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基础医学

大鼠骨髓间充质干细胞exosome提取及其心肌细胞H9C2靶向作用的实验探索

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摘要：

目的 研究缺氧预处理条件下，骨髓间充质干细胞分泌的exosome的特征；探索心肌细胞H9C2是否骨髓间充质干细胞exosome作用的靶细胞之一，以及H9C2对骨髓间充质干细胞exosome的摄取是否随时间有一定的变化特征。方法 采用分步离心结合蔗糖/D2O垫超速离心方法分离提取干细胞分泌的exosome囊体颗粒。采用透射电镜和Western blotting法鉴定提取物是否为exosome。采用图像分析软件Image- Pro Plus 6.0 对透射电镜结果中exosome 数据进行采集，采用excel和统计软件Graph Pad 5.0对采集得到的exosome数据进行统计，以明确exosome的直径分布区间和平均半径等特征。采用绿色荧光染料PKH-67标记exosome，将标记的exosome与H9C2共孵育，观察exosome能否被心肌细胞H9C2摄取。将exosome与H9C2共孵育的不同时间段，观察H9C2对exosome的摄取随时间变化的特征。结果 提取物呈微型囊体结构，形态近似球形或者椭球形，大小均一，均匀分散的分布在视野中，提取物均阳性表达CD63和CD9分子，且较CD63分子，提取物更高表达CD9分子；缺氧预处理条件下，骨髓间充质干细胞exosome直径的集中分布范围是20～60nm, 半径为(17.03 ± 0.40) nm；exosome与心肌细胞H9C2共孵育结果显示，仅实验组H9C2细胞质内可见大量的exosome绿色荧光颗粒；H9C2摄取exosome的时间特征显示，对照组4个亚组中H9C2对exosomes的摄取情况无明显不同。实验组各亚组显示，exosome与H9C2共孵育1h，H9C2细胞质中即开始观察到标记的exosome；共孵育1.5～2.5h，H9C2细胞质中exosome绿色荧光颗粒数量较共孵育其他时间段的多，荧光强度较共孵育其他时间段的强。结论 成功分离提取到了缺氧预处理条件下的大鼠骨髓间充质干细胞exosome；心肌细胞H9C2是骨髓间充质干细胞exosome生物学作用的靶细胞；H9C2对骨髓间充质干细胞exosome的摄取情况随时间的延长有明显的变化。

关键词： 缺氧预处理；骨髓间充质干细胞；exosome；H9C2；心力衰竭；缺血再灌注损伤

Isolation of rat bone marrow mesenchymal stem cell-derived exosome and the uptake of exosome by H9C2

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Abstract:

Objective To study the features such as the diameter distribution and average radius of the hypoxia preconditioned bone marrow mesenchymal stem cells (BMSCs)-derived exosomes; to elucidate whether the exosomes are able to target cardiomyocytes efficiently; to explore the feature of time-dependent uptake of exosomes by H9C2. Methods We collected exosomes from the supernatant of hypoxia preconditioned BMSCs by the combination of step-by-step centrifugations and ultracentrifugation, characterized exosomes by transmission electron microscopy (TEM) and western blotting, statistically analyzed the messages of the diameters from results of TEM by image analysis software Image Pro Plus 6.0, obtained the distribution of diameter and average radius of exosomes by excel and statistical software Graph Pad 5.0, tracked exosomes with PKH-67, incubated PKH-67-dyed exosomes with H9C2 to observe whether rat BMSCs-derived exosomes could be uptaken by H9C2. Further, we incubated PKH-67-labeled exosomes with H9C2 for different periods to explore the relationship between uptake and time. Results We observed that the extract was of micro-capsule structures, approximately spherical or ellipsoidal in shape and homogeneous in size. They scattered neatly in the vision, and positively expressed both CD63 and CD9 molecules. The diameters of

exosomes approximately ranged from 20nm to 60nm, and the radius was (17.03±0.40) nm. In the incubation experiment of exosomes with H9C2, we could only observe green fluorescent-flashing exosome pellets within the cytoplasm of H9C2 in the dyed positive group. The uptake of exosomes by H9C2 was not different between four subgroups of the control group. For the PKH-67-dyed exosomes within H9C2 cytoplasm 1h after the co-culture, during the period of 1.5h to 2.5h, the numbers of green fluorescent-flashing exosome pellets were more and the fluorescence intensity was stronger than any other experimental subgroups. Conclusion We successfully isolated hypoxia preconditioned rat BMSCs-derived exosomes, and proved that cardiomyocytes H9C2 were the biological targets of such exosomes. The uptake of exosomes by H9C2 showed significant changes with the extension of incubating time.

Keywords: Hypoxia preconditioning; BMSCs; Exosome; H9C2; Heart failure; Ischemia-reperfusion injury

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