

REVIEW

Research Progress in Applying Proteomics Technology to Explore Early Diagnosis Biomarkers of Breast Cancer, Lung Cancer and Ovarian Cancer

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Abstract

According to the China tumor registry 2013 annual report, breast cancer, lung cancer, and ovarian cancer are three common cancers in China nowadays, with high mortality due to the absence of early diagnosis technology. However, proteomics has been widely implanted into every field of life science and medicine as an important part of post-genomics era research. The development of theory and technology in proteomics has provided new ideas and research fields for cancer research. Proteomics can be used not only for elucidating the mechanisms of carcinogenesis focussing on whole proteins of the tissue or cell, but also seeking the biomarkers for diagnosis and therapy of cancer. In this review, we introduce proteomics principles, covering current technology used in exploring early diagnosis biomarkers of breast cancer, lung cancer and ovarian cancer

Keywords: Proteomics - biomarkers - breast cancer - lung cancer - ovarian cancer

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Introduction

Proteomics has been widely implanted into every field of life science and medicine as an important part of post-genomics era research. Proteomics is introduced by Wilkins and Williams in 1995, and it refers to a way of all protein in organism and its activities (Wilkins et al., 1996). The products of gene--protein--is the main part of the organism to perform a variety of complex physiological functions. Hence, just know genome is unable to understand life activity process, this is because gene expression levels do not accurately predict protein levels (Davis et al., 2004), due to a lot of mRNA expression product of protein undergo modification after translation (i.e. phosphorylation, glycosylation, oxidation, reduction) or simply shift in rates of synthesis and degradation (Panisko et al., 2002), so it cannot determine protein modification and processing simply by gene sequence, and proteome is constantly changing, determining and reflecting the life activities.

Compared to the genome, the proteome can provide a more dynamic and accurate reflection of both the intrinsic genetic program me of the cell and the impact of its immediate environment (Aebersold et al., 2005). Since proteome analysis can provide the link between gene sequence and cellular physiology (Dove et al., 1995), proteomics is expected to complement gene analyses for evaluating disease development, prognosis, and response to treatment (Clarke et al., 2003). It is said that exploring

specific proteins associated with disease can by comparing the normal and pathological cell and tissue protein expressed in expressing quantity, location and modify status differences, and such proteins can not only provide clues to pathogenesis of the disease, but also can be used as the molecular markers of disease diagnosis, as well as can be used as a target of treatment and drug development.

More than 30% of people will develop some form of cancer during their lifetime, and cancer is the leading cause of the death worldwide (Karimi et al., 2014). Nowadays, cancer is still a problem urgently to be solved in the medical profession, but it cannot be diagnosed at the early stage by traditional methods, this is, early detection is of utmost importance in reducing mortality. Research during the last several decades resulted in the identification of clinically useful cancer biomarkers such as carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP), prostate-specific antigen (PSA), cancer antigen 125 (CA 125), CA15-3 and CA19-0. However most of these biomarkers lack the necessary specificity and sensitivity for screening purpose (Khatcheressian et al., 2006). There is currently a need for discovery of diagnostic methods with improved performance. Proteomics is one of the most effective ways to look for molecular markers of disease and drug targets (Pavlou et al., 2010).

The use of proteomics in cancer biomarker research has two complementary starting points. The first is to directly profile tumor specimens for diagnosis and stratification of patients, for prognosis with or without

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particular therapies, and for clues to mechanism and to circulating biomarkers. The second is to profile proteins in the blood plasma to discover and validate biomarkers for earlier or more specific diagnoses and to apply such biomarkers to predict response to treatment and monitor patients for recurrence or metastasis of the tumor (Omenn et al., 2013). There are also some reports (Andre et al., 2006; Bensalah et al., 2007; Leppert et al., 2007) shows that six different types of biomarkers can be differentiated in cancer: (1) early detection: this biomarker is used for screening patients to discover cancer at an early stage; (2) diagnostic: this biomarker can help identify classical histopathologic characteristics in assessing presence or absence of cancer; (3) prognostic: this biomarker is used to dissect the outcome of patients into different prognostic risk groups thereby allowing individualized management; (4) predictive: this biomarker is used to predict whether the treatment (drug or other therapy) will be effective or to monitor the effectiveness of the treatment. It can help identify the best treatment modality; (5) therapeutic target: this biomarker can help identify patients who will benefit from a particular treatment regimen; (6) surrogate end point: this biomarker is used to substitute for a clinical end point or measure clinical benefit, harm, or lack of benefit or harm lackage.

