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Effect of recombinant human fibronectin polypeptide CH50 on growth and metastasis of melanoma[1]

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KEY WORDS recombinant proteins; fibronectins; laminin; peptides; experimental melanoma; cultured tumor cells; antineoplastic agents

ABSTRACT

AIM: To study the effect of recombinant human fibronectin polypeptide CH50 on murine melanoma growth and metastasis, and its antitumor mechanism. **METHODS**: Mouse and melanoma B16 cell tests were used to observe antitumor effect and mechanism of CH50. **RESULTS**: CH50 markedly inhibited melanoma growth and experimental lung metastasis. The melanoma weight was reduced from (2.3 ± 1.2) g of control group to (0.7 ± 0.8) g of test group ($P < 0.05$). CH50 at 0.125, 0.25, 0.5 mg/mouse reduced melanoma lung metastatic colonies from 87 ± 49 of control group to 34 ± 6, 14 ± 12, 4 ± 2, respectively. CH50 adhered to melanoma B16 cells and inhibited adhesion of B16 cells to laminin. CH50 enhanced the cytotoxicity of macrophages to melanoma B16 cells. **CONCLUSION**: CH50 inhibited tumor growth and metastasis of murine melanoma. The antitumor effect of CH50 is related to its adhesion ability to melanoma B16 cells and enhancing macrophage cytotoxicity.

INTRODUCTION

Fibronectin is a multi-functional glycoprotein which exists in extracellular matrix, basement and plasma. Much attention has been paid recently to the prospective value of recombinant human fibronectin polypeptide on tumor therapy[1,2]. We have prepared bifunctional-domain recombinant peptide CH50 containing Cell I-Hep II domains of human fibronectin (Pro 1239 - Ser1515 connected with Ala1690 - Thr1960 through Met)[3]. In this experiment, we studied the effect of CH50 on the tumor growth and metastasis as well as its antitumor mechanism. (Fig 1)

MATERIALS AND METHODS

Mice Male Kunming mice ($n = 50$, 6 - 8 wk, 22 g ± s 1 g, Grade II, Certificate № 19 - 052) were purchased from Experimental Animal Center, Tongji Medical University.

Tumor cells Melanoma B16/F1 cells were purchased from ATCC Co, USA. Cells were cultured in RPMI-1640 containing 10 % calf serum (Gibco).

Laminin was purchased from Department of Cytology, Basic Medical School, Beijing Medical University. Dexamethasone (Dex) was purchased from Binghu Drug Co (lot № 950606).

Recombinant fibronectin polypeptide CH50 was prepared by Heparin-agarose chromatography (purity 98 %, identified by SDS-PAGE)[3].

Cell-adhesion assay According to the method[1], a 96-well plate was coated with CH50, and PBS was added to the control

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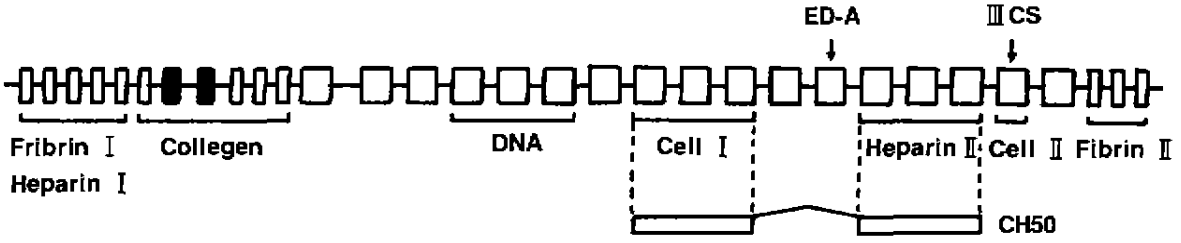

Fig 1. Full length fibronectin polypeptide and its recombinant polypeptide CH50. □: type Ⅰ repeat. ■: type Ⅱ repeat. □: type Ⅲ repeat.

