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硕 士 学 位 论 文

**^{131}I 标记抗 NRP-1 单克隆抗体在荷胶质瘤
裸鼠体内分布和 SPECT/CT 显像研究**

**Biodistribution and Imaging of ^{131}I labeled Anti-
Neuropilin-1 Monoclonal Antibody in Malignant Gliomas**

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摘要

背景介绍

放射免疫显像 (radioimmunoimage, RII) 在肿瘤早期诊断中占据重要地位, FDA 已经批准了五种基于 RII 抗体药物用于临床肿瘤显像。但目前, 现有靶点的 RII 分子探针面临肿瘤显像探测效能不足, 并存在假阴性等诸多难题。开发针对新靶点的分子探针, 是 RII 不断发展成熟的关键所在。神经鞭毛素受体-1 (neuropilin-1, NRP-1), 是 Semaphorin 3A 和血管内皮生长因子 (VEGF) 的共受体, 参与肿瘤血管新生, 肿瘤生长和转移。它在多种肿瘤组织中高表达, 并与肿瘤的恶性程度密切相关, 被认为是一个很有前景的肿瘤显像与治疗靶点。但目前针对 NRP-1 靶点分子显像, 尚缺乏有效的特异性探针, 为了发展靶向 NRP-1 的靶向显像药物, 我们实验室前期通过杂交瘤技术制备了一株靶向 NRP-1 的功能性单克隆抗体---A6, 本课题拟采用碘 ^{131}I 标记的抗神经鞭毛素抗体 (^{131}I -A6-MAb) 做为分子探针, 探讨 ^{131}I -A6-MAb 体外特性、在荷瘤裸鼠中的生物学分布特征及其靶向显像特性。

实验方法

- (1) 我们通过用 rProtein A 亲和柱纯化将抗 NRP-1 抗体从小鼠腹水中纯化, 并且通过 SDS-PAGE 鉴定抗体的纯度, ELISA 方法鉴定抗体的亲和力。
- (2) 采用 Iodogen 法标记碘 ^{131}I 和抗神经鞭毛素 (Neuropilin-1 NRP-1) 单克隆抗体, 制备 ^{131}I -A6-MAb 探针, 凝胶柱分离法纯化 ^{131}I -A6-MAb 探针, 放射性纸层析法 (TLC) 鉴定 ^{131}I -A6-MAb 纯化纯度, Elisa 法鉴定 ^{131}I -A6-MAb 免疫活性, 体外观测法鉴定 ^{131}I -A6-MAb 稳定性。
- (3) 选用 U87 胶质瘤细胞株, 采用细胞摄取实验测定探针结合特异性, 饱和结合实验测定探针结合力, 细胞内吞实验测定探针内吞速率。
- (4) 建立人胶质瘤 U87 细胞裸鼠皮下移植瘤模型, 随机分为 4 组, 每组 4 只, 通过尾静脉注射 ^{131}I -A6-MAb 7.4MBp 与未标记抗体 (0、2.5mg/kg, 5mg/kg、10mg/kg), 并于注射后 24、48、72、96 和 120 h 处死小鼠。取血、心、肝、脾、肺、肾、胃、肠、骨骼、肌肉、甲状腺及肿瘤组织, 测质量及放射性计数, 经时间衰减校正后计算每克组织的放射性摄取率 (%ID/g)。

(5) 将荷 U87 MG 胶质瘤模型裸鼠随机分 2 组, 一组为未阻断组, 另一组为阻断组, 每组 4 只, 经尾静脉注射 $7.4 \text{ MBq } ^{131}\text{I-A6MAb}$ (即未阻断组) 或与 $700 \mu\text{g}$ 未标记抗体同时注射 (即阻断组), 于注射后不同时间点 (12、24、48、72、96、120h) 行 SPECT/CT 显像。

实验结果

(1) SDS-PAGE 检测显示经纯化的抗 NRP-1 抗体 A6 的纯度为 95%, 浓度为 4 mg/mL ; ELISA 检测抗体的亲和力为原腹水结合力的 76%。

(2) 纸层析法结果计算表明, $^{131}\text{I-A6-MAb}$ 标记率为 98%, 放化纯为 98%; 96 h 稳定性测试表明, $^{131}\text{I-A6-MAb}$ 在室温下存放以及在 PBS 溶液中, 其标记率仍然都维持在 85 % 以上, 说明其具有良好的体外稳定性。

