Gene Ther Mol Biol Vol 8, 539-546, 2004

Two dimensional gel electrophoresis analyses of human plasma proteins. Association of retinol binding protein and transthyretin expression with breast cancer

Research Article

Karim Chahed[1,2], Bechr Hamrita[1], Hafedh Mejdoub[3], Sami Remadi[1,4], Anouar Chaïeb[5] and Lotfi Chouchane[1,*]

[1]Laboratoire d'Immuno-Oncologie Moléculaire, Faculté de Médecine de Monastir, Tunisia
[2]Institut supérieur de Biotechnologie de Monastir, Tunisia
[3]Faculté des Sciences de Sfax, Tunisia
[4]Laboratoire Cytopath, Sousse, Tunisia
[5]Service d'Obstétrique et des maladies féminines, Hôpital Universitaire Farhat Hached, Sousse Tunisia.

***Correspondence**: Prof. Lotfi Chouchane, Laboratoire d'Immuno-oncologie Moléculaire, Faculté de Médecine de Monastir, 5019 Monastir, Tunisie; Tel: 216-73-462-200; Fax: 216-73-460-737; e-mail: lotfi.chouchane@planet.tn

Key words: Two dimensional gel electrophoresis. Breast cancer. Acute phase proteins.

Abbreviations: carcinoembryonic antigen, (CEA); cellular retinol, (CRBP); isoelectrofocalisation, (IEF); National Center for Biotechnology Information, (NCBI); prostate specific antigen, (PSA); retinoic acid, (RA); retinol binding protein, (RBP); Serum amyloid P, (SAP); transthyretin, (TTR)

This work was supported by le Ministère de la Recherche Scientifique et de Technologie, le Ministère de l'Enseignement Supérieur and le Ministère de la Santé Publique de la République Tunisienne.

Received: 10 December 2004; Revised: 11 January 2005
Accepted: 17 January 2005; electronically published: March 2005

Summary

The identification of markers for either early diagnosis, treatment response or for survival of breast cancer is of critical importance. The plasma carries an archive of important histological information whose determination may help to improve early disease detection. Using two dimensional gel electrophoresis and protein sequencing we investigated the changes in protein expression profiles derived from analysis of plasma from healthy Tunisian women and patients with breast carcinoma. We have found an association between retinol binding protein, transthyretin expression and breast cancer. The levels of acute phase proteins known to accompany both acute and chronic inflammatory disorders comprising haptoglobin, serum amyloid P, apolipoprotein A1, α1-antitrypsin and α1-acidic glycoprotein were also intimately associated with this neoplastic disease.

I. Introduction

Breast cancer is the most frequent malignancy among women representing a major health problem in many countries. Considering the cellular complexity and the dynamic structures of mammary tumors breast cancer is mainly classified according to the cellular origin of the cancer cells and on the evolution of the disease (Hondermarck, 2003). Current methods used to detect breast tumors are based on mammography. It is a widely used and clinically screening method that is effective in detecting early stage breast cancer before clinical symptoms appear (Brenner, 2002). However, since a tumor should be at least a few millimeters in size, it is already late when breast cancer is detected. So, there is a considerable need for the identification of useful pathological markers that can help not only in early detection but also for typing and treatment.

It is well established that changes that occur in disease versus normal tissues at either the gene (genomic) or protein (proteomic) level are regarded as an appropriate way to identify markers of pathologies that could be correlated with drug response and patient survival.

Proteomics with the recent advances in mass spectrometry has brought with it the hope of discovering novel biomarkers that could be used to diagnose diseases. Probably, the most widely used proteomic technology is the identification of alterations in protein expression between two samples through comparative 2-DE (Conrads et al, 2003). In such investigation a biomarker is defined as a protein having more or less intensity on one gel compared with the other and should be particularly associated with the disease.

The search for biomarkers and specific alterations using proteomic methods largely focus upon plasma or serum (Anderson and Anderson, 1977; Hoogland et al, 1999). These biological fluids are clinically relevant since they could be obtained in sufficient quantities from patients. It is well known that during necrosis and apoptosis content of cells could be released into the plasma. In addition, plasma may contain proteins or peptides that are aberrantly shed or secreted from cells in response to a disease (Adkins et al, 2002). It might be expected that the presence of a disease could be determined by measuring the altered presence or abundance of the constituant molecular species and reinforces the benefits of using a 2-DE approach for identifying biomarkers for disease states (Wrotnowski, 1998).

Blood plasma like cells contains many high abundant proteins. The major constituents include albumine, haptoglobin, immunoglobulins, transferrin, lipoproteins, fibrinogene B and fibrinogene γ. Other very low abundant proteins are commonly present in plasma (Wrotnowski, 1998; Anderson and Anderson, 2002). They represent the low molecular weight plasma proteome and could be generated from larger proteins by proteolysis within the circulatory system or in the environment of the tumors.

