Laminin Interactions with Head and Neck Cancer Cells under Low Fluid Shear Conditions Lead to Integrin Activation and Binding*

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Background: Clinically relevant tumor cell/lymph node interactions are likely initiated in the context of lymphodynamic flow.

Results: β1 integrins mediate interaction with laminins under lymphodynamic shear stress, resulting in a discrete calcium signal.

Conclusion: β1 integrins mediate tumor cell/lymph node interactions active under lymphodynamic flow.

Significance: These novel interactions may drive growth and immunomodulation in this niche.

Lymphatic metastasis of cancer cells involves movement from the primary tumor site to the lymph node, where the cells must be able to productively lodge and grow. It is there that tumor cells encounter cellular and non-cellular constituent elements that make up the lymph node parenchyma. Our work shows that head and neck squamous cell carcinoma (HNSCC) cell lines are able to bind to laminin, fibronectin, vitronectin, and hyaluronic acid, which are extracellular matrix elements within the lymph node parenchyma. HNSCC cell lines bound to laminin under lymphodynamic low shear stress (0.07 dynes/cm²), consistent with lymph flow via β1 integrins, including αvβ1, α3β1, and α6β1. Binding occurred in the presence of shear stress and not in the absence of flow. Additionally, tumor cell binding to laminin under flow did result in calcium signaling. Our data indicate a novel role for β1 integrin-mediated binding of HNSCC cells to laminin under conditions of lymphodynamic flow that results in intracellular calcium signaling within the cancer cell.

Head and neck squamous cell carcinoma (HNSCC) is an unusual tumor in that it metastasizes primarily to the lymph node and less often to distant sites, such as the lung. The presence of tumor cells in the lymph node is associated with a 50% decrease in patient survival (1). Lymph node metastasis in HNSCC has been hypothesized to occur via a multistep process that begins with direct entry of tumors cells into lymphatic vessels associated with the primary tumor mass and is followed in stepwise manner by tumor cell resistance to anoikis and finally the establishment of stable interaction with the lymph node parenchyma. The latter step has been previously thought of as a passive process, with tumor cell growth a result of productive microenvironmental survival signals, such as those driven by cytokine/cytokine receptor interactions. An alternative hypothesis based on known mechanisms of lymphocyte homing and cancer cell targeting of distant sites of metastasis via blood can be posed, where tumor cell receptors active under conditions of lymphodynamic flow could specifically drive tumor cell interactions with respective ligands expressed within the lymph node parenchyma. These interactions (with lymph node constituent cells or the extracellular matrix) would result in the transition from a bulk lymph flow phase to a stable anchored interaction of tumor cells with constituent elements present within the lymphatic compartment. Such interactions could provide specific anchoring for tumor cells within this targeted niche and also provide the initial signals important for establishment of metastases.

Extensive literature has shown that cells in flow are capable of stable binding to stationary targets with interactions that resist shear stress. For example, leukocytes in the bloodstream targeting sites of inflammation involve distinct proteins that mediate tethering, rolling, activation, and firm adhesion, which are critical cell physiologic steps required for cell egress from bulk blood flow into target parenchymal compartments (2, 3). Selectins interacting with their respective tissue-specific ligands are the main mediators of tethering under hemodynamic shear stress. L-selectin is expressed on leukocytes, whereas E- and P-selectins are inducible on endothelial cells. Conversely, rolling interactions are supported by selectins, integrins, and their ligands and by CD44 interactions with hyaluronic acid. Finally, firm adhesion, a process mediated largely by integrins, follows as a consequence of integrin receptor activation, with resultant increased binding affinity and stable interactions between leukocytes and the endothelial wall that fully resist wall shear stress. However, although the lymphatic compartment is similarly a compartment exposed to flow with predicted wall shear stresses that likely impact cell/cell and cell/matrix interactions, these interactions (and the molecules that mediate them) remain little studied. Integrins are the principal...
membrane proteins involved in binding and anchoring cells to extracellular matrix elements, such as collagen, laminin, fibronectin, and vitronectin. Given that lymph nodes are rich in laminin, collagens, fibronectin, and vitronectin, integrins emerge as interesting candidates for mediating interactions within the lymph node lymphatic compartment that may be important for lymph node metastasis.

