Shear stress induces internalization of E-cadherin and invasiveness in metastatic oesophageal cancer cells by a Src-dependent pathway

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Metastatic disease is dependent on tumor cell migration through the venous and lymphatic systems and requires dynamic rearrangement of adherens junctions. Endocytosis of cadherins is a key mechanism to dynamically arrange adherens junctions, signaling, and motility in tumor cells; however, the role of shear in regulating this process in metastatic cells is unknown. In this study, the role of shear in regulating cell surface expression of E-cadherin was investigated. We found that exposure to venous shear (shear rate, 200/s) induced internalization of E-cadherin in adherent metastatic oesophageal tumor cells (OC-1 tumor cell line). Internalized E-cadherin was found localized to Rab5-positive endosomes and was not present in lysosomes. As the Src family of tyrosine kinase have been implicated in regulating cadherin expression, we investigated the role of shear in regulating E-cadherin through Src activity. Pretreatment of OC-1 cells with the specific Src kinase inhibitor 4-amino-5-(4-methylphenyl)-7-(t-butyl)pyrazolo[3,4-d]pyrimidine (PP1) prevented shear-induced internalization of E-cadherin. Direct measurement of Src activity (phosphorylation on Y416) showed that Src is activated in sheared OC-1 cells and that the shear-induced increase in phospho-Src is inhibited by the presence of PP1. Moreover, we show that shear stress significantly increased the invasive capacity of OC-1 cells ($P < 0.001$), a process inhibited by the presence of PP1. These results indicate a novel role for shear in regulating the endocytosis of E-cadherin and invasiveness in metastatic cells. (*Cancer Sci* 2009; 100: 1082–1087)

Metastatic cell survival is dependent on adhesion and migration under shear stress encountered in the lymphatic and venous circulation. A key mechanism in this process involves altering cell surface expression of specific integrins and cadherins. Recent work has shown that both integrins and cadherins enter into early endosomes where they can be recycled back to the plasma membrane or degraded in lysosomes.[1,2] More recently, we[3] and others[4] have shown that exposure to physiological shear rates induces activation, translocation, and clustering of integrins in tumor cells; however, the precise role for shear in modulating cadherin expression in metastatic cells is unknown.

Downregulation of cadherin-based adherens junctions is an important step in epithelial tumor invasion and metastasis,[5] and can occur through transcriptional silencing,[6] protein degradation,[7] or through endocytosis.[8] Although transcriptional repression of the E-cadherin gene can result in loss of E-cadherin expression, more recent evidence suggests that post-translational mechanisms such as endocytosis may be involved in migratory behavior.[9] Accumulating evidence suggests that whenever epithelial cells need to become motile, E-cadherin is rapidly removed from the plasma membrane, where it can be recycled to sites of new cell–cell contacts.[10,11] Recently, Palacios *et al*. have shown that endocytosed E-cadherin enters into early Rab5-positive endosomes where it can be recycled rapidly back to the cell surface (time scale of 15–30 min) without undergoing degradation.[11] The Src family of tyrosine kinases have been implicated in regulating this process as they can phosphorylate tyrosine residues in the short intraplasmic tail of E-cadherin, thereby promoting their internalization by endocytosis.[12] In a recent study by Avizienyte *et al*., elevated Src in colon cancer cells was shown to alter adhesive properties that are associated with the disorganization of E-cadherin-dependent cell–cell contacts.[13] As loss of E-cadherin is associated with cellular invasion,[14] we hypothesized that shear may alter E-cadherin expression in metastatic tumor cells (OC-1 cells) in a Src-regulated manner.

In the present study, the role of shear in the trafficking of E-cadherin within OC-1 cells was investigated. We show that shear induces internalization of E-cadherin into Rab5-positive endosomes, in contrast to static OC-1 cells where E-cadherin localizes specifically to cell–cell contacts. Src inhibition, using the specific inhibitor 4-amino-5-(4-methylpheny1)-7-(t-buty1) pyrazolo[3,4-d]pyrimidine (PP1), disrupts the shear-induced internalization of E-cadherin resulting in relocalization of E-cadherin to adherens junctions. In addition, we show that Src is activated in sheared OC-1 cells and that the shear-induced increase in Src activity is inhibited by the presence of PP1. Finally, we show that shear stress significantly increases OC-1 cell invasion, a process inhibited by the presence of PP1. These results indicate a novel role for shear in regulating the endocytosis of E-cadherin through Src activation.

