

肿瘤乏氧靶向性基因治疗增强放射治疗的实验研究

刘军叶 郭 鹄 郭国祯

(第四军医大学预防医学系放射医学教研室 西安 710032)

乏氧是实体肿瘤组织微环境中的一个重要现象,乏氧肿瘤细胞对放射治疗、化学治疗的耐受性增强,乏氧肿瘤细胞的存在是恶性肿瘤患者治疗失败、复发和转移的重要原因之一^[1]。乏氧细胞的基因表达与正常细胞有着很大的差异,然而,这些差异使针对肿瘤乏氧细胞的靶向性治疗成为可能^[2]。本研究利用乏氧细胞自身的特点,构建乏氧依赖性表达的腺病毒载体,借助该载体引导自杀基因BCD(细菌胞苷脱氨酶,可将无毒性的前体药物5-氟胞嘧啶转变为细胞毒性药物5-氟尿嘧啶)在乏氧肿瘤细胞的特异性表达,观察该系统对放射治疗的增强效应。

借助DNA重组技术将BCD基因置于5个拷贝的乏氧反应元件HRE(Hypoxia-responsive element)的下游,并将5HRE-BCD重组入腺病毒载体,构建成为乏氧依赖性表达的腺病毒载体Ad-5HRE/hCMVmp-BCD。用Ad-5HRE/hCMVmp-BCD感染Hela细胞并给予乏氧处理,借助MTS实验法检测5-FC对细胞的生长抑制效应。建立小鼠的Hela细胞移植瘤,并对肿瘤进行腺病毒Ad-5HRE/hCMVmp-BCD介导的基因治疗、放射治疗或两者联合治疗。

Ad-5HRE/hCMVmp-BCD感染Hela细胞后,BCD在正常氧条件下呈低水平表达,乏氧处理后可显著上调其蛋白表达量;细胞生长抑制实验结果表明,与正常氧条件下相比,乏氧条件下细胞对5-FC的 IC_{50} 下降了20倍;并且,在每一个病毒感染剂量(MOI)下,随着5-FC剂量的增加,细胞的生存数量呈剂量依赖性降低。裸鼠移植瘤生长抑制试验结果显示,单纯腺病毒Ad-5HRE/hCMVmp-BCD+前体药物具有一定的抗肿瘤效应。但将病毒+前体药物的自杀基因疗法与分次放射治疗联合后,其抗肿瘤效应显著增强。PBS对照组、单纯腺病毒Ad-5HRE/hCMVmp-BCD+前体药物、前体药物+对照病毒+放射治疗、前体药物+放射治疗、Ad-5HRE/hCMVmp-BCD+前体药物+放射治疗各组肿瘤的倍增时间依次为8、11、18、19、42 d。

本研究发现乏氧诱导性的腺病毒Ad-5HRE/hCMVmp-BCD自杀基因系统可显著增强放射治疗效果,具有一定的临床应用前景。

关键词 乏氧,腺病毒,基因治疗,肿瘤,细菌胞苷脱氨酶

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Adenovirus-mediated gene therapy targeting tumor hypoxia

LIU Junye GUO Yao GUO Guozhen

(Department of Radiation Medicine, Faculty of Preventive Medicine, Fourth Military Medical University, Xi'an 710032)

The work is to construct a hypoxia-activated adenovirus vector expressing suicide gene BCD and to evaluate anti-tumor effects of the hypoxia-targeted suicide gene therapy system with or without combination radiotherapy. The recombinant adenovirus Ad-5HRE/hCMVmp-BCD was generated by the COS-TPC methods. Hela cells were treated with Ad-5HRE/hCMVmp-BCD infection, followed by incubated with 5FC-containing medium un-

第一作者:刘军叶,女,1972年4月出生,2004年于第四军医大学获博士学位,放射医学专业,讲师
通讯联系人:郭国祯

der the aerobic conditions or hypoxic conditions for 24 h. Cell growth inhibition was determined by MTS assay. Tumor xenografts were established by injecting Hela cells s.c into the right leg of nu/nu BALB/c mice. The tumor-bearing mice were given Ad-5HRE/hCMVmp-BCD-mediated gene therapy or combination therapy with radiotherapy. Western blot analysis clearly indicated that significant hypoxia-induced expression of BCD protein after Hela cells were infected with Ad-5HRE/hCMVmp-BCD. The actual amounts and the ratio of the BCD expression under aerobic and hypoxic conditions were increased with increasing MOI. MTS assay results indicated that hypoxia-induced sensitization to 5FC. Under the hypoxic incubation the IC_{50} is 20 folds lower than that of the aerobic condition. The increased cytotoxicity at each MOI of Ad-5HRE/hCMVmp-BCD infection with increasing 5FC concentration was also observed. Tumor growth delay assay results showed that with single radiation or fractionated radiation, the Ad-5HRE/hCMVmp-BCD+5FC +radiation group produced the longest times to tumor regrowth and average tumor doubling compared with the various control treatment groups. In vitro and in vivo studies demonstrated the combination of Ad-5HRE/hCMVmp-BCD and prodrug 5-FC can obviously inhibit the growth of Hela cells. Using the mouse xenografts model, we demonstrated the Ad-5HRE/hCMVmp-BCD and 5-FC can enhance the antitumor effects of conventional radiation therapy.

KEYWORDS Hypoxia, Adenovirus, Gene therapy, Tumor, Cytosine deaminase

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