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Original Research

# Effect of matrix rigidity on organ-specific capture of tumor cells by flow

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**Abstract:** Accumulating evidences have suggested that tumor metastasis exists prominent organ discrepancy. In this progression, the capture of intravascular tumor cells (TCs) to endothelium in distant tissues and organs plays a decisive role in the organ-specific metastasis formation. However, the mechanism of tumor cells preferentially arrest and adhere to endothelial cells (ECs) of target organ still remains elusive. By using the parallel plate flow chamber and the polyacrylamide gels with different matrix stiffness, we here explored the combined effects of matrix rigidity, shear stress, and chemokine SDF-1 on the capture of circulating tumor cells to ECs in the bloodstream. In addition, the expression and the role of integrin β1 on the tumor cells surface were also detected by SDF-1 treatment. The results show that breast tumor cells MDA-MB-231 display an increasing number of adherent cells on the preferred substrate, which is similar to the matrix rigidity of breast cancer tissue (about 5kPa), under a certain shear stress. Moreover, ECs exacerbates the preferred capture of tumor cells compared with the FN-coated substrate alone. Besides, SDF-1 upregulates the number of adherent tumor cells by responding to matrix stiffness via promoting the expression of integrin β1, which is abolished by blocking of integrin β1. These results may provide a novel point of view for the mechanism of "organ specificity" phenomenon in tumor metastasis, which in turn contribute to a rational development of new drugs for cancer.

***Key words:*** Matrix rigidity; Cell adhesion; Endothelium; Shear stress.

## Introduction

Metastasis of tumor cells is a leading cause of death in cancer patients (1). A large number of clinical studies have found that certain types of tumors tend to form metastases in specific organs. For example, breast and prostate cancers are more susceptible to moving to the lung, liver and bone, while colon cancer is easily transferred to the liver and lung (2,3). The specificity of the tumor cell metastasis to particular distant organs is also known as tissue tropism.

During the development of hematogenous metastasis, tumor cells (TCs) first penetrate the walls of capillary vessels, circulate through the bloodstream to distant tissues and organs, and selectively adhere and reside within the microvasculature, indicating that the capture of intravascular tumor cells to endothelial cells (ECs) depends on the environmental factors of metastatic organ (4,5). However, the factors that regulate the organ-specific activity of intravascular tumor cells in metastatic spreading remain poorly understood.

Recently, many studies had been carried out to clarify this issue and some believed that the activity of intravascular tumor cells was determined by the chemokines and cell adhesion molecules (6-10). In contrast, Ewing et al. advocated that the capture of intravascular tumor cells might possibly be regulated by the flow-induced shear stress and vascular architecture (11). Moreover, paget et al. postulated that tumor cells (seeds) only resided in the microenvironment of a particular target organ (soil), which were suitable for the formation of distant metastases (12). However, up to now, all these are still failed to fully clarify the selective mechanism of tumor cells in vivo, needless to say to test their hypothesis. Thus, these studies are inconclusive and further studies are still necessary.

Different from the previous hypothesis, we suspect that the organ-specific activity of intravascular tumor cells in vivo is not really the regulation of shear stress and chemokines, but is especially the result of matrix mechanics. In vivo, different organs or tissues render the specific matrix mechanical properties (organ specificity). It is also well known that cell is sensitive to the mechanical properties of the attached matrix (13,14). Specifically, ECM rigidity, which defined by its elastic modulus (E) in units of force per area (Pa), has been proved to play an important role in many aspects of cell behavior, including cellular morphology, differentiation, migration, and invasion, which in turn contribute to cancer progression (15-17). Kostic et al. demonstrated that matrix stiffness altered the properties of SCPs cells, such as proliferation and migration required for cancer metastasis. Meanwhile, this regulation of matrix stiffness was decreased dramatically by Fyn gene knockdown, which induced by matrix mechanics (18). Interestingly, Chiung et al. found that nanostructure dimensions of matrix were also an important physical parameter to the adherent activity of tumor cells (19). What is more, recent evidences showed that matrix stiffness affected the cell adhesion behaviors by altering the expression and function of adhesion molecules (20,21). All these results suggested that matrix mechanics might

play a vital part in the regulation of organ-specific capture of intravascular tumor cells to ECs.

Although many researches have been done about the organ-specific metastasis, the mechanism is still not clear. We therefore hypothesize that matrix rigidity, compared with flow-induced shear stress and chemokines, regulates the organ-specific capture of intravascular breast cancer cells to ECs. In this study, we sought to investigate how the combined effects of substrate mechanics, shear stress and SDF-1 modulated the capture of breast cancer cells to ECs. In addition, the expression and role of integrin β1 on the surface of breast cancer cells were also detected by treatment with SDF-1 in vitro.