Materials and Methods

Chemicals and materials. Bovine serum albumin (BSA), phosphate buffered saline (PBS), and human plasma fibronectin (Fn) were purchased from Sigma Aldrich (Wicklow, Ireland); Dulbecco's Modified Eagle Media (DMEM) was from Gibco (Dublin, Ireland); Src inhibitor (PP1) was purchased from Calbiochem (Dublin, Ireland); LysoTracker-red was purchased from Molecular Probes (Dublin, Ireland); Matrigel invasion chambers were from Calbiochem.

Antibodies and cells. Monoclonal antibody directed against E-cadherin was purchased from Calbiochem. Monoclonal antibody against the integrin receptor $\alpha_v\beta_3$ (LM609) was obtained from Chemicon International (Cork, Ireland). Polyclonal antibody against Rab5 was purchased from Santa Cruz (Santa Cruz, CA, USA). Phospho-Src 416 polyclonal antibody was from AbCam (Dublin, Ireland). The metastatic esophageal cancer cell line (OC-1) was established from the ascites of a male patient with metastatic squamous cell cancer of the esophagus.[15]

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Parallel plate flow chamber assays. The interaction between adherent OC-1 cells, Fn, and shear was monitored in a parallel plate flow chamber as described previously.[16] In brief, glass slides were coated with Fn (100 μg/mL) for 2 h at room temperature, blocked with 1% BSA in PBS for 1 h, and then washed three times with PBS prior to use. OC-1 cells were resuspended in serum-free DMEM at a concentration of 1×10^6 cells/mL. Tumor cell suspension (400 μL) was added to each Fn-coated slide and incubated for 1 h at room temperature. To evaluate the effect of shear on adherent cells, OC-1 cells were allowed to adhere to Fn for 30 min and then exposed to a continuous shear rate of 200/s for 30 min at 37°C.

Localization of E-cadherin and integrin αVβ3 in static and sheared conditions. Following static (60 min adhesion to Fn, no shear) or sheared conditions (30 min adhesion to Fn followed by 30 min continuous shear rate of 200/s) adherent OC-1 cells were washed three times with PBS, fixed in 3.7% formaldehyde for 15 min at room temperature, and permeabilized with ice-cold acetone for 2 min. OC-1 cells were then incubated with 5 μg/mL E-cadherin antibody or $\alpha_v\beta_3$ antibody for 1 h at room temperature, washed, and incubated with a secondary rhodamine goat anti-mouse IgG antibody for 10 min. The cells were then washed with PBS and mounted using DAKO (Dublin, Ireland) fluorescent mounting media. Cells were visualized using a LSM510 Axioplan 2 upright confocal microscope (Zeiss, Gottingen, Germany). Fluorescence was detected at 488 nm.

To evaluate the effects of Src inhibition on the distribution of E-cadherin in OC-1 cells in the presence and absence of shear, OC-1 cells were pre-treated with PP1 (10 μM) for 48 h, washed, and resuspended in serum-free DMEM at a concentration of 1×10^6 cells/mL. The OC-1 cells were harvested either resting or following shear and stained to evaluate for the effect of Src inhibition on E-cadherin distribution.

Localization of internalized E-cadherin. Co-localization of internalized E-cadherin with the endosomal compartment and lysosomes was investigated. Lysosomes were labeled with LysoTracker (Molecular Probes) according to manufacturer's instructions. OC-1 cells were incubated in serum-free DMEM at 37°C for 1 h followed by incubation with a 1 : 1000 dilution of LysoTracker-red reagent for 3 h at 37°C. The cells were then washed and incubated in complete medium at 37°C for 2 h to allow the LysoTracker reagent to reach the lysosomes. To investigate if E-cadherin was internalized via endocytosis, OC-1 cells were exposed to static and sheared conditions as described above, fixed, and labeled with antibodies to Rab5 (a marker of early endosomes) and E-cadherin.

