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重组腺病毒介导survivin基因转染树突状细胞对肾细胞癌的免疫效应 点此下载全文

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摘要：

摘 要 目的: 研究重组腺病毒介导survivin基因转染的树突状细胞(DCs)体外诱导特异性抗肾细胞癌的免疫效应。方法： 以携带survivin基因的重组腺病毒（Ad-svv）感染DCs， Western bloting检测转染DCs的survivin的表达，流式细胞术检测DCs表面分子CD83、MHC Ⅱ、CD80、CD86的表达，ELISA法检测DCs培养上清中IL-12 的含量；混合淋巴细胞反应(MLR)测定DCs刺激同种异体淋巴细胞增殖的能力，ELISA法检测DCs刺激淋巴细胞后上清中IFN-γ含量，MTT法检测其诱导的特异的细胞毒性T淋巴细胞(CTLs)免疫效应。结果： 各组Ad-svv转染DCs后均表现出成熟的DCs表型特征，均可见survivin蛋白表达；转染Ad-svv或转染空白腺病毒载体(Ad-CMV)的DCs上清中IL-12均高于对照组(P <0.01)，转染Ad-svv的DCs上清中IL-12高于转染Ad-CMV-DCs组(P <0.01)。MLR中，转染或未转染腺病毒的DCs均能刺激同种异体T淋巴细胞的增殖，Ad-svv-DC刺激T淋巴细胞增殖的能力最强(P <0.01)；MLR后，转染或未转染重组腺病毒的DCs均能刺激T淋巴细胞IFN-γ的分泌，Ad-svv-DC组IFN-γ的分泌能力最强(P <0.01)。Ad-svv-DCs组诱导的CTL对survivin表达阳性的786-0肾癌细胞的杀伤率明显高于无survivin表达的L-02肝细胞(P <0.01)，Ad-CMV-DCs、空白DC对786-0肾癌细胞无杀伤作用。结论： 重组腺病毒介导survivin基因转染能显著提高DCs对肾癌细胞抗原的提呈能力、激活特异性细胞毒性T淋巴细胞、诱导针对 survivin的特异性抗癌免疫效应。

关键词：survivin基因 树突状细胞 肾癌 腺病毒 免疫治疗 基因治疗

Recombinant adenovirus mediated survivin gene transfection of dendritic cells induces immune responses against renal cell carcinoma Download Fulltext

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Abstract:

Abstract Objective:To investigate the anti tumor immune responses against renal cell carcinoma induced by dendritic cells (DCs) transfected with survivin gene mediated by recombinant adenovirus.Methods: The DCs derived from human peripheral blood were infected with recombined adenovirus vector carrying the survivin gene. The expression of survivin protein in the infected DCs was examined by Western blotting assay; the surface expression of CD83, MHC Ⅱ, CD80 and CD86 by flow cytometry (FCM), Interleukin 12 (IL 12) in the supernatants of DCs and IFN γ released by the cytotoxic T lymphocytes (CTLs) by ELISA, the ability of DCs in proliferating allo lymphocytes by mixed lymphocyte reaction (MLR), and specific killing activity of CTLs by MTT assay. Results: The DCs presented mature DCs phenotype after transfection with Ad svv. The expression of survivin protein in transfected DCs was confirmed by Western blotting analysis. The IL 12 level in the supernatant of DCs transfected with Ad svv was significantly higher than that transfected with empty vector (Ad CMV, P <0.01); and both of them were significantly higher than that in the control group(P <0 01). During MLR assay the DCs infected and not infected with adenovirus both stimulated allogeneic lymphocyte proliferation, with DCs infected with Ad svv having the strongest stimulating activity (P <0.01). After MLR the DCs infected and not infected with adenovirus both stimulated IFN γ secretion by T lymphocytes, with DCs infected with Ad svv having the strongest secreting activity (P <0.01). The CTL activity of DCs was significantly higher against renal cancer cell line 786 0 positive for survivin than against hepatic cancer cell line L 02 negative for survivin(P <0.01). DCs in the Ad CMV group and empty control group had not cytotoxic effect on 786 0 cells. Conclusion: Infection with the recombinant adenovirus encoding survivin can greatly enhance the antigen presenting ability of DCs and subsequently induce survivin specific CTL activity and anti tumor effect.

Keywords:survivin gene dendritic cell renal cell carcinoma adenovirus immuno therapy gene therapy

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