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体外扩增对食管癌患者NK细胞表面受体表达及其抑瘤活性的影响 点此下载全文

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摘要：

目的： 探讨食管癌患者NK细胞扩增前后的受体表达及其对肿瘤细胞的杀伤。 方法： 收集福建省肿瘤医院食管癌患者外周血20例，健康供者（对照组）外周血10例。NK细胞培养采用细胞因子（IL-2+IL-12+IL-15+IL-18）组合，流式细胞术检测NK细胞免疫表型及其受体（CD56 +、CD69 +、NKG2D、NKp30、NKp44、NKp46、CD158b、CD159a）的表达，LDH法检测不同效靶比时NK细胞对多种肿瘤细胞株（K562、Raji、Eca-109、TE-1）的杀伤作用。 结果： 与对照组相比，食管癌患者外周血CD3 +、CD4 +细胞比例以及CD4 +/CD8 +T细胞比值明显降低（ P <0.05），NK细胞（CD3 -CD56 +）及调节性T细胞（Treg）比例明显升高（ P <0.05）。经细胞因子IL-2+IL-12+IL-15+IL-18组合定向扩增20 d后，食管癌患者NK细胞比例高达90%以上，NK细胞数扩增达1 000倍以上（ P <0.01）；而CD3 +T细胞、CD4 +、CD8 +T细胞、CD19 +B细胞、Treg细胞及单核巨噬细胞（CD14 +）比例均显著降低（ P <0.01），且食管癌患者与对照组之间无统计学差异（ P >0.05）。经细胞因子体外培养20 d后，NK细胞表面CD69及活化性受体（NKG2D、NKp30、NKp44、NKp46）均明显上调，而抑制性受体（CD158b、CD159a）均明显下调（ P <0.05）。培养20 d后，食管癌患者NK细胞对肿瘤细胞K562、Raji、Eca-109、TE-1的杀伤能力均显著高于培养前\[（69.2±5.1）% vs （42.3±3.0）%，（44.6±3.2）% vs （21.1±2.0）%，（69.7±3.9）% vs （50.3±3.5）%，（67.1±4.5）% vs （41.2±3.3）%；均 P <0.01\]。 结论：细胞因子IL-2+IL-12+IL-15+IL-18组合能有效扩增外周血NK细胞并上调其活并性受体的表达、下调抑制性受体的表达，其数量及功能均能满足临床治疗需要。

关键词：NK细胞 活化性受体 抑制性受体 白细胞介素 肿瘤细胞

Effect of in vitro expansion on expressions of surface receptors and anti-tumor activity of NK cells derived from patients with esophageal cancer Download Fulltext

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Abstract:

Objective:To explore the expressions of receptors before and after NK cell amplification from patients with esophageal cancer and its cytotoxicity to tumor cells. Methods: Peripheral blood was collected from the Fujian Provincial Tumor Hospital, including 20 cases of esophageal cancer patients and 10 cases of healthy donors (control group). NK cells were amplified by combination of IL-2+IL-12+IL-15+IL-18. Cell immunophenotype and NK cell receptor expressions (CD56 +, CD69 +, NKG2D, NKp30, NKp44, NKp46, CD158b and CD159a) were determined by flow cytometry. The cytotoxicity of NK cells to various tumor cell lines (K562, Raji, Eca-109 and TE-1) were detected by lactate dehydrogenase (LDH) assay. Results: Compared with the control group, the ratio of CD3 +, CD4 +and CD4 +/CD8 +T cells were significantly lower in the peripheral blood of patients with esophageal cancer (P <0.05), and the ratios of NK cells (CD56 +) and regulatory T cells (Treg) were significantly higher (P <0.05).The ratio of NK cells (CD3 -CD56 +) increased up to 90% after being cultured in combination of IL-2+IL-12+IL-15+IL-18 for 20 days. NK cell count expanded up to 1 000 times (P <0.01). The ratios of CD3 +, CD4 +T cells, B cells (CD19), monocyte-macrophage cells (CD14) and regulatory T cells (T-reg) were in a significant reduction 20 days after culture (P <0.01), with no significant difference between the patients with esophageal cancer and the control group (P >0.05). CD69 +and NK cell activating receptors (NKG2D, NKp30, NKp44, NKp46) were significantly increased and the inhibitory receptors (CD158b, CD159a) were significantly decreased (P <0.05). NK cells derived from patients with esophageal cancer showed significant increase of cytotoxicity on tumor cells K562, Raji, Eca-109 and TE-1 after culture for 20 days compared to those before culture (\[69.2±5 1\]% vs \[42.3±3 0\]%, \[44.6±3.2\]% vs \[21.1±2.0\]%, \[69.7±3.9\]% vs \[50.3±3.5\]%, \[67.1±4.5\]% vs \[41.2±3.3\]%, P <0.01). Conclusion:The combined cytokines of IL-2+IL-12+IL-15+IL-18 can effectively expand peripheral blood NK cells, up-regulate the expressions of activated receptor and down-regulate the expression of inhibitory receptors. The numbers and functions of the cultured NK cells can both meet the clinical treatment needs.

Keywords:NK cell activating receptor inhibitory receptor interleukin tumor cell

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