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Evaluation of SEREX defined antigens by mRNA expression analysis in breast cancer tissues of Egyptian patients

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ABSTRACT

The identification of human cancer antigens has opened new approaches to the development of cancer vaccines. Characterization of the defined antigens including mRNA expression analysis, protein analysis, antibody response and epitope identification are important to assess the value of those antigens.The aim of the present work is to identify tumor specific or tumor associated antigens by serological analysis of recombinant cDNA expression library (SEREX). Different cancer cell lines and normal testis were used as a source of cDNA library in this study including lung cancer, renal cancer, colon cancer and ovarian cancer in addition to a testicular library. For immunoscreening, autologous as well as allogeneic sera were obtained. By autologous immunoscreening, we previously reported the SEREX analysis of the lung adenocarcinoma cell line OU-LU-6 including the identification of the cancer/testis antigen XAGE-1b. We also reported the SEREX analysis of the lung adenocarcinoma cell lines OU-LU-17 and OU-LU-11. This work focused on the defined SEREX genes by autologous and allogeneic immunoscreening of the renal cancer cell line RC-1. The total number of defined clones was 51 representing 39 different genes named OY-REN-1 through OY-REN-39. The vast majority of clones identified by SEREX are ubiquitously expressed genes. In the renal cancer cell line RC-1, OY-REN-1, 3, 18, 29 and 31 genes were the most frequently isolated (3 of 51), subsequently, OY-REN-2 and 30 were the second most frequent isolated genes, each was represented by 2 of 51 clones. All other genes were represented by a single clone. Within the isolated genes, protein kinase c, Iota (PKCI) which clones had a deletion compared to sequences in the database which might give the chance to identify a new isoform. mRNA expression analysis of PKCI in normal as well as breast cancer tissues showed no cancer specific characteristics compared to the cancer/testis antigen XAGE-1b and c variants. Very interestingly, XAGE-1b antigen showed a significantly high frequency of expression in breast cancer tissues (5/10) taken from Egyptian patients. Using the SEREX defined antigens for mRNA expression in breast cancer tissues may aid the development of diagnostic and immunotherapeutic agents for patients with breast cancer in Egypt.

Key words: SEREX; Lung Cancer; Colon Cancer; Renal Cancer; Testicular Library.

INTRODUCTION

The identification of tumor-associated antigens and their cognate autoantibodies is a promising strategy for diagnosis, monitoring and immunotherapy of human cancer (Christoph et al., 2001). The immune response to the incidence of cancer is elicited in humans, as demonstrated in part by the identification of autoantibodies against a number of tumor-associated antigens in sera from patients with different types of cancer (Desmetz et al., 2009). Since, studies of the cellular and humoral immune response to cancer have revealed an extensive repertoire of tumor antigens recognized by the immune system, collectively termed the cancer immunome

(Sang-Yull et al., 2003). The possibility that cancers could be eradicated by specific immune response and that the immune system could be stimulated to effectively kill tumor or malignant cells is the most desired goal for all studies worldwide (Olivera 2003). The idea is that the effective and specific antitumor response by the host immune system can be induced by immunization with tumor specific antigens is our marginal base to identify tumor antigens, which can work as therapeutic vaccine to stop the growth of existing tumors, prevent the recurrence of cancer by killing or eliminating cancer cells (Howard et al., 2004).

Serological analysis of recombinant cDNA expression libraries (SEREX) using tumor mRNA and autologous patient serum provides a powerful approach to identify immunogenic tumor antigens (Chen et al., 1997, Eldib et al., 2004). Since, SEREX analysis has been identified a large number of antigens in almost all cancers analysed, including cancer/testis (CT) antigen (NY-ESO-1, SSX, SCP-1). CT antigens have received a particular attention, at least in part, because of their restricted expression in normal tissues; therefore, CT antigens are potential targets for vaccine-based immunotherapies (Sang-Yull et al., 2003). In general, CT antigens are expressed in 20–40% of specimens from a given tumor type (Sahin et al., 1998, Tadashi et al., 2011).