Searching for human plasma alterations using 2-DE with regard to neoplastic disease has been extensively investigated. As early as 1974, 2-DE was carried to look for differences between protein patterns of individuals suffering cancer (Wright, 1974). Since, several markers were characterized and are currently used for diagnosis. As an example, increased levels of molecular markers such as prostate specific antigen (PSA) and CA 125 are now routinely used for the detection of cancer in the prostate and ovary respectively (Charrier et al, 2001; Petricoin et al, 2002). Other markers are effective for diagnosing primary or advanced neoplastic diseases. The carcinoembryonic antigen (CEA) is used for detecting colorectal cancer, Her2/neu, CA 15-3 and CA 27-29 for advanced breast cancer (Diamandis, 1996; Buzdor and Hortobagy, 1999). Kallikreins, a family of secreted serine proteases were highly associated with ovarian carcinoma as well as with breast and prostate cancers (Yousef and Diamandis, 2001).

The plasma carries an archive of important histological information whose determination could help to improve early disease detection. In the present study, by using 2-DE investigations of human plasma proteins we have found an association between the levels of retinol binding protein (RBP), transthyretin (TTR) and breast carcinoma among Tunisian women.

II. Materials and methods

A. Patients and controls

Plasma samples were collected from six untreated patients diagnosed with infiltrating breast ductal carcinoma. Control subjects (16) were healthy blood donors having no evidence of any personal or family history of cancer (or other serious illness). Controls and patients were selected from the same population living in the middle coast of Tunisia. All the samples were collected with informed consent according to protocols approved by the institutional review boards of the respective hospitals. Plasma samples were stored at -80°C before analysis.

B. Two dimensional gel electrophoresis and evaluation of 2D data

1. Isoelectrofocalisation of proteins (first dimension) and SDS-PAGE

To the plasma, four volumes of cold acetone (-20°C) were added and the solution was incubated for 1 Hour at -20°C. The pellet was washed with cold acetone (80%), dried under partial vacuum and solubilised in 7.0 M urea, 2.0 M Thiourea, 4% (w/v) CHAPS, 0,5% w/v DTT and 2% ampholytes (1 part pH 3/10, 1 part pH 5/7, 2parts pH 6/8). Acetone precipitation led to a better resolution of abundant plasma proteins, but there has been significant loss in lower molecular weight proteins. Protein contents were determined according to the procedure described by Bradford (Bradford, 1976) and modified by Ramagli and Rodriguez (Ramagli and Rodriguez, 1985). Bovine serum albumin (Fraction V, Sigma) was used as a standard. Analytical 2D-PAGE was carried out in a Bio-Rad system (Miniprotean II). Equal amounts of proteins issued from control or breast cancer samples were subjected simultaneously to isoelectrofocalisation (IEF) and SDS-PAGE analysis. Extraction of proteins, solubilisation, IEF, SDS-PAGE and staining were carried under very similar conditions for the different samples. Each experiment was repeated for at least three times. Focused strips were equilibrated in SDS equilibration buffer and were then loaded onto SDS gel slabs for separation in the second dimension (Laemmli, 1970).

2. Gel staining

After separation in SDS-PAGE gels, the proteins were visualised by a sensitive colloidal coomassie G-250 stain (Neuhoff et al, 1985). The dye solution contained 17% (w/v) ammonium sulfate, 3% (v/v) phosphoric acid, 0,1% (w/v) coomassie G250 and 34% (v/v) methanol. The staining solution was changed once after 12 hours staining and the gel slabs subjected to a 24 hours cycle for increasing dye deposition on low abundance proteins. The detection was then increased by placing the gel into 1% v/v acetic acid for producing a better contrast between spots and gel. Silver staining was done according to Oakley et al, (1980). All coomassie and silver–stained gels were scanned into adobe photoshop 6.0. Alterations in protein levels defined as clear differences in size and/or density of the protein spot on the gel were confirmed through differential analyses using melanie 3.0 software tools. Comparison of the 2D patterns with published human plasma protein 2D-PAGEs of the Swiss-2DPAGE database (Sanchez et al, 1995) allowed characterization of the indicated plasma proteins.

3. N-terminal amino acid sequencing

As the experimental and theoretical positions of a protein may differ significantly, the identity of proteins of interest was confirmed by sequencing. Plasma proteins (500 μg) were

fractionated on 12 cm IEF rod gels (1.5 mm diameter) at 300 volts for 1 hour, 450 volts for 2 hours and 650 volts for 15 hours. SDS-PAGE was performed under constant current intensity (35 mA/gel). Following electrophoresis, proteins were electroblotted on Immobilon P using a semi-dry blotter system (Millipore) and stained with coomassie blue according to the manufacturer's instructions. Spots on Immobilon membranes, corresponding to polypeptides of interest were collected ans subjected to Edman degradation using an applied biosystem modele (Procise). Amino acid sequence analysis and data base search were performed at the National Center for Biotechnology Information (NCBI) and comparison with the Swiss Prot data bases.

