贝伐珠单抗对结直肠癌细胞 VEGFR1 和 VEGFR2 表达的影响

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摘　要:[目的] 探讨贝伐珠单抗对结直肠癌细胞血管内皮生长因子（VEGF）相关受体的影响。[方法] RT-PCR、Western blotting 检测不同浓度贝伐珠单抗对结直肠癌肿瘤细胞 VEGF 相关受体表达水平的影响。[结果] 与对照组相比,贝伐珠单抗药物组结直肠癌细胞的 VEGF 表达水平均明显下降(P<0.05);VEGFR1 和 VEGFR2 表达水均明显上调(P<0.05)。[结论] 贝伐珠单抗可引起了结直肠癌细胞 VEGFR1 和 VEGFR2 表达上调。

关键词:贝伐珠单抗;结直肠癌;血管内皮生长因子 1;血管内皮生长因子受体 2
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The Effect of Bevacizumab on VEGFR1 and VEGFR2 Expression of Colorectal Carcinoma Cells

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Abstract:[Purpose] To investigate the effect of bevacizumab on vascular endothelial growth factor (VEGF) receptors on colorectal carcinoma cells. [Methods] The changes of VEGF receptors expression on colorectal carcinoma cell at different concentrations of bevacizumab were detected by RT-PCR and western blotting. [Results] Compared with the controls,the expressions of VEGF were both decreased on bevacizumab-induced colorectal carcinoma cells(P<0.05). Besides the expressions of VEGFR1 and VEGFR2 were both up-regulated on bevacizumab-induced colorectal carcinoma cells(P<0.05). [Conclusions] Bevacizumab induced the up-regulation of VEGFR1 and VEGFR2 on colorectal carcinoma cells.

Key words:bevacizumab;colorectal carcinoma;VEGFR1;VEGFR2

血管内皮生长因子（vascular endothelial growth factor,VEGF）调控着血管内皮细胞的生长和代谢,在机体正常血管发育、血管病变和肿瘤血管侵袭方面起重要作用[1-3],VEGF 有多种异构体,其中 VEGF-A 占主要成分[4]。与血管生成关系密切的 VEGF 受体主要有两种，即 VEGFR1（vascular endothelial growth factor receptor 1）和 VEGFR2（vascular endothelial growth factor receptor 2),VEGFR1 和 VEGFR2 主要表达于血管内皮细胞上，与 VEGF 一起调控各种血管相关的病变或疾病发生[5]。

贝伐珠单抗是 VEGF 的人源单克隆抗体，可有效地降低晚期肿瘤患者体内 VEGF 水平，很大程度上抑制了肿瘤血管生成,从而抑制肿瘤血管侵袭,可提高治疗效果,改善患者的生活质量[6-8]。贝伐珠单抗和 5-Fu 为基础的联合治疗已成为晚期侵袭性结直肠癌治疗的经典方案[9,10]。但临床肿瘤治疗中贝伐珠

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单抗存在部分患者耐药及治疗后病情加重现象，这降低了贝伐珠单抗肿瘤治疗的效果[11~14]。目前为止，贝伐珠单抗耐药的相关研究较少，耐药机制尚不清楚[15]。据文献报道，VEGFR1 和 VEGFR2 不仅表达于血管内皮细胞，也表达于某些肿瘤细胞[16]。高表达 VEGFR1 的肿瘤细胞具有更强的侵袭能力和促血管生长能力[17,18]；在肺癌临床研究中，高表达 VEGFR2 的患者往往具有较差的预后[19]。本实验主要检测贝伐珠单抗对结直肠癌细胞 VEGFR1 和 VEGFR2 表达的影响，探究临床肿瘤治疗中贝伐珠单抗耐药的可能机制，为临床肿瘤药物治疗及双靶点药物治疗提供一些参考。