Cone plate shearing to assess the direct effect of shear on phospho-Src activity. To evaluate the direct effect of shear alone on phospho-Src activity, OC-1 cells were sheared in a cone plate viscometer (Haake RheoStress RS600, MA, USA) as previously described.[17] Previous work in our laboratory has shown that 15 min exposure to shear at 200/s is sufficient for maximal activation of OC-1 cells. Shearing the cells for longer periods in the cone and plate viscometer results in apoptosis as these cells require matrix–cell interactions to survive. This assay is used primarily to assess the direct effect of shear on the OC-1 cells and to assess invasion after maximal stimulation by shear, which in our case is a time point of 15 min. Src activation at 15 min is therefore representative of the cell activation prior to seeding of the OC-1 cells in the invasion chambers. In brief, OC-1 cells suspended in serum-free DMEM (1×10^6 cells/mL) were placed in the viscometer and exposed to a shear rate of 200/s for 15 min or static conditions for 15 min at 37°C. The cells were pelleted at 100 *g* for 5 min, resuspended in ice-cold Tris-buffered saline (TBS), and pelleted at 100 *g* for 5 min. The pellet was then lysed in lysis buffer (25 mM Tris HCl, pH 7.5, 150 mM NaCl, 5 mM $MgCl_2$, 1% NP-40, 1 mM DTT, and 5% glycerol) on ice for 5 min. OC-1 lysates were centrifuged at 16 000 *g* at 4°C for 15 min. The lysates were separated on 7.5% gel, transferred to nitrocellulose, and incubated with phospho-Src 416 antibody. In additional experiments OC-1 cells, pre-treated with or without PP1 (10 μM) for 48 h, were washed in PBS and resuspended in serum-free DMEM. These cells were then sheared for 15 min via cone plate viscometry and lysed prior to measurement of Src activity or assayed for their ability to invade as described below.

Invasion and migration assay. The effects of shear and Src inhibition on OC-1 cell invasion was determined using a modified Boyden chamber assay (BD BioCoat Matrigel Invasion Chamber; BD Biosciences, Dublin, Ireland). OC-1 tumor cell suspensions (5×10^4 cells/mL in the presence and absence of PP1 were sheared at 200/s for 15 min via cone plate viscometry and added to Matrigel invasion chambers as previously described.[16] In control experiments, non-sheared OC-1 cells pre-treated with and without PP1 or control (untreated) were washed in PBS, resuspended in serum-free DMEM, and added to the invasion chambers. Following the 22-h incubation period, OC-1 cells that had invaded the Matrigel layer were stained with crystal violet and quantified by light microscopy. Cells from 10 randomly chosen fields (10×, 40×, and 63× magnification) on the lower side of the filter were counted and all experiments were run in triplicate.

Cell viability assay. Cell viability was measured by trypan blue exclusion. Cells in 200 μL DMEM were mixed with 50 μL trypan blue stain. The number of live and dead cells was counted using a hemocytometer. Results were expressed as the mean of four independent experiments.

Results

Venous shear reduces cell–cell contacts between OC-1 cells and induces internalization of E-cadherin. In the present study, we investigated the role of shear in the membrane trafficking of E-cadherin in adherent OC-1 cells. Previously, we[16] and others[4] have shown that exposure to shear causes activation, translocation, and clustering of integrins in tumor cells; however, it is unknown if shear induces rearrangement of adherens junctions, in particular, the cadherins in adherent metastatic cells.

Because Fn supports maximal adhesion of OC-1 cells under venous fluid flow,[16] we used Fn as the adhesive matrix component in all parallel plate flow experiments. A period of 30 min enabled maximal interactions of OC-1 cells with Fn. This was followed by exposure to a low venous shear rate of 200/s for 30 min to monitor the acute response of shear on cell–cell contacts and cadherin translocation. In the presence of shear there was a significant reduction in cell–cell contacts in contrast to non-sheared cells (Fig. 1A). This was quantified by measuring the number of single cells (i.e. those that did not form cell–cell contacts) adherent to Fn pre-shear and post-shear. As E-cadherin is largely responsible for cell–cell contacts in these cells, we then looked at the localization of E-cadherin pre-shear and post-shear. Under static conditions, cell surface staining of E-cadherin localized at the cell surface specifically to cell–cell adhesion sites between OC-1 cells (Fig. 1B, no shear). This finding is consistent with current models, which show extracellular homophilic binding of cadherins at the junctions of adjacent epithelial cells.[2] Exposure to a shear rate of 200/s for 30 min resulted in translocation of E-cadherin from the cell periphery to a more central cellular location (Fig. 1B, shear). This shear-induced internalization of E-cadherin was reversed when the sheared cells were allowed to recover for a further 30 min in the absence of shear (data not shown), resulting in E-cadherin relocalizing to areas of cell–cell contact. It was also noted that shear-exposed OC-1 cells exhibited a greater degree of circular ruffling at the surface of the cell (indicated by arrowheads Fig. 1C), in contrast to static cells, which exhibited a smoother cell surface.

