Experimental
Hematology & Oncology

REVIEW

Open Access

Three are better than one: plasminogen receptors as cancer theranostic targets

Patrizia Ceruti[1,2†], Moitza Principe[1,2†], Michela Capello[1,2], Paola Cappello[1,2] and Francesco Novelli[1,2*]

Abstract

Activation of plasminogen on the cell surface initiates a cascade of protease activity with important implications for several physiological and pathological events. In particular, components of the plasminogen system participate in tumor growth, invasion and metastasis. Plasminogen receptors are in fact expressed on the cell surface of most tumors, and their expression frequently correlates with cancer diagnosis, survival and prognosis. Notably, they can trigger multiple specific immune responses in cancer patients, highlighting their role as tumor-associated antigens. In this review, three of the most characterized plasminogen receptors involved in tumorigenesis, namely Annexin 2 (ANX2), Cytokeratin 8 (CK8) and alpha-Enolase (ENOA), are analyzed to ascertain an overall view of their role in the most common cancers. This analysis emphasizes the possibility of delineating new personalized therapeutic strategies to counteract tumor growth and metastasis by targeting plasminogen receptors, as well as their potential application as cancer predictors.

Keywords: Annexin 2, Cytokeratin 8, Alpha-enolase, Plasminogen system, Plasminogen receptors, Cancer, Immunotherapy

Introduction

The plasminogen system is involved in tumor growth, invasion and metastasis [1-5]. Several proteins of this system have now been demonstrated to have a clinical value as diagnostic and prognostic markers in many types of cancer [6-11]. Overexpression of plasminogen receptors has been associated with poor prognosis, shorter patient survival and resistance to chemotherapy. Notably, this over-expression may lead to the production of autoantibodies in many types of cancers, which can be consequently used as tumor biomarkers. Moreover, most plasminogen system components are cell surface-expressed and therefore represent readily accessible targets for cancer therapy. This review aims to analyze the plasminogen receptors Annexin 2 (ANX2), Cytokeratin 8 (CK8) and alpha-Enolase (ENOA) in the most common tumors and to correlate their expression with specific immune responses and/or clinical outcomes.

The plasminogen system

Plasminogen is a 90kDa glycoprotein produced by the liver and present in plasma and extracellular fluids [12-15]. It possesses seven structural domains: an N-terminal activation peptide (1–77 aa), five kringle domains and a serine-protease domain (562–791 aa). The kringle domains mediate plasminogen binding to substrates and to cell surface receptors [13,16] whereas the activation of plasminogen to plasmin is mediated by the proteolytic action of either tissue-type (tPA) or urokinase-type (uPA) plasminogen activators [2,4,17,18]. Plasmin is a serine protease with a broad-spectrum substrate, including fibrin, extracellular matrix components (laminin, fibronectin) and proteins involved in extracellular matrix degradation (matrix metalloproteinases, such as MMP3) [16,19-22]. Binding of plasminogen to surface receptors has profibrinolytic consequences: enhancement of plasminogen activation, protection of plasmin from its inhibitor a2-antiplasmin and enhancement of the proteolytic activity of cell-bound plasmin [4,23-25]. Proteolysis mediated by cell-associated plasmin contributes both to physiological processes, such as tissue remodeling and embryogenesis, and to pathophysiological processes, such as cell invasion, metastasis and inflammatory responses [2-4,26-28]. A noteworthy positive

* Correspondence: franco.novelli@unito.it
†Equal contributors
¹Center for Experimental Research and Medical Studies (CeRMS), Azienda Ospedaliera Città della Salute e della Scienza, Via Cherasco 15, Turin 10126, Italy
²Department of Molecular Biotechnology and Health Science, University of Turin, Turin, Italy

correlation exists between elevated levels of plasminogen activation and malignancy [2,29].

Plasminogen receptors bind plasminogen by carboxy-terminal lysines, and this common recognition motif results in a similar affinity of plasminogen for most of its receptors (Kd ≈ 1μM). Although many plasminogen receptors have been described so far [30-43], the most well-known plasminogen receptors shown to play a role in cancer are ANX2, CK8 and ENOA. Interaction of the plasminogen lysine binding sites with ENOA and CK8 is dependent upon recognition of three C-terminal lysines [23,40]. For ENOA, an additional internal plasminogen binding site, which includes Lys256, has been proposed [44]. On the other hand, ANX2 harbors internal amino acids that mimic C-terminal lysines and therefore requires cleavage prior to binding plasminogen [45].

Molecular features of plasminogen receptors

ANX2 is a 36 kDa peripheral membrane protein (p36) that belongs to the Annexin family, consisting of calcium-dependent phospholipid-binding proteins with various membrane-related functions [46-48]. ANX2 either exists as a monomer, a heterodimer or a heterotetramer, the latter being composed of two copies of a 36 kDa heavy chain (p36) and two copies of an 11 kDa light chain (p11). Formation of the Anx2 heterotetramer facilitates its binding to the plasma membrane [45,48,49].

CK8 is an intermediate filament protein that polymerizes with CK18 to form a component of the epithelial cytoskeleton [50]. In addition to its cytoplasmic localization it can also be expressed on the cell surface [51,52] where it is localized to the blebs of the cell membrane and acts as a plasminogen receptor [53].

ENOA (2-phospho-D-glycerate hydrolase) was initially discovered as a metalloenzyme that catalyzes the dehydration of 2-phospho-D-glycerate (PGA) to phosphoenolpyruvate (PEP) in the glycolytic pathway [54,55]. In vertebrates, the enzyme has three different isoforms (alpha, beta, and gamma) codified by three independent loci. While ENOA is mostly ubiquitous, beta- and gamma-enolase are almost exclusively found in muscle and in neuronal tissues, respectively [54,56,57]. ENOA can form homo- or heterodimers, such as alpha-alpha, alpha-beta and alpha-gamma [58,59]. Both homo- and heterodimers can be expressed on the cell surface where they act as plasminogen receptors.

Interestingly ANX2, CK8 and ENOA amino acid sequences lack a transmembrane portion, and the mechanism by which they are displayed on the cell surface remains unknown. Several mechanisms have been proposed for the cell surface expression, including lipid binding followed by translocation to the outer membrane [60]; penetration and projection through the plasma membrane as a part of a protein complex [52,61]; and non-covalent association to the cell membrane after proteolytic release from cells into the extracellular space [62-64]. In the case of ANX2 and ENOA, post-translational modifications, such as acylation, acetylation, methylation and phosphorylation, particularly common in tumor cells, may also play a role [65-67].

Expression and function of ANX2 in cancer

ANX2 is physiologically expressed on the surface of epithelial cells, vascular endothelial cells and macrophages and is not only involved in plasminogen activation but also in many biological processes that include inflammation, cell proliferation, angiogenesis, cell–cell interactions and membrane bridgings, exocytosis and endocytosis, cell growth and apoptosis [48,68-73]. All these functions are due to its ability to interact with actin filaments in the cytoskeletal system, to locate proteases and other extracellular matrix components (plasminogen-plasmin-tPA) on the cell surface through tetramerization, or by acting as a major substrate for phosphorylation and as a second messenger of growth-mediating receptor [49]. Indeed, ANX2 is phosphorylated on Tyr23, Ser11 and Ser25 residues by c-Src, v-Src and Protein Kinase C (PKC), respectively [74-76] after activation of insulin receptor [77], insulin growth factor regulator [78], platelet-derived growth factor-R [79], fibroblast growth factor (FGF) or epidermal growth factor (EGF) [80]. Therefore, ANX2 may play a role as a second messenger for transduction of growth and differentiation.

During oncogenic transformation, ANX2 is usually up-regulated and is a marker of aggressiveness in the majority of cancer types (Table 1) [68,81-101]. As a result, it is a valuable tool for cancer diagnosis. In fact, in hepato-cellular carcinoma (HCC), ANX2 has been proposed as a differential diagnostic marker, in combination with glypican-3 (GPC3), glutamine synthetase (GS) and heat shock protein 70 (HSP70) [65,85,86,90,93,98,100-104]. ANX2 down-regulation has also been reported in certain cancers, such as laryngeal and esophageal squamous cell carcinoma, head and neck dysplasia [105-109], osteosarcoma [110] and prostate cancer [111-113] (Table 1). In osteosarcoma and oral carcinoma, ANX2 overexpression is generally associated with well-differentiated tumors and, in certain cases, ANX2 down-regulation was observed in poorly-differentiated cancer which may be due to its ability to promote differentiation independently from its plasminogen-binding function [107,110]. Indeed, in oral carcinoma two different groups have reported an increased expression of ANX2 in tumor tissue compared to the surrounding normal mucosa or mucosa from patients without oral cancer [100,107]. Rodrigo et al. also added a clinical correlation analysis in which the lower expression of ANX2 seemed to correlate with a poor prognosis. Even if these studies appear controversial, both groups reported that in poorly differentiated tumors, which usually correlate with a bad prognosis, ANX2 was down-regulated. We

Table 1 Expression, immune response, clinical correlation and function of ANX2 in cancer

