Identification of Tumor Antigens in Lung Cancer Patient by SEREX

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Serological anlysis of recombinant cDNA expression libraries (SEREX) has led to identification of several categories of new antigens recognized by the immune system of cancer patients, which are referred to as the cancer immunome. We analyzed normal testis cDNA expression libraries with serumobtained from non-small lung cancer patient and isolated 40 distinct antigen designated KP-LuT-1 through KP-LuT-40. Among these antigens 20 antigens were previously identified by SEREX analysis of other tumor types, and 20 out of 40 antigens (50%) did not match entries in Cancer Immunome Database and were considered newly identified antigens. Sequencing analysis showed that the antigens comprised 26 functional known proteins and 14 noble/uncharacterized gene products. Of these, the hypothetical protein KP-LuT-6 was shown tissue-restricted. RT-PCR showed it to be expressed strongly only in normal testis. In addition to normal tissues-restricted expression, KP-LuT-6 mRNA was detected in lung tumor samples(3/10), stomach tumor samples(3/10), and breast tumor samples(1/5), whereas not detected in colon tumor samples(0/12). These data suggest that KP-LuT-6 is a cancer/testis (CT)-like antigen as a potential target for cancer immunotherapies.

Key words – SEREX, tumor antigen, cancer/testis(CT) antigen, lung cancer

Introduction

Lung cancer is one of the leading causes of cancer death in the world [1]. Non small cell lung cancer (NSCLC) is a predominant type, and the 5-year survival is poor even in the early stage [10]. Recent progress in tumor immunology based on the molecular identification of tumor antigens may allow immunotherapy to be a promising treatment for lung cancer.

The repertoire of tumor antigens recognized by the immune system is referred to as the cancer immunome [13]. The cancer immunome comprises antigens defined by T cell epitope cloning [27], MHC peptide elution [20] and serological methods such as SEREX (serological analysis of recombinant cDNA expression libraries) [11,21] or SERPA (serological proteome analysis) [14].

The identification of human tumor antigens recognized by the autologous host is yielding an array of target molecules for the diagnosis, monitoring, and immunotherapy of human cancer [9,11,12]. Analysis of serological recombinant expression libraries (SEREX) is a powerfull method of immunoscreening tumor-derived cDNA expression libraries with cancer patient sera in order to identify molecules recognized by high titered IgG antibodies [21]. Approximately 2500 distinct antigens have been defined by SEREX to date, including CT antigens [24], differentiation antigens [8], mutational antigens [18], overexpressed or amplified genes [23], splice variant antigens [15], and viral antigens [26].

Cancer-Testis (CT) antigens are immunogenic proteins expressed in normal testis and in different type of tumors. CT antigens are considered promising candidates for cancer immunotherapy and the identification of novel CT antigens is a prerequisite for the development of cancer vaccines[24]. CT antigens have previously been isolated by various methods. MAGE1 was first isolated by cDNA expression cloning with melanomareactive T cells [27], and NY-ESO-1 was isolated by cDNA expression cloning (SEREX) with serum from a patient with esophageal cancer. CT15, 16, and 17 were isolated by DNA homology search using public gene databases [25], and MAGE-1 was isolated by cDNA subtraction (RDA) between testis cDNA library and normal tissues [5]. In general, CT antigens are expressed in 20-40% of specimens from a given tumor type [24].

Several SEREX studies of lung cancer have been reported on lung cancer [2,10,17]. Following these earlier

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studies, non-small cell lung cancer has been examined by a few groups [2,10]. These studies have defined a new CT antigen CAGE-1 [2] and defined previously unknown transcript variants of another CT antigen, XAGE-1 [10]. Two other genes known to be associated with oncogenesis, namely TP53BP and LBC (lymphoid blast crisis oncogene), were also isolated.

In this study, the SEREX methodology is applied to isolate lung cancer associated antigens. For this purpose, sera from lung cancer patient were used to screen cDNA libraries derived from normal testis and the 40 distinct lung cancer antigens were identified. Among 40 distinct lung cancer antigens, KP-LuT-6 can be considered a novel CT antigen on the basis of its restricted mRNA expression profile.