In this study, we performed the SEREX analysis to identify tumor associated and tumor specific antigens by different cancer types. mRNA expression analysis for XAGE-1 and PKCI was then conducted using breast cancer tissues. Some of our SEREX defined antigens need further analysis to clarify whether they are candidates for diagnosis or therapy.

MATERIALS AND METHODS

Tissues, cell lines and sera

All cell lines were obtained from Immunology Department, Okayama University, Japan. OU-LU-6, 17 and 11 are lung cancer cell lines established from pleural effusion of 3 different patients with adenocarcinoma. RC-1 is a renal cancer cell line obtained from a patient with clear cell carcinoma. The colon cancer cell line AH-C-164 was from a patient with adenocarcinoma. A fresh tissue from normal testis was used to prepare the testicular library. Autologous and allogeneic sera used in the study were obtained from cancer patients in accordance with the Okayama university guidelines after receiving written informed consent. Breast cancer specimens were surgically obtained from patients at Damanhour Oncology Center and at Aldelengat General Hospital. Collection of tissues and sera was agreed upon by patients and healthy donors, after provision of written, informed consent.

Preparation of cDNA libraries

mRNA was purified from each cell line independently, using a Quick Prep mRNA Purification Kit (Amersham Pharmacia, Piscataway, NJ). Each cDNA expression library was prepared in a λZAP Express vector using a cDNA library kit (Stratagene, La Jolla, CA).

Immunoscreening of the cDNA library and characterization of selected immune-reactive clones

Each cDNA expression library was screened with autologous and/or allogeneic patient serum. In brief, serum samples diluted 1:10 were pre-absorbed with lysate from *Escherichia coli* Y1090/Y1089 and bacteriophage-infected Y1090 coupled to sepharose 4B (Bio Dynamics Lab. Inc., Tokyo, Japan). Nitrocellulose membranes containing the phage plaques at a density of about 4,000 pfu/140 mm plate were incubated overnight at room temperature with the pre-absorbed serum diluted 1:200 (Fig. 1). Reacted clones were detected by peroxidase-conjugated goat anti-human IgG (Jackson Immuno Research, West Grove, PA) and visualized with 3-3´-diaminobenzidine (Sigma, St. Louis, MO). The selected immunoreactive clones were tested for reactivity against diluted sera using the same plaque assay. A negative clone randomly chosen was included in each assay as a negative control.

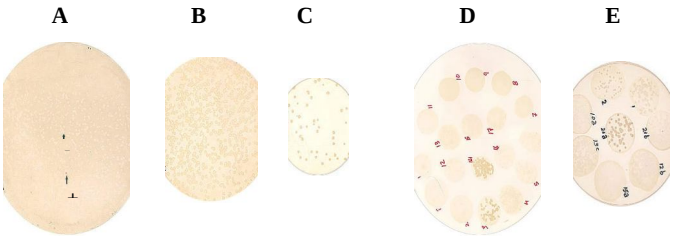


Fig. 1: Sample nitrocellulose membranes of 3 different sizes used for immunoscreening of a cDNA library with cancer patient serum and phage plaque assay for petit serology. Recombinant phages were spread on agar plates at a density of about 4000 pfu, clones were transferred to 140 mm nitrocellulose membranes, then incubated overnight with the pre-absorbed serum diluted 1:200, positive clones were detected by peroxidase-conjugated goat anti-human IgG (Jackson Immuno Research, West Grove, PA) and visualized by 3-3´-diaminobenzidine (Sigma, St. Louis, MO) (A). Positively reacted clones (+) were picked up and subjected to 2nd screening using the same serum on 80 mm membranes (B). A 3rd screening was conducted using positive clones for monoclonality using 40 mm membranes (C). The frequency of antibody response to SEREX-defined antigens in sera from normal individuals and cancer patients were assessed using phage plaque assay (D-E).

