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C2min 2

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［摘要］ 目的 PAMAM-PEG-C2min
方法 - PAMAM C2min ^{1}H NMR
PAMAM-PEG-C2min PC3 LNCaP
siR-M 结果
PAMAM-PEG-C2min PC3 LNCaP PAMAM-PEG-C2min
C2min PAMA-PEGM PAMAM-PEG-C2min
PAMAM-PEG-C2min 2 结论
PAMAM-PEG-C2min

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The C2min aptamer-modified gene delivery system for targeting ADPC/AIPC prostate cancer

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［**Abstract**］ **Objective** To synthesize a novel prostate cancer targeting gene vector PAMAM-PEG-C2min and improve gene transfection efficiency targeting on prostate cancer. **Methods** The aptamer (C2min) and polyamide-amine (PAMAM) were ligated by polyethylene glycol (PEG). The structure of the synthesized PAMAM-PEG-C2min was identified by NMR. The biological characteristics of the nanoparticles were examined by the uptake experiments and gene transfection experiments (the loaded gene was siR-M) with the prostate cancer cells (PC3 and LNCaP). Besides, the *in vivo* targeting was investigated using *in vivo* image system. The *in vivo* targeting results indicated that PAMAM-PEG-C2min can achieve the simultaneous targeting of two prostate cancer tissues. **Results** The PAMAM-PEG-C2min synthesis was confirmed by NMR. Cell uptake experiments showed that the cell uptake efficiency of PAMAM-PEG-C2min was concentration dependent. *In vitro* experiments showed that the PC3 and LNCaP cells transfection efficiency and targeting of PAMAM-PEG modified with C2min were significantly improved compared with the PEG modified PAMAM. **Conclusion** PAMAM-PEG-C2min is a potential targeted drug delivery vehicle. It provides a new technology platform for comprehensive and specific targeting treatment of prostate cancer.

［**Key words**］ adapter targeting drug delivery androgen-dependent prostate cancer androgen-independent prostate cancer gene vector

PCa [1] androgen-dependent prostate cancer, ADPC 1~1.5 ADPC androgen-independent prostate cancer, AIPC [2-3] ADPC AIPC ADPC AIPC [4] ADPC [5] ADPC/AIPC 2

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(LNCaP PC3) CD71 C2min [6] NHS-PEG-MAL - polyamidoamine PAMAM 2 siR-M PAMAM-PEG-C2min/siR-M

1 材料与方法

1.1 主要仪器和试剂

Mercury Plus 300 MHz Varian IX2-RFACA Olympus FACSCalibur BD C2min Ribo Bio pEGFP-N2-Luc pDNA siR-m, Weijin Bio Promega QIAGEN Plasmid Mega Kit Qiagen GmbH, PAMAM (G5) 5% Sulfhydryl Addition Kit DyLight-633 NHS Ester BCA Protein Assay kit Thermo MAL-PEG-NHS 4000 Nektar FAM cy-7 RPMI-1640 Gibco,

1.2 细胞和实验动物

ADPC LNCaP AIPC PC3 BALB/C SCXK 2018-0006

1.3 细胞培养及荷 2 种移植瘤裸鼠模型的建立

LNCaP PC3 5% FBS RPMI-1640 37 ℃ 5% CO_2 [7] 2~3 d 80% PC3 LNCaP 1×10^7 /ml 0.2 ml/ 15~20 d 500 mm^2

1.4 PAMAM-PEG-C2min 的合成与结构鉴定

FAM-NSH PAMAM FAM-PAMAM-PEG-C2min PAMAM (G5) FAM 5:1 4 ℃ 12 h G-25 FAM FAM-PAMAM PEG NHS-PEG-MAL PEG PAMAM 2 : 1 15 min 5000 FAM-PAMAM-PEG C2min FAM-PAMAM-PEG C2min PAMAM 1:1 24 h FAM-PAMAM-PEG-C2min cy7 PAMAM PAMAM-PEG PAMAM-PEG-C2min ^{1}H NMR 3 FAM

1.5 两种前列腺癌细胞对 PAMAM-PEG-C2min 的摄取

PC3 LNCaP 6 6×10^4/ 80% 0.04 0.20 0.40 0.80 1.20 μmol/L FAM-PAMAM-PEG-C2min PC3 LNCaP 1 h PBS 3 2 PBS 2 FAM

