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SA-IL-7融合蛋白的制备及其膀胱内灌注对小鼠表浅性膀胱癌的治疗效应 点此下载全文

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摘要：

目的：制备链亲和素标记的白介素7（streptavidin-tagged interleukin-7, SA-IL-7）融合蛋白，评价其对小鼠表浅性膀胱癌治疗的效果。方法：构建pET24a-SA-IL-7重组质粒，制备SA-IL-7融合蛋白。流式细胞术检测SA-IL-7融合蛋白与生物素化膀胱癌细胞MB49的结合，胸腺细胞增殖法检测SA-IL-7融合蛋白促进胸腺细胞增殖的生物学活性。应用小鼠表浅性膀胱癌模型评价膀胱内灌注SA-IL-7的治疗效果，观察小鼠生存期，免疫组化法检测各组小鼠肿瘤浸润CD8+T细胞。结果：成功制备的SA-IL-7融合蛋白可锚定于生物素化的MB49细胞表面，结合率达（96.6±1.3）%；同时能够促进胸腺细胞的增殖。SA-IL-7融合蛋白能够锚定于小鼠生物素化的膀胱黏膜表面7 d以上，而未生物素化的膀胱黏膜在始终未检测到IL-7的存在。在小鼠表浅型膀胱癌模型中，MB49细胞接种后的第80天，IL-7灌注治疗组90%小鼠死亡，而SA-IL-7组60%小鼠存活且未出现肿瘤（P<0.05）；且SA-IL-7灌注治疗组小鼠肿瘤组织中浸润CD8+T细胞明显增多(P<0.01)。SA-IL-7治愈的15只表浅型膀胱癌模型小鼠中，11只能够抵抗膀胱内MB49细胞的第二次接种，而对照组15只小鼠仅1只幸存（P<0.01）。结论：SA-IL-7融合蛋白膀胱内灌注能够产生抗肿瘤免疫应答和明显的治疗效果，有可能成为免疫治疗浅表性膀胱癌的新方法。

关键词：白细胞介素7 链亲和素 生物素 融合蛋白 表浅性膀胱癌

Preparation of streptavidin-tagged interleukin-7 fusion protein and its therapeutic effect with intravascal instillation on mouse superficial bladder cancer Download Fulltext

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Abstract:

Objective : To prepare streptavidin-tagged interleukin-7 (SA-IL-7) fusion protein and to value its therapeutic effect on mouse superficial bladder cancer. Methods: Recombinant pET24a-SA-IL-7 plasmid was constructed and then SA-IL-7 fusion protein was prepared. The binding of SA-IL-7 to biotinylated bladder cancer MB49 cells was examined by flow cytometry and the bioactivity of SA-IL-7 fusion protein was examined by thymocyte proliferation assay. The mouse superficial bladder cancer model was used to evaluate the therapeutic effect of intravesical instillation of SA-IL-7. Mice were monitored for survival and the tumor-infiltrated CD8+T cells were examined by immunohistochemistry assay. Results: SA-IL-7 fusion protein efficiently anchored on the biotinylated MB49 cell surface, with a binding ratio of (96.6±1 3)%; SA-IL-7 promoted the proliferation of thymocytes. The SA-IL-7 fusion protein anchored on biotinylated bladder mucosal surface for more than 7 d. Whereas, IL-7 was no longer detected in the first day in the unbiotinylation group. After being attacked by bladder cancer MB49 cells for 80 d, 90% mice in the IL-7 group, while 60% mice survived without tumors in the SA-IL-7 group (P<0.05). Moreover, tumor-infiltrated CD8+ T cells in the SA-IL-7 group was significantly higher than those in the control group (P<0.01). Importantly, 11 out of 15 SA-IL-7-cured mice resisted to a second intravesical MB49 cell challenge, whereas only 1 of 15 control mice survived the challenge (P<0.01). Conclusion: Intravesical instillation of SA-IL-7 fusion protein can induce anticancer immunity, which may represent a promising immunotherapy for superficial bladder cancer.

Keywords:interleukin-7 streptavidin biotin fusion protein superficial bladder cancer

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