Integrins are heterodimeric proteins composed of one α subunit and one β subunit. Sequencing of the human genome identified 18 α subunits and eight β subunits. Biochemical and functional studies have characterized 24 productive heterodimeric combinations (4). Integrin heterodimers can exist in a collapsed low affinity state or an elongated, activated, or high affinity state (5). The transition to a high affinity state occurs in response to intracellular signaling affecting kindlins, talins, and other proteins that bind to the intracellular C termini of the β integrins (6, 7). In addition to this “inside-out” activation signaling, integrins can also be activated by signaling as a response to ligand binding via focal adhesion kinase, Src, Akt, and other as yet undefined pathways (8, 9).

We have previously reported that HNSCC cells are able to bind to L-selectin under conditions of lymphodynamic shear (10). Here, we show that HNSCC cells can also bind to laminin under the same lymphodynamic shear stresses. This binding activity is mediated primarily by α2β1, α3β1, and α6β1 integrins and is enhanced by shear. Engagement of β1 integrins furthermore initiates a discrete and measureable calcium signal within cancer cells.

**EXPERIMENTAL PROCEDURES**

**Cells and Antibodies**—HNSCC cell lines JHU-SCC-011 and JHU-SCC-019 were a gift from Dr. James Rocco. The cell lines were derived from HNSCC tumors of the upper aerodigestive tract (11, 12). Cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum under standard cell culture conditions. The following antibodies were used in flow cytometry and stress flow experiments: α1 integrin clone FB12, α2 integrin clone P1E6, α3 integrin clones P1B5 and B3A, α6 integrin clones NKI-GohH3 and 4F10, β4 integrin clones ASC-3 and ASC-9, and β1 integrin clone P5D2 (all from EMD Millipore, Billerica, MA) and β1 integrin clone mAb13 (BD Biosciences). Immunoblots were probed with polyclonal sera to α2, α3, and β1 (EMD Millipore, Billerica, MA) and α6 (R&D Biosystems, Minneapolis, MN) integrins. Immunoprecipitation was performed using anti-β1 integrin monoclonal antibody (clone P5D2).

**Preparation of Laminin Surfaces**—Extracellular matrix surfaces were prepared by overnight incubation of extracellular matrix solution (5, 2.5, or 1 μg/ml in PBS) on standard polystyrene Petri dishes. Fibronectin, vitronectin, collagen I, collagen IV, collagen III, hyaluronic acid, heparin sulfate, and placental laminin were obtained from Sigma-Aldrich. Placental laminin is a partially digested and purified preparation from human placenta. Laminins 511, 211, 111, and 411 (BioLamina, Stockholm, Sweden) and laminin 332 (Abcam, Cambridge, MA) are purified human recombinant full-length proteins from HEK293 cells. Laminin 332 is purified from human foreskin keratinocyte cultures.

**Head and Neck Carcinoma Cell Binding to Laminin**

**Binding Assays**—Shear-dependent binding assays were performed as described previously (10). Briefly, HNSCC cells were harvested using PBS plus 5 mM EDTA. Following washing, the cells were resuspended at 1 × 10⁶/ml in Hanks’ balanced salt solution supplemented with 2 mM CaCl₂ and were pulled into the flow chamber (GlycoTech, Gaithersburg, MD) over laminin (Sigma human placental laminin; 5 μg/ml) at flow rates calculated to result in shear stress of 0.07 dynes/cm². After 4.5 min, a 1-min video was recorded and used for subsequent quantification of rolling velocities and binding. In antibody blocking experiments, antibody was added to the cells, along with 0.5% horse serum, 2 h prior to introducing the cells into the flow chamber and kept on ice until the assay was performed. Each condition was assayed in at least three independent experiments.

