Detection of MAGE-A3 Antigen and HLA-class I Genes Distribution in Lung Cancer

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Abstract: Aim. In order to speculate the ratio of lung cancer patients who can be treated with immunotherapy based on MAGE-A3 antigen, the expression of MAGE-A3 antigen and the distribution of HLA-class I genes were investigated in lung cancer tissues. **Methods.** SDS-PAGE and Western blot were to detect the expression of MAGE-A3 antigen in 63 lung cancer patients. The distributions of HLA-A1, A2 and A24 gene in 70 lung cancer patients and the tissues adjacent were detected with PCR-SSP. **Results.** 1) 31 of 63 lung cancer tissues, and 5 tumor adjacent tissues expressed MAGE-A3 antigen; 2) In 70 lung cancer patients the distribution of HLA-A1, A2 and A24 were 4.28%, 54.28% and 50.0%, respectively; 3) In 70 tumor adjacent tissues the distribution of HLA-A1, A2, A24 were 4.28%, 60.0% and 52.86%, respectively. The total positive rate of HLA-A1, A2, A24 was 80.0% in lung cancer tissues. **Conclusions.** There were about 39% lung cancer patients who could be treated with immunotherapy based on MAGE-A3 antigen. [Life Science Journal. 2006;3(2):27-31] (ISSN: 1097-8135).

Keywords: lung neoplasm; MAGE-A3; HLA-I molecules; immunotherapy

Abbreviations: CTL: cytotoxic T lymphocyte; HLA: human leukocyte antigen; MAGE: melanoma antigen

1 Introduction

The MAGE-A3 is a member of MAGE family, whose antigen is known to be neo-expressed in a large proportion of tumors but who is not detectable in normal tissues, and who could be a target antigen recognized by autologous cytotoxic T lymphocytes (CTLs). Such tumor cells generating MAGE-A3 antigen could be an ideal target to carry out tumor specific immunotherapy[1]. In addition HLA class I molecule exerts very important effect in the process that MAGE-A3 antigen induces the CTLs proliferation[2]. This experiment detected the expression levels of MAGE-A3 antigen in lung cancer patients. At the same time we quested for distribution of three HLA class I alleles (HLA-A1, A2, A24) in lung cancer patients, which could be combined with MAGE-A3 antigen specifically. The percentage was measured of lung cancer patients who were able to be treated with immunotherapy based on MAGE-A3 antigen.

2 Materials and Methods

2.1 Sample collection

70 fresh surgical specimens were obtained from the First Affiliated Hospital of Zhengzhou University, the Second Affiliated Hospital of Zhengzhou University and Henan Province Chest Hospital, repectively. The samples include: 37 squamous cancers, 22 adenocarcinomas, 5 squamous-adenocarcinomas, 2 big cell lung cancers, 3 small cell lung cancers and 1 hybrid lung cancer were mixed big and small cells. All tissues were frozen in liquid nitrogen immediately after surgery and stored at −80℃ until the extraction of protein.

2.2 Protein extraction

The protein was extracted by SDS-PAGE and stored at −20℃.

2.3 DNA extraction

UNIQ-10 genome DNA extraction kit (Shanghai, China). DNA solution was stored at −20℃.

2.4 Western blot

Protein samples were separated by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a nitrocellulose membrane. The membrane was blocked with 1% protein powder (Wuhan BOSTER Ltd., Wuhan, Hubei, China) in PBS for 2 hours at room temperature or overnight at 4 ℃, and incubated with primary antibody − 57B (generously presented by Giulio C. Spagnoli, M. D.) at room temperature for 2 hours; protein bands in membrane were stained with a horseradish peroxidase-conjugated secondary antibody − Goat anti-mouse IgG and counterstained with 3′3-diaminobenzidine (DAB) finally. Pictures were captured by Bio Imaging System (USA).

2.5 PCR with sequence specific primer (PCR-

SSP)

The sequence specific primers of HLA-A1, HLA-A2 and HLA-A24 were designed according to the principle of primer design and their full sequences of primers were showed in Table 1.

