The Journal of Breast Health
meme sağlığı dergisi

The Journal of Breast Health 2010 Vol: 6 • No: 2
Meme Sağlığı Dergisi 2010 Cilt: 6 • Sayı: 2

REVIEW/DERLEME

GENOMICS AND PROTEOMICS METHODS AND THEIR VALUE IN BREAST CANCER DIAGNOSIS

Can Atalay

Genel Cerrahi, Ankara Onkoloji Hastanesi, Ankara

ABSTRACT

Genomics and proteomics are newly developing fields and becoming widely used in cancer research especially in cancer diagnosis. The aim is to review the role of genomics and proteomics to discover new breast cancer biomarkers for diagnosis with higher sensitivity and specificity. Studies reporting on potential biomarkers in breast cancer were included in this review. In the future, platforms in genomics and proteomics will be used for the discovery of tumor specific genes and related proteins, which will lead to development of novel therapeutic targets leading to patient specific therapies.

Key words: cancer, diagnosis, genomics, proteomics

GENOMİK VE PROTEOMİK YÖNTEMLER VE MEME KANSERİ TANISINDAKI YERİ

ÖZET

Genomik ve proteomik yeni gelişen alanlardır ve kanser araştırmalarında, özellikle de kanser tanısında, sıklıkla kullanılır hale gelmektedir. Bu yazıda, meme kanseri tanısında daha yüksek özgünlük ve özgüllüğe sahip yeni belirteçlerin bulunmasında genomik ve proteomikin rolünün gözden geçirilmesi amaçlanmıştır. Meme kanserinde potansiyel önemi olan belirteçleri bildiren çalışmalar bu derlemede incelenmiştir. Gelecekte, genomik ve protemik alanlarında kullanılan yöntemler, hastaya özgün tedaviyi sağlayacak yeni hedeflerin geliştirilmesinde tümöre özgün gen ve proteinlerin bulunması için kullanılacaktır.

Anahtar sözcükler: kanser, tanı; genomik, proteomik

1. Introduction

The biology of cancer remains poorly understood and individual diagnostic factors provide limited information about the diagnosis of the disease. Genomics and proteomics are newly developing fields and becoming widely used in cancer research especially after the completion of human genome sequencing project. Genomic and proteomic technologies have evolved; rapidly accelerating the rate of clinical cancer research. The potential applications of genomics and proteomics in the field of oncology, especially in cancer diagnosis, are virtually unlimited. Systematic investigation of expression patterns of thousands of genes or proteins in tumors and their correlation to specific phenotypes might provide the basis for an improved description of cancer. Although there is a continuing progress in cancer therapy, early diagnosis of cancer undoubtedly remains the most important factor in improving long-term survival of cancer patients. The identification of tumor markers suitable for the early diagnosis of cancer holds great promise to improve the clinical outcome of patients. Emerging technologies in the fields of genomics and proteomics have enabled researchers to understand the biology of cancer and to discover new biomarkers for cancer diagnosis, treatment response, and clinical outcome with higher sensitivity and specificity. Reliable biomarkers will facilitate novel therapeutic discoveries and improve patient selection for clinical trials. Replacement of low throughput techniques such as Northern, Southern, and Western blotting, reverse-transcription polymerase chain reaction, in-situ hybridization, immunohistochemistry, and two-dimensional gel electrophoresis with high throughput techniques such as DNA, RNA, protein, and tissue microarrays, and mass spectrometry has been the mainstay of recent achievements in cancer diagnosis. The role of these new methods in the diagnosis of breast cancer are evaluated in this review.

2. Genomics

Genomics is related to comprehensive analysis of expressions of large number of genes in a single experiment. Recent developments in genomic technologies allow the evaluation of thousands of genes and the assessment of interactions between these genes at the same time. The basic premise of these emerging technologies is the simultaneous quantification of gene expressions and combination of these results into prediction scores that aid to establish clinical diagnosis more accurately than any single gene expression. Genomic technology has made a major impact in the understanding of cancer biology and is now widely used in clinical cancer research. Microarray technology has recently replaced low-throughput techniques such as Southern blot (hybridization of DNA-DNA) and Northern blot (hybridization of RNA-DNA).