well. The plate was blocked with 3 % bovine serum albumin. Trysinized B16/F1 melanoma cells in serum-free RPMI-1640 medium (5×10^8 cells·L^{-1}) was added to the plate, 0.1 mL/well. After incubation at 37 ℃ for 2 h, unattached cells were removed and the remained substrate-bound tumor cells were lysed with 0.1 mL of 1 % NP-40 in PBS, the lactic dehydrogenase (LDH) activity of lysed cells were measured[5]. The concentration that gave 50 % of the maximal number of adhering cells (EC_{50}) was estimated from a concentration-response curve.

Adhesion inhibiting test was carried out as cell adhesion test, laminin (20 mg·L^{-1}) was coated to culture wells, B16 cells were added to culture wells after 2-h incubation with CH50 at 37 ℃.

Macrophage cytotoxicity test According to the method[4], mice peritoneal macrophages ($2 \times 10^8 \cdot L^{-1}$) 0.1 mL and melanoma B16 cells ($10^7 \cdot L^{-1}$) 0.1 mL were added to 96-well culture plate in the presence or absence of CH50 (20 mg·L^{-1}). After incubation at 37 ℃ for 48 h, LDH activity of supernatant of each well was measured and the cytotoxicity of macrophage was expressed as follows:

Cytotoxicity ratio = (A_{570} of sample release − A_{570} of nature release)/(A_{570} of maximal release − A_{570} of nature release) × 100 %

Tumor growth experiment After mice were sc injected melanoma B16 cells (2×10^5/mouse), the test group was injected 0.2 mL CH50 (250 mg·L^{-1}) once every 2 days in the tumor cell injected location for 7 times, the control group was injected with PBS. On d 14 after the inoculation of tumor cells, the mice were killed, and the tumor were surgically removed and weighted.

Tumor metastasis experiment After sc injection of Dex (2.5 mg·kg^{-1}·d^{-1} × 7 d), the mice were iv injected melanoma B16 cells ($4 \times 10^8 \cdot L^{-1}$, 0.5 mL/mouse) suspended in PBS containing CH50 (1000, 500, 250 mg·L^{-1}) for test group; or in PBS for control group. On d 14 after inoculation, the mice were killed and the number of metastatic colonies of melanoma was recorded.

Statistical analysis All data were expressed as $\bar{x} \pm s$ and analyzed by t test.

RESULTS

Adhesion ability of CH50 to melanoma B16 cells The adhesion ability of CH50 was concentration-depended (Fig 2). The value of EC_{50} was 8 (95 % confidence limits 7.8 − 8.2) nmol·L^{-1} which was close to that of native FN[2].

Ability of CH50 inhibiting melanoma B16 cells binding laminin The ability of the cells binding laminin was inhibited and the inhibitory rate was related to the dosage of CH50. At 25, 50, 100 mg·L^{-1}, the inhibition rates were 3.7 % ± 0.9 %, 22.2 % ± 1.6 %, and 40.7 % ± 2.1 %, respectively.

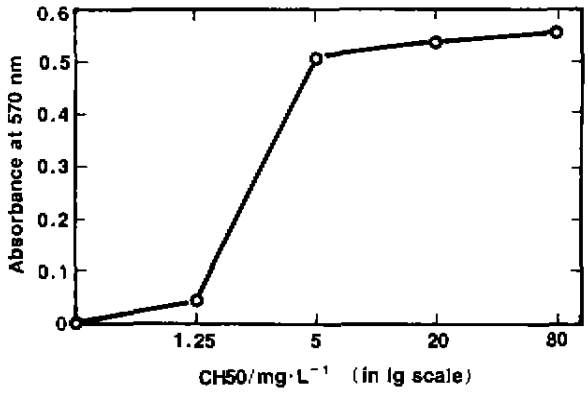

Fig 2. Adhesion of B16 melanoma cells to CH50.

