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- 총 설 -

SEREX: discovery of tumor antigens

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The identification of tumor antigens is essential for the development of anticancer therapeutic vaccines and clinical diagnosis of cancer. SEREX (serological analysis of recombinant cDNA expression library) has been used to identify such tumor antigens by screening sera of cancer patients with cDNA expression libraries. SEREX-defined antigens provide markers for the diagnosis of cancers. SEREX is also a powerful method for the development of anticancer therapeutics. The development of anticancer vaccines requires that tumor antigens can elicit antigen-specific antibodies or T lymphocytes. This review provides information on the application of SEREX for discovery of tumor antigens.

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

Introduction

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 [17], and serological expression cloning [12], 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 [16].

Sahin and his colleagues [18] have introduced a method for the identification of tumor antigens recognized by autologous serum IgG of cancer patients. This method, termed SEREX (serological analysis of recombinant cDNA expression libraries) has led to the identification of a series of provocative cancer antigens that have relevance to the etiology, diagnosis, and therapy of cancer. As annotated in the SEREX database [2]. More than 2,000 different antigens have been defined by SEREX analysis of more than 15 different tumor types to date, including cancer/testis or CT antigens [22], differentiation antigens [6], mutational antigens [15], over expressed or amplified genes [20], splice variant antigens [9], and viral antigens [24].

It is generally accepted that immune recognition involves both cell-mediated and humoral-immunity. The recent development of a new approach to dissect the humoral immune response to cancer has opened the way to establishing a comprehensive picture of the immune repertoire against human cancer antigens. SEREX allows systematic and unbiased search for cancer-specific antigens, and immunogenic proteins based on their reactivity with autologous patient serum.

Serology plays a central role in two phases of SEREX analysis. The first phase is antigen identification. A second phase involves screening panels of serum from normal individuals and cancer patients with the aim of defining antigen panels that demonstrate cancer-restricted immune recognition [3].

The demonstration of cytotoxic T-lymphocytes (CTL) reactive with SEREX defined antigens, and the SEREX identification of antigens that were originally defined as CTL recognized peptides [7] indicate that SEREX detects tumor antigens eliciting CTL immunity as well. Thus, in comparison to the other methods, SEREX is less technically demanding and can yield similar information regarding host immune responses to cancer.

SEREX Approach

The SEREX approach offers following features : i) the use of fresh tumor specimens restricts the analysis to genes that are expressed by the tumor cells in vivo; ii) the use of patients'serum allows for the identification of multiple antigens iii) the screening is restricted to antigens against which patients raised high-titer antibody responses.

SEREX (Fig. 1) employs a bacterophage recombinant cDNA expression library prepared from tumor tissues, tu-

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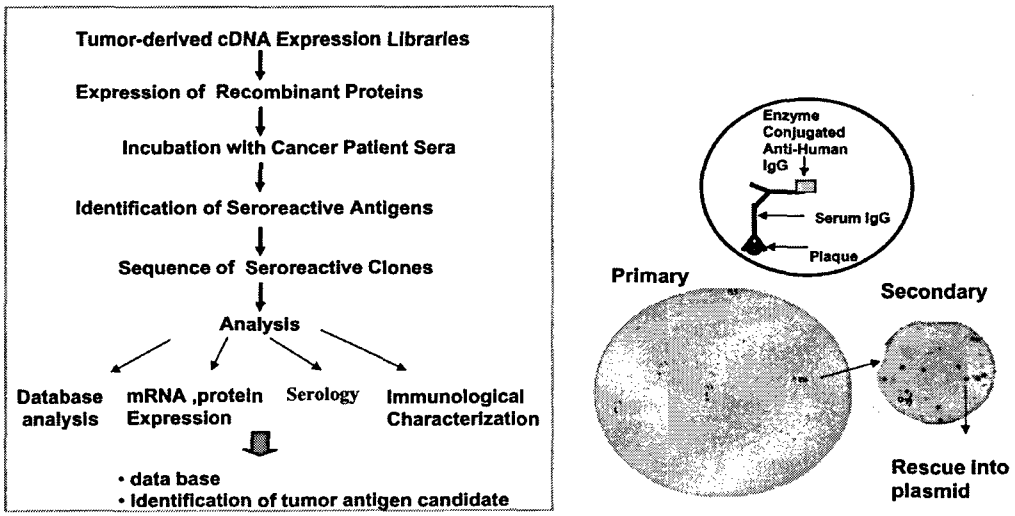