Gönderilme Tarihi: 13 Temmuz 2009 • **Revizyon Tarihi:** 26 Ağustos 2009 • **Kabul Tarihi:** 28 Ağustos 2009

2.1 Platforms in genomics

Low-density and high-density arrays are the principally used methods in the field of genomics. In addition, real time quantitative reverse-transcription polymerase chain reaction (qRT-PCR) helps further defining gene sets described in microarray experiments. DNA microarrays are the primary tools used to perform gene profiling in cancer. Brown et al. have pioneered the development of high-density microarrays, which now have become the key technology in biomedical research (1). The process mainly consists of three steps: (1) array construction, (2) sample preparation and hybridization, and (3) image and data analysis. A microarray is a solid support (nylon or glass) containing thousands of different gene fragments called probes, which consist of either oligonucleotides or complementary DNA (cDNA). Study sample containing a mixture of unknown RNA or DNA is named as target and hybridizes with the probes if it contains the genes present on the microarray. The probe is immobilized on dot blots and microarrays, and, in contrast to this, target is immobilized on Southern and Northern blots. Probes labeled with fluorescence or radioactivity emits signals. These signals, present in each probe, are detected, quantified, and specialized hardware and software are utilized to give gene expression profiles. Data can be used to differentiate between normal and tumoral tissues, to gather information about the prognosis of the patients, and to discover the genes playing key roles in cancer biology for further manipulation in treatment.

The main difference between low-density and high-density arrays lies in the number of genes or samples studied in a single experiment. Dot blots are low-density arrays and are produced manually by spotting gene specific probes on a membrane. Labeled targets are added onto dot blots for hybridization and visualization via autoradiography. With the development of automated systems, spotting thousands of DNA fragments onto a single array became possible. Current technology enables the placement of over 50,000 elements on standard glass microscope slides. The most commonly used DNA microarrays can be categorized according to their method of manufacturing into two groups as robotically spotted microarrays and microarrays produced by synthesizing the DNA probe directly on the supporting material. Early microarrays deposited PCR amplified cDNA clones as probes, which resulted in variability in probe sequence between different clones. Recently developed microarrays utilize synthetic oligonucleotides as probes, but this requires information on gene sequence. In addition, cDNA quantity may change between various prints when spotted cDNA microarrays are used. These microarrays are analyzed by hybridizing two differently labeled targets and one of these targets is a reference RNA sample that is used as an internal control. In contrast, other group of microarrays works on a single sample instead of a mixture of two samples and the ratio of gene expression of two samples is compared. These microarrays contain short (25-mer) oligonucleotides directly synthesized on the substrate with photolithography method (2). Oligonucleotides comprise of probes, which contain a transition at the 13th base used to detect non-specific hybridization. This method of production decreases the possibility of variation due to the printing procedure allowing for one-color detection and comparisons to be made between arrays.

Low-density arrays and RT-PCR are utilized as validation techniques when a limited number of genes are determined to play a role in cancer with high throughput methods. Low-density arrays can be divided into two categories as custom-made spotted cDNA or oligonucleotide arrays with a limited number of genes and high throughput microfluidic cards based on real-time PCR assay. Custom-made low-density arrays resemble high-density arrays with more tailored gene content. On the other hand, high throughput microfluidic cards are composed of 384-well plates containing reagents and probes required for RT-PCR manufactured using automated systems. These arrays provide less experimental variations with higher standardization. However, for low-density arrays to be cost effective, hundreds of genes determined in high-density microarrays should be evaluated.