1 材料与方法

1.1 材 料

HCT116 和 HT29 细胞株购于中国科学院上海生命科学研究院细胞资源中心。DMEM 培养基、胎牛血清、0.25% 胰蛋白酶均购于 Hyclone 公司。抗人 VEGF 抗体购自 Santa Cruz Biotechnology 公司；抗 VEGFR1、抗 VEGFR2、抗 pVEGFR2 抗体购自 Cell Signaling Technology 公司；抗 pVEGFR1 抗体购自 R&D Systems 公司；抗人 β-actin 抗体、羊抗兔抗体(二抗)购自康为公司。

1.2 细胞培养

HCT116 和 HT29 细胞株培养于含 10% 胎牛血清的 DMEM 培养基。放置于 37℃、5%CO_2 培养箱中，1~2d 换液 1 次，待贴壁细胞长满至 80%~90% 时传代。当肿瘤细胞达到对数生长期时开始试验，其中 A 组为 HCT116 细胞，B 组为 HT29 细胞。

1.3 样品处理

先将 A、B 两组处于对数生长期的肿瘤细胞分别铺于 6 孔培养板上，24h 后待细胞完全贴壁后更换为新鲜血清培养基，同时加入终浓度为 0、25μg/L、50μg/L、100μg/L、200μg/L、400μg/L 的贝伐珠单抗药物。37℃温箱孵育 48 h 后，收集培养上清液，然后用冷 PBS 冲洗孔板 2 次，用细胞裂解液冰上裂解细胞 30min，4℃ 12 000r/min 离心 5min，收集上清，BCA 法测量每孔细胞总蛋白，进行总蛋白定量。

1.4 RT-PCR 检测 mRNA 表达

引物序列如下：VEGFA：forward primerTGAGATCGAGTACATCTTCAAGCC，reverse primer CACATTTGTTGTGCTGTAGGAAGC；VEGFR1：forward primer TGAGAAAGAATTTGATACGCACC，reverse primer CGCTGTCCATCTGCTCCTG；VEGFR2：forward primerCGGCAAATGTGTCAGCTTTG，reverse primer CACGTGGAAGGAGATCACCC；β-actin：forward primer GGACCTGACTGACTACCTCATGAAGAT，reverse primer TCGTAGCTCTTCTCCAGGGAGGAGCT。

引物合成于生工生物工程公司(上海)，制备样品，Triozl 法提取总 RNA，AMV 法逆转录 cDNA，进行 PCR 反应，凝胶电泳，分析目的条带。

1.5 Western blotting 法测定目的蛋白表达

制备样品，BCA 法总蛋白定量，煮样，采用 6%~8%梯度聚丙烯酰胺凝胶，上样，电泳，采用 PVDF 膜转膜，封闭慢摇床，浸入稀释的一抗中(1:100)，于摇床上 4 ℃杂交过夜，TBST 洗膜 3 遍。取出 PVDF 膜，洗膜后孵育二抗(1:5000)，摇床上杂交 1 h。取出 PVDF 膜，TBST 洗膜 3 遍。将 ECL 试剂盒内的两种试剂等体积混合后滴在 PVDF 膜上，反应 1~2 min，然后在暗屋中压 x 光片 45min 后曝光，分析结果。采用 Bio-Rad Quantity One 软件进行分析目的条带和净光密度值(IOD)，在 β-actin 为内参标准下分析目的条带的 IOD 值，从而得出相应比值，最后进行统计学分析。

1.6 统计学处理

采用 SPSS15.0 软件进行数据统计分析，数据用 $\bar{x}\pm s$ 表示，采用单因素 t 检验，$P<0.05$ 为差异有统计学意义。

2 结 果

2.1 VEGFA、VEGFR1 和 VEGFR2 的 mRNA 水平变化

加入终浓度 400μg/贝伐珠单抗，48h 后 RT-PCR 法检测肿瘤细胞的 mRNA 水平。和对照组相比，药物组细胞内 VEGFA 表达水平无明显变化；VEGFR1 和 VEGFR2 表达水平出现了上调。相同实验条件下重复实验 3 次，未发现实验结果不一致，见 Figure 1 和 Figure 2。

