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World Chin J Digestol 2003 June;11(6):708-711
世界华人消化杂志 ISSN 1009-3079 CN 14-1260/R

抗肝癌单链免疫毒素基因修饰的PBMCs在动物体内的抑瘤作用

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收稿日期:2002-12-23 接受日期:2003-01-02

In vivo antitumour activity of PBMCs *via* genetic modification of single-chain immunotoxin

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Received:2002-12-23 Accepted:2003-01-02

Abstract

AIM:To investigate *in vivo* antitumour activity of single-chain immunotoxin (sFv-TNF-α fusion protein).

METHODS:HCC-specific killer cells were generated by transducing the recombinant retroviral virus in supernatant of the virus producing cells (C_{22}) into human peripheral blood mononuclear cells (PBMCs). SMMC-7721 xenograft nude mice were given iv either 1×10^6 (0.2 mL) transduced or mock-transduced PBMCs once five days for three weeks and tumour growth was detected.

RESULTS:Tumour growth were (20.8±4.9) mg/d in PBMCs/PST group and (28.5±6.7)mg/d in PBMCs/ pLXSN group, with a significant difference (P <0.05).

CONCLUSION:Genetic modification of PBMCs by single-chain immunotoxin has antitumour activity *In vivo*.

Cheng H, Liu YF, Zhang HZ, Shen WA, Zhang J, Zhang J. *In vivo* antitumour activity of PBMCs *via* genetic modification of single-chain immunotoxin. Shijie Huaren Xiaohua Zazhi 2003;11(6):708-711

摘要

目的:用携带分泌型抗肝癌单链免疫毒素基因(sFv-TNF-α)的重组逆转录病毒感染人外周血单个核细胞(peripheral blood mononuclear cells, PBMCs),使其表达并分泌针对人肝癌细胞的sFv-TNF-α融合蛋白, 观察转导的PBMCs/PST静脉注射后对荷肝癌裸鼠的抑瘤作用.

方法:分离正常人PBMCs并用PHA和IL-2进行体外刺激培养,用感染性重组病毒产生细胞C_{22}(PA317/PST)产生的病毒上清转导后,经尾静脉输入荷肝癌裸鼠体内,注射细胞数为每只1×10^6(0.2 mL),每5 d注射1次,共注射3次.定期观察记录肿瘤生长情况,注射3次后处死,取出瘤组织称质量、记录,进行统计学处理,并制备石蜡切片进行常规HE染色及免疫组化染色观察.

结果:实验组肿瘤生长速率为(20.8±4.9) mg/d,对照组为(28.5±6.7) mg/d,经t检验,P<0.05.

结论:经重组逆转录病毒转导的PBMCs/PST在裸鼠体内对人肝癌细胞系SMMC-7721移植瘤具有一定的生长抑制作用.

程虹,刘彦仿,张惠中,沈万安,张菊,张静. 抗肝癌单链免疫毒素基因修饰的PBMCs在动物体内的抑瘤作用. 世界华人消化杂志 2003;11(6):708-711
http://www.wjgnet.com/1009-3079/11/708.asp

0 引言

肝细胞肝癌(hepatocellular carcinoma, HCC)是人类常见的恶性肿瘤之一,有较高的发病率和死亡率,历有癌王之称.其多发生于亚洲、非洲和地中海流域,全世界每年约有100多万患者死于肝癌,其中我国就有11万人[1-7],且发病率有上升的趋势.迄今为止,肝癌的病因和发病机制仍不十分清楚,更无理想的治疗手段[8-18].随着现代分子生物学和免疫学的发展,自1990年代初迄今,已有百余项肿瘤基因治疗方案获准进入临床试验,但临床疗效与预期的目标之间还存在较大差距,原因之一就是肿瘤细胞的特异性识别尚未解决.肿瘤特异性单链抗体的问世为基因治疗靶向性问题的解决提供了新的手段,近年来成为肿瘤免疫基因治疗中的热点,目前已有多种单链免疫毒素基因治疗计划进入临床试验[19-27].我们在成功克隆抗肝癌单链抗体基础上,将其与TNF-α基因相连构建成分泌型抗肝癌单链免疫毒素基因,细胞学实验表明,该重组基因转入

人外周血单个核细胞(peripheral blood mononuclear cells, PBMCs)后可表达并分泌具有特异性结合活性的抗肝癌 sFv-TNF-α融合蛋白，后者对体外培养的肝癌细胞具有杀伤作用. 为进一步观察分泌型抗肝癌单链免疫毒素在体内的生物学作用，我们用携带分泌型抗肝癌单链免疫毒素基因的逆转录病毒产生细胞 C_{22} 的上清转导人 PBMCs，观察转导的 PBMCs /PST 静脉注射后对荷肝癌裸鼠的抑瘤作用.