## Materials and Methods

### Cell Culture

Breast cancer line MDA-MB231 and human umbilical vein endothelial cells (HUVEC) were generous gift from Third Military Medical University. MDA-MB231 were maintained in high glucose DMEM (gibco, USA) supplemented with 10% Fetal Bovine Serum(TBD, China), 24 mM NaHCO3, 2.3mM L-glutamine,100 mg/mL streptomycin, and 100 units/mL penicillin, while HUVECs were maintained in M199 (hyclone, USA) supplemented with 10% Fetal Bovine Serum(Biological Industries, Australia), 3.1 ng/mL β-ECGF, 0.108 mg/mL Heparin Sodium, 2.5 mg/mL Thymide, 2.3mM L-glutamine,100 mg/mL streptomycin, and 100 units/mL penicillin. Cells were cultured under the condition of 37°C and 5% CO2 for a few days until about 80% confluence.

### Preparation of polyacrylamide substrates

Flexible polyacrylamide substrates were generated on glass coverslips or in 6-well dish as described by Yeung et al (22). Here, the stiffness of polyacrylamide (PA) gels contained 2kPa (premalignant breast tissue), 5kPa (malignant metastatic breast cancer tissue), 10kPa (muscle tissue) and 25kPa (bone tissue), were adopted to simulate biologically relevant substrates. The formula of polyacrylamide gels with different mechanical elasticity was shown in Table 1. After that, a heterobifunctional crosslinker, was diluted to 2 mg/ mL in 50 mM HEPES and pipetted onto the gel surface for irradiating by an ultraviolet lamp for 8 min. Repeat the last step, and rinsed the gels with HEPES buffer for two times. Then, fibronectin (FN) solution (10 mg/ml) was put on the gel surface at a density of 1.0 μg/cm$^2$ and incubated at 4°C overnight. Before cell seeding, each gel was washed by PBS for twice and incubated with complete medium at 37°C for at least 30 minutes.

### Flow chamber

The flow chamber was assembled so that the coverslip formed the lower surface of the chamber (Figure 1). A peristaltic pump (JieHeng, BT-600CA, China) was used to aspirate cell suspension (1.0×105 cells per mL) through the flow chamber. Cell suspension was perfused over the coverslip for 20 min under a shear rate about 2dyne/cm$^2$. After the termination of perfusion, the flow chamber was washed with non cell culture medium for 10 min to remove non-adherent cells. Then take pictures under the microscope, and the adherent cells were counted and analyzed by SPSS software.

**Table 1.** The formula of different polyacrylamide substrates.

| Substrate rigidity (kPa) | 30% acrylamide (ml) | 1% bis-acrylamide (ml) | (3-acrylamidopropyl) trimethylammonium chloride (ml) | dd$H_2O$ (ml) |
|---|---|---|---|---|
| 2 | 0.85 | 0.30 | 0.15 | 3.55 |
| 5 | 1.00 | 0.45 | 0.15 | 3.25 |
| 10 | 1.15 | 0.85 | 0.15 | 2.70 |
| 25 | 1.35 | 1.05 | 0.15 | 2.30 |

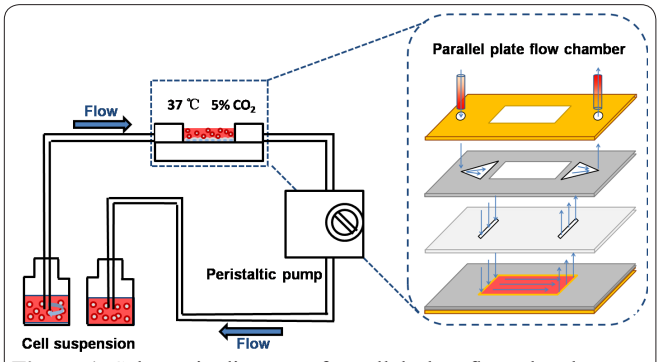


**Figure 1.** Schematic diagram of parallel plate flow chamber system.

### Antibody, Chemokine and Calcein

Here, integrin β1 antibody (Bioss, China) was used to block the role of integrin β1 on the breast cancer cells surface. After digestion of the tumor cells, the antibody was added in cell suspension (1:100) and incubated for 30 min at 37°C. To remove the residual antibody, cells were washed by PBS for twice before applied to flow. Meanwhile, to explore the effect of SDF-1 (invitrogen, USA) on integrin β1 expression of the breast cancer cells surface, partial tumor cells were treated with SDF-1 (20ng/ml,) for 6h. Calcein (invitrogen, USA) was used to distinguish the tumor cells from ECs under the microscope. The tumor cells were stained with calcein (2uM) for 30 min at 37°C in the dark, then washed twice with PBS.