2.2 VEGFA 蛋白表达水平变化

加入不同终浓度贝伐珠单抗 48h 后，Western

blotting 法检测细胞 VEGFA 的表达。和对照组相比，药物组肿瘤细胞 VEGFA 蛋白水平出现了明显降低。相同实验条件下重复实验 3 次，未发现实验结果不一致，见 Figure 3 和 Figure 4。

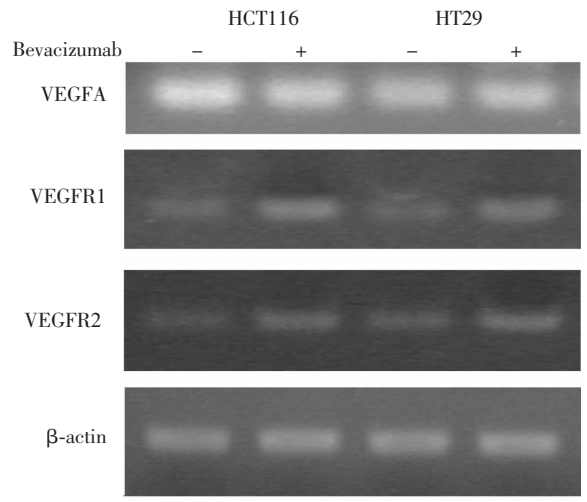


Figure 1　RT-PCR showing expressions of mRNA of colorectal carcinoma cells

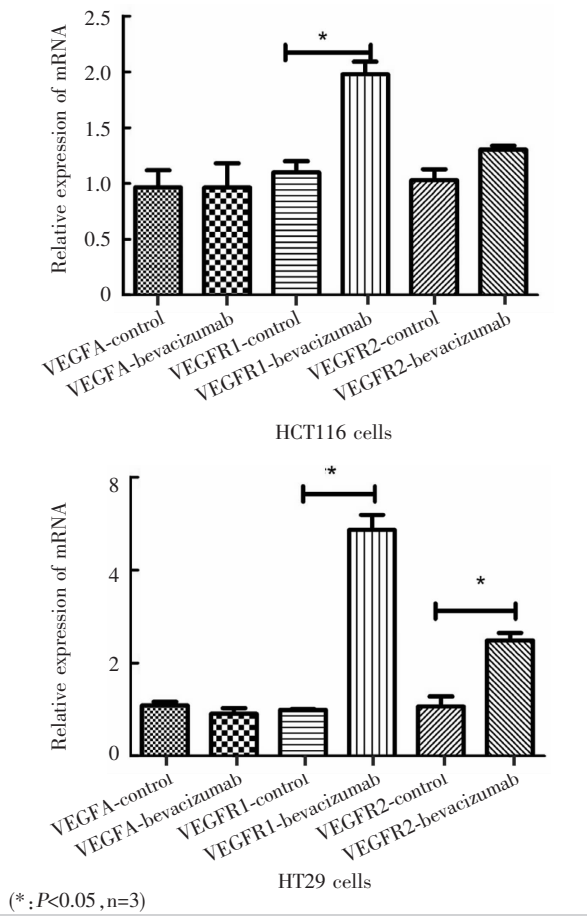


Figure 2　Relative expressions of mRNA of colorectal carcinoma cells

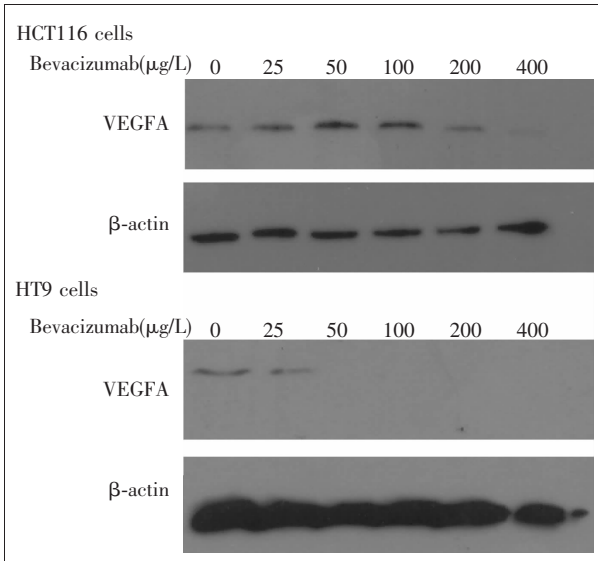


Figure 3　Western blotting showing expressions of VEGFA protein of colorectal carcinoma cells

