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Can the acute-phase reactant proteins be used as cancer biomarkers?

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ABSTRACT: The association between the acute-phase reactant proteins (APRPs) and cancer has long been established. There have been numerous reports correlating altered levels of various APRPs with different types of cancers. However, researchers are often quick to dismiss the use of these APRPs as potential biomarkers for the diagnosis and monitoring of cancer because alterations in APRP concentrations are observed in a wide range of diseases. Recent progress in proteomics studies which profiled the serum proteins of cancer patients and those of normal individuals indicated that the altered APRP expressions were different for distinct types, subtypes, and even stages of cancer. Interestingly, these data are in agreement with those observed earlier using immunochemical and biochemical assays. In view of this compelling association of different patterns of APRPs with various types of cancers and in an apparent shift of paradigm, we present in this review some indications that APRP fingerprinting may be used as complementary cancer biomarkers. (Int J Biol Markers 2010: 25:)

Key words: Acute-phase reactant proteins, Biomarker, Cancer, Diagnosis, Serum

INTRODUCTION

The C-reactive protein (CRP) was the first acute-phase reactant protein (APRP) to be identified. In 1930, Tillett and Francis discovered that the sera of patients suffering from pneumonia reacted with fraction "C", or the C polysaccharide of *Streptococcus pneumoniae* (1). A decade later, it was shown that the "C-reactive" component of human serum is a protein expressed during acute infections which we now know as CRP (2). Since then, extensive research has focused on the changes in CRP and other APRPs in response to various acute and chronic inflammatory diseases or clinical conditions including burns, tissue infarction, infections, and also cancer.

One of the earliest documented works linking APRP to cancer was carried out by Hiramatsu et al in 1976. The researchers demonstrated that the concentrations of serum protease inhibitor, α_1-antitrypsin (AAT), were highly elevated in patients with liver cancer (3). Numerous reports have since linked APRP changes to various types or subtypes of cancer and different stages of the disease. To understand how distinct types of cancer trigger different acute-phase responses, and hence differential APRP expression/secretion (or both) by hepatocytes, the functions of the APRPs during inflammation or the onset of disease, and what regulates APRP release into the blood circulation are also briefly addressed in the following discussion. For the reader's convenience, a list of acronyms for the various APRPs described in this review is presented in Table I.

ACUTE-PHASE RESPONSE

When subjected to an infection or other types of immunological stress, our bodies' first reactions are innate, systemic, and non-specific. This is referred to as the acute-

TABLE I - LIST OF ACRONYMS FOR ACUTE-PHASE REACTANT PROTEINS

Acronym	Acute-phase reactant protein
AAT	α_1-antitrypsin
ABG	α_1-B glycoprotein
ACT	α_1-antichymotrypsin
AHS	α_2-HS glycoprotein
ATR	Antithrombin III
CFB	Complement factor B
CLU	Clusterin
CPL	Ceruloplasmin
CRP	C-reactive protein
HAP	Haptoglobin
KNG	Kininogen
LRG	Leucine-rich glycoprotein
ZAG	Zinc α_2-glycoprotein

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phase response. An acute-phase response precedes the specific immunological response during an infection. It refers to the temporary replacement of stable, homeostatic processes by a different homeostatic state in the face of a changing external environment due to tissue injury or infection. These events are usually proinflammatory, with the aim of removing tissue debris and foreign organisms, and aiding tissue repair (4). Clinically, the acute-phase response is associated with a number of measurable changes characterized by fever, leukocytosis, endocrine changes, muscle proteolysis, and anorexia (5, 6). The acute-phase response may be transient, as with recovery from an infection, or it can be persistent, such as that observed in chronic disease (7). One of the features of the acute-phase response is the changes in the concentrations of serum or plasma proteins, also known as the acute-phase reactant proteins.

ACUTE-PHASE REACTANT PROTEINS

An APRP is defined as a protein whose plasma or serum concentration increases or decreases by at least 25% during inflammatory disorders (8). The levels of most APRPs increase in response to an inflammatory stimulus. These proteins are referred to as the positive APRPs. Conversely, the negative APRPs are those whose concentrations decrease during inflammation. α_2-HS glycoprotein (AHS) and kininogen (KNG) are 2 common examples of negative APRPs.

Based on the kinetics of their concentration changes, the APRPs may be divided into first- and second-line proteins. The first-line APRPs include CRP, α_1-antichymotrypsin (ACT), and serum amyloid A. Their levels rise as early as 4 hours upon induction by an inflammatory stimulus, reaching a peak within 24-72 hours and declining rapidly with elimination half-times between 12 and 18 hours (7). In contrast, most of the second-line APRPs such as haptoglobin (HAP) and AAT begin to increase 24-48 hours after stimulus, reaching a peak in about 7-10 days, and need about 2 weeks to return to normal (7). Generally, the magnitude of the acute-phase response is related quantitatively to the activity or extent of inflammation in the acute situation.

FUNCTIONS OF APRPs

The true functions of APRPs during an acute-phase response are unclear. It is assumed that fluctuations in APRPs are beneficial, based on the known functional capabilities of the proteins and how they may respond during inflammation, healing, or adaptation to a harmful stimulus. That being said, there are APRPs which are relatively unknown or have no known functions, such as leucine-rich glycoprotein (LRG) and α_1-B glycoprotein (ABG). Clusterin (CLU), also known as apoliprotein J, is yet to be assigned any genuine function despite having been cloned since 1989 (9, 10). This is because CLU has been associated with extremely diverse physiological and pathological processes and thus its functions are varied and often contradictory (11).

A number of APRPs play major roles in the host defense (Tab. II). CRP, a component of the innate immune system, binds to phosphocholine and hence recognizes some foreign pathogens as well as damaged cells (12). It can activate the complement system when bound to one of its ligands and can also bind to phagocytic cells, suggesting that it may assist in the elimination of targeted cells via interactions with both humoral and cellular effector systems of inflammation. Aside from CRP, there are many other APRPs that have central proinflammatory roles in immunity. These include the classical components of the complement pathway and also the mannose-binding lectin. Complement activation often leads to chemotaxis and the opsonization of infectious agents and damaged cells (13).

