细胞分子生物力学

整合素对肝癌细胞与 IV 型胶原粘附与趋化行为的影响

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采用微吸管实验技术，测定肝细胞癌（hepatocellular carcinoma，HCC）细胞与 IV 型胶原被衬表面的粘附力；进一步加入针对整合素 alpha1 ~ alpha6（CD49a ~ CD49f）和 beta1 亚单位（CD29）的单克隆抗体（Anti - CD49a ~ Anti - CD49f、Anti - CD29）处理 HCC 细胞，观察 Anti - CD29 对细胞与 IV 型胶原被衬表面的粘附力的影响。采用双微吸管实验法进行 HCC 细胞趋化实验，在两侧微管内加入相同浓度的 IV 型胶原，并引导微管尖端与同一细胞紧密接触，动态观察细胞两侧伪足形成过程；在一侧微吸管内分别加入 Anti - CD49a ~ Anti - CD49f 、Anti - CD29，考察整合素亚单位阻断对 HCC 细胞伪足形成的影响。利用流式细胞仪对 HCC 细胞表面整合素亚单位的表达进行分析。从而可以直观清楚地得到整合素分子阻断对 HCC 与 IV 型胶原被衬表面粘附和整合素分子对介导 HCC 细胞向 IV 型胶原发生趋化行为的影响。

Effects of Molecular Length, Ortientation and Amino Acid Mutation on Kinetics of Selectin/Ligand Interactions

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Receptor/ligand interactions are basic issues to cell adhesion, which are important to many physiological and pathological processes such as lymphocyte - mediated cytotoxicity, tumor metastasis and inflammatory reaction1. Selectin/carbohydrate ligand bindings have been found to mediate the fast rolling of leukocytes on activated endothelial monolayer[1,2]. Kinetic rate and binding affinity constants are essential determinants of cell adhesion.

Selectins contain a single N - terminal lectin (Lec) domain, followed by an epidermal growth factor (EGF) - like domain, a series of short consensus repeats (CRs), a transmembrane region, and a cytoplasmic tail. The functionality of each domain remains unclear[3]. Biochemical measurements revealed that although the Lec/EGF domains could mediate the leukocyte adhesion independently, they were not sufficient to initiate or support stable rolling[4,5]. The length and orientation of selectins play a critical role in leukocyte rolling[3-4,6-8]. Amino acid mutation of selectins and ligands also affect the efficiency of selectin - ligand bindings[3,9-12].

To examine how the molecular structures of selectins affects the features that favor selectin - mediated adhesions, a micropipette aspiration assay was used to measure the binding of selectin/ ligand and data were compared with the predictions using a small system probabilistic model[13-17]. P - and E - selectin constructs were coupled onto the surfaces of healthy human red blood cells (HRBC), which in turn bound to selectin ligands expressing on a human promyelocytic leukemia cell line (HL - 60). Variations of molecular length and orientation were defined by two different coupling modes. In a capture mode, anti - selectin monoclonal antibodies (mAbs) were coated onto the HRBC using CrCl3 protocol[13,16], which then captured the Lec/EGF or extra - cellular selectin constructs. In a directly coating mode, extra - cellular selectin constructs were directly coated onto the HRBC surfaces. Combination of the two modes resulted in the molecular models of different lengths and orientations of selectin constructs. Besides, effect of E - selectin mutated in specific amino acid residues (e. g., residue 264) was also tested[4].

Kinetic rate and binding affinity constants were predicted by comparing the measured data with the probabilistic model[13,16]. Results suggested that the molecular length, orientation and amino acid mutation had large influences on the binding affinities of (2 ~ 4 folds) but not the reverse kinetic rate of selectin - ligand binding. Data presented here were reasonable and consistent with previous works[18]. The outcomes further our understanding of selectin/ligand binding and provide a basis for quantitative descriptions of interactions between flowing cells and the vessel wall under physiological conditions. (Supported by NSFC grants 10072071, 10128205, and 30225027).

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The Effect of Concentration on Confirmational Transition of Fibroin

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Introduction The silk fibers of silkworm have extraordinary mechanical properties. People commonly recognize that the ? - sheet structure in it mainly contributes that. So the formation of silk fiber from fibroin gel should be studied. Fibroin is primarily synthesized in the posterior division of silk gland of fifth - instar silkworm larva and from the posterior part to the anterior part of silk gland, the concentration of solution increases as well as a conformational transition, ? - helix to ? - sheet transition, occurs simultaneously. But little is known about the detailed process of the conformational transition under the influence of the concentration of fibroin gel. The present work studies the conformational transition of fibroin solution when it is diluted.

Materials and Methods The posterior division of silk gland was taken out from fifth - instar tussah. The obtained fibroin solution was diluted with distilled water as a series of times, i. e. 8, 16, 32, 64, 128, 192, 256, 320, 384, 640. The concentration of fibroin solutions was measured by BCA protein assay kit . The content of secondary structures of fibroin were measured and calculated by circular dichromatic spectrum instrument.

Results The relation between contents of all conformations and concentrations of fibroin solutions is presented in Fig. 1. It can be shown that the content of ? - sheet retains about 45% when the solutions are diluted from 2.66 wt% to 0.33 wt%. But the content of ? - sheet increases up to 57% at the concentration of 0.15 wt% and 0.11 wt%. Then it dramatically decreases up to 12.9% when the solutions are diluted up to 0.33 wt%. These results agree with the experimental results of Ayub et al. (1994) and Inoue et al. (2000). It is noteworthy that Ayub et al. and Inoue et al. obtained their solutions by dilution. In contrast with the variation of ? - sheet, the content of ? - helix decreases slightly at first and then increases dramatically when the solution is gradually diluted. It can also be found from Fig. 1 that when initially diluting, the contents of ? - turn and random coil in the solution do not change and then increase slightly in the region of dramatical variation of ? - sheet. So it may be predicted that most of ? - sheet structure converted into ? - helix structure at the lower concentration.

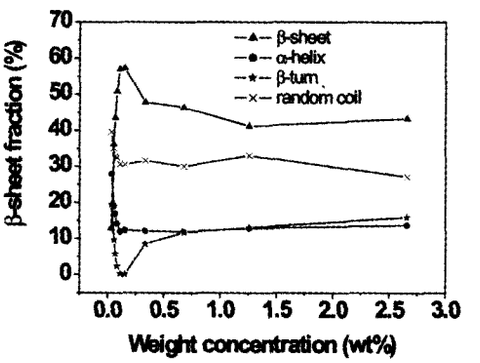

Fig. 1 The relation between the contents of all secondary structures of fibroin solutions and their concentrations.

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