

namics)方法^[9-16]和NAMD程序^[10-17]对选择素P-selectin在经验势场CHARMM22^[18]作用下的去折叠过程进行了模拟。基本步骤如下:(1)初始构象建立:以蛋白质数据库中P-selectin的晶体结构(1G1Q)为起点,保留四聚体中的一个单体,然后用PSFGEN^[18]加氢,VMD加球形水边界^[19],得到包括氢原子坐标在内的系统初始构象;(2)系统驰豫(relaxation):在系统能量极小化后使系统升温至室温,然后自由驰豫至系统温度和位移均方根(RMSD)相对稳定状态(对应涨落足够小);(3)动态模拟:固定P-selectin EGF区(上皮样生长因子区)C末端 α -碳原子,在P-selectin/PSGL-1相互作用的结合位点上施加线性力^[20],使得分子在外力作用下去折叠。

我们比较了不同作用位点、不同拉伸速度等条件下P-selectin的去折叠过程。通过对模拟得到的微观构象变化和对应力谱、位谱、能谱的分析,可得到P-selectin去折叠过程中非共价相互作用的影响和势垒分布,以及P-selectin去折叠受作用位点和/或拉伸速度的影响。作为考察P-selectin/PSGL-1复合物解离的第一步,P-selectin去折叠过程的构象变化将为应用SMD模拟P-selectin/PSGL-1复合物解离提供物理方法和计算基础,同时,其结果还可与应用AFM实验测定P-selectin去折叠和P-selectin/PSGL-1复合物解离的结果相对照。

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Effects of Surface Microtopology and Membrane Stiffness on Kinetics of Selectin/Ligand Interactions by a Modified BFP

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More and more evidences come out to support that the functionality of adhesion molecules are influenced by the surface microtopology of cell carrier or substrate. Adhesive molecules usually express on the microvilli of a cell, providing a well-defined spatial configuration to mediate the adhesions to the counterpart molecules on the apposed surface. In a pioneering work, comparisons were done by using immunoglobulin (IgG) binding to their counterpart receptors CD16 expressed onto the membrane of transfected K562 cells (rough surfaces) and coated onto the surface of human red blood cells (smooth surfaces), respectively^[1]. Here we extended this idea by developing a biomembrane force probe to elucidate the effect of surface microtopology and membrane stiffness on the kinetics of selectin/ligand binding.

A modified protocol was employed to develop a biomembrane force probe^[2-4]. Briefly, commercial polystyrene microsphere (with a radius of 0.56 - 5.6 μm) cross-linked with streptavidin were incubated with biotinylated human red blood cells, and then incubated with biotinylated proteins (P-selectin or P-selectin glycoprotein ligand 1, PSGL-1). This resulted in forming a modified Biomembrane Force Probe (mBFP) with relatively smooth surface and enhanced membrane stiffness.

A human promyelocytic leukemia cell line (HL-60) expressing the carbohydrate ligands was used as a control.

Interactions of selectins and their ligands mediate rolling and tethering of leukocytes to the endothelial wall under flow, which are important to inflammatory response and tumor metastasis cascade^[5,6]. Following the line of kinetic measurements developed before^[7-10], we measured experimentally via a micropipet aspiration assay the binding of P-selectin/PSGL-1 interactions in three categories: 1) A mBFP binds to a HL-60 cell; 2) A mBFP binds to a red cell coated with purified PSGL-1 from neutrophils^[7,8]; 3) A mBFP binds to a microsphere coated with PSGL-1. Upon the well-developed probabilistic model^[7-10], kinetic rates and binding affinities were predicted by fitting the measured data with the model. Comparisons of kinetic parameters were made from the three categories to distinguish the effect of surface microtopology. Force measurements demonstrated that mBFP is the more accurate force transducer compared with RBC itself. We concluded that both microtopology and stiffness impact the interactions of receptors and their ligands. These further the understandings of cell adhesions mediated by adhesive molecules from various microtopological surfaces.

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力学环境因素与骨髓间充质干细胞的增殖与分化

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间充质干细胞具有多向分化功能, 在体外培养时仍可保持其增殖和分化的能力, 可以作为组织工程的一种较为理想的细胞来源。细胞与胞外环境之间以及细胞与细胞之间的相互作用对细胞增殖和分化具有重要影响。对干细胞的研究大多集中于生化因素的影响, 而且一般是在静态的常规细胞培养容器中进行。在本研究中, 尝试在骨髓间充质干细胞的培养过程中, 施加不同的力学环境, 考查不同的力学信号刺激对骨髓间充质干细胞增殖和分化的影响。为此采用两种细胞培养系统:(1)运用三维旋转细胞培养系统对骨髓间充质干细胞进行高密度培养, 旋转培养器可以提供一个三维的、低剪切的环境, 并能保证充分的物质交换。主要考查该培养系统对骨髓间充质干细胞增殖的影响;(2)运用弹性基底动态加载培养系统, 对培养细胞的基底施加不同频率和幅度的动态加载, 考查不同水平的应力刺激对骨髓间充质干细胞增殖和分化的影响。目前获得的实验数据表明, 在三维旋转细胞培养系统内可以实现骨髓间充质干细胞的高密度培养, 在该条件下部分细胞可以三维生长, 培养效率得到提高。增殖后的骨髓间充质干细胞其分化能力与常规培养方法获得的细胞无显著差异。应力刺激可以显著地改变骨髓间充质干细胞的铺展形态, 并影响细胞增殖与分化的进程。

作用力对 TNF- α 抗原/抗体二维反应的影响*

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TNF- α 是一种多功能的细胞因子, 具有杀伤或抑制肿瘤细胞, 提高中性粒细胞吞噬能力等多种重要的生物学功能; 同时也发现各种原因引发的感染性休克、炎症、自身免疫性疾病均表现为 TNF- α 表达水平过高。大量的研究结果表明, TNF- α 参与诸如脓毒症、感染、自身免疫疾病、移植排斥、移植抗宿主病等多种人类疾病。随着 TNF 晶体结构的获得, 目前已着手设计治疗策略以抑制或抵消 TNF- α 的活性。其中, 国内外正在寻找中和 TNF- α 的抗体作为抑制 TNF- α 活性的手段。