### Flow cytometry

MDA-MB-231 were plated onto gels of four different rigidities and two wells of 6-well plates, and followed by culturing three days. Then, the cells were digested and counted to ensure that the number of cells in each tube exceed 10$^6$. After centrifuged at 1,000g for 5 minutes to collect the cells, PE labeled integrin β1 antibody (Abcam , England) was added into the four tubes that the cells was cultured on various gels, and PE labeled IgG antibody solution or PBS buffer were put into the other tubes for isotype control and negative control. After incubation at 4°C for 30minutes in the dark, cells were washed and diluted into 150ul PBS, and then counted using Flow cytometry (BD, USA).

### Cell capture assays

For the cell capture assay, images of adherent cells were acquired using a fluorescent microscope equipped with a CCD camera (Olympus, Japan), and at least 9 independent fields per flow experiment were recorded. All experiments were repeated at least three times independently. Analysis of acquired images was carried out by the image analysis program, Image J software.

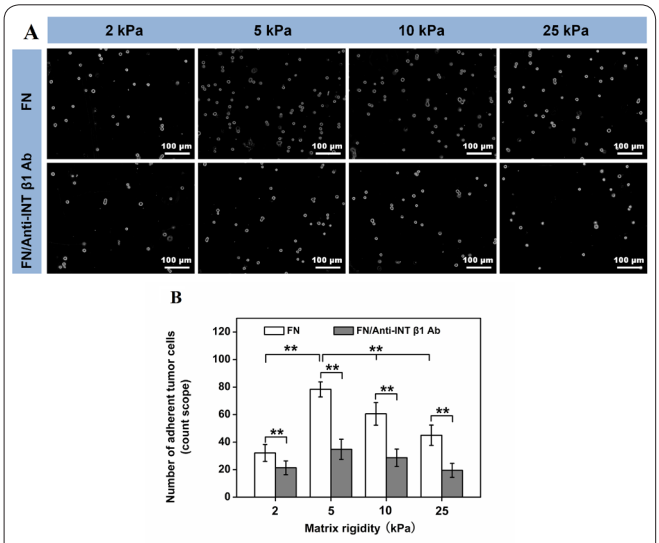


**Figure 2. Effects of matrix rigidity on tumor cells capture by shear stress.** (A) the photomicrographs of the adherent tumor cells, treated and untreated with integrin β1 antibody, captured by FN-coating substrate with different rigidity under flow; and (B) quantification of the data from the pictures, n = 9. ** P < 0.01.

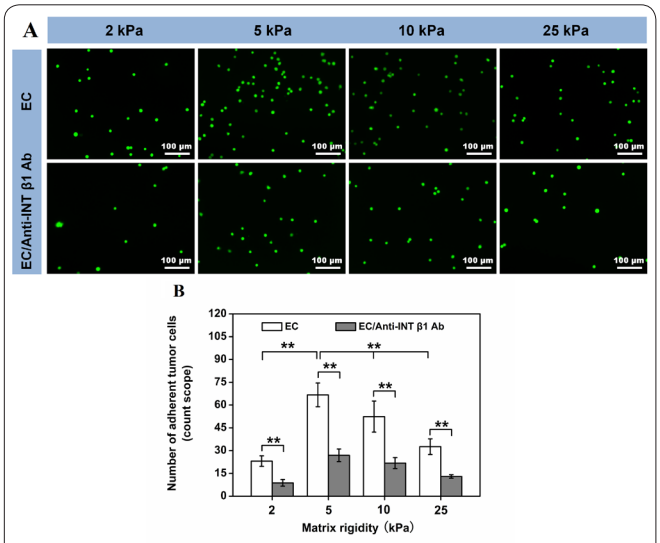


**Figure 3. Effects of ECs with different rigidity substrates on tumor cells capture by shear stress.** (A) the photomicrographs of the adherent tumor cells, treated and untreated with integrin β1 antibody, captured by endothelial cells, which cultured on substrate with different rigidity under flow; and (B) quantification of the data from the pictures, n = 9. ** P < 0.01.