Another platform used in genomics studies is qRT-PCR, which is commonly utilized to validate a small number of genes in a small number of samples. This method requires 5-10 ng of RNA and detects the quantitative relationship between the amount of target sample and PCR product. This relationship can be determined "real-time" and the term "real-time" PCR denotes to the detection of PCR products as they accumulate. Available qRT-PCR systems utilize a set of primers and fluorogenic probes and the amount of fluorescence is measured at each amplification cycle, providing information on "real time" changes in the amplification product. Identification of the PCR cycle where exponential growth phase starts enables accurate quantization of gene expressions in study sample. However, there are risks of cross-contamination and experimental variations whenever this method is applied and this is reduced by optimization of each reaction with new primers. PCR-based low-density arrays can be widely used in laboratories at a reduced cost and time.

2.2 Genomics applications in breast cancer

In breast cancer, three genes were identified to express differentially between abnormal (atypical ductal hyperplasia, ductal carcinoma in situ, and invasive ductal carcinoma) and normal breast cells. Two of these genes are highly expressed in cancer cells and are coding for cytosine-rich intestinal protein 1 and hematological and neurological expressed sequence 1. The other gene is coding for the second epithelium restricted Ets transcription factor and is expressed at lower levels. Although previously mentioned studies suggested a few new biomarkers for breast cancer, the results reported in these studies should be cautiously evaluated due to the lack of standardization in most of them.

3. Proteomics

Proteomics can be defined as the detection, identification, and quantification of all proteins present in a particular tissue, organ, and organism to provide accurate and comprehensive data

about that system. Proteomics elucidates the properties of proteins, which cannot be understood by analyzing gene expressions such as post-translational modifications, compartmentalization of proteins, and formation of multi-protein complexes. Complete sequencing of human genome has led to the assembly of protein databases, which increased the speed of developments in proteomics research. Although there are about 20,000-30,000 genes in the human genome, due to alternative splicing and sequence deletions, human proteom consists of approximately a million different proteins which makes proteomic research even more difficult. However, methods used in proteomics allow the validation of multiple markers at once, greatly decreasing the study time.

3.1 Platforms in proteomics

There are various techniques utilized in the field of proteomics. Recent advances in technology have increased the resolution, accuracy, and speed of separation of peptide and protein mixtures to smaller number of proteins per fraction using chromatographic techniques, labeling and detection of proteins and antibodies using multi-color fluorophores, imaging equipment, computer software, and analyzing clinical samples without any extensive preparation with high throughput capacity (3). Enzyme-linked immunosorbent assay (ELISA) and immunohistochemistry (IHC) are standard methods in clinical laboratories whereas Western blot and immunoprecipitation are frequently used in basic science laboratories. Two-dimensional (polyacrylamide) gel electrophoresis was the most commonly used method to study differences in protein expression between two samples such as cancer and normal tissue. It sequentially separates proteins by their charge and molecular weight, but this technology cannot effectively separate and distinguish proteins below 10 kDa. In addition, it is a very labor-intensive method, and has limited resolution for the analysis of large numbers of proteins. Each protein has to be excised from the gel and the peptide fragments analyzed using mass spectrometry (MS). Although two-dimensional gel electrophoresis cannot separate complex mixture of proteins, subsequent protein identification can be accomplished. In addition, high-performance liquid chromatography, two-dimensional liquid chromatography, or capillary electrophoresis uses columns or multiple capillary loops to separate proteins on the basis of size and charge.

Various ionization techniques such as matrix-assisted laser-desorption and ionization (MALDI), surface-enhanced laser-desorption and ionization (SELDI), and electrospray ionization have revolutionized the detection, identification, and structural characterization of proteins. Although these techniques enable the measurement of small protein molecules, it is possible to measure molecular weights above 200 kDa by first fragmenting the proteins. Rapid expansion of gene and protein databases has allowed the identification of proteins with greater ease. MS techniques analyze peptides, proteins, and polynucleotides as ions and distinguish them based on mass-to-charge ratio (m/z). Protein chips used in SELDI MS contain multiple spots with varied surfaces including hydrophobic, ion exchange, and metal affinity binding surfaces or normal chromatographic surfaces. MS output is shown as a chromatographic pattern with peaks at a given m/z. The resolution of MS unit directly correlates with its sensitivity. MS resolution capability varies between older and newer machines, proteomic chips containing different chromatographic surfaces, and different bioinformatics programs that reveal other discriminatory peaks.

