The Role of Annexin A2 in Tumorigenesis and Cancer Progression

Noor A. Lokman · Miranda P. Ween ·
Martin K. Oehler · Carmela Ricciardelli

Received: 6 January 2011 / Accepted: 21 February 2011 / Published online: 5 March 2011
© Springer Science+Business Media B.V. 2011

Abstract Annexin A2 is a calcium-dependent, phospholipid-binding protein found on various cell types. It is up-regulated in various tumor types and plays multiple roles in regulating cellular functions, including angiogenesis, proliferation, apoptosis, cell migration, invasion and adhesion. Annexin A2 binds with plasminogen and tissue plasminogen activator on the cell surface, which leads to the conversion of plasminogen to plasmin. Plasmin is a serine protease which plays a key role in the activation of metalloproteinases and degradation of extracellular matrix components essential for metastatic progression. We have recently found that both annexin A2 and plasmin are increased in conditioned media of co cultured ovarian cancer and peritoneal cells. Our studies suggest that annexin A2 is part of a tumor-host signal pathway between ovarian cancer and peritoneal cells which promotes ovarian cancer metastasis. Accumulating evidence suggest that interactions between annexin A2 and its binding proteins play an important role in the tumor microenvironment and act together to enhance cancer metastasis. This article reviews the current knowledge on the biological role of annexin A2 and its binding proteins in solid malignancies including ovarian cancer.

Keywords Annexin A2 · p11 protein · t-PA · Plasmin and metastasis

N. A. Lokman · M. P. Ween · M. K. Oehler · C. Ricciardelli (✉)
Discipline of Obstetrics and Gynaecology,
School of Paediatrics and Reproductive Health,
Research Centre for Reproductive Health, Robinson Institute,
University of Adelaide,
Adelaide, South Australia, Australia
e-mail: carmela.ricciardelli@adelaide.edu.au

M. K. Oehler
Department of Gynaecological Oncology,
Royal Adelaide Hospital,
Adelaide, Australia

Introduction

The plasminogen activation system known to be involved in thrombolysis and wound healing plays a major role in cancer progression. The inactive enzyme plasminogen is converted to the active serine protease plasmin by plasminogen activators; tissue plasminogen activator (t-PA) and urokinase plasminogen activator (u-PA). Plasmin is involved in many biological processes besides vascular thrombolysis including angiogenesis, tissue remodeling and also activates metalloproteinases (MMPs) and latent growth factors and the degradation of extracellular matrix (ECM), processes which all contribute to tumor development and metastasis [1]. A well studied protein which is an important mediator in the plasminogen activator system is annexin A2 [2]. The interaction between annexin A2 and t-PA mediates the conversion of plasminogen to plasmin and increases the catalytic efficiency of plasmin generation by up to 60-fold [3].

Annexin A2, a calcium-binding cytoskeletal protein is localized at the extracellular surface of endothelial cells and various types of tumor cells [4, 5]. The increased expression of annexin A2 has been reported in cancers of the breast, liver, prostate and pancreas [6–9]. Additionally, annexin A2 has also been demonstrated to play a role in cancer cell migration, invasion [7, 10] and adhesion [11, 12], processes which are essential for cancer metastasis. This article reviews the current knowledge on annexin A2 expression in various malignancies including ovarian cancer and its role in regulating cancer cell behavior.

The Structure and Function of Annexin A2

Annexin A2 belongs to the annexin family of calcium binding proteins which consists of 12 members (A1-A11 &

A13). Each family member shares the conserved calcium binding motif in the carboxyl domain. However, the individual annexin members have diverse functions due to the highly variable amino-terminal domain [13]. Annexin family members have been reported to play multiple roles in cancer, including signal transduction, angiogenesis, tumour invasion and metastasis and apoptosis (reviewed in [14]). Studies have shown annexin A1, A2, A3, A4, A6, A8 and A11 to be up-regulated whilst annexin A1, A2, A7, A8, A10 and A11 are decreased in different cancer types [14]. Moreover, annexins have also been associated with chemo resistance in breast and ovarian cancer cells [15–18].

