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THE RESEARCH PROGRESSION OF SEROLOGICAL ANALYSIS OF RECOMBINANT cDNA EXPRESSION LIBRARIES SCREENING ANTIGENS OF ATHEROSCLEROTIC DISEASES

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Abstract: The serological analysis of recombinant cDNA expression libraries (SEREX) is an important research method of screening disease-associated antigens, it combines the gene cloning technology with the serum screening technology, the recombinant cDNA expression clone library is screened by autologous serum of patients, thus the relevant target antigen genes are obtained, In turn, serum antibodies of patients can be identified by the target genes. It was initially used to screen tumor-related antigens, then the range of application expanded and gradually applied to screen the antigens of atherosclerotic disease. This article reviews the basic principles, experimental procedures, advantages and disadvantages of SEREX and its application and research progress in screening antigens of atherosclerotic disease.
Keywords: SEREX, Atherosclerosis, Atherosclerotic Disease, Antigen, Antibody

INTRODUCTION

SEREX ,serological analysis of recombinant cDNA expression libraries, it was proposed by Sahin group [1], it combines molecular cloning technology with autotyping technology of serum antibodies to antigens, the target antigens with immunogenicity is screened by detecting the reaction of antigen with serum antibody, and target antigen genes can be determined directly at the molecular level by DNA sequencing technology. The theoretical basis is that the pathogenic antigen proteins induced by the cDNA expression library can be recognized by the serum-derived human B lymphocytes, and partial antigen proteins can trigger high-priced IgG antibody responses, then the antigens associated disease are identified, the target genes in the cDNA expression library are identified by the method of secondary antibody labeling.

THE PROCESSES OF SEREX AND ADVANTAGES AND DISADVANTAGS

Technical process of SEREX

Construction of cDNA expression library： Collect fresh tissue lesions or tumor tissue, total mRNA from fresh tumor tissues or cell lines are extracted and purified and reversely transcribed to cDNA, constructed into phage expression vector Uni-ZAP XR, then Phage transfect E.coli, after proliferation and lysis, the collected Uni-ZAP XR phages are recombinant cDNA expression clone library; Serum pretreatment: The serum is co-incubated with induced products of E. coli loaded with empty carriers, eliminating disease-independent antibodies in serum against somatic proteins; Immunoscreening library: cDNA expression libraries are induced to express target proteins on LB plates, transfering the target protein onto nitrocellulose membrane, the pretreated serum is co-incubated with NC membrane, antibodies in the serum that are abnormally elevated bind to antigens, sheep anti-human lgG antibody labeled with horseradish peroxidase (HRP) is used to mark the antigen-antibody reaction. At last, the positive clones are selected through the color reaction, and then repeat 2 to 3 rounds of screening to obtain the positive monoclones; Positive monoclonal DNA homology analysis: The positive monoclones are transformed into plasmids after internal excision of the monoclones, and DNA sequence detection is performed after plasmid extraction, the homology analysis and gene function analysis of the measured DNA sequences and known genes are performed using Basic Local Alignment Search Tool (BLAST) provided by national Center for Biotechnology Information (NCBI), as shown in the figure below.

Advantages and disadvantages of SEREX

cDNA library is constructed from a wide range of samples, including fresh human aortic endothelial cells, tumor cell lines, and human testicular tissue, it avoids the mutation, deletion or non-expression of target antigen gene caused by in vitro strain construction and overculture of cells, and it affects the positive rate of the reaction of target antigen and

serum antibody. The serum used to detect cDNA expression library can be autologous serum or foreign serum, the antigens screened by experiment are limited to target antigens that can induce strong immune response and have common T cell helper, the serum dilution is 1∶100 ~ 1∶1000, that is high titer IgG antibody, the target antigen obtained by screening is not limited to cell surface antigen, but also included products such as peptide encoded by the target gene, thus the target antigen can be accurately identified; This method is a relatively high throughput analysis method, improving the efficiency of antigen screening, discovering multiple antigens simultaneously[2]; This method is simple and feasible for screening disease-associated antigens, which provides the possibility for the preparation of vaccines and immunotherapy for diseases; The application of cDNA expression library greatly improves the concentration of target antigen proteins, which not only solves the problem of insufficient titer of antigen in previous antigen-antibody reactions, but reveals a large number of related antigens.