Fig. 1. Method of SEREX for discovery of tumor antigen cDNA expression libraries are made from tumor tissues, tumor cell lies, or testis tissues. The obtained cDNA library is cloned into λ ZAP expression vector. cDNA expression libraries are screened with pooled sera of patients with cancers. Immune reactive clones are selected by reacting nitrocellulose membrane containing recombinant clones with pooled sera of patients with cancers followed by incubation with alkaline phosphatase-conjugated secondary antibody. Thus selected clones are subjected to in vivo excision and sequencing to determine identity of each immune reactive clone.

mor cell lines, and testis tissues. The use of tumor cell lines for SEREX analysis has benefits, including the absence of contaminating normal cell types invariably present in tumor specimens, and the elimination of B cells that give rise to false-positive IgG-expressing clones in the expression library. The cDNA expression library is used to transduce *E. coli*. The recombinant protein library is induced and transferred to nitrocellulose membranes. These membranes are then incubated with diluted (1:100-1:1,000) extensively preabsorbed pooled serum from the autologous patient. Clones reactive with high-titer antibodies are identified using an enzyme (Alkaline phosphatase-conjugated secondary antibody specific for human IgG. Positive clones are then subjected to DNA sequencing. Sequence information of DNA insert can be used to determine expression profile of the transcript and to evaluate the incidence of antibody responses to the respective antigens. SEREX has been widely used in identifying antigens in patients with solid tumors [3)] and autoimmune disease, including systemic sclerosis, systemic lupus erythematosus, and Sjogren's syndrome [8].

Classification of the SEREX-defined antigens

A number of SEREX-defined antigens with these cancer-related characteristics have been identified and can be classified into one of the following categories, including differentiation antigens, mutational antigens, over expressed antigens, and cancer/testis antigens.

Mutational Antigens

Several mutational antigens have been isolated by SEREX. Tumor suppressor gene p53 has been identified by SEREX of ovarian cancer, colon cancer [21]. In the case of colon cancer, a single base substitution of p53 (A to G) was identified, confirming this mutation as the basis for the observed immunogenicity. Other examples of mutational antigens in colon cancer were AD034, with a 32 bp frame shift mutation, and CDX2, with a single-base frame shift mutation [5].

Differentiation Antigens

Differentiation antigens are expressed in tumors in a lineage-specific pattern, but also in normal cells of the same origin. The classic example of a differentiation antigen recognized by SEREX is the melanocyte-specific protein tyrosinase [15]. Other examples include NY-BR-1 in breast cancer [6], and glial fibrillary acidic protein (GFAP) in glioma. Normal tissue expression of galectin-4 is restricted to normal colon and small intestine. Because galectin-4 is localized to the leading edge of lamellipodia, it is thought to have a role in cell adhesion [19].

Amplified or Overexpressed Antigens

Many SEREX-defined genes are overexpressed in cancer based on northern blot analysis, and real-time RT-PCR. Amplified or over expressed antigens identified include carbonic anhydrase XII in breast [29], eIF-4 gamma [1] in lung cancer, AKT1, and HER-2/neu [20] in breast cancer. Several mechanisms can account for amplified expression of gene products in cancer, including gene amplification (e.g., eIF-4 gamma), increased steady-state mRNA (e.g., KOC3), and increased protein stability [28].

Cancer/testis antigens (CT)

CT antigens share the following characteristics: (i) predominant mRNA expression in testis, but generally not in other normal somatic tissues, (ii) gene activation and mRNA expression in a wide range of human tumor types, (iii) existence of multigene families, and (iv) with rare exception, localization of coding genes to chromosome X. Table 1 shows partial list of CT antigens identified by various methods, including SEREX. The frequent expression of CT antigens in various types of tumors is an exception to this general rule. It suggests that the CT antigens, most of them with unknown function at present, are a distinct group of proteins in terms of their regulation and possibly their biological function [22,25].