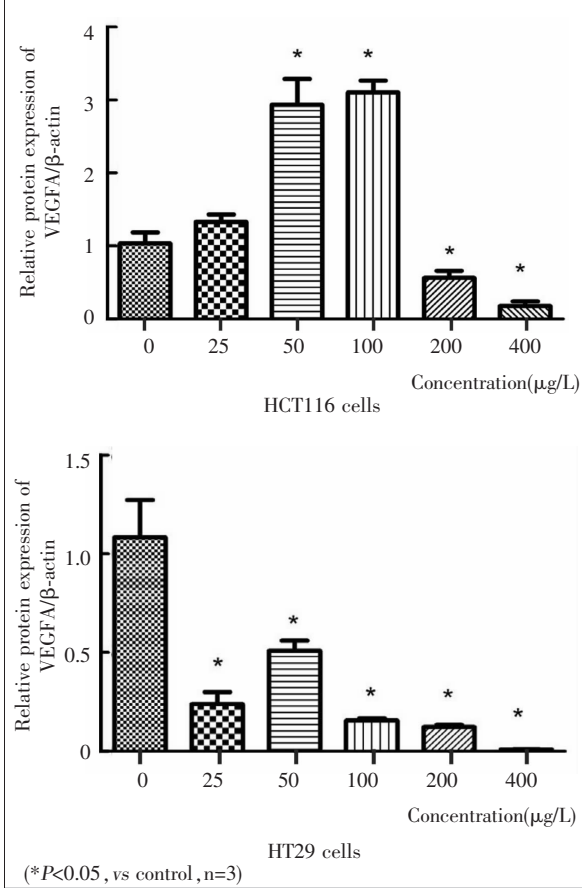


Figure 4　Relative protein expressions of VEGFA of colorectal carcinoma cell

2.3 VEGFR1 和 pVEGFR1 受体表达水平变化

加入不同终浓度贝伐珠单抗 48h 后，Western blotting 法检测细胞 VEGFR1 和 pVEGFR1 受体的表达水平。和对照组相比，药物组 VEGFR1 受体和 pVEGFR1 蛋白水平出现了明显上调（$P<0.05$）。相同实验条件下重复本实验 3 次，未发现实验结果不一致，见 Figure 5 和 Figure 6。

2.4 VEGFR2 和 pVEGFR2 受体表达水平变化

加入不同终浓度贝伐珠单抗 48h 后，Western blotting 法检测细胞 VEGFR2 和 pVEGFR2 受体的表达水平。和对照组相比，药物组细胞 VEGFR2 和 pVEGFR2 表达水平出现了明显上调（$P<0.05$）。相同实验条件下重复本实验 3 次，未发现实验结果不一致，见 Figure 7 和 Figure 8。

3 讨 论

在临床肿瘤治疗中，贝伐珠单抗作为分子靶向药物的经典药物，明显地抑制肿瘤血管新生，抑制肿瘤血管侵袭和转移[20,21]。贝伐珠单抗和 5-氟尿嘧啶为基础的联合治疗显著地提高晚期结直肠患者的

