Expression of E-selectin ligands on circulating tumor cells: cross-regulation with cancer stem cell regulatory pathways?

Monica M. Burdick1,2*, Karissa A. Henson2, Luis F. Delgadillo1, Young Eun Choi3, Douglas J. Goetz2,4, David F. J. Tees2,3 and Fabian Benencia2,4

1 Department of Chemical and Biomolecular Engineering, Russ College of Engineering and Technology, Ohio University, Athens, OH, USA
2 Biomedical Engineering Program, Russ College of Engineering and Technology, Ohio University, Athens, OH, USA
3 Department of Physics and Astronomy, College of Arts and Sciences, Ohio University, Athens, OH, USA
4 Department of Biomedical Sciences, Heritage College of Osteopathic Medicine, Ohio University, Athens, OH, USA

*Correspondence: Monica M. Burdick, Department of Chemical and Biomolecular Engineering, Russ College of Engineering and Technology, Ohio University, Stocker Center 177, Athens, OH 45721, USA. e-mail: burdick@ohio.edu

INTRODUCTION

Distant metastasis is the culmination of an elaborate cascade of events in which cancer cells break away from the primary tumor, intravasate through blood vessel walls, enter the bloodstream, travel throughout the body, and finally extravasate through the vessels of a distant organ to establish a secondary colony. While resident in the blood vasculature, circulating tumor cells (CTCs) must survive biochemical and biophysical assaults including necrosis or apoptosis, plus avoid elimination by immune cells, in order to metastasize. Regardless of their ultimate fate, the clinical interpretation of CTCs arising from solid tumors has been the subject of much debate, with definitive answers yet to emerge as to if, when, and for which cancers these cells offer significant diagnostic, prognostic, or therapeutic value. Despite lack of consensus on their clinical utility, CTCs can still provide a meaningful portrait of a cancer patient’s health, or rather disease, status. CellSearch, a test marketed by Johnson & Johnson’s Veridex division, is FDA-approved to capture and enumerate CTCs in metastatic breast, colon, and prostate cancer patients for prognostic or maintenance purposes (Kim et al., 2008; Riethdorf and Pantel, 2010). More recently, the development of next-generation fluidics-based CTC isolation devices by the Haber and Toner groups, the CTC-chip and herringbone (HB)-chip (Nagrath et al., 2007; Stott et al., 2010; Yu et al., 2011), has generated increased attention to CTCs and the use of “liquid biopsies” or “blood biopsies” to enumerate and capture CTCs for further study.

Although significant progress has been made in the fight against cancer, successful treatment strategies have yet to be developed to combat those tumors that have metastasized to distant organs. Poor characterization of the molecular mechanisms of cancer spread is a major impediment to designing predictive diagnostics and effective clinical interventions against late stage disease. In hematogenous metastasis, it is widely suspected that circulating tumor cells (CTCs) express specific adhesion molecules that actively initiate contact with the vascular endothelium lining the vessel walls of the target organ. This “tethering” is mediated by ligands expressed by CTCs that bind to E-selectin expressed by endothelial cells. However, it is currently unknown whether expression of functional E-selectin ligands on CTCs is related to cancer stem cell regulatory or maintenance pathways, particularly epithelial-to-mesenchymal transition and the reverse, mesenchymal-to-epithelial transition. In this hypothesis and theory article, we explore the potential roles of these mechanisms on the dynamic regulation of selectin ligands mediating CTC trafficking during metastasis.

Keywords: circulating tumor cells, cancer stem cells, epithelial-to-mesenchymal transition, selection, selectin ligands, cell adhesion

As with any portrait, further examination reveals nuances not observed at first glance. For instance, post-capture investigation using RT-PCR in the AdnaTest (AdnaGen) may reveal upregulated pathways related to cancer stem cells (CSCs), metastatic aggressiveness, or responsiveness to treatment (i.e., trastuzumab for HER-2 overexpressing breast cancers) that are impossible to observe through a simple CTC count (Fehm et al., 2007; Dawood et al., 2008; Riethdorf and Pantel, 2008, 2010; Mostert et al., 2009). Though the scientific and medical communities may achieve significant new insights from these blood biopsies, the information itself is static. Cancer is dynamic. How medical professionals interpret a particular patient’s case, as well as predict future outcomes of an ever-changing disease, will depend partially on information gleaned from CTC assessments at single moments in time.

In general, CTCs possessing enhanced survival capabilities will generate metastatic colonies in distant organs, as well as reseed the original primary tumor with more aggressive cells (Kim et al., 2009). Uncovering the molecular mediators by which CTCs initiate adhesion with endothelial cells lining the blood vessel walls of the target site may therefore prove useful in predicting and thwarting metastasis. In particular, stimulated vascular endothelium expressing E-selectin can capture CTCs expressing E-selectin ligands, thereby initiating adhesion and subsequent CTC invasion. However, this statement is a simplification of a tangle of issues underlying functional selectin ligand expression on cancer...
cells. To be qualified as a true selectin ligand, Varki (1997) proposed that the purported ligand must be expressed “in the right place at the right time” among other criteria. So do all CTCs express selectin ligands, or even the “right” selectin ligands? How and when do these selectin ligands arise? Are they modulated by pathways associated with epithelial-to-mesenchymal transition (EMT) or other mechanisms of CSC generation and maintenance, or are they independent of these pathways? In this article, we explore the complex networks through which selectin ligands on CTCs may be regulated and propose working theories based on ongoing studies with breast cancer in our laboratories. New findings from these investigations, coupled with additional discoveries from other labs, will address significant shortcomings in our understanding of the molecular networks promoting cancer metastasis.

CTCs and Cell Adhesion Mediated by E-Selectin and Its Ligands

It has been proposed that the early steps by which CTCs cells leave the bloodstream to invade secondary sites mimic the physiologic trafficking of leukocytes to sites of inflammation and hematopoietic stem cells to bone marrow. Because numerous excellent review articles on cell trafficking have been published through the years (Springer, 1994; Sackstein, 2005; Barthel et al., 2007; Konstantopoulos and Thomas, 2009; Zarbock et al., 2011; Bendas and Borsig, 2012; Chase et al., 2012; Geng et al., 2012), only a general overview is presented here (Figure 1). Circulating cells are first captured or “tethered” from bulk blood flow onto vascular endothelial cells, which is immediately followed by rolling on the endothelium. Tethering and rolling are typically mediated by interactions between ligands expressed on the surface of the circulating cells that recognize E-selectin, an endothelial adhesion molecule upregulated in response to inflammatory stimuli as well as constitutively expressed by bone and dermal endothelial cells (Springer, 1994; Sackstein, 2004). Subsequently, rolling cells firmly adhere and migrate through the vessel wall into the underlying tissue in response to specific cytokines and chemokines.