A considerable number of APRPs function as serine protease inhibitors. Proteases, such as those released by neutrophils during the innate immune response, are useful in degrading cell debris or microorganisms. They also have regulatory functions in local inflammatory processes

TABLE II - FUNCTIONS OF ACUTE-PHASE REACTANT PROTEINS

Function	Acute-phase reactant protein
Host defense	CRP, HAP, secreted phospholipase A_2, lipopolysaccharide-binding protein, IL-1 receptor antagonist, granulocyte CSF, C3, C4, C9, factor B, C1 inhibitor, C4b-binding protein, mannose-binding lectin
Antiproteases	AAT, ACT, ATR, α_1-protease inhibitor, pancreatic secretory trypsin inhibitor, inter-α-trypsin inhibitor
Transportation of metabolites	CPL, HAP, albumin, hemopexin, transferrin, transthyretin, thyroxine-binding globulin
Coagulation & fibrinolysis	KNG, fibrinogen, Hageman factor (Factor XII), plasminogen, urokinase, TPA, protein S, vitronectin, plasminogen-activator inhibitor 1
Role in growth/angiogenesis	AAT, AHS, CPL, HAP, insulin-like growth factor I
Others	AAT, CLU, HAP, ZAG, serum amyloid A, α_1-acid glycoprotein, fibronectin, ferritin, angiotensinogen, α-fetoprotein

like the proteolytic modulation of cytokine bioactivities. However, proteases have deleterious potential due to their ability to degrade the extracellular matrix at the foci of inflammation (14). Hence protease inhibitors are deployed during an acute-phase response to control the activities of the proteases. Such protease inhibitors include AAT and ACT, which are responsible for inhibiting leukocyte and lysosomal proteolytic enzymes (15).

Some APRPs participate in the transportation of metabolites in addition to being antioxidants. For example, ceruloplasmin (CPL) is involved in copper transport, iron metabolism, and antioxidant defense (16). CPL inhibits the copper ion-stimulated formation of reactive oxidants and scavenges hydrogen peroxide and superoxide, thereby protecting host tissues from the toxic oxygen metabolites released from phagocytic cells during the acute-phase response. Another APRP, hemopexin, binds heme (a reactive form of iron participating in oxygen-radical reactions) released from damaged heme-containing proteins. Hemopexin-heme complexes are cleared from the circulation by surface receptors on hepatocytes with the subsequent release of free hemopexin to the bloodstream. Such a mechanism provides for conservation of body iron stores (17).

During an acute-phase response, an APRP may be involved in more than one function. Indeed, most APRPs possess multiple functions, and many of these additional functions do not fall within the categories mentioned above. For example, apart from being a serine protease inhibitor, AAT stimulates the production of hepatocyte growth factor (18) and was found to induce the proliferation of fibroblasts and the synthesis of procollagen (a matrix component) in culture (19). In tumor cell lines, AAT may act as a growth inhibitor (20). Proteolytically modified AAT and AAT in complex with elastase have been demonstrated to have neutrophil chemoattractant activity (21). Elastase-AAT complexes can also stimulate AAT production by macrophages (22).

SYNTHESIS AND REGULATION OF APRPs

APRPs are normally synthesized by the liver. It has been recognized, however, that extrahepatic tissues can also contribute to the synthesis of some of the plasma proteins. For example, cells of the monocyte-macrophage lineage have been shown to produce several complement components and AAT. The significance of this and the extent to which it normally occurs in vivo, particularly at the site of inflammation, is not known (4).

The biosynthesis of APRPs by hepatocytes in turn is regulated by cytokines, in particular interleukin-6 (IL-6). Cytokines are released by a number of different cell types, including activated macrophages, endothelial cells, and stromal cells at the site of inflammation (6). These cytokines may induce changes in APRP concentration from about 50% to as much as 1000-fold in the case of CRP and several complement components. The concentrations of APRPs commonly increase simultaneously, but they do not necessarily increase uniformly in all patients with the same inflammatory disease.

INFLAMMATION, CANCER AND ALTERED APRPs

Inflammatory diseases range from infections and rheumatic disease to cancer and they may either be acute or chronic. Acute inflammatory diseases are usually self-limiting as the production of antiinflammatory cytokines closely follows those that are proinflammatory. In contrast, chronic inflammatory diseases are characterized by persistent inflammation, either in response to unrelenting initiating factors or a failure of mechanisms required for resolving the usual inflammatory response (23). It may be this inability to switch off the inflammatory response that makes chronic inflammation a causative factor in a variety of cancers despite it having many of the same mediators (e.g. cytokines and free radicals) as those generated during acute inflammation (24).

The functional relationship between inflammation and cancer is not new. Virchow hypothesized in 1863 that the origin of cancer was at sites of chronic inflammation (25). It is now accepted that many cancers arise from sites of infection and chronic inflammation and that proliferation of cells alone does not cause cancer.

During an inflammatory response, leukocytes and other phagocytic cells produce an array of cytokines and also reactive nitrogen and oxygen species to fight infection and mediate cell killing (26, 27). As the inflammatory response prolongs and develops chronically, repeated exposure of proliferating epithelium to the highly reactive nitrogen and oxygen species results in permanent DNA damage such as point mutations, deletions or rearrangements. Indeed, it has been shown that p53 mutations in tumors have a similar frequency to those in chronic inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease (28). The strongest association of chronic inflammation with malignant disease is the development of colon cancer in individuals with inflammatory bowel diseases such as chronic ulcerative colitis or Crohn's disease (23).

In the early stages of tumor development, cancer cells often require the presence of specific cytokines or growth factors in order to proliferate. These cells may express growth factor receptors abnormally or undergo cell division instead of differentiation in response to the growth factors and cytokines (24). Some examples of the dependence of tumor cells on cytokines are the growth dependence of AIDS- and EBV-associated B-cell lymphomas, B-cell leukemias, and multiple myeloma on

the inflammatory cytokines IL-6 (29) and IL-15 (30) and the dependence of malignant mesothelioma on platelet-derived growth factor (24). In the presence of cytokines and growth factors during tumorigenesis, it is therefore not surprising that changes in APRP concentrations are observed.

IMMUNOLOGICAL AND BIOCHEMICAL STUDIES OF APRP LEVELS IN PATIENTS WITH CANCER

Changes in the levels of serum or plasma APRPs in relation to cancer have been studied as early as the 1970s. In those early times, APRP level determination was mainly carried out using the classical immunological techniques of radial immunodiffusion, rocket immunoelectrophoresis, and nephelometric immunoassay (Tab. III). Although many investigators perceived the quantitative changes of the APRPs as non-specific because they respond to a wide variety of stimuli including cancer, these findings generated continued interest in establishing the rationale for the alteration of APRPs in cancer patients. Very soon, accumulated data were compiled on the association of altered levels of various APRPs with distinct types of cancer.

From the compiled data (Tab. III), elevated levels of serum or plasma AAT have since been shown in patients with 9 different types of cancers. For some of the cancer types, consistent results were generated by several independent groups of researchers. For example, Bernacka (1988), Stamatiadis (1990) and Solakidi (2004) and their respective coworkers reported that AAT was higher in serum samples of patients with colorectal cancer than in those of control individuals (31-33). Similarly, 2 different groups of researchers also detected higher levels of AAT in serum/plasma samples of patients with cervical cancer (34, 35).