For stationary binding assays, cells were prepared for flow-based assays. Cells (2 × 10⁶) were spotted onto immobilized laminin and allowed to bind for 2–5 min before washing with Hanks’ balanced salt solution supplemented with 2 mM CaCl₂. Photographs were taken before and after washing and used to quantify the extent of binding.

**Immunoprecipitation and Immunoblotting**—Integrin expression was assessed by standard flow cytometry. Assembled β1 dimers were evaluated by immunoprecipitation. Cell extracts were prepared in 10 mM Tris acetate (pH 8.0), 150 mM NaCl, 0.5 mM CaCl₂, and 0.5% Nonidet P-40 with phosphatase and protease inhibitors. Immunoprecipitation was performed with anti-β1 integrin antibody (clone P5D2), followed by nonreducing SDS-PAGE and immunoblotting with the relevant anti-α integrin antibody.

**Intracellular Calcium Measurements**—To measure intracellular calcium, JHU-SCC-019 cells were grown in suspension with constant rocking prior to harvesting by low speed centrifugation. Cells were then resuspended in serum-free RPMI 1640 medium containing 2 μM Fluo-4/AM (Invitrogen) and 0.02% Pluronic F-127 (Invitrogen) and incubated at 37 °C for 30 min for dye uptake. Cells were removed from dye by centrifugation, resuspended in Hanks’ balanced salt solution supplemented with 2 mM CaCl₂, and incubated at room temperature for 20–30 min prior to use. Thapsigargin (3 μM) was added to cells at least 20 min prior to use when desired. To measure intracellular calcium levels at the time of attachment, cells were introduced into the parallel plate flow chamber for the standard binding assay while photographs were recorded at 1-s intervals. Intracellular calcium concentrations were calculated using the formula [Ca²⁺]i = 345(F – Fₘᵦₓ)/(Fₘᵦₓ – F).

**RESULTS**

**HNSCC Cells Bind to Extracellular Matrix Elements under Lymphodynamic Shear Stress**—We examined the ability of HNSCC cells to bind purified extracellular matrix elements known to be present in the lymph node. Placental laminin, collagen I, collagen III, collagen IV, hyaluronic acid, fibronectin, vitronectin, and heparan sulfate were spotted onto plastic dishes and used as substrates for interaction under flow conditions consistent with lymphodynamic shears. Using a parallel plate flow chamber, three HNSCC cell lines (JHU-SCC-011, JHU-SCC-012, and JHU-SCC-019) were introduced over extra-
cellular matrix elements at 0.07 dynes/cm². All three cell lines bound to L-selectin under shear conditions, as we have reported previously (10). However, differential binding to vitronectin, fibronectin, and placental laminin was observed (Fig. 1). JHU-SCC-011 cells bound to placental laminin significantly more than to the other matrix elements; JHU-SCC-012 cells bound to placental laminin and vitronectin more than to the others; and JHU-SCC-019 cells bound to placental laminin and fibronectin more than to the others. All three cell lines also exhibited rolling interactions on hyaluronic acid in a CD44-dependent manner (Fig. 1) (data not shown).

Because all three cell lines exhibited robust binding only to placental laminin, we examined this activity more closely using the JHU-SCC-019 cell line. We evaluated the ability of our cells to bind purified full-length recombinant laminins 111, 211, 411, 511, and 332, as well as the purified native placental laminin preparation. Although recombinant laminins 111 and 332 did not show any appreciable binding under flow, laminins 211 and 511 bound at a level equal to the placental laminin, and laminin 411 was able to bind at a reduced level (Fig. 1D). Because laminin 511 is the major laminin isoform present in the placental laminin preparation and because it is also present in the lymph node in both basement membranes and stromal fibers (13), we pursued more detailed studies using this laminin preparation.