Cancer tissue	Expression	Immune response	Clinical correlation	Function
Bone	protein [110]			Metastasis [110]
Brain	protein [97,98]		Diagnosis [98]	
Breast	mRNA [99]			Chemoresistance, Growth, Metastasis [99,114-116,118]
Colon	mRNA, protein [86,89,96]		Prognosis [86]	
Gastric	protein [85]		Prognosis [85]	
Head and neck	mRNA, protein [100] [105-109]	Ab [119,120]	Prognosis [100]	
Hematopoietic system	mRNA [95] protein [68,91]			Growth, Metastasis [68,91]
Kidney	protein [84,101]		Survival, Prognosis [101]	Metastasis [121]
Liver	mRNA, protein [88,90,92,93]		Diagnosis, Prognosis [90,93,103]	
Lung	protein [82]	Ab [122]		Growth, Metastasis [117]
Ovary		Ab [123]		
Pancreas	mRNA, protein [81,87]	Ab [65,124]	Survival, Prognosis [65,104]	Chemoresistance, EMT, Metastasis [65,104]
Prostate	protein [102,111-113]		Prognosis [102]	Growth, Metastasis [47,102]
Skin		T cells [125]		
Thyroid	mRNA [83,94]			

can therefore speculate that ANX2 is up-regulated even in oral carcinoma, but microenvironment and inflammatory responses related to the anaplastic condition induce down-regulation of ANX2 during cancer progression. Opposite trends reported ANX2 as a suitable prognostic marker in HCC, renal (CRCC), colorectal and gastric carcinomas, where its expression negatively correlates with a favorable clinical outcome [85,93,96,100,101]. In fact, when up-regulated, ANX2 contributes to cancer invasion and metastasis by acting as a co-receptor for plasminogen, tPA and pro-cathepsin B [49]. ANX2-dependent plasmin generation is required for invasion, metastasis and angiogenesis in several cancer types [47,65,68,114-117]. For example, it has been demonstrated that in pancreatic cancer (PDAC), tyrosine 23 phosphorylation is necessary for cell-surface localization of ANX2. This translocation is critical for the Transforming Growth Factor beta (TGFbeta)-induced, Rho-mediated epithelial-to-mesenchymal transition (EMT) [65]. Moreover, in PDAC, ANX2 overexpression is a predictor of rapid recurrence after surgery in patients who have undergone gemcitabine-adjuvant chemotherapy [104]. In conclusion, we can hypothesize that in tumors where ANX2 is overexpressed, its role as a plasminogen receptor predominates, and is responsible for the metastatic process, while in tumors where ANX2 is down-regulated, plasminogen-independent mechanisms favor the malignant state through anaplastic transformation.

Expression and function of CK8 in cancer

Several mechanisms have been proposed for the expression of CK8 on the cell surface. In transformed cells, surface expression could be due to insufficient incorporation into intermediate filaments due to over-production of CK8 [64]. Indeed, CK8 is over-expressed both at the mRNA and the protein level in various carcinomas (Table 2) and is present at the cell surface of several human cancers and established tumor cell lines [51,52,64,88,126-129]. By contrast, healthy human tissues do not express CK8 at the cell surface, with the exception of a sporadic and weak CK8 membrane localization in the liver and heart [127,130].

When present on the cancer cell surface, CK8 binds plasminogen and promotes its activation through plasminogen activators. In breast cancer cells it also works as a receptor for uPA [134], suggesting a model in which CK8, in complex with uPA, plasminogen and fibronectin constitutes a signaling platform capable of modulating cell adhesion and growth. Indeed, increased expression of CK8/CK18 has been shown at the invasive front of certain tumors [145]. Of particular note, over-expression of CK8 on the tumor cell surface and in the cytoplasm correlates, both *in vitro* and *in vivo,* with increased invasiveness and metastatic properties [144]. In breast and endometrial cancer, a clear correlation exists between CK8 expression and tumor stage [133,144], and CK8 confers drug resistance to chemotherapeutic treatment

Table 2 Expression, immune response, clinical correlation and function of CK8 in cancer

Cancer tissue	Expression	Immune response	Clinical correlation	Function
Breast	protein [52,64,127,131]	Ab [132]	Prognosis, Survival, Diagnosis [131,133]	Chemoresistance, Growth, Metastasis [134-136]
Colon		Ab [137]		
Gastric	mRNA [128]	Ab [138]		
Head and neck	protein [51]	Ab [51,139]		
Liver	mRNA, protein [52,88]	Ab [140,141]	Prognosis [130]	
Lung	mRNA, protein [126,129]		Survival [126,142]	
Skin				Metastasis [143]
Uterus			Prognosis [144]	Metastasis [144]

in breast cancer cell lines [135,136]. The effect of CK8 over-expression on survival remains controversial as in non-small cell lung carcinomas (NSCLC), increasing serum levels of CK8 were significantly associated with tumor progression and lower patient survival [126,142], whereas breast cancer patients with low expression of CK8 and high expression of Human Epidermal Growth Factor Receptor 2 (HER2) have a higher risk of recurrence and death within 5 years (Table 2) [131].

Expression and function of ENOA in cancer

ENOA over-expression is associated with tumor development through a process known as aerobic glycolysis or the Warburg effect [146], which determines an increase in anaerobic glycolysis in hypoxic conditions, a very common feature in most solid tumors, but also in the presence of normal levels of oxygen [147-149]. The ENOA promoter contains a hypoxia responsive element [150,151] that leads to self up-regulation at both the mRNA and protein levels (Table 3) [130,152-173]. However, in a small fraction of NSCLC [174], ENOA expression is down-regulated and, interestingly, is a gene that can be homozygously absent in tumors [175]. In PDAC, ENOA is not only up-regulated but is also subjected to specific post-translational modifications, namely acetylation, methylation and phosphorylation which do not occur in normal tissue [67,176].

Moreover, ENOA is expressed on the surface of hematopoietic cells, such as monocytes, T cells, B cells and neutrophils according to their activation status or pathophysiological conditions [54]. ENOA is localized on the cell surface of PDAC, breast and lung cancers

Table 3 Expression, immune response, clinical correlation and function of ENOA in cancer

Cancer Tissue	Expression	Immune response	Clinical correlation	Function
Brain	mRNA [152]			
Bone	protein [170]		Prognosis [170]	
Breast	mRNA, protein [164,167,177]	Ab [178,179]	Prognosis, Survival [167,178]	Chemoresistance, Metastasis [167,177,178]
Colon	mRNA, protein [152,158,168]			
Gastric	mRNA, protein [152,162,169]	T cells [180]		
Head and neck	mRNA, protein [155,166,171]	Ab [119,120]	Prognosis [166]	Metastasis [166]
Hematopoietic system	protein [160]	Ab [181]		
Liver	mRNA, protein [130,152,156,165]		Prognosis [156,165]	Growth, Metastasis [156]
Lung	mRNA, protein [152,159,172,174,182]	Ab [159,172,178,183,184]	Prognosis, Survival [172,174,178]	
Ovary	mRNA, protein [152,153]			
Pancreas	mRNA, protein [152,154,161,163]	Ab and T cells [154,185,186]	Survival [185]	
Prostate	mRNA, protein [152,187]			
Skin	mRNA [173]	Ab [173,188]		

[67,154,172,189] (Table 3), while in melanoma and NSCLC it is also secreted by exosomes [190,191]. On cell surfaces, ENOA is part of a multi-protein complex, together with uPA receptor (uPAR), integrins and certain cytoskeletal proteins, collectively known as a *metastasome*, responsible for adhesion, migration and proliferation, as clearly demonstrated in ovarian cancer cells [192]. Moreover, since ENOA interacts with the cytoskeleton in muscle cells, its over-expression is likely to promote migration of tumor cells by providing ATP [29]. Noteworthy, in human follicular thyroid carcinoma cells, retinoic acid causes a decrease in ENOA levels that coincides with their reduced motility [193], while cell surface ENOA is enhanced in breast cancer cells rendered super-invasive following paclitaxel treatment [194].

The prognostic value of ENOA expression has been observed in several tumors (Table 3) [156,165-167,170, 172,174,178,185]. In breast cancer, enhanced ENOA expression correlates with a greater tumor size, poor nodal status and a shorter disease-free interval. Higher expression of ENOA increases the risk of longer distance relapse when compared to loco-regional relapse in postsurgical 4-Hydroxytamoxifen-treated Estrogen Receptor-positive breast cancer patients. Therefore, down-regulation of ENOA could be a novel pharmacological approach for overcoming 4-Hydroxytamoxifen resistance in breast cancer therapy [167]. In head and neck cancer and NSCLC, ENOA levels increase with cancer progression and negatively correlate with patients' overall- and progression-free survival [166]. In HCC, ENOA expression is up-regulated in poorly differentiated tumors compared to well-differentiated ones, and its levels positively correlate with tumor size and venous invasion [156,165]. Even if ENO1 are not currently used in clinical routine, the altered expression or the induced immune response, as show later, may have a prognostic/diagnostic value, especially in combination with gold-standard markers. For example, in PDAC, the detection of serum antibodies against phosphorylated ENOA, in combination with the commonly used serum marker CA19.9, enhances the ability to discriminate between control and cancer patients [185]. Taken together, these findings determine that ENOA is a good biomarker to monitor tumor progression and to predict clinical outcome.

Plasminogen receptors elicit immune responses in cancer

Activation of the immune system is an early event during tumorigenesis, as illustrated by the detection of high titers of autoantibodies in patients with early-stage cancer, and correlates with the progression of malignant transformation [119,195,196]. The presence of an immune response to the plasminogen receptors ANX2, CK8 and ENOA has been reported in patients with different types of cancer.