Therefore, this multi-step model indicates that CTCs must initially tether on endothelial cells, presumably through E-selectin ligand recognition of E-selectin, in order to trigger the series of events necessary for metastatic growth. These adhesive interactions occur under hydrodynamic shear stresses generated by blood flow (post-capillary venule and bone marrow endothelial venule wall shear stress ranges from 0.5 to 4.0 dyn/cm²; Jones et al., 1991; Mazo et al., 1998), enabled by the hallmark catch-slip bonds and rapid bond formation/breakage kinetics of selectins and their ligands (Dembo et al., 1988; Marshall et al., 2003; Zhu and McEver, 2005; Evans and Calderwood, 2007; Ham et al., 2007; McEver and Zhu, 2007). E-selectin has been established as a mediator of colon and prostate cancer adhesion and distant metastasis (Khabib et al., 2002; Barthel et al., 2007, 2009), and there is clinical and in vitro evidence for the role of E-selectin in promoting metastasis of several other cancers, including breast, pancreatic, and head and neck cancers (Weizel et al., 1995; Eshel et al., 2000; Barthel et al., 2007; Geng et al., 2012). The other two members of the selectin family, P-selectin expressed by activated platelets and activated endothelium and L-selectin expressed by most leukocytes, also have been proposed to participate in cancer metastasis (Laubbé and Borsig, 2016; St Hill, 2012).

Notably, the expression levels of the minimal selectin-binding epitope sialyl Lewis X (sLeX, NeuAc₂,3Galβ(1,4)[Fucα(2,3)]GlcNAc) and its stereoisomer sialyl Lewis A (sLeA, NeuAc₂,3Galβ(1,3)[Fucα(1,4)]GlcNAc) on certain glycoproteins and glycolipids increase progressively from normal tissue to early stage cancer to metastatic disease, consistent with aberrant glycosylation rendering altered cell adhesion molecules relative to normal tissue in most cancers, including breast, bladder, and colon cancers (Izumi et al., 1995; Klopocki et al., 1996; Renkonen et al., 1997; Skorstengaard et al., 1999; Kajiwara et al., 2005). Transfer of sialic acid (NeuAc) onto a terminal galactose (Gal) residue occurs through the action of α(2,3) sialyltransferases. The enzymes directing α(1,3) fucosylation for sLeX production are multiple fucosyltransferases (Fts) III, IV, V, VI, and VII while FTII and FTIV are also α(1,4) Fts involved in the production of sLeA (Edlböck et al., 1997; de Vries et al., 2003; Dupay et al., 2004).
Clearly, these enzymes must be (dys)regulated in cancer cells through the transition from primary tumor to advanced stage cancer to result in the observed upregulation of sLe^âã in tissues and thus selectin ligands (Benkó and Mészáros, 1998). Although the tumor stroma and hypoxic conditions are known to influence tumor cell glycosylation (Oakey et al., 2010; Shroff et al., 1995), the exact biochemical (or biophysical) regulators of cancer glyclosylation are unknown. Nevertheless, the presence of sialofucosylated moieties such as sLe^âã is significant in that upregulated expression of functional selectin ligands may indicate their role in promoting CTC adhesion during metastasis (Burdick et al., 2001, 2006; Barthel et al., 2007; Shirure et al., 2011). Thus, it is necessary to identify the core proteins or lipids presenting sialofucosylated glycans to better characterize roles for specific selectin ligands.

To date, several major tumor cell surface glycoprotein selectin ligands that may fulfill the criteria of “real” selectin ligands have been identified, most prominently the specialized CD44 glycoform HCELL as an E-/L-/P-selectin ligand on colon cancer cells (Hanley et al., 2005, 2006; Burdick et al., 2006), and an E-selectin ligand on prostate and breast cancer cells (Barthel et al., 2009; manuscript in preparation). Carcinoembryonic antigen (CEA, MUC-1, CD43, and PSGL-1, have also been proposed as selectin ligands. Contributions roles have also been identified for colon, prostate, breast, and head and neck cancer sialofucosylated glycolipids in adhesion to endothelial E-selectin (Burdick et al., 2003; Dunstroff et al., 2004; Barthel et al., 2007; Shirure et al., 2011; Geng et al., 2012). Though the understanding of infections and their ligands is growing, it is imperative to consider their functionalities in the wider context of biochemical and biophysical factors encountered by CTCs in transit.

**CTC TRANSIT THROUGH CAPILLARIES**

The ability of cancer cells to enter small vessels such as capillaries (as well as to roll in larger vessels such as post-capillary venules described above) depends critically on the mechanical deformability of the cells. Capillaries range from 2 to 8 μm in diameter (Doerschuk et al., 1993) and cancer cells, which tend to be large and stiff, may not be able to deform enough to enter at least some portions of the capillary bed (Loetta, 1987; Weiss et al., 1998; Chambers et al., 1992; Lafrentz et al., 1993). Organs with small vessels that are susceptible to metastasis include the lung microcirculation (which is particularly important because it is the first capillary bed that a metastasizing cancer cell entering the venous circulation will encounter after passing through the first two chambers of the heart), bone marrow and liver sinusoids, and the kidney microcirculation. The mechanical properties of cancer cells surely play a role in transit: if certain CTCs are stiff and resistant to deformation, then the possibility of sequestration at the entrance of small vessels should be large.

Conversely, if CTCs are less stiff (more deformable), then their potential to pass through the microcirculation and metastasize could be enhanced. Furthermore, it is possible that deformation is not a by-stander process for the cell; deformation itself may induce changes affecting molecular and mechanical phenotype, perhaps in a manner that promotes CTC survival and metastasis.

Protocols to quantify cellular mechanical properties have existed for nearly 30 years, and parameters for models of cell mechanics have been measured using many experimental techniques: micropipette aspiration (Figure 2), magnetic twisting rheometry, cell stretching with optical tweezers or mechanical stretching devices, nanoscale indentation with probes or AFM tips, particle tracking microrheology, etc. (Mason and Weitz, 1995; Shroff et al., 1995; Choquet et al., 1997; Mason et al., 1997; Thoumine and Ott, 1997; Bausch et al., 1999; Yap and Kamm, 2005; Sirghi et al., 2006). As a result of these efforts, much is known about the deformability of red and white blood cells (which are known to undergo massive deformations in the normal course of circulation) and a sampling of other cell types. On the basis of these collective works (Mason and Weitz, 1995; Shroff et al., 1995; Choquet et al., 1997; Mason et al., 1997; Thoumine and Ott, 1997; Bausch et al., 1999; Yap and Kamm, 2005; Sirghi et al., 2006), it was found that the major distinction in cell rheological properties is whether the cell behaves like a liquid drop with a cortical tension (as white blood cells clearly do) or as a viscoelastic solid (most other cell types). Devices to identify cell subsets based on differences in cellular mechanical properties are in early development stages (Oakley et al., 2010; Sraj et al., 2010), and these methodologies are being considered for identifying and isolating normal healthy mesenchymal stem cells (MSCs) for use as therapeutics and in regenerative medicine (Porada et al., 2006; Pardeepadan and Mîldî, 2010). These cells lack unique cell surface molecules through which they can be easily isolated from their sources (e.g., bone marrow, umbilical cord; Porada et al., 2006; Pountos et al., 2007) but have distinct mechanical properties compared to their counterparts.
differentially daughter cells. These differences are currently being explored as specific identifying MSC characteristics (Darling et al., 2008; Tam et al., 2008; Xu et al., 2010). Similarly, benign versus tumorigenic cancer cells have been explored for differing traits (Kim et al., 2008; Hou et al., 2010). However, much more work needs to be performed to understand CTC metastatic potential attributable to inherent or alterable molecular and mechanical properties. It is tantalizing to speculate a role for biophysical modula- tion of CTC properties, including effects on selectin ligand expression or function.

