miR-520a-3p靶向ABCG2增强乳腺癌细胞对多西他赛敏感性的机制研究

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Title: **miR-520a-3p enhances the sensitivity of human breast cancer cells to docetaxel by suppressing ABCG2 expression**

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关键词: 乳腺癌; miR-520a-3p; ABCG2; 多西他赛; 化疗敏感性

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摘要: 目的：研究miR-520a-3p对乳腺癌细胞多西他赛敏感性的影响，并探索潜在的分子机制。方法：收集2015年4月至2017年10月在我院乳腺外科确诊的45例乳腺癌标本，实时荧光定量PCR法检测乳腺癌组织中miR-520a-3p的表达水平。分析miR-520a-3p与乳腺癌化疗耐受和肿瘤生物学特征的相关性。采用实时荧光定量PCR检测乳腺癌细胞MCF-7、MCF-7/Doc和MDA-MB-231中miR-520a-3p和三磷酸腺苷结合盒转运蛋白（ABCG2）mRNA的表达，采用蛋白质免疫印迹实验检测ABCG2的表达。转染miR-520a-3p mimic后，采用细胞毒性实验观察乳腺癌细胞对多西他赛敏感性的变化。采用实时荧光定量PCR和蛋白质免疫印迹实验观察miR-520a-3p升高后，ABCG2 mRNA和蛋白的表达变化。进一步采用双荧光素酶活性实验验证miR-520a-3p对ABCG2的靶向作用。结果：化疗耐药组新辅助化疗后肿瘤原发部位的miR-520a-3p表达水平明显降低（P < 0.05），同时相关性分析发现，miR-520a-3p低表达与高TNM分期（III期）和淋巴结转移相关（P < 0.05）。miR-520a-3p在MCF-7/Doc和MDA-MB-231细胞中表达较在MCF-7细胞中下降（P < 0.05），而ABCG2的表达水平则明显升高（P均<0.05）。上调miR-520a-3p后，MCF-7和MCF-7/Doc细胞对多西他赛的敏感性增强（P < 0.05），而ABCG2在mRNA和蛋白水平的表达均下降（P < 0.05）。双荧光素酶报告基因结果则证实ABCG2是miR-520a-3p的靶基因。结论：miR-520a-3p可逆转MCF-7/Doc对多西他赛的耐药性，这一作用可能与负调控肿瘤耐药相关蛋白ABCG2的表达从而抑制药物外排有关。

Abstract: Objective:To investigate the role of miR-520a-3p on the sensitivity of breast cancer cells and the possible regulatory mechanisms.Methods:From April 2015 to October 2017,45 surgical specimens were collected.There expression of miR-520a-3p at miRNA level in breast cancer tissues were quantified by quantitative real-time PCR.The correlation between miR-520a-3p and the biological features of breast cancer as well as the sensitivity to docetaxel was analyzed.The expression of miR-520a-3p in breast cancer was detected by real time PCR.The miR-520a-3p was overexpressed by Lipofectamine 2000 transfection with miR-520a-3p mimics.The effects of miR-520a-3p on the sensitivity to docetaxel were detected by CCK-8 assay.The protein expression of ABCG2 was determined by Western blot.The expression relationship between ABCG2 and miR-520a-3p in MCF-7/Doc cells was detected by dual-luciferase reporter assay.Results:In the present study,in qRT-PCR results demonstrated that miR-520a-3p was weakly expressed in chemoresistant breast cancer tissues (P < 0.05).The expression of miR-520a-3p was lower in both MCF-7/Doc and MDA-MB-231 cells than in MCF-7 cells (P < 0.05,respectively),while ABCG2 expression was opposite to that (P < 0.05,respectively).By upregulation of miRNA-520a-3p,chemosensitivity of MCF-7 and MCF-7/Doc cells to docetaxel was improved (P < 0.05,respectively).The expression of ABCG2 in MCF-7 cells was inversely changed after upregulation or

downregulation of miR-520a-3p was improved(P < 0.05,respectively).The result of dual-luciferase-3'-UTR reporter assay confirmed that miR-520a-3p binds to the 3'-UTR of ABCG2.Conclusion:The findings of our study demonstrate that miR-520a-3p over-expression in MCF-7/Doc cells enhances docetaxel sensitivity by inhibiting ABCG2 expression.

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