Sequence analysis

The positive clones were sub cloned to monoclonality, purified, and excised *in vivo* to pBK-CMV plasmid forms (Stratagene). Plasmid DNA was prepared using a Quantum Prep Plasmid Miniprep Kit (Bio-Rad, Hercules, CA). The nucleotide sequence of cDNA inserts was determined by an ABI PRISM R310 Genetic Analyzer (PerkinElmer), and sequence alignments were performed with BLAST software and compared with sequences in the GenBank and EST databases http://blast.ncbi.nlm.nih.gov/Blast.cgi and HUGO Gene Nomenclature Committee http://www.genenames.org/index.html.

mRNA expression analysis by RT-PCR

Using specific primers of some SEREX defined antigens, and tumor tissues from Egyptian cancer patients. To amplify cDNA segments from tumor and normal tissues, primers for the respective *XAGE-1b and XAGE-1c* transcripts were used (Eldib et

al., 2004). For PKCI, specific sense and antisense primers were designed (Fig. 2).

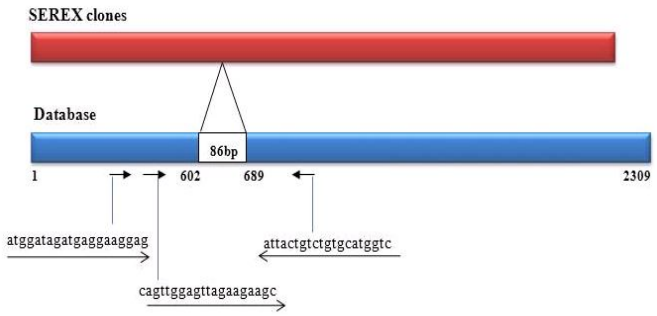


Fig. 2: protein kinase c, Iota which SEREX defined clones had a 86 bp deletion compared to sequences in the database.

Total RNA was isolated from different tumor tissues using an RNeasy Mini Kit (Qiagen, Hilden, Germany). The isolated RNA was reverse-transcribed into a single-strand cDNA using Moloney murine leukemia virus reverse transcriptase (Ready-To-Go You-Prime First-Strand Beads, Amersham Pharmacia) and oligo (DT) $_{15}$ as a primer. A normal tissue panel obtained commercially (Clontech, Palo Alto, CA) was also used for PCR reaction. RT-PCR was performed using 30 cycles at an annealing temperature of 60ºC, and the products were analysed by agarose gel electrophoresis. cDNA was tested for integrity by amplification of *G3PDH* in a 30-cycle reaction.

RESULTS

Molecular characterization of SEREX defined antigens

Schematic representation and a brief summary of SEREX (Serological identification of antigens by recombinant expression cloning) using autologous and/or allogeneic sera and evaluation of tumor antigens are shown in (Table 1). SEREX analysis was performed using several cancer cell lines including 3 lung cancer cell lines (OU-LU-6, OU-LU-17 and OU-LU-11), a renal cancer cell line (RC-1), a colon cancer cell line (AH-C-164), an ovarian cancer cell line (O-32) in addition to normal testis as sources of cDNA libraries (Table 1). As shown in table 1, a total number of 202 serum reactive clones representing 144 distinct antigens were totally obtained in autologous and allogeneic immunoscreening.
From the lung cancer cell lines, we isolated a panel of antigens, previously reported (Eldib, et al., 2004, Tadashi et al., 2011). Using those three lung adenocarcinoma cell lines, we isolated a total number of 79 positive clones representing 44 different genes including the cancer/testis XAGE-1b and c transcript variants.