**CSCs, EMT, AND MESENCHYMAL-TO-EPITHELIAL TRANSITION**

The discovery and identification of leukemic stem cells (LSCs) effectively ushered in a new era of cancer research (Lapidot et al., 1994; Bonnet and Dick, 1997). LSCs share the properties of self-renewal and pluripotency with their normal hematopoietic stem cell brethren, but are also leukemogenic. LSCs are particularly dangerous in that they can survive chemotherapy (Costello et al., 2000; Graham et al., 2002; Holtz et al., 2002), leading to relapse with LSCs even more aggressive than their previous incarnation (Oravecz-Wilson et al., 2009). Shortly after LSC identification, a groundbreaking report by Al-Hajj et al. (2003) found that breast cancers similarly harbor deadly CSCs, which exhibited a much greater propensity for tumor formation than cells of a different phenotype. These breast CSCs were putatively characterized by the expression levels of glycophosphat markers on the surface of the cell: high expression of CD44, little to no expression of CD24, high expression of epithelial-specific antigen (ESA), and lack of lineage markers (lin), or CD44+/CD24−/low/ESA−/lin− (Al-Hajj et al., 2003). These CSCs were able to form heterogeneous tumors from a relatively small number of cells. Specifically, only 200 CD44+/CD24−/low/ESA−/lin− breast cancer cells, isolated from patient primary tumors, could regenerate and expand to form secondary tumors that also contained CSCs, in as little as 12 weeks in mice (Al-Hajj et al., 2003). In contrast, as many as 20,000 cells of alternate phenotypes from the same tumor origin as the CD44+/CD24−/low/ESA−/lin− cells were unable to form new tumors. Thus, the breast CSCs were capable of self-renewal and differentiation, two general properties possessed by normal stem cells, and the ability to generate new tumors (Al-Hajj et al., 2003; Ponti et al., 2005; Fillmore and Kuperwasser, 2008).

Since this initial breast cancer study, CSCs have reportedly been found in nearly all solid cancers, with a specific molecular phenotype for each type of cancer. However, the cancer research community continues to debate the true nature of CSCs (Campbell and Polyak, 2007; Gupta et al., 2009; Badve and Nakshatri, 2012; Liu et al., 2012; Magere et al., 2012), including whether CSCs are tumor-initiating or metastasis-initiating cells (Kelly et al., 2007; Adams and Strasser, 2008; Fillmore and Kuperwasser, 2008). The reasons for the extended scientific discussion are many and are outlined in a comprehensive review from the Morrison lab (Magere et al., 2012).

Perhaps some of the confusion and seemingly contradictory findings surrounding CSCs will be allayed by the growing evidence demonstrating that CSCs are not a single population of cells identified by one specific molecular signature. Rather, while all CSCs possess general stem cell properties, CSGs are actually comprised of heterogeneous subpopulations with multiple molecular and functional phenotypes that are generated through different pathways (Liu et al., 2012; Magere et al., 2012). It is becoming abundantly clear for breast cancer that such heterogeneity exists in its CSCs. Breast CSCs that are CD44+/CD24− (the simplified breast CSC phenotype) are the result of cytokine-induced EMT (Mani et al., 2008; Morel et al., 2008; Blick et al., 2010, Liu et al., 2012), a process by which cells lose epithelial characteristics (E-cadherin expression, cell–cell contacts, polarity) and become more mesenchymal (N-cadherin expression, mesenchymal morphology, enhanced migration abilities; Oder et al., 2008; Zeisberg and Neilson, 2009). Many of the properties EMT confers are normally helpful to development (Kalluri and Weinberg, 2009), but EMT can also contribute to cancer progression in adult tissue (Mani et al., 2008; Oder et al., 2008). Often, cancer cells at the invasive front of a primary tumor have a mesenchymal phenotype (Kalluri and Weinberg, 2009). Interestingly, in breast cancer patients with metastases, CTCs have been found to express markers of EMT in addition to stem cell traits (Aktaş et al., 2009; Bonnomet et al., 2010; Kallergi et al., 2011). It is important to note that EMT is reversible, such that cells can undergo mesenchymal-to-epithelial transition (MET). The Wicha group reported that CSCs can exist in an MET state (Liu et al., 2012) as well as an EMT state previously found by the Weinberg group (Mani et al., 2008; Liu et al., 2012). MET CSCs actively self-renew and express aldehyde dehydrogenase (ALDH, a marker uniquely identified as a CSC indicator in several types of cancer (Günther et al., 2007; Clay et al., 2010; Silva et al., 2011; Kryczek et al., 2012), epithelial cell adhesion molecule (EpCAM, the same molecule that forms the basis for the capture of CTCs by CellSearch and the CTC- and HB-chips), and CD49f (integrin subunit) in contrast to quiescent yet invasive CD44+/CD24−/EpCAM+/CD49f− EMT CSCs (Liu et al., 2012). Given the interconversions between CSC states, which are regulated by microRNAs (miRNAs), it is not surprising that there exists a subpopulation of CD44+/CD24− and ALDH+ cells (Liu et al., 2012). However, studies linking CSCs with properties facilitating CTC lodgment at sites of metastasis (i.e., selectin ligands and cell mechanical properties) are lacking.