1 材料和方法

1.1 材料　人肝癌细胞系 SMMC-7721 为本室保存，RPMI1640 常规传代培养. 转染的逆转录病毒产生细胞系 C_{22} 由本室构建[28]，用 DMEM 常规传代培养. DMEM，G418，Superscript™ 逆转录试剂盒和 Trizol Reagent 为 Gibco 公司产品，Polybrene 为 Sigma 公司产品，MTT 为 Serva 公司产品，PHA 购自 Sigma 公司，超级小牛血清购自杭州四季青生物工程材料研究所. 重组人 IL-2为上海生物技术研究所产品. 淋巴细胞分离液购自上海试剂二厂.

1.2 方法[28-30] 消化对数生长期的 SMMC-7721 细胞，无血清 RPMI1640 培养液洗涤 2 次，计数活细胞数，调整细胞数，将细胞重悬于 PBS 中. 在无菌条件下，将 0.2 mL 细胞悬液接种于裸鼠胸部皮下，含活细胞数为每只 5×10^6，共 10 只. 接种后置无特殊病原体饲养室饲养，定期观察记录肿瘤生长情况. 抽取正常人外周血 50 mL，肝素抗凝，加入淋巴细胞分离液 50 mL，室温 1 400 g 离心 30 min，吸取单个核细胞层悬液，40 mL 无血清 RPMI1640 洗涤细胞 3 次(1 000 g 离心 10 min)，计数，用含 200 mL/L 小牛血清的完全 RPMI1640 培养液稀释为活细胞 1×10^9/L，加入 5×10^5/L IL-2 及 5×10^3 μg/L PHA，于 37 ℃，50 mL/L CO_2 培养 3 d，换含 5×10^5/L IL-2，200 mL/L 小牛血清的完全 RPMI1640 培养液继续培养 3 d 后，进行逆转录病毒的转导. 用 C_{22} 细胞制备重组逆转录病毒上清，取 C_{22} 细胞上清(不含 G418) 5 mL 及 8 mg/L Polybrene 感染 1×10^6 个 PBMCs，置 37 ℃，50 mL/L CO_2 孵箱内培养 6 h，换普通完全 RPMI1640 培养液，连续感染 3 d，转导的 PBMCs 命名为 PBMCs/PST. 对照组 PBMCs 用 PA317/pLXSN(空白载体)细胞上清感染，转导的 PBMCs 命名为 PBMCs/ pLXSN. 接种 7 d 的荷肝癌裸鼠随机分为实验组 5 只和对照组 5 只. 分别离心收集连续感染 3 d 的 PBMCs/PST 和 PBMCs/ pLXSN 细胞，计数，无血清 RPMI1640 培养液洗涤 2 次后重悬于生理盐水，从尾静脉注射入裸鼠体内，实验组注射 PBMCs/PST，对照组注射 PBMCs/pLXSN，注射细胞数为每只 1×10^6(0.2 mL)，每 5 d 注射 1 次，共注射 3 次. 注射后置无特殊病原体饲养室饲养，定期观察记录肿瘤生长情况. 注射 3 次后处死各组荷瘤裸鼠，取出瘤组织称重、记录，进行统计学处理，并固定，制备石蜡切片，常规 HE 染色、免疫组化染色观察.

2 结果

2.1 荷肝癌裸鼠模型的建立 接种人肝癌细胞系 SMMC-7721 的 10 只裸鼠 7 d 后均成瘤，移植瘤直径约为 0.58 cm.

2.2 PBMCs/PST的体内抑瘤作用 经尾静脉注射PBMCs/PST 3 次后，肉眼观察接种 SMMC-7721 细胞的实验组裸鼠移植瘤体积较小，处死后取出瘤组织称质量并计算肿瘤生长速率，实验组为(20.8±4.9) mg/d，对照组为(28.5±6.7) mg/d，经 t 检验，$P<0.05$. HE 染色可见肿瘤细胞呈巢状分布，纤维组织分隔，瘤细胞大小不等，核大且深染，核分裂相多见(图 1)，符合肝细胞肝癌的组织学形态. 以鼠抗人 TNF-α多克隆抗体进行免疫组化染色，可见瘤组织中少数细胞有强、弱不等的阳性信号，分布于细胞膜上(图 2)，对照组肿瘤组织免疫组化染色未见阳性信号.

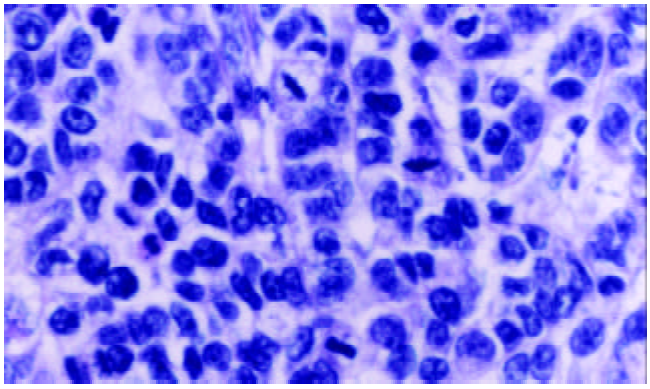

图 1 接种肝癌细胞 HHCC 的裸鼠移植瘤组织 HE 染色 × 400.

