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Medical Applications of High-throughput Protein Arrays

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Abstract: *A solution containing the ligand is incubated with an immobilized protein of interest on a solid support. Unbound ligands are washed away, and bound ligands are detected. Protein affinity analysis can be used to characterize protein-protein interactions and to screen for inhibitors. Despite being low-throughput, protein microarray/S.E.L.D.I. mass spectrometry (Ciphergen, Fremont, CA, U.S.A.) has been used for differential analysis and protein marker discovery in infectious diseases and cancer. Protein sequencing methods can accurately differentiate ovarian cancer from non-cancers in serum by using proteomic approaches for high-risk and general populations. It can be used to screen for cancer and autoimmune diseases like systemic lupus erythematosus. Thus, high-throughput sequencing technology is widely used in various fields of laboratory medicine, and if some methods and techniques are combined, test results can be accurate and specific.*

Keywords: *High-throughput protein arrays; Molecular biology; Proteomics; Immunological assays*

1. Introduction

The field of molecular biology has been incredible advancements in this century, with new sequencing technologies and updated polymerase chain reaction instruments. Molecular biology has seen incredible advancements in this century. New sequencing technologies and updated polymerase chain reaction instruments have allowed major breakthroughs in the field. These advancements have led to a better understanding of the molecular basis of disease and the development of new treatments and therapies.

However, the concept of high-throughput protein came into being with the introduction of proteomics. High-throughput protein sequences are also known as high-throughput protein chips, and high-throughput protein arrays allow miniaturization and parallel analysis of many diagnostic markers in complex samples. Automated colony picking and gridding is a popular method for expressing and screening cDNA or antibody libraries as clone arrays. This method has the advantage of being high throughout, meaning that many colonies can be processed quickly and efficiently. Protein arrays offer high sensitivity and specificity, which makes them ideal for applications that require accurate detection, such as antigen detection and tumor marker screening. Therefore, protein array technology can be used in many areas of medical testing, such as immunoassays, biochemical tests, and histopathological tests. In this essay, we present the application of this technology in different fields.

2. Application of high-throughput protein sequencing technology to immunological assays

The human immunological network is complex and known to play roles in several disease states, including bacterial and viral pathogenesis, tumorigenesis and metastasis, and autoimmune disorders. [1,2,3] Upon invasion of the body by a pathogen, T cells are involved in cellular immunity that rapidly recognizes antigens; B lymphocytes are involved in humoral immunity, producing antibodies and generating an antigen-antibody response. It also induces the release of immune factors, such as interleukins and interferons. These molecules help to regulate viral replication and also promote tumor cell death. In addition, this protein can also help to regulate the inflammatory response. Ultimately, these properties make this protein an important tool in inhibiting tumorigenic growth.[4]

In past studies, proteomic and proteomic interactions in the adaptive immune response were determined using protein sequencing techniques. High-throughput proteomic sequencing technology significantly impacts immunological examinations and is used for serological analysis in antibody

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reactions. This research has ranged from infectious diseases to cancer to autoimmune diseases.[5] The current approach is to perform high-throughput PCR amplification of each predicted open reading frame, homologous recombinant expression of the gene vector in vivo, and imprinting them onto nitrocellulose membranes to form the proteome sequences. This method is used to analyze serum antibody specificity in subjects inoculated with cowpox virus for high throughput assessment of humoral immunity; and the reaction of Mycobacterium tuberculosis with the proteome. [6] However, this technique has limitations, as it can only provide information on the protein in a sample. They cannot provide information on the function of these proteins.

