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论著

E1A基因对人鼻咽癌动物模型放射增敏的实验研究

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摘要

目的：探讨腺病毒E1A基因对人鼻咽癌动物模型放射增敏的实验研究。 方法：取对数生长期的CNE-2Z细胞5×105 /0.2 mL，接种于4周龄左右裸鼠右前肢腋部皮下致瘤，第7天裸鼠皮下肿瘤长至直径0.6～0.8 cm时，随机分为6组(n=10)：PBS组, Ad-β-gal组，放射组，Ad-β-gal+放射组,Ad-E1A组，Ad-E1A+放射组。Ad-β-gal组/Ad-E1A组在第2周开始给予荷瘤裸鼠皮下肿瘤内滴度为5×109 PFU/ 50μL的Ad-E1A/ Ad-β-gal注射，每周2次，连续2周，放射组在第3周给予6MV-X照射，每天2 Gy,连续5 d。Ad-β-gal+放射组/Ad-E1A＋放射组在第2周开始，给予荷瘤裸鼠皮下肿瘤内滴度为5×109 PFU/ 50μL 的Ad-E1A/ Ad-β-gal注射，每周2次，连续2周，在第3周给予6MV-X照射，每天2 Gy,连续5 d。第1次治疗后，每隔4天用卡尺测量1次肿瘤的体积，记录其直径，裸鼠的生存时间。当肿瘤的直径超过2 cm时，处死裸鼠，分离肿瘤组织，采用免疫组织化学方法分析血管内皮生长因子(vascular endothelial growth factor, VEGF)和CD34表达，TUNEL分析细胞凋亡。 结果：Ad-E1A+放射组较其他组能明显延缓移植瘤生长时间, Ad-E1A+放射组裸鼠的肿瘤平均体积比单独放射组小4.7倍，比单独用Ad-E1A组的小5.3倍。Ad-E1A+放射组的裸鼠生存率显著高于其他治疗组。Ad-E1A+放射组的VEGF蛋白表达和微血管密度较其他各组明显下降（P＜0.01)。TUNEL分析结果显示Ad-E1A组和Ad-E1A+放射组及放射组的肿瘤组织内均可明显观察到凋亡细胞。并且Ad-E1A+放射组肿瘤细胞凋亡数目明显高于Ad-E1A组或者放射组。结论：E1A基因通过抑制肿瘤血管的形成和诱导肿瘤细胞的凋亡来提高人鼻咽癌细胞对放射的敏感性。

关键词　鼻咽癌；放射治疗；E1A；血管内皮生长因子；CD34

分类号

Effect of adenovirus-E1A gene therapy on in vivo radiosensitivity to nasopharyngeal cancer

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Abstract

ObjectiveTo determine the effect of Ad-E1A gene therapy on in vivo radiosensitivity to nasopharyngeal carcinoma. MethodsCNE-2Z cells (2×105) were subcutaneously injected into nude mice to develop tumor (1～3 mm) 6 days later. The tumor-bearing mice were then randomly divided into 6 groups (10 mice per group) for PBS treatment or treatment with radiotherapy, Ad-E1A, or Ad-β-gal alone or radiotherapy in combination with Ad-E1A or Ad-β-gal. The mice were treated with Ad-E1A or Ad-β-gal (5×109 plaque forming units) by intratumoral injection twice weekly for 2 weeks at beginning of week 2. The mice treated with radiotherapy in combination with Ad-E1A or Ad-β-gal received 2 Gy radiotherapy daily for 5 days following the first week of treatment with Ad-E1A or Ad-β-gal. Control mice received PBS therapy or radiotherapy only after tumor cells were injected. When the size of tumor exceeded 2 cm， the mice were killed and the tumors underwent immunohistochemical analysis for VEGF and CD34 expression and TUNEL assay for apoptosis.Results The growth delay time was longest in the Ad-E1A plus radiotherapy group. Tumors treated with Ad-E1A plus radiotherapy were 4.7-fold smaller than those treated with radiotherapy alone and 5.3-fold smaller than those treated with Ad-E1A alone. The survival rate of tumor-bearing mice treated with Ad-E1A plus radiotherapy was significantly higher than that of other treatment groups. The vessel density and the VEGF expression were significantly lower in tumors treated with Ad-E1A plus radiotherapy than those treated with radiotherapy alone, Ad-E1A alone, Ad-β-gal alone, or Ad-β-gal plus radiotherapy (P<0.01). TUNEL staining revealing apoptosis can be detected in the Ad-E1A group, radiotherapy group, Ad-E1A plus radiotherapy group, and more apoptosis can be detected in tumors treated with Ad-E1A plus radiotherapy than those of other treatment groups.ConclusionE1A gene therapy can effectively enhance the nasopharyngeal carcinoma sensitivity to the radiotherapy by down-regulating VEGF expression and inducing apoptosis.

Key words nasopharyngeal cancer radiation therapy E1A VEGF CD34

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