Materials and Methods

Human tissues and cell lines

Human tumor tissues and sera were obtained from Department of Surgery and made a diagnosis and staging by Department of Pathology, Pusan National University Hospital. The tissues were frozen in liquid nitrogen and stored at -80°C untill use.

Preparation of sera for immunoscreening

Each serum from a lung cancer patient was used independently, at a dilution of 1:200with 0.2% NFDM (Non-Fat Dry Milk in TBS buffer), to immunoscreen the testis cDNA libraries.

To remove serum antibodies reactive with *E.coli* / bacteriophage-related antigens, sera were absorbed against *E.coli* /bacteriophage lysatesas described by Lee *et al.* [17]. Briefly, wild-type lambda ZAP Express bacteriophage at a concentration of 5,000 plaque forming unit (pfu) per 15cm plate was amplified in *E.coli* XL1 Blue MRF' overnight in NZCYM mixed 0.7% agarose. Ten milliliters of binding buffer (0.1M $NaHCO_3$, pH 8.3) was then added to the plates, and the plates were gently agitated at 4°C for 15 hr. The resulting supernatants were collected and residual *E. coli* pellet was lysed by sonication. The lysate was coupled to CNBr-Sepharose 4B (Amersham Pharmacia Biotech, Piscataway, NJ) as per manufacturer's instructions. Patient sera (1:10 dilution) were absorbed by batch absorption with Sepharose 4B coupled *E. coli/* phage lysates, followed by 15 hr incubation with nitrocellulose filters (PROTRAN BA 85, 0.45 µm, Schleicher & Schuell) precoated with proteins derived from *E. coli* and *E.coli* / phage lysates.

Total RNA extraction from tissues and cell lines

Total RNA was isolated from human tissue samples and human tumor cell lines using standard TRIzol reagent (Life Technologies, Gaithersburg, MD) and RNA isolation Kit (RNeasy Maxi Kit, QIAGEN) following the manufacturer's instructions. The amount of isolated RNAs was measured by spectrophotometer (Ultrospec 2000, Phamacia biotech) at 260nm.

Normal tissue RNA preparations were purchased from Clontech laboratories Incorporated (CA, USA) and Ambion Incorporated (Texas, USA).

SEREX analysis of cDNA expression libraries

Poly(A)+ mRNA from normal testis tissues was prepared using the Fast Track mRNA purification Kit (Invitrogen, Life Technologies, Carlsbad, CA). Testis cDNA libraries were constructed in the ZAP Express vector (Stratagene, La Jolla, CA) according to manufacturer's instructions using 5 mg mRNA. Libraries containing 1-2x10^6 primary recombinants were obtained and were not amplified for immunoscreening.

To remove serum antibodies reactive with vector-related antigens, sera was absorbed against *E. coli*/bacteriophage lysates prepared in the following manner. Wild-type lambda ZAP Express bacteriophage at a concentration of 5,000 plaque forming unit(pfu) per 15x15cm plate was amplified in *E. coli* XL1 Blue MRF' overnight in NZY/0.7% agarose. Ten milliliters of binding buffer (0.1M $NaHCO_3$, pH 8.3) was then added to the plates, and the plates were gently agitated at 4°C, for 15 hr. The resultant supernatants were collected and residual *E. coli* were lysed by sonication. The lysates were then coupled to CNBr-Sepharose 4B (Amersham Pharmacia Biotech, Piscataway, NJ) as per manufacturer's instructions. Patient sera (1:10 dilution) were absorbed by batch absorption with Sepharose 4B coupled *E. coli*/phage lysates, followed by 15 hr incubation with nitrocellulose filters precoated with proteins derived from *E. coli* and *E. coli*/phage lysates.

Library screenings were performed as previously described [16,17]. One serum from patient with non-small cell lung cancer was used at a dilution of 1:200, to immunoscreen the normal testis cDNA libraries (2.0x10^5 recombinants screened per cDNA library). A total of 2.0X 10^5recombinants were screened per serum/cDNA library

combination. Serum reactive phage clones were converted to plasmid forms and subjected to DNA sequencing using standard techniques (Macrogen Co. Korea).