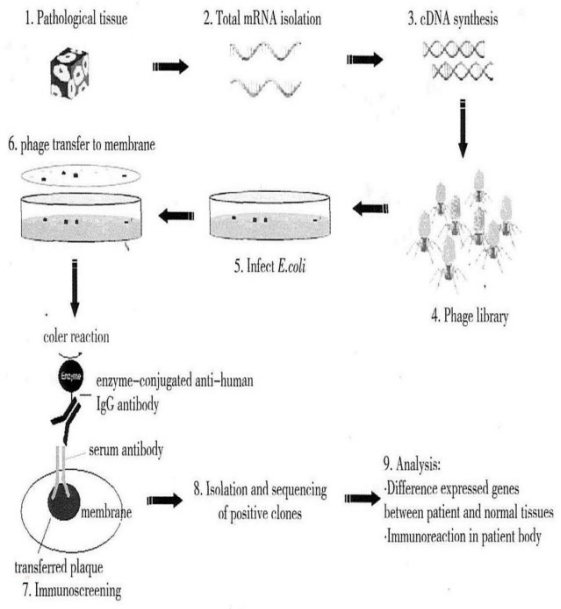

Figure 1. Steps for the SEREX technique[1]

The approach also has limitations, because the cDNA library is expressed in lambda ZAP II phage prokaryotic system, therefore, the absence of post-translational modification of proteins makes it impossible to screen out antigenic determinants that requires post-translational modification and undergoes structural changes when expressed in bacteria[2]; If the time of taking fresh tissue specimens is not consistent with the expression stage of the antigen, some antigens expressed at different stages of tissue development may be missed[3];In the screening of cDNA expression libraries, high level of antigens in libraries are generally obtained, which make some low expressive antigens easy to be missed[2]; Some of the identified antigens are patient-specific antigens or antigens expressed in normal tissues rather than disease-specific antigens, which have no general significance for clinical diagnosis; Antibodies against E.coli somatic proteins and phage antigens may also produce false positive results.

The improvement of SEREX

Testicular tissue cDNA expression library rather than human aortic endothelial cell cDNA expression library is used as antigen source, increasing the category of defined antigens; Improving the quality of cDNA library, the quality of cDNA library is mainly reflects in the representation of library and the sequence integrity of recombinant cDNA fragment, the representativeness of the library can be measured by the library capacity, it is required that the amount of cDNA library screened by serum must reach more than 1×10^6 clones ,ensuring that all the target gene fragments in the cDNA library have the opportunity to react with the serum. The sequence integrity of the recombinant cDNA fragment is another important factor, so it is required that the recombinant cDNA fragment in the library should reflect the structure of the natural gene as completely as possible; SEREX was used to screen the antigens of atherosclerotic diseases, not all the disease antigens screened by this technique were atherosclerotic disease antigens, therefore, it is necessary to test its serum reactivity to normal people and patients with various atherosclerotic diseases, evaluating whether it is associated with atherosclerotic diseases. In addition, there may be a common antigen among various atherosclerotic diseases, for example, MMP1 exists in both diabetic patients and cerebral infarction patients, so serum of patients with different atherosclerotic diseases should be strictly classified for screening; if the recombinant library is expressed on fungal yeast cells, it can improve the defects of bacterial post-transcriptional modification and have some degree of glycosylation and other modifications[3].

SEREX AND ATHEROSCLEROTIC DISEASE ANTIGENS

SEREX, as a direct serological screening method for proteins, is increasingly being used to screen serum markers that predict ischemic attacks in patients with atherosclerotic diseases [4], atherosclerosis is the main cause of atherosclerotic diseases such as ischemic stroke and coronary atherosclerotic diseases. Atherosclerosis is not only an inflammatory disease of the arteries, but also an autoimmune disease[5,6],the immune system is involved in the development of atherosclerosis [7-9], The process of atherosclerosis caused by dyslipidemia, obesity and infection as well as the progression of atherosclerotic

plaque is an immune response process, so the application of SEREX is of great significance for screening the antigens of atherosclerotic disease[10]. At present, the main applications in atherosclerotic diseases are ischemic cerebrovascular diseases and cardiovascular diseases (CVD).