1.6 PAMAM-PEG-C2min/pDNA 纳米复合物的制备及表征

PAMAM PAMAM-PEG PAMAM-PEG-C2min 12 μg/ml PAMAM DNA N/P 1:1 5:1 10:1 15:1 20:1 25:1 30 s PAMAM\pDNA PAMAM-PEG\pDNA PAMAM-PEG-C2min\pDNA Z90 Malvern N/P PAMAM-PEG\pDNA Zeta *n*=3

1.7 PAMAM-PEG-C2min/pDNA 体外转染

PC3 LNCaP 24 2×10^4/ “1.6” N/P 3 μg pDNA / 37 ℃ 5% CO_2 2 h 48 h 488 nm 525 nm [8]

1.8 纳米复合物的体内靶向性效果

cy7-PAMAM-PEG-C2min “1.4” 0.5 2

6 12 24 h

1.9 统计学处理

SPSS 18 *t*

$\bar{x} \pm s$

2 结果

2.1 PAMAM-PEG-C2min 的结构鉴定

NHS-PEG-MAL NHS PAMAM PAMAM-PEG PAMAM-PEG-MAL PEG MAL C2min PAMAMA-PEG-C2min ^{1}H NMR D_2O 4.7 ppm PAMAM 2.2 3.4 ppm 1A PEG 3.6 ppm 8.3 ppm 1B PEG 2.4 C2min PAMAM 2.2 3.4 ppm 1C 8.3 ppm C2min PAMAM-PEG PAMAM-PEG-C2min

2.2 两种前列腺癌细胞对 PAMAM-PEG-C2min 的摄取

PAMAM-PEG-C2min PC3 LNCaP LNCaP PC3 2A PAMAM-PEG-C2min 0.04 μmol/L 0.12 μmol/L LNCaP 10.31±0.38 % 82.34±3.83 % PC3 8.75±0.47 % 85.37±3.25 % LNCaP PC3 (2B)

