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K-ras基因突变通过调节E-cadherin/β-catenin/p120蛋白复合体形成和RhoA蛋白活性对结肠癌细胞株Caco-2转移的影响

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Effects of K-ras Gene Mutation on Colon Cancer Cell Line Caco-2 Metastasis by Regulating E-cadherin/β-catenin/p120 Protein Complex Formation and RhoA Protein Activity

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摘要 摘要：目的 观察K-ras基因突变通过调节E-cadherin/β-catenin/p120蛋白复合体形成和RhoA蛋白活性对结肠癌细胞株Caco-2转移的影响，探讨其促进结肠癌转移的相关机制。 方法 将培养的结肠癌细胞株Caco-2分别给予空白质粒phr-GFP转染（对照组）、K-ras基因突变型质粒phr-K-ras（Val12）转染（转染组）、phr-K-ras（Val12）质粒转染+丝裂原活化蛋白激酶（MAPK）途径特异性抑制剂PD98059处理（MAPK抑制组）和phr-K-ras（Val12）质粒转染+磷脂酰肌醇3激酶（PI-3K）途径特异性抑制剂LY294002处理（PI-3K抑制组），Transwell细胞迁移实验检测细胞迁移率，细胞免疫荧光检测E-cadherin、β-catenin蛋白表达及其在细胞内定位，Western blot检测细胞内p120蛋白的表达，免疫沉淀检测与E-cadherin结合的β-catenin蛋白水平，Pull-down实验检测RhoA蛋白活性。结果 转染组Caco-2细胞的迁移率为（19.8±5.6）%，明显高于对照组的（14.0±4.2）%（P=0.001）和MAPK抑制组的（15.8±1.2）%（P=0.044），但与PI-3K抑制组的（17.5±2.8）%差异无统计学意义（P=0.095）。细胞免疫荧光检测结果显示，转染组细胞膜上的E-cadherin和β-catenin蛋白表达减少。Western blot检测结果显示，转染组和PI-3K抑制组细胞内p120总蛋白表达减少。免疫沉淀检测结果显示，转染组和PI-3K抑制组细胞内与E-cadherin结合的β-catenin蛋白水平下降。Pull-down实验检测结果显示，转染组细胞内的RhoA蛋白活性增加。结论 K-ras基因突变可以促进结肠癌细胞株Caco-2的转移，其可能是通过MAPK途径减少E-cadherin/β-catenin/p120蛋白复合体形成，增强RhoA蛋白活性来实现的。

关键词： K-ras基因 突变 E-cadherin/β-catenin/p120蛋白复合体 RhoA蛋白 结肠癌 转移

Abstract： ABSTRACT:Objective To explore the effects of K-ras gene mutation on colon cancer cell line Caco-2 metastasis by regulating E-cadherin/β-catenin/p120 protein complex formation and RhoA protein activity. Methods K-ras wild-type colon cancer cell line Caco-2 was transiently transfected by phr-GFP vector (control group), transfected by mutant K-ras gene phr-K-ras (Val12) vector (transfection group), transfected by mutant K-ras gene phr-K-ras (Val12) vector and treated by specific MAPK pathway inhibitor PD98059 (MAPK inhibition group), or transfected by mutant K-ras gene phr-K-ras (Val12) vector and treated by specific PI-3K pathway inhibitor LY294002 (PI-3K inhibition group), respectively. Cell migration was tested by Transwell experiment. E-cadherin and β-catenin protein expression and intracellular location were detected by cell immunofluorescence method. Intracellular p120 protein expression was detected by Western blot. β-catenin protein level which combined with E-cadherin was detected by immunoprecipitation. RhoA activity was analyzed by Pull-down assay. Results The Caco-2 cell migration rate was (19.8±5.6)% in transfection group, which was significantly higher than that in control group ［(14.0±4.2)%］ (P=0.001) and in MAPK inhibition group ［（15.8±1.2）%］ (P =0.044), but was not significantly different from that in PI-3K inhibition group ［（17.5±2.8）%］ (P=0.095). Immunofluorescence method showed that the E-cadherin and β-catenin stain located in the cell membrane decreased in transfection group. Western blot showed that the total intracellular p120 preotein decreased in transfection group and PI-3K inhibition group. Immunoprecipitation data showed that β-catenin protein level combined with E-cadherin decreased in transfection group and PI-3K group. Pull-down test showed that RhoA protein activity was up-regulated in transfection group. Conclusion K-ras gene mutation stimulates the migration of colon cancer cell Caco-2, which may be achieved by decreasing the E-cadherin/β-catenin/p120 protein complex formation via MAPK pathway and increasing the RhoA protein activity.

Keywords： K-ras cene mutation e-cadderin/β-catenin/p120 protein complex rdoa protein colon cancer metastasis

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