We investigated the range of force under which cells were able to bind. JHU-SCC-019 cells were introduced in flow over placental laminin adherent to a stable plastic surface using a range of shear stress conditions, representative of physiologic lymphodynamic stress to venous shear stress (Fig. 2A). As shear stress was increased, binding was reduced exponentially, with cells binding at a very low frequency at 1 dyne/cm² (equivalent to venular shear levels). Integrins are the primary receptors for laminin binding and can be experimentally activated to their high affinity conformation by replacing calcium with manganese (14, 15). To determine whether prior activation of the integrins would enable the cells to bind to laminin under higher shear stress, we substituted 1 mM MnCl₂ for the CaCl₂ present in our cell suspension and running solutions. Binding of these cells containing activated integrins displayed the same shear-sensitive characteristics as that of calcium-treated cells at shear stresses >0.05 dynes/cm² (Fig. 2A).

Binding of JHU-SCC-019 cells to placental laminin under shear stress showed elements of rolling and firm adhesion. Using real-time video, we measured the deceleration of the cells by measuring the distance traveled by individual cells in successive intervals before they ceased forward motion. The cells gradually slowed, until they ceased all forward motion for >10 s (Fig. 2C). The transition from rolling to firm adhesion is similar to the characteristics of binding of JHU-SCC-019 cells to L-selectin. Binding to laminin (placental, 511, and 211), once established at 0.07 dynes/cm², resulted in firm adhesion and was resistant to increases in shear stress as measured by detachment assay. Greater than 90% of the cells remained stably

FIGURE 1. HNSCC cells bind to extracellular matrix elements under low shear stress. HNSCC cell lines JHU-SCC-019 (A), JHU-SCC-011 (B), and JHU-SCC-012 (C) were assayed for their ability to bind to matrix elements immobilized on plastic at the low shear stress level of 0.07 dynes/cm². Matrix elements were vitronectin (5 μg/ml), collagen I (50 μg/ml), collagen IV (50 μg/ml), collagen III (50 μg/ml), fibronectin (5 μg/ml), placental laminin (5 μg/ml), hyaluronic acid (250 μg/ml), or heparan sulfate (25 μg/ml). The L-selectin/Fc chimera was used at 1 μg/ml. D, JHU-SCC-019 cells were assayed for their ability to bind to laminins 111, 211, 411, 511, and 332 (5 μg/ml). Error bars represent the S.E. of at least three assays. *, statistically significant (p < 0.05) differences from the FBS control; #, statistically significant differences from vitronectin. **, significantly different from placental control; *, significantly different from both placental control and lam411.
attached even at a shear stress of 10 dynes/cm² (data not shown).

We next measured binding under static non-flowing conditions to assess whether shear is required for binding. Cells were applied in suspension directly onto the placental laminin-coated surface, incubated statically for 2 min, and then washed. Under stationary conditions, calcium-treated cells showed no binding, whereas Mn²⁺/H₁₁₀₀₁-activated cells showed robust binding (Fig. 2 B). Therefore, integrin activation is required for cell binding to laminin in the absence of shear stress, yet under conditions of force consistent with lymphodynamic flow, activation is not previously required. When recombinant laminins 211 and 511 were used in a similar fashion, it became apparent that at higher concentrations of laminin (5 𝜇g/ml), cells could bind under stationary conditions as well as under shear stress, but as the concentration of laminin was decreased, the ability to bind under stationary conditions was lost, whereas the binding under shear stress remained (2.5 𝜇g/ml) (Fig. 3). At the lowest concentration of laminin (1 𝜇g/ml), all binding was lost.

**FIGURE 2.** HNSCC cell/laminin interaction supports tethering, rolling, and firm adhesion under shear stress. A, JHU-SCC-019 cells were introduced into the flow chamber and allowed to bind to placental laminin at various shear stress levels in the presence of either 2 mM CaCl₂ (solid line) or 1 mM MnCl₂ (dashed line). The number of cells that bound and accumulated after 4.5 min was quantified. The inset shows an expansion of the low end of the scale between 0 and 0.1 dynes/cm². B, under static conditions, Mn²⁺ was required for binding to laminin. JHU-SCC-019 cells were applied to a laminin-coated surface in Hanks’ balanced salt solution with either Ca²⁺ or Mn²⁺ present, incubated for 2 min, and then washed. The number of remaining cells was scored as bound cells. Error bars represent the S.E. of at least three assays.