**PUTTING IT ALL TOGETHER: HYPOTHESIZED BREAST CANCER MODELS LINKING REGULATION OF CSCs, CTCs, AND E-SELECTIN LIGANDS**

Arguably, CSGs and CTCs from breast cancer are the most well-studied among all cancers, thereby easing efforts aimed at uncovering crosstalk between CSC regulatory pathways, CTC characteristics, and expression of functional selectin ligands. Such investigations may aid in diagnosing breast cancer at an early stage, when it is largely considered curable, or assist in identifying new therapeutic targets or treatment modalities for those women diagnosed at the metastatic stage, for whom the 5-year survival rate is ~20% (DeSantis et al., 2011). Most commonly, breast cancer metastases are found in the lungs and bone marrow (Moore, 2001; Minn et al., 2005; Balic et al., 2006; Reithdorf and Pantel, 2010), exhibiting a tropism not explainable by circulation pattern alone (Minn et al., 2005; Talmadge and Fidler,
Recently, it has been reported that disseminated breast cancer cells in human bone marrow are largely CD44+/CD24− (Abraham et al., 2005; Balic et al., 2006), corresponding to EMT CSCs. These CD44+/CD24− are also resistant to radiotherapy and chemotherapy (Diehn and Clarke, 2006; Phillips et al., 2006; Reim et al., 2009). It is therefore necessary to understand the reasons for CD44+/CD24− breast cancer cells in bone: whether CTCs are CD44+/CD24− CSCs that preferentially migrate and establish metastases, or if non-CD44+/CD24− CTCs are induced to the CD44+/CD24− phenotype in the bone marrow.

As mentioned previously, E-selectin is constitutively expressed on bone marrow endothelium (Keelan et al., 1994; Schweitzer et al., 1996), and breast cancer cells have been shown to express E-selectin ligands on their surface (Tozeren et al., 1995; Narita et al., 1996; Zen et al., 2008; Julien et al., 2011; Shirure et al., 2011, 2012). Previous studies have also demonstrated the E-selectin-dependence of binding interactions between commercially available breast cancer cell lines and human umbilical vein endothelial cells (HUVECs; Giavazzi et al., 1993, Narita et al., 1996; Julien et al., 2011; Shirure et al., 2011, 2012). As the expression levels of the minimal selectin-binding epitopes sLeX and sLeA increase progressively from normal tissue to early stage breast cancer to metastatic disease (Renkonen et al., 1997), it may be hypothesized that CTCs retain expression of selectin ligands that were generated in the primary site, then upregulate such ligands during transit to the metastatic site. Altogether, these findings imply that E-selectin and its ligands are likely to comprise important elements of breast cancer metastasis in vivo. Since breast cancer cells at the invasive front of a primary tumor tend to be mesenchymal (Kalluri and Weinberg, 2009) and breast CTCs have been found to express markers of EMT in addition to stem cell traits (Aktas et al., 2009; Bonnomet et al., 2010), it would seem a logical extension of the hypothesis that E-selectin ligands are upregulated with EMT and the corresponding CD44+/CD24− CSC phenotype. However, our studies with human breast cancer cell lines revealed surprising results: non-CD44+/CD24− cells expressed much greater E-selectin ligand activity than CD44+/CD24− cells (Figure 3 and Table 1; Shirure et al., 2011, 2012; manuscript in preparation). These findings imply that lower expression of E-selectin ligands correlates with CD44+/CD24− breast CSCs arising from EMT. Notably, the bone marrow microenvironment is enriched in TGF-β, a cytokine that is well-known to induce EMT (Brown et al., 2004; Lee et al., 2008; Mani et al., 2008; Lenferink et al., 2010), and production of TGF-β by microenvironment stromal cells may be responsible for CD44+/CD24− breast cancer cells in bone (Abraham et al., 2005; Balic et al., 2006). Thus, it may be speculated that soluble TGF-β decreases the expression of E-selectin ligands either before or during CTC engagement with bone marrow endothelium (Figure 4), thus throwing into doubt the relevance of E-selectin ligands on breast CTCs in establishing bone metastases. Studies in which EMT is induced in breast cancer cells need to be performed, with coordinated monitoring of glycosylation machinery, core E-selectin ligand protein and lipid expression, E-selectin ligand activity under flow conditions, and EMT and CSC markers, in order to verify or refute mechanistic links between functional E-selectin ligand expression and transition/maintenance of CD44+/CD24− CSCs. Ultimately, it may be found that downregulation of E-selectin ligands is not dependent on EMT per se, since E-selectin ligand activity fails to decrease consistently from the least mesenchymal luminal to the...
somewhat mesenchymal basal A to the most mesenchymal basal B cells (Table 1). Instead, persistent suppression of E-selectin ligands in CD44+/CD24- CSCs may be controlled by EMT pathways.

Alternatively, the MET state of CSCs (indicated by ALDH expression but not necessarily CD44+/CD24- cells; Liu et al., 2012) may regulate E-selectin ligand expression or function. Interestingly, a recent study of all cell lines in Table 1 except Hu578T revealed that BT-20 and MDA-MB-468 cells, both CD44+/CD24- cell lines of the basal A type with relatively high E-selectin ligand

activity (Figure 3 and Table 1), possessed the highest percentage of cells with ALDH activity (Deng et al., 2009). CSCs in the MET state may thus maintain or potentially upregulate E-selectin ligands, in contrast to CSCs in EMT. This notion merits further investigation, in that CTCs from breast cancer patients can simultaneously express mesenchymal and stem cell markers in addition to epithelial markers, and not just EMT markers (Aktas et al., 2009; Bonnomet et al., 2010; Armstrong et al., 2011; Kallergi et al., 2011). Moreover, the bone resident chemokine stromal derived factor-1 (SDF-1, Figure 4), known to mediate HSC homing and breast cancer migration through ligation of CXCR4, has been shown to regulate miRNAs in breast cancer cells and stromal cells that control breast cancer cell tumorigenicity and quiescence (Lim et al., 2011; Rhodes et al., 2011a,b). It may only be a matter of time until it is shown that SDF-1 also regulates miRNAs associated with EMT-MET phenotypes of CSCs. Thus, bone microenvironmental expression of E-selectin, TGF-$\beta$, and SDF-1 may be a certain type of CTC to establish a metastatic colony: a circulating CSC already equipped to infiltrate the bone parenchyma, or else another cell, CSC or otherwise, that will undergo EMT or MET as needed to attach to endothelium, invade, and grow.

