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幽门螺杆菌外膜泡对人脐静脉内皮细胞的促凋亡作

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Title: Outer membrane vesicles from *Helicobacter pylori* promote apoptosis in human umbilical vein endothelial cells

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关键词: 幽门螺杆菌; 外膜泡; 动脉粥样硬化; 人脐静脉内皮细胞; 细胞凋亡

Keywords: *Helicobacter pylori*; outer membrane vesicles; atherosclerosis; human umbilical vein endothelial cells; apoptosis

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摘要: 目的　探讨幽门螺杆菌（*Helicobacter Pylori*, Hp）外膜泡（outer membrane vesicles，OMVs）对人脐静脉内皮细胞（human umbilical vein endothelial cells, HUVECs）的作用及其机制。　方法　Skirrow氏液体培养基培养幽门螺杆菌，经超速离心法提取HP-OMVs后用负染透射电镜和Western blot进行鉴定，将HP-OMVs与HUVECs共培养后观察其对HUVECs凋亡及增殖的影响。　结果　负染透射电镜显示HP-OMVs呈圆形小泡状结构，大小20~300 nm，HP-OMVs中有CagA和VacA 表达。HP-OMVs抑制HUVECs的增殖，并呈时间和浓度依赖性。流式细胞仪和TUNEL染色检测显示：HP-OMVs处理组细胞凋亡比例分别高达(27.86±2.89)%和(25.4±4.65)%，较对照组显著升高（$P<0.05$）。HP-OMVs处理组Cleaved-caspase-3蛋白表达显著升高（$P<0.05$）。HP-OMVs处理组Bcl-2家族的促凋亡蛋白Bax表达亦显著升高（$P<0.05$），而抗凋亡蛋白Bcl-2表达显著降低（$P<0.05$）。　结论　幽门螺杆菌OMVs能够抑制HUVECs的增殖并促进其凋亡，其凋亡的发生可能与caspase-3的激活及Bcl-2家族的Bcl-2/Bax表达比例失衡有关。

Abstract: Objective To determine the effect of outer membrane vesicles (OMVs) derived from *Helicobacter Pylori* (HP) on the human umbilical vein endothelial cells (HUVECs). Methods After HP was cultured in the Skirrow's liquid medium, OMVs were obtained by ultracentrifugation, which were further identified with electron-microscopy and Western blotting. Then the obtained OMVs were co-cultured with HUVECs (CRL-2922 EA.hy926) to observe the effects of OMVs on the apoptosis and proliferation of HUVECs by flow cytometry, TUNEL staining and CCK-8 assay. Results Transmission electron microscopy indicated that the obtained OMVs were round vesicles in a size of 20 to 300 nm. Western blotting showed that CagA and VacA, commonly used as markers for OMVs were expressed. OMVs inhibited the proliferation of HUVECs in a time- and concentration-dependent manner. Flow cytometry and TUNEL staining showed that the percentage of apoptotic cells was accounted for (27.86±2.89)% and (25.4±4.65)% respectively in OMVs treated cells (P<0.05). OMVs treatment also resulted in significantly increased expression of cleaved-caspase-3 (P<0.05) and Bax, a pro-apoptotic protein of the Bcl-2 family (P<0.05), while decreased expression of Bcl-2, an anti-apoptotic protein (P<0.05). Conclusion HP-derived OMVs inhibit the proliferation and promote the apoptosis of HUVECs, which might be due to the activation of caspase-3 and the imbalance between Bcl-2 and Bax expression.

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