Ischemic cerebrovascular disease

Transient ischemic attack (TIA) and cerebral infarction (CI) are both clinically common ischemic cerebrovascular disease. Specific miRNA have shown significant regulatory effects prior to the onset of acute and irreversible brain injury, and they may enable early diagnosis of ischemic stroke or identification before it progresses to acute cerebral infarction[11]. HaoWang[12] et al. found that MMP1、CBX1 and CBX5 are candidate antigens in TIA and CI patients by SEREX, there are antibodies against these antigens in the serum. Serum levels of antibody against MMP1, CBX1 and CBX5 may be useful tools for diagnosing TIA and predicting the onset of aCI. TIA is a predictor of cerebral infarction (CI), and the early diagnosis of TIA is of great significance for preventing CI. MMP1、CBX1 and CBX5 may be related to the pathophysiological mechanisms of TIA and CI [13]. YoichiYoshida[14]et al. screened the human aortic endothelial cell cDNA library through SEREX, and found 9 antigens: RPA2、TUBB2C、 ATP2B4、BMP-1、MMP1、DHPS、SH3BP5、PDCD11、CBX1, RPA2 and PDCD11 respectively suggest atherosclerotic lesions[14] and cerebral ischemia, the level of elevated PDCD11-Abs in serum is an independent predictor of TIA[15], elevated serum antibody levels of anti-replication protein A2 antibodies (RPA2Abs) and anti-programmed cell death 11 antibodies (PDCD11Abs) may be diagnostic markers for TIA or CI. ToshioMachida[14] et al. screened cDNA expression library and identified six candidate antigens: BMP1, LGALS9, PPP1R15A, RPA2, SC65, and WD36, among them, the RPA2-Abs have higher correlation with ischemic stroke. The level of RPA2-Abs is an independent and strongly correlated risk factor for ischemic stroke, and RPA2-Abs may be a biomarker for the assessment of the risk of ischemic stroke. Takaki Hiwasa[16] et al. found that adiponectin secreted by adipocytes has a multipotent and anti-atherosclerosis effect, and circulating adiponectin levels are helpful to evaluate the progress of CI.

Cardiovascular disease (CVD)

Atherosclerosis is the primary cause of cardiovascular disease and occurs to a large extent after atherosclerotic plaque damage and/or rupture. UlfdeFaire [17] et al. have demonstrated by SEREX that anti-PC IgM is negatively correlated with the development of atherosclerosis in patients with hypertension, low level of anti-PC IgM independently predict the development of CVD, anti-PC IgM may become a new risk marker for CVD; JohanFrostegard[18]et al. found that anti-PC IgM has anti-inflammatory properties, and low level of anti-PC IgM can lead to the occurrence of chronic inflammatory diseases, Such as atherosclerosis, in which oxidation and/or inflammatory phospholipids play a role, low level of anti-PC IgM can predict the development of myocardial infarction, and the level of anti-PC IgM may be a new diagnostic tool for atherosclerotic disease; HyeSeonJeong [19]et al. found that circulating miR-212 serves as a marker of atherosclerosis, miR-212 has a synergistic effect with the three standard cardiovascular risk markers HbA1c, HDL-C and lipoprotein (a), Mir-212 improves the evaluation ability of the combined three cardiovascular risk markers[20].Cardiovascular risk markers HbA1c, HDL-C, and lipoprotein (a) are independent predictors [21], and miR-212 is a useful circulatory marker that independently or collaboratively improves the ability to assess the presence of atherosclerosis; In the patients with cardiovascular disease, RPA2、TUBB2C、ATP2B4、BMP-1、MMP1、DHPS、SH3BP5, The levels of serum antibodies against these antigens were significantly higher than that of healthy donors[14]. Circulating adiponectin levels are helpful to predict coronary artery disease(CAD) progression, and Serum level of anti-adiponectin antibody may be a common marker of atherosclerotic diseases[16].

