Cloning and Sequencing of the Tumor Antigen MAGE-12 Gene

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Abstract: Objective. To clone the MAGE-12 gene, preparing for researching its biological effects on MAGE-12 positive malignant tumors. **Methods.** mRNA was extracted from human lung cancer specimen. MAGE-12 gene was amplified by reverse transcriptase polymerase chain reaction(RT-PCR) and was identified by enzyme cutting with Pvu Ⅱ. The PCR fragment was inserted into pGEM-T easy plasmid, and then was transformed into JM109. After the selection of blue/white screening, the primers designed with T7/SP6 sequence of pGEM-T easy plasmid were applied to identify the recombinant. Further more, DNA sequence of the recombinant was analyzed. **Results.** The length of the DNA fragment RT-PCR amplified by mRNA was 944 base pairs. which was conformed by enzyme Pvu Ⅱ cutting identification. The correctness of linking between MAGE-12 and pGEM-T easy was verified through selection of blue/white screening and identified by T7/SP6 primers, and the sequences of DNA fragment were homology with corresponding sequences published in GenBank, which indicated that the target gene had been inserted into pGEM-T easy successfully. **Conclusion.** The MAGE-12 gene was cloned successfully, which made the foundation of biological treatment by using MAGE-12 gene. [Life Science Journal. 2006;3(1):72-74] (ISSN: 1097-8135).

Keywords: MAGE-12; tumor antigen; gene

1 Introduction

Melanoma antigen-1(MAGE-1) gene, expressed by malignant melanoma, was first found by van der Bruggen et al with cloning technique in 1991. And it's encoding antigen MZ2-E, expressed in variant degree not only in melanoma but also in other tumors, however, never expressed in normal tissue(except testicle and placenta). The finding of tumor specific antigen established a strong base for the active specific immunotherapy of human tumor vaccine. Tumor specific antigen, as a basis for human tumor specific immunotheraphy, should be paid more attention nowadays especially when biotheraphy of tumor developing is so fast. From studying and analyzing, the authors cloned MAGE-12 gene successfully, and which made the foundation of biological effect in malignant tumor and producing tumor gene vaccine by investigating MAGE-12.

2 Materials and Methods

2.1 Materials

All specimen of lung cancer tissue were from the First Affiliated Hospital of Zhengzhou University, plasmid vector pGEM-T easy, restriction enzyme Pvu Ⅱ, AMV-reverse transcriptase, Taq DNA polymerase 6-mers random primer, T4 DAN joinase were Promega products. TRIZOL reagent was Invitrogen product; gelatin of Wharton reclaiming kit was V-Gene product; isopropy-β-D-thiogalactoside (ITPG), 5-bromo-4-chloro-3-indolyl-β-D-galactoside(X-Gal) were Takara products; *E. coli*. JM109 were from our department's preservation.

2.2 Methods

2.2.1 The design and synthesis of primer

According to MAGE-12 gene sequences published in GenBank, designed the primer. Ecto-supra primer M1: 5′-GCACTAGCTCCTGCCCACAC-3′; ecto-infra primer M2: 5′-TGGGCCTCATGTCACACGAC-3′; ento-supra primer M3: 5′-ACTCGAGGCCACCATGCCACTTGAGCAGAGGAG-3′; partake Xho Ⅰ enzyme cutting site and translation initiation codon; ento-infra primer M4: 5′-TGGTA CCCTCTTCCCCCTCTCTAAAAG-3′ partake Kpn Ⅰ enzyme cutting site. According to the T7, SP6 promoter sequence, which are on both sides of multiple clone site in plasmid pGEM-T easy, design the consensus primer T7: 5′-TAATACGACTCACTATAGGGAGA-3′; SP6: 5′-CATACGTATTAGGTGACACTA TAG-3′. All primers we used were synthetized by Shanghai Sangon

Biotechnology Co., Ltd.