(3) 细胞实验显示, $^{131}\text{I-A6-MAb}$ 可特异性结合 U87MG 细胞表面 NRP-1 抗原, 并在 1h 时达到高峰为 $15.80 \pm 1.30\%$; 其与细胞表面抗原亲和力(K_D)为 $1.67 \pm 0.14 \text{ nM}$, 与抗原结合后的细胞内吞速率为 $25.7 \pm 3.0\%$ (8h), 表明了 $^{131}\text{I-A6-MAb}$ 能特异性、高亲和力地结合于 U87 MG 细胞表面 NRP-1 受体。

(4) 生物学分布实验显示, $^{131}\text{I-A6-MAb}$ 可特异性靶向肿瘤组织, 未阻断组与阻断组 (2.5、5、10mg/kg), 裸鼠肿瘤组织摄取在 24h 摄取值分别为 6.0 ± 1.2 、 8.8 ± 1.7 、 11.4 ± 2.0 、 $8.2 \pm 1.4 \text{ ID\%/g}$, 阻断后可提高肿瘤摄取 $^{131}\text{I-A6-MAb}$ ($P < 0.05$), 其中用 5mg/kg 时肿瘤摄取 $^{131}\text{I-A6-MAb}$ 最高, 而用 10mg/kg 时肿瘤摄取 $^{131}\text{I-A6-MAb}$ 就受至抑制; 肝脏摄取在 24h 分别为 7.6 ± 1.5 、 6.4 ± 0.9 、 5.8 ± 0.7 、 $6.0 \pm 0.8 \text{ ID\%/g}$, 阻断组与为阻断组肝脏摄取 $^{131}\text{I-A6-MAb}$ 取虽然被抑制, 但差别无统计学意义 ($P > 0.05$); $^{131}\text{I-A6-MAb}$ 体内放射性剂量随时间延长逐渐下降, 其中在血液中清除最快, 120h 时各组血浆中放射性浓度分别为 0.8 ± 0.1 、 1.2 ± 0.3 、 2.0 ± 0.3 、 $6.0 \pm 1.3 \text{ ID\%/g}$; 肿瘤/血浆比值 (T/B) 在 120h 达到最高, 为 1.8 ± 0.50 、 2.3 ± 0.7 、 2.4 ± 0.6 、 0.9 ± 0.2 , 表明肿瘤组织特异性结合 $^{131}\text{I-A6-MAb}$, 正常组织不结合或很少特异性摄取 $^{131}\text{I-A6-MAb}$ 。

(5) SPECT/CT 显像结果显示, 尾静脉注射 $^{131}\text{I-A6-MAb}$ 后 72 h 肿瘤显影, 随时间的延长, 肿瘤影像越清楚, 至 120 h 时最清晰, 而在 $700 \mu\text{g}$ 未标记抗体阻断后, 肿瘤影像明显受抑制。提示 $^{131}\text{I-A6-MAb}$ 能特异性结合于肿瘤组织。

结论

(1) 我们建立抗 NRP-1 抗体亲和层析纯化方法，获得一批高亲和力的抗 NRP-1 抗体 A6。

(2) 我们成功制备 ^{131}I -NRP-1A6b 分子探针， ^{31}I -A6-MAb 的标记方法简单，易行，标记率高，稳定性好，具有良好的 NRP-1 的靶向性和特异性，有望成为一种新型肿瘤诊断和治疗的药物。

(3) 阻断正常组织摄取可提高肿瘤组织摄取，而 5mg/kg 可能为最佳阻断剂量。

【关键词】 脑胶质瘤；神经鞭毛素受体-1；单克隆抗体；放射性核素显像；裸鼠

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Abstract

Background

Radioimmunoimagine (RII) plays an important role in early tumor diagnosis. To date, there were only five RII drugs approved by FDA in clinical studies. However, the use of RII can be limited by the poor diagnostic efficiency and high false negative results. Therefore, it is a cornerstone for RII to further develop a novel antibody probe targeting tumor. Neuropilin-1 (NRP-1), a co-receptor for Semaphorin 3A (Sema 3A) and vascular endothelial growth factor (VEGF), plays an important role in neoplastic processes of angiogenesis, growth and metastatic spread. Overexpression of NRP-1 has been frequently detected in a wide range of human tumors, for example, lung cancer, gastric cancer, colon cancer, pancreas cancer, gliomas, etc. Furthermore, increasing evidence has demonstrated that there is a correlation between NRP-1 overexpression and poor prognosis, and short survival for some cancer types. Therefore, NRP-1 has become an attractive target for cancer molecular imaging and therapy. In the study, to further develop an anti-NRP-1 antibody-based probe for future clinical translation, an monoclonal antibody--A6 targeting to NRP-1 was labeled with ^{131}I by Iodogen method, The resulting probe, ^{131}I -A6-MAb, was then evaluated in tumor models bearing U87 MG cell to study whether the SPECT probe can be used for imaging of NRP-1 positive tumor in vivo.