Identification of renal cancer antigens by SEREX

A cDNA library of $2.5x10^6$ primary clones was constructed from RC-1 cell line. Four different sera were prepared for the SEREX analysis, including the autologous serum of RC-1. Serum preparation included E.coli lysates absorption treatment and screening with a non-related coda from mouse library. The used sera are RC-1 as an autologous serum, and three allogeneic sera 9-K-b, 9-A-1, and 11-A-9. A total number of about $2.8x10^5$ RC-1 clones were immunoscreened consisting of $1x10^5$ clones with the autologous serum, and $0.6x10^5$ clones with each allogeneic serum (Table 1).

Table 1: Summary of autologous and allogeneic immunoscreening of SEREX analysis using cDNA libraries from cancer cell lines and normal testis.

cDNA library	OU-LU-6*	OU-LU-17**	OU-LU-11**	RC-1			Testicular			AH-C-164	O-32
cDNA source cell line/tissue	Lung cancer cell line	Lung cancer cell line	Lung cancer cell line	Renal cancer cell line			Normal tissue			Colon cancer cell line	ovarian cancer Cell line
Histology	Adeno.	Adeno.	Adeno.	Clear cell			Normal			Adeno.	Adeno.
Stage of disease	Stage IV	Stage IV	Stage IV	Stage IV			Normal			Stage IV	Stage IIIC
Primary clones no. of cDNA library	$1.5x10^6$	$3x10^6$	$1.2x10^6$	$2.5x10^6$			$2.4x10^6$			$2x10^6$	$1x10^6$
Serum for screening	Auto.[1]	Auto.[1]	Auto.[1]	Auto.[1]	Allo. A[2]	Allo. B[2]	Allo. C[2]	Allo.[3] AH-C-164	Allo.[4] KC-18	Allo.[5] RC-1	Auto.[1]	Auto.[1]
Total no. of screened clones	$2.4x10^5$	$2x10^5$	$1.6x10^5$	$1x10^5$	$0.6x10^5$	$0.6x10^5$	$0.6x10^5$	$2x10^5$	$2x10^5$	$2x10^5$	$1.6x10^5$	$1.2x10^5$
Total no. of positive clones	38	20	21	19	16	12	4	11	11	5	31	14
Total no. of defined antigens	16	9	19	14	14	7	4	11	7	5	24	14

*We previously reported the SEREX analysis of the lung adenocarcinoma cell line OU-LU-6 , *Int. J. Cancer*: 108, 558-563 (2004).

* * We also reported the SEREX analysis of the lung adenocarcinoma cell lines OU-LU-17 and OU-LU-11 , *Cancer Letters*: 301, 57-62 (2011).

[1]Auto. : autologous serum [2] Allo. A (9-K-b), Allo. B (9-A-1) and Allo.C (11-A-9): are 3 allogenic sera from different renal cancer patients

[3]Allo. AH-C-164 and [4]Allo. KC-18: Allogenic sera from two colon cancer patients.

[5]Allo. RC-1: Allogenic serum from a renal cancer patient screened with the testicular library.

Table 2: Genes identified from the renal cancer cell line Rc-1 by autologous serum screening.