In recent years, high-throughput proteome sequencing technologies can be used in the antigen detection of coronavirus. Unlike the traditional way of using ELISA to determine specific antibodies and antigens, high-throughput protein sequencing is more specific and sensitive. Traditional ELISA methods can only determine the presence or absence of antigens, a general detection strategy; high-throughput protein sequencing can detect the presence or absence of antigens, the type of viral spike, etc., a complete detection strategy. Based on this detection strategy, clinicians can further characterize the antigens and provide more precise treatment to patients. As the level of diagnostics has evolved, some researchers have combined ELISA and high-throughput protein sequencing technologies to integrate a fluorescent assay analyzer. Not only can the antigen of SARS-CoV-2 be measured, but also immunoglobulins such as IgM and IgG. This multiplex assay has been evaluated for medical devices in China and C.E. marked in Europe.[7] Using high throughput, multiplex screening assay, [8,9]it is possible to classify different virus subtypes and measure surface antigens such as S and N proteins.

3. Application of high-throughput protein sequencing technology to hematology assays

High-throughput protein sequencing technology can also be used in hematology tests, often in diagnosing leukemia and assessing treatment effectiveness. Leukemia, also known as blood cancer, is a clinically malignant disease. The pathogenesis of the disease is usually due to abnormalities in the cloning of hematopoietic stem cells, the proliferation of white blood cells in the blood, and abnormal differentiation due to viral, chemical, radioactive, and genetic factors. The disease can be divided into acute leukemia and chronic leukemia, depending on the progression of the disease, granulocytic leukemia, and lymphocytic leukemia, depending on the type of white blood cells. Different types of leukemia depend on the chromosomal karyotype.

Nowadays, China is faced with an aging population, and it is expected that by 2035, China will enter a severe stage of population aging. Among elderly leukemia patients, the most common is chronic lymphocytic leukemia (CLL). Chronic lymphocytic leukemia(CLL) is a low-grade B cell malignancy characterized by the accumulation of mature CD5+/CD19+/CD23+ lymphocytes with low surface expression of an immunoglobulin monoclonal antibody.[10]The majority of patients develop recurrent disease after treatment and are considered refractory to standard therapies. Patients with this cancer usually have no symptoms or require only minor medical care. In a past study, several researchers used next-generation sequencing technologies [11,12]to study the genome of chronic lymphocytic leukemia (CLL). These studies have improved our understanding of the genetic changes that occur in CLL and how these changes contribute to the development and progression of the disease. Studies into the genetic changes of CLL have been incredibly beneficial in understanding how the disease develops and progresses. The changes that occur in the genes of those with chronic lymphocytic leukemia (CLL) contribute to the disease in a major way, and by studying these changes, we can learn more about how to treat and prevent the disease from occurring or progressing. CLL is a type of cancer that affects the blood and bone marrow, and it is the most common type of leukemia in adults. CLL's cause is unknown, but it is thought to be due to a combination of genetic and environmental factors. There is no cure for CLL, but treatments are available to help control the disease. By revealing altered protein sequence mutations that lead to the emergence of an unusual form of genomic complexity, this form is chromosome rupture [13], and chromosome rupture ends up with new genetic damage. [14] The resulting loss of chromosomes causes a disorder called chromosomal instability, which can cause disease in humans. This disorder occurs when two or more chromosomes are damaged together. It also happens when one or more genes are mutated, causing a defect in D.N.A. replication. In some cases, it can be caused by a combination of these events, such as a change in diet, stress, or age.

High-throughput techniques can also be used to diagnose and treat acute myeloid leukemia. The method of using high-throughput technology is informative for grading treatment and drug selection for patients. In addition, artificial intelligence (A.I.) has recently garnered attention in cancer therapy due to its ability to enhance drug discovery, development, and administration.[15] The application of genomics,

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epigenomics, transcriptomics, proteomics, and metabolomics in targeting leukemia with high-throughput technologies provides precise data for treatment and enables clinically accurate therapy. Discovery of genomic alterations by genome sequencing. Identifying all-trans retinoid acid (ATRA) and arsenic trioxide therapy for acute promyelocytic leukemia (A.P.L.) is a successful example. The majority (~95%) of the A.P.L. is characterized by ant(15; 17) (q22; q21) translocation resulting in the promyelocytic leukemia retinoid acid receptor alpha (PML-RAR-α) transcript of the fusion gene. The discovery of this translocation has clinical significance due to its responsiveness to arsenic trioxide and ATRA, which promote the degradation of PML-RAR-α. [16] ATRA and arsenic trioxide therapy can achieve complete remission in 85% to 90% of patients with not only newly diagnosed but also relapsed A.P.L. [17]