An autoantibody response against ANX2 occurs in the early stages of gingivo-buccal carcinogenesis and in PDAC, ovarian, and lung cancer (Table 1) [65,119,120,122,123]. It has been demonstrated that in melanoma, the ANX2 (208–223) peptide induces antigen-specific T cells, which can recognize cancer cells over-expressing the ANX2 molecule. This peptide may therefore be useful in immunotherapy for recruiting CD4+ type 1 helper cells locally active in the tumor environment [125]. Moreover, phase II clinical trial PDAC patients [124] showed an increased anti-ANX2 antibody response after administration of the GM-CSF (granulocyte-macrophage colony-stimulating factor) PDAC-specific vaccine, which positively correlated with prolonged survival [65].

CK8/18 complexes elicit humoral responses in breast, gastric, colon, head and neck and liver carcinomas [51,132,137-141]. In squamous cell carcinoma (SCC), elevated titers of CK8-specific serum antibodies are detectable in early stage patients, while a weaker humoral response has been observed in advanced patients, probably due to immunosuppression by tumor cells. In several adenocarcinomas, patients develop an immune response against tumoral neo-epitopes of CK8 [137], while head and neck and lung cancer patients develop autoantibodies against post-translationally modified variants of the protein (Table 2) [139,142]. The induction of specific antibodies against CK8 can be explained by its ectopic expression on the cancer cell surface that makes CK8 a potential candidate for antibody-based cancer therapy.

A humoral and/or T cell immune response against ENOA has been observed in patients with breast, head and neck, gastric, lung cancer, chronic myeloid leukemia, melanoma and PDAC (Table 3) [119,120,154,159,172,173, 178-181,183-186,188]. Notably, detection of autoantibodies against ENOA combined with expression of carcinoembryonic antigen (CEA) and CK19 fragment enhances the sensitivity for differential diagnosis of NSCLC [184]. High levels of antibodies to phosphorylated ENOA were found in PDAC, and their combination with the serological marker CA19.9 discriminates patients from controls [185]. The presence of the humoral response to phosphorylated ENOA also correlates with a longer progression-free survival upon gemcitabine treatment and overall survival [185] and notably, with the presence of a specific T cell response [154,180,186,197]. Conversely, a marked decrease in basal levels of ENOA-autoantibodies is a common event in late lung and breast cancers, proposing ENOA-autoantibodies as a prognostic marker to monitor disease progression in these patients [178].

Plasminogen receptors as therapeutic targets in cancer

Despite heterogeneous numbers of plasminogen receptors existing in eukaryotic cells, only ENOA, CK8 and ANX2 have a recognized role in human cancer

progression by promoting plasmin-dependent tumor invasion. ANX2 also behaves as a tPA receptor, whilst ENOA and CK8 do not directly bind plasminogen activators, but most likely form a multi-protein complex with uPAR and integrins [64,67]. Nevertheless, the contribution of these three receptors to cancer development is not only linked to their plasminogen-binding capacity. Indeed ANX2, ENOA and CK8 all interact with the actin cytoskeleton and such an association could promote the migration of tumor cells independently from plasminogen-binding [29]. These three receptors are present on the surface of the majority of human neoplasms, and in some cases, such as breast and colon, they are expressed altogether. All of these receptors elicit an antibody immune response in cancer patients that can be potentially boosted for therapeutic purposes. Regarding the potential risk of inducing an autoimmune reaction, CK8 would probably be the best candidate as it is aberrantly expressed only on the tumor cell surface. However, even the ANX2 and ENOA are frequently over-expressed by tumor cells and exposed on the cell surface. This strengthens the hypothesis for utilizing them as potential targets of therapeutic monoclonal antibodies. Notably, data obtained in animal models show a therapeutic effect of anti-ANX2 monoclonal antibody (mAb) treatment in breast, pancreatic and lung cancer by inhibiting tumor growth, suppressing metastases and prolonging survival (Table 1) [65,114,117]. Thus, blocking plasminogen receptors is a promising strategy to counteract metastasis development.

A recent paper has shown that a DNA-based vaccination against human ENOA, followed by electroporation, was able to significantly prolong the median of life expectancy of mice that spontaneously develop PDAC [186]. Indeed, the ENOA DNA vaccine induced both a humoral and cellular response. Autoantibodies against ENOA were able to bind the cell surface of PDAC murine cells and mediate their killing by complement-dependent cytotoxicity. This "immunogenic" death might be responsible for the greater influx of CD3 into tumor and for their activation toward Th1/Th17 phenotype. Cytokines produced by these Th populations strongly supported the "effecto" switching of anti-ENOA autoantibodies [186]. Moreover, the ENOA-DNA vaccine significantly decreased suppressor cells such as myeloid-derived suppressor cells and regulatory T cells [186].

Conclusions

Overall, with very few exceptions, all three plasminogen receptors reported here display performant diagnostic and prognostic values. Since one single receptor type is not expected to account for the entire plasminogen binding on tumor cells, a combined therapy targeting all these molecules would be desirable. Nevertheless, further studies are necessary in order to better understand the involvement of these three receptors in cancer progression, so that more effective strategies for cancer treatment may be developed.

References
1. Kwaan HC, McMahon B: **The role of plasminogen-plasmin system in cancer.** *Cancer Treat Res* 2009, **148**:43–66.
2. Andreasen PA, Egelund R, Petersen HH: **The plasminogen activation system in tumor growth, invasion, and metastasis.** *Cell Mol Life Sci* 2000, **57**:25–40.
3. Dano K, Behrendt N, Hoyer-Hansen G, Johnsen M, Lund LR, Ploug M, Romer J: **Plasminogen activation and cancer.** *Thromb Haemost* 2005, **93**:676–681.
4. Plow EF, Herren T, Redlitz A, Miles LA, Hoover-Plow JL: **The cell biology of the plasminogen system.** *FASEB J* 1995, **9**:939–945.
5. McMahon B, Kwaan HC: **The plasminogen activator system and cancer.** *Pathophysiol Haemost Thromb* 2008, **36**:184–194.
6. Shin SJ, Kim KO, Kim MK, Lee KH, Hyun MS, Kim KJ, Choi JH, Song HS: **Expression of E-cadherin and uPA and their association with the prognosis of pancreatic cancer.** *Jpn J Clin Oncol* 2005, **35**:342–348.
7. Xue A, Scarlett CJ, Jackson CJ, Allen BJ, Smith RC: **Prognostic significance of growth factors and the urokinase-type plasminogen activator system in pancreatic ductal adenocarcinoma.** *Pancreas* 2008, **36**:160–167.
8. Tang L, Han X: **The urokinase plasminogen activator system in breast cancer invasion and metastasis.** *Biomed Pharmacother* 2013, **67**:179–182.

9. Zhang W, Ling D, Tan J, Zhang J, Li L: **Expression of urokinase plasminogen activator and plasminogen activator inhibitor type-1 in ovarian cancer and its clinical significance.** *Oncol Rep* 2013, **29**:637–645.

10. Markl B, Renk I, Oruzio DV, Jahnig H, Schenkirsch G, Scholer C, Ehret W, Arnholdt HM, Anthuber M, Spatz H: **Tumour budding, uPA and PAI-1 are associated with aggressive behaviour in colon cancer.** *J Surg Oncol* 2010, **102**:235–241.

11. Berger DH: **Plasmin/plasminogen system in colorectal cancer.** *World J Surg* 2002, **26**:767–771.

12. Derechin M: **Purification of human plasminogen.** *Biochem J* 1962, **82**:241–247.

13. Petersen TE, Martzen MR, Ichinose A, Davie EW: **Characterization of the gene for human plasminogen, a key proenzyme in the fibrinolytic system.** *J Biol Chem* 1990, **265**:6104–6111.

14. Hayes ML, Castellino FJ: **Carbohydrate of the human plasminogen variants. I. Carbohydrate composition, glycopeptide isolation, and characterization.** *J Biol Chem* 1979, **254**:8768–8771.

15. Hayes ML, Castellino FJ: **Carbohydrate of the human plasminogen variants. III. Structure of the O-glycosidically linked oligosaccharide unit.** *J Biol Chem* 1979, **254**:8777–8780.

16. Vassalli JD, Sappino AP, Belin D: **The plasminogen activator/plasmin system.** *J Clin Invest* 1991, **88**:1067–1072.

17. Castellino FJ, Ploplis VA: **Structure and function of the plasminogen/ plasmin system.** *Thromb Haemost* 2005, **93**:647–654.

18. Paciucci R, Tora M, Diaz VM, Real FX: **The plasminogen activator system in pancreas cancer: role of t-PA in the invasive potential in vitro.** *Oncogene* 1998, **16**:625–633.

19. Lijnen HR, Van Hoef B, Lupu F, Moons L, Carmeliet P, Collen D: **Function of the plasminogen/plasmin and matrix metalloproteinase systems after vascular injury in mice with targeted inactivation of fibrinolytic system genes.** *Arterioscler Thromb Vasc Biol* 1998, **18**:1035–1045.

20. Plow EF, Felez J, Miles LA: **Cellular regulation of fibrinolysis.** *Thromb Haemost* 1991, **66**:32–36.

21. Sato H, Takino T, Okada Y, Cao J, Shinagawa A, Yamamoto E, Seiki M: **A matrix metalloproteinase expressed on the surface of invasive tumour cells.** *Nature* 1994, **370**:61–65.

22. Vassalli JD, Pepper MS: **Tumour biology. Membrane proteases in focus.** *Nature* 1994, **370**:14–15.

23. Miles LA, Dahlberg CM, Plescia J, Felez J, Kato K, Plow EF: **Role of cell-surface lysines in plasminogen binding to cells: identification of alpha-enolase as a candidate plasminogen receptor.** *Biochemistry* 1991, **30**:1682–1691.