**FIGURE 3.** HNSCC cell binding to laminin 511 under flow or stationary conditions is concentration-dependent. Laminin 511 was bound to the solid surface at 5, 2.5, and 1 𝜇g/ml. For binding under flow, JHU-SCC-019 cells were introduced in flow over laminin at 0.07 dynes/cm². Stationary binding was measured after JHU-SCC-019 cells were applied to a laminin-coated surface, incubated for 2 min, and then washed. The number of remaining cells was scored as bound cells. Error bars represent the S.E. of at least three assays.

We next measured binding under static non-flowing conditions to assess whether shear is required for binding. Cells were applied in suspension directly onto the placental laminin-coated surface, incubated statically for 2 min, and then washed. Under stationary conditions, calcium-treated cells showed no binding, whereas Mn²⁺-activated cells showed robust binding (Fig. 2B). Therefore, integrin activation is required for cell binding to laminin in the absence of shear stress, yet under conditions of force consistent with lymphodynamic flow, activation is not previously required. When recombinant laminins 211 and 511 were used in a similar fashion, it became apparent that at higher concentrations of laminin (5 𝜇g/ml), cells could bind under static conditions as well as under shear stress, but as the concentration of laminin was decreased, the ability to bind under stationary conditions was lost, whereas the binding under shear stress remained (2.5 𝜇g/ml) (Fig. 3). At the lowest concentration of laminin (1 𝜇g/ml), all binding was lost.
Integrin Subunits Responsible for Binding to Laminin under Shear—Integrin heterodimers known to bind to laminin include α1β1, α2β1, α3β1, α6β1, α7β1 (in muscle), and α6β4. We evaluated the expression of each of these monomers in two of our HNSCC cell lines (JHU-SCC-019 and JHU-SCC-011) using standard flow cytometric staining of non-permeabilized cells. Integrins α2, α3, and α6 (and, to a lesser extent, α1, α5, and αv) were readily expressed on the cell surface (Fig, 4A). Both of the laminin-binding β subunits β1 and β4 were detected. Integrin α4 was present in the JHU-SCC-011 cell line but absent in the JHU-SCC-019 cell line. Co-immunoprecipitation with antibody to β1 integrin and subsequent immunoblotting with antibodies to detect the α subunits showed that α2β1, α3β1, and, to a lesser degree, α6β1 heterodimers were present in both cell lines (Fig. 4B).

We next sought to determine which integrin heterodimers mediate binding activity. Inhibitory antibodies for each of the subunits of interest were added to the JHU-SCC-019 cells prior to assay by flow cytometry for binding to placental laminin using the parallel plate chamber assay. The anti-β1 integrin antibody was the only antibody that significantly inhibited binding in isolation. Antibodies against β4, α1, α2, α3, and α6 did not affect binding when used in isolation. Inhibition of β4 integrin did have an additive effect if β1 integrin was concomitantly inhibited (Figs. 5 and 6). Inhibition of α2, α3, and α6 did have a significant effect when all were inhibited simultaneously, indicating the α2β1, α3β1, and α6β1 receptors all have the capacity to mediate binding of tumor cells to placental laminin under shear stress. The results were similar when recombinant laminin 511 was used instead of placental laminin, although the inhibition by anti-β1 integrin antibody was no longer significant.

Using a detachment assay, we examined the effect of blocking α2β1, α3β1, and α6β1 integrin receptors on the strength of binding. Cells were bound to placental laminin at a shear stress of 0.07 dynes/cm², and detachment was measured as the percentage of cells that remained bound following the transition to a 10-fold higher shear stress (0.7 dynes/cm²). Untreated cells showed strong attachment and were not detached under these conditions. Inhibition of the β1 integrin and combinations of α integrins by preincubation of the cells using functional blocking antibodies decreased the number of cells that remained bound when stress levels were increased (Fig. 6). Therefore, β1 integrin is involved in mediating and maintaining cell binding to placental laminin under shear stress. On laminin 511, the effect of anti-β1 integrin antibody blockage was present but less pronounced, and the effect of anti-α3 integrin antibody was more pronounced.