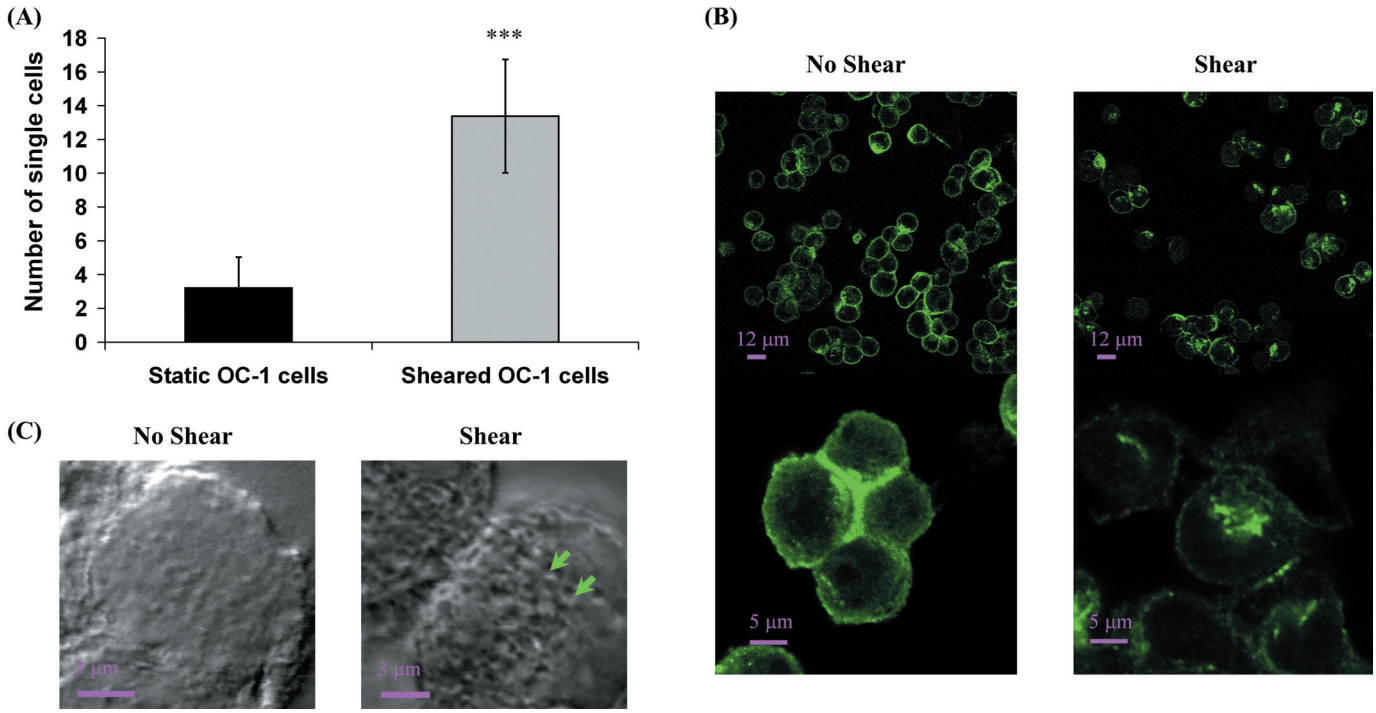

Fig. 1. Venous shear reduces OC-1 cell–cell contacts and induces internalization of E-cadherin. Adherent OC-1 cells were exposed to static (no shear) or sheared conditions (shear rate, 200/s) in a parallel plate flow chamber, fixed for cell–cell contact quantification, and finally labeled for E-cadherin (green). (A) Under static conditions, OC-1 cells exhibited maximal cell–cell contacts; however, in the presence of shear there was a reduction in cell–cell contacts resulting in an increase in single OC-1 cells. The results are quantified from 25 separate micrographs pre-shear and post-shear. To investigate the activity of E-cadherin at OC-1 cell–cell contacts, we compared the localization of E-cadherin in OC-1 cells pre-shear and post-shear. Under static conditions (B, no shear), cell surface staining of E-cadherin was localized to the cell membrane and was maximal at cell–cell adhesion sites between OC-1 cells. Exposure to a shear rate of 200/s for 30 min (B, shear) resulted in movement of E-cadherin from cell junctions into intracellular vesicles. (C) Static cells retained a smoother cell surface (left differential interferance contrast microscopy (DIC) image) in contrast to sheared OC-1 cells (right DIC image), which exhibited increased circular ruffle formation (see arrowheads). The results shown in B and C are representative of three independent experiments. DIC, differential interferance contrast microscopy.