2.3 不同 N/P 比 PAMAM-PEG-C2min/pDNA 纳米复合物粒径和 Zeta 电位

3 PAMAM-PEG-C2min/pDNA N/P Zeta PAMAM

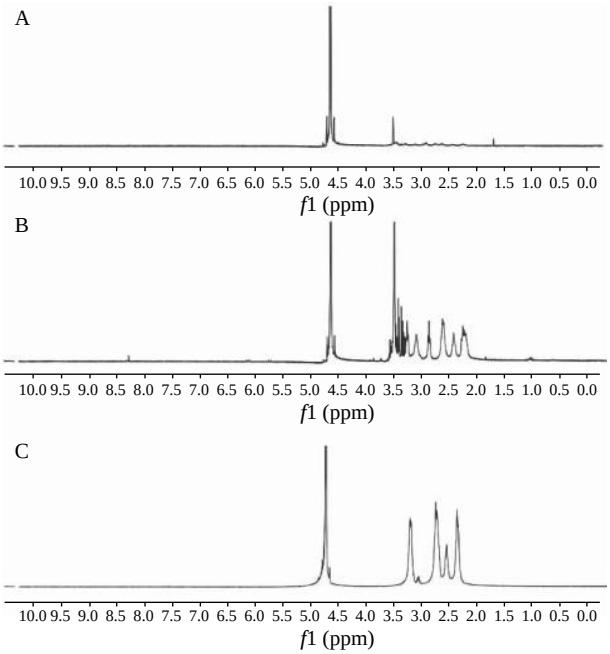

图 1 PAMAM (A)、PAMAM-PEG (B)、PAMAM-PEF-C2min (C) 的^{1}HNMR 图谱

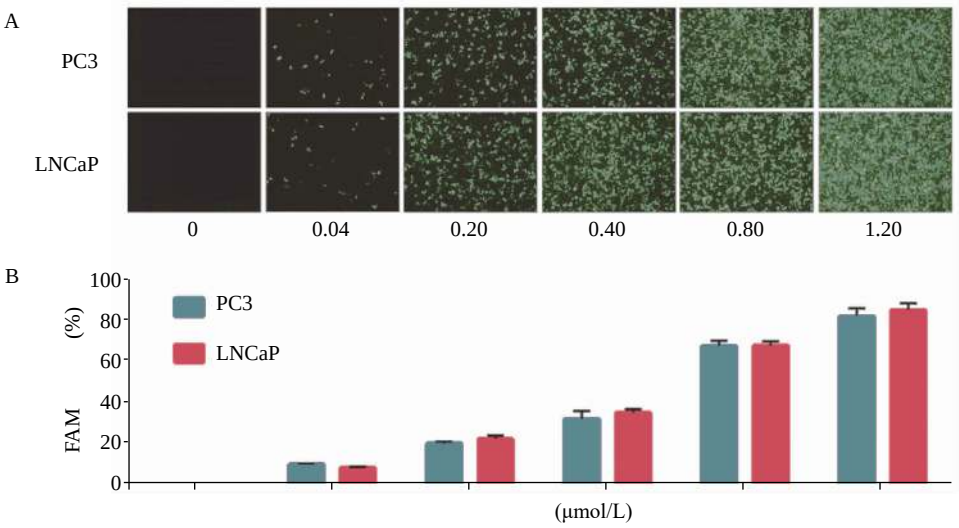

图 2 PC3 和 LNCaP 对不同浓度的 PAMAM-PEG-C2min 的摄取情况（*n*=4）

A. B.

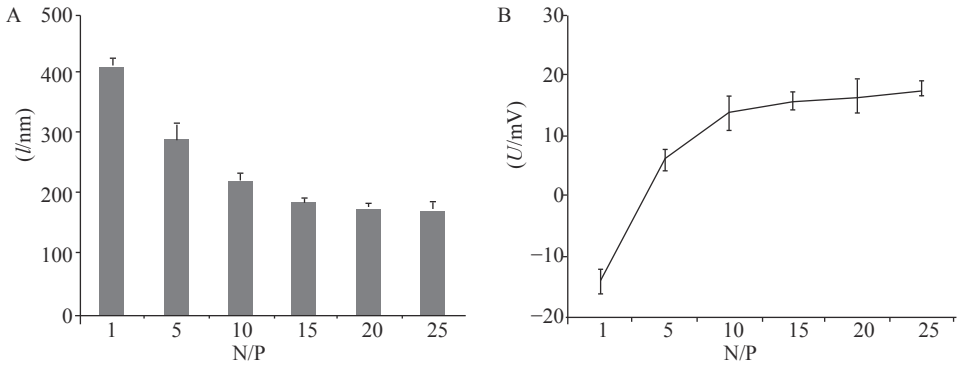

图 3　不同 N/PPAMAM-PEG-C2min/pDNA 复合物粒径和 Zeta 电位（*n*=3）

A.　　B.

2.4　PAMAM-PEG-C2min/pDNA 体外转染

PAMAM-PEG-C2min/pDNA　　N/P　　4A　　4B　　N/P　　PAMAM-PEG　　C2min　　2

PAMAM-PEG-C2min

2　　ADPC　　AIPC

2.5　纳米复合物的体内靶向性效果

5　　Cy7-PAMAM-PEG-C2min　　0.5 h　　2 h　　12 h　　24 h　　C2min

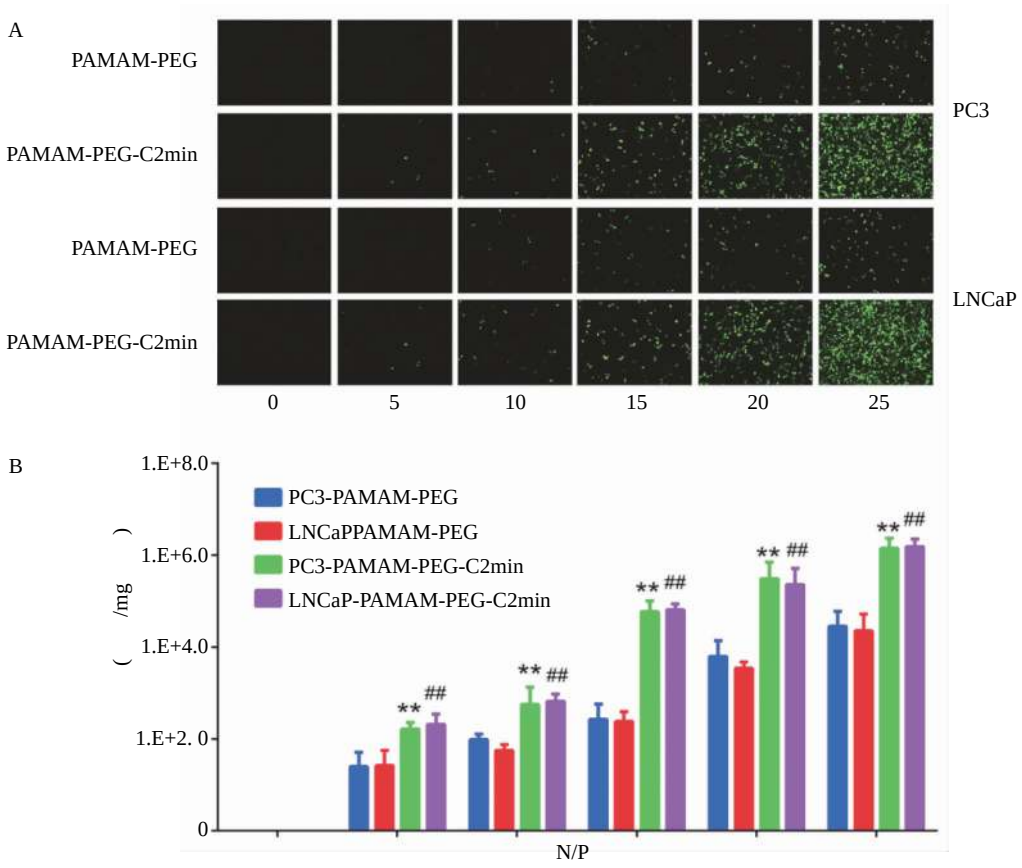

图 4　荧光显微镜（A）、生物发光检测仪（B）观察不同纳米复合物的细胞表达情况（*n*=4）