Proteomics Platform & Challenge

Proteomics is an emerging technology that can identify low molecular weight molecules in a high-throughput, nonbiased discovery approach using patient serum, plasma, urine, or other secretions such as ascites.

During the last several years, the field of proteomics has evolved considerably. With the integrated application of sophisticated biology, physic, chemical and informatics, proteomics research also gradually become an analysis process with high production, high precision. Today, the main application technologies in the proteome research include: two-dimensional (2D) PAGE, surface-enhanced laser desorption (SELDI), mass spectrometry (MS), laser capture micro dissection (LCM), Database Settings, ELISA, Immunohistochemistry, western blotting, and more recently, tissue microarray (TMA) and protein microarray (PMA), and utilize these tools to discover novel cancer biomarkers and validate them in clinical trails.

To date, analyses of protein levels in cancer have been performed primarily using two-dimensional (2D) PAGE or SELDI mass spectrometry (Everley et al., 2004). SELDI in particular has been used to demonstrate altered protein expression patterns in cancer but does not provide easy identification of individual proteins (Wulfkuhle et al., 2003). TMA is the most used proteomics approach in oncology since it was first published in 1998. TMA can simultaneously analyze a new protein marker or a group of 'protein signature' markers in hundreds to millions of cylindrical fragments of clustered tumor samples, collected from original paraffin blocks. TMA associated with IHC allows the performance of trails in the same technical conditions with promptness and viable costs, making it a powerful tool in investigative pathology (Bertucci

et al., 2008). Protein arrays are similar approaches to c-DNA-microarray. Arrangement of several protein probes on solid surfaces is included for the evaluation of interactions with specific proteins of complex samples. The antibody-microarray, in which the targeted proteins are specific antibodies printed on solid surface, is the most advanced format of this technique (Haab et al., 2005). Protein and antibody-arrays may provide information on PTM (posttranslational modifications) of specific proteins.

Advances in mass spectrometry (MS) have extended the sensitivity, accuracy and speed of analysis to now routinely enable the identification of several thousand protein per experiment. The introduction of MD methods for accurate relative and absolute protein quantification and the large-scale analysis of PTM, such as phosphorylation and ubiquitylation, have allowed truly functional proteomics to be carried out (Kolch et al., 2010). MS is now joined by antibody and protein-protein interaction arrays (Wolf-Yadlin et al., 2009), fluorescence- and flow cytometry-based detection of proteins and PTM (Schulz et al., 2007), and optical spectroscopic methods of proteome analysis (Faley et al., 2009; Fournier et al., 2009). These latter techniques are promoted by an ever-increasing repertoire of specific antibodies against protein and PTM, and bring single-cell proteomics into reach (Kolch et al., 2010).

Multiple reaction monitoring (MRM) is a targeted MS approach for protein quantification and it is emerging as a bridge for gap between biomarker discovery and clinical validation (Cho, 2014). MRM assays are highly multiplexed and can verify many candidates simultaneously. This facilitates the development of biomarker panels with increased specificity. The development of scheduled MRM now enables hundreds of candidate biomarkers to be rapidly quantified and validated in a single MS analysis without the use of antibodies (Chambers et al., 2014).

Clearly the availability for discovery and validation of bio specimens that are highly relevant to the intended clinical application and have been collected, processed, and stored with the use of standard operating procedures is of crucial importance to the successful application of proteomics to the development of biomarkers for cancer.