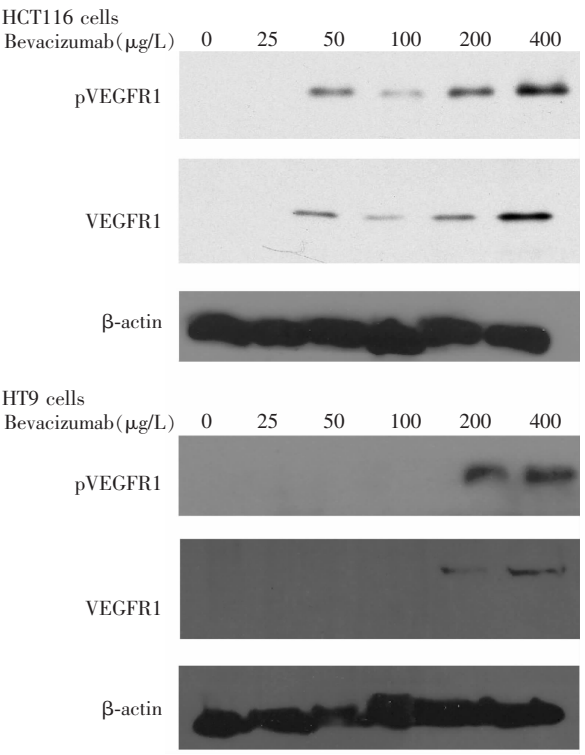
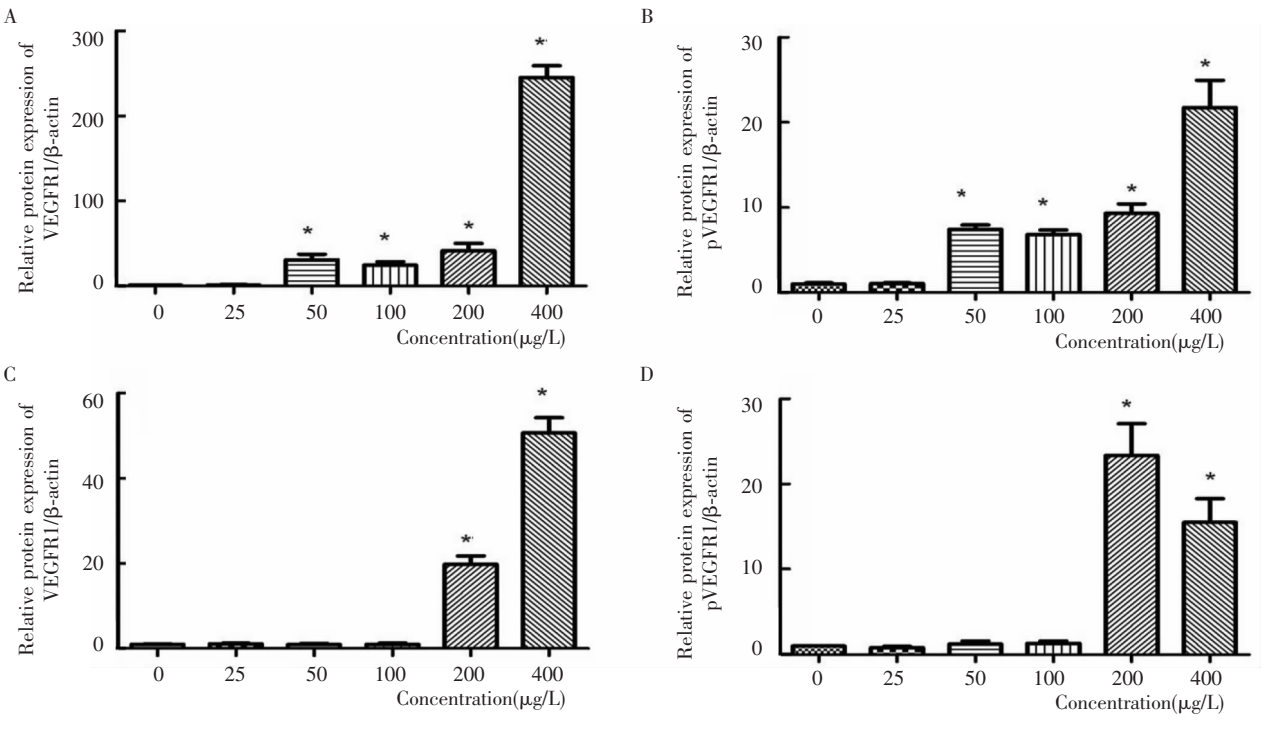