Reverse Transcriptase- PCR (RT-PCR) analysis

The cDNA preparations used as templates for RT-PCR reactions were prepared using 1 mg of total RNA in conjunction with the Superscript first strand synthesis kit (Invitrogen Life Technologies, Carlsbad, CA). PCR primers specific for amplifying KP-LuT-6 were: forward; 5'- gcacgacgtaagcaaagtga-3', and reverse; 5'-cggctattctgagaccttgc-3'. The cDNA templates used were normalized on the base amplification of GAPDH (BD Bioscience Clontech). For PCR, 25 μl reaction mixtures were utilized, consisting of 2 μl cDNA, 0.2 mM dNTP, 1.5 mM $MgCl_2$, 0.25 M gene specific forward and reverse primers, and 2.5 U Platinum Taq DNA polymerase (Invitrogen Life Technologies). Reaction mixes were heated to 94°C for 2 min., followed by 35 cycles of 94°C for 30 seconds, 60°C for 30 seconds and 72°C for 1 minute (final cycle: 72°C for 5 minute) using a GeneAmp PCR System 9700 thermocycler (Applied Biosystems). Amplified products were analyzed on 1.5% Agarose/Tris-Acetate-EDTA gels stained with ethidium bromide.

Results

Identification of human lung cancer antigens by SEREX

Serum sample from one individual with lung cancer was used to immunoscreening normal testis-derived cDNA expression libraries using SEREX. The primary screening of approximately 4×10^5 clones of testis cDNA expression libraries with one lung cancer patient serum led to the isolation of 45 immunoreactive cDNA clones. All positive clones were examined two times with serum. After preparation and sequencing, 40 different DNA sequences could be determined (some clones were found several times). With the data base comparison (www.ncbi.nlm.nih.gov) homologies could be found for all DNA fragments. Table 1 shows all positively identified clones, the established homologies as well as the frequency, with which the clones were found. This resulted in the identification of cDNA clones for 40 distinct antigens designated KP-LuT-1 through KP-LuT-40 (Table 1).

Comparison with the SEREX-database

When the cDNA sequences encoding the 40 lung cancer

Table 1. Lung Cancer Antigens defined by Serological analysis of Testis cDNA expression libraries

KP-LuT-#	Gene Identity / Unigene Cluster[A]	Clone #	SEREX match[B]	KP-LuT-#	Gene Identity / Unigene Cluster	Clone #	SEREX match
1	CEBPG / Hs.429666	1	No	21	PHKG2 / Hs.196177	1	No
2	RBM27 / Hs.61441	1	No	22	SPACA4 / Hs.122599	1	Yes
3	BRAP / Hs.530940	4	No	23	NEK3 / Hs.409989	1	No
4	LOC127540 / Hs.127736	1	Yes	24	RPS3A / Hs.356572	1	Yes
5	NSEP1 / Hs.473583	1	Yes	25	ALMS1 / Hs.184720	1	No
6	C6orf10 / Hs.57692	1	No	26	MGC41945 / Hs.148250	1	Yes
7	LOC57228 / Hs.206501	1	No	27	MIS12 / Hs.267194	1	Yes
8	ARPM1 / Hs.135411	2	No	28	ANKRD7 / Hs.371820	1	Yes
9	GHITM / Hs.352656	1	No	29	AL137026 / No*	1	No
10	NME5 / Hs.519602	1	Yes	30	PRM1 / Hs.2909	1	Yes
11	TUVLI / No*	1	No	31	KIAA1191 / Hs.519783	1	No
12	VPS24 / Hs.255015	1	Yes	32	PPM1G / Hs.17883	1	Yes
13	RPS12 / Hs.546289	1	Yes	33	MAD2L1BP / Hs.122346	1	No
14	C9orf23 / Hs.15961	1	Yes	34	PRM2 / Hs.2324	1	No
15	KIAA1838 / Hs.369522	1	Yes	35	AL031906 / No*	1	No
16	MDH1B / Hs.147816	1	Yes	36	AJ842751No*	2	Yes
17	AC002467 / No*	1	No	37	AL359260 / No*	1	Yes
18	C21orf70 / Hs.410830	1	No	38	C1orf33 / Hs.463797	1	No
19	MARK3 / Hs.35828	1	Yes	39	HES1 / Hs.250666	1	Yes
20	KHDRBS3 / Hs.444558	1	No	40	AP000753 / No*	1	Yes