4. Application of high-throughput sequencing technology in histopathology

In traditional cancer diagnosis, pathological tissue diagnosis is the only gold standard for detecting tumors. However, this traditional diagnosis method only diagnoses cancer at the tissue and cellular level. It may make the diagnosis less accurate because of the inadequacy in the level of pathology technology. With the advancement of molecular diagnostic techniques, pathological diagnosis is not limited to the traditional way. The advancement of molecular diagnostic techniques has led to a change in how pathological diagnosis is conducted. Oncogenes can now be diagnosed by protein sequencing technology, which is more accurate and reliable than traditional methods. This new technology is changing how oncogenes are being diagnosed and providing more accurate and reliable results.

High-throughput protein sequencing technology can be used to find gene loci in disease-causing genes in tumors. Tissue microarray is a common method for tumor diagnosis in pathology, and the opening of tissue microarray solves the limitations of traditional techniques and enables "genome-scale" molecular pathology.[18] The tissue microarray is used for diagnostic and drug target discovery in genomics and in most cases, for research in the field of cancer. It is used in oncology research to analyze the frequency of molecular alterations in tumor specimens, to explore tumor progression, to determine prediction and prognosis, and to deliver targeted therapies. In the conventional pathological diagnosis, doctor stage and score tumors based on the size and extent of adjacent tissue involvement, lymph node involvement, and distant metastases. Using the tissue microarray(T.M.A.), we can quickly find tissue points, obtain digital images, and score quickly. In the previous studies, In previous studies, several researchers have demonstrated a good correlation between manual and automated scoring of HER-2 oncoprotein staining intensity on breast cancer T.M.A.s.[19]

The FISH technique can also be combined with tissue microarray to analyze mutations, translocations, and other abnormalities in gene expression, but it is more cumbersome to score tumors using the signal from this technique. Some researchers developed a confocal fluorescence microscope-based system with associated image analysis algorithms for automatically scoring FISH results on T.M.A. slides.[20]The system was used to score FISH results from two different T.M.A. studies involving prostate cancer and the other bladder cancer. The system was accurate in scoring the FISH results from both studies, and the researchers believe that it could be used to score FISH results from other types of cancer as well.

5. Summary

Sequencing the human genome provides a large number of targets for drugs[21] and promotes the joint development of the medical and pharmaceutical industries, achieving a win-win situation. Based on the array order library, we can see that cDNA clone arrays are used for high-throughput expression and analysis of unknown proteins.[22] According to using the above methods, antibodies were screened and selected from phage display libraries using bacterial clone arrays.[23]Protein affinity assays are used to analyze interactions between proteins such as antibodies, receptors, or enzymes with other proteins, peptides, low molecular weight compounds, oligosaccharides, or D.N.A. In order to perform an assay, the protein of interest is immobilized on a solid support and incubated with a solution containing the ligand. After binding, the unbound ligand is washed away, and the bound ligand is detected. Protein affinity assays are useful for the characterization of protein-protein interactions and can be used to screen for inhibitors of these interactions. The Protein Chip/S.E.L.D.I. (surface-enhanced laser desorption/ionization) mass-spectrometry technology (Ciphergen, Fremont, CA, U.S.A.) is still low-throughput but has been used for differential profiling and protein marker discovery in the realm of infectious diseases and cancer.[24]Proteomic modalities may provide a more complete, proven cancer screening method for high-risk and general populations, such as using protein sequencing methods that can completely distinguish between cancer in the ovary and non-cancer in the serum.[25]High-

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throughput protein array technology can be used to screen for cancer and autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis. Therefore, high-throughput sequencing technology is used a lot in various fields of laboratory medicine, and if some methods and techniques are combined, the test results can be specific and accurate.