There are many limitations of current modalities for cancer detection. At present the detection of particular common cancers relies heavily on procedures, notably imaging, that are specific to each cancer type, such as computerized axial tomography (CT) scan for lung cancer, mammograms for breast cancer. Advances in imaging technology have allowed improved detection of small lesions. These advances have also led to increases in false-positive findings, necessitating invasive procedures to make a definitive diagnosis (Crowell et al., 2010; Chubak et al., 2010). An important issue to consider in developing biomarkers for cancer detection using proteomics is the status of currently available modalities. However, proteomics research itself also has some difficulties. Firstly, the diversity of proteins. Gene as the carrier of genetic information, no matter under what conditions, it is always the same. However, for different types of cells or the same cells in different active state, proteins is

diversity. Secondly, proteome is dynamic. An individual's genome from birth to death, always remain the same. But, proteome, as the main executor of the metabolism, always changes constantly in individual's life activities. Furthermore, because the stability of proteins is much worse than nucleic acids, degradation or loss may occur in the process of preparation. In addition, the distinction between the different proteins usually use two-dimensional gel electrophoresis to detect, but because of the limitation of its resolution, two-dimensional electrophoresis is difficult to detect trace amount of protein. Hence, accurate determination of differences in the quality of protein is a difficult task. Thirdly, proteome has timeliness and spatiality. DNA is located in the nucleus, and remain stable, so the determination of DNA sequence is not affected by time and space. For transcription of mRNA, time is the main factor of reference. In the different stages of development or cell activity at different time, mRNA expression is not the same. Therefore, mRNA research need consider the time, but usually do not need to consider the effect of space. In proteomics study, not only the influence of time but the space should be in view. Different protein distribution in different parts of the cell, their factions closely related with their spatial orientation. Additionally, many proteins in cells are not stationary, and they often play a role in cells through moving in the different subcellular environment.

In the current review, many protein peaks have been reported to bear significant diagnostic, prognostic or predictive value, however, only few candidate markers have been structurally identified yet. In addition, although of pivotal importance in preventing over fitting of data and systematic bias by pre-analytical parameters, validation of biomarker candidates by other, quantitative, methods and/or in new populations is very limited. Moreover, none of the identified candidate biomarkers has been investigated for their utility as cancer markers in large, prospective, clinical setting. As such, the candidate biomarkers discussed in this overview have not all been validated sufficiently to be used for clinical patient care.

Breast Cancer

Breast cancer is women's highest morbidity and mortality of malignant tumor, in which more than 1 million new cases occur every year, and it is the first cause of death in women aged 40-59 years old (Galvao et al., 2011). The recurrence and metastasis is still the leading cause of death in patients with breast cancer (Chaffer et al., 2011; Jemal et al., 2011). Breast cancer is an acquired or inherited genetic disorder influenced by environmental, behavioral, and reproductive factors. The most significant risk factors are gender (being a woman) and age (growing older). Most breast tumors are of epithelial origin, and therefore the large majority of malignant breast tumors are classified as carcinomas (malignant epithelial tumors) (Hondermarck, 2003). Early detection is of paramount importance in reducing mortality, yet the diagnosis of breast cancer is hampered by the lack of an adequate detection method. Hence, better makers for early diagnosis, accurate prognosis and prediction of response to treatment are

warranted to improve breast cancer care.

Current method used to detect breast tumors, either benign or malignant, are based on mammography. In the last few decades, the survival rate improved due to advances in mammography and adjuvant therapy (Galvao et al., 2011). However, there are intrinsic limitations to mammography. First, there are suggestions that X-rays can potentially induced carcinogenesis. Second, it is clear that to be detected in mammography, a breast tumor should be at least a few millimeters in size (Hondermarck, 2003). However, a tumor of that size already contains several hundred million tumor cells. From the cellular point of view, a single cell can lead to the development of a whole tumor (clonal origin of cancer), it is late when a breast tumor is detected by mammography. In clinical practice, after the surgical removal of a tumor, tumor size, inflammation, histoprognostic grading, and node involvement are used to decide treatment and prognosis. However, breast cancer is not an homogeneous disease and there are various types. Moreover, short of prevention, detection at an early stage remains the best route to decrease breast cancer related mortality. Hence, there is a critical need to find new biomarkers not only for detection, but also for typing and treatment of breast cancer. Since proteomics can bridge the gap between the genetic alterations underlying cancer and cellular physiology, much is expected from proteome analyses for the detection of better protein biomarkers.

