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Basic fibroblast growth factor enhanced LAK cell cytotoxicities against human bladder neoplasm cells[1]

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KEY WORDS basic fibroblast growth factor; lymphokine-activated killer cells; cell division; interleukin-2; bladder neoplasms; immunologic cytotoxicity

ABSTRACT

AIM: To study the effects of basic fibrolast growth factor (bFGF) on the proliferation of lymphokine-activated killer (LAK) cells from patients with bladder cancer and LAK cells cytolysis against bladder tumor cells. **METHODS**: LAK cell proliferation was assayed in the presence of various concentrations of bFGF combined with interleukin-2 (IL-2) by cell count. Cytotoxicity of LAK cells against bladder cancer cell line EJ cells and bladder tumor cells (BTC) from patients was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays. **RESULTS**: The proliferation of peripheral blood monocytes (PBMC) was inhibited by bFGF 5 $\mu g \cdot L^{-1}$. bFGF did not affect the stimulation of LAK cells induced by IL-2. The LAK cell numbers in the combination of IL-2 with bFGF were not significantly different compared with that treated with IL-2 alone. bFGF enhanced cytotoxicity of LAK cells against bladder cancer cell line EJ cells or BTC, respectively. **CONCLUSION**: Although the proliferation of PBMC was inhibited by bFGF, bFGF increased LAK cell cytotoxicity against bladder neoplasm cells.

INTRODUCTION

The results of using lymphokine-activated killer (LAK) cells in clinical cancer therapy are not very satisfactory. Therefore, it is very important to investigate the factors influencing antitumor activities of LAK cells.

The basic fibroblast growth factor (bFGF) is a potent factor stimulating endothelial cell proliferation, migration, and differentiation to form nevasculature and promoting the synthesis of collagen and fibronectin[1]. In addtion, bFGF is also a potent autocrine and paracrine positive growth regulator involved in malignant cellular proliferation[2]. The presence of bFGF transcripts in some human T cell clones indicats that bFGF may be involved in the regulation of immunity[3].

It is not clear what role bFGF may play in the development of bladder neoplasm and antitumor immunity. This study was to investigate the roles of bFGF in the modulation of LAK cell proliferation from patients with bladder cancer and its antitumor activities against bladder cancer cells.

MATERIALS AND METHODS

Reagents bFGF were provided by Torita Biotechnology Ltd (Zhuhai, China). RPMI-1640 medium was obtained from Gibco. 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) and *L*-glutamine were obtained

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from Sigma. Recombinant interleukin-2 (IL-2) was provided by Changchun Institute of Biological Products (Ministry of Public Health, China).

Culture of LAK cells Peripheral blood monocytes (PBMC) obtained from 19 patients with pathologically diagnosed transitional cell carcinoma of bladder were isolated by Ficoll-paque (Shanghai 3rd Chemical Reagents Ltd) density-gradient centrifugation. Interface cells were aspirated and washed 3 times with Hanks' solution. The PBMC (1×10^9 cells·L^{-1}) were suspended in complete medium (CM) consisting of RPMI-1640, benzylpenicillin 100 kU·L^{-1}, streptomycin 100 kU·L^{-1}, gentamicin 50 kU·L^{-1}, *L*-glutamine 2 mmol·L^{-1}, sodium pyruvate 1 mmol·L^{-1} and 15 % heat-inactivated fetal calf serum (FCS). The cells were allowed to settle in 25-cm^2 tissue culture flasks at 37 ℃ in 5 % CO_2 for 2 h. The single suspension PBMC (2×10^8 cells·L^{-1}) were further cultivated in CM supplemented with IL-2 1 MU·L^{-1} for 96 h.

LAK cell proliferation assay The nonadherent PBMC (15×10^3 cells/well in 300 μL CM + IL-2) were plated in 96-well plates in the presence of either bFGF (1, 5, or 10 μg·L^{-1}). Each concentration had 4 wells. The cells were serially counted in a hemocytometer. The tests were repeated for 4 independent experiments and mean cell numbers of 4 wells were calculated in each experiment.

Cytotoxicity assay Bladder tumor cells (BTC) from patients with transitional cell carcinoma of bladder were prepared[4]. Human bladder transitional cell carcinoma cell lines EJ (kindly provided by the Institute of Urology, Beijing Medical University) cultured in wells for 24 h and BTC were used as the target cells. LAK cells pretreated with bFGF (5 μg·L^{-1}) for 48 h and washed with Hanks' for 3 times were added to target cells in effector/target (E/T) ratios = 30:1 in CM 250 μL. The cytotoxicity of LAK was determined by MTT assay[5] and repeated by 4 independent experiments (4 wells each). The absorbance (A) at 570 nm in each well was determined by microplate autoreader (Huadong Electric Ltd, Nanjing, China). The % of lysis = [1 − (*A* of traget cell plus LAK − *A* of LAK cell)/*A* of target cell] × 100 %.

Statistical analysis LAK cell proliferation experiments were analyzed with ANOVA. All LAK cell cytotoxicity against tumor cells was analyzed by the unpaired Wilcoxon's *u* test.