Methods

(1) An anti-NRP-1 mAb-A6 was obtained by rProtein A column purification from the ascites, and its affinity was identified by ELISA methods.

(2) A6 was labeled with ^{131}I by Iodogen method under the optimum labeling conditions, then the labeling efficiency and the stability were detected in vivo by TLC.

(3) The bioactivity of ^{131}I -A6 in U87 MG cells was measured by cell uptake assay, binding test, internalization assay.

(4) The nude mice bearing human U87 MG glioma cells were randomly divided into 4 groups with 4 in each group. The nude mice were sacrificed by cervical

dislocation and dissected at 24,48,72,96,120 h respectively after intravenous injection of 7.4 MBq ^{131}I -A6. The biodistribution of the agent was measured as % ID/g..

(5) SPECT/CT imaging was performed in 8 mice including unblocking group (n=4) and blocking group (n=4) at different time (12、24、48、72、96、120h) after injection of 7.4 MBq ^{131}I -A6 MAb with or without a 100-fold (700 μg) unlabeled A6 MAb.

Results

(1) The purity of purified anti-NRP-1 mAb -A6 was more than 95%, concentration was 4 mg/mL.

(2) The Paper chromatography showed that the labeling efficiency and the radiochemical purity of ^{131}I - labeled A6 were 98%. The stability of ^{131}I -A6-MAb in PBS buffer solution were maintained over 85% in 96 h;

(3) ^{131}I -A6-MAb had rapid accumulation in U87 MG cells and reached the highest value of $15.80 \pm 1.30\%$ at 1 h, and it bound to NRP-1 with low nanomolar affinity ($K_D = 1.67 \pm 0.14 \text{ nM}$) in U87 MG cells. The internalization rate of ^{131}I -A6-MAb reached highest $25.7 \pm 3.0\%$ at 8h

(4) The in-vivo biodistribution study showed that ^{131}I -A6-MAb displayed relatively high levels of radioactivity accumulation in U87 MG tumors. The value of tumor uptake for ^{131}I -A6-MAb were 6.0 ± 1.2 、 8.8 ± 1.7 、 11.4 ± 2.0 、 $8.2 \pm 1.4 \text{ ID\%/g}$ at 24h in unblocking and blocking (2.5、5、10mg/kg) group, respectively. Furthermore, in 5mg/kg blocking group, the tumor uptake of ^{131}I -A6-MAb was the most highest, however in 10mg/kg blocking group, the tumor uptake decreased. The liver uptake were 7.6 ± 1.5 、 6.4 ± 0.9 、 5.8 ± 0.7 、 $6 \pm 0.8 \text{ ID\%/g}$ respectively. As time progressed, the radioactivity in the tumor and other normal tissue (except thyroid) was decreased, but a faster clearance of ^{131}I -MAb was observed in the blood. At 120h p.i., the radioactivity in the blood were 0.8 ± 0.1 、 1.2 ± 0.3 、 2.0 ± 0.3 、 $6.0 \pm 1.3 \text{ ID\%/g}$, and the tumor:blood ratios (T/B) were 1.8 ± 0.5 、 2.3 ± 0.7 、 2.4 ± 0.6 、 0.9 ± 0.2 ;

(5) The SPECT imaging showed the tumor uptake of ^{31}I -A6-MAb increased from 12 h to 120 h gradually after injection. Co-injection of an excess (700 μg) of unlabeled A6 antibody resulted in significant reduction in tumor uptake.

Conclusion

(1) We developed a anti-NRP-1 antibody A6 with high affinity and purity a with an affinity chromatography purification method

(2) ^{131}I -A6-MAb can be easily synthesized by Iodogen method with high radiochemical purity. The specific tumor uptake of ^{131}I -A6-MAb, which correlates with NRP-1 expression in gliomas, makes it a new promising tumor targeted radiotracer.

(3) Saturation the uptake of ^{131}I -A6-MAb in normal tissue could increase the tumor uptake, and 5mg/kg may be the optimal dose .