In the recent years, many proteomics technologies have been applied, with varying success, to the study of tissue samples of breast carcinoma for expression profiling in order to discover protein biomarkers suitable for: characterization and subtyping of tumors; early diagnosis, and both prognosis and prediction of outcome of chemotherapy. As is known to all, CA15.3 is a FDA approved biomarker used to detect breast cancer, and its sensitivity and specificity is 58.2% and 96.0%, respectively (Polanski et al., 2007). CA 27.29 is also approved by FDA now (Aktas et al., 2013). But their sensitivity and specificity is not high enough to accurately diagnose breast cancer at early stage. There are a large number of scholars devote themselves to researching more sensitive and specificity protein-based biomarkers for diagnosis of breast cancer at early stage. Studies on the proteome in breast cancer have used tissue samples as well as biological fluids including serum, plasma, saliva, nipple aspirate, and cerebrospinal fluid in search for the detection of diagnostic, predictive, and/or prognostic biomarkers.

J Sohn, et al. (Sohn et al., 2013), used functional proteomics to determine the molecular characteristics of residual triple receptor-negative breast cancer (TNBC) patients after neoadjuvant systemic chemotherapy (NCT). Multivariable analysis using the top 25 proteins from univariable analysis at a false discovery rate (FDR) of 0.3 showed that AKT, IGF2BP2, LKB1, S6 and Stathmin were predictors of recurrence-free survival (RFS), and they found a five-protein model that independently predicted RFS risk in patients with residual TNBC disease. The P13K pathway may represent potential therapeutic targets in this resistant disease.

Washam et al. (2011) used surface enhanced laser

desorption time-of-light mass spectrometry (SELDI-TOF MS) to research breast cancer patient plasma which is associated with bone metastasis, and the study is showed that PTHrP (12-48) is a novel plasma biomarker of breast cancer bone metastasis (sensitivity: 97%, specificity: 82%). Bone metastasis is a devastating and often incurable phase of breast cancer progression that significantly compromises patient morbidity and mortality. Plasma samples were collected from a total of 110 breast

cancer patients, consisting of 38 breast cancer patients with clinical evidence of bone metastasis, and 38 with no evidence of a bone metastasis from time of diagnosis to clinical outcome. The independent validation cohort consisted of 34 breast cancer patients with an unknown bone metastasis classification. SELDI-TOF MS was used to analyze, and a Random Forest classifier was used to identify.

Tang and Mackey, et al. (Tang et al., 2013) studied

Table 1. Protein-Based Biomarkers in Detection of Lung Cancer: Currently Available

