二、实验设计论坛

医学免疫学、医学病原生物学与感染性疾病

**葡萄糖在靶向免疫检查点关键分子PD-1治疗恶性肿瘤中的作用研究**

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**摘要：立论依据** 程序性细胞死亡蛋白1（PD-1）是具有负向免疫调节作用的免疫检查点关键分子，靶向PD-1的免疫治疗已在临床治疗多种恶性血液肿瘤中显示良好效果，但实体瘤治疗中仍受到肿瘤微环境（TME）中诸如营养物质的利用等问题的限制。目前普遍认为，肿瘤细胞是肿瘤微环境中葡萄糖的主要消耗者。然而，最新研究表明，在TME中髓系免疫细胞摄取葡萄糖的能力最强，其次是T细胞和癌细胞。据此推测，通过添加某种浓度葡萄糖可能影响TME中免疫细胞的数量、比例、活性及代谢信号通路，增强免疫细胞抗瘤能力，从而抑制肿瘤的发生发展；**设计思路** 制备多种小鼠肿瘤模型，在PD-1抗体免疫治疗中，灌胃不同浓度的葡萄糖，对比分析葡萄糖添加对小鼠生存期及肿瘤大小的影响，并通过流式细胞术分析及单细胞测序分析探讨可能的作用机制；**实验内容** 选取MC38、LLC1、4T1、SGC7901四种细胞株分别建立小鼠的肿瘤模型。每种肿瘤模型中，随机分为6组，模型对照组、纳武利尤单抗注射液组（抗PD-1抗体）、纳武利尤单抗注射液加浓度为0.25g/ml、0.5g/ml、0.75g/ml、1g/ml的葡萄糖溶液组。监测小鼠体重和生存期、瘤体直径、重量等。制备肿瘤组织单细胞悬液，采用流式细胞术检测各种免疫细胞的数量、比例及活性；利用单细胞测序技术检测TME中细胞聚类情况及细胞代谢相关基因的表达情况。综合分析在PD-1治疗中葡萄糖干预对免疫细胞的激活作用及可能的机制；**材料** 细胞系：MC38、LLC1、4T1、SGC7901；实验动物：C57BL/6小鼠； 药物：葡萄糖、纳武利尤单抗注射液等；流式抗体：17A2、GK1.5、eBio1D3 (1D3)等；**预实验结果** 灌胃葡萄糖浓度较高的组，其最终肿瘤组织中免疫细胞所占比例以及活性有一定的提高；**可行性** 1、目前葡萄糖应用于肿瘤治疗的作用尚无定论，有进行相关实验的必要性。2、有文献表明葡萄糖的供应可增强免疫细胞的活性；**创新性** 1、研究抗PD-1抗体免疫治疗与葡萄糖的联合作用；2、不同于以往的肿瘤饥饿疗法，对肿瘤的辅助治疗提出了新的设想，突破了传统思维的限制。

**关键词:** 肿瘤；葡萄糖；免疫治疗；PD-1

**Study on the role of glucose in the treatment of malignant tumors by targeting the key molecule of immune checkpoints PD-1**

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**Abstract:** **Argument basis** Programmed cell death protein 1 (PD-1) is a key molecule of immune checkpoint with negative immunomodulatory effect. Immunotherapy targeting PD-1 has shown good results in the clinical treatment of various malignant hematological tumors. However, the treatment of solid tumors is still restricted by problems such as the utilization of nutrients in the tumor microenvironment (TME). It is generally believed that tumor cells are the main consumers of glucose in the tumor microenvironment. However, the latest research shows that in TME, myeloid immune cells have the strongest ability to take up glucose, followed by T cells and cancer cells. According to the above literature, we speculated that adding a certain concentration of glucose may affect the number, proportion, activation and metabolic signaling pathways of immune cells in TME, enhance the anti-tumor ability of immune cells, and thereby inhibit the occurrence and development of tumors; **Design ideas** Prepare a variety of mouse tumor models. In PD-1 antibody immunotherapy, different concentrations of glucose were administered to the stomach, and the effects of glucose addition on the survival time and tumor size of mice were compared and analyzed. The possible mechanism of action was explored through flow cytometry analysis and single-cell sequencing analysis; **Experiment contents** Four cell lines MC38, LLC1, 4T1, SGC7901 were selected to establish tumor models in mice. Each tumor model was randomly divided into 6 groups, the model control group, nivolumab injection group (anti-PD-1 antibody), and nivolumab injection with a concentration of 0.25g/ml, 0.5g/ml, 0.75g/ml, 1g/ml glucose solution group. Monitor mice’s weight, survival period, tumor diameter, tumor weight, etc. Prepare tumor tissue single cell suspension. Then the number, ratio and activity of various immune cells were detected by flow cytometry; Single cell sequencing technology was used to detect cell clustering in TME and the expression of cell metabolism-related genes. Comprehensive analysis of the activation effect and possible mechanism of glucose intervention on immune cells in PD-1 treatment; **Materials** Cell lines: MC38, LLC1, 4T1, SGC7901; Experimental animals: C57BL/6 mice; Drugs: glucose, nivolumab injection, etc.; Flow antibodies: 17A2, GK1.5, eBio1D3 (1D3), etc.; **Pre-experiment results** In the group with higher glucose concentration, the proportion and activity of immune cells in the final tumor tissue were increased to a certain extent; **Feasibility** 1. At present, the role of glucose in tumor treatment is still inconclusive, and it is necessary to conduct related experiments. 2. There are documents showing that the supply of glucose can enhance the activity of immune cells; **Innovation** 1. Research on the combined effect of anti-PD-1 antibody immunotherapy and glucose; 2. Different from the previous tumor starvation therapy, it puts forward new ideas for tumor adjuvant therapy, breaking through the limitations of traditional thinking.

**Keywords:** tumor; glucose; immunotherapy; PD-1