24. Miles LA, Hawley SB, Baik N, Andronicos NM, Castellino FJ, Parmer RJ: **Plasminogen receptors: the sine qua non of cell surface plasminogen activation.** *Front Biosci* 2005, **10**:1754–1762.

25. Plow EF, Freaney DE, Plescia J, Miles LA: **The plasminogen system and cell surfaces: evidence for plasminogen and urokinase receptors on the same cell type.** *J Cell Biol* 1986, **103**:2411–2420.

26. Lopez-Alemany R, Longstaff C, Hawley S, Mirshahi M, Fabregas P, Jardi M, Merton E, Miles LA, Felez J: **Inhibition of cell surface mediated plasminogen activation by a monoclonal antibody against alpha-Enolase.** *Am J Hematol* 2003, **72**:234–242.

27. Dano K, Andreasen PA, Grondahl-Hansen J, Kristensen P, Nielsen LS, Skriver L: **Plasminogen activators, tissue degradation, and cancer.** *Adv Cancer Res* 1985, **44**:139–266.

28. Felez J: **Plasminogen binding to cell surface.** *Fibrinolysis Proteolysis* 1998, **12**:183–189.

29. Liu KJ, Shih NY: **The role of Enolase in tissue invasion and metastasis of pathogens and tumor cells.** *J Cancer Mol* 2007, **3**:45–48.

30. Borza DB, Morgan WT: **Acceleration of plasminogen activation by tissue plasminogen activator on surface-bound histidine-proline-rich glycoprotein.** *J Biol Chem* 1997, **272**:5718–5726.

31. Das R, Burke T, Plow EF: **Histone H2B as a functionally important plasminogen receptor on macrophages.** *Blood* 2007, **110**:3763–3772.

32. Dudani AK, Cummings C, Hashemi S, Ganz PR: **Isolation of a novel 45 kDa plasminogen receptor from human endothelial cells.** *Thromb Res* 1993, **69**:185–196.

33. Dudani AK, Ganz PR: **Endothelial cell surface actin serves as a binding site for plasminogen, tissue plasminogen activator and lipoprotein(a).** *Br J Haematol* 1996, **95**:168–178.

34. Gonzalez-Gronow M, Kaczowka S, Gawdi G, Pizzo SV: **Dipeptidyl peptidase IV (DPP IV/CD26) is a cell-surface plasminogen receptor.** *Front Biosci* 2008, **13**:1610–1618.

35. Havre PA, Abe M, Urasaki Y, Ohnuma K, Morimoto C, Dang NH: **The role of CD26/dipeptidyl peptidase IV in cancer.** *Front Biosci* 2008, **13**:1634–1645.

36. Herren T, Burke TA, Das R, Plow EF: **Identification of histone H2B as a regulated plasminogen receptor.** *Biochemistry* 2006, **45**:9463–9474.

37. Kanalas JJ, Makker SP: **Identification of the rat Heymann nephritis autoantigen (GP330) as a receptor site for plasminogen.** *J Biol Chem* 1991, **266**:10825–10829.

38. Lighvani S, Baik N, Diggs JE, Khaldoyanidi S, Parmer RJ, Miles LA: **Regulation of macrophage migration by a novel plasminogen receptor Plg-R KT.** *Blood* 2011, **118**:5622–5630.

39. Phipps KD, Surette AP, O'Connell PA, Waisman DM: **Plasminogen receptor S100A10 is essential for the migration of tumor-promoting macrophages into tumor sites.** *Cancer Res* 2011, **71**:6676–6683.

40. Redlitz A, Fowler BJ, Plow EF, Miles LA: **The role of an enolase-related molecule in plasminogen binding to cells.** *Eur J Biochem* 1995, **227**:407–415.

41. Strickland DK: **A new plasminogen receptor.** *Blood* 2010, **115**:1315–1316.

42. Winram SB, Lottenberg R: **The plasmin-binding protein Plr of group A streptococci is identified as glyceraldehyde-3-phosphate dehydrogenase.** *Microbiology* 1996, **142**(Pt 8):2311–2320.

43. Andronicos NM, Chen EI, Baik N, Bai H, Parmer CM, Kiosses WB, Kamps MP, Yates JR 3rd, Parmer RJ, Miles LA: **Proteomics-based discovery of a novel, structurally unique, and developmentally regulated plasminogen receptor, Plg-RKT, a major regulator of cell surface plasminogen activation.** *Blood* 2010, **115**:1319–1330.

44. Kang HJ, Jung SK, Kim SJ, Chung SJ: **Structure of human alpha-enolase (hENO1), a multifunctional glycolytic enzyme.** *Acta Crystallogr D Biol Crystallogr* 2008, **64**:651–657.

45. Kang HM, Choi KS, Kassam G, Fitzpatrick SL, Kwon M, Waisman DM: **Role of annexin II tetramer in plasminogen activation.** *Trends Cardiovasc Med* 1999, **9**:92–102.

46. Liemann S, Lewit-Bentley A: **Annexins: a novel family of calcium- and membrane-binding proteins in search of a function.** *Structure* 1995, **3**:233–237.

47. Shiozawa Y, Havens AM, Jung Y, Ziegler AM, Pedersen EA, Wang J, Lu G, Roodman GD, Loberg RD, Pienta KJ, Taichman RS: **Annexin II/annexin II receptor axis regulates adhesion, migration, homing, and growth of prostate cancer.** *J Cell Biochem* 2008, **105**:370–380.

48. Waisman DM: **Annexin II tetramer: structure and function.** *Mol Cell Biochem* 1995, **149–150**:301–322.

49. Gerke V, Moss SE: **Annexins: from structure to function.** *Physiol Rev* 2002, **82**:331–371.

50. Moll R, Divo M, Langbein L: **The human keratins: biology and pathology.** *Histochem Cell Biol* 2008, **129**:705–733.

51. Gires O, Andratschke M, Schmitt B, Mack B, Schaffrik M: **Cytokeratin 8 associates with the external leaflet of plasma membranes in tumour cells.** *Biochem Biophys Res Commun* 2005, **328**:1154–1162.

52. Hembrough TA, Li L, Gonias SL: **Cell-surface cytokeratin 8 is the major plasminogen receptor on breast cancer cells and is required for the accelerated activation of cell-associated plasminogen by tissue-type plasminogen activator.** *J Biol Chem* 1996, **271**:25684–25691.

53. Kralovich KR, Li L, Hembrough TA, Webb DJ, Karns LR, Gonias SL: **Characterization of the binding sites for plasminogen and tissue-type plasminogen activator in cytokeratin 8 and cytokeratin 18.** *J Protein Chem* 1998, **17**:845–854.

54. Pancholi V: **Multifunctional alpha-enolase: its role in diseases.** *Cell Mol Life Sci* 2001, **58**:902–920.

55. Lohman K, Meyerhof O: **Über die enzymatische Umwandlung von Phosphoglyzerinsäure in Brenztraubensäure und Phosphorsäure (Enzymatic transformation of phosphoglyceric acid into pyruvic and phosphoric acid).** *Biochem Z* 1934, **273**:60–72.

56. Giallongo A, Oliva D, Cali L, Barba G, Barbieri G, Feo S: **Structure of the human gene for alpha-enolase.** *Eur J Biochem* 1990, **190**:567–573.

57. Marangos PJ, Zis AP, Clark RL, Goodwin FK: **Neuronal, non-neuronal and hybrid forms of enolase in brain: structural, immunological and functional comparisons.** *Brain Res* 1978, **150**:117–133.

58. Kato K, Asai R, Shimizu A, Suzuki F, Ariyoshi Y: **Immunoassay of three enolase isozymes in human serum and in blood cells.** *Clin Chim Acta* 1983, **127**:353–363.

59. Royds JA, Parsons MA, Taylor CB, Timperley WR: **Enolase isoenzyme distribution in the human brain and its tumours.** *J Pathol* 1982, **137**:37–49.