Cell surface antigens

To date, relatively few surface-associated antigens have been identified using SEREX. Nevertheless, the increasingly detailed annotation of the human genome and the availability online of details of the function and subcellular distribution of gene products render the deliberate search for surface antigens by SEREX . By applying this approach, we identified two antigens (NY-SAR-35 and NY-TLU -57) that are putatively exposed on the cell surface and might thus serve as targets for monoclonal antibody-based immunotherapy [12,13]. Of particular interest is NY-SAR-35, which represents a recently defined CT antigen apparently expressed exclusively in normal testis, as well as melanoma, sarcoma, lung cancer, esophageal cancer, ovarian cancer, and breast cancer. Like several other CT antigens, NY-SAR-35, maps to chromosome X, but in contrast to other CT antigens , NY-SAR-35 is not a member of a multigene family. The presence of a signal sequence, a putative transmembrane domain, and a Trefoil domain suggests that NY-SAR-35 may be an extracellular or plasma membrane associated protein. Thus, in addition to being a potential cancer vaccine target, NY-SAR-35 may also be a target for of therapeutic antibodies [12]. The gonad specific anion transport protein SLCO6A1 (NY-TLU-57) was shown to be tissue-restricted. RT-PCR showed it to be expressed strongly only in normal testis, and weakly in spleen, brain, fetal brain, and placenta. In addition, NY-TLU-57 mRNA was found in lung tumor samples and lung cancer cell lines, as well as bladder and esophageal tumor samples. These data suggest that SLCO6A1 is a putative cancer/testis (CT) cell surface antigen of potential utility as a target for antibody-based therapy for a variety of tumor types [13].

Table 1. Examples of cancer/testis antigens encoding immunogenic product

CT antigens gene family	Number of gene / Function	Chromosome location	Immune response
MAGEA	12/ translation	Xq28	cellular and humoral
MAGEB	4/ unknown	Xp21-22	humoral
BAGE	2/ unknown	13	cellular
GAGE	8/ unknown	Xp11	cellular
SSX	5/ unknown	Xp11	cellular and humoral
NY-ESO-1	3/ unknown	Xq28	cellular and humoral
SCP-1	3/spematogenesis	1p12-p13	humoral
MAGE-C1	2/ unknown	Xq26	humoral
OY-TES-1	1/ spematogenesis	12p	humoral
NY-SAR-35	1/ unknown	Xq28	humoral
cTAGE-1	2/ unknown	Xq27	humoral
CAGE	1/ helicase	Xp22	humoral
FATE	1/ unknown	Xq28	humoral
TPTE	1/ phosphtase	21	humoral
NY-TLU-57	1/ transport	21	humoral

*humoral and cellular denotes identification of cancer/testis antigens by SEREX and T cell epitope cloning

Biomarker of SEREX-defined antigens; SADA

The identification of biomarkers for diagnosis, prognosis, and therapy of human cancer has been a long-standing challenge in cancer research. With regard to serum markers for cancer, a limited number of clinically beneficial antigenic markers have been defined, such as carcinoembryonic antigen in gastrointestinal cancers, fetoprotein in hepatoma and germ cell tumors, CA125 in ovarian cancer, and prostate-specific antigen in prostate cancer [26]. SEREX analysis has defined a subset of tumor antigens that react exclusively with serum antibodies derived from multiple cancer patients but do not react with sera from normal individuals. Extensions of the petit serology have been devised by Scanlan et al.,[23], and given the name SADA (serial analysis of defined antigen). This involves arranging the phage clones on filters in a "dot blot" fashion, thus allowing simultaneous testing of a large panel of antigens (Fig. 2). This method works very well for clones that show strong sero-reactivity.

Evaluation of antibody responses to the SEREX-defined antigens using allogeneic sera from cancer patients would offer valuable cancer diagnostics employing antibody-based screening. Aside from SADA (serial analysis of defined antigens), two new screening methods are established and named sero GRID and SMARTA (serological mini-arrays of recombinant tumor antigens [10,11]. These assays would provide alternatively reliable method for analyzing a panel of recombinant antigens at a large scale.

Conclusion

In the past two decades, the number of SEREX-defined antigens has been greatly increasing. Identification of these antigens makes it possible for early detection and the development of anticancer vaccines. However, SEREX has its own problems that need resolution. In the conventional SEREX approach, the antigens are expressed in bacterial- and phage-based systems, which are generally not capable of naturally folding antigenic proteins and modifying them posttranslationally. The conformation and posttranslational modifications play an important role in the proper function of proteins and also affect their immunogenicity. This problem can be solved by employing eukaryotic expression vector systems such as yeast [14]. Thus, modifications of SEREX have been tried to minimize the identification of irrelevant antibodies and increase the number of cancer-related antibodies [14].