Mass spectrometers are generally composed of three devices: (1) an ionization device which volatizes and ionizes the sample, (2) a mass analyzer which separates ions depending on their m/z ratios, and (3) a detector which detects ions after separation. After the separation of proteins or peptides, they must be ionized into a gas phase before MS analysis. Liquids can undergo electrospray ionization and MS analysis. On the other hand, solids can be ionized by MALDI or SELDI. MALDI technique utilizes a small volume of sample (<1µL) and digested proteins are mixed with an organic acid matrix. Molecular ratio of matrix to sample should be in the range of 5000-10000 for a better analysis. Upon drying, sample and matrix co-crystals form and change into an ionized form when irradiated by an ultraviolet laser. As a result, singly charged ion species are formed and ions accelerate through an electrical field in a time of flight MS which seperate them by their m/z. As the ions reach the detector at different times, a peptide mass profile is created reflecting the protein composition of the sample. MALDI analysis is well suited for resolution of proteins <20 kDa. One way of identification of proteins is by comparing the peptide mass profile to masses published in protein databases. However, there are certain limitations with MALDI technique such as signal background problems resulting from contaminants, which have hindered it from being used as a high-throughput screening tool for proteins in complex biological samples.

In order to overcome the limitations related to MALDI, SELDI method has recently been developed for protein analysis and it was first described by Hutchens and Yip (4). The SELDI method captures proteins from various body fluids on diverse biochip surfaces using modified chromatographic techniques. Biochip surfaces can vary due to their chemical properties as hydrophobic, ionic, and immobilized metal affinity capture and due to biochemical properties as antibody, DNA, enzyme, receptor, and drug covered surfaces (5). Proteins are combined with energy absorbing molecules at the surface of the biochip and pulsed with a laser into a time of flight MS. Proteins interact differently with different surfaces and this results in different mass spectra. A disadvantage of SELDI method is its inability to identify individual proteins from mass spectra and this is because SELDI method can only analyze small intact proteins. As a whole, advantages of MS technology are faster evaluation of small amount of protein samples, simple sample preparation, analysis of complex mixtures, and better data analysis.

On the other hand, electrospray ionization analyzes samples in solution instead of on platforms. Pushing the sample solution through a thin needle biased at positive voltage, which faces a grounded sampling skimmer electrode forms ions. Spraying proc-

ess forms very small droplets which progressively desolvate liberating ions. For proteomic analysis, sources are operated with flow rates between 0.5-1.5 µL/s and 0.02-0.5 µL/s. In electrospray ionization, m/z distribution for ions is below 4000 and around 800-1000. Ions produced by electrospraying carry multiple charges, which makes the analysis of complex mixtures difficult.

Another tool used to analyze proteins is protein-microarrays, which rely on the same principles as their DNA counterparts. This method principally depends on binding of antibodies and/or antigens to glass microarrays allowing the simultaneous assessment of thousands of proteins. Protein binding is measured by comparative fluorescence, providing a high throughput ELISA (6). However, there is a need for specific and high-quality antibodies against proteins of interest, and specificity of these antibodies must be validated by Western blotting before use. These protein arrays can utilize biologic samples such as serum or plasma, nipple aspiration fluid, cell lysates, or the surface membranes of microdissected cells. Protein microarrays are categorized as forward-phase and reverse-phase arrays. In forward-phase arrays, capture molecules such as antibodies, nucleic acids, or peptides are immobilized onto glass surfaces, membranes, or hydrogels similar to DNA microarrays. Biofluid containing the target protein is incubated with the prefixed antibodies and detected using labeled secondary antibodies. Forward-phase arrays have the advantage of detecting many different proteins in one experiment. In reverse-phase arrays, a target sample containing a mixture of proteins is immobilized onto a glass slide and specific antibodies against target proteins are incubated over the slide. This method allows the detection of target proteins in a large number of samples as a high throughput assay. Depending on the specificity of antibody binding and sensitivity of labeling methods, detection of target proteins in the concentration range equivalent to 10 cells is possible.