The human annexin A2 gene spans approximately 40 kb on the long arm of chromosome 15 (15q21) [19]. This region has been shown to be associated with allelic imbalance in advanced breast cancer [20]. However, no annexin A2 mutations have been described to date in cancer or other diseases. Annexin A2 has a functional N-terminal domain with p11 proteins and t-PA binding sites and a C-terminal domain consist of binding sites for calcium, phospholipids and actin filaments (Fig. 1). Annexin A2 exists both as a monomer and a heterotetramer. The annexin A2 monomer is an intracellular 38 kDa protein whilst, the annexin A2 heterotetramer consisting of two subunits of annexin A2 monomers and two subunits of p11 proteins (also known as S100A10) localized to the plasma membrane [21]. The translocation of annexin A2 monomers from the cytoplasm to the cell surface occurs as a result of heat stress [22], tyrosine phosphorylation [23], and interaction with heat shock protein 90 alpha [24, 25]. In endothelial cells, translocation of annexin A2 to the plasma membrane due to heat stress is dependent on the expression of p11 proteins and phosphorylation of tyrosine 23 [22]. Annexin A2 also assists in the rearrangement of the actin cytoskeleton, maintaining the plasticity of the actin cytoskeleton and regulating membrane trafficking events including endocytosis and exocytosis [26–28].

Expression of Annexin A2 in Cancer

Many studies have reported increased expression of annexin A2 in cancer tissues compared with normal tissues

[6, 7, 29–31] (Table 1). The up-regulation of annexin A2 expression in pancreatic, colorectal, and brain tumors was directly correlated with advanced clinical stage [31–33]. Higher annexin A2 expression was also observed in metastatic breast cancer and colon cancer cells compared with the non-metastatic cells [7, 34]. In prostate cancer however, reports regarding annexin A2 have been conflicting. Whilst several studies reported a reduction of annexin A2 expression in prostate cancer tissues [35–38], a study by Banerjee et al. (2003) showed high focal membrane staining of annexin A2 in high grade prostate cancers and the prostate carcinoma cell lines, PC3 and DU145 [39]. Furthermore, a more recent study demonstrated a correlation between high annexin A2 levels and a more aggressive prostate cancer phenotype [9].

Conflicting results on annexin A2 expression have also been observed in head and neck cancers. A study by Wu et al. (2002) using 2D gel-electrophoresis of two head and neck squamous cell carcinoma cell lines derived from primary and metastatic tumors from the same patient, found annexin A2 to be up-regulated in the cell line established from the metastatic cancer cell line [40]. In contrast, Pena-Alonso et al. (2008) observed a significant down-regulation of annexin A2 mRNA and protein levels in poorly differentiated carcinomas of the head and neck by real time-PCR and immunohistochemistry [41]. These contradictory findings may be explained by different experimental techniques utilised to examine annexin A2 expression or differences between primary tumors and metastatic lesions. The different annexin A2 levels identified by Wu et al. (2002) may reflect annexin A2 protein modifications detected by mass spectrometry which are not detectable by real-time PCR or immunohistochemistry [40]. Despite various studies having confirmed either an up or down regulation of annexin A2 in various tumour types, the exact mechanism that regulate annexin A2 expression at both gene and protein level are poorly understood.

Annexin A2 apart from being a cell surface protein, has also been shown to be secreted. Deora et al. (2003) reported that annexin A2 is secreted via a non-classical secretory pathway as it does not have a signal secretion sequence [22]. Secreted annexin A2 was also found in the conditioned media of co-cultured human keratinocytes and

Fig. 1 Annexin A2 domain structure. The N-terminal domain consists of the p11 proteins and tissue plasminogen activator (t-PA) binding sites. The C-terminal domain consists of four repeating domains which each contains annexin A2 consensus sequence and the binding sites for calcium, phospholipids and F-actin