Identifying the complete repertoire of immunogenic gene products in human cancer is now an achievable goal for tumor immunology. Since the establishment of the SEREX database in 1997, 2593 sequences derived from 2169 clones have been deposited. Many of the genes have been isolated repeatedly by SEREX, from the same and/or from

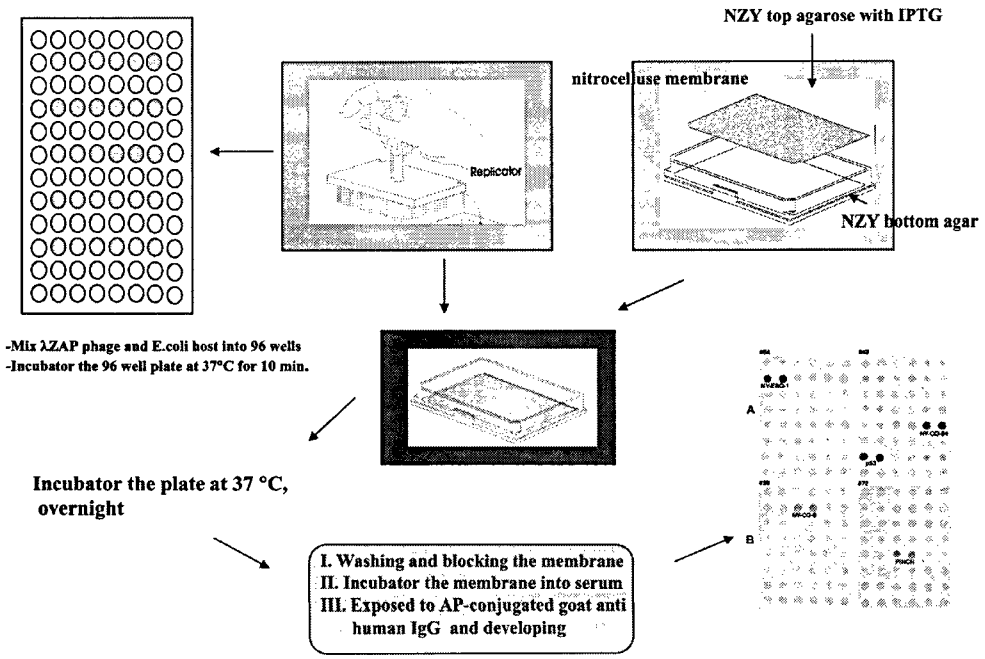

Fig. 2. Analysis of SADA (serial analysis of defined antigens). (see reference 12, 23 for detailing method)

different tumor types, indicating that these gene products are highly immunogenic in the human host. In our recent study of sarcoma and lung cancer [12,13] only about one third of the isolated genes were already in the database, suggesting that the pool of immunogenic cancer antigens, although apparently finite in size, is still far from completely defined.

Thus, we propose that continued SEREX-based searches for cancer antigens should be undertaken to provide as wide an option as possible for the design of novel therapies.