III. Results and Discussion

Plasma proteomic analysis of six malignant breast cancer samples and 16 samples from human healthy donors were compared by high resolution two dimensional gel electrophoresis. Several proteins were up-regulated in all of the breast cancer samples compared to that of healthy controls. The majority of the protein identifications appeared to represent differences in overall abundance. 2-DE investigations showed elevated levels of acute phase proteins such as haptoglobin (β-chain), serum amyloid P, α1-antitrypsin, α1-antichymotrypsin and α1-acidic glycoprotein in plasma from patients diagnosed with breast cancer (**Figure 1**). Two other proteins, highly elevated in cancer plasma, were identified as RBP and TTR.

The first group of proteins designed as positive acute phase proteins is known to accompany both acute and chronic inflammatory disorders (Doherty et al, 1998). During tumoral growth, acute phase proteins have also been described to accumulate at high levels and could be used to distinguish tumor type and prognosis (Negishi et al, 1987; Schmid et al, 1995; Alaiya et al, 2000). This is well described for prostate cancer where the association of antichymotrypsin and PSA is well investigated to help in the differential diagnosis of prostate cancer from benign prostate hyperplasia (Charrier et al, 2001). In a recent study, Cho W C et al, (2004) identified serum amyloid A as a serum biomarker that could be useful in the diagnosis of relapse in nasopharyngeal cancer.

As our investigation, several studies have examined aspects of the acute phase response in which many high abundant plasma proteins increase or decrease following a range of inflammatory insults or cancer (Bini et al, 1992). In acute inflammatory responses and in rheumatoid arthritis, differences in the levels of 19 acute phase proteins were reported to be affected. These studies, based on quantitative serum analysis, showed that high abundant acute phase–related proteins could be good prognostic markers of inflammation (Doherty et al, 1998). Gianazza and co-workers (Miller et al, 1999, Eberini et al, 2000) identified, using 2-DE, 34 proteins with human homologues showing changes in protein abundance and were associated with inflammatory diseases. Several other 2-DE studies have examined aspects of the acute phase response following an inflammatory insult. Changes in haptoglobin levels were reported in duchenne muscular dystrophy (John and Purdom, 1989), human gonadotropin isoforms in patients with trophoblastic tumors (Hoemann et al, 1993) and ApoA-1 during parturition (Del Piore et al, 1991) and heart disease (Cassler et al, 1992). The levels of other acute phase proteins such as serum amyloid A were altered after a severe head injury (Choukaite et al, 1989) or viral infections (Bini et al, 1996). By comparative proteome analysis, Vejda et al (2002) found elevated levels of degradation products of antiplasmin and laminin γ-chain in cancer samples. They also found significantly elevated levels of the acute phase proteins α1-acidic glycoprotein, α1-antitrypsin, α1-antichymotrypsin and haptoglobin. The α1-antitrypsin and laminin γ-chain were described as being anti-apoptotic factors (Yoshida et al, 2001; Vejda et al, 2002). Kuhajda et al, (1989) reported that haptoglobins could be associated with phenotypically aggressive neoplasia and serve as mediators of some malignant processes in breast cancer. They were also found to stimulate collagen synthesis in fibroblasts from cancerous body fluids (Viellard et al, 1974). Detection and quantification of haptoglobins could also be a useful diagnostic procedure in cancer. A recent study of haptoglobin polymorphism in breast cancer patients demonstrates that haptoglobin 1 and 2 alleles were over-represented in patients with familial and non familial breast cancer respectively (Awadallah and Atoum, 2004). Other studies provided evidence that the haptoglobin α subunit is specifically increased in sera of ovarian cancer patients. It has been postulated that Hpα might affect the immune response as a potent immunosuppressant (Bini et al, 2003). The study we carried out showed an increase in the levels of haptoglobin (β chain) in all breast cancer samples (**Figure 1**). However, we were unable to draw conclusions concerning α1 and α2 chains because of the genetic polymorphism associated with the corresponding gene. In the case of two other protein family members, α1-acidic glycoprotein and α1-antitrypsin, showing elevated levels in our breast cancer samples, a direct anti-apoptotic mode of action has been demonstrated on tumor necrosis factor-induced apoptosis of hepatocytes (Van Molle et al, 1997). The increase in the levels of protease inhibitors in plasma such as α1-antitrypsin and α1-antichymotrypsin could be related to the high proteolytic activity mediated by proteases such as plasmin in cancer samples (Anderson et al, 1993; Vejda et al, 2002). These two related glycoprotein protease inhibitors, present in plasma could also neutralize proteases released by leucocytes in response to trauma and inflammatory stimuli (Bergman et al, 1993). Serum amyloid P (SAP), a plasma glycoprotein, shows also a slight increase in breast cancer samples (**Figure 1**). This pentraxin protein has been shown to bind chromatin in apoptotic and necrotic cells, thus preventing antinuclear auto-immunity (Bickerstaff et al, 1990). The SAP protein recognizes ligands from necrotic cells, binds to late apoptotic cells and is involved in their phagocytosis by human monocyte derived macrophages (Familian et al, 2001; Bijl et al, 2002).

