miR-199-3p通过对FGF2的调控抑制肝癌细胞MHCC97H的增殖、迁移作用及机制

《现代肿瘤医学》[ISSN:1672-4992/CN:61-1415/R] 期数: 2019年13期 页码: 2250-2257 栏目: 基础研究 出版日期: 2019-05-31

Title: Study of the regulation effect of miR-199-3p on FGF2 to inhibit the proliferation, migration and progression of hepatocellular carcinoma cells MHCC97H

作者: 韩银淑; 徐艺丹
厦门医学院医学技术系，福建 厦门 361023

Author(s): Han Yinshu; Xu Yidan
Department of Medical Technology, Xiamen Medical College, Fujian Xiamen 361023, China.

关键词: 肝癌; miR-199-3p; FGF2; MHCC97H

Keywords: liver cancer; miR-199-3p; FGF2; MHCC97H

分类号: R735.7

DOI: 10.3969/j.issn.1672-4992.2019.13.006

文献标识码: A

摘要: 目的：探讨miR-199-3p通过靶向调控抑制靶基因FGF2从而抑制肝癌MHCC97H细胞的增殖和迁移及机制。方法：qRT-PCR检测癌旁正常组织及肝癌肿瘤组织中miR-199-3p的表达水平；Transwell细胞侵袭实验检测肝癌细胞株Huh7、MHCC-97L、SMMC7721、HepG2和MHCC97H株其侵袭能力；qRT-PCR检测miR-199-3p在5株肝癌细胞系中的表达量；通过生物信息软件预测靶基因FGF2 mRNA 3' UTR的碱基存在miR-199-3p可能互补结合的位点并用荧光报告基因法及WB进行验证；通过qRT-PCR、免疫组化及Western blot检测FGF2在肝癌肿瘤组织和癌旁正常组织及各类肝癌细胞株表达的影响；Transwell、划痕、CCK8及流式细胞法检测miR-199-3p与FGF2对肝癌MHCC97H细胞增殖、侵袭、迁移及周期S期聚集能力的调控；采用MHCC97H细胞构建肝癌肿瘤异种移植小鼠模型进行肿瘤观测及免疫组化实验验证miR-199-3p对肝癌的调控作用。结果：miR-199-3p的表达水平与肝癌细胞侵袭转移能力相关；miR-199-3p能够通过抑制FGF2的mRNA翻译，抑制FGF2蛋白水平表达；FGF2与肝癌细胞侵袭转移能力相关；miR-199-3p可以负调控FGF2抑制肝癌MHCC97H细胞的生物行为；miR-199-3p 可抑制肝癌细胞中阳性信号，细胞增殖水平显著降低。结论：miR-199-3p通过抑制FGF2抑制肝癌细胞MHCC97H的增殖和迁移。

Abstract: Objective: In this study, miR-199-3p inhibits the occurrence and development of liver cancer through targeted regulation and inhibition of target gene FGF2, suggesting that this study may be a new target for liver cancer.Methods: The expression level of miR-199-3p was detected by qRT-PCR.Transwell cell invasion assay detected the invasiveness of hepatocellular carcinoma cell lines Huh7, MHCC-97L, SMMC7721, HepG2 and MHCC97H.The expression of miR-199-3p in 5 hepatocellular carcinoma cell lines was detected by qRT-PCR.The bases of the target gene FGF2 mRNA 3' UTR were predicted by bioinformatics software for the presence of possible complementary binding sites of miR-199-3p and verified by fluorescent reporter gene method and WB.The effect of FGF2 on the expression of liver cancer tumor tissues and paracancerous normal tissues and various liver cancer cell lines was detected by qRT-PCR, immunohistochemistry and Western blot.The regulation of miR-199-3p and FGF2 on proliferation, invasion, migration and aggregation in cycle S of MHCC97H cells of liver cancer was detected by Transwell, scratch, CCK8 and flow cytometry.MHCC97H cells were used to construct a hepatocellular carcinoma xenograft mouse model for tumor observation and immunohistochemistry experiments to verify the regulatory effect of miR-199-3p on hepatocellular carcinoma.Results: The expression level of miR-199-3p was correlated with the invasion and metastasis of hepatocellular carcinoma cells.miR-199-3p can inhibit the expression of FGF2 protein by inhibiting the translation of FGF2 mRNA.FGF2 is related to the invasion and metastasis of hepatocellular carcinoma cells.miR-199-3p can negatively regulate the inhibition of the biological behavior of HCC MHCC97H cells by FGF2.miR-199-3p can significantly inhibit positive signals and cell proliferation in hepatocellular carcinoma cells.Conclusion: miR-199-3p inhibits the biological behavior of hepatocellular carcinoma cells MHCC97H by inhibiting FGF2.

