Abstract: Metastasis is the main cause of cancer death, and tumor cells mainly disseminate to the distal organs through the blood circulation, in which they experience considerable levels of fluid shear stress. CTCs are heterogeneous with diverse subpopulations of distinct genotypes and phenotypes and the frequency of CTCs is correlated with poor prognosis and overall survival in cancer patients. Less than 0.01% of them may eventually generate metastatic tumors in secondary sites, indicating the inefficiency of metastasis. Nevertheless, metastasis accounts for over 90% of cancer-related deaths, suggesting that a subpopulation of CTCs are able to survive the metastatic process and form metastases. To target metastasis, it is thus essential to understand the roles of various factors during dissemination in the survival and functions of CTCs. However, the effects of hemodynamic shear stress on biophysical properties and functions of CTCs in suspension are not fully understood. This study was to investigate the effect of hemodynamic shear stress on the survival and anti-chemotherapy ability of suspended circulating tumor cells during metastasis, and the effect of actomyosin activity on this regulation. In this study, we developed a circulatory system to generate physiologic levels of hemodynamic shear stress, which mimicked certain important aspects of the CTC microenvironment in blood circulation. The survival of tumor cells in suspension, as a model for real CTCs, under different shear stress and circulation duration was examined. We found that the majority of breast tumor cells s in suspension can be eliminated by hemodynamic shear stress. The surviving cells exhibit unique biophysical properties, including significantly retarded cell adhesion, mesenchymal-like cell morphology, and reduced F-actin expression and cellular stiffness. Cancer stem cells which has been reported in other papers have lower stiffness compared with conventional tumor cells showed significantly higher survival in blood flow. Importantly, low actomyosin activity promotes the survival of CTCs in blood shear flow while high actomyosin activity inhibits tumor cells surviving shear stress treatment. These findings might be explained by the up- and down-regulation of the anti-apoptosis genes. Soft surviving tumor cells held survival advantages in shear flow and higher resistance to chemotherapy. Metastasis is closely linked with chemoresistance. However, the underlying mechanisms have not been fully understood, in particular, the roles of hemodynamic shear stress and actomyosin-dependent cell mechanics in drug resistance of CTCs remain unclear. Inhibiting actomyosin activity in suspended tumor cells enhanced chemoresistance, while activating actomyosin suppressed this ability. These findings might be associated with the corresponding changes in multidrug resistance related genes. Our study unveils the regulatory roles of actomyosin in the survival and drug resistance of circulating tumor cells in hemodynamic shear flow, which imply the importance of fluid shear stress and actomyosin activity in tumor metastasis. Our findings reveal a new mechanism by which circulating tumor cells are able to survive hemodynamic shear stress and chemotherapy and may offer a new potential strategy to target circulating tumor cells in shear flow and combat chemoresistance through actomyosin.

Acknowledgments: We acknowledge the support from National Natural Science Foundation of China (11672255), Shenzhen Science and Technology Innovation Commission (JCYJ20170303160515987, JCYJ20170413154735522), Early Career Scheme from Research Grants Council of the Hong Kong Special Administrative Region, China (PolyU 252094/17E), and the internal grant from the Hong Kong Polytechnic University (1-ZE4Q, 1-ZVJ8).

E-选择素调控白细胞跨内皮转运中内皮细胞骨架重组动力学

黄丹丹[1,2]， 龚一心[1,2]， 章 燕[1,2*]， 龙 勉[1,2*]
(1.中国科学院力学研究所,生物力学与生物工程中心/工程化构建与力学生物学北京市重点实验室,
中国科学院微重力重点实验室，北京 100190; 2 中国科学院大学工程科学学院，北京 100049)
E-mail: zhangyan@imech.ac.cn, mlong@imech.ac.cn