60. Asch HL, Mayhew E, Lazo RO, Asch BB: **Lipids noncovalently associated with keratins and other cytoskeletal proteins of mouse mammary epithelial cells in primary culture.** *Biochim Biophys Acta* 1990, **1034**:303–308.
61. Vidrich A, Gilmartin ME, Mitchell J, Freedberg IM: **Postsynthetic modifications of epithelial keratins.** *Ann N Y Acad Sci* 1985, **455**:354–370.
62. Chan R, Rossitto PV, Edwards BF, Cardiff RD: **Presence of proteolytically processed keratins in the culture medium of MCF-7 cells.** *Cancer Res* 1986, **46**:6353–6359.
63. Chou CF, Riopel CL, Rott LS, Omary MB: **A significant soluble keratin fraction in 'simple' epithelial cells. Lack of an apparent phosphorylation and glycosylation role in keratin solubility.** *J Cell Sci* 1993, **105**(Pt 2):433–444.
64. Hembrough TA, Kralovich KR, Li L, Gonias SL: **Cytokeratin 8 released by breast carcinoma cells in vitro binds plasminogen and tissue-type plasminogen activator and promotes plasminogen activation.** *Biochem J* 1996, **317**(Pt 3):763–769.
65. Zheng L, Foley K, Huang L, Leubner A, Mo G, Olino K, Edil BH, Mizuma M, Sharma R, Le DT, *et al*: **Tyrosine 23 phosphorylation-dependent cell-surface localization of annexin A2 is required for invasion and metastases of pancreatic cancer.** *PLoS One* 2011, **6**:e19390.
66. Cooper JA, Esch FS, Taylor SS, Hunter T: **Phosphorylation sites in enolase and lactate dehydrogenase utilized by tyrosine protein kinases in vivo and in vitro.** *J Biol Chem* 1984, **259**:7835–7841.
67. Capello M, Ferri-Borgogno S, Cappello P, Novelli F: **alpha-Enolase: a promising therapeutic and diagnostic tumor target.** *FEBS J* 2011, **278**:1064–1074.
68. Bao H, Jiang M, Zhu M, Sheng F, Ruan J, Ruan C: **Overexpression of Annexin II affects the proliferation, apoptosis, invasion and production of proangiogenic factors in multiple myeloma.** *Int J Hematol* 2009, **90**:177–185.
69. Chiang Y, Rizzino A, Sibenaller ZA, Wold MS, Vishwanatha JK: **Specific down-regulation of annexin II expression in human cells interferes with cell proliferation.** *Mol Cell Biochem* 1999, **199**:139–147.
70. Creutz CE: **The annexins and exocytosis.** *Science* 1992, **258**:924–931.
71. Hajjar KA, Krishnan S: **Annexin II: a mediator of the plasmin/plasminogen activator system.** *Trends Cardiovasc Med* 1999, **9**:128–138.
72. Hajjar KA, Menell JS: **Annexin II: a novel mediator of cell surface plasmin generation.** *Ann N Y Acad Sci* 1997, **811**:337–349.
73. Ling Q, Jacovina AT, Deora A, Febbraio M, Simantov R, Silverstein RL, Hempstead B, Mark WH, Hajjar KA: **Annexin II regulates fibrin homeostasis and neoangiogenesis in vivo.** *J Clin Invest* 2004, **113**:38–48.
74. Gould KL, Woodgett JR, Isacke CM, Hunter T: **The protein-tyrosine kinase substrate p36 is also a substrate for protein kinase C in vitro and in vivo.** *Mol Cell Biol* 1986, **6**:2738–2744.
75. Hayes MJ, Moss SE: **Annexin 2 has a dual role as regulator and effector of v-Src in cell transformation.** *J Biol Chem* 2009, **284**:10202–10210.
76. Hubaishy I, Jones PG, Bjorge J, Bellagamba C, Fitzpatrick S, Fujita DJ, Waisman DM: **Modulation of annexin II tetramer by tyrosine phosphorylation.** *Biochem* 1995, **34**:14527–14534.
77. Biener Y, Feinstein R, Mayak M, Kaburagi Y, Kadowaki T, Zick Y: **Annexin II is a novel player in insulin signal transduction. Possible association between annexin II phosphorylation and insulin receptor internalization.** *J Biol Chem* 1996, **271**:29489–29496.
78. Jiang Y, Chan JL, Zong CS, Wang LH: **Effect of tyrosine mutations on the kinase activity and transforming potential of an oncogenic human insulin-like growth factor I receptor.** *J Biol Chem* 1996, **271**:160–167.
79. Brambilla R, Zippel R, Sturani E, Morello L, Peres A, Alberghina L: **Characterization of the tyrosine phosphorylation of calpactin I (annexin II) induced by platelet-derived growth factor.** *Biochem J* 1991, **278**(Pt 2):447–452.
80. Zhao WQ, Chen GH, Chen H, Pascale A, Ravindranath L, Quon MJ, Alkon DL: **Secretion of Annexin II via activation of insulin receptor and insulin-like growth factor receptor.** *J Biol Chem* 2003, **278**:4205–4215.
81. Chen R, Brentnall TA, Pan S, Cooke K, Moyes KW, Lane Z, Crispin DA, Goodlett DR, Aebersold R, Bronner MP: **Quantitative proteomics analysis reveals that proteins differentially expressed in chronic pancreatitis are also frequently involved in pancreatic cancer.** *Mol Cell Proteomics* 2007, **6**:1331–1342.
82. Cole SP, Pinkoski MJ, Bhardwaj G, Deeley RG: **Elevated expression of annexin II (lipocortin II, p36) in a multidrug resistant small cell lung cancer cell line.** *Br J Cancer* 1992, **65**:498–502.
83. Delys L, Detours V, Franc B, Thomas G, Bogdanova T, Tronko M, Libert F, Dumont JE, Maenhaut C: **Gene expression and the biological phenotype of papillary thyroid carcinomas.** *Oncogene* 2007, **26**:7894–7903.
84. Domoto T, Miyama Y, Suzuki H, Teratani T, Arai K, Sugiyama T, Takayama T, Mugiya S, Ozono S, Nozawa R: **Evaluation of S100A10, annexin II and B-FABP expression as markers for renal cell carcinoma.** *Cancer Sci* 2007, **98**:77–82.
85. Emoto K, Sawada H, Yamada Y, Fujimoto H, Takahama Y, Ueno M, Takayama T, Uchida H, Kamada K, Naito A, *et al*: **Annexin II overexpression is correlated with poor prognosis in human gastric carcinoma.** *Anticancer Res* 2001, **21**:1339–1345.
86. Emoto K, Yamada Y, Sawada H, Fujimoto H, Ueno M, Takayama T, Kamada K, Naito A, Hirao S, Nakajima Y: **Annexin II overexpression correlates with stromal tenascin-C overexpression: a prognostic marker in colorectal carcinoma.** *Cancer* 2001, **92**:1419–1426.
87. Esposito I, Penzel R, Chaib-Harrireche M, Barcena U, Bergmann F, Riedl S, Kayed H, Giese N, Kleeff J, Friess H, Schirmacher P: **Tenascin C and annexin II expression in the process of pancreatic carcinogenesis.** *J Pathol* 2006, **208**:673–685.
88. Guedj N, Zhan Q, Perigny M, Rautou PE, Degos F, Belghiti J, Farges O, Bedossa P, Paradis V: **Comparative protein expression profiles of hilar and peripheral hepatic cholangiocarcinomas.** *J Hepatol* 2009, **51**:93–101.
89. Guzman Aranguez A, Olmo N, Turnay J, Lecona E, Perez Ramos P, Lopez De Silanes I, Lizarbe MA: **Differentiation of human colon adenocarcinoma cells alters the expression and intracellular localization of annexins A1, A2, and A5.** *J Cell Biochem* 2005, **94**:178–193.
90. Ji NY, Park MY, Kang YH, Lee CI, Kim DG, Yeom YI, Jang YJ, Myung PK, Kim JW, Lee HG, *et al*: **Evaluation of annexin II as a potential serum marker for hepatocellular carcinoma using a developed sandwich ELISA method.** *Int J Mol Med* 2009, **24**:765–771.
91. Liu Y, Wang Z, Jiang M, Dai L, Zhang W, Wu D, Ruan C: **The expression of annexin II and its role in the fibrinolytic activity in acute promyelocytic leukemia.** *Leuk Res* 2011, **35**:879–884.
92. Liu Y, Zhu X, Zhu J, Liao S, Tang Q, Liu K, Guan X, Zhang J, Feng Z: **Identification of differential expression of genes in hepatocellular carcinoma by suppression subtractive hybridization combined cDNA microarray.** *Oncol Rep* 2007, **18**:943–951.
93. Mohammad HS, Kurokohchi K, Yoneyama H, Tokuda M, Morishita A, Jian G, Shi L, Murota M, Tani J, Kato K, *et al*: **Annexin A2 expression and phosphorylation are up-regulated in hepatocellular carcinoma.** *Int J Oncol* 2008, **33**:1157–1163.
94. Musholt TJ, Hanack J, Brehm C, von Wasielewski R, Musholt PB: **Searching for non-RET molecular alterations in medullary thyroid carcinoma: expression analysis by mRNA differential display.** *World J Surg* 2005, **29**:472–482.
95. Olwill SA, McGlynn H, Gilmore WS, Alexander HD: **Annexin II cell surface and mRNA expression in human acute myeloid leukaemia cell lines.** *Thromb Res* 2005, **115**:109–114.
96. Pei H, Zhu H, Zeng S, Li Y, Yang H, Shen L, Chen J, Zeng L, Fan J, Li X, *et al*: **Proteome analysis and tissue microarray for profiling protein markers associated with lymph node metastasis in colorectal cancer.** *J Proteome Res* 2007, **6**:2495–2501.
97. Reeves SA, Chavez-Kappel C, Davis R, Rosenblum M, Israel MA: **Developmental regulation of annexin II (Lipocortin 2) in human brain and expression in high grade glioma.** *Cancer Res* 1992, **52**:6871–6876.
98. Roseman BJ, Bollen A, Hsu J, Lamborn K, Israel MA: **Annexin II marks astrocytic brain tumors of high histologic grade.** *Oncol Res* 1994, **6**:561–567.
99. Zhang J, Guo B, Zhang Y, Cao J, Chen T: **Silencing of the annexin II gene down-regulates the levels of S100A10, c-Myc, and plasmin and inhibits breast cancer cell proliferation and invasion.** *Saudi Med J* 2010, **31**:374–381.
100. Zhong LP, Wei KJ, Yang X, Zhang L, Zhou XJ, Pan HY, Li J, Chen WT, Zhang ZY: **Increased expression of Annexin A2 in oral squamous cell carcinoma.** *Arch Oral Biol* 2009, **54**:17–25.
101. Zimmermann U, Woenckhaus C, Pietschmann S, Junker H, Maile S, Schultz K, Protzel C, Giebel J: **Expression of annexin II in conventional renal cell carcinoma is correlated with Fuhrman grade and clinical outcome.** *Virchows Arch* 2004, **445**:368–374.
102. Inokuchi J, Narula N, Yee DS, Skarecky DW, Lau A, Ornstein DK, Tyson DR: **Annexin A2 positively contributes to the malignant phenotype and secretion of IL-6 in DU145 prostate cancer cells.** *Int J Cancer* 2009, **124**:68–74.
103. Longerich T, Haller MT, Mogler C, Aulmann S, Lohmann V, Schirmacher P, Brand K: **Annexin A2 as a differential diagnostic marker of hepatocellular tumors.** *Pathol Res Pract* 2011, **207**:8–14.