## Statistical Analysis

All data were collected from at least three independent experiments and the data are expressed as mean ± SEM The statistical significance of differences between experimental groups was evaluated by student's t-test and ANOVA, using the OriginPro (version 7.5) program. $P<0.05$ was considered to be statistically significant.

## Results

### Effects of matrix rigidity on tumor cells capture by shear stress

As shown in Fig 2.A, tumor cells more likely adhered to the 5kPa FN-coating gels, whose stiffness closer to the breast cancer tissue. Noticeably, the number of attached cells was 5kPa>10kPa>25kPa>2kPa, and the 5kPa group was almost two times as much as that on the 2kPa (Fig 2.B). Moreover, the treatment with integrin β1 antibody significantly decreased the number of adherent cells for all rigidity groups. Especially, the number of attached cells on 5kPa was reduced to about 40% with integrin β1 antibody treatment, when compared with non-specific antibody treatment. These results demonstrated that integrin β1 played a critical role in the process of tumor cells capture by flow, and matrix rigidity might regulate the arrest and adhesion of tumor cells by changing the active state of FN, which in turn affected the binding of FN to integrin β1.

### Effects of ECs with different rigidity substrates on tumor cells capture by shear stress

As we know, vascular cavity inner surface covered with a layer of ECs. Almehdi et al. used the in vivo video microscopy techniques and found that the metastatic tumor cells were captured by ECs instead of the vessel wall in the capillaries of the lungs of rats (23). In the present study, we cultured ECs on different stiffness substrates for 3 days, and then challenged with shear stress. The effects of ECs on the capture of tumor cells were analyzed by immunofluorescence assay. The results showed that the number of attached tumor cells was markedly higher in the 5kPa gel groups than the other groups ($P<0.01$), and the number of adherent cells in 2kPa gel groups was the least ($P<0.01$) (Figure 3.A). After treating with integrin β1 antibody, the attached tumor cells in each group were all significantly decreased (Figure 3.B). These results indicated that ECs, culturing on different rigidity substrates, affected the capture of tumor cells in bloodstream through the integrin β1.

### Effects of SDF-1 on tumor cells capture by shear stress

To investigate whether SDF-1 regulated the capture behavior of tumor cells, the flow chamber experiments were performed with the tumor cells by SDF-1 (20 ng/ml for 6 hr) treatment. As expected, SDF-1 treatment dramatically augmented the tropism of tumor cells to endothelial cells which cultured on different rigidity substrates ($P<0.01$), and the number of attached cells was still the most in the 5kPa groups (Fig 4.A), consistent with the above results. Also, we found that the integrin β1 antibody blocked the SDF-1-induced augmentation of tumor cells capture (Fig 4.B). These results suggested that SDF-1 might enhance the preferentially capture of tumor cells via improving integrin β1 receptors. Then, we further determine whether SDF-1 affect the integrin β1 expression of tumor cells with different matrix rigidity.

### Effects of SDF-1 on integrin β1 expression of tumor cells by matrix rigidity

Recent studies uncovered that SDF-1 upregulated the expression of integrin β1 and β3, two molecules closely related with cancer adhesion and metastasis (24). To determine whether SDF-1 promote the adhesion of MDA-MB231 to varying stiffness substrates by cell adhesion molecules, we detected the level of integrin β1 expression. The results appeared that the expression of integrin β1, on the surface of breast cancer cells, was very high (Fig 5.A). Without SDF-1 treatment, there was significant difference in the expression of integrin

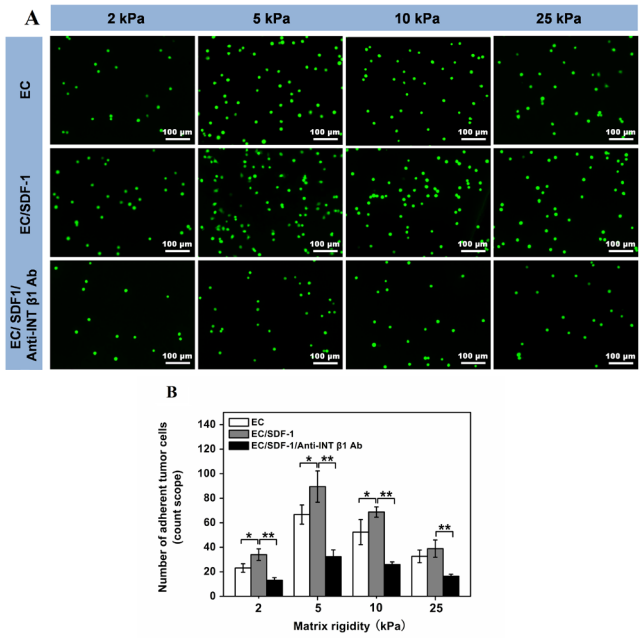


**Figure 4. Effects of SDF-1 on tumor cells capture by shear stress.** (A) the photomicrographs of the adherent tumor cells (labeling with Calcein), treated and untreated with SDF-1 or integrin β1 antibody, captured by ECs, which cultured on different rigidity substrate under flow; and (B) quantification of the data from the pictures, n = 9. ** P < 0.01, * P < 0.05.