Tissue microarray is another method, which is modernized recently and utilized in proteomics research (7). Tissue microarray has a lower cost and can be developed using archival tumor tissues. In this method, a new block of tissue is formed containing different tissue cores with a diameter of 0.6-2 mm and sections from tens to hundreds of paraffin embedded tissue specimens can be combined on single glass slide. Tissue microarrays prepared by this technique can be used for histochemical and immunohistochemical staining or in situ hybridization. Tissue microarrays eliminate slide-to-slide experimental variation, reduce the amount and the cost of antibodies, and scoring time for pathologists. In contrast to gene microarrays, only protein products of one gene can be studied at a time using tissue microarrays. However, tissue microarrays can be used as an adjunct to cDNA microarrays to understand the correlations between gene and protein expressions. There are a few points to be taken into account when using tissue microarrays. During the construction of tissue microarrays, representative parts of paraffin blocks should be chosen and, in heterogeneous tumors, positive staining parts can be easily missed. In-situ and invasive lesions cannot be easily distinguished due to the lack of surrounding structures. In addition, candidate proteins with specific and high quality antibodies should be known. During the scoring process of tissue microarrays, automated tools can be used to decrease the variations between the pathologists (8). When formalin fixed tissues are used, recovery of intact or good quality genomic and proteomic material is difficult due to intense cross-linking between biomolecules induced by formalin. For this reason, tissue microarrays were constructed from frozen tissues recently and are called cryo-tissue microarrays.

3.2 Proteomics applications in breast cancer

Proteomics technology utilizes samples of serum, plasma, seminal plasma, saliva, urine, cerebrospinal fluid, nipple aspirate, ductal lavage fluids, and tumor tissue in the clinics in order to find novel biomarkers for cancer diagnosis with higher sensitivity and specificity. Protein profiling can be performed on complex mixtures from tissue extracts or biofluids. For cancer diagnosis, protein profiles are obtained from control and patient samples and compared to detect significant protein patterns unique to each group. Protein profiling has greater power to discriminate between cancer patients and healthy individuals than identifying specific disease-related proteins.

Breast cancer is another cancer type evaluated for new biomarkers. The diagnosis of proliferative or pre-invasive lesions such as atypical hyperplasia or in situ carcinoma in the breast places the patients in a high-risk group, although the progression to invasive cancer is expected to occur in a small proportion of these patients. Defining the patients with high risk in a better way with recently developed molecular techniques will decrease the screening costs alleviating the anxiety of the individuals. SELDI-TOF MS was widely used to analyze proteins secreted by epithelial cells of the ductal system in the breast. Previous studies have reported different protein profiles for patients with and without breast cancer (9-11). In those studies using serum samples, diagnostic protein profiles showed sensitivities and specificities ranging between 76-93% and 90-93%, respectively (11-13). Li et al. studied 169 serum samples from patients with breast cancer, benign breast diseases, and healthy controls and identified three protein peaks that separate breast cancer patient from non-cancer individuals with 93% sensitivity and 91% specificity (11). These biomarkers were validated in a different set of serum samples from patients with breast cancer, benign breast diseases, and healthy controls and two of these biomarkers were identified to be complement component $C3a_{desArg}$ and a C-terminal-truncated form of $C3a_{desArg}$ (14). Proteomic analysis of normal breast tissue and ductal carcinoma in situ revealed a difference in protein profiles between the two tissues suggesting that ductal carcinoma in situ is a pre-invasive lesion (15). Varnum et al. identified 15 proteins that had been reported as potential biomarkers for breast cancer, but had not been previously identified in nipple aspirate fluid (16). Paweletz et al., similarly studying nipple aspirate fluid, detected two proteins unique to breast cancer and two proteins unique to normal samples (10). Sauter et al. reported on five differentially expressed proteins in nipple aspirate fluid samples and these proteins were

