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Title: Fusion protein TAP-SSL5 suppresses binding of platelet microparticles to THP-1 cells and activation of Mac-1

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关键词: [血小板微粒](#); [金黄色葡萄球菌超抗原样蛋白-5](#); [蛭抗凝血肽](#); [融合蛋白](#); [THP-1细胞](#); [Mac-1](#)

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摘要: 目的 探讨融合蛋白TAP-SSL5对血小板微粒 (platelet microparticles, PMPs) 与人单核细胞株THP-1细胞结合及Mac-1活化的影响。 方法 以二磷酸腺苷 (adenosine diphosphate, ADP) 激活人血小板并获取PMPs。采用流式细胞仪 (flow cytometry, FCM) 及PE标记的抗CD62P单克隆抗体、FITC标记的Annexin V检测PMPs, 以FITC标记的抗CD41单克隆抗体和PE标记的抗CD154 (CD40L) 单克隆抗体检测PMPs的表面特征。采用JC-1试剂盒检测血小板线粒体膜电位。采用FCM检测PMPs与THP-1细胞的结合, 以及PMPs诱导THP-1细胞表面 Mac-1 (CD11b/CD18, $\alpha\text{M}\beta_2$) 的活化情况, 并研究TAP-SSL5的干预作用。 结果 PMPs呈现CD62P和Annexin V双阳性, 且 CD41和CD40L的阳性率分别达到50.8%和44.0%。JC-1检测显示, ADP对血小板线粒体膜电位无明显影响 ($P>0.05$)。PMPs与THP-1细胞的结合率为 (24.80±5.16)%, PMPs 诱导THP-1细胞Mac-1的活化率为 (21.17±5.92)%, THP-1细胞经10 mg/L TAP-SSL5预处理后, PMPs的结合率下降至 (13.67±2.15)% ($P<0.05$), Mac-1的活化率下降至 (0.99±0.62)% ($P<0.01$)。 结论 TAP-SSL5可抑制PMPs与THP-1细胞的结合及THP-1细胞表面Mac-1的活化。

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Abstract: Objective To investigate the effect of anticoagulant and anti-inflammatory fusion protein TAP-SSL5 (tick anticoagulant peptide and staphylococcal superantigen like protein-5) on the binding of platelet microparticles (PMPs) to human acute monocytic leukemia cell line THP-1 and the activation of Mac-1.

Methods PMPs were generated by adenosine diphosphate (ADP) activating human platelets. Flow cytometry (FCM) was used to identify PMPs by PE labeled mouse anti-human CD62P monoclonal antibody and FITC-Annexin V, and to check the activation and phosphatidylserine (PS) by FITC labeled mouse anti-human CD41 monoclonal antibody and PE labeled mouse anti-human CD151 monoclonal antibody (CD40L). The mitochondrial membrane potential in human platelets was checked with JC-1 kit. The binding rates of PMPs to THP-1 cells and the conformation change of Mac-1 (CD11b/CD18, αMB_2) after co-incubation with PMPs were assayed by FCM.

Results Both CD62P and PS were positive on PMPs. The positive rates of CD41 and CD40L on the ADP-induced PMPs were 50.8% and 44.0% respectively. While there was no significant change on the mitochondrial membrane potential in ADP activated platelets ($P>0.05$). The binding rates of PMPs to THP-1 cells and the activation rates of Mac-1 on THP-1 cells were $(24.80 \pm 5.16)\%$ and $(21.17 \pm 5.92)\%$ respectively, which decreased to $(13.67 \pm 2.15)\%$ and $(0.99 \pm 0.62)\%$ after the THP-1 cells were pre-incubated with 10 mg/L TAP-SSL5 ($P<0.05$ and $P<0.01$).

Conclusion TAP-SSL5 directly inhibits the binding of PMPs to THP-1 cells, and subsequently inhibits the activation of Mac-1 on THP-1 cells.

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