2.2.2 Extraction of total RNA

Put lung cancer tissue specimen just sliced into grinder and levigate it, add 1 ml TRIZOL reagent, misce bene and transfer the mixture into 1.5 ml centrifuge tube; standing at room temperature for 5 minutes to make sure ribosome lyzed completely; add chloroform of 0.2 ml, freezing centrifuged with 12000 rpm at 4℃ for 5 minutes; shift the supernatant into another centrifuge tube and add dimethylcarbinol of 0.5 ml; misce bene lightly, freezing centrifuged with 12000 rpm at 4℃ for 10 minutes; dislodge supernanant and add 75% ethyl alocohol of 1 ml; freezing centrifuged with 7,500 rpm at 4℃ for 5 minutes; dislodge supernanant; air drying at room temperature for 10 minutes; add DEPC liquor of 30 μl, put into bain-marie at 55℃ for 10 minutes. Then get total RNA and reverse transcribe target gene fragment immediately.

2.2.3 RT-PCR and amplification of target gene fragment

Preparation of cDNA: reaction system(30 μl): 5 × Buffer 6 μl, 4 × dNTP(2.5 mmol/l dNTP) 2 μl, 6-mers random primer 1 μl, AMV 1 μl, template(the total RNA we extracted) 5 μl, DEPC liquor 15 μl; reaction condition: aqueous bath at 42℃ for 1 hour, aqueous bath at 72℃ for 5 minutes to inactivate enzyme AMV. Amplification of target gene with PCR: reaction system of the first amplication: 10 × Buffer 3 μl, 4 × dNTP 2 μl, M1 0.5 μl, M2 0.5 μl, Taq DNA polymerase 0.5 μl, Mini-H_2O 18.5 μl, cDNA 5 μl; reaction condition: predict apomorphosis at 94℃ is 5 minutes, apomorphosis at 94℃ for 45 seconds, renaturation at 55℃ for 45 seconds, elongation at 72℃ for 60 seconds, 35 reaction circulations, and elongation at 72℃ for 5 minutes the last time. Reaction system of the second amplification: 10 × Buffer 3 μl, 4 × dNTP 2 μl, M3 0.5 μl, M4 0.5 μl, Taq DNA polymerase 0.5 μl, Mini-H_2O 18.5 μl, product of the first amplication 5μl; reaction condition: predict apomorphosis at 94℃ is 5 minutes, apomorphosis at 94℃ for 45 seconds, renaturation at 55℃ for 45 seconds, elongation at 72℃ for 60 seconds, 35 reaction circulations, and elongation at 72℃ for 5 minutes the last time. Take suction of amplified sample for 5 μl and identify with 1.5% agarose gel electrophoresis, preserve the product of the second amplification into frige at −20℃.

2.2.4 Verification of enzyme Pvu Ⅱ cutting target gene and gelatin of Wharton reclaiming target gene

Reaction system: Mini-H_2O 6.5 μl, target gene product of ultimum amplification 1.5 μl, 10 × Buffer 1 μl, Pvu Ⅱ 1 μl; reaction condition: aqueous bath at 37℃ for 2 hours, aqueous bath at 72℃ for 5 minutes to inactivate enzyme Pvu Ⅱ. Product and target gene, from enzyme cutting, which were identified with 1.5% agarose gel electrophoresis. Then reclaiming product of the second PCR amplification with V-Gene gelatin reclaiming kit.

2.2.5 Construction and sequence analysis of human MAGE-12 gene cloning vector

Ligate MAGE-12 gene that gelatin reclaimed with vector Pgem-T easy, The ligation get through the night at 4℃, then have the ligation transfected into *E. coli*. JM109. After the selection of blue/white screening, select the white colony, then amplified by polymerase chain reaction(PCR). After identifing the positive cloning, sent the product for PCR to sequence analysis.

3 Results

3.1 The product of PCR and identification of enzyme cutting

Taking the cDNA as template, M1, M2 as the primer, amplified by RT-PCR. Electrophorese the product with 1.5% agarose gel. The stripe we get is located at about 1000 bp contrasting with DNA marker(Figure. 1), this is close to our anticipation. Electrophorese the product digested by PVU Ⅱ with 1.5% agarose gel, the stripe is at about 500 bp contrasting with DNA marker(Figure 2). In the abstract, PVU Ⅱ enzyme cutting site of the MAGE-12 gene is at 3434 bp, after the enzyme cutting, the lengths of the gene fragment are 474bp and 470 bp. The result is consistent with the abstract. The product amplified by RT-PCR is our anticipating gene.