参考文献/REFERENCES

[1] Liang ZK，Cheng JT，Hu ZX，et al.miR-150-5p inhibited the migration and invasion of hepatocellular carcinoma cells and its mechanism [J] .Chinese Journal of General Surgery，2016，25(8)：1175-1179.[梁治坤，程几天，胡走肖，等.miR-150-5p抑制肝癌细胞的迁移和侵袭及其机制 [J] .中国普通外科杂志，2016，25(8)：1175-1179.]
[2] Sato-Matsubara M，Matsubara T，Daikoku A，et al.Fibroblast growth factor 2 (FGF2) regulates cytoglobin expression and activation of human hepatic stellate cells via JNK signaling [J] .J Biol Chem，2017，292(46)：18961-18972.
[3] Uriarte I，Latasa MU，Carotti S，et al.Ileal FGF15 contributes to fibrosis-associated hepatocellular carcinoma development [J] .In J Cancer，2015，136(10)：2469-2475.
[4] Li H，Li J，Jia S，et al.miR675 upregulates long noncoding RNA H19 through activating EGR1 in human liver cancer [J] .Oncotarget，2015，6(31)：31958-31984.
[5] Liu F，Kong X，Lv L，et al.MiR-155 targets TP53INP1 to regulate liver cancer stem cell acquisition and self-renewal [J] .FEBS Lett，2015，589(4)：500-506.
[6] Lei ZW，Zhang Y，Zhang X.Role of miR-181a in human malignant tumors [J] .Academic Journal of Chinese Pla Medical School，2016，37(11)：1204-1207.[雷振伟，张瑜，张旭.miR-181a在人类恶性肿瘤中作用机制的研究进展 [J] .解放军医学院学报，2016，37(11)：1204-1207.]
[7] Su W，Lin Y，Liang L，et al.The role of miR-135a in regulating multi-drug resistance of small cell lung cancer [J] .Chinese Journal of Cancer Biotherapy，2015，22(3)：304-309.
[8] Cui H，Seubert B，Stahl E，et al.Tissue inhibitor of metalloproteinases-1 induces a pro-tumourigenic increase of miR-210 in lung adenocarcinoma cells and their exosomes [J] .Oncogene，2015，34(28)：3640-3650.
[9] Huang H，Hu M，Li P，et al.MiR-152 inhibits cell proliferation and colony formation of CD133+ liver cancer stem cells by targeting KIT [J] .Tumour Biology，2015，36(2)：921-928.
[10] Sun B，Li J，Shao D，et al.Adipose tissue-secreted miR-27a promotes liver cancer by targeting FOXO1 in obese individuals [J] .Oncotargets & Therapy，2015，8(default)：735-744.
[11] Zhou XF，Jiang JX.Advances in the study of microrna-29c in tumors [J] .Journal of Guizhou Medical University，2016，41(9)：993-996.[周显飞，江建新.microRNA-29c在肿瘤中的研究进展 [J] .贵州医科大学学报，2016，41(9)：993-996.]
[12] Zheng N，Wei W，Wang Z.Emerging roles of FGF signaling in hepatocellular carcinoma [J] .Transl Cancer Res，2016，5(1)：1-6.
[13] Cui G，Martin RC，Jin H，et al.Up-regulation of FGF15/19 signaling promotes hepatocellular carcinoma in the background of fatty liver [J] .J Exp Clin Cancer Res，2018，37(1)：136.
[14] Feng X，Jiang J，Shi S，et al.Knockdown of miR-25 increases the sensitivity of liver cancer stem cells to TRAIL-induced apoptosis via PTEN/PI3K/Akt/Bad signaling pathway [J] .Int J Oncol，2016，49(6)：2600-2610.
[15] Fu WM，Tang LP，Xiao Z，et al.MiR-218-targeting-Bmi-1 mediates the suppressive effect of 1，6，7-trihydroxyxanthone on liver cancer cells [J] .Apoptosis，2015，20(1)：75-82.
[16] Liu C，Wang C，Wang J，et al.miR-1297 promotes cell proliferation by inhibiting RB1 in liver cancer [J] .Oncol Lett，2016，12(6)：5177-5182.

备注/Memo: 福建省高校重点实验室技能与临床转化课题(编号：JNYLC1809)

更新日期/Last Update: 2019-05-31