$^{**}P$<0.01　　N/P　　PC3-PAMAM-PEG-C2min　　PC3-PAMAM-PEG　　$^{\#\#}P$<0.01　　N/P　　LNCaP-PAMAM-PEG-C2min　　LNCaP-PAMAM-PEG

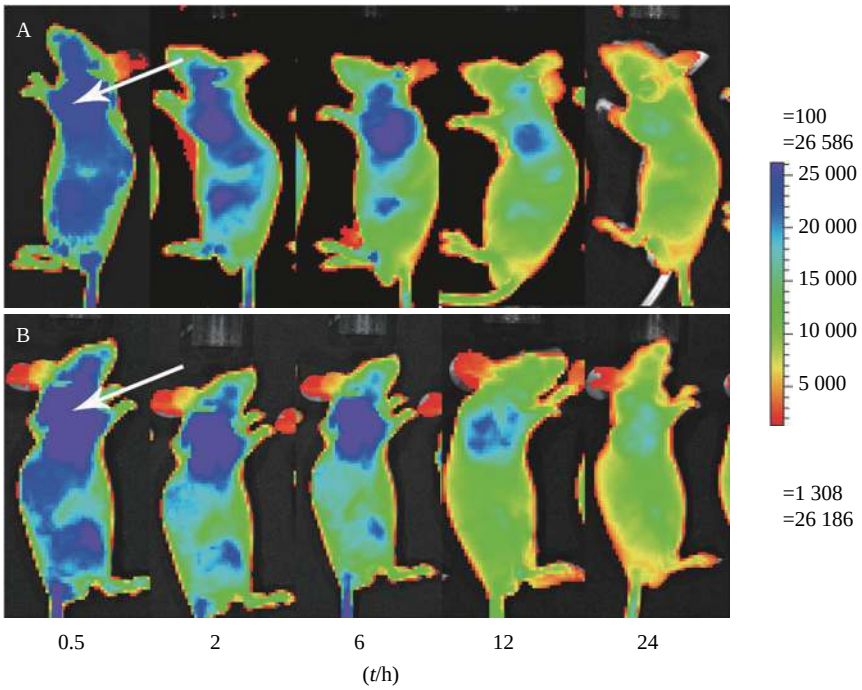

图 5 动物活体成像

A. PC3 B. LNCaP

3 讨论

PAMAM

[9]

[10-12]

[6, 13]

LNCaP PC3 CD71

C2min CD71 [14]

C2min 43 (43 nt)

C2min

2

PAMAM-PEG-C2min

C2min PC3 LNCaP

PAMAM-PEG-C2min-pDNA

N/P

[15] N/P

2

C2min

PAMAM-

PEG-C2min 2

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表 2 防暑清热饮总黄酮和总多糖含量测定结果 (*n*=3，εg/ml)

20181210	855.2	5 085
20181212	869.2	5 108
20190107	828.0	4 420

3 讨论

65%

5 h 4 500 r/min 10 min

5 10 20 h

508 nm 487 nm

1

5 10 20

508 nm 5

A_{508} 0.3~0.7 5

40 min

1

50 100 487 nm 100

A_{487} 0.3~0.7 100

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