Table 1. Sequence specific primers

Gene	Region	Primer sequence	PCR fragment (bp)
HLA-A1	Ex2: 113~130	5′-CGACGCCGCGAGCCAGAA-3′	557
	Ex3:216~232	5′-AGCCCGTCCACGCACCG-3′	
HLA-A2	Ex2: 149~167	5′-GTGGATAGAGCAGGAGGGT-3′	489
	Ex3:110~128	5′-CCAAGAGCGCAGGTCCTCT-3′	
HLA-A24	Ex2: 166~184	5′-GGCCGGAGTATTGGGACGA-3′	629
	Ex3: 195~213	5′- CCTCCAGGTAGGCTCTCTG-3′	

DNA was extracted from lung cancer and cancer adjacent tissues according to the manufacture instructions. DNA purifying and concentrating were measured by spectrophotometry. 100 ng DNA was used for PCR-SSP (total volume: 30 μL). Whatman Biometra Tgradient 96 PCR (Germany) was used to carry out the PCR, with a program of preheating for 5 min at 95 ℃, followed by 5 cycles of 30 sec at 95 ℃, 50 sec at 65 ℃, and 50 sec at 72 ℃; 5 cycles of 30 sec at 95 ℃, 50 sec at 60 ℃, and 50 sec at 72 ℃. Last 20 cycles was carried out of 30 sec at 95 ℃, 50 sec at 57 ℃, and 50 sec at 72 ℃. The final step was 5 min at 72 ℃. All PCR products were analyzed by electrophoresis in agarose with TAE buffer (0.04 M Trisacetate, 0.001 M EDTA, pH 8.0).

2.6 Statistics analysis

Experimental data was analyzed by SPSS software (10.0). Difference between two independent ratios was determined with Chi-square test. Significance was assumed at $P<0.05$. If the number of samples was less than 40 ($n<40$) the rank sum test would be used for analysis.

3 Results

3.1 The relationship of MAGE-A3 antigen and different clinical pathologic types and stages (Table 2, Figure 1)

Table 2. The expression of MAGE-A3 antigen in lung cancer tissues and adjacent tissues

Tissue type	Cancer tissue			Cancer adjacent		
	positive	negative	total	positive	negative	total
SC[a]	15	15	30	3	27	30
GC[b]	10	12	22	1	21	22
GSC[c]	4	1	5	1	4	5
NDC[d]	2	4	6	0	6	6
Total	31	32	63	5	58	63

a: squamous cancer; b: adenocarcinoma; c: squamous-adenocarcinoma; d: non-differential cancer

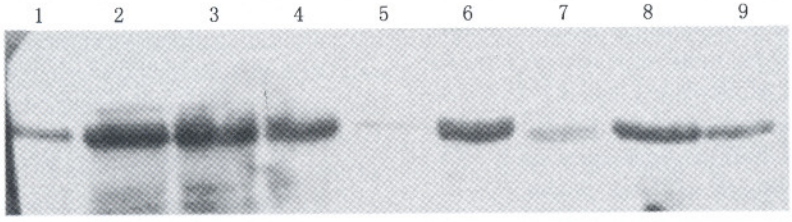

Figure 1. Western blot analysis on the expression of MAGE-A3 antigen in lung cancer patients
Lane 1, 3, 5, 7: cancer adjacent tissues; Lane 2, 4, 6, 8, 9: cancer tissues

Expression of MAGE-A3 antigenic protein: Among 63 samples MAGE-A3 antigen was expressed in 31 cases. The ratio of MAGE-A3 antigen expression was 49.21% in lung cancer. There was no significance between different pathological clinical types and different pathological stages. In addition, 5 samples of tumor adjacent tissues also expressed MAGE-A3 antigen as well as their cancer tissue.

3.2 The results of PCR-SSP (Figures 2, 3, 4)

The distribution of HLA-class I molecules by DNA typing: In tumor tissues the positive rates of

HLA-A1, HLA-A2 and HLA-A24 were 4.28% (3/70), 54.28% (38/70) and 50.0% (35/70), respectively. In para-tumor tissues the positive rates were 4.28% (3/70), 60.0% (42/70) and 52.86% (37/70), respectively. The total positive rate of HLA-A1, A2, A24 was 80.0% (56/70) in lung cancer patients.