A; UniGene cluster of isolated antigens (http://www.ncbi.nlm.nih.gov/). *; Cluster is not founded. B Sequences were compared with those contained in the SEREX database of the Ludwig Institute for Cancer Research (http://www2.licr.org).

Table 2. SEREX-defined lung cancer antigen ; The antigens were found in SEREX database*

KP-LuT-#	Gene Identity	Cancer Patient Seroreactivity*	Unigene Cluster	Chromosome Location
4	LOC127540	TST, MEL, HCC, CC, SRC, BC	Hs.127736	1p35.1
5	NSEP1	TST, SRC	Hs.473583	1p34
10	NME5	HCC, CC,MEL, SRC	Hs.519602	5q31
12	VPS24	HCC, MEL, CC, SRC,	Hs.255015	2p24
13	RPS12	HCC, SRC, MEL, RC, BR, TST, CC, ST	Hs.546289	6q23.2
14	C9orf23	CC, HCC, SRC, PAN, RC, TST	Hs.15961	9p13.3
15	KIAA1838	HCC, SRC, MEL, BR, TST, CC, ST	Hs.369522	6q27
16	MDH1B	MEL, HCC,SRC, TST, CC, BR	Hs.147816	2q33.3
19	MARK3	PC	Hs.35828	14q32.3
22	SPACA4	HCC, BR, SRC, MEL, TST, CC, ST	Hs.122599	19q13.33
24	RPS3A	SKBR3, SRC, CC, MEL, HCC, BR, TST	Hs.356572	4q31.2-q31.3
26	MGC41945	MEL, HCC, TST, BR, CC, SRC, ST	Hs.148250	9p13.3
27	MIS12	HCC, SRC, MEL, CC, BR, TST	Hs.267194	17p13.2
28	ANKRD7	HCC, MEL, BR, TST, SRC, CC, ST	Hs.371820	7q31
30	PRM1	HCC, SRC, MEL, TST, CC, BR	Hs.2909	16p13.2
32	PPM1G	ST	Hs.17883	2p23.3
36	AJ842751	BR, HCC, ST, TST, MEL, OC, BC, SRC, MGC	No	mitochondrion
37	AL359260	MEL, HCC, BR, TST, CC, SRC, ST	No	10q25
39	HES1	HCC, MEL, BR, TST, SRC, CC, ST	Hs.250666	3q28-q29
40	AP000753	HCC, MEL, BR, TST, CC, Hodgkin, BC, SRC, ST	No	unknown

*Note: Determined by sequence comparisons with the SEREX database (http://www2.licr.org/)
Abbreviation: BC, breast cancer; CC, colon cancer; CO, colon adenocarcinoma;GL, glioma; HCC, hepatocellular carcinoma; HN, head and neck cancer; LC, lung cancer; MEL, melanoma; OC, ovarian cancer; PC,prostate cancer; PN, pancreatic cancer; RC, renal cancer; SRC, sarcoma UN, Unclasificable; STC, Stomach cancer; HOD, Hodgkin's lymphoma

antigens were compared to those deposited in the Cancer Immunome DB, it was found that 20 of the 40 lung cancer antigens identified (50 %) had been previously identified by SEREX analyses with any cDNA/serum combination, whereas 20 of the antigens (50%) had not been previously reported (Table 1). The immunomic pattern of the tumor antigens which previously identified in SEREX by other groups were analyzed (Table 2).

The 20 antigen identified through SEREX database are known to be associated with other tumor type including breast, colon, esophageal, gastric, ovarian, prostate, thyroid , renal cancer, glioma, melanoma, sarcoma, and hodgkins disease. However, these antigens have not been known to be associated with lung cancers (Table 2).