Shear induces the shuttling of E-cadherin from cell–cell junctions to Rab5-positive endosomes. We investigated if shear-internalized E-cadherin was localized to Rab5-positive vesicles or lysosomes. Rab5 is a key regulator of early endosomes, where it is involved in clathrin-coated vesicle formation, fusion between early endosomes, and endosomal motility.[18] E-cadherin was visualized for colocalization with Rab5 by immunofluorescence using secondary antibody conjugated with FITC (E-cadherin) and Rhodamine (Rab5). However, for visual communication, we have colored the Rab5-positive endosomes in blue. As shown in the merged fluorescence of Figure 2(A), E-cadherin localized to a population of Rab5-positive endosomes (colocalization is shown in light blue); however, E-cadherin was also found localized to a subset of unidentified vesicles. The merged differential interferance contrast microscopy (DIC) on the right in Figure 2(A) shows E-cadherin (green) localizing to the Rab5 (blue) circular endosomes.

To investigate if shear was targeting E-cadherin to lysosomes for degradation, we examined if E-cadherin localized to lysosomes. Colocalization studies confirmed that E-cadherin was not present in lysosomes (spherical red vesicles, Fig. 2B), suggesting that shear-internalized E-cadherin was not being degraded.

To determine if the internalization of E-cadherin was a specific response to shear, the localization of integrin $\alpha_v\beta_3$ in OC-1 cells in the presence and absence of shear was examined. In resting adherent cells, the integrin $\alpha_v\beta_3$ localized to both the cytosol and membrane of the cell under static conditions. In contrast, shear induced clustering of the integrin at the cell edge and it did not internalize into vesicles, as seen with E-cadherin (Fig. 2C). Thus the internalization of E-cadherin in response to shear is a specific event.

Src inhibition disrupts shear-induced internalization of E-cadherin. Src-mediated phosphorylation of E-cadherin is required for endocytosis.[12] To characterize the role of shear in Src activation, OC-1 cells that were pretreated in the presence and absence of PP1 were sheared using a cone and plate viscometer and lysed for subsequent analysis of phospho-Src activity (Fig. 3A). Corresponding static OC-1 cells with and without PP1 were used as controls. Static OC-1 cells contained a basal level of phospho-Src activity, which increased in the presence of shear. The shear-induced increase in Src activation was inhibited by the presence of PP1.

We then investigated whether Src inhibition could disrupt shear-induced internalization of E-cadherin. Adherent OC-1 cells, which were pretreated with PP1, were sheared in a parallel plate flow chamber at a shear rate of 200/s for 30 min. Non-sheared cells with and without PP1 were used as controls. Our results show that cells pre-treated with the specific Src inhibitor PP1 and subject to shear maintained a similar membrane distribution of E-cadherin as that observed in static non-treated PP1 cells (Fig. 3B).

Sheared OC-1 cells invade in a Src-dependent manner. Our results demonstrate that shear induces internalization of E-cadherin through a Src-mediated signaling mechanism. Therefore, to investigate whether this had any functional consequences on the behavior of OC-1 cells, we evaluated invasion in sheared cells in the presence and absence of the inhibitor PP1. A BioCoat Matrigel Invasion Chamber assay was used. This assay utilizes a mixture of extracellular matrix components coated on a filter as a model of the basement membrane. Matrix and growth factor components were kept constant throughout the invasion