Table 1 Annexin A2 expression in cancer

Cancer	Annexin A2 Expression	Ref
Pancreatic cancer	Over-expression of cell surface annexin A2 in pancreatic cell lines	[10]
	Five human pancreatic cell lines had 5–15 fold higher levels of annexin A2 compared with normal cells	[6]
	Over-expression of annexin A2 on surface of gemcitabine resistance pancreatic cells	[81]
Renal cell carcinoma	Annexin A2 expression correlates with tumor differentiation and clinical outcome	[111]
	Increased annexin A2 expression in metastatic tumor of clear cell renal carcinoma compared with the primary tumor	[29]
Prostate cancer	Annexin A2 expression is lost in prostate cancer tissues	[35]
	Expression annexin A2 is reduced in prostate cancer cells	[36]
	Down-regulation of annexin A2 at mRNA and protein level in metastatic prostate cancer	[37]
	Reduced annexin A2 levels in high grade tumor	[38]
	Increased expression of annexin A2 in high grade tumor and metastatic cell lines	[39]
Breast cancer	Increased expression of annexin A2 in invasive breast cancer cell lines compared with non-invasive breast cancer cells	[7]
	Annexin A2 is a potential predictor of response to neoadjuvant chemotherapy in breast cancer	[18]
Head and neck cancer	Up-regulation of annexin A2 in metastatic cancer cells compared to cells derived from primary tumors	[40]
	Down-regulation of annexin A2 expression in moderately and poorly differentiated cancer	[41]
Esophageal Carcinoma	Decreased expression of annexin A2 in esophageal cancer cell lines and cancer tissues	[98]
Angiosarcoma	Increased annexin A2 in benign and malignant tissues	[99]
Colorectal cancer	Annexin A2 over-expressed in colorectal cancer tissues	[100]
Brain	Increased annexin A2 expression in high grade glioma cells	[33, 101, 102]
Oral squamous carcinoma	Increased annexin A2 protein and mRNA levels in cancer cells and tissues	[79, 103]
Cervical carcinoma	Up-regulated annexin A2 in tumor tissue compared with non-tumorous tissue	[104, 105]
Acute myeloid leukaemia	Up-regulation of annexin A2 at the mRNA level in promyelocytic cell line compared with the early myeloid cell line	[106]

fibroblasts [42]. Annexin A2 secretion has been also reported for various tumor types. Davis et al. (1995) demonstrated high levels of secreted annexin A2 in a human pancreatic adenocarcinoma cell line (Capan-2) [43]. Zhao et al. (2003) have reported that annexin A2 secretion by rat adrenal pheochromocytoma (PC12) cells in vitro is induced by activation of both the insulin and insulin like growth factor receptor followed by reduction of intracellular annexin A2 [44]. A study by Ji et al. (2009) reported that annexin A2 was up-regulated in the serum of hepatocellular carcinoma patients compared with the serum of healthy patients by ELISA [45]. A recent study using a proteomic approach investigating the secretome of the gastric cancer cell line, SGC7901 identified annexin A2 as a secreted phosphoprotein [46]. Together these studies suggest that annexin A2 has potential diagnostic and prognostic value for several different carcinomas which needs to be further examined.

Role of Annexin A2 and Its Binding Proteins in Cancer

The co-localization of annexin A2 with its binding proteins including t-PA, p11 protein, tenascin C and cathepsin B

facilitates the proteolytic cascade leading to the activation of pro-enzymes and selective degradation of ECM components which regulate cancer cell properties and enhance metastasis (reviewed in [5]). Several studies have demonstrated the co-expression of annexin A2 and p11 protein in tumor and endothelial cells. Annexin A2 co-localizes with p11 protein at the surface of tumor cells where plasminogen activation occurs [47]. Puisieux et al. (1996) showed a co-expression of annexin A2 and p11 protein in colon, renal and liver carcinoma cell lines [48]. Moreover, over-expression of annexin A2 and p11 protein was observed in renal cell carcinoma tissues when compared with normal kidney [31]. Zhang et al. (2010) also reported over-expression of annexin A2 and p11 protein in the invasive breast cancer cell lines compared with the non-invasive breast cancer cell lines [47].

Annexin A2 also binds to tenascin C (reviewed in [5]), a large ECM molecule which is expressed in the tumor microenvironment and plays an important role in tumorigenesis (reviewed in [49]). Esposito et al. (2006) reported annexin A2 and tenascin C to be over-expressed at both the mRNA and protein levels in pancreatic cancers compared with normal pancreas tissues [50]. Moreover, the interaction between annexin A2 and tenascin C has been shown to

regulate cell migration and to enhance cell proliferation of endothelial cells [51]. Tenascin C has also been shown to be over-expressed in malignant ovarian tumor and to regulate adhesion and migration of ovarian cancer cells [52].

Cathepsin B, a lysosomal cysteine protease on the cell surface of various tumor cells (reviewed in [5]) also binds to annexin A2. Mai et al. (2000) reported that human pro-cathepsin B interacts with the annexin A2 heterotetramer which is co-localized on the surface of human breast carcinoma and glioma cells [53]. Cavallo-Medved et al. (2009) recently reported that the co-localization of cathepsin B and annexin A2 heterotetramer were involved in ECM degradation in endothelial cells during in vitro tube formation [54].