Characterization of the tumor-associated antigens

Among the 40 isolated antigens, 26 antigens were functionally identified as known and predicted proteins. The antigens fall into several functional group; for example, DNA /RNA processing related genes (KP-LuT-1, -2, -5, -13, -20), regulatory/signal transduction (KP-LuT-3, -10, -19, -21, -23, -25, -32, -33), mitochondria related genes (KP-LuT -11, -36, -37), sperm related genes (KP-LuT-22, -30, -34), and others (KP-LuT-8, -9, -12, -16, -39) (Table 3).

A last group with unknown function was defined with 14 uncharacterized gene products (KP-LuT-4, -6, -7, -14, -15, -17, -18, -26, -28, -29, 31, -35, -38, -40), including sequences designated in the databases as ESTs, KIAA series clones, FLJ series clones, MGC series clones, DKFZ series clones and anonymous ORFs. The protein sequences are only hypothetical and the functions therefore are mostly unknown.

The mRNA expression profiles of KP-LuT-6 gene by RT-PCR

A preliminary in silico mRNA expression profile and characterization of gene products identified in this study was undertaken based on the tissue distribution of expressed sequence tags (ESTs) [6], SAGE tags [4] in the Cancer Genome Anatomy Database (CGAP: http://cgap.nci.nih. gov/), Massively Parallel Signature sequencing tags (MPSS: https://www2.licr.org/BRAIN/) and the information contained in the GeneCards database (http://bioinfo.weizmann.ac.il/cards-bin/).

According to the bioinformatics program(digital gene expression file), One antigen (KP-LuT-6) was identified as

Table 3. Functional classification of lung cancer antigens identified by SEREX

Functional Group	KP-Lu-#	Gene Identify	Unigene Cluster	Localization
DNA / RNA Processing Gene	1	CEBPG: CCAAT/enhancer binding protein	Hs.429666	19q13.11
	27	MIS12: MIS12 homolog	Hs.267194	17p13.2
	2	RBM27: RNA binding motif protein 27	Hs.61441	5q32
	5	NSEP1: Nuclease sensitive element binding protein 1	Hs.473583	1p34
	13	RPS12: Ribosomal protein S12	Hs.546289	6q23.2
	20	KHDRBS3: KH domain containing, RNA binding,	Hs.444558	8q24.23
	24	RPS3A: Ribosomal protein S3A	Hs.356572	4q31.2-q31.3
Regulatory/ Signal transduction	3	BRAP: BRCA1 associated protein	Hs.530940	12q24.12
	10	NME5: Non-metastatic cells 5, protein expressed in (nucleoside-diphosphate kinase)	Hs.519602	5q31
	19	MARK3: MAP/microtubule affinity-regulating kinase 3	Hs.35828	14q32.3
	21	PHKG2: Phosphorylase kinase, gamma 2 (testis)	Hs.196177	16p11.2
	23	NEK3: NIMA (never in mitosis gene a)-related kinase 3	Hs.409989	13q14.3
	25	ALMS1: Alstrom syndrome 1	Hs.184720	2p13.1
	32	PPM1G: Protein phosphatase 1G	Hs.17883	2p23.3
	33	MAD2L1BP: MAD2L1 binding protein	Hs.122346	6p21.1
Mitochondria related gene	11	TUVLI mitochondrion	No cluster	mitochondrion
	36	AJ842751 mitochondrion	No cluster	mitochondrion
	37	AJ842751 mitochondrion	No cluster	mitochondrion
Sperm relating gene	22	SPACA4: Sperm acrosome associated 4	Hs.122599	19q13.33
	30	PRM1: Protamine 1	Hs.2909	16p13.2
	34	PRM2: Protamine 2	Hs.2324	16p13.13
Other functions	8	ARPM1: Actin related protein M1	Hs.135411	3q26.2
	9	GHITM: Growth hormone inducible transmembrane protein	Hs.352656	10q23.1
	12	VPS24: Vacuolar protein sorting 24 (yeast)	Hs.255015	2p24
	16	MDH1B: Malate dehydrogenase 1B	Hs.147816	2q33.3
	39	HES1: Hairy and enhancer of split 1	Hs.250666	3q28-q29

cancer/testis(CT)-like protein, which could thus potentially serve as targets for immunotherapy.