Epithelial-to-mesenchymal transition and MET are, by their very names, dynamic transitional regulators of cellular phenotypes and behaviors. Thus far, we have proposed that these pathways modulate E-selectin ligands on breast CTCs and CSCs. However, it is valid to explore the potential roles of E-selectin and its ligands as regulators of EMT and MET. The proper E-selectin ligands expressed at the right time in the right place (e.g., HCELL, Mac-2bp, and/or glycolipids; Burdick et al., 2006; Shirure et al., 2011, 2012; manuscript in preparation) on a breast CTC in the vasculature at the metastatic site could facilitate EMT- or MET-generated/maintained CSCs in response to microenvironmental cues. E-selectin-primed cells may then effectively establish metastatic colonies. If a CTC encounters a small capillary rather than a larger venule, another possibility arises. Assuming the CTC can sufficiently deform to enter the capillary, which can be tested...
Although this article has been focused on presenting the potential relationships between CSCs, CTCs, and E-selectin ligands in hematogenous distant metastasis, the pathways mediating metastasis in total are far more extensive. Restricting the discussion to the selectins, CTCs may engage P-selectin expressed on blood vascular endothelial cells (Ludwig et al., 2004; Laubli and Borsiog, 2010; St Hill, 2012), which is arguably less understood than endothelial E-selectin-mediated pathways. CTCs in the bloodstream may also form multicellular aggregates with platelets and/or leukocytes (Borsiog et al., 2002), and the presence of these other cells can alter the manner in which CTCs interact with the vascular endothelium (Kim et al., 1999; Burdick and Konstantopoulos, 2004; Liang and Dong, 2008; Gong et al., 2012). The initial formation of heterotypic aggregates presumably occurs through engagement of P-selectin on platelets or L-selectin on leukocytes with their respective ligands on CTCs (Mannori et al., 1995; Jadhav et al., 2001; McCarty et al., 2002), such as the aforementioned HCELL (Hanley et al., 2005, 2006; Burdick et al., 2006; Burdick et al., 2009), sulfated glycosaminoglycans or proteoglycans (Ma and Geng, 2002; Monzavi-Karbasi et al., 2007; Couney et al., 2011), or sulfatides (Neddham and Schnei, 1993). Multicellular aggregation induces CSC phenotype(s). This theory warrants further investigation, given the discovery in HB-chips of CTC aggregates indicating prior CTC-leukocyte engagement (Mott et al., 2010), and a recent publication revealing that platelet–cancer cell contact can induce EMT in breast and colon cancer cells (Labelle et al., 2011). Alternatively, completely novel mechanisms of CTC–CSC regulation may be encountered in lymph node metastasis, considering the vastly different biochemical and biophysical environment of the lymphatic system compared to the blood vasculature (Lund and Swartz, 2010; Swartz and Lund, 2012). Thus, other compelling models of CTC–CSC regulation may be proposed and tested, which could lead to new ways to inhibit cancer metastasis.

CONCLUSION

A full understanding of how a cancer cell progresses from primary tumor cell to CTC to disseminated tumor cell remains elusive. Although EMT, MET, and stem cell pathways are clearly relevant, their effects relative to selectin ligands (and vice versa) on CTCs remain to be determined. On their directed journey to establish new metastatic colonies, CTCs are subject to the influences of a bevy of biochemical and biophysical stressors that may change their phenotype at specific times and at specific locations. CTCs captured from the blood of cancer patients by CellSearch, CTC-or HB-chips, AdnaTest, and other devices reflect only a single temporal data point from which inferences about disease status, treatment strategies, and survival predictions are extrapolated. While this information from blood biopsies is extraordinarily important, some caution is warranted. Molecular markers and phenotypes serving as the basis of capture in these assays have limitations, and information derived from these assays may have further shortcomings in light of CTC-dynamics. Therefore, novel CTC capture techniques and therapeutic strategies currently in development must respect the changing epithelial, mesenchymal, CSC-associated, etc., markers and functional phenotypes (e.g., expression of selectin ligands) to be truly meaningful for patients. Ultimately, collective efforts to elucidate the molecular descriptors of CTCs, including selectin ligands and their regulators such as CSC generation/maintenance pathways, will greatly improve the clinical utility of CTCs as diagnostics, prognostics, therapeutic indicators, or therapeutic targets.

ACKNOWLEDGMENTS

This work was supported by CBET-1106118 (to Monica M. Burdick, Fabian Benencia, David F. J. Tees), CBET-1106119 (to Douglas J. Goetz, Monica M. Burdick, Fabian Benencia, David F. J. Tees), and BES-0547165 (to David F. J. Tees) from the National Science Foundation, IR11CA161830-01 from the National Institutes of Health (to Monica M. Burdick), and a seed grant from the Ohio Cancer Research Associates (to Monica M. Burdick). For helpful discussions and assistance with manuscript preparation, we wish to thank our graduate students Mr. Grady Carlson, Ms. Tiantian Liu, Mr. Eric Martin, Ms. Amenoh-Mohammadpour, Mr. John O’Brien, Mr. Venketh Shirure, and Ms. Chengkai Xiong, as well as our undergraduate students Ms. Emily Blaha, Mr. Aaron Burdette, Mr. Chaz Cuckler, Ms. Jisqalyn Hawes, and Mr. Nate Reynolds.

REFERENCES

Abraham, B. K., Fuss, P., McAd- lan, M., Hauertrop, P., Athelo- ge, M., and Bruch, H. (2005). Prevalence of CD44+/CD10− cells in breast cancer may not be associated with clinical outcome but may favor distant metastasis. Clin. Cancer Res. 11, 1154– 1159.

Adams, J. M., and Strasser, A. (2008). Is tumor growth sustained by rare cancer stem cells or dominant clones? Cancer Res. 68, 4018–4021.

Agüero, S., Ramos, C. L., Hafizi- Moghaddam, A., Lawrence, M. B., Fredericks, J., Ahvogt, P., and Ley, K. (1998). CD24 mediates rolling of breast carcinoma cells on P-selectin. J. Exp. Med. 182, 12131218.

Alkan, B., Sivas, M., Fehm, T., Hauch, S., Kimmig, R., and Koerner-Raue, S. (2007). Stem cell and epithelial-mesenchymal transition markers are frequently overexpressed in circulating tumor cells of metastatic breast cancer patients. Breast Cancer Res. Treat. 11, 846.

Al-Hajj, M., Wicha, M. S., Benito- Hernández, A., Morrison, S. J., and Clarke, M. F. (2003). Prospective identification of tumorigenic breast...
Bausch, A. R., Möller, W., and Sack-Barthel, S. R., Gavino, J. D., Descheny, C., Burdick et al. (2012). CTCs, CSCs, and E-selectin ligands...
circulation. Ann. Rev. Biochem. 80, 790–805.

Garraway, L. A., Fregodina, M., Donis, R., and Romanzio, A. (1995). Rolling and adhesion of human tumor cells on vascular endothelium under physiologic flow conditions. J. Clin. Invest. 95, 3038–3044.

Gneist, C., Har, M. H., Charafe-Jauffret, E., Monderou, F., Duchet, J., Brown, M., Jaquemien, J., Vauth, P., Klaus, C. G., Lin, S., Schmitt, A., Hays, D., Brinbaum, D., Weich, M. S., and Donis, G. (2007). ADH1A is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. Carcinogenesis 28, 553–567.

Gong, L. M., Hu, H. J., Zhu, H., Zhou, X., and Yang, H. (2012). Selectin-mediated platelet activation promotes adhesion of non-small cell lung carcinoma cells on vascular endothelial cells under flow. Mol. Biol. Rep. 39, 935–942.

Graham, S. M., Jorgensen, H. G., Allan, W. D., Napier, S. L., Burdick, M. C. T., Jadhav, S., Bochner, B. S., and Konstantinou, K. (2011). Selectin ligands on colon carcinoma variant C701–C707 define ovarian cancer stem cells. Am. J. Pathol. 179, 177–201.