Signaling in Response to Binding—Given the capacity of integrin receptors to initiate signaling in response to binding, we examined whether HNSCC cell binding to placental laminin under the lymphodynamic shear stress conditions results in intracellular signaling. Using the parallel plate binding assay, we measured changes in intracellular calcium levels in response to binding in real time. Cells were loaded with Fluo-4/AM before being introduced into the chamber at 0.07 dynes/cm². As the cells bound to the laminin-coated surface, they were monitored for changes in fluorescence as an indicator of changes in intracellular signaling. There was no change in intracellular calcium levels associated with cell rolling or immediately following the establishment of firm adhesion. However, there was a distinct rise in intracellular calcium levels soon after establishing firm adhesion (5–15 s), indicating signaling via changes in intracellular calcium levels. Two general patterns of calcium flux were observed: a distinct rise followed by a gradual decrease (Fig. 7A) and a clear oscillatory pattern with two to four peaks before a low/basal calcium level was restored (Fig. 7B). Cells pretreated with thapsigargin did not increase their intracellular calcium levels following laminin binding (Fig. 7C), indicating that the intracellular rise in calcium was due to a release of calcium from intracellular stores and not from extracellular sources. Under stasis or β1 integrin inhibition, binding did not occur, which prevented similar calcium signaling analysis.
DISCUSSION

Clinical medicine has recognized the consequences of lymphatic metastasis for decades. Patients with epithelial tumors that have metastasized to regional lymph nodes have a 50%
Head and Neck Carcinoma Cell Binding to Laminin

FIGURE 7. Binding to laminin under shear stress induces calcium release from intracellular stores. JHU-SCC-019 cells were allowed to bind to placental laminin under low shear stress (0.07 dynes/cm²) while intracellular calcium levels were monitored using the Fluo-4/AM indicator dye. Changes in intracellular calcium followed a pattern of a calcium increase followed by a slow decrease (A) or an oscillating and decreasing pattern following binding (time 0) (B). C, cells were treated with thapsigargin to empty intracellular stores of calcium for 30 min prior to binding to laminin. The changes in the intracellular calcium level from the initial value at the time of binding are plotted for untreated (solid line) and thapsigargin-treated (dashed line) cells. The shaded areas represent the region of the S.E.

The linchpin for cellular braking is the “catch bond,” which increases the strength of interaction in response to force, rather than the traditional bond, which breaks apart when force is applied (20, 21). It is the catch bond that allows selectins to catch and bind their ligands as they engage each other. Several reports have recently suggested, however, that integrin/ligand interactions can also be activated by shear stress and operate as catch bonds. Previous studies have been conducted under high shear stresses of 0.5 dynes/cm² and higher, characteristic of the interactions within blood flow rather than the low shear hemodynamic conditions of our studies.

The majority of studies have focused on the binding of leukocytes to endothelial cells to study the mechanisms underlying binding. Under stress conditions, leukocytes are able to bind to laminin via α6β1 (22) and to intercellular adhesion molecule 1 (ICAM-1) via LFA if the cells are activated by cytokines (23) or if the αL integrin involved in leukocyte LFA/ICAM-1 interactions is forced into the open conformation (24). Most recently, mechanical studies have directly addressed the integrin catch bond designation of those interactions enhanced by shear stress. The integrin α5β1/fibronectin interaction has been described as a catch bond based on studies that applied a mechanical force (25) or used atomic force microscopy (26), and the LFA-1/ICAM-1 interaction exhibited catch bond characteristics using a biomembrane force probe (27). Studies using other cell types, including breast epithelial cells and colon carcinoma cells, confirmed a role for β4 and β1 integrins in cell