A second set of proteins designed as negative acute phase proteins comprising TTR and retinol binding protein (RBP) displayed interestingly increased intensities in all the breast cancer samples (**Figure 1**). A lower amount of RBP and TTR was found in all control samples regardless to the age. This result suggests that the association

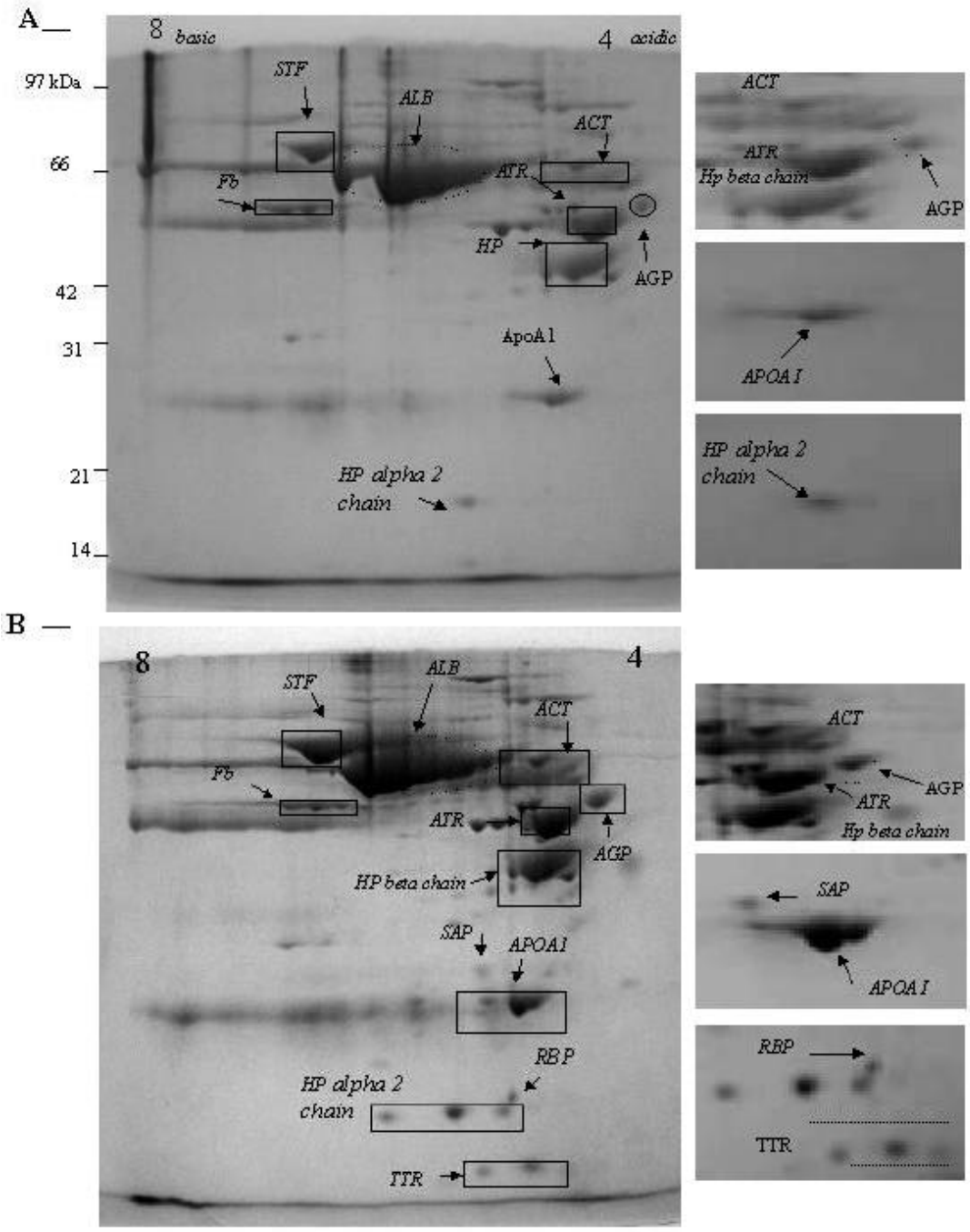

Figure 1. Two dimensional gel electrophoresis analyses of plasma proteins derived from (**A**) a healthy donor and (**B**) a breast cancer patient. Partial 2-DE images from a control gel (**A**) and from a breast cancer sample (**B**) are shown. Abr: STF: serotransferrin ; ALB: albumin ; ACT: anti-chymotrypsin ; ATR: anti-trypsin ; AGP: acidic glycoprotein ; Hp: haptoglobin ; Fb: fibrinogen beta chain ; ApoAI: apoAI lipoprotein ; SAP: serum amyloid P ; RBP: retinol binding protein.

between RBP and TTR expression in plasma and breast cancer is unlikely to be related to the age.