Greiner, A. M., Binns, R. M., and Haskard, D. O. (1994). Characterization of E-selectin ligand expression on colon carcinoma variant C701–C707. Cell Biol. Int. 18, 352–355.

H. W. Hou, H. W., Bhagat, A. A., Chong, A. G., Mao, P., Tan, S. W., Han, J., and Lian, C. T. (2012). Deformability based cell margination—a simple microfluidic device for making infected erythrocyte supernatant. Lab Chip 10, 2605–2613.

Iannini, Y., Yamagishi, Y., Tani, T., Smith, C. W., Nakamura, S., Fuller, E. L., and Irimura, T. (1995). Characterization of human colon carcinoma variant selected for stably La+ carbohydrate antigen: liver colonization and adhesion to vascular endothelial cells. Exp. Cell Res. 216, 218–222.

Jalilian, S., Bochner, B. R., and Konstantinou, K. (2005). Hydrodynamic shear regulates the kinetics and receptor specificity of polymorphonuclear leukocyte selectin-caricinoma cell adhesive interactions. J. Immunol. 167, 5896–5905.

Jonas, R. J., Ambinder, R. F., Piantadosi, S., and Santoro, G. W. (1993). Evidence of agrin versus lymphocyte function associated with allogeneic bone marrow transplantation. Blood 77, 649–653.

Julien, S., Brot, A., Gregoireaul, A., Qi, Z., Burford, B., Sperotto, D., Pasco, G., Gillett, C., Papp, S. L., Schaffer, L., Tati, A., Infante, Pampaunrathevi, J., Pines, S. E., and Burdick, M. C. T. (2011). Selectin ligand expression regulates adhesion of human cancer cells to vascular endothelium under physiologic flow conditions. J. Cell Sci. 124, 317–329.

Kaji, H., Yuraku, M., Konaka, N., Shikahama, T., and Shumaker, Y. (2005). Expression of carbohydrate antigen: liver colonization and metastasis of human tongue cancer cell lines. Carcinogenesis 26, H278–H290.

Karoui, K., and Weinberg, R. A. (2009). Cancer stem cells: mirage or reality? Nat. Med. 15, 105–113.

Kam, A. S., Guett, D. J., Kibonov, A. L., and Laurencin, M. B. (2007). Microplate adhesive dynamics and rolling mediated by selectin-specific antibodies under flow. J. Biomech. Eng. 129, 796–807.

Kantarci, H. S., Cengel, I. S., and Konstantinou, K. (2005). CD44 on Lewis tumor colon cancer cells regulates their migration activity. Cancer Res. 65, 5012–5017.

Kashyap, A. N., Wang, C., Lierlich, M. M., Scham, L. B., Sarkisken, R., and Konstantinou, K. (2006). Variant isofoms of CD44 are P- and selectin ligands on colon carcinoma cells. J. Cell Sci. 119, 357–369.

Kohls, M. S., Slovak, M. L., Zhang, F., Saverio, C. L., Foreman, S. J., and Bharia, R. (2002). Invasive murine breast tumors exhibit selectin mediate metastatic behavior through tumor cell extracellular matrix interactions. J. Natl. Cancer Inst. 94, 577–584.

Krichevsky, I., Lioz, S., Bob, M., Vetan, Y., Suldiga, W., Wei, S., Banevici, M., Mao, Y., Koteruik, I., Wicha, M. S., Liu, R., and Zhu, W. (2012). Expression of aldehyde dehydrogenase and CD133 defines ovarian cancer stem cells. J. Cell Sci. 125, 239–244.

Konstantinou, K., and Thomou, S. N. (2009). Cancer cells in transit: the vascular interactions of tumors cells. Annu. Rev. Bioeng. 11, 177–201.

Krysiak, I., Liu, S., Roh, M., Vetan, Y., Suldiga, W., Wei, S., Banevici, M., Mao, Y., Koteruik, I., Wicha, M. S., Liu, R., and Zhu, W. (2012). Expression of aldehyde dehydrogenase and CD133 defines ovarian cancer stem cells. J. Cell Sci. 125, 239–244.

Kuno, K., and Weinberg, R. A. (2005). The basics of epithelial-mesenchymal transition. J. Clin. Invest. 119, 1420–1427.

Kumar, R. (2004). Molecular mechanisms for cancer-associated induction of stably Lewis X and stably Lewis A expression-The Warburg effect revisited. Carcinogenesis 25, 177–202.

Kumar, R., Iwan, W., Moloke, T., Mikan, K., and Komiya, N. (2004). Carbohydrate-modulated cell adhesion in cancer metastasis and angiogenesis. Cell Mol. Life Sci. 61, 577–584.

Koepsell, D., Magee, J. A., Piskounova, E., and Morrison, S. F. (2011). Biomechanical analysis of cancer and normal cells based on bulk generation in a microfluidic device. Analyt. Chem. 83, 1432–1439.

Kym, Y. I., Bresolin, L., Han, H. L., Varki, N. M., and Varki, A. (1995). Distinct selectin ligands on colon carcinoma mucins can mediate pathological interactions among platelets, leukocytes, and endothelium. Am. J. Pathol. 146, 411–422.

Kupesic, S., Krep-Wwartan, A., D., and Uganci, M. (1999). Adhesion of human uroepithelial cells to E-selective: possible involvement of multifunctional Lewis x-glycoprotein. J. Exp. Med. 189, 239–244.

Lafrenie, R. M., Buchanan, M. R., Laufer, M., and Ugorski, M. (1996). Adhesion of human uroepithelial cells to E-selectin. Am. J. Physiol. 271, F702–F709.

Liang, S., and Dong, C. (2008). Integrin-VE-cadherin-staphylococcal negative ligands adhesion to and extravasation through the endothelium under low flow conditions. Am. J. Physiol. Cell Physiol. 295, C704–C707.

Lim, P. K., Rhoo, S. A., Paid, S. A., Taberger, M., Davis, M. A., Gregory, L. A., Gruca, S. J., Bryan, M., Patel, P. S., and Rameswaran, P. (2011). Gap junction-mediated export of microRNA from bone marrow stromal cells can elicit cell cycle quiescence in breast cancer cells. Exp. Cell Res. 317, 1550–1560.

Litwack, L. A. (1987). Biochemical mechanisms of tumor invasion and metastasis. Clin. Biochem. 20, 177–199.

Liu, S., Cloethoven, S. G., and Weich, M. S. (2012). Role of microRNAs in the regulation of breast cancer stem cells. J. Mammary Gland Biol. Neoplasia 17, 15–21.

Ludwig, R. B., Boshus, B., Podila, M., Henschel, R., Jareg, E., Tandi, C., Boshnek, W. H., Zollert, T. M., Kaufmann, R., and Gile, J. (2004). Endothelial P-selectin as a target of herapin action in experimental melanoma lung metastasis. Cancer Res. 64, 2743–2750.