Figure 5 Western blotting showing expressions of VEGFR1 and pVEGFR1 of colorectal carcinoma cells



(A:HCT116 cells;B:HCT116 cells;C:HT29 cells;D:HT9 cells. *$P<0.05$, *vs* control, n=3)

Figure 6 Relative protein expressions of VEGFR1 and pVEGFR1 of colorectal carcinoma cells

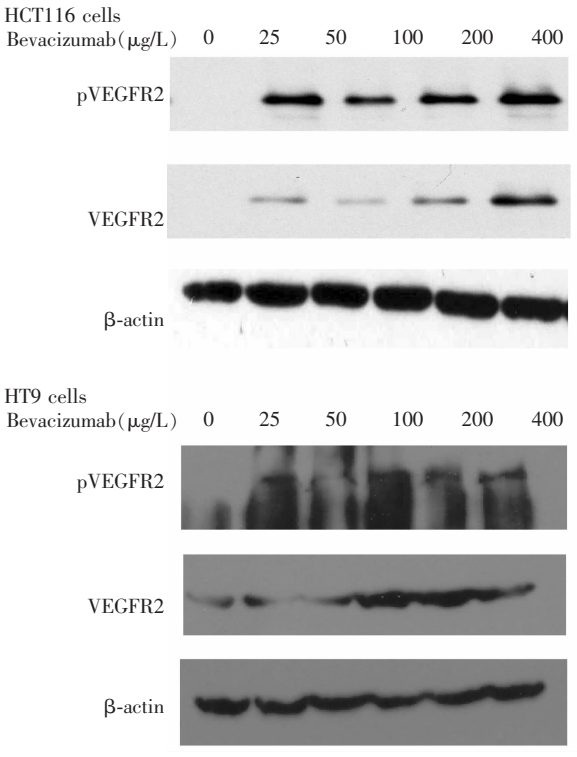
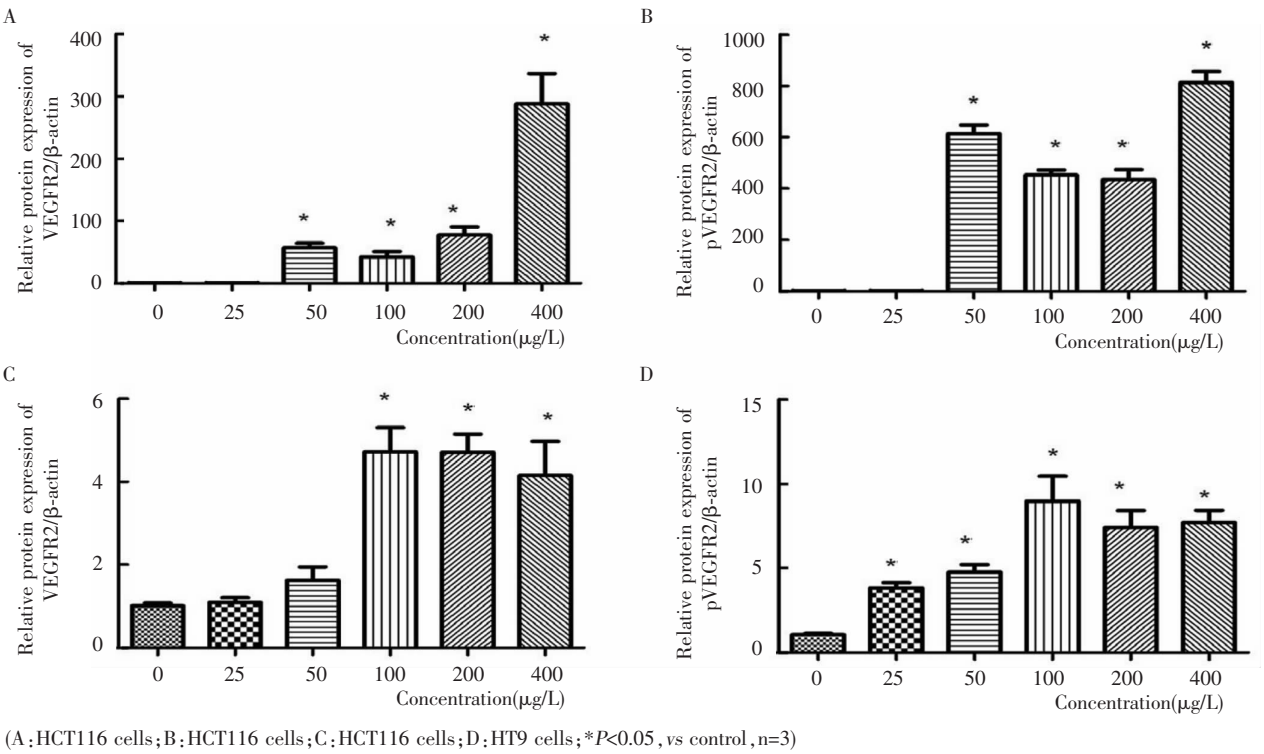