Atherosclerosis is the pathological basis of atherosclerotic diseases such as ischemic stroke and coronary heart disease,under the influence of risk factors such as dyslipidemia, diabetes, hypertension, obesity and infection, arterial intima is damaged, the accumulation of lipids and complex sugars followed, Subsequently, fibrous tissue hyperplasia and calcareous deposition, and then gradual degeneration and calcification of the middle layer of the artery, and atherosclerosis develops into atheromatous plaque, because of the long-term presence of risk factors, local atheromatous plaque and systemic immune responses are still present for a long time, most plaques continue to develop and cause thickening, hardening, and narrowing of the arteries, Finally, it leads to cardiovascular and cerebrovascular events. Almost every link of the occurrence of coronary heart disease is closely related to immune response, these responses include cellular immunity、 humoral immunity and all the links of the immune system, coronary heart disease is not only a circulatory disease, but also a disease of the immune system[10]. Therefore, it is of great significance to use SEREX to screen the antigens ,which serve as markers for early diagnosis of such diseases ,taking timely preventive measures to prevent the occurrence of serious cerebrovascular and cardiovascular events.

SUMMARY AND PROSPECT

SEREX has been come out since 1995, It has been continuously enriched and improved by many researchers.Application of SEREX are becoming more widespread and mature, it was originally used in oncology, then applied in the field of autoimmune diseases, Nowadays, it has been applied to atherosclerotic diseases and many new disease antigens have been obtained, these disease antigens as biomarkers have a good prospect in the early diagnosis of atherosclerotic diseases, the immune response in the progression of atherosclerotic disease provides a theoretical basis for the development of new serological detection methods, atherosclerotic disease antigen genes screened and identified by SEREX can be used as molecular targets for early diagnosis and precision therapy of atherosclerotic diseases,it provides a possibility for developing specific guidance of individualized treatment plan.

Analysis of antigen-antibody responses to screening for disease-associated antigens, it is expected that high sensitivity and high specificity diagnostic chips can be developed in the future for the early detection of atherosclerotic diseases, such as acute cerebral infarction(aCI) and acute myocardial infarction (AMI), it can increase the diagnostic methods of atherosclerotic diseases in clinical application, using this diagnostic chip to screen high-risk groups, to a certain extent, the early immunological diagnosis and early prevention of atherosclerotic diseases such as aCI and AMI can be achieved,and thus suppress or slow down the occurrence of the above diseases. In addition, inspired by "The combination of tumor-associated antigens can optimize the sensitivity and specificity of oncological diagnosis in specific tumor patients[22,23]", using existing discovered atherosclerotic disease antigens,it is a good direction to select the most favorable combination of antigens to detect the early onset of diseases,it can improve the sensitivity and specificity of diagnosis.The specific antigens or specific antigen combinations of atherosclerotic diseases may be used to prepare vaccines, and the screened autoantigens are also expected to develop new immunotherapy methods, which have a good application prospect, but further research is needed.