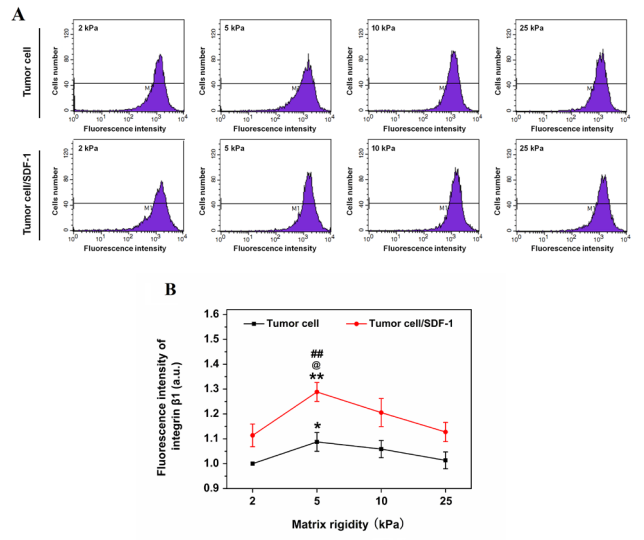


**Figure 5. Effects of SDF-1 on the expression of integrin β1 on tumor cell surface by matrix rigidity**. (A) the flow cytometry pictures of integrin β1 on tumor cell surface in different groups; and (B) quantification of the data from the pictures, n = 4. * p<0.05 and **p<0.01 compared with 2 kPa group; @ p<0.05 compared with 10 kPa group; and ## p<0.01 compared with 25 kPa group.

β1 between 5kPa group and 2kPa group, but no difference was detected with other groups (Fig 5.B). Interestingly, SDF-1 had an important impact in the integrin β1 of tumor cells. First, similar to the untreated groups, the expression of integrin β1 in 5kPa group was also the highest, and there was significant difference between the 5kPa groups and the other groups ($P$<0.01, $P$<0.05). Second, SDF-1 promoted the expression of integrin β1 in 5kPa group more drastically than the other groups (Fig 5.B). Our results suggested that SDF-1 not only enhanced the expression of integrin β1 of tumor cells with different matrix rigidity, but also enlarged the differences of tumor cells capture in each other groups.

## Discussion

Understanding the mechanisms of cancer progression will facilitate the development of novel therapeutic strategies for unrespectable tumor. Tumor metastasis is the process of invasion and growth of malignant tumor cells from primary site to the distant organs. Many factors lead to the organ specificity of tumor metastasis. Among them, matrix rigidity is a vital component of the cancer microenvironment, and it has been proved as an important cause for the "tissue tropism". In our study, we mainly investigated the capture activity of circulating tumor cells to different stiffness substrates. The results demonstrated that breast tumor cells displayed an increasing number of adherent cells on the preferred substrate (about 5kPa) by a certain shear stress. Moreover, SDF-1 exacerbated the preferred capture of tumor cells. Besides, the preferred capture of tumor cells was in response to matrix stiffness via promoting the expression of integrin β1, and the increase was abolished by blockading the integrin β1.

Mechanical forces are powerful regulators of tumor biology, and are key in the steps of cancer metastasis. Ghodsnia et al. found that tumor cells were round and showed radial spreading under static conditions, and the extent of axial spreading was strengthened under flow conditions (25). In particular, the selective adhesion of circulating cancer cells is highly dependent upon the flow induced shear stress. Liang et al. showed that shear stress changed the cell adhesion via regulating kinetic rates and affinity of integrin β2 on melanoma cells and ICAM-1 on neutrophils (26). Using a parallel-plate flow chamber, we also found that shear stress had an effect on the preferred capture of MDA-MB-231 cells to suitable stiffness substrates.