present in 75-84% of breast cancer patients in contrast to 0-9% in healthy controls (9). In another study, nipple aspirate fluids from breast cancer patients were compared and no significant differences were identified in protein expressions between the breast with intact breast carcinoma and the contralateral non-cancerous breast (17). However, nipple aspirate fluid analysis revealed several peaks that differentiate between both breasts of the cancer patients and healthy individuals. Li et al., in another study, using nipple aspirate and ductal lavage fluids, identified three protein peaks, which differentiate breast cancer patients from high-risk women. These peaks were found to correspond to human neutrophil peptides 1 to 3 and persistent elevation of these peptides in high-risk women may imply early onset of breast cancer (18). Besides their functional activities in antimicrobial immunity, human neutrophil peptide expression has been shown in various tumor tissues and cell lines affecting tumor growth in a concentration dependent manner (18). Acetyl-LDL receptor is another biomarker related to early diagnosis of breast cancer. Its decreased concentration in nipple aspirate fluid compared to normal breasts indicates a strong likelihood of breast cancer or precancerous lesions. Similarly, a concentration difference of this protein between the two breasts of an individual may indicate the presence of breast cancer in the breast with lower concentration. Recently, protein profiling from serum samples was reported to differentiate highly suspicious lesions on mammography, which will result in a decrease in the number of unnecessary breast biopsies (19).

4. Conclusion

There are several obstacles to be addressed before genomics and proteomics reach an optimal yield and be beneficial for the patients. The requirement of fresh or frozen tissue samples to protect and obtain high quality genetic material to use in high-throughput techniques limits their wide spread use. The facilities for immediate freezing of tissue samples are not readily available in all hospitals. Establishment of high quality sample banks with databases containing information about all clinical and histopathological characteristics of the patients will help to collect uniform samples and data in clinical trials. Developing better techniques in order to utilize paraffin-embedded tumor tissue can be another way to circumvent this problem. Several groups are working on isolating RNA from paraffin-embedded tissue sections and studying gene expression by microarrays and RT-PCR (20,21). Recently developed LCM technique is used to obtain tissue samples from paraffin-embedded tissue sections and it increases the probability of getting more homogenous cell populations for genomic and proteomic studies (22). During this procedure, tumor cells can be isolated from other cells in the tissue and tumor cell specific gene expressions are shown by microarrays. However, with LCM, data from stromal and other surrounding cells should be analyzed separately, increasing the cost of the study. LCM also increases tissue processing time and manipulation. It is feasible to reach the appropriate results without employing LCM, if a molecular signature to use as a biomarker is investigated. However, if the goal of the study is to identify the biological differences between pre-malignant, pre-invasive, and invasive cells, LCM is necessary for precision on cell type (23). Although LCM method can overcome the heterogeneity of cancer tissue samples, an alternative way could be to compare expression profiles of macro dissection samples with those of cell lines representing the different cancer types, namely virtual micro dissection (24). In addition, the amount of material required for the experiments should be kept to a minimum due to limited sources and this could be achieved as improvements in nanotechnology provide better instruments. Besides from technical issues, careful experimental design, clearly defined outcomes, and a large enough sample size for independent validation of the data will help to overcome the problems encountered in genomic and proteomic research. In addition, there are several limitations specific to proteomics platforms. Tissue and protein microarrays can only be used if specific antibodies for the candidate proteins are available. Proteomics is an evolving field for which procedures and equipments lack the type of standardization, which was achieved in microarray gene expression studies.