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References

1. Brass, N., D. Heckel, U. Sahin, M. Pfreundschuh, G. W. Sybrecht, E. Meese. 1997. Translation initiation factor eIF-4gamma is encoded by an amplified gene and induces an immune response in squamous cell lung carcinoma. *Hum. Mol. Genet.* **6**, 33-39.
2. Cancer Immunome database: http://www2.licr.org/CancerImmunomeDB
3. Chen, Y. T., M. J. Scanlan, Y. Obata and L. J. Old(2000) Identification of human tumor antigens by serological expression cloning. *In*:S. A. Rosenberg (ed.). Principles and Practice of Biologic Therapy of Cancer, pp. 557- 570. Philadelphia: Lippincott Williams & Wilkins.
4. Fernandez-Madrid, F., P. J. VandeVord, X. Yang, R. L. Karvonen, P. M. Simpson, M. J. Kraut, J. L. Granda and J. E. Tomkiel. 1999. Antinuclear antibodies as potential markers of lung cancer. *Clin. Cancer Res.* **5**, 1393-1400.
5. Ishikawa, T., T. Fujita, Y. Suzuki, S. Okabe, Y. Yuasa, T. Iwai and Y. Kawakami. 2003. Tumor-specific immunological recognition of frameshift-mutated peptides in colon cancer with microsatellite instability. *Cancer Res.* **63**, 5564-5572.
6. Jager, D., M. Unkelbach, C. Frei, F. Bert, M. J. Scanlan, E. Jager, L. J. Old, Y. T. Chen and A. Knuth, 2002. Identification of tumor-restricted antigens NY-BR-1, SCP-1, and a new cancer/testis-like antigen NW-BR-3 by serological screening of a testicular library with breast cancer serum. *Cancer Immun.* **2**, 5-9.
7. Jager, E., Y. T. Chen, J. W. Drijfhout, J. Karbach, M. Ringhoffer, D. Jager, M. Arand, H. Wada, Y. Noguchi, E. Stockert, L. J. Old and A. Knuth. 1998. Simultaneous humoral and cellular immune response against cancer-testis antigen NY-ESO-1: definition of human histocompatibility leukocyte antigen (HLA)-A2-binding peptide epitopes. *J. Exp. Med.* **187**, 265-270.
8. Jeoung, D. I., E. B. Lee, S. Lee, Y. Lim, D. Y. Lee, J. Kim, H. Y. Kim and Y. W. Song. 2002. Autoantibody to DNA binding protein B as a novel serologic marker in systemic sclerosis. *Biochem. Biophys. Res. Commun.* **299**, 549-554.
9. Koslowski, M., O. Tureci, C. Bell, P. Krause, H. A. Lehr, J. Brunner, G. Seitz, F. O. Nestle, C. Huber and U. Sahin. 2002. Multiple splice variants of lactate dehydrogenase C selectively expressed in human cancer. *Cancer Res.* **62**, 6750-6755.
10. Krause, P., O. Tureci, P. Micke, R. Buhl, C. Huber and U. Sahin. 2003. SeroGRID: an improved method for the rapid selection of antigens with disease related immunogenicity. *J. Immunol. Methods* **283**, 261-267.
11. Lagarkova, M. A., E. P. Koroleva, D. V. Kuprash, V. E. Boitchenko, U. A. Kashkarova, S. A. Nedospasov and Y. V. Shebzukhov. 2003. Evaluation of humoral response to tumor antigens using recombinant expression-based serological mini-arrays (SMARTA). *Immunol. Lett.* **85**, 71-74.
12. Lee, S. Y., Y. Obata, M. Yoshida, E. Stockert, B. Williamson, A. A. Jungbluth, Y. T. Chen, L. J. Old and M. J. Scanlan. 2003. Immunomic analysis of human sarcoma. *Proc. Natl. Acad. Sci. USA.* **100**, 2651-2656.
13. Lee, S. Y., B. Williamson, O. L. Caballero, Y. T. Chen, M. J. Scanlan, G. Ritter, C. V. Jongeneel, A. J Simpson and L. J. Old. 2004. Identification of the gonad-specific anion transporter SLCO6A1 as a cancer/testis (CT) antigen expressed in human lung cancer. *Cancer Immun.* **17**, 4:13.
14. Mischo, A., A. Wadle, K. Watzig, D. Jager, E. Stockert, D. Santiago, G. Ritter, E. Regitz, E. Jager, A. Knuth, L. Old, M. Pfreundschuh and C. Renner. 2003. Recombinant antigen expression on yeast surface (RAYS) for the detection of serological immune responses in cancer patients. *Cancer Immun.* **3**, 5.
15. Novellino, L., N. Renkvist, F. Rini, A. Mazzocchi, L. Rivoltini, A. Greco, P. Deho, P. Squarcina, P. F. Robbins, G. Parmiani and C. Castelli. 2003. Identification of a mutated receptor-like protein tyrosine phosphatase kappa as a novel, class II HLA-restricted melanoma antigen. *J. Immunol.* **170**, 6363-6370.
16. Old, L. J. 2003. Cancer vaccines 2003: opening address. *Cancer Immun.* **3(2)**, 1.
17. Pascolo, S., M. Schirle, B. Guckel, T. Dumrese, S. Stumm, S. Kayser, A. Moris, D. Wallwiener, H. G. Rammensee, S. A. Stevanovic. 2001. MAGE-A1 HLA-A A*0201 epitope identified by mass spectrometry. *Cancer Res.* **15**, 4072-4077
18. Sahin, U., O. Tureci, H. Schmitt, B. Cochlovius, T. Johannes, R. Schmits, ,F. Stenner, G. Luo, I. Schobert and M. Pfreundschuh, 1995. Human neoplasms elicit multiple specific immune responses in the autologous host. *Proc. Natl. Acad. Sci. USA.* **92**, 11810-11813.
19. Sakakura, C., K. Hasegawa, K. Miyagawa, S. Nakashima, T. Yoshikawa, S. Kin, Y. Nakase, S. Yazumi, H. Yamagishi, T. Okanoue, T. Chiba and A. Hagiwara. 2005. Possible involvement of RUNX3 silencing in the peritoneal metastases of gastric cancers. *Clin. Cancer Res.* **11**, 6479-6488.
20. Salazar, L. G., J. Fikes, S. Southwood, G. Ishioka, K. L.