Lund, A. W., and Swardt, M. A. (2010). Role of lymphatic vessels in tumor immunity: passive conduits or active participants? J. Mammary Gland Biol. Neoplasia 15, 341–352.

Ma, T. Q., and Gong, I. G. (2002). Obligatory requirement of sialylation for P-selectin binding to human sodium chloride gland carcinoma A-375-MC cells and breast carcinoma ZB-75-50 cells. J. Immunol. 168, 1090–1096.

Maggs, J. A., Paukovice, E. J., and Morris, S. J. (2012). Cancer stem cells.
impact, heterogeneity, and uncertainty. Cancer 21, 21–29.
Mam, S. S., Gao, W., Liao, M. J., Eaton, E. N., Arjunan, A., Zhou, A. Y., Brooks, M., Reiszfeld, E., Zhang, C. C., Shipston, M., Campbell, L. L., Poljak, R., Binkin, L., Yang, J., and Weinberg, R. A. (2008). The epithelial–mesenchymal transition generates cells with properties of stem cells. Cell 134, 704–715.
Manners, G., Cottrell, P., Caocci, O., Hanakata, E., Ansell, A., Nolan, R. M., Varka, A. and Berdica, M. P. (1995). Differential colony cancer cell adherence E-selectin–L-selectin role of mucin-type glycoproteins. Cancer Res. 55, 4452–4553.
Burdick, M. M. (2012). Mac-2 binding protein as a breast cancer stem cell marker. Breast Cancer Res. 18, 423–429.
Zhao, Y., Shen, J., Zhu, C. (2003). Direct observation of individual cancer cells in the multistep paradigm. Curr. Opin. Hematol. 12, 444–445.
Shriver, M. D., Cannon, R. L., Nimrichter, L. and Burdick, M. M. (2012). Selection of cancer stem cells from the population with multiple applications. Curr. Stem Cell Res. Ther. 1, 365–389.
Fumagalli, L. K. and Solano, R. L. (1991). The HNK-1 reactive sulfoglycolipid are ligands for L-selectin and P-selectin in breast cancer. Prog. Natl Acad Sci USA 88, 1393–1397.
Neve, R. M., Chin, K., Fridlyand, J., Yeh, M., Burrell, K. S., Lapuk, A., Wang, N. J., Kuo, W. L., Lander, E. S., andounge, D. B. (2005). Immunoselection of breast cancer stem cells from breast cancer cell lines. Nat. Med. 11, 87–117.
Oudry, T. T., Gupta, P. R., Mani, S. A., Yang, Y., Lander, E. S., and Weinberg, R. A. (2008). Loss of E-cadherin promotes metastasis via multiple downstream transcriptional pathways. Cancer Res. 68, 3645–3654.
Orange Wilson, K. I., Phillips, S. Y., Tilma, O. H., Amin, M. H., Li, L., Cravedi, B. D., Gurung, A. M., Iacca, F. C., Sitwala, K., Downing, J. R., Morrison, S. J. and Ross, T. S. (2009). Persistence of leukemia-maintaining cells in a conditional knockin model of an murine-receptor tyrosine-proliferative disease. Cancer Cell 16, 137–148.
Parekkadan, B. and Miblad, M. (2010). Mesenchymal stem cells as therapeutics. Annu. Rev. Biomed. Eng. 12, 87–117.
Phillips, T. M., McBeath, W. H. and Payen, F. (2006). The response of CD24+ -low/CD44+ breast cancer initiating cells to radiation. J. Natl. Cancer Inst. 98, 1777–1780.
Prini, D., Costa, A., Zaffaroni, N., Pratini, G., Petracchi, G., Ceradi, D., Pilotto, S., Pavone, A., and Daldone, M. G. (2005). Isolation and in vitro propagation of tumorigenic breast cancer cells with stem/progenitor cell properties. Cancer 96, 3506–3511.
Porada, C. D., Zanetti, E. D., and Almeda-Potlal. G. (2006). Adult bone marrow-derived stem cells: a pluripotent population with multiple applications. Curr. Stem Cell Res. Ther. 1, 365–389.
Saner, D. R., Lal, S., Bevilacqua, M. P. and Miller, L. L., Polyak, K., Brisken, C., Zhang, C. C., Shipitsin, M., Camp-