摘要: 目的 以血管稳态及重建为切入点,研究 E-选择素(E-selectin) 通过调节微丝骨架网络的动态重组来调控内皮细胞低阻

抗区的形成,从而导致白细胞跨膜迁移增强的信号调控网络和关键分子机制。方法 采用 Lifeact_GFP 转染人脐静脉内皮细胞(Human Umbilical Vein Endothelial Cells, HUVEC) ,可使内皮细胞纤维状肌动蛋白(F-actin) 可视化,并实时动态观察白细胞跨内皮迁移过程内皮细胞骨架重组动力学。采用荧光漂白后恢复技术(Fluorescence Recovery After Photobleaching, FRAP) 量化 E-选择素及其配体交联下,内皮细胞不同区域的细胞骨架重组能力。采用 RNA 干扰技术降低 HUVEC 上 E-selectin 表达,并利用 AFM 检测 E-selectin 干扰前后 HUVEC 各区域硬度分布,结合小分子抑制剂考察 E-selectin 调控细胞骨架重组从而介导白细胞跨内皮迁移的分子机制。结果 白细胞跨内皮迁移过程,内皮细胞上可形成孔洞、且白细胞倾向于旁细胞迁移。FRAP 结果表明内皮细胞胞体上微丝蛋白恢复速度更快,E-selectin 与其配体交联可改变微丝蛋白骨架重组能力。干扰 HUVEC 内 E-selectin 表达可降低胞间连接的硬度,并增加胞间间隙的面积,有利于白细胞跨内皮迁移。采用 Cyto D 破坏细胞骨架、CK666 抑制 Arp2/3 活性均可增加白细胞在对照组内皮细胞上的迁移,但是干扰 E-selectin 后,这些小分子抑制剂的作用消失。结论 E-选择素通过 Arp2/3 调节内皮细胞骨架的重组,降低胞间连接的硬度,增加胞间间隙的面积,并最终在胞间连接处形成低阻抗区,进而促进白细胞迁移。

关键词: E-选择素; Arp2/3; 细胞骨架重组; 低阻抗区; 白细胞迁移
致谢: 国家自然科学基金项目(31627804,91642203,11772345, 91539119)

VEGF 可变剪切在细胞外基质硬度调控神经母细胞瘤功能中的作用

鲍 敏, 刘继婷, 陈 毅, 汪文斌, 齐颖新*
(上海交通大学 生命科学技术学院,力学生物学研究所, 上海 200240)
E-mail: qiyx@ sjtu.edu.cn

摘要: 目的 肿瘤在发生发展过程中,细胞外基质(extracellular matrix,ECM) 中大量蛋白质沉积交联从而引起 ECM 硬度增大,影响肿瘤组织增殖、血管新生能力。血管内皮生长因子(Vascular Endothelial Growth Factor,VEGF) 对肿瘤血管新生有重要调控作用,它可变剪切会产生不同功能的亚型。本研究旨在探讨不同 ECM 硬度是否调控 VEGF 可变剪切及其机制。方法与结果 通过 Western Blot、qPCR 检测,揭示不同硬度的聚丙烯酰胺凝胶(1 kPa、8 kPa、30 kPa、Plastic) 调控神经母细胞瘤中 VEGF 剪切体和可变剪切分子 SRSF1 的表达。siRNA 干扰 SRSF1 之后,VEGF 各亚型 mRNA 表达水平均呈一定程度下降。免疫荧光与核质分离实验发现不同 ECM 硬度条件下 SRSF1 没有发生明显的核质转移。通过 Western Blot 检测发现 YAP、转录因子 RUNX2 在不同硬度基质胶上与 SRSF1 呈相同表达趋势,同时 YAP 在不同 ECM 硬度条件下发生核质转移。通过 siRNA 干扰以及 YAP 过表达质粒的构建证明 YAP 通过 RUNX2 调控 SRSF1 的表达。不同 ECM 硬度条件下的肿瘤细胞增殖能力以及共培养条件下的 HUVEC 迁移能力发生显著性变化。通过 siRNA 分别干扰 YAP、SRSF1 之后,肿瘤细胞增殖能力明显降低。结论 不同 ECM 硬度调控神经母细胞瘤细胞可变剪切分子 SRSF1 及其下游 VEGF 各亚型的表达,同时 YAP、转录因子 RUNX2 在不同 ECM 硬度刺激下与 SRSF1 出现同样的变化趋势。不同 ECM 硬度条件刺激下的肿瘤细胞增殖能力以及共培养条件下的 HUVEC 迁移、血管新生能力发生改变。综上,不同 ECM 硬度可能通过 YAP 调控 SRSF1 的表达、VEGF 的可变剪切,进而影响神经母细胞瘤的增殖能力。

关键词: 神经母细胞瘤; 细胞外基质硬度; 可变剪切; 血管新生
致谢: 国家自然科学基金项目(11625209)