siRNA沉默Rac1表达促进增生期血管瘤内皮细胞内质网应激和抑制细胞增殖的作用与机制研究

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Title: siRNA silencing Rac1 expression promotes endothelial cell endoplasmic reticulum stress and inhibits cell proliferation in proliferative hemangioma

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摘要: 目的:研究慢病毒介导Rac1沉默对增生期血管瘤内皮细胞内质网应激及Caspase-12活化水平的影响。方法:用Rac1 siRNA慢病毒载体和阴性对照慢病毒载体感染人增生期血管瘤内皮细胞，用Realtime PCR和Western blot检测细胞中Rac1表达变化。MTT法测定细胞增殖能力，PI单染法检测细胞周期变化，Annexin V-FITC/PI双染法检测细胞凋亡变化，Western blot检测细胞中周期蛋白p21、细胞周期蛋白D1（Cyclin D1）和凋亡蛋白活化的Caspase-3（C-Caspase-3）表达水平，同时检测细胞中内质网应激相关蛋白CCAAT/增强子结合蛋白同源蛋白(CHOP)、活化的Caspase-12（C-Caspase-12）表达水平。结果:Rac1 siRNA慢病毒载体感染后的血管瘤内皮细胞中Rac1表达水平明显降低，阴性对照慢病毒对细胞中Rac1表达水平没有影响。沉默Rac1后的增生期血管瘤内皮细胞增殖能力下降，细胞G0/G1比例升高，p21蛋白水平升高，Cyclin D1蛋白水平下降，凋亡率升高，C-Caspase-3蛋白水平升高，CHOP、C-Caspase-12蛋白水平也升高，与没有感染慢病毒的细胞比较，差异有统计学意义（P＜0.05）。阴性对照慢病毒感染对血管瘤内皮细胞增殖、凋亡、细胞周期及细胞中p21、Cyclin D1、C-Caspase-3、CHOP、C-Caspase-12蛋白水平没有影响。结论:慢病毒介导Rac1沉默诱导增生期血管瘤内皮细胞凋亡，阻滞细胞周期，诱导内质网应激发生，诱导Caspase-12活化。

Abstract: Objective:To investigate the effect of lentivirus mediated Rac1 silencing on endoplasmic reticulum stress and Caspase-12 activation in proliferative hemangioma endothelial cells.Methods:Human proliferative hemangioma endothelial cells were infected by Rac1 siRNA lentiviral vector and negative control lentivirus vector.The expression of Rac1 in cells was detected by Realtime PCR and Western blot.MTT assay was used to determine cell proliferation,and PI single staining was used to detect cell cycle changes.Annexin V-FITC/PI double staining was used to detect apoptosis,and the expression levels of cyclin p21,Cyclin D1 and apoptotic protein C-Caspase-3 were detected by Western blot.Meanwhile,the expression levels of endoplasmic reticulum stress related protein CHOP and C-Caspase-12 were detected.Results:The expression level of Rac1 in the endothelial cells of hemangioma after Rac1 siRNA lentivirus carrier infection was significantly decreased,and the negative control lentivirus had no effect on the expression of Rac1 in the cells.The proliferative ability of proliferative hemangioma after Rac1 silence decreased,the proportion of G0/G1 in cells increased,the level of p21 protein elevated,the level of Cyclin D1 protein decreased,the rate of apoptosis increased,the level of C-Caspase-3 protein elevated,the levels of CHOP and C-Caspase-12 protein also increased.Compared with the cells that have not infected the lentivirus,the difference was statistically significant (P＜0.05).Negative control lentivirus infection has no effect on the proliferation,apoptosis,cell cycle and the level of p21,Cyclin D1,C-Caspase-3,CHOP and C-Caspase-12 in the endothelial cells of hemangioma.Conclusion:Lentivirus mediated Rac1

silencing induces apoptosis in proliferative hemangioma endothelial cells,blocks cell cycle,induces endoplasmic reticulum stress,and the activation of Caspase-12 is induced.

参考文献/REFERENCES

[1] He X,Liu Y,Li K,et al.Sildenafil suppresses the proliferation and enhances the apoptosis of hemangioma endothelial cells [J] .Experimental & Therapeutic Medicine,2017,13(6):2645-2650.

[2] Bischoff J.Monoclonal expansion of endothelial cells in hemangioma:An intrinsic defect with extrinsic consequences [J] ?Trends in Cardiovascular Medicine,2002,12(5):220-224.

[3] Vial E,Sahai E,Marshall CJ.ERK-MAPK signaling coordinately regulates activity of Rac1 and RhoA for tumor cell motility [J] .Cancer Cell,2003,4(1):67-79.

[4] Walmsley MJ,Ooi SK,Reynolds LF,et al.Critical roles for Rac1 and Rac2 GTPases in B cell development and signaling [J] .Science,2003,302(5644):459-462.

[5] Kissil JL,Walmsley MJ,Hanlon L,et al.Requirement for Rac1 in a K-ras induced lung cancer in the mouse [J] .Cancer Research,2007,67(17):8089-8094.

[6] Liu J,Liu ZY.Expression andsignifieance of Tiam1 and Rac1 in Human Dermal Hemangiomas [J] .Chinese Journal of Histochemistry and Cytochemistry,2013,22(6):466-470.