In the case of CPL, increased levels of the APRP were consistently reported for 8 different types of cancer by 10 independent groups of researchers. Patients with gastrointestinal cancer appear to express high levels of serum CPL as reported by 3 separate groups of investigators using different assay techniques. When bound to copper, CPL acts as a molecular switch for activating proangiogenic factors (36, 37). The elevated CPL in cancer patients was thought to induce angiogenesis, which supports the growth of tumors.

Aside from AAT and CPL, consistent results were also observed for ACT and HAP between studies. While ACT

TABLE III - IMMUNOLOGICAL AND BIOCHEMICAL STUDIES OF ACUTE-PHASE REACTANT PROTEIN LEVELS IN CANCER

APRP	Type of cancer	Response	Method	Reference
AAT	Liver cancer	↑	Radial immunodiffusion	3
	Cervical cancer	↑	Immunochemical assay	34
			Trypsin inhibitory capacity; immunodiffusion	35
	Colorectal cancer	↑	Immunological methods and nephelometry	31-33
	Gastric cancer	↑	Immunological method and nephelometry	31, 33
	Lung cancer	↑	Immunological methods	38, 39
	Multiple myeloma	↑	Nephelometry	40
	Ovarian cancer	↑	Radial immunodiffusion	41
	Pancreatic cancer	↑	Radial immunodiffusion	42, 43
	Prostate cancer	↑	Radial immunodiffusion	39, 44
ACT	Colorectal cancer	↑	Rocket immunoelectrophoresis	31
	Gastric cancer	↑	Rocket immunoelectrophoresis	31
	Lung cancer	↑	Immunological methods	38
	Pancreatic cancer	↑	Radial immunodiffusion	42
AHS	Liver cancer	↓	Radial immunodiffusion	45
CLU	Urinary bladder cancer	↑	ELISA	46
CPL	Cervical cancer	↑	Radial immunodiffusion	47
			Nephelometry	48
	Chronic lymphocytic leukemia	↑	Nephelometry	49
	Endometrial cancer	↑	Radial immunodiffusion	47
	Gastrointestinal cancer	↑	Immunological methods and nephelometry	50-52
	Kidney and urinary tract cancer	↑	Radial immunodiffusion	53
	Lung adenocarcinoma	↑	Biochemical assays	54
	Melanoma	↑	Nephelometry	55
	Solid malignant tumors	↑	Nephelometry	56
HAP	Cervical cancer	↑	Nephelometry	57
	Ovarian cancer	↑	Immunochemical assay	34
			ELISA	58
KNG	Gastrointestinal cancer	↓	Immunochemical assay	59

↑ = increase in expression; ↓ = decrease in expression

was found to be increased in 4 different types of cancer by 4 independent groups of researchers, elevated serum HAP was detected in 2 types of cancer by 3 separate groups of scientists (34, 57, 58). On the other hand, only a single group of investigators reported upregulation of serum CLU in cancer patients. This was demonstrated using ELISA in patients with urinary bladder cancer (46).

In contrast to the positive APRPs, AHS and KNG were both found to be decreased in serum/plasma samples of patients with liver and gastrointestinal cancers (45, 59). This is consistent with their classification as negative APRPs. AHS is known for its antigrowth activity (60, 61). It is therefore not surprising to find that the serum levels of AHS were reduced in patients with hepatocellular carcinoma.

While the reported findings on the altered levels of APRPs in various types of cancer were highly consistent among independent studies, they were rather inconclusive. The reasons why different cancers triggered distinct acute-phase responses as seen from the different altered APRPs detected in the cancers are not known. A further confounding factor is that investigators were often content to catalogue changes of the proteins in a wide variety of cancers with little attention to tumor size or patient performance. Many studies have also utilized indirect assays such as the measurement of enzyme inhibitor activity, binding capacity, or partially purified serum fractions instead of directly quantifying the APRPs.

PROTEOMICS ANALYSES OF APRP EXPRESSION IN PATIENTS WITH CANCER

The direct simultaneous analysis of serum or plasma protein levels is now possible through proteomics technology. Proteomics generally involves the separation of proteins using gel- or liquid-based techniques and their subsequent identification by mass spectrometry and database search. When coupled with densitometry, fluorescence labeling or incorporated with isotopic labels, the expressed proteins may be quantified and compared between various biological samples (62). In line with its function, proteomics is currently a popular tool to profile serum or other clinically relevant body fluids in the quest for new biomarkers for cancer. Indeed, the diagnosis of all cancers is in dire need of complementary biomarkers, particularly those that may be used for early detection (63, 64).

Initial attempts at using proteomics in the search for novel cancer biomarkers have not been successful. At the early stages of cancer, the amount of tumor-specific proteins produced by cancer cells may not be detectable as they are substantially diluted upon secretion into the blood circulation. This is further compounded by the limitation that gel-based proteomics can only detect serum proteins of high abundance. Attempts have been made to deplete albumin and other high-abundance proteins from the serum samples in order to reveal serum proteins of lower abundance (65-68). However, this only reduced the dynamic range of the serum proteome by 2 to 3 orders of magnitude and is still far from being able to detect the low-abundance proteins (69). It is therefore not surprising that changes of the same serum proteins are detected in studies using depletion techniques and those that use neat whole serum samples (Tab. IVA). In addition, the highly abundant albumin and immunoglobulins are known to interact non-specifically with many serum proteins including those that have been clinically or experimentally used as biomarkers (70). Losing these serum proteins in the depletion process certainly affects the interpretation of the data obtained from these experiments.

Despite their not being able to detect tumor-specific proteins, the proteomics analyses of serum or plasma samples highlight the different aberrant expression of selective proteins in patients with various cancers (Tab. IVA and IVB). Because of their altered concentration in serum, these proteins are categorized by definition as APRPs. In many of the proteomics analyses performed on patients with various types of cancers, which involved neat serum samples or even those that had been subjected to albumin depletion, the concentrations of many APRPs were affected. Many of these are apparently proteins of high abundance.

The high-abundance APRPs whose altered concentrations were commonly and consistently detected in numerous cancer patients using the proteomics approach include AAT, ACT, AHS, complement factor B (CFB), CLU, CPL, HAP, KNG, LRG and zinc α_2-glycoprotein (ZAG). With the exception of AHS and KNG, whose expression was reduced in selective cohorts of cancer patients as compared to negative control subjects, most of the other serum proteins were positive high-abundance APRPs. Interestingly, many of these proteomics findings confirmed previously reported observations from studies using the immunological and biochemical methods discussed earlier.