104. Takano S, Togawa A, Yoshitomi H, Shida T, Kimura F, Shimizu H, Yoshidome H, Ohtsuka M, Kato A, Tomonaga T, et al: **Annexin II overexpression predicts rapid recurrence after surgery in pancreatic cancer patients undergoing gemcitabine-adjuvant chemotherapy.** *Ann Surg Oncol* 2008, **15**:3157–3168.

105. Pena-Alonso E, Rodrigo JP, Parra IC, Pedrero JM, Meana MV, Nieto CS, Fresno MF, Morgan RO, Fernandez MP: **Annexin A2 localizes to the basal epithelial layer and is down-regulated in dysplasia and head and neck squamous cell carcinoma.** *Cancer Lett* 2008, **263**:89–98.

106. Qi YJ, He QY, Ma YF, Du YW, Liu GC, Li YJ, Tsao GS, Ngai SM, Chiu JF: **Proteomic identification of malignant transformation-related proteins in esophageal squamous cell carcinoma.** *J Cell Biochem* 2008, **104**:1625–1635.

107. Rodrigo JP, Lequerica-Fernandez P, Rosado P, Allonca E, Garcia-Pedrero JM, de Vicente JC: **Clinical significance of annexin A2 downregulation in oral squamous cell carcinoma.** *Head Neck* 2011, **33**:1708–1714.

108. Zhang X, Zhi HY, Zhang J, Wang XQ, Zhou CN, Wu M, Sun YT, Liu ZH: **Expression of annexin II in human esophageal squamous cell carcinoma.** *Zhonghua Zhong Liu Za Zhi* 2003, **25**:353–355.

109. Zhi H, Zhang J, Hu G, Lu J, Wang X, Zhou C, Wu M, Liu Z: **The deregulation of arachidonic acid metabolism-related genes in human esophageal squamous cell carcinoma.** *Int J Cancer* 2003, **106**:327–333.

110. Gillette JM, Chan DC, Nielsen-Preiss SM: **Annexin 2 expression is reduced in human osteosarcoma metastases.** *J Cell Biochem* 2004, **92**:820–832.

111. Chetcuti A, Margan SH, Russell P, Mann S, Millar DS, Clark SJ, Rogers J, Handelsman DJ, Dong Q: **Loss of annexin II heavy and light chains in prostate cancer and its precursors.** *Cancer Res* 2001, **61**:6331–6334.

112. Liu JW, Shen JJ, Tanzillo-Swarts A, Bhatia B, Maldonado CM, Person MD, Lau SS, Tang DG: **Annexin II expression is reduced or lost in prostate cancer cells and its re-expression inhibits prostate cancer cell migration.** *Oncogene* 2003, **22**:1475–1485.

113. Yee DS, Narula N, Ramzy I, Boker J, Ahlering TE, Skarecky DW, Ornstein DK: **Reduced annexin II protein expression in high-grade prostatic intraepithelial neoplasia and prostate cancer.** *Arch Pathol Lab Med* 2007, **131**:902–908.

114. Sharma M, Blackman MR, Sharma MC: **Antibody-directed neutralization of annexin II (ANX II) inhibits neoangiogenesis and human breast tumor growth in a xenograft model.** *Exp Mol Pathol* 2012, **92**:175–184.

115. Sharma M, Ownbey RT, Sharma MC: **Breast cancer cell surface annexin II induces cell migration and neoangiogenesis via tPA dependent plasmin generation.** *Exp Mol Pathol* 2010, **88**:278–286.

116. Sharma MR, Koltowski L, Ownbey RT, Tuszynski GP, Sharma MC: **Angiogenesis-associated protein annexin II in breast cancer: selective expression in invasive breast cancer and contribution to tumor invasion and progression.** *Exp Mol Pathol* 2006, **81**:146–156.

117. Sharma MR, Rothman V, Tuszynski GP, Sharma MC: **Antibody-directed targeting of angiostatin's receptor annexin II inhibits Lewis Lung Carcinoma tumor growth via blocking of plasminogen activation: possible biochemical mechanism of angiostatin's action.** *Exp Mol Pathol* 2006, **81**:136–145.

118. Zhang F, Zhang L, Zhang B, Wei X, Yang Y, Qi RZ, Ying G, Zhang N, Niu R: **Anxa2 plays a critical role in enhanced invasiveness of the multidrug resistant human breast cancer cells.** *J Proteome Res* 2009, **8**:5041–5047.

119. Shukla S, Govekar RB, Sirdeshmukh R, Sundaram CS, D'Cruz AK, Pathak KA, Kane SV, Zingde SM: **Tumor antigens eliciting autoantibody response in cancer of gingivo-buccal complex.** *Proteomics Clin Appl* 2007, **1**:1592–1604.

120. Shukla S, Pranay A, D'Cruz AK, Chaturvedi P, Kane SV, Zingde SM: **Immunoproteomics reveals that cancer of the tongue and the gingivobuccal complex exhibit differential autoantibody response.** *Cancer Biomark* 2009, **5**:127–135.

121. Ohno Y, Izumi M, Kawamura T, Nishimura T, Mukai K, Tachibana M: **Annexin II represents metastatic potential in clear-cell renal cell carcinoma.** *Br J Cancer* 2009, **101**:287–294.

122. Brichory FM, Misek DE, Yim AM, Krause MC, Giordano TJ, Beer DG, Hanash SM: **An immune response manifested by the common occurrence of annexins I and II autoantibodies and high circulating levels of IL-6 in lung cancer.** *Proc Natl Acad Sci USA* 2001, **98**:9824–9829.

123. Barua A, Edassery SL, Bitterman P, Abramowicz JS, Dirks AL, Bahr JM, Hales DB, Bradaric MJ, Luborsky JL: **Prevalence of antitumor antibodies in laying hen model of human ovarian cancer.** *Int J Gynecol Cancer* 2009, **19**:500–507.

124. Lutz E, Yeo CJ, Lillemoe KD, Biedrzycki B, Kobrin B, Herman J, Sugar E, Piantadosi S, Cameron JL, Solt S, et al: **A lethally irradiated allogeneic granulocyte-macrophage colony stimulating factor-secreting tumor vaccine for pancreatic adenocarcinoma. A Phase II trial of safety, efficacy, and immune activation.** *Ann Surg* 2011, **253**:328–335.

125. Heinzel S, Rea D, Offringa R, Pawelec G: **The self peptide annexin II (208–223) presented by dendritic cells sensitizes autologous CD4+ T lymphocytes to recognize melanoma cells.** *Cancer Immunol Immunother* 2001, **49**:671–678.

126. Fukunaga Y, Bandoh S, Fujita J, Yang Y, Ueda Y, Hojo S, Dohmoto K, Tojo Y, Takahara J, Ishida T: **Expression of cytokeratin 8 in lung cancer cell lines and measurement of serum cytokeratin 8 in lung cancer patients.** *Lung Cancer* 2002, **38**:31–38.

127. Imai S, Nagano K, Yoshida Y, Okamura T, Yamashita T, Abe Y, Yoshikawa T, Yoshioka Y, Kamada H, Mukai Y, et al: **Development of an antibody proteomics system using a phage antibody library for efficient screening of biomarker proteins.** *Biomaterials* 2011, **32**:162–169.

128. Kim TM, Jeong HJ, Seo MY, Kim SC, Cho G, Park CH, Kim TS, Park KH, Chung HC, Rha SY: **Determination of genes related to gastrointestinal tract origin cancer cells using a cDNA microarray.** *Clin Cancer Res* 2005, **11**:79–86.

129. Wikman H, Kettunen E, Seppanen JK, Karjalainen A, Hollmen J, Anttila S, Knuutila S: **Identification of differentially expressed genes in pulmonary adenocarcinoma by using cDNA array.** *Oncogene* 2002, **21**:5804–5813.

130. Teramoto R, Minagawa H, Honda M, Miyazaki K, Tabuse Y, Kamijo K, Ueda T, Kaneko S: **Protein expression profile characteristic to hepatocellular carcinoma revealed by 2D-DIGE with supervised learning.** *Biochim Biophys Acta* 2008, **1784**:764–772.

131. Livasy CA, Karaca G, Nanda R, Tretiakova MS, Olopade OI, Moore DT, Perou CM: **Phenotypic evaluation of the basal-like subtype of invasive breast carcinoma.** *Mod Pathol* 2006, **19**:264–271.

132. Hamrita B, Chahed K, Kabbage M, Guillier CL, Trimeche M, Chaieb A, Chouchane L: **Identification of tumor antigens that elicit a humoral immune response in breast cancer patients' sera by serological proteome analysis (SERPA).** *Clin Chim Acta* 2008, **393**:95–102.