Gene	No. of Clones	Identity/similarities	UniGene cluster	HGNC ID	Chromosome	Expression
OY-REN-1	3/51	Immunoglobulin (CD79A) binding protein 1	Hs.496267	5461	Xq13.1-q13.3	Ubiquitous
OY-REN-2	2/51	La ribonucleoprotein domain family, member7	Hs.713663	24912	4q25	Ubiquitous
OY-REN-3	3/51	Acetyl-CoA acetyltransferase 2 (ACAT2)	Hs.571037	94	6q25.3-q26	Ubiquitous
OY-REN-4	1/51	PHD finger protein 10 (PHF 10)	Hs.435933	18250	6q27	Ubiquitous
OY-REN-5	1/51	Zinc finger, MYM-type 5 (ZMYM5)	Hs.530988	13029	13q12	Ubiquitous
OY-REN-6	1/51	Xeroderma pigmentosum, complementation A	Hs.654364	12814	9q22.3	Ubiquitous
OY-REN-7	1/51	Chromosome 20 ORF 11 (C20orf11)	Hs.353013	15857	20q13.33	Ubiquitous
OY-REN-8	1/51	TATA element modulatory factor 1(TMF1)	Hs.267632	11870	3p21-p12	Ubiquitous
OY-REN-9	1/51	Myosin light chain kinase (MYLK)	Hs.477375	7590	3q21	Ubiquitous
OY-REN-10	1/51	Retinoblastoma binding protein 6 (RBBP6)	Hs.188553	9889	16p12.2	Ubiquitous
OY-REN-11	1/51	Rab9 effector protein with kelch motifs	Hs.19012	16896	9q33.1-q33.3	Ubiquitous
OY-REN-12	1/51	Heat shock protein family B (small), member 11	Hs.525462	25019	1p32	Ubiquitous
OY-REN-13	1/51	Dual specificity phosphatase 1(DUSP1)	Hs.171695	3064	5q35.1	Ubiquitous
OY-REN-14	1/51	Cell division cycle 27 homolog (S. cerevisiae)	Hs.463295	1728	17q21.32	Ubiquitous

The Identity was performed using the Blast software: http://blast.ncbi.nlm.nih.gov/Blast.cgi

HGNC: HUGO Gene Nomenclature Committee http://www.genenames.org/index.html

Table 3: Genes identified from the renal cancer cell line RC-1 by screening with allogeneic serum (A).

Gene	No. of Clones	Identity/similarities	UniGene cluster	HGNC ID	Chrom.	Expression
OY-REN-15	1/51	CDC5 cell division cycle 5-like (S. pombe)	Hs.485471	1743	6p	Ubiquitous
OY-REN-16	1/51	Sec1 family domain containing 1	Hs.369168	20726	14q12	Ubiquitous
OY-REN-17	1/51	protein phosphatase1, regulatory (inhibitor) subunit 14B	Hs.523760	9057	11q13	Ubiquitous
OY-REN-18	3/51	protein kinase C, iota	Hs.478199	9404	3q26.3	Ubiquitous
OY-REN-19	1/51	chromosome 7 open reading frame 36	Hs.83313	24857	7	Ubiquitous
OY-REN-20	1/51	G patch domain and ankyrin repeats 1	Hs.247478	13920	6p21.3	Ubiquitous
OY-REN-21	1/51	KLRAQ motif containing 1	Hs.654619	30595	2p16.3	Ubiquitous
OY-REN-22	1/51	RNA binding motif, single stranded interacting protein1	Hs.470412	9907	2q24.2	Ubiquitous
OY-REN-23	1/51	BMI1 polycomb ring finger oncogene (BMI1)	Hs.380403	1066	10p13	Ubiquitous
OY-REN-24	1/51	RNA binding motif protein 41	Hs.139053	25617	Xq22.3	Ubiquitous
OY-REN-25	1/51	microtubule-associated protein 1B	Hs.335079	6836	5q13	Ubiquitous
OY-REN-26	1/51	UTP15, U3 small nucleolar ribonucleoprotein	Hs.406703	25758	5q13.2	Ubiquitous
OY-REN-27	1/51	talin 1	Hs.471014	11845	9p23-p21	Ubiquitous
OY-REN-28	1/51	La ribonucleoprotein domain family, member 7	Hs.713663	24912	4q25	Ubiquitous

The Identity was performed using the Blast software: http://blast.ncbi.nlm.nih.gov/Blast.cgi

HGNC: HUGO Gene Nomenclature Committee http://www.genenames.org/index.html

The total number of positive clones isolated from RC-1 library screening with the four renal cancer sera was 51clones representing 39 genes named OY-REN-1 through OY-REN-39 (Tables 2-5). The database search showed ubiquitous expression characters for all RC-1 genes.