References

[1] Dunn, G. P., Old, L. J., & Schreiber, R. D. (2004). The immunobiology of Cancer Immunosurveillance and immunoediting. Immunity, 21(2), 137–148. https://doi.org/10. 1016/j. immuni. 2004.07.017

[2] Vesely, M.D. et al. (2011) "Natural innate and adaptive immunity to cancer," Annual Reviewof Immunology, 29(1), pp. 235–271. Available at: https://doi.org/10.1146/annurev-immunol-031210-101324.

[3] Stewart, T.J. and Abrams, S.I. (2008) "How tumours escape mass destruction," Oncogene, 27(45), pp. 5894–5903. Available at: https://doi.org/10.1038/onc.2008.268.

[4] Weaver, W.M. et al. (2013) Advances in high-throughput single-cell microtechnologies, Current Opinion in Biotechnology. Elsevier Current Trends. Available at: https://www.sciencedirect. com/science/article/pii/S0958166913006654?via%3Dihub (Accessed: October 28, 2022).

[5] Kingsmore, S.F. (2006) "Multiplexed protein measurement: Technologies and applications of protein and antibody arrays," Nature Reviews Drug Discovery, 5(4), pp. 310–321. Available at: https:// doi.org/10.1038/nrd2006.

[6] Reddy, S.T. and Georgiou, G. (2011) "Systems analysis of adaptive immunity by utilization of high-throughput technologies," Current Opinion in Biotechnology, 22(4), pp. 584–589. Available at: https://doi.org/10.1016/j.copbio.2011.04.015.

[7] Lin, Q. et al. (2020) "Microfluidic immunoassays for sensitive and simultaneous detection of IGG/IGM/antigen of SARS-COV-2 within 15 min," Analytical Chemistry, 92(14), pp. 9454–9458. Available at: https://doi.org/10.1021/acs.analchem.0c01635.

[8] de Assis, R.R. et al. (2020) "Analysis of SARS-COV-2 antibodies in COVID-19 convalescent blood using a coronavirus antigen microarray." Available at: https://doi.org/10.1101/2020.04.15.043364.

[9] *Khan, S. et al. (2020) "Analysis of serologic cross-reactivity between common human coronaviruses and SARS-COV-2 using coronavirus antigen microarray." Available at: https://doi.org/10. 1101/2020.03.24.006544.*

[10] M.P.; P.T.L.S. (no date) Chronic lymphocytic leukemia: Prognostic factors and impact on treatment, Discovery medicine. U.S. National Library of Medicine. Available at: https://pubmed.ncbi.nlm.nih.gov/21356166/

[11] Puente, X.S. et al. (2011) "Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukaemia," Nature, 475(7354), pp. 101–105. Available at: https://doi.org/10. 1038/ nature10113.

[12] Wang, L. et al. (2011) "sf3b1and other novel cancer genes in chronic lymphocytic leukemia," New England Journal of Medicine, 365(26), pp. 2497–2506. Available at: https://doi.org/10. 1056/ nejmoa1109016.

[13] Stephens PJ;Greenman CD;Fu B;Yang F;Bignell GR;Mudie LJ;Pleasance E.D.;Lau KW;Beare D;Stebbings LA;McLaren S;Lin ML;McBride DJ;Varela I;Nik-Zainal S;Leroy C;Jia M;Menzies A;Butler AP;Teague JW;Quail MA;Burton J;Swerdlow H;Carter NP;Morsberger LA;Iacobuzio-D (no date) Massive genomic rearrangement acquired in a single catastrophic event during cancer development, Cell. U.S. National Library of Medicine. Available at: https://pubmed.ncbi. nlm.nih. gov/21215367/

[14] Landau, D.A. and Wu, C.J. (2013) "Chronic lymphocytic leukemia: Molecular heterogeneity revealed by high-throughput genomics," Genome Medicine, 5(5), p. 47. Available at: https://doi.org/ 10.1186/gm451.