Biomarkers	Diagnosis	Therapy monitoring	Prognosis monitoring	Details	References
CEA	AdenoCA, LCLC (>10ug/L)	AdenoCA, Advanced NSCLC	AdenoCA, NSCLC	“Use in combination with CYFRA. Often elevated in smokers.”	“(Kulpa et al., 1999; Molina et al.2003; Molina et al., 2005; Holdenrieder et al., 2004;Ardizzoni et al., 2006) (Salgia et al., 2000; Sun et al., 2000;Muley et al., 2002; Pollan et al., 2003; Barak et al., 2004; Barlési et al., 2004; Muley et al., 2004; Okada et al., 2004; Sakao et al., 2004; Tomita et al., 2004; Okamoto et al., 2005; Lee et al., 2005) “
CYFRA21-1	NSCLC, SCC (Sensitivity for NSCLC varies between 23 and 70%)	Advanced NSCLC	NSCLC, SCC	Often elevated in patients with benign lung disease	“(Kulpa, et al., 1999; Molina, et al., 2003; Molina, et al., 2005; Holdenrieder, et al., 2004; Ardizzoni, et al., 2006) (Schneider, et al., 2002; Buccheri, et al., 2003; Barak, et al., 2004) (Hatzakis, et al., 2002;Muley, et al., 2002; Barlési, et al., 2004) (Sun, et al., 2000; Kashiwabara , et al., 2000; Pujol, et al., 2001;Hatzakis, et al., 2002; Reinmuth, et al., 2002;Kulpa, et al., 2002; Merle, et al., 2003; Vollmer, et al., 2003; Pujol, et al., 2003; Pujol, et al., 2004;Barlesi, et al., 2005;)”
ProGRP	SCLC (>200ng/L=Highly suspicious) (Sensitivity for SCLC range 47-86%)	SCLC	-	Increased in renal failure and some benign lung disease. Use in combination with NSE	(Bonner, et al., 2000; Shibayama, et al., 2001; Schneider, et al., 2002; Satoh, et al., 2002; Schneider, et al., 2002; Massacesi, et al., 2002; Molina, et al., 2003; Muley, et al., 2004; Molina, et al., 2004; Schneider, et al., 2006)
TPA	NSCLS, SCC		NSCLC		(Kulpa et al., 1999; Bonner et al., 2000; Maeda et al., 2000; Pujol et al., 2001; Kulpa et al., 2002; Hatzakis et al., 2002; Buccheri et al., 2003; Bremnes et al., 2003;Ferrigno et al., 2003; Barak et al., 2004; Pujol et al., 2004; Ando et al., 2004; Lee et al., 2005)
NES	SCLC (>100ug/L probability) (Sensitivity for SCLC as high as 74%)	SCLC	SCLC	Use in combination with ProGRP May correlates with short survival Increased in inflammat	(Maeda et al., 2000; Bremnes et al., 2003; Ferrigno et al., 2003; Ando et al., 2004)
KPNA2					(Yu et al., 2013)
CD98, fascin, sPIgR4, and 14-3-3 η	LC (Sensitivity =96%)				(Xiao et al., 2005)

the proteomics changes that accompany the HER2 gene amplification to identify potential new therapeutic targets and biomarkers. Bio-triplicate proteome samples extracted from wild-type MCF-7 human breast cancer cells were analyzed, and their isogenic stably overexpressing HER2 (amplified) transfectants. Differential isotope labeling LC-MS was used to profile the proteomes of two cell lines. In total, 2455 unique proteins were quantified with 1278 differentially expressed proteins. Select biomarker candidates of particular interest were validated by western blotting, and evaluated for clinical relevance by the immunohistochemical assessment of protein abundance in breast tumor biopsies. HER2 transfection produced marked changes in proteins related to multiple aspects of cancer biology, and identified expression patterns were recapitulated in the clinical samples. The Nek2C splice variant of the serine/threonine kinase Nek2 is involved in breast cancer development; Nek2C inhibition may be a potential therapeutic approach to targeting some types of human breast tumors (Liu et al., 2012).

Lung Cancer

Lung cancer is one of the most prevalently occurring and the most life-threatening neoplasia in most part of the world. It has an incidence of 1.2 million people in worldwide, and accounted for about 25% of all cancer deaths (Sung et al., 2008), and among the deadliest cancers with a 5-year survival rate of 15% (Indovina et al., 2013). In china, lung cancer is the leading cause of cancer incidence of a disease. Among men the number of lung cancer deaths nearly triples the number of prostate cancer deaths, and among women, the number of lung cancer deaths is almost double the number of breast cancer deaths (Granville et al., 2005). This disease is clinically divided in two subtypes, small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), and NSCLC accounts for 85%-90% of all cases and can be further histologically subdivided in adenocarcinoma (AD), squamous cell carcinoma (SC), large cell carcinoma (LCC) and "others" (Pavlou et al., 2010). Numerous potential DNA biomarkers such as hypermethylations of the promoters and mutations in K-ras, p53, and protein biomarkers: carcinoembryonic antigen (CEA), CYFRA21-1, plasma kallikrein B1 (KLKB1), Neuron-specific enolase, etc. (Table 1) have been discovered as lung cancer biomarkers. But they are not ideal tools in detection of lung cancer in clinical due to their low specificity and/or sensitivity. Other diagnostic tools include computed tomography (CT) scans, bronchoscopy and sputum analysis, which demonstrate

limited efficiency, judging from the percentage (>60%) of patients diagnosed with last stage disease. Hence, there is an urgent need for better markers for its diagnosis and prognosis.