133. Bonin S, Brunetti D, Benedetti E, Dotti I, Gorji N, Stanta G: **Molecular characterisation of breast cancer patients at high and low recurrence risk.** *Virchows Arch* 2008, **452**:241–250.

134. Obermajer N, Doljak B, Kos J: **Cytokeratin 8 ectoplasmic domain binds urokinase-type plasminogen activator to breast tumor cells and modulates their adhesion, growth and invasiveness.** *Mol Cancer* 2009, **8**:88.

135. Liu F, Chen Z, Wang J, Shao X, Cui Z, Yang C, Zhu Z, Xiong D: **Overexpression of cell surface cytokeratin 8 in multidrug-resistant MCF-7 /MX cells enhances cell adhesion to the extracellular matrix.** *Neoplasia* 2008, **10**:1275–1284.

136. Liu F, Fan D, Qi J, Zhu H, Zhou Y, Yang C, Zhu Z, Xiong D: **Co-expression of cytokeratin 8 and breast cancer resistant protein indicates a multifactorial drug-resistant phenotype in human breast cancer cell line.** *Life Sci* 2008, **83**:496–501.

137. Ditzel HJ, Strik MC, Larsen MK, Willis AC, Waseem A, Kejling K, Jensenius JC: **Cancer-associated cleavage of cytokeratin 8/18 heterotypic complexes exposes a neoepitope in human adenocarcinomas.** *J Biol Chem* 2002, **277**:21712–21722.

138. Pfaff M, O'Connor R, Vollmers HP, Muller-Hermelink HK: **Human monoclonal antibody against a tissue polypeptide antigen-related protein from a patient with a signet-ring cell carcinoma of the stomach.** *Cancer Res* 1990, **50**:5192–5198.

139. Gires O, Munz M, Schaffrik M, Kieu C, Rauch J, Ahlemann M, Eberle D, Mack B, Wollenberg B, Lang S, et al: **Profile identification of disease-associated humoral antigens using AMIDA, a novel proteomics-based technology.** *Cell Mol Life Sci* 2004, **61**:1198–1207.

140. Le Naour F, Brichory F, Misek DE, Brechot C, Hanash SM, Beretta L: **A distinct repertoire of autoantibodies in hepatocellular carcinoma identified by proteomic analysis.** *Mol Cell Proteomics* 2002, **1**:197–203.

141. Li L, Chen SH, Yu CH, Li YM, Wang SQ: **Identification of hepatocellular-carcinoma-associated antigens and autoantibodies by serological proteome analysis combined with protein microarray.** *J Proteome Res* 2008, **7**:611–620.

142. Gharib TG, Chen G, Wang H, Huang CC, Prescott MS, Shedden K, Misek DE, Thomas DG, Giordano TJ, Taylor JM, et al: **Proteomic analysis of cytokeratin isoforms uncovers association with survival in lung adenocarcinoma.** *Neoplasia* 2002, **4**:440–448.

143. Yamashiro Y, Takei K, Umikawa M, Asato T, Oshiro M, Uechi Y, Ishikawa T, Taira K, Uezato H, Kariya K: **Ectopic coexpression of keratin 8 and 18**

promotes invasion of transformed keratinocytes and is induced in patients with cutaneous squamous cell carcinoma. *Biochem Biophys Res Commun* 2010, **399**:365–372.

144. Kabukcuoglu S, Ozalp SS, Yalcin OT, Colak E, Abubakar AA: **Comparison of histopathologic classification and surgical stage by cytokeratin 8 and cytokeratin 18 in endometrial cancer.** *Eur J Gynaecol Oncol* 2010, **31**:641–644.

145. Schaafsma HE, Van Der Velden LA, Manni JJ, Peters H, Link M, Rutter DJ, Ramaekers FC: **Increased expression of cytokeratins 8, 18 and vimentin in the invasion front of mucosal squamous cell carcinoma.** *J Pathol* 1993, **170**:77–86.

146. Warburg O, Wind F, Neglers E: *The metabolism of tumors.* London: Constable & Co.; 1930.

147. Crowther M, Brown NJ, Bishop ET, Lewis CE: **Microenvironmental influence on macrophage regulation of angiogenesis in wounds and malignant tumors.** *J Leukoc Biol* 2001, **70**:478–490.

148. Lukashev D, Ohta A, Sitkovsky M: **Hypoxia-dependent anti-inflammatory pathways in protection of cancerous tissues.** *Cancer Metastasis Rev* 2007, **26**:273–279.

149. Semenza GL: **Hypoxia-inducible factor 1: oxygen homeostasis and disease pathophysiology.** *Trends Mol Med* 2001, **7**:345–350.

150. Sedoris KC, Thomas SD, Miller DM: **Hypoxia induces differential translation of enolase/MBP-1.** *BMC Cancer* 2010, **10**:157.

151. Semenza GL, Jiang BH, Leung SW, Passantino R, Concordet JP, Maire P, Giallongo A: **Hypoxia response elements in the aldolase A, enolase 1, and lactate dehydrogenase A gene promoters contain essential binding sites for hypoxia-inducible factor 1.** *J Biol Chem* 1996, **271**:32529–32537.

152. Altenberg B, Greulich KO: **Genes of glycolysis are ubiquitously overexpressed in 24 cancer classes.** *Genomics* 2004, **84**:1014–1020.

153. Cao L, Li X, Zhang Y, Peng F, Yi H, Xu Y, Wang Q: **Proteomic analysis of human ovarian cancer paclitaxel-resistant cell lines.** *Zhong Nan Da Xue Xue Bao Yi Xue Ban* 2010, **35**:286–294.

154. Cappello P, Tomaino B, Chiarle R, Ceruti P, Novarino A, Castagnoli C, Migliorini P, Perconti G, Giallongo A, Milella M, *et al*: **An integrated humoral and cellular response is elicited in pancreatic cancer by alpha-enolase, a novel pancreatic ductal adenocarcinoma-associated antigen.** *Int J Cancer* 2009, **125**:639–648.

155. Govekar RB, D'Cruz AK, Alok Pathak K, Agarwal J, Dinshaw KA, Chinoy RF, Gadewal N, Kannan S, Sirdeshmukh R, Sundaram CS, *et al*: **Proteomic profiling of cancer of the gingivo-buccal complex: Identification of new differentially expressed markers.** *Proteomics Clin Appl* 2009, **3**:1451–1462.

156. Hamaguchi T, Iizuka N, Tsunedomi R, Hamamoto Y, Miyamoto T, Iida M, Tokuhisa Y, Sakamoto K, Takashima M, Tamesa T, Oka M: **Glycolysis module activated by hypoxia-inducible factor 1alpha is related to the aggressive phenotype of hepatocellular carcinoma.** *Int J Oncol* 2008, **33**:725–731.

157. Hennipman A, Smits J, van Oirschot B, van Houwelingen JC, Rijksen G, Neyt JP, Van Unnik JA, Staal GE: **Glycolytic enzymes in breast cancer, benign breast disease and normal breast tissue.** *Tumour Biol* 1987, **8**:251–263.

158. Katayama M, Nakano H, Ishiuchi A, Wu W, Oshima R, Sakurai J, Nishikawa H, Yamaguchi S, Otsubo T: **Protein pattern difference in the colon cancer cell lines examined by two-dimensional differential in-gel electrophoresis and mass spectrometry.** *Surg Today* 2006, **36**:1085–1093.

159. Li C, Xiao Z, Chen Z, Zhang X, Li J, Wu X, Li X, Yi H, Li M, Zhu G, Liang S: **Proteome analysis of human lung squamous carcinoma.** *Proteomics* 2006, **6**:547–558.

160. Lopez-Pedrera C, Villalba JM, Siendones E, Barbarroja N, Gomez-Diaz C, Rodriguez-Ariza A, Buendia P, Torres A, Velasco F: **Proteomic analysis of acute myeloid leukemia: Identification of potential early biomarkers and therapeutic targets.** *Proteomics* 2006, **6**(Suppl 1):S293–299.

161. Mikuriya K, Kuramitsu Y, Ryozawa S, Fujimoto M, Mori S, Oka M, Hamano K, Okita K, Sakaida I, Nakamura K: **Expression of glycolytic enzymes is increased in pancreatic cancerous tissues as evidenced by proteomic profiling by two-dimensional electrophoresis and liquid chromatography-mass spectrometry/mass spectrometry.** *Int J Oncol* 2007, **30**:849–855.

162. Qi Y, Chiu JF, Wang L, Kwong DL, He QY: **Comparative proteomic analysis of esophageal squamous cell carcinoma.** *Proteomics* 2005, **5**:2960–2971.

163. Shen J, Person MD, Zhu J, Abbruzzese JL, Li D: **Protein expression profiles in pancreatic adenocarcinoma compared with normal pancreatic tissue and tissue affected by pancreatitis as detected by two-dimensional gel electrophoresis and mass spectrometry.** *Cancer Res* 2004, **64**:9018–9026.

164. Somiari RI, Sullivan A, Russell S, Somiari S, Hu H, Jordan R, George A, Katenhusen R, Buchowiecka A, Arciero C, *et al*: **High-throughput proteomic analysis of human infiltrating ductal carcinoma of the breast.** *Proteomics* 2003, **3**:1863–1873.

165. Takashima M, Kuramitsu Y, Yokoyama Y, Iizuka N, Fujimoto M, Nishisaka T, Okita K, Oka M, Nakamura K: **Overexpression of alpha enolase in hepatitis C virus-related hepatocellular carcinoma: association with tumor progression as determined by proteomic analysis.** *Proteomics* 2005, **5**:1686–1692.