Another issue is the processing of the large amount of data obtained from the use of high-throughput techniques in both genomics and proteomics. Statistical data analyses are tremendously challenging. The number of measured variables always outnumbers the number of samples evaluated. In order to minimize the problems in statistical analyses, acquired data must be filtered according to the goals of the researcher. Development of methods for statistical evaluation, normalization, and filtering of the data are all areas of active research. Although the development of specialized software is continuing, there is also a need for expert statisticians in this field. Collaborations should be established between researchers across disciplines for producing, storing, analyzing, and interpreting the data obtained from various experiments. Formation of centralized databases containing information on molecular characteristics of individual tumor types will help to reach already available data and may save time and resources during research.

Finally, cost of the high-throughput techniques is another prohibitive factor, which prevents them from gaining access to most of the laboratories. The accessibility to these tools is increasing rapidly and complexity and cost are likely to improve with the development of next generation of tools. Exquisite quality controls are required to optimize the results. Currently, advances in cancer diagnostic methods are integrated into clinical practice in a slow and uneven fashion.

Genomics and proteomics should be recognized as complementary fields of investigation in cancer diagnosis and strengths and weaknesses of each individual technology should be balanced to obtain maximum benefit. From all the work done until today, it is clear that genomics and proteomics have generated a considerable amount of data for breast cancer diagnosis. However, results obtained from previous studies must be validated, refined, and extended and the relevance of these data for clinical practice still has to be established. Integration of genomics and proteomics technology into clinical trials and practice could lead to individu-

alized patient care. Biomarkers used for early detection of breast cancer can be targets of new drugs individualizing treatment and increasing success.

Multi-center clinical trials are required to validate the available data and to set the standards in various methods utilized in genomics and proteomics for breast cancer. These trials will help to find the best ways to integrate genomics and proteomics in patient care. In this context, phase I studies will aim to define the predictive gene set, establish the prediction rules, and determine assay cutoffs in a well-defined patient population. In phase II studies, the predictors will be tested on independent cases, and the reproducibility and reliability of the assays will be determined and phase III studies will prove their efficacy in cancer diagnosis in prospective randomized trials (25). In the future, methods used in genomics and proteomics will be useful for the discovery of tumor specific marker genes and related proteins in breast cancer, but traditional methods will be applied in daily clinical practice.