Taguchi A and Liotta LA and their colleagues (Liotta et al., 2011; Taguchi et al., 2011) identify plasma-based biomarkers with an integrative proteomics approach. Secreted proteins of cancer cells can be shed into blood. Their serum levels may increase in the early stage of cancer, and correlate with cancer cell proliferation and/or secreted protein overexpression. Thus, secreted proteins may be used as potential serum biomarkers of cancer (Welsh et al., 2003; Wu et al., 2005). Well known secreted protein serving as serum biomarkers include CEA for colon cancer, prostatic acid phosphatase (PSA) for prostate cancer (Kosari et al., 2002; Balk et al., 2003). On the basis of these examples, Ling-Jin Huang and colleagues (Huang, et al., 2006) identified secreted protein A549 for lung cancer using MS and database search. Fourteen human proteins were identified, and DDH was selected for further analysis using RT-PCR, immunoblotting, immunohistochemical staining and ELISA in NSCLC patients. Compared with normal lung tissues, higher DDH mRNA and protein expression level were found in 15 NSCLC cancer tissues ($p < 0.05$). DDH overexpression was identified to be located in cytoplasm and cell membrane by immunohistochemical (n=64) than nonmalignant lung tumor (n=20) and healthy controls (n=20) ($p < 0.05$). The results show that DDH was one of the secreted proteins in NSCLC, and it can serve as a tissue marker and a novel serological marker of NSCLC. Planque et al. (Planque et al., 2009) have presented one of the most comprehensive proteomics analyses of cell secretome; Using a two-dimensional LC-MS/MS strategy on the conditioned media of four lung cancer cell lines of different histological backgrounds (non-small cell lung cancer: H23 (adenocarcinoma), H520 (squamous cell carcinoma), and H460 (large cell carcinoma) were separated. 1830 proteins were identified of which 38% were classified as extracellular or membrane bound. The findings were evaluated based on existing literature data, tissue specificity assays, functional classifications, and existing proteomics data on lung-related diseases. Five novel lung cancer candidates, ADAM-17, osteoprotegerin, pentraxin 3, follistatin, and tumor necrosis factor receptor superfamily member 1A were preliminarily validated in the serum of patients with lung cancer and healthy controls.

Table 2. Potential Biomarkers for Detection of Ovarian Cancer: Current Research

Biomarker	Technology	Reference
LAMP-1	MS	(Tiss et al., 2014)
Three-protein panel: TAP1, SERPINB1, SRPRB	Immunohistochemistry, ELISA	(Byrd et al., 2014)
STIP1	MS, flow cytometry, siRNA silencing, protein blotting	(Van et al., 2014)
CLIC4, TPM2, TPM3, TPM4	LC-MS/MS, Immunoaffinity, SDS-PAGE, Data processing, Label-free GeLC-MRM	(Tang et al., 2013)
COL11A1	Immunoblot	(Teng et al., 2014)
Mesothelin, FLT4, AGP	MS, Immunoassay	(Aktas et al., 2013; Collinson et al., 2013)
L1CAM	ELISA	(Leung et al., 2013)
FOLR1	ELISA	(Ludwig et al., 2005)

C C Wu and colleagues (Wu et al., 2010) analyzed the secretomes of 23 human cancer cell lines derived from 11 cancer types using one-dimensional SDS-PAGE and nano-LC-MS/MS performed on an LTQ-Orbitrap mass spectrometer to generate a more comprehensive cancer cell secretome. A total of 31,180 proteins were detected, 55.8% of the proteins appeared to be released or shed from cells using protein secretion-predictive algorithms. Protein expression profiles in the Human Protein Atlas was examined to identify biomarker candidates that were simultaneously detected in the secretomes and highly expressed in cancer tissues. This analysis yielded 6-137 marker candidates selective for each tumor type and 94 potential pan-cancer markers. CD14 (for liver cancer), stromal cell-derived factor 1 (for lung cancer), and cathepsin L1 and interferon-induced 17-kDa protein (for nasopharyngeal carcinoma) were selectively validated as potential serological cancer markers.