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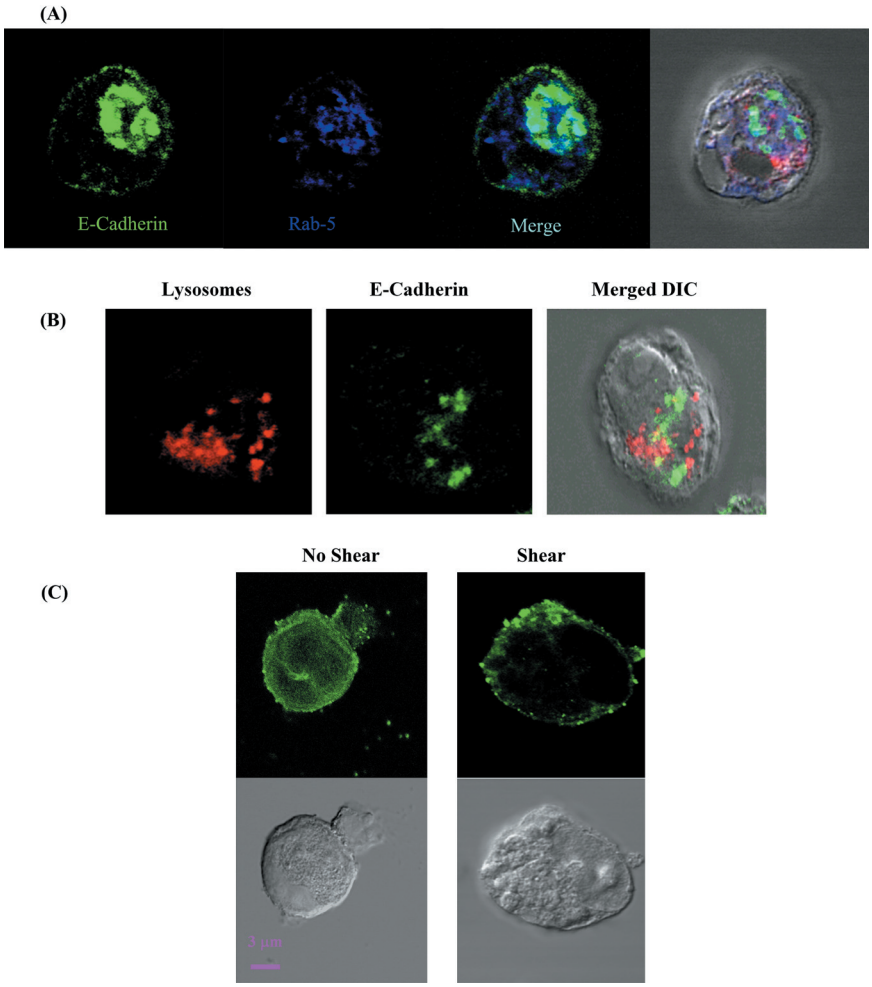

Fig. 2. Shear induces translocation E-cadherin into Rab5-positive endosomes. By contrast, shear induces clustering of $\alpha_v\beta_3$ to the cell edge. (A) Adherent OC-1 cells were exposed to shear (shear rate 200/s for 30 min) and labeled for E-cadherin (green) and Rab5 (blue). Colocalization of E-Cadherin and Rab5 is shown in light blue. E-cadherin localized to Rab5-positive endosomes following exposure to shear as seen in the merged differential interferance contrast microscopy (DIC) (E-Cad, green; Rab5, blue; lysosomes, red). (B) OC-1 cells were pre-incubated with LysoTracker to label lysosomes (red) and were exposed to the same shear conditions as described above. E-cadherin (green) did not translocate to lysosomes (red). (C) In separate experiments, adherent OC-1 cells were exposed to static and sheared conditions (shear rate, 200/s) and labeled for integrin $\alpha_v\beta_3$ (green). The integrin $\alpha_v\beta_3$ was localized to both the cytosol and periphery of the cell under static conditions. However, in the presence of shear, clustering of the integrin occurred at the OC-1 cell edge and did not cluster into Rab5-positive endosomes. DIC, differential interferance contrast microscopy.