lower survival rate than patients with similar primary tumors that have not metastasized to lymph nodes, after controlling for distant metastases and treatment. Based on this observation, regional lymph node metastasis forms a part of all clinical staging algorithms used for determining the expected outcome as well as to define therapy. To date, however, we do not understand the mechanisms underlying this important association. We hypothesized that tumor cell/lymph node interactions are important mediators of this physiologic association and not just a marker of poor tumor biology. We have thus established in vitro models of flow-based interactions under conditions of hemodynamic shear stress as a way to study interactions that may be critical for the initial step of tumor cell lodgment within the lymph node microenvironment. Our work presented here identified the α2β1, α3β1, and α6β1 integrin receptors as specific receptors that mediate interactions between tumor cells and laminin under conditions consistent with hemodynamic flow. Under flow conditions, the tumor cells did not bind to laminin 111 (which is predominantly embryonic and not present in the lymph node) or to laminin 332 (which is present predominantly in epidermal junctions and present in the lymph node in only limited locations), but the cells did bind to the much more widely expressed laminins 511 and 211, which are widely distributed in the lymph node. Interestingly, the receptors required flow for full function within this set of conditions, a novel finding for integrin receptors. Their engagement under flow also resulted in discrete calcium signals within tumor cells, suggestive of physiologic consequences of this activity.

Integrins have long been known to be the principal agents for mediating cell adhesion to the extracellular matrix under static as well as hemodynamic flow conditions. The latter activity has been examined primarily in the setting of leukocytes and platelets and their adhesion to endothelial cells, although similar activity has also been identified with cancer cells (16–19). The prevailing model depicts cells in flow establishing interactions with endothelial cells via rolling. Such rolling interactions, generally mediated by selectins, allow for integrin-integrin ligand interactions to engage and result in integrin activation. The current model for integrin activation preceding firm adhesion is that talin and kindlin interaction with the cytoplasmic tail of the integrin dimer causes the transmembrane regions of the two subunits to move farther apart, resulting in the extracellular domain assuming the high affinity state for binding of the ligand (7).
binding to extracellular matrix elements or laminin under shear stress of 1 dyne/cm² (16–18). Subsequent studies (23–25) determined that applying shear force enhanced integrin/ligand interactions.

Our studies are significant, as they demonstrated that HNSCC cell binding to laminin is dependent on β1 integrin and is enhanced under the extremely low stress levels (0.07 dynes/cm²) that we used to model lymphodynamic flow. The binding of β1 integrins to laminin in our studies was dependent on shear stress, as evidenced by a clear threshold for binding and the lack of binding under stationary conditions. Notably, maximal binding occurred at shear stresses ~10-fold lower than those previously described and consistent with shear stresses estimated within the lymph node lymphatic compartment. We interpret our findings to indicate that interactions under force can lead to high affinity binding of integrin receptors in the absence of additional external triggers. However, we cannot distinguish between rapid activation of integrin receptors as result of integrin receptor engagement during the initial rolling, resulting in talin/kindlin activation and then in turn driving high affinity integrin binding, versus direct conformational changes to the integrins as a result of the force applied to the integrin–laminin bond. The rolling interactions of the cells on laminin surfaces lasted >10 s in our experimental system, which may allow for inside-out signaling to talin and/or kindlin via integrins or other binding molecules, a hypothesis that will require future experimentation. The level of force we have applied is very low, but it is still required for the firm adhesion of the HNSCC cells to the laminin. Thus, tumor cells entering lymph nodes via afferent lymphatic flow do not require additional activation by cytokines to establish firm adhesion. The force interaction that results from tumor cells encountering laminin under lymphodynamic shear stress conditions is sufficient to initiate integrin receptor activation with resultant physiologic downstream signaling as evidenced by our findings of intracellular calcium signaling. It is tempting to speculate that the intracellular signaling pathways triggered by calcium fluxes described here in response to tumor cell engagement of laminin under lymphodynamic flow may be critical for downstream tumor cell growth or survival within the lymph node microenvironment. Further work will be required to better understand the relevant signals important for tumor biology in this niche.

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