The enhanced levels of serum ACT, CFB, CPL, LRG and ZAG and the reduced levels of AHS and KNG detected in selective cancers via proteomics analyses were rather consistent. However, the altered patterns – in terms of which APRPs were involved – differed greatly between the broad spectra of cancers studied (Tab. IVA and IVB). This is similarly observed in the experiments performed using non-proteomics approaches (Tab. III). Serum CLU, HAP and AAT expressions were upregulated in a few of the cancers studied. Notable exceptions were the reports on the decrease or loss of pre-surgery serum CLU in esophageal squamous cell carcinoma by Zhang et al (81), lowered expression of HAP α_2 chain in pooled serum samples of breast cancer patients by Huang et al (83), and differences in results of AAT expression obtained

TABLE IVA - PROTEOMICS STUDIES OF APRP EXPRESSION IN CANCER: DATA FROM OTHER LABORATORIES

APRP	Type of cancer	Response	Method	Reference
AAT	Breast cancer (IDC)	↑	*2-DE; MALDI-MS	71
	Colorectal cancer	↑	SELDI-MS; Western blot	72
	Lung cancer (NSC)	↑	2D-DIGE; MALDI-MS; ELISA	73
	Pancreatic cancer	↑	*2-DE ; MALDI-MS	74
	Pancreatic ductal adenocarcinoma	↑	Solid-phase extraction fractionation; MALDI-MS	75
ACT	Pancreatic cancer	↑	*Immunoaffinity HPLC; 2D DIGE; MALDI-MS	76
	Pancreatic ductal adenocarcinoma	↑	Solid-phase extraction fractionation; MALDI-MS	75
AHS	Acute myeloid leukemia	↓	*2-DE; MALDI-MS; ESI-MS	77
	Lung cancer (SCC)	↓	*2D-DIGE; MALDI-MS; LC-MS/MS	78
CFB	Pancreatic cancer	↑	*2-DE; MALDI-MS; LC-MS/MS	79
CLU	Colorectal cancer	↑	Lectin-affinity purification; 2-DE; MALDI-MS	80
	Esophageal cancer (SCC)	↓	2-DE; MALDI-MS	81
CPL	Pancreatic cancer	↑	ESI-MS	82
HAP	Breast cancer	↓	*2D-DIGE; MALDI-MS	83
	Head and neck cancer (SCC)	↑	2-DE; ion-trap LC-MS/MS; PCR	84
	Liver cancer (AC)	↑	Lectin affinity purification; 2-DE	85
	Lung cancer (AC)	↑	2-DE; MALDI-MS	86
	Lung cancer (SCC)	↑	*2D-DIGE; MALDI-MS; LC-MS/MS	78
	Ovarian cancer	↑	SELDI-MS; ELISA	87
			*2-DE; MALDI-MS; ESI-MS/MS	68
LRG	Lung cancer (AC)	↑	Multilectin chromatography; LC-ESI-MS/MS	88
	Pancreatic cancer	↑	2-DE	89
			*2D-DIGE; LC-MS/MS	90
ZAG	Prostate cancer	↑	LC-MS/MS	91

↑ : increase; ↓ : decrease; *analyses of samples depleted of high-abundance proteins
IDC, infiltrating ductal carcinoma; SCC, squamous cell carcinoma; AC, adenocarcinoma; NSC, non-small cell (lung cancer)

TABLE IVB - PROTEOMICS STUDIES OF APRP EXPRESSION IN CANCER: DATA FROM OUR LABORATORY

Type of cancer	Relative expression		Reference
	Increased	Decreased	
Breast cancer	ACT, CFB, CLU	AAT, KNG	92
Nasopharyngeal cancer	CPL	-	93
Endometrial cancer	ABG, ATR, CLU, LRG	AAT, KNG	94
Cervical cancer (SCC & AC)	ABG, ATR, ZAG	AAT, KNG	94
Germ-line ovarian cancer	AAT, CLU, CLP, HAP, LRG	AHS	95
Epithelial ovarian cancer	AAT, ACT, CLU, HAP, LRG	-	95

SCC, squamous cell carcinoma; AC, adenocarcinoma

in our laboratory (92, 94) as compared to those of others. The results obtained from the analyses of CLU, HAP and AAT performed using biochemical and immunological methods are all in agreement with the main consensus.

The difference in expression of CLU may be due to its diverse and contradictory roles that were mentioned earlier, while the α_2 chain of HAP is known to vary between individuals (96). In the case of AAT, the results from the proteomics analyses demonstrated that the levels of this serum protease inhibitor were consistently enhanced in patients with breast, colorectal, lung and pancreatic cancers (Tab. IVA), and these results are compatible with the data obtained using biochemical and immunological means (Tab. III). However, different results were reported on the levels of AAT in sera of patients with breast or cervical cancers, and the levels of this serum protease inhibitor were also reported to be significantly reduced in patients with endometrial carcinoma (Tab. IVB). These discrepancies may be due to the differences in how serum protein concentrations are expressed; for example, AAT is expressed relative to the total detectable proteins by our laboratory rather than as its true serum concentration. This is a significant consideration because the expression of other serum proteins is greatly enhanced in gel-based proteomics studies.

When the proteomics data obtained from patients with various types of cancers were compiled and compared, uniform differential patterns of APRP expression were