Chinese researchers (Xin-Ju Li et al., 2013) employed proteomics profiling to identify lung squamous cell carcinoma markers, and they found that fibrinogen alpha chain may be a potential diagnosis marker.

Autoantibodies against tumor antigens represent one type of biomarker that may be assayed in serum for detection of cancer and monitoring of disease progression. PHe, T Naka, et al. (He et al., 2007) detected autoantibodies against a-enolase in subset of NSCLC patients' sera by combining two-dimensional electrophoresis, western blotting, and mass spectrometry with enzyme-linked immunosorbent assay technology. The prevalence of this autoantibody was 27.7% in patients with NSCLC (26/94), 1.7% in healthy control subjects (1/60), when 'Mean OD healthy control sera +3 SD healthy control sera' was used as the cut-off point, and not detectable in sera from 15 patients with small cell lung cancer, 18 patients with gastrointestinal cancer and 9 patients with Mycobacterium avium complex infection of lung. a-enolase was increased in cancer tissues of NSCLC patients, and flow cytometric analysis confirmed the expression of a-enolase at the surface of cancer cells. Autoantibodies against a-enolase may constitute a promising biomarker for NSCLC by combined detection of autoantibodies against a-enolase, carcinoembryonic antigen and cytokeratin 19 fragment (CYFRA 21-1) enhanced sensitivity for the diagnosis of NSCLC.

Ovarian Cancer

Ovarian cancer (OC) ranks as the fourth most frequent cause of cancer-related death in the same population and is considered the most fatal and feared of the gynecological malignancies. Approximately 4% of all cancers occurring in women is ovarian cancer and more than 90% of ovarian cancer originate from surface epithelial cells (Ardekani et al., 2004). Ovarian cancer is associated with a heavy burden of morbidity and a high case-fatality rate despite by surgical resection alone (Fotopoulou et al., 2013). The high fatality rate is attributed to late diagnosis, since 65% of patients are diagnosed with advanced cancer (Ardekani et al., 2004) and this has led to a 5-year relative survival of only 28% of patients with ovarian cancer (Ardekani et al.,

2004). However, when the diagnosis and treatment is made at an early clinical and specific test for early detection of ovarian cancer, about 70% of all epithelial ovarian cancer present as stage III or IV disease (Wakeley et al., 2000). One of the difficulties of early detection of ovarian cancer is a failure to recognize a premalignant phase. It is thought that epithelial ovarian cancer begin in the cells of germinal epithelium inclusion cysts that are formed when the surface epithelium of ovary grows into the area of the follicle following ovulation and the epithelial cells become entrapped within the ovary. Perhaps atypical metaplastic changes in inclusion cyst epithelial cells represent a premalignant change (Wakeley et al., 2000).

To date, there are no effective screening tests for EOC early detection, despite extensive evaluation, imaging with transvaginal ultrasound, and the serum marker CA-125 have not resulted in acceptable sensitivity and specificity levels (Daly et al., 2002). Serum CA-125 currently is the best clinical marker for ovarian papillary serous adenocarcinoma in postmenopausal patients. In premenopausal women, non-serous histologies, and in early stage cancer, its performance as a tumor marker is less impressive (Nosov et al., 2009). Only about 50% of early stage ovarian cancers will be associated with elevated serum CA-125 (Bast et al., 1997). Transvaginal ultrasound allows for detailed imaging of the ovaries and the detection of morphological changes that may signify a developing malignancy. A number of studies considered ultrasound methodology as a candidate-screening tool for the early detection of ovarian cancer (Levine et al., 1992; Bailey et al., 1998; Valentin et al., 2003). Nowadays, routine screening tests, such as serum CA-125, human epididymis protein-4 (HE4) (Ludwig et al., 2005), ultrasound, or in combination, aimed at the early detection of ovarian cancer, when it still can potentially be cured, are neither specific nor sensitive enough. Proteomics techniques have yielded new putative biomarkers for ovarian cancer that may be of significant clinical importance.