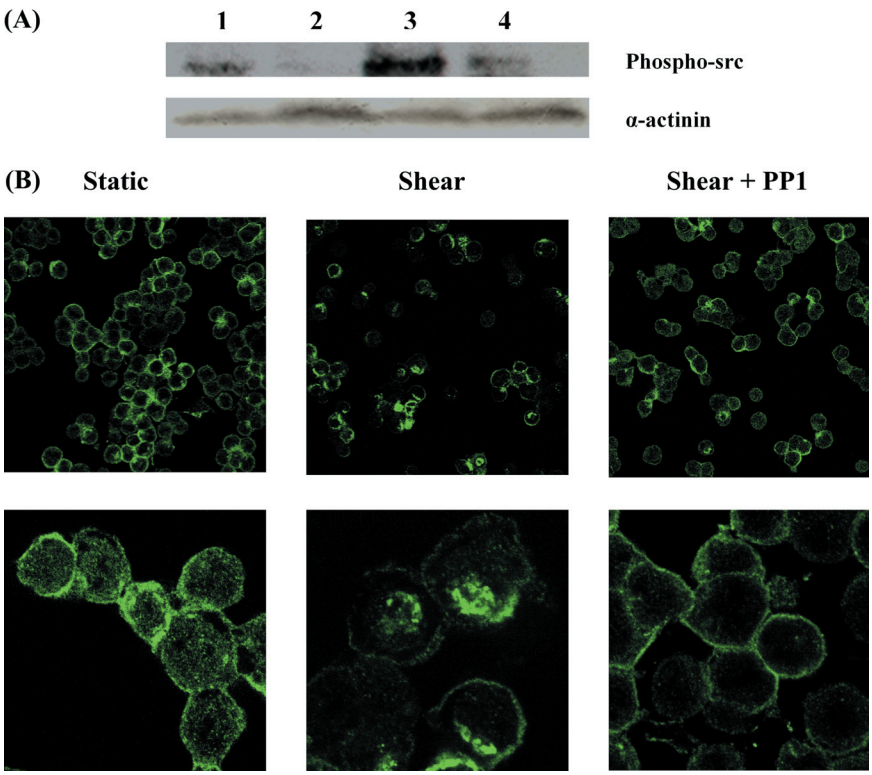

Fig. 3. Src inhibition disrupts shear-induced internalization of E-cadherin. (A) OC-1 cells, pretreated with the Src inhibitor 4-amino-5-(4-methylphenyl)-7-(t-butyl)pyrazolo[3,4-d]pyrimidine (PP1) (10 μM) for 48 h, were sheared at 200/s for 15 min in a cone and plate viscometer and lysed prior to measurement of phospho-Src via western blotting. Static OC-1 cells contained a basal level of phospho-Src activity, which increased in the presence of shear. However, pre-treating OC-1 cells with PP1 resulted in a significant decrease in the shear-induced activation of phospho-Src. A representative immunoblot is shown. 1, static OC-1; 2, static OC-1 + PP1; 3, sheared OC-1; 4, sheared OC-1 + PP1. (B) Adherent OC-1 cells, pre-treated with PP1, were exposed to shear in a parallel plate flow chamber and labeled for E-cadherin. In static OC-1 cells (B, static), E-cadherin localized specifically to cell–cell contacts, whereas in the presence of shear (B, shear) E-cadherin internalized into endosomes. In sheared OC-1 cells pre-treated with PP1 (B, shear + PP1), E-cadherin re-established at the adherens junctions, in a similar distribution to that observed in static OC-1 cells.

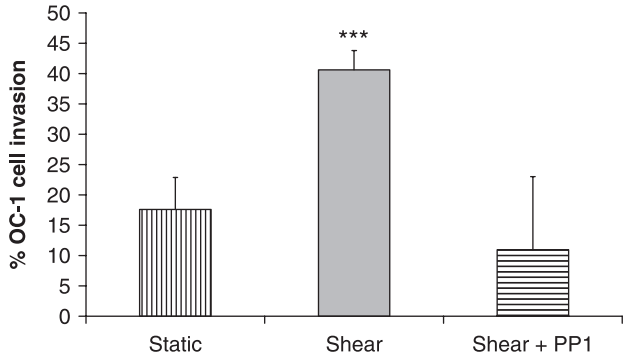

Fig. 4. Sheared OC-1 cells invade in a Src-dependent pathway. OC-1 cells with or without 4-amino-5-(4-methylphenyl)-7-(t-butyl)pyrazolo [3,4-d]pyrimidine (PP1) were sheared (200/s for 15 min via cone plate viscometry) and seeded onto a matrix coating. Penetration of the extracellular matrix barrier was determined after 22 h. Sheared OC-1 cells were significantly more invasive (***$P < 0.001$) in contrast to non-sheared samples; however, pre-treatment with PP1 inhibited the enhanced invasiveness of sheared OC-1 cells.

experiments, to assess the direct effect of shear alone and PP1 drug treatment. A standard time of 22 h was used to assess invasion.[16]

OC-1 cells that were pre-treated with or without PP1 were sheared in the cone and plate viscometer for 15 min and then seeded onto the matrix coating. Non-sheared OC-1 cells with and without PP1 were seeded as controls. Invasion into the extra-cellular matrix barrier was then determined. Analysis revealed that sheared OC-1 cells are significantly more invasive ($^{***}P < 0.001$) in contrast to non-sheared cells (Fig. 4). The enhanced invasiveness of sheared OC-1 cells was abrogated in the presence of PP1. By contrast, there were no significant differences in tumor invasion between static and PP1-treated static cells (data not shown).