3.3 The relationship of HLA-A1, A2, A24 and different clinical pathological types and stages of lung cancer (Tables 3, 4, 5)

There were no significant differences of lung cancer tissues and cancer adjacent tissues of these three alleles ($P>0.05$). In addition no significant differences in different pathological types and different pathological stages of lung cancer were found. The positively expressed MAGE-3 antigen and HLA-class I (A1, A2, A24) molecules were about 39.36% (80.0% × 49.21%) in lung cancer patients.

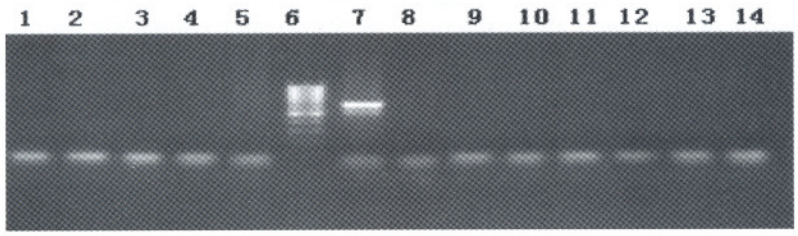

Figure 2. The result of HLA-A1 PCR-SSP in lung cancer patients

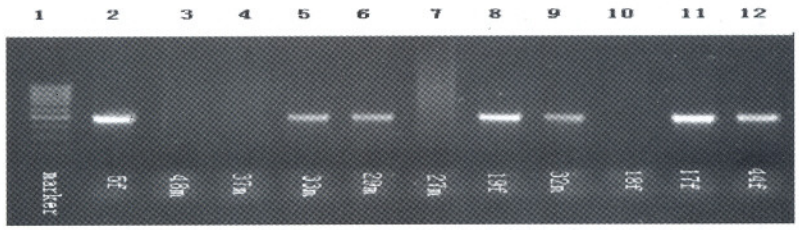

Figure 3. The result of HLA-A2 PCR-SSP in lung cancer patients

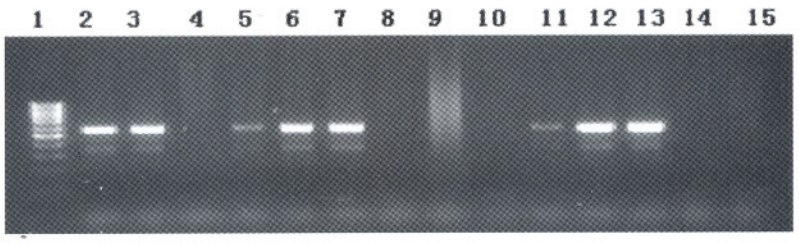

Figure 4. The result of HLA-A24 PCR-SSP in lung cancer patients

4 Discussion

4.1 The expression of MAGE-A3 antigen in lung cancer

The human MAGE-A3 gene family consists of a large number of chromosome-X-linked genes originally identified because they encode the products that can be recognized by autologous cytotoxic T cells. For the MAGE genes don't express in normal adult tissues except testis while express in a large variety of neoplastic lesions, so they are considered as tumor-specific antigens and ideal targets for cancer immunotherapy. Numerous CTL epitopes from the MAGE-A3 antigen have been identified, which were found to be restricted by commonly found MHC class I alleles such as HLA-A1, HLA-A2, HLA-A24, HLA-B37 and HLA-B44. As a tumor specific antigen it can act with a lot of immune cells such as antigen process cells (APCs) and autologous cytotoxic T lymphocyte etc. The interaction between various cells determined the fate of tumor cell at a large extent.

Currently, there are a lot of reports about the expression of MAGE-A3 in tumor tissues at home and abroad, but much concentrated on its mRNA level[3], few on its protein level and the result dis-