【Key word】 Gliomas; Neuropilin-1; Monoclonal antibody; Radionuclide imaging; nude mice

目 录

摘 要	I
Abstract	IV
前 言	1
一、放射免疫显像	1
二、Neuropilin1-简介	2
2.1 Neuropilin-1 起源	3
2.2 NRP-1 蛋白结构	3
三、NRP1 正常组织中表达及其生物学作用	3
3.1 NRP-1 在正常组织中表达	3
3.2 NRP-1 与生长发育	4
3.3 NRP-1 与免疫调节	4
四、NRP1 与肿瘤	4
4.1 NRP1 在肿瘤组织中表达	4
4.2 NRP-1 与肿瘤血管新生	5
4.3 NRP-1 与肿瘤生长	6
4.4 NRP-1 与肿瘤转移	7
4.5 NRP1 与肿瘤干细胞	7
4.6 NRP1 表达与肿瘤进展机制	7
五、NRP1 共受体	8
5.1 VEGFR	8
5.2 C-MET	9
5.3 PDGFRS	9
5.4 EGFR	9
六、NRP-1 靶点治疗现状	10
6.1 单克隆抗体	10
6.2 小分子肽	10
6.3 可溶性 NRP1 (SNRP-1)	10
6.4 干扰 RNA	11
6.5 Sema3A	11
七、NRP-1 靶点分子显像现状	11

八、本研究目的与内容.....	12
第一章 ^{131}I-A6-MAb 的制备、纯化及鉴定	13
前言	13
一、实验材料	13
1.1 抗体腹水	13
1.2 主要试剂及耗材	13
1.3 主要仪器及设备	14
1.4 主要试剂的配制	14
二、实验方法	16
2.1 抗体纯化	16
2.2 ^{131}I -A6-MAb 制备	19
2.3 ^{131}I -A6-MAb 纯化	19
2.4 ^{131}I -A6-MAb 放射性化学纯度测定	19
2.5 ^{131}I -A6-MAb 体外稳定性观察	19
2.6 ^{131}I -A6-MAb 免疫活性测定	19
三、实验结果	20
3.1 A6 抗体 SDS-PAGE 分析	20
3.2 A6 抗体间接 ELISA	21
3.3 ^{131}I -A6-MAb 标记率	22
3.4 ^{131}I -A6-MAb 放射性化学纯度	23
3.5 ^{131}I -A6MAb 免疫活性	23
3.6 ^{131}I -A6-MAb 体外稳定性	24
四、讨论	24
第二章 ^{131}I-A6-MAb 体、内外特性及 SPECT/CT 显像..	25
前言	25
一、实验材料	25
1.1 实验用细胞株	25
1.2 主要试剂及耗材	25
1.3 主要实验仪器	25
1.4 主要试剂配制	26
二、实验方法	27
2.1 细胞培养	27

2.2 ^{131}I -A6-Mab 细胞摄取实验	28
2.3 ^{131}I -A6-MAb 结合力 (K_D) 测定	28
2.4 ^{131}I -A6-MAb 细胞内吞试验	29
2.5 ^{131}I -A6-MAb 移植瘤裸鼠生物学分布	29
2.6 ^{131}I -A6-MAb 裸鼠 SPECT/CT 显像	30
三、实验结果	30
3.1 ^{131}I -A6-MAb 细胞摄取	30
3.2 ^{131}I -A6-MAb 亲和力(K_D)	31
3.3 ^{131}I -A6-MAb 细胞内吞速率	31
3.4 ^{131}I -A6-MAb 裸鼠体内生物学分布	32
3.5 ^{131}I -A6-MAb 荷瘤裸鼠 SPECT/CT 显像	32
四、实验讨论	34
第三章 ^{131}I-A6-MAb 裸鼠体内阻断实验	36
前言	36
一、实验材料	36
1.1 细胞株及实验动物	36
1.2 实验仪器	36
1.3 实验溶液配制	36
二、实验方法	36
2.1 肿瘤模型制作	36
2.2 阻断组荷瘤裸鼠生物学分布	36
2.3 阻断组裸鼠 SPECT/CT 显像	37
三、实验结果	37
3.1 ^{131}I -A6-MAb 各组裸鼠血浆代谢	37
3.2 ^{131}I -A6-MAb 裸鼠血液放射性分布	38
3.3 ^{131}I -A6-MAb 各组裸鼠 24h 生物学分布	39
3.4 ^{131}I -A6-MAb 不同时间点各组荷瘤裸鼠肿瘤代谢	40
3.5 各组裸鼠不同时间点肿瘤/血浆比值	41
3.6 ^{131}I -A6-MAb 各组裸鼠 48hSPECT/CT 显像	41
四、实验讨论	43
五、讨 论	44
结 论	47