Autologous serum screening led to the isolation of 19 positive clones representing 14 different antigens (Table 2). Of note, OY-REN-1 and 3 genes were the most frequently isolated (3 clones each), subsequently, OY-REN-2 was represented by 2 clones while the rest of genes were represented by a single clone for each (Table 2).

As shown in Tables 3, 4 and 5 using the allogeneic sera these are allogeneic serum A (9-K-b), allogeneic serum B (9-A-1) and allogeneic serum C (11-A-9) from 3 different renal cancer patients for immunoscreening led to the isolation of 16, 12 and 4 positive clones representing 14, 7 and 4 different genes,

Table 4: Genes identified from the renal cancer cell line RC-1 by screening with allogeneic serum (B).

Gene	No. of Clones	Identity/similarities	UniGene cluster	HGNC ID	Chrom.	Expression
OY-REN-29	3/51	ubiquitin B	Hs.356190	12463	17p12-p11.2	Ubiquitous
OY-REN-30	2/51	heat shock 70kDa protein 4	Hs.90093	5237	5q31.1	Ubiquitous
OY-REN-31	3/51	zinc finger protein 549 (ZNF549)	Hs.132562	26632	19q13.43	Ubiquitous
OY-REN-32	1/51	ubiquitin C	Hs.524832	12468	12q24.3	Ubiquitous
OY-REN-33	1/51	leucine rich repeat interacting protein 1	Hs.471779	6702	2q37.3	Ubiquitous
OY-REN-34	1/51	bromodomain adjacent to zinc finger domain,1B	Hs.728963	961	7q11.23	Ubiquitous
OY-REN-35	1/51	cyclin-dependent kinase 6	Hs.119882	1777	7q21-q22	Ubiquitous

The Identity was performed using the Blast software: http://blast.ncbi.nlm.nih.gov/Blast.cgi

HGNC: HUGO Gene Nomenclature Committee http://www.genenames.org/index.html

Table 5: Genes identified from the renal cancer cell line RC-1 by screening with allogeneic serum (C).

Gene	No. of Clones	Identity/similarities	UniGene cluster	HGNC ID	Chrom.	Expression
OY-REN-36	1/51	enoyl-CoA delta isomerase 2	Hs.15250	14601	6p24.3	Ubiquitous
OY-REN-37	1/51	ubiquitin specific peptidase 8	Hs.443731	12631	15q21.1	Ubiquitous
OY-REN-38	1/51	topoisomerase (DNA) II alpha	Hs.156346	11989	17q21-q22	Ubiquitous
OY-REN-39	1/51	ring finger and CCCH-type domains 2	Hs.533499	21461	9q34	Ubiquitous

The Identity was performed using the Blast software: http://blast.ncbi.nlm.nih.gov/Blast.cgi

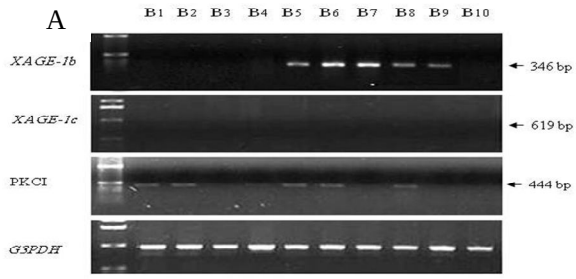
HGNC: HUGO Gene Nomenclature Committee http://www.genenames.org/index.html

respectively. Among the genes defined by the allogeneic serum A, OY-REN-18, the protein kinase C Iota was the most frequently isolated 3 clones (Table 3). All other genes were represented by one clone each. The interesting point in our SEREX research is the presence of a deletion in the sequence of the PKCI clones compared to the sequence in the database, which might give a chance to identify a new isoform. The deletion shifts the ORF and may give a new putative protein (Fig. 2).