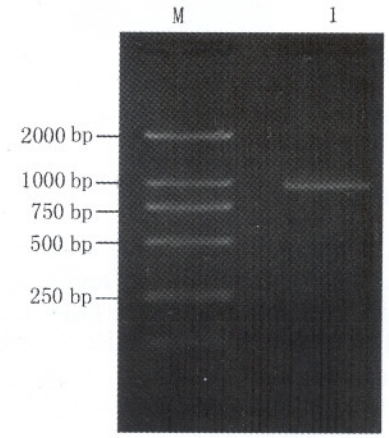

Figure 1. The product of RT-PCR(1.5% agarose gel electrophoresis). Lane M: DNA marker; Lane 1: the product of PCR

3.2 Cloning and analysis of the target gene

After reclaiming target gene with gelatin, link the MAGE-12 gene with the vector pGEM-T easy.

Transfect into E. coli. JM109, select 4 stochastic bacteria *colanys* to identified by PCR. The amplified gene fragments are about 1000 bp contrasting with DNA marker the stripe locating at about 100bp is the empty plasmid colonies (Figure 3). The result is consistent with the abstract. This suggests that we have cloned the MAGE-12 gene successfully.

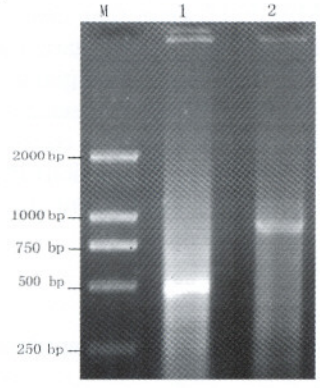

Figure 2. The result of identified by PVU Ⅱ (1.5% agarose gel electrophoresis). Lane M: DNA marker; Lane 1: product digested by PVU Ⅱ; Lane 2: MAGE-12 gene

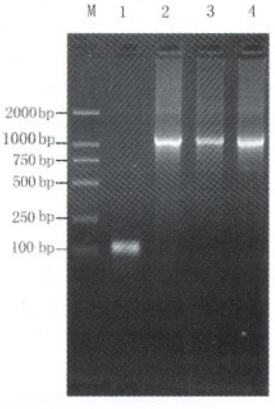

Figure 3. Identification of recombinant vector PGEM-T easy-MAGE-12
Lane M: DNA marker; Lane 1: amplified product of negative clone; Lane 2~4: amplified product of positive clones.

4 Discussion

In 1991, van der Bruggen et al found the first melanoma antigen(MAGE-1). From then on, people found that MAGE family at least included six sub-family: MAGE-A, B, C, D, E, F. MAGE-A is constituted by 12 genes. At first, these genes were named MAGE-1 ~ MAGE-12, and later some people suggested that these genes were called MAGE-A1 ~ MAGE-A12. The MAGE-12 gene was found by Ding M et al in DM150 melanoma cell line, which was homology with MAGE-2, MAGE-3. These genes are all belong to tumor special antigen (TSA), which have MHC-Ⅰ restriction and are mainly expressed in malignancy tumor, and never expressed in normal tissues except testicle and placenta. Therefore, MAGE-12 is a perfect target antigen in anti-tumor immunity. Yanqiu Li and Yuzhang Wu doped out the MAGE-12 epitope identified by CTL, which was restricted by HLA-A2, using the immunology methods. The CTL identified epitod is located at about 271-279 (FLWGPRALV) remnant-radix. In our country, the literature about the MAGE-12 gene in human lung cancer was rare.

The authors cloned the MAGE-12 gene successfully using the reverse transcriptase polymerase chain reaction (RT-PCR), and constructed pGEM-T easy-MAGE-12 cloning vector. After the sequence analysis, the sequences of DNA fragment were homology with corresponding sequences published in GenBank. The authors constructed the pGEM-T easy-MAGE-12 cloning vector, and made the foundation for constructing the eukaryotic expression vector and developing the MAGE-12 gene vaccine.

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