There are also other proteins which have been reported in the literature to bind to annexin A2. Annexin A2 receptor protein which has been characterized in osteoclasts [55] plays an important role in promoting prostate cancer progression [11]. Annexin A2 binds to collagen I in epithelial cells in a calcium dependent manner [56] and annexin A2 also interacts with the transcription factor, STAT6 in the metastatic prostate cancer cells [57]. Annexin A2 also binds to progastrin in colon cancer cells [58] and the interaction between annexin A2 and progastrin mediate growth effects of progastrin in gastrointestinal cancers (reviewed in [59]). Furthermore, annexin A2 has also been reported to form complexes with other S100 proteins such as S100A4 in endothelial cells [60] and S100A6 in pancreatic cancer cells [61].

Role of Annexin A2 in the Plasminogen Activator System

Emerging evidence indicates that annexin A2 plays an important role in the plasminogen activator system which regulates cancer metastasis and angiogenesis (reviewed in [62, 63]). The interaction between annexin A2 and t-PA mediates the conversion of plasminogen to plasmin [3, 64, 65] which facilitates MMP activation and ECM degradation leading to enhanced cancer cell migration and invasion [10, 66]. These findings suggest that annexin A2 is likely to play an important role in the early dissemination model which has been recently described by Coghlin and Murray [67].

Kinetic studies have demonstrated that binding of annexin A2 to t-PA on the cell surface of endothelial cells increases the catalytic efficiency of plasmin generation by up to 60-fold [3]. The co-localization of annexin A2 and t-PA has been demonstrated in breast and pancreatic cancer cells [10, 66, 68]. Roda et al. (2003) reported that the annexin A2 binding site for t-PA, the LCKLSL peptide motif (amino acid 8–13), is vital for the interaction between annexin A2 and t-PA to mediate plasmin production [69].

This was confirmed by Diaz et al. (2004) who reported activation of plasminogen via specific interaction of t-PA and annexin A2 in pancreatic cancer cells using the LCKLSL annexin A2 peptide [10].

Over-expression of annexin A2 resulted in higher levels of plasmin production in acute promyelocytic leukaemia cells [70] and increased plasmin levels were also observed in invasive breast cancer cell lines when compared with non-invasive cell lines [47, 66]. Recently, the interaction between heat shock protein 90 alpha (Hsp90a), annexin A2, t-PA and plasminogen in the exosomes of human breast, glioma and fibrosarcoma cancer cell lines resulted in increased cancer cell motility due to increased plasmin production [24]. Thus, these studies suggest increased annexin A2 expression enhances plasmin generation in the cancer cells via the t-PA dependent plasminogen activation pathway.

Role of Annexin A2 in Cancer Cell Adhesion, Invasion, and Migration

Several studies suggest that annexin A2 plays an essential role in regulating cancer cell adhesion, invasion, proliferation and migration [10, 36, 66, 71]. The effects of annexin A2 on different cancer cells are summarised in Table 2. Sharma et al. (2006) demonstrated that the highly invasive breast cancer cell line, MDA-MB231 expressed high annexin A2 levels and in the presence of plasminogen resulted in plasmin generation which could be blocked by a monoclonal antibody to annexin A2 [7]. However, neither plasmin production nor annexin A2 expression was observed in the non-metastatic MCF-7, breast cancer cell line [7]. Additionally, annexin A2 expression was shown to be up-regulated in MDA-MB231 breast cancer cells by basic fibroblast growth factor (bFGF) which is known to accelerate tumor growth and angiogenesis [7]. A later study by Sharma et al. (2009) confirmed the importance of annexin A2 and t-PA interactions on the cell surface of MDA-MB231 cells and demonstrated that the t-PA dependent plasmin generation was essential for cancer cell migration and neo-angiogenesis [66]. In contrast, Gillette et al. (2004) showed that over-expression of annexin A2 in osteosarcoma cells did not alter their metastatic properties such as motility, adhesion, or proliferation. This study suggested however that annexin A2 decreases osteosarcoma aggressiveness by inducing a more differentiated state [72]. Furthermore, Jung et al. (2007) demonstrated using both in vivo and in vitro studies that the annexin A2 heterotetramer regulates adhesion of hematopoietic stem cells to osteoblasts and bone marrow endothelial cells [73]. Recently, Shiozawa et al. (2008) also reported that annexin A2 regulates adhesion and migration of prostate cancer cells to osteoblasts and endothelial cells [11].