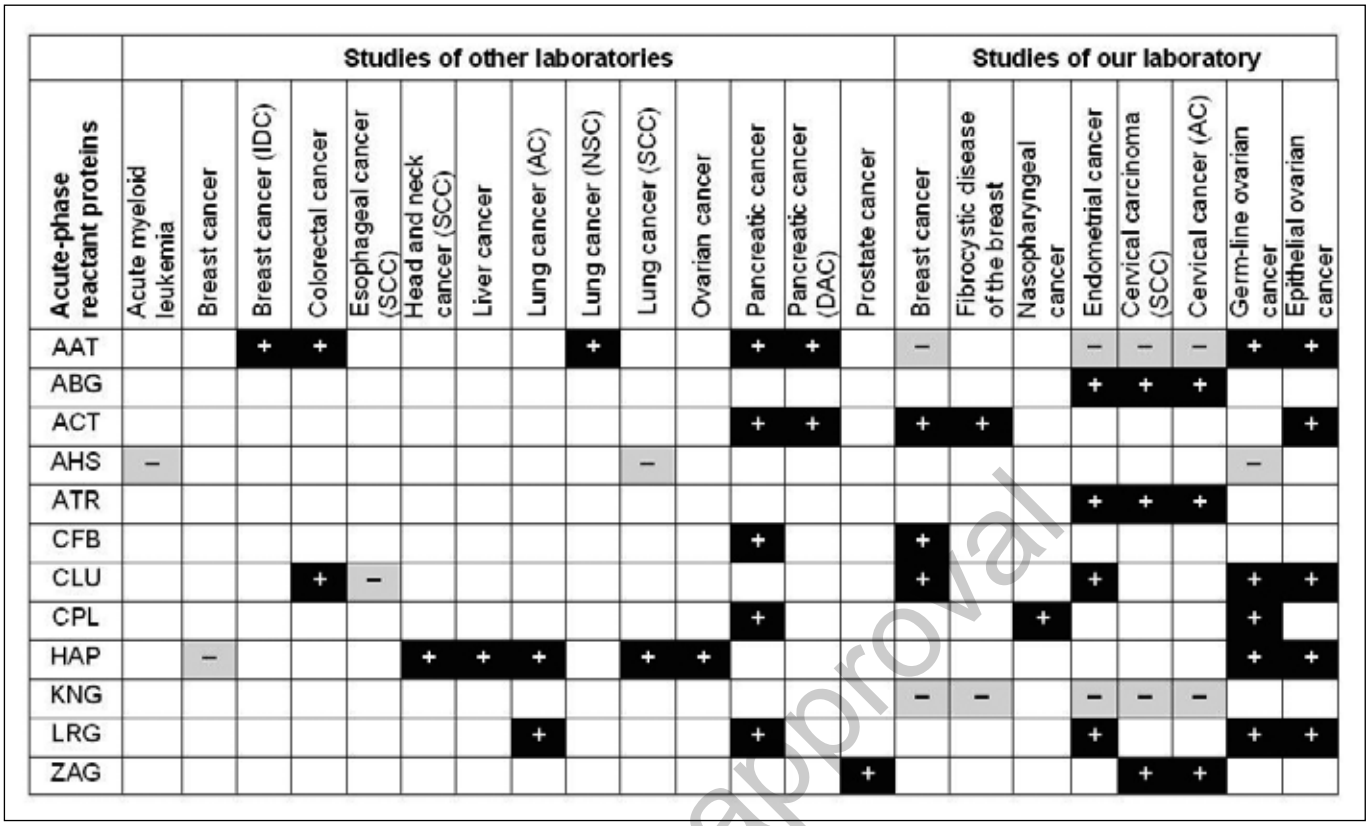

Fig. 1 - *Checkered diagram of acute-phase reactant protein expression in cancer.*
(+) indicates overexpression and (–) indicates underexpression of APRPs in serum of patients with cancer as compared to negative controls. IDC, infiltrating ductal carcinoma; SCC, squamous cell carcinoma; AC, adenocarcinoma; NSC, non-small cell (lung cancer); DAC, (pancreatic) ductal adenocarcinoma. For references, please see Tables III, IVA and IVB.

observed. Figure 1, a simple checkered diagram, demonstrates the accumulated proteomics analyses of APRP expression in patients with different cancers. The proteomics data were compiled from results generated in our laboratory as well as others. While many of the proteomics analyses involved serum samples that were depleted of albumin and other high-abundance proteins or fractionation procedures (Tab. IVA), the experiments performed in our laboratory involved the use of neat serum samples from cancer patients (Tab. IVB). Our data were also expressed in percentage of volume contribution of a protein relative to the total proteins detected, with the levels of some of the common APRPs subsequently validated using competitive ELISA (92-95).

PUTATIVE APRP FINGERPRINTS IN CANCER PATIENTS

From the overall pattern that was derived from the compiled data, the altered serum APRP profiles of patients appear to be unique for each type of cancer (Fig. 1). For example, pancreatic cancer is associated with upregulated serum AAT, ACT, CFB and LRG, while breast cancer is associated with enhanced levels of ACT, CFB and CLU and decreased KNG. Endometrial adenocarcinoma is characterized by enhanced levels of ABG, antithrombin III (ATR), CLU and LRG, and reduced levels of KNG, while cervical cancer is also associated with reduced KNG but with increased levels of ABG, ATR and ZAG. In the case of ovarian cancer, an altered pattern involving enhanced levels of CLU, CPL, HAP and LRG, and lowered levels of AHS is suggestive of germ-line cancer, while epithelial ovarian carcinoma is indicated by elevated levels of serum ACT, CLU, HAP and LRG.

The distinct patterns of altered serum APRP in patients with different cancers suggest that they may be used as protein fingerprints to distinguish cancer types, facilitate their diagnosis, and/or monitor disease. For example, breast cancer may be suspected if the early screening of a patient's serum demonstrates enhanced levels of ACT, CFB and CLU and decreased KNG, while a pattern showing upregulated serum AAT, ACT, CFB and LRG is indicative of pancreatic cancer. However, APRP fingerprinting may not be helpful for cancers like nasopharyngeal carcinoma because the concentration of only one of the high-

abundance proteins is altered.

In epithelial ovarian carcinoma we observed a positive correlation between the expression of ACT, HAP and AAT and progression of the 3 initial stages of cancer, while a negative correlation was found between the expression of LRG and CLU and cancer progression (unpublished data). A positive correlation of increasing AAT and HAP precursor with stages I, II and III of breast cancer has also been observed (71). These results underline the potential prognostic significance of selective high-abundance serum APRPs for breast and epithelial ovarian cancers.

FUTURE DIRECTIONS IN THE STUDY OF APRPs IN PATIENTS WITH CANCER

Any implications of the use of serum APRP patterns as fingerprints for cancer diagnostics and monitoring are still preliminary considering that the number of patients involved in most of the reported proteomics analyses was relatively small and the cohorts of patients involved in the studies were not quite appropriately standardized. However, the importance of carrying out a large-scale validation analysis of the serum APRP expressions in different types and subtypes of cancer is greatly emphasized by the compelling evidence for the diagnostic and/or prognostic potential of the method. This can perhaps not be done with the classical proteomics techniques, which are laborious and time consuming, but is readily possible in light of the new developments in high-throughput methodologies like protein arrays, multiplexed protein assays, and targeted chip-based proteomic assays. The levels of the serum APRPs of interest, which have already been identified by previous studies, may be assessed simultaneously in different cohorts of cancer patients using these state-of-the-art approaches, with the patients grouped not only on the basis of the different types or subtypes of cancer but also according to cancer stage, tumor load, treatment, and performance status.