While ovarian cancer remains the most lethal gynecological malignancy in some countries, there are no biomarkers available that are able to predict therapeutic responses to ovarian malignancies. In order to detect ovarian cancer in a state of hyperproliferation, Marzinke, et al. (Marzinke et al., 2013) analyzed the implications of molecular signaling cascades in the ovarian cancer cell line OVCAR3 in a temporal manner, using a MS-based proteomics approach. OVCAR3 cells were treated with EGF, and the time course of cell progression was monitored based on Akt phosphorylation and growth dynamics. Validation studies were performed on one of the differentially regulated proteins, lysosomal-associated membrane protein (LAMP-1), in human tissue lysates and ovarian tumor tissue sections. Further study demonstrated that tissue microarray analysis was performed to demarcate LAMP-1 expression across different stages of epithelial ovarian cancer. These data support the use of this approach for the efficient identification of tissue-based markers in tumor development related to specific signaling pathways. LAMP-1 is a promising biomarker for studies of the progression of EGF-stimulated ovarian cancers and might be useful in predicting treatment response involving

tyrosine kinase inhibitors or EGF receptor monoclonal antibodies.

Tiss and Timms, et al. (Tiss et al., 2014) used combining MS analysis of serum peptidome with data collected over a period of 7 years from the UK 295 patients with ovarian cancer, 290 with benign neoplasm, and 2236 postmenopausal healthy controls to improve early diagnosis of OC. The results showed that OC could be accurately predicted up to 15 months before its clinical diagnosis, based a combination of two MS peaks with CA-125 clinical test. An overall sensitivity of 94.8% (96.6% specificity) was obtained when comparing malignancies versus healthy postmenopausal controls. High classification accuracies were also obtained for early stage cancer (93.5% sensitivity). MS discriminatory peaks were identified as connective tissue-activating peptide III (CTAP III) and platelet factor 4 (PF4), platelet-derived chemokines, suggesting a link between platelet function and tumor development. Those markers might be promising for clinical use in cancer early detection and treatment. Elevated CA-125 in predicts tumor burden in woman's body, especially in the ovary, but cannot differentiate between malignant or benign. Li Li, et al. (Li et al., 2012) used intensive modern proteomics approaches to research it, and they verified A-4 (APOA 4) and natural resistance-associated macrophage serum proteins in the serum of women with elevated CA-125.

Byrd and colleagues (2014) have used immunohistochemistry, ELISA or data from The Cancer Atlas to validate a biomarker panel. In-depth proteomics analysis of archival, OC patients tissues stratified by long (mean: 10.4 yrs, n=15) versus short-term (mean: 1.2 yrs, n=28) survivorship revealed protein abundance changes indicating more aggressive disease characteristics in short versus long-term ovarian cancer patients. Correlation of differentially abundant protein candidates with survivorship revealed a three-protein panel comprising TAP1, SERPINB1 and SRPRB that exhibited mean hazard-ratios of accuracy of 0.761. This candidate biomarker panel correlates with disease survival in OC patients and warrants further investigation as a signature of disease prognosis in ovarian cancer.

Conclusion

Cancer is one of the most lethal diseases in the world. The majority of deaths are due to a lack of early diagnosis and timely treatment. The discovery of cancer biomarkers for early detection and diagnosis is a possible solution to tackle this problem (Gupta et al., 2014). Early detection and accurate disease classification are key components of most cancer treatment programs and this drives the desire for more effective biomarkers (Indovina et al., 2013). However, quite a number of patients are diagnosed at advanced stage of cancer due to few sensitive and specific cancer biomarkers being available for clinical care.

Recent proteomics studies have discovered a large number of potential protein biomarkers. However, the lack of follow-up validation studies remains a major challenge in translation of these biomarkers into routine clinical setting. To translate the identified protein biomarkers into

clinical use, fully developed tools and workflows with high robustness, sensitivity, specificity and throughput are needed. Nevertheless, the discovery of proteomic understanding of cancer biology and rapid development of more advanced proteomics technologies, some of these novel protein-based biomarkers will have considerable potential to translate into routine clinical practice.

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