Table 2 Summary of the effects of annexin A2 on cancer cells

Function	Observations	Ref
Motility	Enhanced migration of hepatocellular carcinoma cells in vitro via annexin A2 interaction with HAb18G/CD187	[76]
	Annexin A2 binds to S100A6 protein enhances cell migration in pancreatic cancer cells	[61]
	The interaction of annexin A2 and t-PA induced cell migration in human breast cancer cell line	[66]
	Annexin A2 expression is vital in prostate cancer cell migration	[36]
Invasion	Annexin A2 mediates plasmin generation in human breast cancer cells which enhances cell invasion	[7]
	Interaction of annexin A2 and t-PA promotes pancreatic cancer cell invasion in vitro	[10]
	Knockdown of annexin A2 expression in pancreatic cancer cells inhibits cell invasion	[32]
	Annexin A2 promotes invasion of hepatocellular carcinoma via interaction with HAb18G/CD187 in vitro	[76]
	Annexin A2 enhances invasiveness of multidrug resistant breast cancer cell lines	[107]
Adhesion	Annexin A2 regulates the adhesion of prostate cancer cells	[11]
	Annexin A2 regulates cell adhesion of RAW 117 cells (large cell lymphoma cells) to the endothelial cells	[12]
	Phosphorylation of annexin A2 mediates baby hamster kidney cell adhesion	[108]
Apoptosis	Annexin A2 plays a role in p53 induced apoptosis	[109]
	Annexin A2 cleavage leads to cell apoptosis	[86]
Proliferation	Annexin A2 affects proliferation of multiple myeloma cells	[71]
	Annexin A2 regulates proliferation of dasatinib sensitive melanoma cells	[82]
	Knockdown of annexin A2 down regulates p11 proteins expression which decreases proliferation of invasive breast cancer cell lines	[47]
	Annexin A2 induces proliferation of hepatocytes	[110]

Annexin A2 has also been demonstrated to play an important role in regulating cytoskeleton structures and actin remodeling which both are essential for cancer cell migration [74, 75]. A recent study by Pu et al. (2009) reported that annexin A2 interaction with HAb18G/CD187 (a member of immunoglobulin family) promotes invasion and migration of human hepatocellular carcinoma cells (FHCC-98 cells) in vitro [76]. siRNA studies have also confirmed that knockdown of annexin A2 expression decreases cell migration in human glioma cells [77] and decreases the invasive ability of multiple myeloma cells [71]. The silencing of annexin A2 expression in hepatocellular carcinoma cells results in a significant decrease of both MMP-2 and MMP-9 levels which decreases cancer cell invasiveness [76]. Bao et al. (2009) also showed that the loss of annexin A2 expression leads to reduced levels of pro-angiogenic molecules including MMP-2, MMP-9, MTI-MMP, and TIMP2 in multiple myeloma cell lines (U266 and RPMI18226) [71]. These pro-angiogenic molecules are essential for tumor cell properties such as angiogenesis, proliferation and invasion [71]. Annexin A2 and Hsp90alpha released in the exosomes of the tumor cells activates plasminogen and pro-MMP2 which increase plasmin production to enhance ECM remodeling and increased tumor cell motility and invasion in the tumor microenvironment [24]. These studies demonstrate that annexin A2 is a key factor in regulating MMP secretion and activation which are essential for ECM degradation and metastatic progression.

Hou et al. (2008) reported that annexin A2 regulates p11 protein and plasmin levels in a mouse lymphoma (L5178Y) cell line [78]. Annexin A2 knockout in L5178Y cells exhibited reduced cell motility and invasion in vitro and in vivo [78]. In a recent study by Zhang et al. (2010) showed that the silencing of annexin A2 expression resulted in an inhibition of breast cancer cell proliferation and invasion which was also associated with a down-regulation of p11 protein and plasmin levels [47]. Together these studies indicate that annexin A2 plays an important role in promoting adhesion, migration and subsequent metastasis of cancer cells.