REFERENCES

1. U. Sahin, O. Tureci, H. Schmitt, B. Cochlovius, T. Johannes, R. Schmits et al.Human neoplasms elicit multiple specific immune responses in the autologous host.Proc Natl Acad Sci U S A,1995,92:11810-11813.
2. Shanfen Hao.SEREX technology and its application in autoimmune disease research. Clinical Medical Engineering,2017,24:583-584.
3. Axel Mischo, Andreas Wadle, Kristin Wätzig, Dirk Jäger, Elisabeth Stockert, Darren Santiago et al.Recombinant antigen expression on yeast surface (RAYS) for the detection of serological immune responses in cancer patients.Cancer immunity,2003,3:5-5.
4. Toshio Machida, Motoo Kubota, Eiichi Kobayashi, Yasuo Iwadate, Naokatsu Saeki, Akira Yamaura et al.Identification of stroke-associated-antigens via screening of recombinant proteins from the human expression cDNA library (SEREX).Journal of translational medicine,2015,13:71-71.
5. E. Matsuura, F. Atzeni, P. Sarzi-Puttini, M. Turiel, L. R. Lopez ,M. T. Nurmohamed.Is atherosclerosis an autoimmune disease?BMC medicine,2014,12:47.
6. P. Libby, P. M. Ridker ,G. K. Hansson.Progress and challenges in translating the biology of atherosclerosis.Nature,2011,473:317-325.
7. F. Schaftenaar, V. Frodermann, J. Kuiper ,E. Lutgens.Atherosclerosis: the interplay between lipids and immune cells.Curr Opin Lipidol,2016,27:209-215.
8. M. Lacy, D. Atzler, R. Liu, M. De Winther, C. Weber ,E. Lutgens.Interactions between dyslipidemia and the immune system and their relevance as putative therapeutic targets in atherosclerosis.Pharmacol Ther,2019,193:50-62.
9. A. Gisterå ,G. K. Hansson.The immunology of atherosclerosis.Nat Rev Nephrol,2017,13:368-380.
10. Ke fei Dou. Advances in the immunological mechanism reasearch of coronary heart disease. Chinese Circulation Journal,2003,75-77.
11. C. Eyileten, Z. Wicik, S. De Rosa, D. Mirowska-Guzel, A. Soplinska, C. Indolfi et al.MicroRNAs as Diagnostic and Prognostic Biomarkers in Ischemic Stroke-A Comprehensive Review and Bioinformatic Analysis.Cells,2018,7:
12. Hao Wang, Xiao-Meng Zhang, Go Tomiyoshi, Rika Nakamura, Natsuko Shinmen, Hideyuki Kuroda et al.Association of serum levels of antibodies against MMP1, CBX1, and CBX5 with transient ischemic attack andcerebralinfarction.Oncotarget,2017,9:5600-5613.
13. Hao Lu,Hao Wang,Xiaomeng Zhang,Jiyue Liu,Yalan Li,Takaki Hiwasa et al. Identification of serological antigens by recombinant cDNA expression cloning for TIA. Journal of Jinan University Natural Science & Medicine Edition, 2018 . 39(1): 61 − 65.
14. Y. Yoshida, T. Hiwasa, T. Machida, E. Kobayashi, S. Mine, J. Matsushima et al.Elevation of Autoantibody in Patients with Ischemic Stroke.Neurologia medico-chirurgica,2018,58:303-310.
15. Yoichi Yoshida, Hao Wang, Takaki Hiwasa, Toshio Machida, Eiichi Kobayashi, Seiichiro

Mine et al.Elevation of autoantibody level against PDCD11 in patients with transient ischemic attack.Oncotarget,2017,9:8836-8848.
16. T. Hiwasa, X. M. Zhang, R. Kimura, M. Ohno, P. M. Chen, E. Nishi et al.Elevated Adiponectin Antibody Levels in Sera of Patients with Atherosclerosis-Related Coronary Artery Disease, Cerebral Infarction and Diabetes Mellitus.Journal of circulating biomarkers,2016,5:8.
17. Ulf De Faire ,Johan Frostegård.Natural antibodies against phosphorylcholine in cardiovascular disease.Annals of the New York Academy of Sciences,2009,1173:292-300.
18. Johan Frostegård.Low level natural antibodies against phosphorylcholine: a novel risk marker and potential mechanism in atherosclerosis and cardiovascular disease.Clinical immunology (Orlando, Fla.),2010,134:47-54.
19. [Hye Seon Jeong, Jee-Yeon Kim, Seo Hyun Lee, Junha Hwang, Jong Wook Shin, Kyu Sang Song et al.Synergy of circulating miR-212 with markers for cardiovascular risks to enhance estimation of atherosclerosis presence.PloS one,2017,12:e0177809-e0177809.
20. Mark A. Hlatky, Philip Greenland, Donna K. Arnett, Christie M. Ballantyne, Michael H. Criqui, Mitchell S. V. Elkind et al.Criteria for evaluation of novel markers of cardiovascular risk: a scientific statement from the American Heart Association.Circulation,2009,119:2408-2416.
21. J. A. Jarmul, M. Pignone ,M. J. Pletcher.Interpreting Hemoglobin A1C in Combination With Conventional Risk Factors for Prediction of Cardiovascular Risk.Circ Cardiovasc Qual Outcomes,2015,8:501-507.
22. Carlos A. Casiano, Melanie Mediavilla-Varela ,Eng M. Tan.Tumor-associated antigen arrays for the serological diagnosis of cancer.Molecular & cellular proteomics : MCP,2006,5:1745-1759.
23. Xiaoxiao Zuo, Ling Chen, Lifeng Liu, Zhe Zhang, Xiaojin Zhang, Qing Yu et al.Identification of a panel of complex autoantigens (LGALS3, PHB2, MUC1, and GK2) in combination with CA15-3 for the diagnosis of early-stage breast cancer.Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine,2016,37:1309-1317.