To examine the distribution of KP-LuT-6 gene expression in detail, RT-PCR was performed using mRNA from normal tissues, tumors, and cancer lines. Among normal tissues, KP-LuT-6 was expressed strongly only in normal testis (Fig. 1). In addition, KP-LuT-6 mRNA expression was detected in 3/10 lung tumors (Figure 2a), 3/10 stomach tumors (Fig. 2b), 1/5 breast tumor (Figure 2d), whereas not detected in 0/12 colon tumor (Fig. 2c).

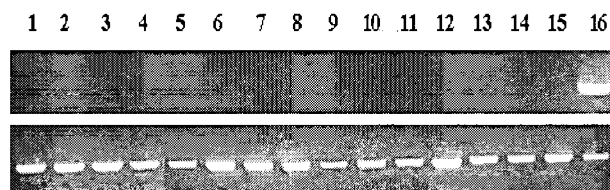

Fig 1. RT-PCR of KP-LuT-6 in normal tissues. KP-LuT-6 was found by RT-PCR to be expressed strongly in normal testis. From the left direction, Spleen(1), Thymus(2), Prostate(3), Ovary(4), Small Intestine(5), Colon(6), Leukocyte(7), Heart(8), Brain(9), Placenta(10), Lung(11), Liver(12), Skeletal(13), Kidney(14), Pancreas(15), Testis (16). The cDNA templates used were normalized using GAPDH as shown at the bottom of each panel.

Discussion

A number of powerful methodologies are being utilized to define the complete repertoire of human cancer antigens, which we have termed the human cancer immunome. The immunome comprises antigens defined by T-cell epitope cloning [27], MHC peptide elution [20], and serological expression cloning [16,17], which are able to identify tumor targets recognized by CD8+ T cells and antibodies, respectively. These approaches are now being supplemented by bioinformatics, transcriptomics and proteomics-based assays to accelerate the definition of the cancer immunome [19].

In the present study, we employed normal testis cDNA librariesas the antigen source for a SEREX analysis using serum from non-small lung cancer patient. This resulted in

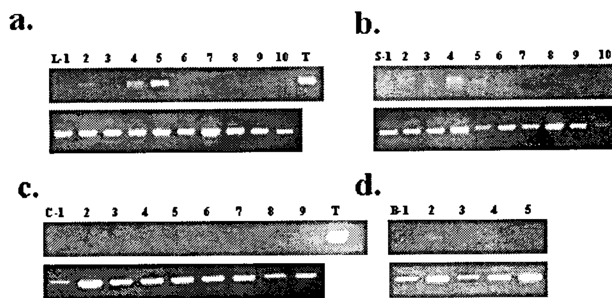

Fig. 2. The expression profile of KP-LuT-6 in cancers. KP-LuT-6 mRNA was detected in a variety of tumor specimens, including in 3/10 lung tumors (a), 3/10 stomach tumors (b) , 0/9 colon tumors (c) and 1/5 breast tumors (d). The cDNA templates used were normalized using GAPDH as shown at the bottom of each panel.

the isolation of 40distinct cDNAs encoding tumor antigens, which were designated from KP-LuT-1 to KP-LuT-40. The 20 antigen identified through SEREX database are known to be associated with other tumor type including breast, colon, esophageal, gastric, ovarian, prostate, thyroid , renal cancer, glioma, melanoma, sarcoma, and hodgkins disease. However, these antigens have not been known to be associated with lung cancers (Table 2).

Furthermore, we found that twenty of the isolated 40 antigens (50%) had not been previously reported (Table 1)and were considered newly identified antigens in this study. These results are consistent with recent reports [16,17] of the identification of sarcoma and lung cancer antigens by SEREX, where 74 of 113 sarcoma antigens (65%) and 51 of 82 lung cancer antigens (62%) were newly identified. About 20% of the isolated sarcoma and lung cancer antigens were uncharacterized genes. Recent report described that SEREX defined cancer immunome of which only one third has been defined to date [16]. With current study, SEREX thus remains a powerful tool for cancer antigen identification and the repeated screening of existing cDNA expression libraries with sera from additional patients as well as the construction and screening of a number of additional libraries would be worthwhile.

