miR-509-5p通过靶向HMGA2调控非小细胞肺癌H1299细胞增殖、迁移和侵袭及其机制探讨

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Title: **miR-509-5p regulates proliferation，migration and invasion of non-small cell lung cancer H1299 cells through targeting HMGA2**

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摘要: 目的：探讨miR-509-5p通过靶向HMGA2对非小细胞肺癌H1299细胞增殖、迁移和侵袭的调控作用及其机制。方法：体外培养人正常肺细胞株CCD-19LU和肺腺癌细胞株A549、H1299，采用qRT-PCR检测miR-509-5p的表达；转染miR-509-5p mimics构建miR-509-5p过表达的H1299细胞后，采用MTT和克隆形成实验检测细胞的增殖能力，Transwell小室实验检测细胞的迁移和侵袭能力，Western blot检测TWIST1、β-catenin和AXIN1蛋白的表达。通过生物信息学分析预测HMGA2可能是miR-509-5p的靶点，并采用双荧光素酶报告基因系统和Western blot检测miR-509-5p和HMGA2的靶向关系。转染干扰质粒载体pLL2G-shHMGA2构建HMGA2沉默的H1299细胞，采用MTT实验、克隆形成实验和Transwell小室实验检测细胞的增殖、迁移和侵袭能力，Western blot检测TWIST1、β-catenin和AXIN1蛋白的表达。结果：与CCD-19LU细胞相比，A549和H1299细胞中miR-509-5p表达明显降低（P<0.05），且在H1299细胞中低于在A549细胞中的表达（P<0.05）。与miR-NC组相比，miR-509-5p组细胞的增殖、迁移和侵袭能力均明显减弱（P<0.05），TWIST1和β-catenin蛋白的表达明显降低（P<0.05），而AXIN1蛋白的表达明显升高（P<0.05）；野生型miR-509-5p组细胞的荧光素酶活性明显低于miR-NC组（P<0.05），而野生型anti-miR-509-5p组细胞的荧光素酶活性明显明显高于anti-miR-NC组（P<0.05）；miR-509-5p过表达可抑制HMGA2表达，反之则促进其表达。沉默HMGA2表达后，H1299细胞的变化趋势与miR-509-5p过表达的结果相一致。结论：miR-509-5p可通过靶向HMGA2调控非小细胞肺癌H1299细胞的增殖、迁移和侵袭，其作用机制可能与抑制Wnt/β-catenin信号通路有关。

Abstract: Objective：To investigate the regulatory effect of miR-509-5p on proliferation，migration and invasion of non-small cell lung cancer H1299 cells through targeting HMGA2 and its mechanism.Methods：Human normal lung cell line CCD-19LU and lung adenocarcinoma cell line A549 and H1299 were cultured in vitro，and the expression of miR-509-5p was detected by qRT-PCR.After transfection of miR-509-5p mimics to construct miR-509-5p overexpressing H1299 cells，the ability of cell proliferation was checked by MTT assay and colony formation assay，and cell migration and invasion were tested by Transwell cell assay，and the expressions of TWIST1，β-catenin and AXIN1 proteins were measured by Western blot.Bioinformatics analysis predicted that HMGA2 might be the target of miR-509-5p.The target relationship between miR-509-5p and HMGA2 was verified by the dual luciferase reporter gene system and Western blot.After transfecting plasmid vector pLL2G-shHMGA2 to construct HMGA2 silent H1299 cells，the proliferation，migration and invasion abilities of the cells were examed by MTT experiment，clone formation experiment and Transwell chamber experiment，and the expressions of TWIST1，β-catenin and AXIN1 proteins were detected by Western blot.Results：Compared with CCD-19LU cells，miR-509-5p in A549 and H1299 cells were decreased significantly（P<0.05），and H1299 cells were lower than A549 cells（P<0.05).Compared with the miR-NC group，the proliferation，migration and invasion abilities of cells in the miR-509-5p group were significantly decreased（P<0.05），and the expressions of TWIST1 and β-catenin proteins were reduced significantly（P<0.05），while the expression of AXIN1 protein

was increased significantly（P<0.05).The luciferase activity of the wild type miR-509-5p group was significantly lower than that of the miR-NC group（P<0.05），while the luciferase activity of the wild type anti-miR-509-5p group was obviously higher than that of the anti-miR-NC group（P<0.05），and the expression of HMGA2 was inhibited by the overexpression of miR-509-5p and the expression of HMGA2 was promoted on the contrary.After silencing HMGA2 expression，the change trend of H1299 cells was consistent with the results of miR-509-5p overexpression.Conclusion：miR-509-5p can regulate the proliferation，migration and invasion of H1299 cells in non-small cell lung cancer by targeting HMGA2，and its mechanism may be related to the inhibition of Wnt/β-catenin signaling pathway.

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备注/Memo: -

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