Further characterization of the RBP and TTR spots was performed by protein sequencing. The two spots were electroblotted on immobilon P and subjected to N-terminal amino acid sequence analyses. The deduced sequences (*RBP*:[1]ERDCRVSSFRVKENFDKARF[20];*TTR*:[1]GPTGTGE SKCPLMVKVLDAV[20]) were compared with the Swiss Prot data bases and found to correspond, respectively, to retinol binding protein and transthyretin.

The high levels of RBP and TTR found in plasma of Tunisian patients with breast cancer as revealed by 2D-PAGE were not reported for other populations (Mehta et al, 1987; Basu et al, 1988;1989; Russell et al, 1988; Vejda et al, 2002). This may be attributable to differences in study design, to the analyzed populations, as well as to the presence of different confounding factors.

The retinol binding protein is a member of the lipocalins family and has been used as a marker of diseases associated with inflammation and cancer (Xu and Venge, 2000). It is synthesized predominantly by the liver and is the principal carrier of all-trans retinol (vitamin A) in the blood stream (Goodman, 1984). Transthyretin acts as a transport protein for thyroxin T4 and is the primary

indirect carrier of vitamin A through its interaction with retinol binding protein. It is well established that the metabolism of RBP and TTR is highly associated with that of vitamin A (Rosales et al, 2000). In the blood, the retinol/ RBP complex further binds to transthyretin at a ratio of 1:1:1 and is then transported to the target cells. Retinol is then metabolized to its active form, retinoic acid (RA), which is an important transcription modulator that acts in the regulation of proliferation and differenciation of many cell types (Blomhoff, 1994). Retinoids act also as cancer chemopreventive and chemotherapeutic agents (Honk and Sporn, 1997). They were also reported to inhibit the growth of several breast cancer cell lines (Chen et al, 1997). The action of retinol and RA is mediated by their binding to cellular retinol (CRBP) and retinoic acid binding proteins (CRABPI and CRABPII) and through two different families of nuclear RA receptors. The latters behave as ligand-activated–trans-acting transcription factors that can regulate the expression of several retinoid-responsive genes and hence alters the growth of normal and cancer cells (Mangelsdorf et al, 1994).

Recent studies indicate that the metabolism of retinol to retinoids is greatly reduced in several human carcinoma cell lines and tumor specimens (Guo and Gudas, 1998; Guo et al, 2000, 2001). Carcinoma cells from the breast showed a decrease in their ability to esterify retinol to retinyl esters (Chen et al, 1997). It has been suggested that this could lead to an inappropriate growth and to the loss of normal differenciation processes (Mira-Y-Lopez et al, 2000). Another frequent event in a subset of human breast cancers is the loss of CRBP expression. This protein is postulated to regulate the formation of retinyl esters and the synthesis of retinoic acid (Ong et al, 1994). It was suggested that CRBP down regulation occurs through DNA hypermethylation in human breast cancer and contributes to breast tumor progression (Arapshian et al, 2004). The decrease in the levels of CRBP leads to a restriction of the effects of intracellular vitamin A levels on breast cells (Kuppumbatti et al, 2000; Arapshian et al, 2004). Furthermore, it has been shown that increasing the levels of CRABPII, an intracellular lipid-binding protein that associates with retinoic acid, in mammary carcinoma cells (MCF7) strongly enhances their sensitivity to retinoic acid-induced growth inhibition (Budhu and Noy, 2002).

These data provide the evidence that increasing the intracellular levels of retinyl esters in malignant cells could be a good approach to treat patients with breast cancer. The increase in the plasma levels of RBP and TTR could thus be linked to the lack of sufficient internal retinyl ester stores necessary to regulate retinoid responsive genes in malignant cells.

In conclusion, the present study showing the high production of RBP and TTR in plasma of patients with breast cancer suggests that overproduction of these proteins could correlate with a decrease of retinyl esters in tumor cells.

Acknowledgments

This work was supported by le Ministère de la Recherche Scientifique et de Technologie, le Ministère de l'Enseignement Supérieur and le Ministère de la Santé Publique de la République Tunisienne.

References

Adkins JN, Varnum SM, Auberry KJ, Moore RJ, Angell NH, Smith RD, Springer DL and Pounds JG **(2002)** Toward a human blood serum proteome: Analysis by multidimensional separation coupled with mass spectrometry. **Mol Cell Proteomics** 1, 917-955.

Alaiya AA, Franzen B, Hagman A, Slitversward C, Moberger B, Linder S and Aner G **(2000)** Classification of human ovarian tumors using multivariate data analysis of polypeptide expression patterns. **Int JCancer** 86, 731-736.

