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[1]陈懿,王晓波,张俊勇,等·超声微泡携MAGL-shRNA靶向释放对大鼠肝细胞肝癌转移作用的实验研究[J].第三军医大学学报,2013,35(16):1708-1712.

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Title: Effect of ultrasound microbubble carrying MAGL-shRNA

on metastasis of hepatocellular carcinoma in rats

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关键词: 肝细胞肝癌; 单酰基甘油脂肪酶; 超声微泡; 转移

Keywords: hepatocellular carcinoma; monoacylglycerol lipase; ultrosound

microbubble; metastasis

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摘要: 目的 观察超声微泡携单酰基甘油脂肪酶沉默基因

(monoacylglycerol lipase short hairpin RNA, MAGL-shRNA)在大鼠肝细胞肝癌(hepatocellular carcinoma, HCC)组织中转染及对HCC转移的作用。 方法 建立大鼠肝细胞肝癌模型,病理解剖和二维超声验证肝脏成瘤情况。40只SD大鼠完全随机分成4组,分别为PBS液组、MAGL-shRNA质粒微泡组(MAGL-shRNA+microbubble, MB)、空白质粒微泡+超声辐照组(microbubbles+ultrasound, MB+US)、MAGL-shRNA质粒微泡+超声辐照组(MAGL-shRNA+MB+US)。每只注射1 mL,对

MB+US组和MAGL-shRNA+MB+US组大鼠肝区同时给予超声辐照,辐照

导航/NAVIGATE

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条件为300 kHz,2 W/cm²,辐照10 s,间隔10 s,共20 min。Western blot检测大鼠HCC组织MAGL蛋白的表达,免疫组化检测MAGL和基质金属蛋白酶2(matrixmetalloproteinase-2, MMP-2)的表达。比较各组动物的肿瘤转移情况。 结果 MAGL在HCC组织中表达明显高于正常肝组织(P<0.05);微泡携MAGL-shRNA可以在HCC组织被超声辐照击破后靶向释放,在各组HCC组织中均有MAGL蛋白的表达,其中MAGL-shRNA+MB+US组表达量明显低于其他组(P<0.01);免疫组化检测MAGL-shRNA+MB+US组MMP-2表达均低于其他组(P<0.01);各组动物均见肿瘤转移,但MAGL-shRNA+MB+US组转移率最低(P<0.05)。 结论 超声辐照可破坏携MAGL-shRNA的微泡使之靶向释放并增强了MAGL-shRNA的转染效率,MAGL-MMP-2通路可能与HCC的转移相关。

Abstract:

Objective To determine the effect of silencing monoacylglycerol lipase by short hairpin RNA (shRNA) on the metastasis of hepatocellular carcinoma in rats by using ultrasoundtargeted microbubble destruction. Methods Liver tumor models of rats were established by intragastrically injection of 0.2% DEN in 5 times per week for 14 weeks. The successful establishment of tumor model was testified by pathologic biopsy and two-dimensional ultrasongraphy. Totally 40 rats with liver tumor were randomly divided into 4 groups, phosphate-buffered solution (PBS) group, lipid microbubbles loaded MAGL-shRNA (MAGL-shRNA+microbubble group), pure lipid microbubbles+ultrasound (MB+US group), and lipid microbubbles loaded MAGL-shRNA+ultrasound (MAGL-shRNA+MB+US group). The microbubbles of 1 mL containing MAGL-shRNA plasmid or not were injected through tail vein. Ultrasound radiation was applied on the rats of MB+US and MAGL-shRNA+MB+US groups after the injection of target gene, with the radiation frequency of 1 kHz, sound intensity of 2 W/cm², with the pulse irradiation of 10 s and interval time of 10 s for totally 20 min. The expression of MAGL protein was detected in the tumor mass by Western blotting. Protein expression of MAGL and matrix metalloproteinase-2 (MMP-2) was detected by immunohistochemistry (IHC). Tumor metastasis were observed and compared among different groups. The protein expression of MAGL was significantly higher in HCC