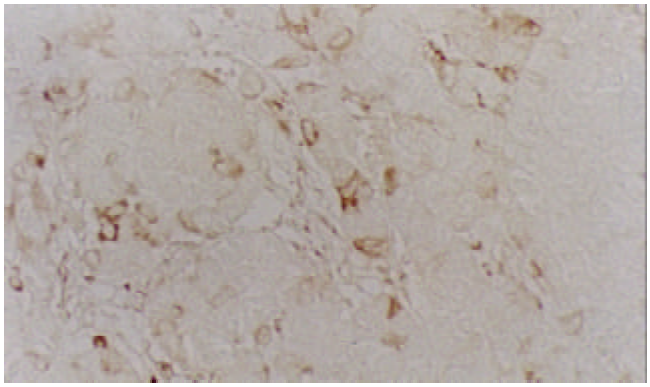

图 2 实验组裸鼠移植瘤免疫组化染色细胞膜阳性 × 400.

3 讨论

要实现基因治疗的目的，最终必须将目的基因转入靶细胞. 实现人类实体瘤基因治疗的途径主要有 2 种,(1)在体靶细胞的转染(*in vivo*)，即直接基因治疗途径，例如将细胞因子或肿瘤抗原的基因表达载体直接体内注射或将 MHC-I 基因直接注射入瘤体内等治疗肿瘤; (2)离体靶细胞的转染(*ex vivo*)，即间接基因治疗途径，必须分离并培养离体的靶细胞，并在接受基因转移后移植回人体以治疗疾病，例如应用体外基因修饰的 TIL、瘤苗、成纤维细胞等体内回输或接种的方法治疗肿瘤[31-34]. 我们选择逆转录病毒介导的体外基因转移方法进行基

因治疗的研究，要求基因转移的靶细胞既要容易获得，又要容易回输到人体，因此，我们采用PBMCs作为基因转移的靶细胞.由于目前所常用的基因转移方法[35-41](包括逆转录病毒载体-包装细胞转移系统)效率均不高，获得大量的靶细胞是重要的先决条件，因此，要求该细胞易于体外培养，而且具有一定的分裂和增生能力.此外，逆转录病毒载体对处于活跃分裂状态的细胞感染率和基因转移率较高，而对未分化的分裂不十分活跃的细胞感染率和基因转移率较低，因此，基因治疗中应尽量选择处于活跃分裂状态的细胞作为靶细胞. PBMCs具备上述的各项条件，目前是一种较理想的基因治疗靶细胞[42-44]. 虽然有研究表明，TIL抗肿瘤的活性是PBMCs的50-100倍，而且具有良好的肿瘤病灶趋向性和浸润性[45-49]，但由于其不易分离和培养，在某些肿瘤如肝癌中TIL数量极少，无法满足基因治疗中反复操作的要求，因而其应用受到很大的限制.

我们分离出正常人PBMCs，并用PHA和IL-2进行体外刺激培养，用携带分泌型抗肝癌单链免疫毒素基因的重组逆转录病毒感染，使其表达并分泌针对人肝癌细胞的sFv-TNF-α融合蛋白，再经尾静脉输入荷肝癌裸鼠体内，观察转导的PBMCs/PST对荷瘤裸鼠体内肿瘤生长的抑制作用.本实验结果表明，经重组逆转录病毒转导的PBMCs/PST在裸鼠体内对人肝癌细胞系SMMC-7721移植瘤具有一定的生长抑制作用，这种抑瘤作用可能是由于转导的PBMCs/PST作为外源目的基因的载体，在体内能够不断表达并分泌针对人肝癌细胞的免疫毒素-TNF-α融合蛋白，这种靶向性融合蛋白分子在肝癌移植瘤局部富集，TNF-α通过与肝癌细胞表面的受体结合而导致跨膜信号的传导，表现出TNF-α的生物学活性作用，从而直接或间接发挥对肝癌细胞的抑制作用[50-53]. 此外，PBMCs/PST分泌的靶向性sFv-TNF-α融合蛋白的抑瘤作用不仅针对原发灶肿瘤细胞，而且对转移至身体任何部位甚至进入血管及淋巴管的肿瘤细胞同样具有生长抑制作用，是集免疫、基因治疗、靶向治疗和过继免疫治疗为一体的一种新的免疫基因治疗方法，为肝癌的靶向基因治疗提供一条新的途径.

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• 编委来信 •

总编:寄来的 2003 年第 5 期中英文杂志收到，一口气读完，真有点爱不释手. 两本杂志，从内容到形式，堪称精品. 英文版增加到 244 页，刊登了 51 篇高水平文章；中文版 190 页，刊登了 61 篇文章，信息容量显著增加，既突出了我国在消化系肿瘤和病毒性肝炎方面研究的优势和特色，又进一步缩短了文章的刊出周期. 同时，新一届编委会成员大部分都承担各类课题，为保证杂志论文质量提供了更好的基础.

在栏目设置方面，有好的临床研究方面的论文可专门刊登在《临床研究》专栏中，以推动和提高我国临床研究的水平.

另外，通过电子邮件寄送的录用通知已经收到，今天已按规定将版面费寄出，请查收.相信杂志会越办越好!

江学良编委 2003-05-26