Anderson C, Gelin J, Iresjo BM and Lundholm K **(1993)** Acute phase proteins in response to tumor growth. **J Surg Res** 55, 807-814.

Anderson L and Anderson N.G **(1977)** High resolution two-dimensional gel electrophoresis of human plasma proteins. **Proc Natl Acad Sci USA** 74, 5421-5425.

Anderson NL and Anderson NG **(2002)** The human plasma proteome. **Mol Cell Proteomics**, 845-867.

Arapshian A, Bertran S, Kuppumbatti YS, Nakajo S and Lopez RM **(2004)** Epigenetic CRBP down regulation appears to be an evolutionarily conserved (human and mouse) and oncogene specific phenomenon in breast cancer. **Molecular Cancer**, 3-13.

Awadallah SM and Atoum MF **(2004)** Haptoglobin polymorphism in breast cancer patients from jordan. **Clin Chim Acta** 341, 17-21.

Basu TK and Sasmal P **(1988)** Plasma vitamin A, retinol-binding protein, and prealbumin in postoperative breast cancer patients. **Int J Vitam Nutr Res** 58 , 281-3.

Basu TK, Hill GB, Ng D, Abdi E and Temple N **(1989)** Serum vitamins A and E, beta-carotene, and selenium in patients with breast cancer. **J Am Coll Nutr** 8, 524-529.

Bergman D, Kadner SS, Cruz MR, Esterman AL, Tahery MM, young BK and Finlay TH **(1993)** Synthesis of α1-antichymotrypsin and α1-antitrypsin by human trophoblast. **Pediatr Res** 34, 312-7.

Bickerstaff MC, Botto M, Hutchinson WL, Herbert J, Tennent GA, Bybee A, Mitchell DA, Cook HT, Butler PJ, Walport MJ and Pepys MB, **(1990)** Serum amyloid P component controls chromatin degradation and prevents antinuclear autoimmunity. **Nat Med** 5, 694-697.

Bijl M, Horst G, Bootsma H, Limburg PC and Kallenberg CGM **(2002)** Serum amyloid P component (SAP) binds to late apoptotic cells and mediates their phagocytosis by macrophages. **Arthritis Res** 4, 8.

Bini L, Magi B, Cellesi C, Rossolini A and Pallini V **(1992)** Two dimensional electrophoresis analysis of human serum proteins during the acute-phase response. **Electrophoresis** 13, 743-746.

Bini L, Nagi B, Marzocchi B, Celiesi C, Berti B, Raggiaschi R, Rossolini A and Pallini V **(1996)** Two dimensional electrophoretic patterns of acute-phase human serum proteins in the course of bacterial and viral diseases. **Electrophoresis** 17, 612-616.

Bini Ye, Cramer DW, Skates SJ, Gygi SP, Pratomo V, Fu LF, Horick NK, Licklider LJ, Schorge JO, Berkowitz RS and Mok SC **(2003)** Haptoglobin α-subunit as potential biomarker in ovarian cancer. **Clinical Cancer Res** 9, 2904-2911.

Blomhoff R, **(1994)** Overview of vitamin A metabolism and function, in Blomhoff R (Ed)-Vitamin A in health and disease, Marcel Dekker, New York, 1-35.

Bradford M **(1976)** A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. **Anal Biochem** 72, 248-254.

Brenner DJ, Sawant SG, Hande MB, Miller RC, Elliston CD, Fu Z, Randers-pehrson G and Marino SA **(2002)** Routine screening mammography: how important is the radiation-risk of the benefit-risk equation?. **Int J radia Biol** 78, 1065-1067.

Budhu AS and Noy N **(2002)** Direct channeling of retinoic acid between cellular retinoic acid-binding protein II and retinoic acid receptor sensitizes mammary carcinoma cells to retinoic acid-induced growth arrest. **Mol Cell Biol** 22, 2632-2641.

Buzdor AU and Hortobagy CN **(1999)** Breast cancer: in Pinedo HM, Londo DL, Chabner BA eds- Cancer chemotherapy and biological response modifiers. Annual 18, Amsterdam. Elsevier-Sciences B V, 435-69.

Cassler AB, Johansen JJ and Kendrick NC **(1992)** Two dimensional gel analysis of serum apolipoprotein A-1 isoforms. Preliminary analysis suggests altered ratios in individuals with heart disease. **Appl Theor Electrophoresis** 3, 41-46.

Charrier JP, Tournel C, Michel C, Comby S, Reynaud CJ, Passagot J, Alban PD, Chauvard D and Jolivet M **(2001)** Differential diagnosis of prostate cancer and benign prostate hyperplasia using two dimensional electrophoresis. **Electrophoresis** 22, 1861-1866.