Immunoscreening the RC-1 library with the allogeneic serum B led to the isolation of 7 genes represented by 12 clones. OY-REN-29 and 31 were represented by 3 clones each; OY-REN-30 was represented by 2 clones while other genes were represented by one clone each. Using the allogeneic serum C led to the isolation of 4 positive clones representing 4 genes.

XAGE-1b, c and PKCI mRNA expression in breast cancer and normal tissues

For comparison, mRNA expression of XAGE-1b and c transcripts and PKCI was investigated in 10 breast cancer tissues using specific PCR primers (Fig. 3A). RT-PCR was performed at 30 cycles. As shown in Fig. 3A, using the common primer pair X-1 and X-2, a PCR product of 346 bp in length was observed in 5/10 breast cancer tissues. Using X-4 and X-2 for XAGE-1c showed no PCR product. While very faint products of 444 bp in length (4/10) were observed after using PKCI specific primers. In adult normal tissues, PKCI expression was observed ubiquitously (Fig. 3B). The results indicated that XAGE-1b is the predominantly expressed antigen in breast cancer tissues.



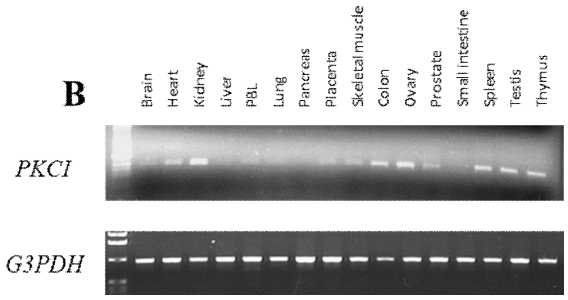


Fig. 3: RT-PCR analysis (30 cycles) for mRNA expression of XAGE-1b and c transcript variants and the SEREX defined antigen, protein kinase C-iota (PKCI) in breast cancer (B1-B10) tissues (A). Primers used are (X-1 and X-2) for XAGE-1b, (X-4 and X-2) for XAGE-1c, Int. J. Cancer: 108, 558-563 (2004). Primers used for (PKCI) are spanning nt 445-888 sense 5`-ATGGATAGATGAGGAAGGAG-3` and anti-sense 5`-ATTACTGTCTGTGCATGGTC-3`. Using the same primer pair for PKCI expression in a normal tissue panel (B). G3PDH was used as internal control.

DISCUSSION

In order to identify human tumor antigens that eliciting high-titer IgG antibodies, serological analysis of recombinant cDNA expression libraries (SEREX) was developed to combine serological analysis with antigen cloning techniques (Sahin et al., 1995) and (Chen et al., 2000). SEREX has contributed greatly to our understanding of the humoral immune response to cancer (Chen 2004). It has led to the identification of a variety of tumor specific and tumor associated antigens, including cancer/testis (CT) antigens (Chen et al., 1997 and Türeci et al., 1998), mutational antigens (Gordan et al., 1998), over-expressed antigens (Brass et al., 1999 and Türeci et al., 1998), differentiation antigens (Scanlan et al., 1999), splice-variant antigens (Jäger et al., 1999), and viral antigens (Türeci et al., 1997). We have done SEREX analysis using several cancer cell lines including 3 lung cancers and a renal cancer cell line, a colon cancer cell line, an ovarian cancer cell line and normal testis. In lung cancer cell lines, a panel of different antigens was isolated, among them XAGE-1 was originally identified by EST database mining (Brinkmann et al.,1999) and found to be highly expressed in normal testis and Ewing's sarcoma (Liu et al., 2000). The XAGE-1 gene is located on chromosome Xp11.21 - Xp11.22, consists of 4 exons and shows homology to GAGE/PAGE genes (Shimono et al., 2007). It has been reported that there are 4 transcript variants, XAGE-1a, b, c, and d. Within those, XAGE-1b was shown to be the major transcript (Nakagawa et al., 2005-Koisumi et al., 2005). A higher expression of XAGE-1b mRNA was observed in Ewing's sarcoma and metastatic lesions of melanoma compared to XAGE-1a (Zendman et al., 2002).

