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四种NK细胞体外扩增方案的比较 点此下载全文

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摘要：

目的： 通过对4种NK细胞体外培养方案扩增后产物的细胞免疫表型、扩增倍数以及杀伤活性进行比较，确定一种高效的NK细胞体外扩增方案。 方法: 建立4种NK细胞体外培养方案：方案1为经典的NK细胞体外扩增方案(IL-2+ IL-15)；方案2为IL-2+IL-15+IL-18；方案3为IL-2+IL-15+IL-7；方案4为新型NK培养基（IL-2+OKT3）。收集天津医科大学附属肿瘤医院生物治疗科2012年2月至2012年4月间10例晚期实体瘤患者的PBMC，按照4种方案进行体外扩增。在体外扩增的0、5、10、15 d，采用流式细胞仪检测各淋巴细胞亚群（尤其是NK细胞）的比例；比较各方案体外扩增15 d后的NK细胞的扩增倍数、淋巴细胞亚群比例变化，并采用LDH法检测各方案扩增产物对人白血病K562细胞的杀伤活性。结果： 上述4种NK细胞培养方案体外扩增15 d后，细胞总数分别扩增（40.1±20.00）、（44.08±22.09）、（44.82±23.67）、（46.82±25 02）倍，其中NK细胞的扩增倍数分别为（75.86±28.57）、（93.32±32.16）、（88.66±24.94），（58.88±41.53）倍。NK细胞比例由0 d的（20.44±2.23）%分别扩增至15 d的（48.30±13.90）%、（54.72±12.25）%、（55.94±12.70）%和(54.5±14.93)%；各培养方案组在总细胞扩增倍数、NK细胞扩增倍数上的差异均无统计学意义（ P >0.05）。前3种培养方案体外扩增产物的杀伤活性明显高于方案4\[（63.40±5 00）%、（77.30±9.40）%、（62.17±5.60）% vs （37.39±10.42）%，均 P <0 05\]，而方案1、2、3之间体外扩增产物杀伤活性的差异则无统计学意义（ P >0.05）。 结论： 细胞因子组合对于体外大规模培养NK细胞有着一定的优势，但不同的细胞因子组合（方案1中是否加入IL-18或IL-7）对NK细胞体外大规模扩增的影响差异并不显著；但前3个方案扩增产物对K562细胞的杀伤活性明显强于方案4。

关键词：NK细胞 IL-2 IL-15 IL-18 IL-7 扩增 K562细胞 杀伤活性

Comparison of four kinds of NK cell *in vitro* expansion methods Download Fulltext

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Abstract:

Objective:By comparing the cell immunophenotype, the expansion fold and the cytotoxic activity of the expansion products in various cell culture methods to identify a more effective solution for the *in vitro* expansion of NK cells. Methods: Four methods for expansion of NK cells from peripheral blood were established, including method one, a classical culture protocol for NK cells (IL-2+IL-15), method two (IL-2+IL-15+IL- 18), method three (IL-2+IL-15+ IL-7), and method four, a novel NK-specific culture medium (IL- 2+OKT3). 10 patients with advanced solid tumors in Department of Biotherapy, Cancer Hospital Affiliated to Tianjin Medical University from February 2012 to April 2012 were obtained, and PBMCs from those patients were isolated by Ficoll-Hypaque density gradient centrifugation. The proportion of different lymphocyte subsets (especially for NK cells) were detected by flow cytometry on 0, 5, 10 and 15 days. The changes of NK cell expansion fold and the proportion of different lymphocyte subsets were detected among 4 groups after expansion *in vitro* for 15 days. The anti-tumor ytotoxicity against human K562 cell line among 4 groups were measured using LDH assay. Results: After expansion for 15 days, the expansion folds of total cells in 4 groups were (40.1±20 00), (44.08±22.09), (44.82±23.67) and (46.82±25.02), respectively. The proportion of NK cells in 4 groups increased from (20.44±2.23)% on day 0 to (48.30±13.90)%, (54.72±12.25)%, (55.94±12.70)% and (54.5±14.93)% on day 15, respectively. The expansion folds of NK cells in 4 groups were (75.86±28.57), (93.32±32.16), (88.66±24.94) and (58.88±41 53), respectively. No significant difference was found on the total cell expansion, NK cell expansion folds among the 4 groups (P <0.05). The cytotoxic activity of the expansion products in methods one, two and three were higher than that of method four *in vitro* (\[63.40±5.00\]%, \[77.30±9 40\]%, [62.17±5.60\]% vs \[37.39±10.42\]%, P <0.05). There was no significant difference among the first 3 groups (P >0.05). Conclusion:NK-specific cytokines have great influence on the expansion of NK cells *in vitro* . However, no significant difference is found among various cytokine combinations. The cytotoxic activity of the expansion products in methods one, two and three against K562 cells are significantly higher than that of method four.

Keywords:NK cell IL-2 IL-15 IL-18 IL-7 expansion K562 cell cytotoxic activity

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