In Vivo 12, 1157–1164.
Zhu, C. (2007). Direct observation of circulating carcinoma cells. J. Pathol. 212, 6506–6511.
Perk, T., Gossels, D., Emery, P., and Giannada, P. V. (2005). Mesenchymal stem cell tissue engineering: techniques for isolation, expansion and application. In: Stem Cells, vol. 1, pp. 523–535.
Raus, F., Berndtsson, R., Richter, C., Buttman, M., Hanier, S., Osad- nik, M., Kockscherhuber, M., Boer, P., Dietl, J., Becker, C., Hing, A. and Wiecheln, J. (2009). Immunoselection of breast and ovarian cancer cells with trastuzumab and natural killer cells selective capture of CD44+ CDEC2CD24− breast cancer stem cells. Cancer Res. 69, 8806–8816.
Rakonczay, J., Pálfy, T., and Rácz, K. (2007). Endothelial and epithelial expression of sialyl Lewis(a) in lesions of breast carcinoma. J. Clin. Cancer 74, 2581.
Rhinos, L. V., Bratton, M. R., Zhu, C., Tilmann, S. L., Sobol-Delmate, T., Horton, L. W., Sara-Matac, S., Collins-Bruns, B. M., Wadebrook, S., Beirman, B. S., Wolof, E., Pots, S. T., Wronstuk, S., and Gratama, J. W. (2009). Circulating receptor CD44 mediates estrogen independent tumorigenesis, metastasis, and resistance to endocrine therapy in human breast cancer. Cancer Res. 71, 605–613.
Kathof, S., and Patell, K. (2008). Disseminated tumor cells in bone marrow and circulating tumor cells in blood of breast cancer patients: current state of detection and characterization. Pathobiology 75, 140–148.
Kathof, S., and Patell, K. (2010). Advancing personalized cancer therapy by detection and characterization of circulating tumor cells. Annu. N. Y. Acad. Sci. 1205, 66–77.
Sackstein, R. (2004). The bone marrow is akin to skin HECEL and the biology of hematopoietic stem cell homing. J. Bone Marrow 122, 1061–1069.
Sackstein, R. (2005). The lympho- cyte-choosing mechanisms: insights from the multidкуп-parallel system. Curr. Opin. Immunol. 17, 444–450.
Schweitzer, K. M., Drager, A. M., van der Valk, P., Thijssen, S. E., Zeeven, A., van der Schoot, C. E., and Langenhuijsen, M. J. (1996). Characterization of the selectin-mediated adhesion of E-selectin and vascular cell adhesion molecule-1 on endothelial cells of hematopoietic tissues. Ann. J. Hematol. 58, 166–175.
Shriver, M. D., Cannon, R. L., Nimrichter, L. and Burdick, M. M. (2010). Gangliosides expressed on breast cancer cells are E-selectin ligands. Biochim. Biophys. Acta 1803, 423–429.
Shriver, M. D., Reynolds, N. M., and Burdick, M. M. (2011). Ganglioside expressed on breast cancer cells are E-selectin ligands. Biochim. Biophys. Acta 1803, 423–429.
Shriver, M. D., Reynolds, N. M., and Burdick, M. M. (2011). Ganglioside expressed on breast cancer cells are E-selectin ligands. Biochim. Biophys. Acta 1803, 423–429.
Shriver, M. D., Reynolds, N. M., and Burdick, M. M. (2011). Ganglioside expressed on breast cancer cells are E-selectin ligands. Curr. Opin. Immunol. 18, 423–429.
Shriver, M. D., Reynolds, N. M., and Burdick, M. M. (2011). Ganglioside expressed on breast cancer cells are E-selectin ligands. Curr. Opin. Immunol. 18, 423–429.
Shriver, M. D., Reynolds, N. M., and Burdick, M. M. (2011). Ganglioside expressed on breast cancer cells are E-selectin ligands. Curr. Opin. Immunol. 18, 423–429.
Shriver, M. D., Reynolds, N. M., and Burdick, M. M. (2011). Ganglioside expressed on breast cancer cells are E-selectin ligands. Curr. Opin. Immunol. 18, 423–429.
Shriver, M. D., Reynolds, N. M., and Burdick, M. M. (2011). Ganglioside expressed on breast cancer cells are E-selectin ligands. Curr. Opin. Immunol. 18, 423–429.
migrate measured at atomic force microscopy. Am. J. Physiol. 296, C238–C292.
Silva, I. A., Bai, S., McLean, K., Yong, K., Griffith, K., Thomas, D., Ginotstein, C., Johnston, C., Kaucz, A., Reynolds, R. K., Wika, M. S., and Bucknarrow, R. J. (2011). Aldehyde dehydrogenase in combination with C2D3 defines angiogenic ovarian cancer stem cells that potentiate poor patient survival. Cancer Res. 71, 3991–4001.
Simon, S., Schöning, M., Selander, C., Borg, L., and Bendall, G. (2010). Analysis of SM1 sulfide as a Pt-selective ligand using model membranes. Biophys. Chem. 156, 68–104.
Singh, L., Kralj, O., Gilliland, D., Cocco, G., and Ross, P. (2006). Cleaning and hydrophobilization of atomic force microscopy silicon probes. J. Phys. Chem. B 110, 29579–29581.
Skordengard, K., Ventergard, E. M., Langkilde, N. C., Christensen, L. L., Wold, H., and Omrøe, T. E. (1999). Laminin antagonized mediated adhesion of freshly removed human bladder tumors to E-selectin. J. Urol. 161, 1356–1362.
Simpson, M. A., and Lund, A. W. (2012). The Warburg effect revisited. Exp. Cell Res. 270, 24–31.
Snurr, B., Shooter, S., Wiley, T. S., and Formby, B. (2001). Hyaluronidase can modulate expression of CD44. Exp. Cell Res. 266, 187–187.
Stott, S. L., He, C. H., Taik, T. D., Yu, M., Miyamoto, D. T., Walters, B. A., Rothenberg, S. M., Shah, A. M., Smas, M. E., Koret, G. K., Floyd, F. F., Gilman, A. J., Lord, J. B., Winokur, D., Springer, S., Inman, D., Nagrath, S., Sequet, L. V., Lee, R. J., Reifhzzler, K. J., Mahowald, S., Hafen, D. A., and Tomer, M. (2010). Isolation of circulating tumor cells using a microvortex generating hornpipe-chip. Proc. Natl. Acad. Sci. U.S.A. 107, 18092–18097.
Swartz, M. A., and Lund, A. W. (2012). Force microscopy silicon probes. J. Biomed. Optics 17, 3233–3251.
Thomas, S. N., Schnaar, R. L., Leong, K. W., and Liao, K. (2008). Lactate stimulates fibroblast expression of hyaluronan and CD44: the functional stretchers. J. Biomed. Optics 13, 9-40.
Tollaksen, A., Kleinman, H. K., Grant, D. S., Moraleda, D., Murchie, A. M., and Bier, S. W. (1993). E-selectin-mediated dynamic interactions of breast- and colon-cancer cells with endothelial-cell membranes. Nat. J. Cancer 60, 426–431.
Tone, A., Kleinman, H. K., Grant, D. S., Moraleda, D., Murchie, A. M., and Bier, S. W. (1993). E-selectin-mediated dynamic interactions of breast- and colon-cancer cells with endothelial-cell membranes. Nat. J. Cancer 60, 426–431.
Varlotta, A. (1997). Selectin ligands will the real ones please stand up? J. Clin. Invest 99, 158–162.
Wang, L., Ott, F. W., and Honn, K. V. (1988). Interactions of cancer cells with the microvasculature during metastases. FASEB J. 2, 12–21.
Wood, C. T., Fisher, B. L., and Ridl, W. Z. (1995). Adhesion of head and neck squamous cell carcinoma to endothelial cells. The missing links. Arch. Otolaryngol. Head Neck Surg. 121, 1279–1286.
Yang, B., and Kamm, R. D. (2005). Mechanical deformation of neutrophils into narrow channels induces pseudopod projection and changes in biomechanical properties. J. Appl. Physiol. 98, 1930–1939.
Yu, H., Tung, C. Y., Luong, W. S., Tan, C. C., Liao, K., and Tan, L. P. (2010). Mechanical behavior of human mononuclear stem cells during adipogenic and osteogenic differentiation. J. Biomech. Res. 39, 110–115.
Yi, M., Steck, S., Steck, M., Mack, H., Hafen, D. A., and Haber, D. A. (2011). Circulating tumor cells: Approaches to isolation and characterization. J. Cell Biol. 192, 373–382.
Yin, Z., Lu, D. Q., Guo, Y. L., Wang, C., Sun, J., Fang, M., Zhang, C. Y., and Liu, Y. (2008). CD44 is a major E-selectin ligand that mediates breast cancer cell transendothelial migration. PLoS ONE 3, e2926. doi: 10.1371/journal.pone.0002926.
Zin, K., Liu, D. Q., Guo, Y. L., Wang, C., Sun, J., Fang, M., Zhang, C. Y., and Liu, Y. (2008). CD44 is a major E-selectin ligand that mediates breast cancer cell transendothelial migration. PLoS ONE 3, e2926. doi: 10.1371/journal.pone.0002926.
Zhu, C., and McEver, R. P. (2005). Catch bonds: physical models and biological functions. Mol. Cell Biomech. 2, 91–104.