We have identified 39 antigens by SEREX analysis of renal cancer represent a diverse group of proteins, including immunoglobulin binding protein (e.g., OY-REN-1), heat shock proteins (e.g., OY-REN-12, 30), RNA binding proteins (e.g., OY-REN-22, 24), microtubule associated proteins (OY-REN-25), and cell division-associated proteins (e.g., OY-REN-14). OY-REN-29, 32 and 37 may be involved in the ubiquitin pathway. Protein kinase C, Iota was identified from a renal cancer cell line (RC1) through immunoscreening of its library with an allogeneic serum in SEREX analysis. Protein Kinase C, Iota was isolated through screening the library with the allogeneic serum 9-K-b, and represented by 3 clones. Sequence analysis of these two clones revealed almost the whole length of the gene. PKC family exists as a family of 9 related gene products that are differentially expressed and that show differences in their co-factor requirements. It is therefore likely that the different isoforms serve different physiological roles (Steinberg et al., 1995).

The functional diversity correlates with a number of structural features predicted from the amino acid sequence of the various isoforms. The PKC family is divided into three major classes: the classical PKCs (a, b, and g isoforms), the novel PKCs (d, e, h, and q isoforms), and the atypical group (z, and i) (Fields et al., 2007). The amino acid sequence of PKC iota showed greatest homology to PKC zeta, with 72% identity overall rising to 84% in the catalytic domain. In contrast, the homology of PKC iota to the other isoforms was less pronounced, with < 53% identity even in the highly conserved catalytic region. Further similarities between PKC zeta and PKC iota included a highly conserved pseudo substrate sequence, the absence of an apparent Ca (2+)-binding region, and the presence of only one cysteine-rich, zinc finger-like domain. PKC iota is included in the atypical subgroup of PKCs whose definitive member is PKC zeta (Murray et al., 2011). The interesting point in our SEREX research was the presence of a deletion in the sequence of the Iota clones compared to the sequence in the database which might give a chance to identify a new isoform. A homology search in GenBank database revealed that 2 genes showed no strong homology indicating novel genes OY-CO-7and 19. Further prospective studies may help for identifying the function of these 2 genes. Serological analysis for antigens was performed on the basis of their reactivity with sera from healthy donors and cancer patients. All examined antigens were reacted with sera from a subset of healthy donors and cancer patients by phage plaque assay. No RC-1 antigens showed a cancer-restricted recognition pattern (data not shown). In order to examine the status of XAGE-1 and PKCI mRNA expression and validate it as a potential diagnostic and therapeutic target, mRNA expression analysis in breast cancer was conducted.

In mRNA expression analysis in breast cancer tissues using the known cancer/testis antigen XAGE1b and c variants and a ubiquitous gene PKCI, the results showed high frequency for XAGE1b while XAGE1c and PKCI showed no expression. These results prove the idea that cancer/testis (CT) antigens are immunogenic proteins expressed predominantly in gametogenic tissue and cancer; they are considered promising target molecules for cancer vaccines (Matthew et al., 2002).

Generally, breast cancers are difficult to distinguish based on histological markers. The results of the present study indicate that XAGE-1b may have prognostic utility and may be a promising molecular target for diagnosis and treatment immunotherapy of breast cancer. This is the first report for mRNA expression profile analysis using cancer/testis antigens (XAGE-1b) in Egyptian patient`s malignant tissues (breast cancer). Such expression profile is a very important step for Egyptian patients. Characterization of those antigens which considered candidate targets for

immunotherapy of tumors will open the door for cancer patients in Egypt in the future for such immunotherapeutic agents.

Further characterization of mRNA and protein analysis governing these and other cancer specific antigens using malignant tissues and samples from Egyptian patients may reveal novel control points leading to development of rational molecular and/or immune-based therapies for breast cancer and other cancer types.

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