Post Translational Modifications and Proteolytic Cleavage of Annexin A2 in Cancer Cells

Several studies have reported post-translational modification and proteolysis of annexin A2 in cancer. Multiple isoforms of annexin A2 were identified by 2D gel electrophoresis and mass spectrometry in colorectal and oral carcinomas [79, 80]. Phosphorylation at tyrosine 23 in the N-terminal domain of annexin A2, has been shown to be present in liver [8] and pancreatic cancer tissues but not in corresponding normal tissues [32]. Takano et al. (2008) also observed post-translational modifications of annexin A2 in a chemotherapy-resistant pancreatic cancer cell line compared with chemosensitive cells [81]. Zheng et al. (2009) recently reported that phosphorylation of annexin A2 at tyrosine 23 was essential for localisation of annexin A2 at the cell surface and its

contribution to the invasive properties of pancreatic cancer cells [32]. Eustace et al. (2011) recently reported increased phosphorylated forms of annexin A2 in dasatinib sensitive melanoma cell lines (WM-115) compared with the dasatinib resistant cell line (WM-2660-4) [82].

Annexin A2 can also undergo proteolysis. N-terminal processing of annexin A2 in human peripheral monocytes [83] and endothelial cells [84] induced by plasmin resulted in the loss of the first 27 amino acid residues. Tomonaga et al. (2007) reported cleavage of annexin A2 in the C-terminal end of annexin A2 in the healthy bowel mucosa but not in the colorectal cancer samples [80]. Tsunezumi et al. (2008) demonstrated that treatment of human colon cancer and breast cancer cell lines with MMP-7 (matrilysin) can result in specific cleavage of annexin A2 at the N-terminal region between Lys9 and Leu10 within the t-PA binding motif [85]. This study additionally demonstrated that the first nine amino acid residues in the N-terminal domain of annexin A2 bound preferentially to t-PA over intact annexin A2 on the surface of colon cancer cells [85]. Annexin A2 cleavage induced by glycogen synthase-3 was also observed in a time-dependent manner in serum-free conditioned media of human lung epithelial cells and human epidermoid cells [86]. Whilst several studies have demonstrated post-translational modifications of annexin A2, including phosphorylation changes and annexin A2 cleavage by cancer cells, the regulation of these modifications is poorly understood. Further studies are required to evaluate the significance of these modified forms of annexin A2 in cancer.

Annexin A2 and Ovarian Cancer

To date, there is limited knowledge about the importance of annexin A2 in ovarian cancer. A proteomic ovarian cancer biomarker study by Wang et al. (2004) using 2D liquid phase separation followed by reverse-phase high performance liquid chromatography (RP-HPLC) and matrix-assisted laser desorption or ionisation-time of flight (MALDI-TOF) mass

spectrometry, identified annexin A2, amongst other proteins at three different pI values. Annexin A2 at pI 7.10, was markedly elevated in ovarian cancer cell lines when compared with normal ovarian surface epithelial cells, suggesting that a post-translational modification of annexin A2 occurs in ovarian cancer cells [87]. Furthermore, Gagne et al. (2007) also found annexin A2 to be up-regulated in the highly metastatic, TOV-112D epithelial ovarian cancer cell line compared with the TOV-81D ovarian cancer cells with low malignant potential, using iTRAQ (isobaric tag for relative and absolute quantitation) and 2D gel electrophoresis coupled with liquid chromatography and tandem mass spectrometry [88]. A more recent microarray study by Tchagang et al. (2008) reveal increased annexin A2 gene expression in ovarian cancer tissues when compared with normal ovarian tissue [89]. Moreover, several proteomic studies have also identified annexin A2 in the secretome of human ovarian cancer cell lines [90, 91].

We have recently explored the interaction between ovarian cancer and peritoneal cells using an in vitro co culture system [92]. We compared proteins in the secretome of ovarian cancer (OVCAR-5) and peritoneal cells (LP-9) cultured separately (Fig. 2a) and co-cultured together (Fig. 2b). We identified annexin A2 to be up-regulated in the secretome of co-cultured ovarian cancer and peritoneal cells by 2D-gel electrophoresis and mass spectrometry (Fig. 2b). Furthermore, we have recently reported increased plasmin levels during the co culture of ovarian cancer and peritoneal mesothelial cells in vitro [92]. Preliminary findings have demonstrated high annexin A2 immunostaining in serous ovarian cancer tissues compared with benign serous cystadenomas (Fig. 3). Our studies suggest that increased plasmin production and increased annexin A2 secretion is part of a tumor-host signal pathway between ovarian cancer and peritoneal cells which promotes ovarian cancer metastasis. Ongoing studies in our laboratory are currently investigating the importance of annexin A2 in ovarian cancer metastasis and its potential as a novel therapeutic target for this malignancy.