The current SEREX analysis of non-small lung cancer led to the isolation of 26 known and expected functional proteins together with 14 uncharacterized gene products, including sequences designated in the databases as ESTs, KIAA series clones, FLJ series clones, MGC series clones, DKFZ series clones and anonymous ORFs. A preliminary *in silico* mRNA expression profile and characterization of gene products identified in this study was undertaken based on the tissue distribution in the Cancer Genome Anatomy Database[6], Massively Parallel Signature sequencing tags and the information contained in the Gene Cards database.

According to the bioinformatics program (digital gene expression file), KP-LuT-6 was identified as cancer/testis(CT)-like protein, which could thus potentially serve as targets for immunotherapy. Nucleotide of KP-LuT-6 was partially identified as hypothetical protein C6ORF10, which function and expression has never been determined. To examine the distribution of KP-LuT-6 gene expression in detail, RT-PCR was performed using mRNA from normal tissues, tumors, and cancer lines. Among normal tissues, KP-LuT-6 was expressed strongly only in normal testis but was absent from all other tissues (Fig. 1). In addition, KP-LuT-6 expression was detected in a variety of cancer type, lung tumors, stomach tumor, and breast tumors (Fig. 2). Based on its expression, it is reasonable that KP-LuT-6 can be cancer/testis(CT) like antigen.

Cancer/testis (CT) antigens are the products of transcripts present only in developing germ cells and human cancers of diverse origins [22] that elicit spontaneous cellular and humoral immune responses in some cancer patients[24]. In general, CT antigens are expressed in 20-40% of specimens from a given tumor type. One exception to this is synovial sarcoma, in which 80% of specimens express the CT antigens, NY-ESO-1 and MAGE [3].

In conclusion, we have used a combined bioinformatics and SEREX based screening for cancer antigens and identified at least one novel potential target for cancer therapy, hypothetical protein C6ORF10, KP-LuT-6. This gene is aberrantly expressed in lung, stomach and breast cancers and is thus potentially of widespread utility. Although current study led to 40 lung cancer antigens, the value of monitoring the humoral immune response of normal and cancer patients to the antigen should be further explored for its diagnostic potential. Given the continued high productivity of SEREX, and the success of this approach in the present instance, we propose that continued SEREX based searches for cancer antigens should be undertaken to be provided as wide an option as possible for the design of novel therapies.

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초록 : 폐암 환자에서 면역항원유전자의 혈청학적 동정

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혈청학적 유전자 검색 방법(SEREX)은 암 환자의 면역계를 인식하는 종양 면역유전체(Cancer Immunome)를 형성하는 수많은 종양항원의 발견을 이끌어왔다. 본 연구는 정상인의 고환 조직으로 만들어진 cDNA liabary을 사용하여 폐암환자의 혈청으로부터 40개의 종양항원을 동정하여 그 항원들을 KP-LuT-1부터 KP-LuT-40까지 명명하였다. 이들 항원 중에서 20개는 기존의 다른 종류의 암에서 분리된 것이며 20개는 본 실험에서 새롭게 동정된 항원들이었다. 유전자 분석을 통하여 분리된 26개의 항원들은 그 단백질의 기능이 알려진 것이었고 14개의 항원들은 기능이 분석되지 않은 유전체의 산물이었다. 이들 항원 중에서 hypothetic단백질 KP-LuT-6는 정상조직에서 제한적으로 발현되었다. RT-PCR에 의한 발현분석 결과에서 16개의 정상조직 중 고환에서만 강력하게 발현하였고 다른 조직에서는 발현되지 않으나 폐암(3/10), 위암 (3/10) 과 유방암(1/5)들에서 발현 하였다. 이 결과는 KP-LuT-6의 항원이 암 면역치료를 위한 잠재적 유전자로 사용될 수 있는 Cancer/Testis(CT) 항원과 비슷한 유전자로 사료된다.