It is well known that an EC monolayer lines the lumen or inside of blood vessels, and tumor cells are actually captured by endothelial cells. Tumor cell adhesion to vascular endothelium is a central component of the metastatic cascade. A wide variety of publications have demonstrated that ECM can regulate the morphology, cytoskeleton reorganization, stress fiber distribution and adhesion molecule expression of endothelial cells (27,28). Moreover, recent studies indicated that cell had much more traction forces on substrates of preferred rigidity (29,30). The altered mechanical property of tumor microenvironment increased integrin clustering and addition of focal adhesion proteins, and following by a change in cytoskeletal organization (31,32). From our data, we also found matrix-dependent difference on the capture of circulating tumor cells to ECs by shear tress. The number of adherent cells on the 5kPa FN-coating gel was the most, then was the 10kPa gel, and at last was the 2kPa gel. The number of cells in 5kPa group was even almost two times as much as that in 2kPa. It reminds that the circulating tumor cells are more likely to adhere to the substrates, whose stiffness is closer to the breast cancer tissue.

The integrins, a family of adhesion receptors, are key molecules in tumorigenesis, invasion and metastasis of cancer (33).Many studies have shown that integrin β1

has been linked to tumor cell adhesion to endothelial cells or ECM (34-37). Our data further identified that the inhibition of integrin β1 was very effective in blocking MDA-MB-231 cells adhesion to FN or ECs. After using the integrin β1 antibody, the number of adherent cells was reduced by 60%.

Notably, current evidence showed that SDF-1 strongly contributed to cancer progression (38,39). Much had been done to explore the response of the tumor cells to SDF-1. Sun et al. reported that SDF-1 transfection increased the invasive ability of MDA-MB-231, and revealed that CXCL12-CXCR4 axis promoted the natural selection of breast cancer cell metastasis (40). Furthermore, recent studies appeared that SDF-1 enhanced the expression of integrin on the transcriptional level in tumor cells (41,42). To test the effect of SDF-1 and substrates rigidity on the expression of integrin β1, we cultured MDA-MB-231 on top of the 3-dimensional polyacrylamide substrates with different rigidities for 3 days. It was noteworthy that SDF-1 upregulated the expression of integrin β1 in every group, and the expression of integrin β1 in 5kPa group was higher than any of the other groups ($P< 0.05$) after stimulating tumor cells with SDF-1 for 6 hours. The findings indicated that SDF-1 could strengthen matrix-dependent differences through promoting the expression level of integrin β1 in various rigidity groups.

In summary, the study demonstrated that matrix rigidity played a critical role in promoting the preferred capture of breast cancer cell by flow. Suitable substrates rigidity facilitated the tumor cells adhesion to ECs. Noticeably, the increasing number of adherent cells was abolished by blockading of integrin β1. In addition, SDF-1 improved the capture of tumor cells to ECs via upregulating the expression level of integrin β1. Generally, matrix rigidity, endothelial cells and SDF-1 significantly contributed to the organ-specific metastasis of tumor cells. The exploration of the capture activity of breast tumor cells in response to different substrate stiffness may provide novel views into the mechanism of "organ specificity" phenomenon in cancer metastasis, which contributes to a rational development of innovative anti-cancer drugs in the future.

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## References

1. Chaffer CL, Weinberg RA. A perspective on cancer cell metastasis. Science 2011; 331:1559-64.

2. Kerstin S, Peter G, Andreas E, Timo K, Andre HB, Jens H, Jörg H. Organ- specific metastatic tumor cell adhesion and extravasation of colon carcinoma cells with different metastatic potential. Am J Pathol 2006; 169:1064-73.

3. Ursini SJ, Siegel PM. The influence of the pre-metastatic niche on breast cancer metastasis. Cancer Lett 2016; 380:281-8.

4. Galie PA, van Oosten A, Chen CS, Janmey PA. Application of multiple levels of fluid shear stress to endothelial cells plated on polyacrylamide gels. Lab Chip 2015; 15:1205-12.

5. Burdick MM, McCaffery JM, Kim YS, Bochner BS, Konstantopoulos K. Colon carcinoma cell glycolipids, integrins, and other glycoproteins mediate adhesion to HUVECs under flow. Am J Physiol Cell Physiol 2003; 284:977-87.

6. Mitchell B, Leone D, Feller JK, Bondzie P, Yang S, Park HY, Mahalingam M. Correlation of chemokine receptor CXCR4 mRNA in primary cutaneous melanoma with established histopathologic prognosticators and the BRAF status. Melanoma Res 2014; 24:621-5.