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REFERENCES

1. Tillett WS, Francis T Jr. Serological reactions in pneumonia with a non-protein somatic fraction of Pneumococcus J Exp Med 1930; 52: 561-71.
2. Abernethy TJ, Avery OT. The occurrence during acute infections of a protein not normally present in the blood: I. Distribution of the reactive protein in patients' sera and the effect of calcium on the flocculation reaction with C-polysaccharide of Pneumococcus. J Exp Med 1941; 73: 173-82.
3. Hiramatsu S, Kojima J, Okada TT, Inai S, Ohmori K. The serum protein profile in chronic hepatitis, cirrhosis and liver cancer. Acta Hepatogastroenterol (Stuttg) 1976; 23: 177-82.
4. Thompson D, Bird HA. Acute phase response. In: Isenberg DA, Maddison PJ, Woo P, Glass D, Breedveld FC, eds. Oxford textbook of rheumatology. 3rd ed. Oxford: Oxford University Press 2004; 473-8.
5. Gruys E, Toussaint MJ, Niewold TA, Koopmans SJ, van Dijk E, Meloen RH. Monitoring health by values of acute phase proteins. Acta Histochem 2006; 108: 229-32.
6. Kushner I, Ganapathi M, Schultz D. The acute phase response is mediated by heterogeneous mechanisms. Ann N Y Acad Sci 1989; 557: 19-29; discussion: 30.
7. Mackiewicz A. Acute phase proteins and transformed cells. Int Rev Cytol 1997; 170: 225-300.
8. Kushner I, Mackiewicz A. Acute phase proteins as disease markers. Dis Markers 1987; 5: 1-11.
9. Jenne DE, Tschopp J. Molecular structure and functional characterization of a human complement cytolysis inhibitor found in blood and seminal plasma: identity to sulfated glycoprotein 2, a constituent of rat testis fluid. Proc Natl Acad Sci U S A 1989; 86: 7123-7.
10. Kirszbaum L, Sharpe JA, Murphy B, et al. Molecular cloning and characterization of the novel, human complement-associated protein, SP-40,40: a link between the complement and reproductive systems. EMBO J 1989; 8: 711-8.
11. Trougakos IP, Gonos ES. Clusterin/apolipoprotein J in human aging and cancer. Int J Biochem Cell Biol 2002; 34: 1430-48.

12. Volanakis JE. Acute phase proteins in rheumatic disease. In: Koopman WJ, ed. Arthritis and allied conditions: a textbook of rheumatology. 13 ed. Baltimore: Williams & Wilkins 1997; 505-14.
13. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med 1999; 340: 448-54.
14. Bank U, Ansorge S. More than destructive: neutrophil-derived serine proteases in cytokine bioactivity control. J Leukoc Biol 2001; 69: 197-206.
15. Cooper EH, Ward AM. Acute phase reactant proteins as aids to monitoring disease. Invest Cell Pathol 1979; 2: 293-301.
16. Samokyszyn VM, Miller DM, Reif DW, Aust SD. Inhibition of superoxide and ferritin-dependent lipid peroxidation by ceruloplasmin. J Biol Chem 1989; 264: 21-6.
17. Gutteridge JM, Smith A. Antioxidant protection by haemopexin of haem-stimulated lipid peroxidation. Biochem J 1988; 256: 861-5.
18. Kikuchi T, Abe T, Yaekashiwa M, et al. Secretory leukoprotease inhibitor augments hepatocyte growth factor production in human lung fibroblasts. Am J Respir Cell Mol Biol 2000; 23: 364-70.
19. Dabbagh K, Laurent GJ, Shock A, Leoni P, Papakrivopoulou J, Chambers RC. Alpha-1-antitrypsin stimulates fibroblast proliferation and procollagen production and activates classical MAP kinase signalling pathways. J Cell Physiol 2001; 186: 73-81.
20. Graziadei I, Gaggl S, Kaserbacher R, Braunsteiner H, Vogel W. The acute-phase protein alpha 1-antitrypsin inhibits growth and proliferation of human early erythroid progenitor cells (burst-forming units-erythroid) and of human erythroleukemic cells (K562) in vitro by interfering with transferrin iron uptake. Blood 1994; 83: 260-8.
21. Banda MJ, Rice AG, Griffin GL, Senior RM. The inhibitory complex of human alpha 1-proteinase inhibitor and human leukocyte elastase is a neutrophil chemoattractant. J Exp Med 1988; 167: 1608-15.
22. Joslin G, Fallon RJ, Bullock J, Adams SP, Perlmutter DH. The SEC receptor recognizes a pentapeptide neodomain of alpha 1-antitrypsin-protease complexes. J Biol Chem 1991; 266: 11282-8.
23. Coussens LM, Werb Z. Inflammation and cancer. Nature 2002; 420: 860-7.
24. Shacter E, Weitzman SA. Chronic inflammation and cancer. Oncology (Williston Park) 2002; 16: 217-26, 229; discussion 230-2.
25. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet 2001; 357: 539-45.
26. Kuper H, Adami HO, Trichopoulos D. Infections as a major preventable cause of human cancer. J Intern Med 2000; 248: 171-83.
27. Wahl LM, Kleinman HK. Tumor-associated macrophages as targets for cancer therapy. J Natl Cancer Inst 1998; 90: 1583-4.
28. Yamanishi Y, Boyle DL, Rosengren S, Green DR, Zvaifler NJ, Firestein GS. Regional analysis of p53 mutations in rheumatoid arthritis synovium. Proc Natl Acad Sci U S A 2002; 99: 10025-30.
29. Aoki Y, Yarchoan R, Braun J, Iwamoto A, Tosato G. Viral and cellular cytokines in AIDS-related malignant lymphomatous effusions. Blood 2000; 96: 1599-601.
30. Trentin L, Cerutti A, Zambello R, et al. Interleukin-15 promotes the growth of leukemic cells of patients with B-cell chronic lymphoproliferative disorders. Blood 1996; 87: 3327-35.
31. Bernacka K, Kuryliszyn-Moskal A, Sierakowski S. The levels of alpha 1-antitrypsin and alpha 1-antichymotrypsin in the sera of patients with gastrointestinal cancers during diagnosis. Cancer 1988; 62: 1188-93.
32. Stamatiadis AP, St Toumanidou M, Vyssoulis GP, Manouras AJ, Apostolidis NS. Value of serum acute-phase reactant proteins and carcinoembryonic antigen in the preoperative staging of colorectal cancer. A multivariate analysis. Cancer 1990; 65: 2055-7.
33. Solakidi S, Dessypris A, Stathopoulos GP, Androulakis G, Sekeris CE. Tumour-associated trypsin inhibitor, carcinoembryonic antigen and acute-phase reactant proteins CRP and alpha1-antitrypsin in patients with gastrointestinal malignancies. Clin Biochem 2004; 37: 56-60.
34. Paternoster D, Cartei G, Campagnutta E, Maggino T, Valente S, Ongaro G. Acute reactive stage proteins in gynaecologic cancers. Eur J Gynaecol Oncol 1980; 1: 94-8.
35. Bhattacharyya J, Chaudhuri L. Alpha 1-antitrypsin: a possible tool for diagnosis of cervical cancer. Biochem Med Metab Biol 1990; 43: 243-5.
36. Nasulewicz A, Mazur A, Opolski A. Role of copper in tumour angiogenesis--clinical implications. J Trace Elem Med Biol 2004; 18: 1-8.
37. Itakura J, Ishiwata T, Friess H, et al. Enhanced expression of vascular endothelial growth factor in human pancreatic cancer correlates with local disease progression. Clin Cancer Res 1997; 3: 1309-16.
38. Zelvyte I, Wallmark A, Piitulainen E, Westin U, Janciauskiene S. Increased plasma levels of serine proteinase inhibitors in lung cancer patients. Anticancer Res 2004; 24: 241-7.
39. El-Akawi ZJ, Al-Hindawi FK, Bashir NA. Alpha-1 antitrypsin (alpha1-AT) plasma levels in lung, prostate and breast cancer patients. Neuro Endocrinol Lett 2008; 29: 482-4.
40. Merlini G, Perfetti V, Gobbi PG, et al. Acute phase proteins and prognosis in multiple myeloma. Br J Haematol 1993; 83: 595-601.
41. Koebl H, Tatra G, Bieglmayer C. A comparative study of immunosuppressive acidic protein (IAP), CA 125 and acute-phase proteins as parameters for ovarian cancer monitoring. Neoplasma 1988; 35: 215-20.
42. Buamah PK, Skillen AW. Concentrations of protease and anti-protease in serum of patients with pancreatic cancer. Clin Chem 1985; 31: 876-7.
43. Tountas Y, Sparos L, Theodoropoulos C, Trichopoulos D. Alpha 1-antitrypsin and cancer of the pancreas. Digestion 1985; 31: 37-40.
44. Kuvibidila S, Rayford W. Correlation between serum prostate-specific antigen and alpha-1-antitrypsin in men without and with prostate cancer. J Lab Clin Med 2006; 147: 174-81.
45. Kalabay L, Jakab L, Prohaszka Z, et al. Human fetuin/alpha2HS-glycoprotein level as a novel indicator of liver cell function and short-term mortality in patients with liver

