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Title: MiR-224 promotes cell migration by targeting HOXD10 in Hep3B cells

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关键词: 肝细胞肝癌; miRNA-224; HOXD10; 迁移

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摘要: 目的 探讨miR-224影响肝癌细胞迁移的可能机制。 方法 采用双荧光素酶实验、载体构建和Western blot实验等对预测的miR-224靶基因进行验证。qRT-PCR检测正常肝细胞株L02和肝癌细胞株Hep3B中miR-224的表达; 划痕实验观察miR-224对肝癌细胞迁移的影响。 结果 qRT-PCR结果显示,与正常肝细胞株L02比较,miR-224在肝癌细胞株Hep3B中明显高表达(相对表达倍数1.679 2, P<0.05);划痕实验显示,与miR-224 mimics阴性对照组(迁移率: 56.43%~58.33%)以及空白组(迁移率: 60.48%~66.94%)比较,miR-224 mimics组(迁移率: 80.12%~82.02%)的Hep3B细胞迁移能力明显增强(P<0.05),反之亦然,提示miR-224表达水平和肝癌细胞的迁移能力成正相关; 双荧光素酶实验结果显示:与对照组比较,miR-224 mimics组细胞的荧光信号明显下降(P<0.05); Western blot实验结果显示:与对照组比较,上调miR-224的表达水平后HOXD10表达下降,下调其表达水平HOXD10表达上升(P<0.05)。 结论 miR-224通过调控靶基因HOXD10的表达,参与调节肝癌细胞的迁移。

Abstract: Objective To investigate the possible underlying mechanism that miR-224 affects the migration of liver cancer cells. Methods A luciferase reporter assay was used to confirm that the gene HOXD10 was one direct target of miR-224. qRT-PCR, Western blotting, and scratch wound migration assay were used to clarify the molecular mechanism of miR-224 in the regulation of cell migration in human hepatocellular carcinoma line Hep3B. Results qRT-PCR indicated the expression of miR-224 was significantly stronger in Hep3B cells than in human hepatic L02 cells (relative expression ratio: 1.679 2, P<0.05).

Scratch wound migration assay showed that transfection of miR-224 resulted in significantly increased ratio of migration (80.12% to 82.02%, $P<0.05$) in Hep3B cells than the cells transfected by miR-224 mimic negative control (56.43% to 58.33%) and by blank vector (60.48% to 66.94%). Compared with the miR-224 negative control , the luciferase activity was significantly decreased in 293 cells with miR-224 mimic (0.633 2). miR-224 affected the migration ability of HCC via directly targeting HOXD10. Conclusion miR-224 is involved in the regulation of hepatoma cell migration via directly targeting HOXD10.

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