References

1. Brown PO, Botstein D. Exploring the new world of the genome with DNA microarrays. Nat Genet 1999;21:33-7. (PMID: 9915498)
2. Fodor SP, Read JL, Pirrung MC, et al. Light-directed, spatially addressable parallel chemical synthesis. Science 1991;251:767-73. (PMID: 1990438)
3. Chung CH, Levy S, Chaurand P, Carbone DP. Genomics and proteomics: Emerging technologies in clinical cancer research. Crit Rev Oncol Hematol 2007;61:1-25. (PMID: 17015021)
4. Hutchens T, Yip T. New desorption strategies for the mass spectrometric analysis of macromolecules. Rapid Commun Mass Spectrom 1993;7:546-80. (PMID:)
5. Gretzer MB, Partin AW, Chan DW, et al. Modern tumor marker discovery in urology: surface enhanced laser desorption and ionization (SELDI). Rev Urol 2003;5:81-9. (PMID: 16985625)
6. Haab BB, Dunham MJ, Brown PO. Protein microarrays for highly parallel detection and quantitation of specific proteins and antibodies in complex solutions. Genome Biol 2001;2:1-13. (PMID: 11182887)
7. Kononen J, Bubendorf L, Kallioniemi A, et al. Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med 1998;4:844-7. (PMID: 9662379)
8. Bauer KD, de la Torre-Bueno J, Diel IJ, et al. Reliable and sensitive analysis of occult bone marrow metastases using automated cellular imaging. Clin Cancer Res 2000;6:3552-9. (PMID: 10999743)
9. Sauter ER, Zhu W, Fan XJ, et al. Proteomic analysis of nipple aspirate fluid to detect biologic markers of breast cancer. Br J Cancer 2002;86:1440-3. (PMID: 11986778)
10. Paweletz CP, Trock B, Pennanen M, et al. Proteomic patterns of nipple aspirate fluids obtained by SELDI-TOF: potential for new biomarkers to aid in the diagnosis of breast cancer. Dis Markers 2001;17:301-7. (PMID: 11790897)
11. Li J, Zhang Z, Rosenzweig J, et al. Proteomics and bioinformatics approaches for identification of serum biomarkers to detect breast cancer. Clin Chem 2002;48:1296-304. (PMID: 12142387)
12. Vlahou A, Laronga C, Wilson L, et al. A novel approach toward development of a rapid blood test for breast cancer. Clin Breast Cancer 2003;4:203-9. (PMID: 14499014)
13. Hu Y, Zhang S, Yu J, et al. SELDI-TOF-MS: the proteomics and bioinformatics approaches in the diagnosis of breast cancer. Breast 2005;14:250-5. (PMID: 16085230)
14. Li J, Orlandi R, White CN, et al. Independent validation of candidate breast cancer serum biomarkers identified by mass spectrometry. Clin Chem 2005;51:2229-35. (PMID: 16223889)
15. Wulfkuhle JD, Sgroi DC, Krutzsch H, et al. Proteomics of human breast ductal carcinoma in situ. Cancer Res 2002;62:6740-9. (PMID: 12438275)
16. Varnum SM, Covington CC, Woodbury RL, et al. Proteomic characterization of nipple aspirate fluid: identification of potential biomarkers of breast cancer. Breast Cancer Res Treat 2003;80:87-97. (PMID: 12889602)
17. Pawlik TM, Fritsche H, Coombes KR, et al. Significant differences in nipple aspirate fluid protein expression between healthy women and those with breast cancer demonstrated by time-of-flight mass spectrometry. Breast Cancer Res Treat 2005;89:149-57. (PMID: 15692757)
18. Li J, Zhao J, Yu X, et al. Identification of biomarkers for breast cancer in nipple aspiration and ductal lavage fluid. Clin Cancer Res 2005;11:8312-20. (PMID: 16322290)
19. Shin S, Cazares L, Schneider H, et al. Serum biomarkers to differentiate benign and malignant mammographic lesions. J Am Coll Surg 2007;204:1065-71. (PMID: 17481542)
20. Finke J, Fritzen R, Ternes P, et al. An improved strategy and a useful housekeeping gene for RNA analysis from formalin-fixed, paraffin-embedded tissues by PCR. Biotechniques 1993;14:448-53. (PMID: 7681300)
21. Stanta G, Bonin S. RNA quantitative analysis from fixed and paraffin-embedded tissues: membrane hybridization and capillary electrophoresis. Biotechniques 1998;24:271-6. (PMID: 9494729)
22. Emmert-Buck MR, Bonner RF, Smith PD, et al. Laser capture microdissection. Science 1996;274:998-1001. (PMID: 8875945)
23. Ma XJ, Salunga R, Tuggle JT, et al. Gene expression profiles of human breast cancer progression. Proc Natl Acad Sci USA 2003;100:5974-9. (PMID: 12714683)
24. Bertucci F, Birnbaum D, Goncalves A. Proteomics of breast cancer: principles and potential clinical applications. Mol Cell Proteomics 2006;5:1772-86. (PMID: 16733261)
25. Pusztai L, Mazouni C, Anderson K, et al. Molecular classification of breast cancer: limitations and potential. Oncologist 2006;11:868-77. (PMID: 16951390)

Corresponding

Can Atalay
E-mail : atalay_can@hotmail.com
Phone : +90 336 09 09 ext. 5605
Fax : +90 312 215 19 24