RESULTS

Effect of bFGF on LAK cell proliferation PBMC were treated with different concentrations of bFGF alone, IL-2 alone, or bFGF (1, 5 or 10 μg·L^{-1}) + IL-2 1 MU·L^{-1} for 96 h. The proliferation of PBMC was inhibited by bFGF 5 μg·L^{-1} ($P < 0.01$). bFGF did not affect the stimulation of LAK cells induced by IL-2. The LAK cell numbers in the combination of IL-2 with bFGF were not different from that treated with IL-2 alone (Tab 1).

Tab 1. Proliferation of LAK cells from patients with bladder cancer treated with bFGF for 48 h. $n = 4$ wells/group and repeated for 4 independent experiments. $\bar{x} \pm s$. $^cP < 0.01$ *vs* Notreatment, $^dP > 0.05$, $^fP < 0.01$ *vs* IL-2 1 MU·L^{-1} alone.

Treatment	Cell number/10^{-8} × cells·L^{-1}
Notreatment	9.42 ± 0.21
IL-2 1 MU·L^{-1} alone	12.24 ± 0.63^c
bFGF 5 μg·L^{-1} alone	7.81 ± 0.24^{cf}
IL-2 1 MU·L^{-1} + bFGF 1 μg·L^{-1}	11.83 ± 0.22^{cd}
IL-2 1 MU·L^{-1} + bFGF 5 μg·L^{-1}	12.21 ± 0.48^{cd}
IL-2 1 MU·L^{-1} + bFGF 10 μg·L^{-1}	11.12 ± 0.21^{cd}

Role of bFGF on cytotoxicity of LAK cells against tumor cells The LAK cells from patients with transitional cell cancer of bladder were pretreated with IL-2 plus bFGF 5 μg·L^{-1}. bFGF enhanced cytotoxicity of LAK cells against EJ cells or BTC from the patients (Tab 2, $P <$

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0.05). The treatment of EJ or BTC with bFGF 5 $\mu g \cdot L^{-1}$ for 4 h did not affect the *A* of MTT.

Tab 2. Bladder tumor cell cytolysis (%) by LAK cells stimulated by bFGF for 48 h. *n* = 4 wells/group and repeated for 4 independent experiments. $^cP < 0.01$ *vs* IL-2 1 $MU \cdot L^{-1}$ alone.

	BTC	EJ
IL-2 1 $MU \cdot L^{-1}$ alone	38.0 ± 2.6	73.9 ± 1.6
IL-2 1 $MU \cdot L^{-1}$ + bFGF 5 $\mu g \cdot L^{-1}$	54.7 ± 2.3^c	80.0 ± 1.5^c

DISCUSSION

FGF is abundant in sites of immune activation[6], providing a pathway linking these events to the immune systems. The tests presented here showed that LAK cell cytotoxicity against bladder cancer cells were enhanced by pretreatment of LAK cell with bFGF. Although bFGF alone inhibited the proliferation of PBMC, LAK cell proliferation was not inhibited in combination of bFGF with IL-2. These studies are a necessary forerunner to understand the possible mechanism of cytokins or growth factors involved in cancer immunotherapy and use of combining immunotherapy with cytokin in bladder cancer.

The biologic functions of FGF are mediated by binding to a family of high affinity cell surface receptors (FGFR) with tyrosine kinase activity. Some human T cells expressed high affinity FGFR respond to FGF[12]. In the presence of anti-CD3 exogenous FGF-1 *functions as a costimulator* for these T cells, while FGF-1 alone does not induce T cell proliferation. Neverthless, mechanism for the difference between effects of bFGF on cancer cells and LAK cells proliferation and cytotoxicity maintains unclear.

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276 ~ 278

碱性成纤维细胞生长因子增强淋巴因子激活的杀伤细胞对人膀胱肿瘤细胞的细胞毒性[1]

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关键词 碱性成纤维细胞生长因子；淋巴因子激活的杀伤细胞；细胞分裂；白细胞介素-2；膀胱癌；免疫细胞毒性

目的：探讨白细胞介素Ⅱ（IL-2）和碱性成纤维细胞生长因子（bFGF）对膀胱癌患者淋巴因子激活的杀伤细胞（LAK细胞）的作用。**方法**：用细胞计数观察不同浓度bFGF对LAK细胞增殖的影响。以膀胱癌细胞系EJ及新鲜分离患者自体肿瘤细胞（BTC）为靶细胞，用MTT法测定LAK细胞对膀胱癌细胞的细胞毒作用。**结果**：虽然外周血单核细胞（PBMC）的增殖可被bFGF 5$\mu g \cdot L^{-1}$所抑制，IL-2所诱导的LAK细胞的增殖却不受bFGF的影响，bFGF明显加强LAK对EJ细胞和BTC的细胞毒作用。**结论**：虽然bFGF抑制PBMC的增殖，但bFGF又增强膀胱癌患者LAK细胞对肿瘤细胞的细胞毒性。

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