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IL-2+IL-15组合培养方案对乳腺癌患者外周血中NK细胞体外扩增的效果

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摘要：

目的：观察IL-2+IL-15组合体外培养方案对于NK、NKT和T细胞亚群的比例、表型、杀伤肿瘤细胞活性与黏附活性的影响，并初步探讨其作用机制。方法：采集天津医科大学附属肿瘤医院生物治疗科2012年5月至2012年7月期间收治的5例乳腺癌患者的外周血单个核细胞（peripheral blood mononuclear cell，PBMC），用IL-2+IL-15 联合培养方案进行培养，观察培养15 d后细胞的扩增倍数和淋巴细胞亚群比例变化，流式细胞术检测细胞免疫表型、细胞表面受体的表达，LDH释放法和CD107a释放法检测不同细胞亚群对于HLA匹配或不匹配的靶肿瘤细胞系的杀伤活性，活细胞工作站检测总扩增产物对于不同靶肿瘤细胞系的黏附作用。结果：与扩增前相比，IL-2+IL-15培养方案扩增15 d后，NK细胞\[（36.74±17 10）% vs （16.34±3.12）%， P <0.05\]、NKT细胞\[（24.88±12.34）% vs （4.06± 2.05）%， P <0.05\]比例显著增加，CD4 + T细胞和Treg细胞比例显著降低（ P <0.05），CD8 + T细胞比例显著升高（ P <0.05）；NK细胞表面活化受体NKp30\[（ 92.38±7.19）% vs （32.14±17.64）%， P <0.05\]、NKp44\[（74.26±16.28）% vs （1.94±1.60）%， P <0.05\]、NKG2D \[（ 98.58±1 28）% vs （66 50±24.84）%， P <0.05\] 的表达率均显著升高，CD16表达率显著降低\[（ 85.12±7.66）% vs （95.60±2 53）%， P <0.05\]； NKT细胞、T细胞表面活化受体DNAM-1和NKG2D明显升高（ P <0.05）。总扩增产物、NK细胞和NKT细胞对HLA不匹配的靶肿瘤细胞的杀伤率均显著高于HLA匹配靶细胞系的杀伤（ P <0.05）；在共孵育至84 min时，与HLA匹配的靶肿瘤细胞系相比，总扩增产物细胞与HLA不匹配的靶细胞系黏附结合数目显著增多\[（ 4.80±0.53） vs （2.25±0 35）个， P <0.05\]。结论：IL-2+IL-15组合方案在扩增NK细胞的同时，也能够有效扩增NKT细胞，即可以扩增CD56 +细胞群。并且，扩增产物主要以不受HLA限制的NK细胞杀伤活性为主来杀伤肿瘤细胞。

关键词：IL-2 IL-15 NK细胞 扩增 乳腺癌

Effect of combinatorial culture protocol (IL-2 and IL-5) on the proliferation of NK cells in the peripheral blood of breast cancer patients in vitro

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Abstract:

Objective:To observe the effect of culture with IL-2 and IL-15 in vitro on the proportion, the cell phenotype, the cytotoxic activity against tumor cells and the adhesion activity of NK cells, NKT cells and T cells subsets, and to discuss the possible mechanism. Methods:Five patients with breast cancer were obtained in the Department of Biotherapy, Tianjin Medical University Cancer Institute and Hospital from May 2012 to July 2012, and PBMCs of those patients were isolated by and cultured with the NK cells proliferation scheme contained IL-2+IL-5. The cell expansion fold and the proportion of different lymphocyte subsets were observed after cultured by IL-2 and IL-15 for 15 d. The changes of the cell immunophenotype and cell surface receptors were detected by flow cytometry. The anti-tumor cytotoxicity against HLA-match or HLA-mismatch cancer cells among three lymphocyte subsets were measured using LDH cytotoxicity assay and CD107a release assay. Adhesion between the total expansion products and HLA-match or HLA-mismatch tumor cells was detected by Live Cell Station. Results: After expansion by IL-2+IL-15 for 15 days, compared with preamplification, the proportions of NK cells (\[36.74±17.10\]% vs \[16.34±3.12\]%, P <0.05) and NKT cells (\[24.88±12.34\]% vs \[4.06±2.05\]%, P <0.05\] were significantly increased; the proportions of CD4 +T cells and Treg cells were significantly decreased (P <0.05), the proportions of CD8 + T cells were significantly increased (P <0.05); the expressions of surface activation receptors NKp30 (\[92.38±7.19\]% vs (32.14±17.64)%, P <0.05\], NKp44 \[(74.26±16 28\]% vs \[1.94±1.60\]%, P <0.05\] and NKG2D (\[98.58±1.28)% vs (66.50±24.84)%, P <0.05\] expressed on NK cells were increased significantly, while CD16 was obviously decreased (\[85.12±7.66\]% vs \[95.60±2 53\]%, P <0.05\]; the activation receptors DNAM-1 and NKG2D expressed on both NKT cells and T cells were significantly increased (P <0.05). The killing rates of total expansion cells, NK cells and NKT cells against HLA-mismatched tumor cells were significantly higher than those against HLA-matched tumor cells (P <0.05). In the 84th minute of co-incubation, the adherent number of total expansion cells combined to HLA-mismatched tumor cells were significantly higher than that of HLA-matched tumor cells (\[4.80±0.53\] vs \[2.25±0.35\], P <0.05\].
Conclusion: Not only NK cells but also NKT cells can be amplificated by cultured with IL-2 and IL-15, indicating that it can be used to expand CD56 + cells. Expansion products kill the tumor cells mainly by the NK cells killing activity without HLA-restricted.

Keywords:IL-2 IL-15 NK cell expansion breast cancer