166. Tsai ST, Chien IH, Shen WH, Kuo YZ, Jin YT, Wong TY, Hsiao JR, Wang HP, Shih NY, Wu LW: **ENO1, a potential prognostic head and neck cancer marker, promotes transformation partly via chemokine CCL20 induction.** *Eur J Cancer* 2010, **46**:1712–1723.

167. Tu SH, Chang CC, Chen CS, Tam KW, Wang YJ, Lee CH, Lin HW, Cheng TC, Huang CS, Chu JS, *et al*: **Increased expression of enolase alpha in human breast cancer confers tamoxifen resistance in human breast cancer cells.** *Breast Cancer Res Treat* 2010, **121**:539–553.

168. Wong CS, Wong VW, Chan CM, Ma BB, Hui EP, Wong MC, Lam MY, Au TC, Chan WH, Cheuk W, Chan AT: **Identification of 5-fluorouracil response proteins in colorectal carcinoma cell line SW480 by two-dimensional electrophoresis and MALDI-TOF mass spectrometry.** *Oncol Rep* 2008, **20**:89–98.

169. Zhao J, Chang AC, Li C, Shedden KA, Thomas DG, Misek DE, Manoharan AP, Giordano TJ, Beer DG, Lubman DM: **Comparative proteomics analysis of Barrett metaplasia and esophageal adenocarcinoma using two-dimensional liquid mass mapping.** *Mol Cell Proteomics* 2007, **6**:987–999.

170. Zhou H, Chen CB, Lan J, Liu C, Liu XG, Jiang L, Wei F, Ma QJ, Dang GT, Liu ZJ: **Differential proteomic profiling of chordomas and analysis of prognostic factors.** *J Surg Oncol* 2010, **102**:720–727.

171. Ito S, Honma T, Ishida K, Wada N, Sasaoka S, Hosoda M, Nohno T: **Differential expression of the human alpha-enolase gene in oral epithelium and squamous cell carcinoma.** *Cancer Sci* 2007, **98**:499–505.

172. Chang GC, Liu KJ, Hsieh CL, Hu TS, Charoenfuprasert S, Liu HK, Luh KT, Hsu LH, Wu CW, Ting CC, *et al*: **Identification of alpha-enolase as an autoantigen in lung cancer: its overexpression is associated with clinical outcomes.** *Clin Cancer Res* 2006, **12**:5746–5754.

173. Suzuki A, Iizuka A, Komiyama M, Takikawa M, Kume A, Tai S, Ohshita C, Kurusu A, Nakamura Y, Yamamoto A, *et al*: **Identification of melanoma antigens using a Serological Proteome Approach (SERPA).** *Cancer Genomics Proteomics* 2010, **7**:17–23.

174. Chang YS, Wu W, Walsh G, Hong WK, Mao L: **Enolase-alpha is frequently down-regulated in non-small cell lung cancer and predicts aggressive biological behavior.** *Clin Cancer Res* 2003, **9**:3641–3644.

175. Muller FL, Colla S, Aquilanti E, Manzo VE, Genovese G, Lee J, Eisenson D, Narurkar R, Deng P, Nezi L, *et al*: **Passenger deletions generate therapeutic vulnerabilities in cancer.** *Nature* 2012, **488**:337–342.

176. Zhou W, Capello M, Fredolini C, Piemonti L, Liotta LA, Novelli F, Petricoin EF: **Mass spectrometry analysis of the post-translational modifications of alpha-enolase from pancreatic ductal adenocarcinoma cells.** *J Proteome Res* 2010, **9**:2929–2936.

177. Hennipman A, van Oirschot BA, Smits J, Rijksen G, Staal GE: **Glycolytic enzyme activities in breast cancer metastases.** *Tumour Biol* 1988, **9**:241–248.

178. Shih NY, Lai HL, Chang GC, Lin HC, Wu YC, Liu JM, Liu KJ, Tseng SW: **Anti-alpha-enolase autoantibodies are down-regulated in advanced cancer patients.** *Jpn J Clin Oncol* 2010, **40**:663–669.

179. Mojtahedi Z, Safaei A, Yousefi Z, Ghaderi A: **Immunoproteomics of HER2-positive and HER2-negative breast cancer patients with positive lymph nodes.** *OMICS* 2011, **15**:409–418.

180. Sato N, Nabeta Y, Kondo H, Sahara H, Hirohashi Y, Kashiwagi K, Kanaseki T, Sato Y, Rong S, Hirai I, *et al*: **Human CD8 and CD4 T cell epitopes of epithelial cancer antigens.** *Cancer Chemother Pharmacol* 2000, **46**(Suppl): S86–90.

181. Zou L, Wu Y, Pei L, Zhong D, Gen M, Zhao T, Wu J, Ni B, Mou Z, Han J, *et al*: **Identification of leukemia-associated antigens in chronic myeloid leukemia by proteomic analysis.** *Leuk Res* 2005, **29**:1387–1391.

182. Huang LJ, Chen SX, Luo WJ, Jiang HH, Zhang PF, Yi H: **Proteomic analysis of secreted proteins of non-small cell lung cancer.** *Ai Zheng* 2006, **25**:1361–1367.

183. Dot C, Guigay J, Adamus G: **Anti-alpha-enolase antibodies in cancer-associated retinopathy with small cell carcinoma of the lung.** *Am J Ophthalmol* 2005, **139**:746–747.

184. He P, Naka T, Serada S, Fujimoto M, Tanaka T, Hashimoto S, Shima Y, Yamadori T, Suzuki H, Hirashima T, *et al*: **Proteomics-based identification of alpha-enolase as a tumor antigen in non-small lung cancer.** *Cancer Sci* 2007, **98**:1234–1240.

185. Tomaino B, Cappello P, Capello M, Fredolini C, Sperduti I, Migliorini P, Salacone P, Novarino A, Giacobino A, Ciuffreda L, *et al*: **Circulating autoantibodies to phosphorylated alpha-enolase are a hallmark of pancreatic cancer.** *J Proteome Res* 2011, **10**:105–112.

186. Cappello P, Rolla S, Chiarle R, Principe M, Cavallo F, Perconti G, Feo S, Giovarelli M, Novelli F: **Vaccination With ENO1 DNA Prolongs Survival of Genetically Engineered Mice With Pancreatic Cancer.** *Gastroenterology* 2013.

187. Rehman I, Azzouzi AR, Catto JW, Allen S, Cross SS, Feeley K, Meuth M, Hamdy FC: **Proteomic analysis of voided urine after prostatic massage from patients with prostate cancer: a pilot study.** *Urology* 2004, **64**:1238–1243.

188. Forgber M, Trefzer U, Sterry W, Walden P: **Proteome serological determination of tumor-associated antigens in melanoma.** *PLoS One* 2009, **4**:e5199.

189. Seweryn E, Pietkiewicz J, Bednarz-Misa IS, Ceremuga I, Saczko J, Kulbacka J, Gamian A: **Localization of enolase in the subfractions of a breast cancer cell line.** *Z Naturforsch C* 2009, **64**:754–758.

190. Mears R, Craven RA, Hanrahan S, Totty N, Upton C, Young SL, Patel P, Selby PJ, Banks RE: **Proteomic analysis of melanoma-derived exosomes by two-dimensional polyacrylamide gel electrophoresis and mass spectrometry.** *Proteomics* 2004, **4**:4019–4031.

191. Yu X, Harris SL, Levine AJ: **The regulation of exosome secretion: a novel function of the p53 protein.** *Cancer Res* 2006, **66**:4795–4801.

192. Saldanha RG, Molloy MP, Bdeir K, Cines DB, Song X, Uitto PM, Weinreb PH, Violette SM, Baker MS: **Proteomic identification of lynchpin urokinase plasminogen activator receptor protein interactions associated with epithelial cancer malignancy.** *J Proteome Res* 2007, **6**:1016–1028.

193. Trojanowicz B, Winkler A, Hammje K, Chen Z, Sekulla C, Glanz D, Schmutzler C, Mentrup B, Hombach-Klonisch S, Klonisch T, *et al*: **Retinoic acid-mediated down-regulation of ENO1/MBP-1 gene products caused decreased invasiveness of the follicular thyroid carcinoma cell lines.** *J Mol Endocrinol* 2009, **42**:249–260.

194. Dowling P, Meleady P, Dowd A, Henry M, Glynn S, Clynes M: **Proteomic analysis of isolated membrane fractions from superinvasive cancer cells.** *Biochim Biophys Acta* 2007, **1774**:93–101.

195. Zitvogel L, Tesniere A, Kroemer G: **Cancer despite immunosurveillance: immunoselection and immunosubversion.** *Nat Rev Immunol* 2006, **6**:715–727.

196. Disis ML, Pupa SM, Gralow JR, Dittadi R, Menard S, Cheever MA: **High-titer HER-2/neu protein-specific antibody can be detected in patients with early-stage breast cancer.** *J Clin Oncol* 1997, **15**:3363–3367.

197. Kondo H, Sahara H, Miyazaki A, Nabeta Y, Hirohashi Y, Kanaseki T, Yamaguchi A, Yamada N, Hirayama K, Suzuki M, *et al*: **Natural antigenic peptides from squamous cell carcinoma recognized by autologous HLA-DR8-restricted CD4+ T cells.** *Jpn J Cancer Res* 2002, **93**:917–924.