本研究存在的问题	47
参 考 文 献	48
致 谢	53

厦门大学博硕士论文摘要库

CONTENTS

Abstract in Chinese	I
Abstract in English	IV
Introduction	1
I、 RII.....	1
II、 Neuropilin-1 intruoduction.....	2
2.1 Neuropilin-1	3
2.2 NRP-1 structure	3
III、 NRP-1expression in normal tissue and function.....	3
3.1 NRP-1 epression in normal tissue	3
3.2 NRP-1 and embryonic development.....	4
3.3 NRP-1 and imunity	4
IV、 NRP-1and tumor.....	4
4.1 NRP-1 expression in tumor	4
4.2 NRP-1 and tumor anjiojenesis.....	5
4.3 NRP-1 and tumor development	6
4.4 NRP-1 and tumor metastasis.....	7
4.5 NRP-1 and tumor stem cell	7
4.6 NRP-1 expression and tumor progression	7
V、 NRP-1 co-receptor.....	8
5.1 VEGFR.....	8
5.2 C-MET.....	9
5.3 PDGFRS.....	9
5.4 EGFR.....	9
VI、 NRP-1 and tumor targeting therapy	10
6.1 monoclonal antibody	10
6.2 peptides.....	10
6.3 SNRP-1.....	10
6.4 siRNA	11
6.5 Sema3A	11
VII、 NRP-1 and tumor targeting imagine	11

VIII、 Purpose and cotents of this reaserch.....	12
---	----

Chapter I Production and characteristics of anti-NRP-1

mAb.....	13
-----------------	-----------

Introduction.....	13
-------------------	----

I Materials.....	13
------------------	----

1.1 A6 monoclonal antibody.....	13
---------------------------------	----

1.2 Main reagents	13
-------------------------	----

1.3 Main instruments.....	14
---------------------------	----

1.4 Main solutions.....	14
-------------------------	----

II Methods.....	16
-----------------	----

2.1 Monoclonal antibody purification	16
--	----

2.2 ¹³¹ I-A6-MAb prearetion.....	19
---	----

2.3 ¹³¹ I-A6-MAb purification	19
--	----

2.4 ¹³¹ I-A6-MAb purification detection.....	19
---	----

2.5 ¹³¹ I-A6-MAb stability in-vivo	19
---	----

2.6 ¹³¹ I-A6-MAb immuno reactivity	19
---	----

III Results	20
-------------------	----

3.1 SDS-PAGE analysis	20
-----------------------------	----

3.2 Indirect ELISA analysis.....	21
----------------------------------	----

3.3 ¹³¹ I-A6-MAb labelling rate	22
--	----

3.4 ¹³¹ I-A6-MAb radio purification	23
--	----

3.5 ¹³¹ I-A6-MAb immuno reactivity	23
---	----

3.6 ¹³¹ I-A6-MAb stability	24
---	----

IV Discussion	24
---------------------	----

Chapter II ¹³¹I-A6-MAb charactertion and SPECT/CT

imagine.....	25
---------------------	-----------

Introduction.....	25
-------------------	----

I Materials.....	25
------------------	----

1.1 Cells	25
-----------------	----

1.2 Main reagents	25
-------------------------	----

1.3 Main instruments.....	25
---------------------------	----

1.4 Main solutions.....	26
II Methods.....	27
2.1 Cell culture	27
2.2 ¹³¹ I-A6-MAb cell uptake assay	28
2.3 ¹³¹ I-A6-MAb <i>K_D</i> assay.....	28
2.4 ¹³¹ I-A6-MAb internalization assay	29
2.5 ¹³¹ I-A6-MAb biodistribution in mice	29
2.6 ¹³¹ I-A6-MAb SPECT/CT imagine.....	30
III Results	30
3.1 ¹³¹ I-A6-MAb cell uptake	30
3.2 ¹³¹ I-A6-MAb <i>K_D</i>	31
3.3 ¹³¹ I-A6-MAb internalization rate.....	31
3.4 ¹³¹ I-A6-MAb biodistribution	32
3.5 ¹³¹ I-A6-MAb SPECT/CT imagine.....	32
IV Discussion	34
Chapter III ¹³¹I-A6-MAb blocking assay in tumor-bearing mice	36
Introduction.....	36
I Materials.....	36
1.1 Cell and animals	36
1.2 Main reagents	36
1.3 Main instruments.....	36
II Methods.....	36
2.1 Tumor models	36
2.2 Biodistribution in blocking groups	36
2.3 SPECT/CT imagine in blocking groups	37
III Results	37
3.1 ¹³¹ I-A6-MAb radioactivity in blood.....	37
3.2 ¹³¹ I-A6-MAb distribution in blood	38
3.3 ¹³¹ I-A6-MAb biodistribution in blocking groups.....	39
3.4 ¹³¹ I-A6-MAb radioactivity in tumor	40
3.5 The tumor/blood ratio	41
3.6 ¹³¹ I-A6-MAb SPECT/CT imagine in blocking groups	41
IV Discussion	43

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