[15] Ho, D. (2020) "Artificial Intelligence in cancer therapy," Science, 367(6481), pp. 982–983. Available at: https://doi.org/10.1126/science.aaz3023.

[16] Zhang, X.-W., Yan, X.-J., Zhou, Z.-R., Yang, F.-F., Wu, Z.-Y., Sun, H.-B., Liang, W.-X., Song, A.-X., Lallemand-Breitenbach, V., Jeanne, M., Zhang, Q.-Y., Yang, H.-Y., Huang, Q.-H., Zhou, G.-B., Tong, J.-H., Zhang, Y., Wu, J.-H., Hu, H.-Y., de Thé, H., … Chen, Z. (2010). Arsenic trioxide controls the fate of the PML-RARA oncoprotein by directly binding PML. Science, 328(5975), 240–243. https://doi.org/10. 1126/science.1183424

[17] Zhou, G.-B., Zhao, W.-L., Wang, Z.-Y., Chen, S.-J., & Chen, Z. (2005). Retinoic acid and arsenic for treating acute promyelocytic leukemia. PLoS Medicine, 2(1). https://doi.org/10.1371/ journal. pmed.0020012

ISSN 2706-6819 Vol.5, Issue 1: 34-38, DOI: 10.25236/IJFM.2023.050106

[18] Kallioniemi, O.-P. (2001). Tissue microarray technology for high-throughput molecular profiling of cancer. Human Molecular Genetics, 10(7), 657–662. https://doi.org/10.1093/hmg/10.7.657
[19] Bucher, C., Tohorst, J., Kononen, J., Haas, P., Askaa, J., Godtfredsen, S.E., Bauer, K.D., Seelig, S., Kallioniemi, O.P. and Sauter, G. (2000) Automated, high-throughput tissue microarray analysis for assessing the significance of Her-2 involvement in breast cancer. J. Clin. Oncol., Annual Meeting, 2338.
[20] Bubendorf, L., Kononen, J., Barlund, M., Kallioniemi, A., Grigorian, A., Sauter, G., Dougherty, E.R. and Kallioniemi, O.P. (1999) Tissue microarray FISH and digital imaging: Towards automated analysis of thousands of tumors with thousands of probes. Am. J. Hum. Genet, 65 (suppl.), 316.
[21] Walter, G., Büssow, K., Lueking, A., & Glökler, J. (2002). High-throughput protein arrays: Prospects for Molecular Diagnostics. Trends in Molecular Medicine, 8(6), 250–253. https://doi.org/10.1016/s1471-4914(02)02352-3
[22] Walter, G., Büssow, K., Cahill, D., Lueking, A., & Lehrach, H. (2000). Protein arrays for gene expression and molecular interaction screening. Current Opinion in Microbiology, 3(3), 298–302. https://doi.org/10.1016/s1369-5274(00)00093-x
[23] de Wildt, R. M., Mundy, C. R., Gorick, B. D., & Tomlinson, I. M. (2000). Antibody arrays for high-throughput screening of antibody–antigen interactions. Nature Biotechnology, 18(9), 989–994. https://doi.org/10.1038/79494
[24] Fung, E. T., Thulasiraman, V., Weinberger, S. R., & Dalmasso, E. A. (2001). Protein biochips for differential profiling. Current Opinion in Biotechnology, 12(1), 65–69. https://doi.org/10.1016/s0958-1669(00)00167-1
[25] Petricoin, E. F., Ardekani, A. M., Hitt, B. A., Levine, P. J., Fusaro, V. A., Steinberg, S. M., Mills, G. B., Simone, C., Fishman, D. A., Kohn, E. C., & Liotta, L. A. (2002). Use of proteomic patterns in serum to identify ovarian cancer. The Lancet, 359(9306), 572–577. https://doi.org/10.1016/s0140-6736(02) 07746-2