Chen AC, Guo X, Derguini F and Gudas LJ **(1997)** Human breast cancer cells and normal mammary epithelial cells: retinol metabolism and growth inhibition by the retinol metabolite 4-oxoretinol. **Cancer Res** 57, 4642-4651.

Cho WC, Yi PC, Yip V, Thulasiraman V, Ngan RK, Yip TT, Lan WH, Au JS, Law SC, Cheng WW, Nja VW and Lim CK **(2004)** Identification of serum amyloid A protein as a potentially useful bio-marker to monitor relapse of nasopharyngeal cancer by serum proteomic profiling. **Clin Cancer Res** 10, 45-52.

Choukaite A, Visvikis S, Steihmetz J, Calteau MM, Kabbaj O, Ferard G, Melais R and Siest G **(1989)** Two dimensional electrophoresis of plasma proteins and high density lipoproteins during inflammation. **Electrophoresis** 10, 781-784.

Conrads TP, Zhou M, Petricoin EF, Liotta L and Veenstra TD **(2003)** Cancer diagnosis using proteomic patterns. **Expert Rev Mol Diagn** 3, 411-420.

Del Piore G, Chatterton R, Lee C, Silver R, Berg L and Lee MJ **(1991)** Comparison of mononuclear cell proteins and plasma proteins before and during parturition by two dimensional electrophoresis. **J Perinat Med** 19, 373-377.

Diamandis EP **(1996)** Prognostic markers in breast cancer. **Clin Lab News** 22, 235-9.

Doherty NS, Lillman BI, Reilly K, Swindell AC, Buss JM and Anderson NI **(1998)** Analysis of changes in acute-phase plasma proteins in an acute inflammatory response and in rheumatoid arthritis using two dimensional gel electrophoresis. **Electrophoresis** 19, 355-363.

Eberini I, Agnallo D, Miller I, Villa P, Fratelli M, Ghezzi P, Gemeiner M, Chau J, Aebersold R and Gianazza E **(2000)** Protein of rat serum V: Adjuvant arthritis and its modulation by non steroidal anti-inflammatory drugs. **Electrophoresis** 21, 2170-2179.

Familian A, Zwart B, Huisman HG, Rensink I, Roem D, Hordijk PL, Aarden LA and Harck CE **(2001)** Chromatin-independent binding of serum amyloid P Component to apoptotic cells. **J Immunol** 167, 647-654.

Goodman DS, **(1984)** In th retinoids (Sporn MB, Roberts AB and Goodman DS eds). Academic Press, New York 2, 41-88.

Guo X and Gudas LJ **(1998)** Metabolism of all-trans retinol in normal human cell strains and squamous cell carcinoma (SCC) lines from the oral cavitiy and skin: reduced esterification of retinol in SCC lines. **Cancer Res** 58, 166-176.

Guo X, Nanus DM, Ruiz A, Rando RR, Lorraine DB and Gudas LJ **(2001)** Reduced levels of retinyl esters and vitamin A in human renal cancers. **Cancer Res** 61, 2774-2781.

Guo X, Ruiz A, Rando RR, Bok D and Gudas LJ **(2000)** Esterification of all-trans-retinol in normal human epithelial cell strains and carcinoma lines from the oral cavity, skin and breast: reduced expression of lecithin retinol acyltransferase (LRAT) in the carcinoma lines. **Carcinogenesis** (Lond) 21, 1925-1933.

Hoemann R, Spoetti C, Crossmann M, Saller B and Mann K **(1993)** Molecular heterogeneity of human chorionic gonadotropin in serum and urine from patients with trophoblastic tumors. **Clin Investig** 71, 953-960.

Hondermarck H (**2003**) Breast cancer: when proteomics challenges biological complexity. **Mol Cell Proteomics**, 281-291.

Honk WK and Sporn MB **(1997)** Recent advances in chemoprevention of cancer. **Science** 278, 1073-1077.

Hoogland C, Sanchez JC, Tonella L, Bairoch A, Hochstrasser OF and Appel RD **(1999)** The Swiss 2D-PAGE database: what has changed during the last year. **Nucl Acid Res** 27, 289-291.

John H and Purdom IF **(1989)** Elevated plasma levels of haptoglobin in duchenne muscular dystrophy: electrophoretic variants in patients with a severe form of the disease. **Electrophoresis** 10, 489-493.

Kuhajda FP, Katumuluwa AI and Pasternack GR **(1989)** Expression of haptoglobin-related protein and it's potential rôle as a tumor antigen. **Proc Natl Acad Sci USA** 86, 1188-1192.

Kuppumbatti YS, Bleiweiss IJ, Mandeli JP, Waxman S and Lopez RMY **(2000)** Cellular retinol-binding protein expression and breast cancer. **J Natl Cancer Inst** 92, 475-480.

Laemmli UK **(1970)** Cleavage of structural proteins during the assembly of the head of bacteriophage T4. **Nature** 227, 680-685.