Knutson, T. A. Gooley, K. Schiffman and M. L. Disis. 2003. Immunization of cancer patients with HER-2/neu-derived peptides demonstrating high-affinity binding to multiple class II alleles. *Clin. Cancer Res.* **9**, 5559-5565.
21. Scanlan, M. J, I. Gout, C. M. Gordon, B. Williamson, E. Stockert, A. O. Gure, D. Jager, Y. T. Chen, A. Mackay, M. J. O'Hare and L. J. Old. 2001. Humoral immunity to human breast cancer: antigen definition and quantitative analysis of mRNA expression. *Cancer Immun.* **1**, 4.
22. Scanlan, M. J., A. J. Simpson and L. J. Old, 2004. The cancer/testis genes: review, standardization, and commentary. *Cancer Immun.* **4**, 1.
23. Scanlan, M. J., S. Welt, C. M. Gordon, Y. T. Chen, A. O. Gure, E. Stockert, A. A. Jungbluth, G. Ritter, D. Jager, E. Jager, A. Knuth and L. J. Old. 2002. Cancer-related serological recognition of human colon cancer: identification of potential diagnostic and immunotherapeutic targets. *Cancer Res.* **62**, 4041-4047.
24. Schiavetti, F., J. Thonnard, D. Colau, T. Boon and P. G. Coulie, 2002. A human endogenous retroviral sequence encoding an antigen recognized on melanoma by cytolytic T lymphocytes. *Cancer Res.* **62**, 5510-5516.
25. Simpson, A. J, O. L Caballero, A. Jungbluth, Y. T. Chen and L. J. Old. 2005. Cancer/testis antigens, gametogenesis and cancer. *Nat Rev Cancer.* **5**, 615-625.
26. Thomas, C.M., C.G. Sweep, Int. J. Biol. 2001. Markers, Serum tumor markers: past, state of the art, and future. *Int J Biol Markers.* **16**, 73-86. Review.
27. van der, B. P., Y. Zhang, P. Chaux, V. Stroobant, C. Panichelli, E. S. Schultz, J. Chapiro, B. J. Van Den Eynde, F. Brasseur and T. Boon, 2002. Tumor-specific shared antigenic peptides recognized by human T cells. *Immunol. Rev.* **188**, 51-64.
28. van Nimwegen, M.J., M. Huigsloot, A. Camier, I.B. Tijdens, B. van de Water 2006. Focal adhesion kinase and protein kinase B cooperate to suppress doxorubicin-induced apoptosis of breast tumor cells. *Mol. Pharmacol. 70*, 1330-1339.
29. Watson S. A, T. M Morris, D. F McWilliams, J Harris, S Evans, A Smith, P. A. Clarke, 2003. Potential role of endocrine gastrin in the colonic adenoma carcinoma sequence. *Br. J. Cancer* **88**, 1065-1070. **R**

초록 : 종양 항원의 발견:SEREX

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(부산대학교 의과대학 생화학 교실)

종양항원의 동정과 발견은 암 백신 및 진단개발에 매우 중요하다. 암환자의 혈청에서 종양 항원를 동정하는 SEREX가 개발되어왔다. SEREX에서 동정된 종양항원은 진단의 분자 지표 뿐 만아니라 항암 백신 개발에 응용되고 있다. 따라서 SEREX는 종양항원 동정에 사용되어지는 매우 강력한 방법이다. 항암 백신의 개발은 동정된 종양항원이 체 또는 T cell에 기초하여 작동하는지 해명하는 것이 중요한 요소이다. 이 논문은 강력한 종양항원의 동정 방법인 SEREX 의 응용에 관하여 고찰 할 것이다.