7. Ejaeidi AA, Craft BS, Puneky LV, Lewis RE, Cruse JM. Hormone receptor-independent CXCL10 production is associated with the regulation of cellular factors linked to breast cancer progression and metastasis. Exp Mol Pathol 2015; 99:163-72.

8. Singh AK, Arya RK, Trivedi AK, Sanyal S, Baral R, Dormond O, Briscoe DM, Datta D. Chemokine receptor trio: CXCR3, CXCR4 and CXCR7 crosstalk via CXCL11 and CXCL12. Cytokine Growth Factor Rev 2013; 24:41-9.

9. Izumi D, Ishimoto T, Miyake K, Sugihara H, Eto K, Sawayama H, Yasuda T, Kiyozumi Y, Kaida T, Kurashige J, Imamura Y, Hiyoshi Y, Iwatsuki M, Iwagami S, Baba Y, Sakamoto Y, Miyamoto Y, Yoshida N, Watanabe M, Takamori H, Araki N, Tan P, Baba H. CXCL12/CXCR4 activation by cancer-associated fibroblasts promotes integrin b1 clustering and invasiveness in gastric cancer. Int J Cancer 2015; 138:1207-19.

10. Yang Z, Zhou X, Liu Y, Gong C, Wei X, Zhang T, Ma D, Gao Q. Activation of integrin β1 mediates the increased malignant potential of ovarian cancer cells exerted by inflammatory cytokines. Anticancer Agents Med Chem 2014; 14:955-62.

11. Ewing J. Neoplastic diseases, a treatise on tumors. Can Med Assoc J 1924; 14: 466.

12. Paget S. The distribution of secondary growths in cancer of the breast. Cancer Metastasis Rev 1989; 8:98-101.

13. Discher DE, Janmey P, Wang YL. Tissue cells feel and respond to the stiffness of their substrate. Science 2005; 310:1139-43.

14. Engler AJ, Shamik S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. Cell 2006; 126:677-89.

15. Ko P, Kim D, You E, Jung J, Oh S, Kim J, Lee KH, Rhee S. Extracellular Matrix Rigiditydependent Sphingosine-1-phosphate Secretion Regulates Metastatic Cancer Cell Invasion and adhesion. Sci Rep 2016; 15:e21564.

16. Jerrell RJ, Parekh A. Matrix rigidity differentially regulates invadopodia activity through ROCK1 and ROCK2. Biomaterials 2016; 84:119-29.

17. Ali MY, Anand SV, Tangella K, Ramkumar D, Saif TA. Isolation of primary human colon tumor cells from surgical tissues and culturing them directly on soft elastic substrates for traction cytometry. J Vis Exp 2015; 4:e52532.

18. Kostic A, Lynch CD, Sheetz MP. Differential matrix rigidity response in breast cancer cell lines correlates with the tissue tropism. PLoS ONE 2009; 4:e6361.

19. Chiung WK, Chueh DY, Chen P. Investigation of size–dependent cell adhesion on nanostructured interfaces. J Nanobiotechnology 2014; 12:e54.

20. Schmitz J, Benoit M, Gottschalk KE. The Viscoelasticity of Membrane Tethers and Its Importance for Cell Adhesion. Biophys J 2008; 95:1448-59.

21. Wu L, Xiao BT, Jia XL, Zhang Y, Lü SQ, Chen J, Long M. Impact of carrier stiffness and microtopology on two- dimensional kinetics of P-selectin and P-selectin glycoprotein ligand- 1 (PSGL-1) interactions. J Biol Chem 2007; 282:9846-54.

22. Yeung T, Georges PC, Flanagan LA, Marg B, Ortiz M, Funaki M, Zahir N, Ming W, Weaver V, Janmey PA. Effects of Substrate Stiffness on Cell Morphology, Cytoskeletal Structure, and Adhesion. Cell Motil Cytoskeleton 2005; 60:24-34.

