICAM-1/ICAM-2 (third step) results in strong and sustained attachment. EC Ca<sub>2+</sub> is also required for cell surface expression of both GMP-140 and ICAM-1, and modulates cell-cell adhesion via this mechanism (9, 11). Furthermore, neutrophils respond with a transient Ca<sub>2+</sub> rise upon contact with EC (12) and the increase in neutrophils Ca<sub>2+</sub> enhances the binding affinity of the leukocyte integrin CD18/11a (29, 30). In a recent study, it was shown that in a dynamic flow system comparable to ours, initial adhesion of B16 melanoma cells to unactivated mouse and human endothelial cells is mediated by the interaction between glycosphingolipid GM3( NeuAca2-3Galb1-4Glcbl-Cer), and lactosylceramide (LacCer, Galb1-4Glcbl-Cer), expressed on TC and EC, respectively (2). Thus, the latter and the present study suggest that EC activation is not necessary for specific melanoma cell adhesion. In addition, under static conditions, adhesion to BAPTA-AM-treated endothelial cells was reduced only at 3 and 5 min, while in the dynamic flow system, adhesion was inhibited also at 25 min (Fig. 5). This result may be caused by an effect of the rapid increase in Ca<sub>2+</sub> on mechanisms of cell–cell adhesion that lead to EC activation and expression of adhesion-enhancing molecules such as ICAMs and selectins.

Furthermore, in our dynamic flow system, TC attachment to EC resembled leukocyte adhesion, since TC “rolling” occurred. This “rolling” phenomenon may result from interaction between glycosphingolipids present either on TC or EC, as recently described (2). Under low shear stress melanoma cells were able to “roll,” but not to attach firmly to BAPTA-AM-treated EC monolayers. Thus, by blocking EC Ca<sub>2+</sub> rise, we were able to inhibit further strengthening of adhesion.

In conclusion, unactivated EC Ca<sub>2+</sub> may play a crucial role in tumor cell adhesion by reinforcing the initial weak interaction and mechanisms of “juxtacrine activation” similar to those described in leukocyte–EC adhesion may also mediate TC–EC adhesion.

References


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