agreement with each other. In this experiment we found that the positive expression of MAGE-A3 antigen was 49.21%, higher than Liu's report[4], in accordance with Bolli's report[5]. The result that there was no significant difference in different pathological types and different pathological stages of lung cancer was in accordance with the result of most reports, which indicated that the expression of MAGE-A3 antigen was an independent index in above-mentioned clinical pathological characteristic. Different pathological types and different pathological stages of lung cancer patient can't affect the tumor specific immune therapy based on MAGE-A3 antigen. We found that the MAGE genes expressed in not only lung cancer tissues but also some normal lung tissues adjacent to cancers (16.13%, 5/31). And by pathological examination these normal lung tissues appeared hyperplasia of fibrous tissues and epithelial tissues (namely heterogeneous histiocytes), which suggested that the activation of MAGE genes could occur at a very early stage of lung carcinogenesis. This result was in accordance with a few reports[6,7] but opposite to most reports. It may be because some of cancer adjacent tissues have already occurred malignant conversion in the early stage in which we couldn't find any typical cancer cells by pathological examination. Furthermore, it showed that MAGE-A3 antigen gene may have already been activated before malignant conversion of normal cells. Some articles reported that MAGE-A3 gene's activation, transcription and expression were determined by the promoter's degree of methylation[8]. The carcinogen may activate MAGE-A3 gene through changing its promoter's methylation. In our opinion, the activation of MAGE was a common phenomenon in carcinogen-exposed lung tissue as well as in lung cancer tissue. Further work is needed about the expression of MAGE-A3 in lung cancer adjacent tissues.

Table 3. The distribution of HLA-A1, A2, A24 in lung cancer and cancer adjacent (n)

Genotype	Cancer			Cancer adjacent		
	Positive	Negative	Total	Positive	Negative	Total
HLA-A1	3	67	70	3	67	70
HLA-A2	38	32	70	42	28	70
HLA-A24	35	35	70	37	33	70

Table 4. The distribution of HLA- A2 in different pathological types of lung cancer (n)

Pathologic type	Cancer			Cancer adjacent		
	Positive	Negative	Total	Positive	Negative	Total
SC	17	19	36	20	17	37
GC	13	10	23	14	8	22
G-SC	4	1	5	4	1	5
NDC	4	2	6	4	2	6
Total	38	32	70	42	28	70

Table 5. The distribution of HLA- A24 in different pathological types of lung cancer (n)

Pathologic type	Cancer			Cancer adjacent		
	Positive	Negative	Total	Positive	Negative	Total
SC	17	19	36	19	18	37
GC	13	10	23	12	10	22
G-SC	1	4	5	2	3	5
NDC	4	2	6	4	2	6
Total	35	35	70	37	33	70

4.2 The distribution and absence of HLA-A1, A2 and A24

The antigen that HLA class I gene codes is distributed in the human body extensively. During tumor immune process HLA class I antigen plays an important role in processing and presenting MAGE-A3 antigen. That is the basic point of tumor cell immune response inducement and adjustment. Many studies reported that HLA class I antigen was absent in many types of tumor[9]. Presently it has been reported that HLA-A1, A2, A3, A24, A29, B18, B35, B40 and B44 can be particularly combined with different MAGE-A3 antigenic determinant (epitope) respectively[10-15]. Among them, the HLA-A2 and HLA-A24 have very high distribution level in China[16]. With DNA typing method, our experiment results showed that for the distribution of HLA-class I molecule in tumor tissues the positive rates of HLA-A1, HLA-A2 and HLA-A24 were 4.28% (3/70), 54.28% (38/70)

and 50.0% (35/70), respectively. However, in tumor adjacent tissues the positive rates of HLA-A1, HLA-A2 and HLA-A24 were 4.28% (3/70), 60.0% (42/70) and 52.86% (37/70), respectively. The total positive rate of HLA-A1, A2 and A24 was 80.0% (56/70) in lung cancer patients. The result was in accordance with previous reports. This indicated that the antigenic determinant (epitope) (that can combine specifically with HLA-A1, A2 and HLA-A24 antigen) should be an ideal candidate for tumor specific vaccine.

In addition, our statistical results showed that there was no significant difference between lung cancer and cancer adjacent of these three alleles (P = ns). No significant differences were in different pathological types and different pathological stages of lung cancer (P = ns). This reminded that the lung cancer had all kinds of absence of HLA-A antigens but its gene structure was stable. Methods should be taken to induce these alleles' re-expression: such as interferon-γ[17], 5-aza-2′-deoxycytidine[18], even virus like newcastle disease virus[19].

5 Conclusion

Our research showed that the positive rate of MAGE-A3 antigen protein expression was 49.21%; HLA-A1, A2 and A24 total distribution was 80%. About 39% patients had HLA-A1 or A2 or A24 distribution and MAGE-A3 antigen expression. That means about 39% lung cancer patients may be treated with tumor specific vaccine based on MAGE-A3 antigen.

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