脂肪干细胞外泌体传递miR-145调控增生性瘢痕成纤维细胞生物学特性的实验研究

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 [摘要]目的：探究脂肪干细胞（Adipose-derived stem cells，ADSCs）来源的外泌体（Exosome，Exo）传递miR-145对增 生性瘢痕成纤维细胞（hypertrophic scar fibroblasts，HSF）生物学功能的影响。方法：从增生性瘢痕组织中分离培养 HSF细胞，从脂肪组织中分离培养ADSCs，流式细胞术检测其表面标记物表达情况；采用含miR-145过表达慢病毒及阴性对照 miR-NC慢病毒感染ADSCs，实时荧光定量PCR测定转染效果，并提取各转染组ADSCs上清液中Exo，获得含miR-145过表达的 ADSCs来源Exo，利用透射电镜观察其形态、纳米颗粒跟踪分析仪检测粒径分布以及Western blot检测表面标志性蛋白CD63、 CD81、CD9和TSG101的表达情况对提取的外泌体进行鉴定；添加外泌体培养HSF细胞，具体分组包括对照组、Exo组、miR-NCExo组、miR-145-Exo组，PKH26荧光标记的ADSC来源的Exos与HSF细胞培养，共聚焦荧光显微镜观察外泌体能否被细胞摄取， EdU检测各组HSF细胞的增殖能力，Annexin Ⅴ-FITC/PI法检测各组HSF细胞的凋亡水平，Western blot检测各组细胞内凋亡 相关蛋白（cleaved-caspase-3、Bax、Bcl-2）及Ⅰ型胶原、Ⅲ型胶原（COL-Ⅰ、COL-Ⅲ）的蛋白表达水平。结果：成功分 离到ADSCs，其表面标志物CD44、CD90及CD105均呈阳性表达；经miR-145过表达的慢病毒转染的miR-145-ADSCs组细胞中miR145相对表达量升高，差异有统计学意义（P＜0.05）。分离到直径约100 nm、呈双层膜结构的杯状或球状以及外泌体标志蛋 白CD63、CD81、CD9和TSG101均为阳性表达的ADSCs来源Exo；将ADSCs来源Exo与HSF细胞共培养后，Exo可被HSF细胞摄取， miR-145过表达的ADSCs来源Exo能够明显抑制HSF细胞增殖（P＜0.05），促进其凋亡（P＜0.05），上调促凋亡蛋白cleavedcaspase-3和Bax的蛋白相对表达量（P＜0.05），抑制抗凋亡蛋白Bcl-2及COL-Ⅰ、COL-Ⅲ的蛋白相对表达量（P＜0.05）。 结论：脂肪干细胞来源的外泌体可通过传递miR-145抑制增生性瘢痕皮肤中成纤维细胞的增殖活性并促进其凋亡，其分子机 制可能与上调cleaved-caspase-3和Bax的蛋白表达以及抑制Bcl-2、COL-Ⅰ、COL-Ⅲ的蛋白表达有关。

[关键词]增生性瘢痕；脂肪干细胞；外泌体；miR-145；成纤维细胞

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Experimental Study on the Biological Characteristics of Hypertrophic Scar Fibroblasts Regulated by Exosome Delivery of Adipose Stem Cells MiR-145 Alimire

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Abstract: Objective To explore the eff ect of miR-145 delivered by exosomes (Exo) derived from adipose-derived stem cells (ADSCs) on the biological functions of hypertrophic scar fi broblasts (HSF). Methods HSF cells were isolated and cultured from hypertrophic scar tissue, and ADSCs were isolated and cultured from adipose tissue, and the expression of surface markers was detected by fl ow cytometry; miR-145 overexpression lentivirus and negative control miR-NC lentivirus were used Infected with ADSCs, real-time fl uorescent quantitative PCR was used to determine the transfection eff ect, and Exo from the supernatant of ADSCs in each transfection group was extracted to obtain Exo from ADSCs containing miR-145 overexpression. The morphology was observed by transmission electron microscope, the particle size distribution detected by nanoparticle tracking analyzer, and the expression of surface marker proteins CD63, CD81, CD9 and TSG101 were detected by Western blot; HSF cells were cultured with exosomes, and the specifi c groups included the control group, Exo group, miR-NC-Exo group, miR-145-Exo group, PKH26 fl uorescently labeled ADSC-derived Exos and HSF cells were cultured, and the confocal fl uorescence microscope was used to observe whether the exosomes could be taken up by the cells, EdU was used to detect the proliferation ability of HSF cells in each group, Annexin Ⅴ-FITC/PI method was used to detect the apoptosis level of HSF cells in each group, Western blot were used to detect the protein expression levels of apoptosis-related proteins (cleaved-caspase-3, Bax, Bcl-2), type Ⅰ collagen and type Ⅲ collagen (COL-Ⅰ, COL-Ⅲ) in each group. Results ADSCs were successfully isolated, and the surface markers CD44, CD90 and CD105 were all positively expressed; The relative expression of miR-145 in the miR-145-ADSCs group cells transfected with the miR-145 overexpression lentivirus increased (P＜0.05). Isolated ADSCs source Exo with a diameter of about 100 nm, a double-layer membrane structure, and exosomal marker proteins CD63, CD81, CD9 and TSG101. After co-culturing Exo from ADSCs with HSF cells, Exo can be taken up by HSF cells. Exo from ADSCs overexpressing miR-145 can signifi cantly inhibit HSF cell proliferation (P＜0.05), promote their apoptosis (P＜0.05), and upregulate the relative expression of pro-apoptotic proteins cleaved-caspase-3 and Bax (P＜0.05), inhibit the relative expression of anti-apoptotic proteins Bcl-2, COL-Ⅰ and COL-Ⅲ (P＜0.05). Conclusion Adipose-derived stem cell-derived exosomes can inhibit the proliferation of fi broblasts in hypertrophic scar skin and promote their apoptosis by delivering miR-145. The molecular mechanism may be related to up-regulating the protein expression of cleaved-Caspase-3 and Bax, and inhibiting the protein expression of Bcl-2, COL-I and COL-Ⅲ.

Key words: hypertrophic scars; adipose-derived stem cells; exosomes; miR-145; fi broblasts