cirrhosis and liver cancer. Eur J Gastroenterol Hepatol 2002; 14: 389-94.
46. Stejskal D, Fiala RR. Evaluation of serum and urine clusterin as a potential tumor marker for urinary bladder cancer. Neoplasma 2006; 53: 343-6.
47. Kirchheimer JC, Kolbl H, Tatra G, Binder BR. Relationship between plasma levels of components of the fibrinolytic system and acute-phase reactants in patients with uterine malignancies. Eur J Clin Invest 1990; 20: 79-84.
48. Arumanayagam M, Wong FW, Rogers M, Swaminathan R. Serum ceruloplasmin, plasma copper concentration and copper to ceruloplasmin ratio in cervical carcinoma. Gynecol Obstet Invest 1993; 35: 175-8.
49. Gundogdu M, Kaya H, Gulcin I, et al. Oxidase activity of ceruloplasmin and some acute phase reactant and trace element concentrations in serum of patients with chronic lymphocytic leukemia. Scott Med J 2007; 52: 24-7.
50. Tsavaris N, Kosmas C, Kopterides P, et al. Retinol-binding protein, acute phase reactants and Helicobacter pylori infection in patients with gastric adenocarcinoma. World J Gastroenterol 2005; 11: 7174-8.
51. Boz A, Evliyaoglu O, Yildirim M, Erkan N, Karaca B. The value of serum zinc, copper, ceruloplasmin levels in patients with gastrointestinal tract cancers. Turk J Gastroenterol 2005; 16: 81-4.
52. Agroyannis B, Dalamangas A, Dardouphas K, et al. Serum transferrin and ceruloplasmin in patients with cancer of the gastrointestinal and other systems. Anticancer Res 1994; 14: 2201-3.
53. Pejovic M, Djordjevic V, Ignjatovic I, Stamenic T, Stefanovic V. Serum levels of some acute phase proteins in kidney and urinary tract urothelial cancers. Int Urol Nephrol 1997; 29: 427-32.
54. Esme H, Cemek M, Sezer M, et al. High levels of oxidative stress in patients with advanced lung cancer. Respirology 2008; 13: 112-6.
55. Ros-Bullon MR, Sanchez-Pedreno P, Martinez-Liarte JH. Serum ceruloplasmin in melanoma patients. Anticancer Res 2001; 21: 629-32.
56. Senra Varela A, Lopez Saez JJ, Quintela Senra D. Serum ceruloplasmin as a diagnostic marker of cancer. Cancer Lett 1997; 121: 139-45.
57. Avall Lundqvist E, Blad E, Xiao L, Sjovall K, Eneroth P. Pretreatment serum levels of C-reactive protein, alpha 1-antitrypsin, haptoglobin, alpha 1-acid glycoprotein and tissue polypeptide antigen in cervical carcinoma. Eur J Gynaecol Oncol 1991; 12: 375-83.
58. Zhao J, Patwa TH, Qiu W, et al. Glycoprotein microarrays with multi-lectin detection: unique lectin binding patterns as a tool for classifying normal, chronic pancreatitis and pancreatic cancer sera. J Proteome Res 2007; 6: 1864-74.
59. Roeise O, Sivertsen S, Ruud TE, Bouma BN, Stadaas JO, Aasen AO. Studies on components of the contact phase system in patients with advanced gastrointestinal cancer. Cancer 1990; 65: 1355-9.
60. Srinivas PR, Wagner AS, Reddy LV, et al. Serum alpha 2-HS-glycoprotein is an inhibitor of the human insulin receptor at the tyrosine kinase level. Mol Endocrinol 1993; 7: 1445-55.
61. Kalabay L, Chavin K, Lebreton JP, Robinson KA, Buse MG, Arnaud P. Human recombinant alpha 2-HS glycoprotein is produced in insect cells as a full length inhibitor of the insulin receptor tyrosine kinase. Horm Metab Res 1998; 30: 1-6.
62. Raghothama C, Akhilesh P. Quantitative proteomics for identification of cancer biomarkers. Proteomics - Clinical Applications 2007; 1: 1080-9.
63. Simpson RJ, Bernhard OK, Greening DW, Moritz RL. Proteomics-driven cancer biomarker discovery: looking to the future. Curr Opin Chem Biol 2008; 12: 72-7.
64. Cho WC. Contribution of oncoproteomics to cancer biomarker discovery. Mol Cancer 2007; 6: 25.
65. Adkins JN, Varnum SM, Auberry KJ, et al. Toward a human blood serum proteome: analysis by multidimensional separation coupled with mass spectrometry. Mol Cell Proteomics 2002; 1: 947-55.
66. Wang YY, Cheng P, Chan DW. A simple affinity spin tube filter method for removing high-abundant common proteins or enriching low-abundant biomarkers for serum proteomic analysis. Proteomics 2003; 3: 243-8.
67. Pieper R, Gatlin CL, Makusky AJ, et al. The human serum proteome: display of nearly 3700 chromatographically separated protein spots on two-dimensional electrophoresis gels and identification of 325 distinct proteins. Proteomics 2003; 3: 1345-64.
68. Ahmed N, Barker G, Oliva KT, et al. Proteomic-based identification of haptoglobin-1 precursor as a novel circulating biomarker of ovarian cancer. Br J Cancer 2004; 91: 129-40.
69. Granger J, Siddiqui J, Copeland S, Remick D. Albumin depletion of human plasma also removes low abundance proteins including the cytokines. Proteomics 2005; 5: 4713-8.
70. Veenstra TD, Conrads TP, Hood BL, Avellino AM, Ellenbogen RG, Morrison RS. Biomarkers: mining the biofluid proteome. Mol Cell Proteomics 2005; 4: 409-18.
71. Hamrita B, Chahed K, Trimeche M, et al. Proteomics-based identification of alpha1-antitrypsin and haptoglobin precursors as novel serum markers in infiltrating ductal breast carcinomas. Clin Chim Acta 2009; 404: 111-8.
72. Ward DG, Suggett N, Cheng Y, et al. Identification of serum biomarkers for colon cancer by proteomic analysis. Br J Cancer 2006; 94: 1898-905.
73. Patz EF Jr, Campa MJ, Gottlin EB, Kusmartseva I, Guan XR, Herndon JE 2nd. Panel of serum biomarkers for the diagnosis of lung cancer. J Clin Oncol 2007; 25: 5578-83.
74. Sun ZL, Zhu Y, Wang FQ, et al. Serum proteomic-based analysis of pancreatic carcinoma for the identification of potential cancer biomarkers. Biochim Biophys Acta 2007; 1774: 764-71.
75. Koomen JM, Shih LN, Coombes KR, et al. Plasma protein profiling for diagnosis of pancreatic cancer reveals the presence of host response proteins. Clin Cancer Res 2005; 11: 1110-8.
76. Yu KH, Rustgi AK, Blair IA. Characterization of proteins in human pancreatic cancer serum using differential gel electrophoresis and tandem mass spectrometry. J Proteome Res 2005; 4: 1742-51.
77. Kwak JY, Ma TZ, Yoo MJ, et al. The comparative analysis of serum proteomes for the discovery of biomarkers for acute