Inhibition of melanoma experimental lung metastasis The metastatic colonies of test groups were less than those of control group (87 ± 49). CH50 at 0.125, 0.25, and 0.5 mg/mouse reduced the lung metastatic colonies to 34 ± 6, 14 ± 12, and 4 ± 2, respectively.

Macrophage cytotoxicity After macrophages were incubated with CH50 20 mg·L^{-1}, their cytotoxicity to B16 cells (effector : targetor ratio was 20:1) was markedly enhanced (Tab 1).

Tab 1. Effects of CH50 on tumor growth and macrophage cytotoxicity. $\bar{x} \pm s$.
$^{b}P < 0.05$, $^{c}P < 0.01$ *vs* control.

Group/mg·L^{-1}	Tumor weight/g (n = 6 mice)	Cytotoxicity of macrophage/% (n = 10 wells)
Control	2.3 ± 1.2	33 ± 14
CH50 250	0.7 ± 0.8^{b}	
CH50 20		58 ± 13^{c}

Tumor growth Melanoma B16 cells were inoculated subcutaneously to mice, after CH50 50 mg · L^{-1} was injected to tumor growth location, the tumor weight was markedly reduced (Tab 1).

DISCUSSION

Recombinant human fibronectin fragment CH271 (Cell I-Hep II recombinant polypeptide of human fibronctin) had the function of antitumor metastasis[1]. The combinant human fibronectin polypeptide CH50 which was constructed and purified by our group has the same domains as CH271, and can reduce the number of lung metastatic colonies when it was co-injected with melanoma B16 cells. On the other hand, our results showed that the tumor weight was reduced significantly when CH50 was sc co-injected with melanoma B16 cells. At the same time, we found that CH50 enhanced the cytotoxicity of macrophages to melanoma B16 cells. These results suggest that CH50 possessed not only the ability of inhibiting the metastasis of melanoma cells, but also that of augmenting the immune system.

Native human fibronectin could promote murine peritoneal macrophages producing NO and induce its cytotoxicity to tumor cell in the presence of interferon-γ, FN alone had no effect on murine peritoneal macrophage NO production and cytotoxicity to tumor cells and did not induce bioactive tumor necrosis factor-α secretion from murine peritoneal macrophages[5], recombinant fragment of FN had much stronger effect on monocytes than that of native FN[6]. Our results showed that CH50 enhanced the function of macrophages in the absence of interferon-γ, suggesting that recombinant fragment of FN be more active than native FN.

The recurrence and metastasis are the 2 difficult problems in tumor therapy. Recombinant human fibronectin polypeptide can not only regulate the body immuno-function, enhance murine peritoneal macrophage cytotoxicity to melanoma B16 cells, but also adhere to B16 cells and inhibit B16 cells' adhesion to laminin and melanoma experimental lung metastasis. FN contains cell-binding domain which can interact with VLA-5 intergrin and induce monokine (such as IL-1 and TNF-α) production of human

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monocyte[7], suggesting that CH50 could be a prospective bio-product in tumor therapy.

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重组人纤连蛋白 CH50 对黑色素瘤生长及转移的影响[1]

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关键词 重组蛋白; 纤连蛋白; 层粘蛋白; 肽类; 实验性黑色素瘤; 培养的肿瘤细胞; 抗肿瘤药

目的: 研究重组人纤连蛋白 CH50 对小鼠黑色素瘤生长的影响及其抗肿瘤机制. **方法**: 进行小鼠动物实验及黑色素瘤 B16 细胞实验. **结果**: CH50 在体内明显抑制黑色素瘤生长及实验性肺转移. CH50 在体外能粘附黑色素瘤 B16 细胞, 抑制 B16 细胞粘附层粘蛋白并明显提高腹腔巨噬细胞对 B16 细胞的杀伤活性. **结论**: CH50 抑制小鼠黑色素瘤的生长和转移, CH50 的抗肿瘤机制与其粘附肿瘤细胞、提高巨噬细胞杀瘤活性有关.

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