[7] Rao GZ,Li A,Zhang YC,et al.Skin hemangioma endothelial cells original generation of isolated culture and appraisal [J] .J Mod Lab Med,2009,24(5):64-66.

[8] Timokhina I,Kissel H,Stella G,et al.Kit signaling through PI3-kinase and Src kinase pathways:An essential role for Rac1 and JNK activation in mast cell proliferation [J] .Embo Journal,2014,17(21):6250-6262.

[9] Song XH,Xin L,Zhou JS,et al.Study on the relationship between Rac1 expression and the proliferation and differentiation of neural precursor cells [J] .Journal of Soochow University:Medical,2009,29(2):201-203. [宋雪慧,辛莲,周俊松,等.Rac1蛋白表达与神经前体细胞增殖分化的关系 [J] .苏州大学学报:医学版,2009,29(2):201-203.]

[10] Hu LP,Lu H,Bai YH,et al.Function and its significance of Rac1 in aristolochic acid-induced renal tubular epithelial cellular injury [J] .Journal of Wenzhou Medical University,2013,43(12):775-778. [胡丽萍,陆红,白永恒,等.小G蛋白Rac1在马兜铃酸诱导肾小管上皮细胞损伤中的作用及意义 [J] .温州医科大学学报,2013,43(12):775-778.]

[11] Liliental J,Sun YM,Lesche R,et al.Genetic deletion of the tumor suppressor gene promotes cell motility by activation of Rac1 and Cdc42 GTPases [J] .Current Biology,2000,10(7):401-404.

[12] Zhong Z,Liu B,Wu X.RhoGDI2 up-regulates P-glycoprotein expression via Rac1 in gastric cancer cells [J] .Cancer Cell International,2015,15(1):1-7.

[13] Gao F,Wang FG,Liu RR,et al.Epigenetic silencing of miR-130a ameliorates hemangioma by targeting tissue factor pathway inhibitor 2 through FAK/PI3K/Rac1/mdm2 signaling [J] .International Journal of Oncology,2017,50(5):1821-1831.

[14] Endresen PC,Prytz PS,Aarbakke J.A new flow cytometric method for discrimination of apoptotic cells and detection of their cell cycle specificity through staining of F-actin and DNA [J] .Cytometry Part A,2010,20(2):162-171.

[15] Wong SCC,Chan JKC,Lee KC,et al.Differential expression of p16/p21/p27 and cyclin D1/D3,and their relationships to cell proliferation,apoptosis,and tumour progression in invasive ductal carcinoma of the breast [J] .Journal of Pathology,2001,194(1):35-42.

[16] Sharif M,Whitehouse A,Sharman P,et al.Increased apoptosis in human osteoarthritic cartilage corresponds to reduced cell density and expression of caspase-3 [J] .Arthritis Rheum,2014,50(2):507-515.

[17] Wilhelm S,Wagner H,Ha¤Cker G.Activation of caspase-3-like enzymes in non-apoptotic T cells [J] .European Journal of Immunology,2010,28(03):891-900.

[18] Matos P,Jordan P.Rac1,but not Rac1B,stimulates RelB-mediated gene transcription in colorectal cancer cells [J] .Journal of Biological Chemistry,2006,281(19):13724-13732.

[19] Wang H,Linghu H,Wang J,et al.The role of Crk/Dock180/Rac1 pathway in the malignant behavior of human ovarian cancer cell SKOV3 [J] .Tumour Biol,2010,31(1):59-67.

[20] Han XM,Sun YH,Zhu XY,et al.Activation of RAC1 mediates apoptosis of human umbilical vein endothelial cells [J] .J Clin Rehabil Tis Eng Res,2009,13(20):3853-3856. [韩秀敏,孙英慧,朱鲜阳,等.RAC1活化介导缺氧的人脐静脉内皮细胞凋亡 [J] .中国组织工程研究,2009,13(20):3853-3856.]

[21] Oyadomari S,Koizumi A,Takeda K,et al.Targeted disruption of the Chop gene delays endoplasmic reticulum stress-mediated diabetes [J] .Journal of Clinical Investigation,2002,109(4):525-532.

[22] Banerjee A,Ahmed H,Yang P,et al.Endoplasmic reticulum stress and IRE-1 signaling cause apoptosis in colon cancer cells in response to andrographolide treatment [J] .Oncotarget,2016,7(27):41432-41444.

[23] Qiang Z,Liu J,Chen S,et al.Caspase-12 is involved in stretch-induced apoptosis mediated endoplasmic reticulum stress [J] .Apoptosis,2016,21(4):432-442.

[24] Goswami P,Gupta S,Biswas J,et al.Endoplasmic reticulum stress plays a key role in rotenone-induced apoptotic death of neurons [J] .Molecular Neurobiology,2016,53(1):285-298.

[25] Dong Z,Yang N,Chen Z,et al.Regulation of sericin on retinal endoplasmic reticulum stress specific caspase-12 dependent pathway and inhibition on apoptosis in diabetic rat [J] .Chinese Journal of Experimental Ophthalmology,2017,35(12):1067-1072.

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