Mangelsdorf DJ, Umesono K and Evans RM **(1994)** The retinoids receptors, sporn MB, Roberts AB, Goodman DS eds. The retinoids: Biology, chemistry and medicine, Raven Press, 319-350.

Mehta RR, Hart G, Beattie CW and Das Gupta TK **(1987)** Significance of plasma retinol binding protein levels in recurrence of breast tumors in women. **Oncology** 44 (6) 350-5

Miller I, Haynes P, Eberini I, Gemeiner M, Aebersold R and Gianazza E **(1999)** Proteins of rat serum. III.Gender-related differences in protein concentration under base line conditions and upon experimental inflammation as evaluated by two dimensional electrophoresis. **Electrophoresis** 20, 836-845.

Mira-Y-Lopez R, Zheng WL, Kuppumbatti YS, Rexer B, Jing Y, Ong DE **(2000)** Retinol conversion to retinoic acid is impaired in breast cancer cell lines relative to normal cells. **J Cell Physiol** 185, 302-9.

Negishi Y, Furukawa T, Oka T, Sakamodo M, Hirata T, Okabe K, Matayoshi K, Akiya K and Soma H **(1987)** Clinical use of CA125 and its combination assay with other tumor markers in patients with ovarian carcinoma. **Gynecol Obstet Investig** 23, 200-207.

Neuhoff V, Stamm R and Elbl H **(1985)** Clear background and highly sensitive protein staining with coomassie blue dyes in polyacrylamide gels: a systematic analysis. **Electrophoresis** 6, 427-448.

Oakley BR, Kirsch DR and Morris NR **(1980)** A simplified ultra-sensitive silver stain for detecting proteins in polyacrylamide gels. **Anal Biochem** 105, 361-363.

Ong DE, Newcomer ME and Chytil F **(1994)** Cellular retinoid-binding proteins, Sporn MB, Roberts AB, Goodman DS eds. The retinoids: Biology, chemistry and medicine, Raven Press, 283-317.

Petricoin LI, Ardekani AM, Hitt BA, Levine PJ, Fusaro VA, Steinberg SM, Mills GB, Simone C, Fishman DA, Kohn EC and Liotta LA **(2002)** Use of proteomic patterns in serum to identify ovarian cancer. **Lancet** 359, 572-577.

Ramagli LS and Rodriguez LU **(1985)** Quantitation of microgram amounts of proteins in two-dimensional polyacrylamide gel electrophoresis sample buffer. **Electrophoresis** 6, 559-563.

Rosales JF, Topping JD, Smith JE, Shankar AH and Ross C **(2000)** Relation of serum retinol to acute phase proteins and malarial morbidity in papua new guinea children. **Am J Clin Nutr** 71, 1582-8.

Russell MJ, Thomas BS and Bulbrook RD **(1988)** A prospective study of the relationship between serum vitamins A and E and risk of breast cancer. **Br J Cancer** 57, 213-5.

Sanchez JC, Appel RD, Golaz O, Pasquali C, Ravier F, Bairoch A and Hochstrasser DF **(1995)** inside swiss 2D-PAGE data base. **Electrophoresis** 16, 1131-1151.

Schmid HR, Schmiller D, Blum P, Miller M and Vonderschmill D **(1995)** Lung tumor cells. A multivariate approach to cell classification using two dimensional protein paterns. **Electrophoresis** 16, 1961-1968.

Van Molle W, Libert C, Fiers W and Brouckaert P **(1997)** α1-antitrypsin inhibit TNF-induced but not anti-Fas induced apoptosis of hepatocytes in mice. **J Immunol** 159, 3555-3564.

Vejda S, Posovszky C, Zelzer S, Peter B, Bayer E, Gelbmann D, Hermann RS and Gerner C **(2002)** Plasma from cancer patients featuring a characteristic protein composition mediated protection against apoptosis. **Mol Cell Proteomics**, 387-393.

Viellard A, Lonbart C, Borel JP and Jayle MF **(1974)** In-vitro influence of haptoglobin and its complex with hemoglobin on the biosynthesis of collagen. **Pathol Biol** 22, 741-742.

Wright GL **(1974)** Two dimensional acrylamide gel electrophoresis of cancer patient serum proteins. **Ann Lab Sci** 4, 281-293.

Wrotnowski C **(1998)** the future of plasma proteins. **Genet Engl News**, 18 14.

Xu S and Venge P **(2000)** Lipocalins as biochemical markers of disease. **Biochim Biophys Acta** 1482, 298-307.

Yoshida Y, Hosokawa K, Dantes A, Kotsuji F, Kleinman HK and Amsterdam **(2001)** A Role of laminin in ovarian cancer tumor growth and metastasis via regulation of mdm2 and Bcl2 expression. **Int J Oncol**. 18, 913-921.

Yousef GM and Diamandis FP **(2001)** The new human tissue kallikrein gene family: structure, function and association to disease. **Endocr Rev** 22, 148-204.