Discussion

We have previously shown that shear stress enhances the mobility and invasiveness of a metastatic esophageal cancer cell line (OC-1 cells) in a Rho-kinase- and Ras-dependent manner.[16] In the current investigation we describe a novel role for shear in the internalization of E-cadherin. Specifically we show that: (1) venous shear induces the internalization of E-cadherin from cell–cell junctions into Rab5-positive endosomes; (2) Src inhibition disrupts shear-induced internalization of E-cadherin; and finally that (3) sheared OC-1 cells invade in a Src-dependent manner.

Adhesion of tumor cells to the vasculature of host organs is a prerequisite for blood-borne metastatic spread.[19] To adhere under the physiological flow of blood, metastatic cells must undergo alterations in cell–cell and cell–extracellular matrix adhesion properties. These changes can occur through altered integrin expression and downregulation of homophilic cadherin interactions. It has been shown that exposure of endothelial cells to shear stress causes partial disassembly of adherens junctions that involves transient dispersal of catenins and VE-cadherin.[20] Endosome recycling at the cell surface is most likely to result in the internalization of many different proteins; however, this current study was primarily interested in molecules involved in cell adhesion and motility, specifically that of cadherins and integrins. We[16] and others[4] have shown that exposure to shear stress causes activation, translocation, and clustering of integrins in tumor cells. We hypothesize that clustering of integrins may aid in the attachment of cells under shear, an adaptive mechanism to enhance focal contacts and counteract the stresses encountered in fluid flow. Previous studies have shown that integrin-mediated functions may involve MMP-9, as integrins regulate MMP-9 secretion in some immortalized and malignantly transformed cells.[21] Other studies propose that vesicles carrying integrins are exocytosed to supply integrins for newly forming focal contacts at the leading edge of migrating cells.[22,23] Although exposure to fluid shear has been shown to activate integrins in tumor cells, the role of shear in modulating cadherin activity in metastatic cells is unknown.

Previous studies have focused on the transcriptional regulation of E-cadherin;[24] however, it has been proposed that E-cadherin endocytosis and recycling are an equally powerful mechanism for maintaining the dynamics of the epithelial monolayer. Recently, Palacios *et al*. have shown that E-cadherin is internalized into rab5 and rab7 endosomes before undergoing degradation in the lysosome.[25] In our study, we found that exposure of OC-1 cells to venous shear rates results in the translocation of E-cadherin from adherens junctions into early endosomes but not lysosomes. Although we show that E-cadherin localizes to Rab5-positive endosomes, this does not exclude the possibility that E-cadherin may also traffic to recycling or late endosomes.

The activity of the Src family of non-receptor kinases is upregulated in a variety of epithelial cancers. Moreover, there appears to be a distinct correlation between levels of Src activity and malignant potential.[26] A number of lines of evidence suggest that Src kinases can induce the remodeling of adherens junctions through Src-mediated phosphorylation of the catenins[27] and activation of Rab5 and Rab7 GTPases.[23] Elevating Src catalytic activity in KM12C colon cancer cells has been shown to disrupt E-cadherin-associated cell–cell contacts.[28] Recently, Src-mediated phosphorylation of E-cadherin has been shown to be required for tagging of the protein with ubiquitin and its subsequent endocytosis.[12] Because Src is activated in response to shear stress, we hypothesized that Src inhibition might prevent the shear-induced internalization of E-cadherin in OC-1 cells. Our results show that pre-treatment of OC-1 cells with the specific Src inhibitor PP1 prevents the internalization of E-cadherin in sheared cells. Furthermore, we show that sheared OC-1 cells invade via a Src-dependent pathway.

In conclusion, this study provides new insights into the dynamic regulation of E-cadherin under physiological shear, which may help us elucidate the mechanisms by which tumor cells exit the vasculature. Internalizing E-cadherin in response to blood flow may be an adaptive metastatic process to promote motility and invasion. Therefore, targeting shear-sensitive pathways that promote E-cadherin internalization, such as those mediated through Src, may act as a potential therapeutic target to prevent further metastatic spread.

Acknowledgments

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