23. Al-Mehdi AB, Tozawa K, Fisher AB, Shientag L, Lee A, Mu-

schel RJ. Intravascular origin of metastasis from the proliferation of endothelium-attached tumor cells: a new model for metastasis. Nat Med 2000; 6:100-2.
24. Yu Y, Shi X, Shu Z, Xie T, Huang K, Wei L, Song H, Zhang W, Xue X.Stromal cell-derived factor-1 (SDF-1)/CXCR4 axis enhances cellular invasion in ovarian carcinoma cells via integrin β1 and β3 expressions. Oncol Res 2013; 21:217-25.
25. Chotard-Ghodsnia R, Haddad O, Leyrat A, Drochon A, Verdier C, Duperray A. Morphological analysis of tumor cell /endothelial cell interactions under shear flow. J Biomech 2007; 40:335-44.
26. Liang SL, Fu CL, Wagner D, Guo HG, Zhan DY, Dong C, Long M. Two- dimensional kinetics of 2- integrin and ICAM- 1 bindings between neutrophils and melanoma cells in a shear flow. Am J Physiol Cell Physiol 2008; 294:743-53.
27. Galie PA, van Oosten A, Chen CS, Janmey PA. Application of multiple levels of fluid shear stress to endothelial cells plated on polyacrylamide gels. Lab Chip 2015; 15:1205-12.
28. Weng S, Fu J. Synergistic regulation of cell function by matrix rigidity and adhesive pattern. Biomaterials 2011; 32:9584-93.
29. McGrail DJ, Kieu QM, Iandoli JA, Dawson MR. Actomyosin tension as a determinant of metastatic cancer mechanical tropism. Phys Biol 2015; 12:e026001.
30. Mih JD, Marinkovic A, Liu F, Sharif AS, Tschumperlin DJ. Matrix stiffness reverses the effect of actomyosin tension on cell proliferation. J Cell Sci 2012; 125:5974-83.
31. Levental KR, Yu H, Kass L, Lakins JN, Egeblad M, Erler JT, Fong SF, Csiszar K, Giaccia A, Weninger W, Yamauchi M, Gasser DL, Weaver VM. Matrix crosslinking forces tumor progression by enhancing integrin signaling. Cell 2009; 139:891-906.
32. Ulrich TA, de Juan Pardo EM, Kumar S. The mechanical rigidity of the extracellular matrix regulates the structure, motility, and proliferation of glioma cells. Cancer Res 2009; 69:4167-74.
33. Desgrosellier JS, Cheresh DA. Integrins in cancer: Biological implications and therapeutic opportunities. Nat Rev Cancer 2010; 10:9-22.
34. Saito Y, Sekine W, Sano R, Komatsu S, Mizuno H, Katabami K, Shimada K, Oku T, Tsuji T. Potentiation of cell invasion and matrix metalloproteinase production by alpha3beta1 integrin-mediated adhesion of gastric carcinoma cells to laminin-5. Clin Exp Metastasis 2010; 27:197-205.
35.Chen CN, Chang CC, Lai HS, Jeng YM, Chen CI, Chang KJ, Lee PH, Lee HY. Connective tissue growth factor inhibits gastric cancer peritoneal metastasis by blocking integrin alpha3beta1-dependent adhesion. Gastric Cancer 2015; 18:504-15.
36. Sanz-Rodríguez F, Hidalgo A, Teixidó J. Chemokine stromal cell-derived factor-1alpha modulates VLA-4 integrin-mediated multiple myeloma cell adhesion to CS-1/fibronectin and VCAM-1. Blood 2001; 97:346-51.
37. Luo J, Li C, Xu T, Liu W, Ba X, Wang X, Zeng X. PI3K is involved in beta1 integrin clustering by PSGL-1 and promotes beta1 integrin-mediated Jurkat cell adhesion to fibronectin. Mol Cell Biochem 2014; 385:287-95.
38. Orimo A, Gupta PB, Sgroi DC, Seisdedos FA, Delaunay T, Naeem R, Carey VJ, Richardson AL, Weinberg RA. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell 2005; 121:335-48.
39. Wald O, Izhar U, Amir G, Kirshberg S, Shlomai Z, Zamir G, Peled A, Shapira OM. Interaction between neoplastic cells and cancer-associated fibroblasts through the CXCL12/CXCR4 axis: role in non-small cell lung cancer tumor proliferation. J Thorac Cardiovasc Surg 2011; 141:1503-12.
40. Sun YN, Mao XY, Fan C, Liu C, Guo A, Guan S, Jin Q, Li B, Yao F, Jin F. CXCL12-CXCR4 axis promotes the natural selection of breast cancer cell metastasis. Tumour Biol 2014; 35:7765-73.
41. Wang B, Wang W, Niu W, Liu E, Liu X, Wang J, Peng C, Liu S, Xu L, Wang L, Niu J. SDF-1/CXCR4 axis promotes directional migration of colorectal cancer cells through upregulation of integrin alphavbeta6. Carcinogenesis 2014; 35:282-91.
42. Jiang YP, Wu XH, Xing HY, Du XY. Role of CXCL12 in metastasis of human ovarian cancer. Chin Med J (Engl) 2007; 120:1251-5.