myeloid leukemia. Exp Hematol 2004; 32: 836-42.
78. Dowling P, O'Driscoll L, Meleady P, et al. 2-D difference gel electrophoresis of the lung squamous cell carcinoma versus normal sera demonstrates consistent alterations in the levels of ten specific proteins. Electrophoresis 2007; 28: 4302-10.
79. Bloomston M, Zhou JX, Rosemurgy AS, Frankel W, Muro-Cacho CA, Yeatman TJ. Fibrinogen gamma overexpression in pancreatic cancer identified by large-scale proteomic analysis of serum samples. Cancer Res 2006; 66: 2592-9.
80. Rodriguez-Pineiro AM, de la Cadena MP, Lopez-Saco A, Rodriguez-Berrocal FJ. Differential expression of serum clusterin isoforms in colorectal cancer. Mol Cell Proteomics 2006; 5: 1647-57.
81. Zhang LY, Ying WT, Mao YS, et al. Loss of clusterin both in serum and tissue correlates with the tumorigenesis of esophageal squamous cell carcinoma via proteomics approaches. World J Gastroenterol 2003; 9: 650-4.
82. Hanas JS, Hocker JR, Cheung JY, et al. Biomarker identification in human pancreatic cancer sera. Pancreas 2008; 36: 61-9.
83. Huang HL, Stasyk T, Morandell S, et al. Biomarker discovery in breast cancer serum using 2-D differential gel electrophoresis/MALDI-TOF/TOF and data validation by routine clinical assays. Electrophoresis 2006; 27: 1641-50.
84. Chen CB, Su YC, Huang TT, et al. Differentially expressed serum haptoglobin alpha chain isoforms with potential application for diagnosis of head and neck cancer. Clin Chim Acta 2008; 398: 48-52.
85. Ang IL, Poon TC, Lai PB, et al. Study of serum haptoglobin and its glycoforms in the diagnosis of hepatocellular carcinoma: a glycoproteomic approach. J Proteome Res 2006; 5: 2691-700.
86. Maciel CM, Junqueira M, Paschoal ME, et al. Differential proteomic serum pattern of low molecular weight proteins expressed by adenocarcinoma lung cancer patients. J Exp Ther Oncol 2005; 5: 31-8.
87. Ye B, Cramer DW, Skates SJ, et al. Haptoglobin-alpha subunit as potential serum biomarker in ovarian cancer: identification and characterization using proteomic profiling and mass spectrometry. Clin Cancer Res 2003; 9: 2904-11.
88. Heo SH, Lee SJ, Ryoo HM, Park JY, Cho JY. Identification of putative serum glycoprotein biomarkers for human lung adenocarcinoma by multilectin affinity chromatography and LC-MS/MS. Proteomics 2007; 7: 4292-302.
89. Deng R, Lu Z, Chen Y, Zhou L, Lu X. Plasma proteomic analysis of pancreatic cancer by 2-dimensional gel electrophoresis. Pancreas 2007; 34: 310-7.
90. Kakisaka T, Kondo T, Okano T, et al. Plasma proteomics of pancreatic cancer patients by multi-dimensional liquid chromatography and two-dimensional difference gel electrophoresis (2D-DIGE): up-regulation of leucine-rich alpha-2-glycoprotein in pancreatic cancer. J Chromatogr B Analyt Technol Biomed Life Sci 2007; 852: 257-67.
91. Bondar OP, Barnidge DR, Klee EW, Davis BJ, Klee GG. LC-MS/MS quantification of Zn-alpha2 glycoprotein: a potential serum biomarker for prostate cancer. Clin Chem 2007; 53: 673-8.
92. Doustjalali SR, Yusof R, Yip CH, Looi LM, Pillay B, Hashim OH. Aberrant expression of acute-phase reactant proteins in sera and breast lesions of patients with malignant and benign breast tumors. Electrophoresis 2004; 25: 2392-401.
93. Doustjalali SR, Yusof R, Govindasamy GK, Bustam AZ, Pillay B, Hashim OH. Patients with nasopharyngeal carcinoma demonstrate enhanced serum and tissue ceruloplasmin expression. J Med Invest 2006; 53: 20-8.
94. Abdul-Rahman PS, Lim BK, Hashim OH. Expression of high-abundance proteins in sera of patients with endometrial and cervical cancers: analysis using 2-DE with silver staining and lectin detection methods. Electrophoresis 2007; 28: 1989-96.
95. Chen Y, Lim BK, Peh SC, Abdul-Rahman PS, Hashim OH. Profiling of serum and tissue high abundance acute-phase proteins of patients with epithelial and germ line ovarian carcinoma. Proteome Sci 2008; 6: 20.
96. Langlois MR, Delanghe JR. Biological and clinical significance of haptoglobin polymorphism in humans. Clin Chem 1996; 42: 1589-600.

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