Figure 7 Western blotting showing expressions of VEGFR2 and pVEGFR2 of colorectal carcinoma cells

生存率及患者生活质量,已成为晚期侵袭性结直肠癌治疗的经典方案[22]，在其他实体肿瘤的临床二期实验,如肾细胞癌、卵巢癌及胃腺癌中也具有不错的临床治疗效果[23-25]。但贝伐珠单抗也存在治疗缺点,如在部分患者中治疗效果不佳或者耐药；治疗效果一过性及极少数患者治疗后病情加重，这限制了贝伐珠单抗的临床应用[26,27]。因此探究贝伐珠单抗的耐药机制显得尤为必要,目前其耐药机制尚不清楚[28,29]。

本实验采用贝伐珠单抗药物诱导结直肠癌细胞,48h 后我们检测发现肿瘤细胞 VEGF 的表达出现了明显下降，此外我们进一步还发现肿瘤细胞 VEGFR1 和 VEGFR2 的表达出现了明显上调。因而我们推测贝伐珠单抗导致了 VEGF 表达水平的下降，这进而导致了肿瘤细胞的 VEGF 相关受体的上调。文献研究表明 VEGF 与其相关受体参与了肿瘤细胞的信号传递,比如 VEGFR1 与 VEGFR2[2]。高表达 VEGFR1 的肿瘤细胞具有更强的肿瘤侵袭和血管新生能力[30];而 VEGFR1 与 VEGFR2 拮抗剂导致了肿瘤细胞增殖受到明显抑制，这均表明了 VEGFR1 与 VEGFR2 很有可能直接参与肿瘤细胞增殖及血管侵袭[31-33]。因此我们推测贝伐珠单抗诱导了



(A:HCT116 cells;B:HCT116 cells;C:HCT116 cells;D:HT9 cells;*P<0.05, *vs* control, n=3)

Figure 8 Relative protein expressions of VEGFR2 and pVEGFR2 of colorectal carcinoma cells

肿瘤细胞的 VEGFR1 和 VEGFR2 的明显上调，而 VEGFR1、VEGFR2 的上调可能直接促进了肿瘤血管新生和侵袭能力，这减弱或抵消了贝伐珠单抗的抑制血管新生及抗肿瘤作用，进而导致了临床耐药，但还需要更多实验来证明。

总之，本实验显示贝伐珠单抗诱导了肿瘤细胞 VEGFR1、VEGFR2 受体的上调，并推测贝伐珠单抗的耐药现象很可能与肿瘤细胞 VEGFR1、VEGFR2 的上调有密切关系，这为探究贝伐珠单抗的临床耐药提供一些帮助，也为双靶点药物联合抗肿瘤提供一些参考[34-36]。

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