Fig. 2 A. 2D-gel electrophoresis of proteins in the secretome of ovarian cancer (OVCAR-5) and peritoneal cells (LP-9) cultured separately. B. 2D-gel electrophoresis gel of proteins in the secretome of OVCAR-5 and LP-9 co culture. Circled spots that were up-regulated in the ovarian cancer peritoneal co-culture but absent in the single culture were identified to be annexin A2 by mass spectrometry

Fig. 3 Annexin A2 expression in human ovarian tumor tissues. **a.** Benign serous cystadenoma **b.** Serous ovarian carcinoma. Annexin A2 immunohistochemistry using mouse anti-annexin A2 (BD Biosciences, 1: 500), with a citrate buffer microwave retrieval. Bar=100 μ m

Annexin A2 as a Potential Therapeutic Target in Cancer

Several research groups have targeted annexin A2 to inhibit cancer progression and metastasis. Monoclonal antibodies against annexin A2 have been reported to be an effective therapeutic approach against Lewis lung carcinoma xenografts [93]. Jacovina et al. (2001) reported that a polyclonal antibody against the N-terminal domain of annexin A2 was able to block plasmin production in rat adrenal pheochromocytoma (PC-12) cells in vitro [94]. Annexin A2 has also been shown to bind to angiostatin, a powerful anti-angiogenic molecule that is generated from plasminogen processing [95]. Tuszyński et al. (2002) reported that angiostatin binding to the lysine binding domain of annexin A2 in endothelial cells results in an anti-angiogenic effect [95]. Moreover, the interaction between annexin A2 and angiostatin resulted in reduced plasmin generation in Lewis lung carcinoma cells [93]. These findings indicate that plasminogen and angiostatin may bind to the same annexin A2 binding site and the

anti-angiogenic action of angiostatin is mediated via interactions with annexin A2 [95]. These studies suggest angiostatin-like compounds that block annexin A2 binding to plasminogen could be promising cancer therapeutics. More recently, Braden et al. (2009) showed that polymeric nanoparticles combined with annexin A2 siRNA vector to allow long term annexin A2 silencing could inhibit prostate cancer growth in mice [96]. Annexin A2 has also been identified as a molecular target for TM601 (a peptide with tumor-targeting and anti-angiogenic effects) in glioma, lung and pancreatic cancer cells [97]. Together these studies indicate that annexin A2 has a great potential as therapeutic target to inhibit cancer progression and metastasis.

Conclusions and Future Directions

In conclusion, annexin A2 has been shown to play an important role in cancer cell migration, invasion, adhesion

Fig. 4 Proposed mechanism of annexin A2 promoting cancer metastasis in the plasminogen activation system. Annexin A2 heterotetramer on the cell surface binds to t-PA and activates plasminogen conversion to plasmin. Plasmin results in activation of MMPs and lead to ECM degradation. Increased annexin A2 expression results in increased plasmin generation and enhances cancer invasion and metastasis

and angiogenesis processes which are essential for cancer metastasis. Emerging evidence indicates that annexin A2 plays an important role in the plasminogen activator system. The interaction between annexin A2 and t-PA mediates the conversion of plasminogen to plasmin which facilitates ECM degradation leading to enhanced cancer cell migration and invasion (Fig. 4). Annexin A2 over-expression and proteolytic cleavage has also been demonstrated in different malignancies. The processing of annexin A2 by MMPs and other proteases results in several annexin A2 isoforms but no studies so far have investigated the significance of these isoforms in tumor progression. Additional studies are needed to further explore the interactions between annexin A2 and its binding proteins and the importance of post-translational modifications of annexin A2 to promote tumor invasion and metastasis. A greater understanding of these mechanisms could potentially lead to the development of novel therapeutics to inhibit annexin A2 function and tumor progression.

Acknowledgement This research was supported by the Ovarian Cancer Research Foundation (OCRF).

References

- Ranson M, Andronikos NM (2003) Plasminogen binding and cancer: promises and pitfalls. *Front Biosci* 8:s294–304
- Hajjar KA, Krishnan S (1999) Annexin II: A mediator of the plasmin/plasminogen activator system. *Trends Cardiovasc Med* 9:128–138
- Cesarman GM, Guevara CA, Hajjar KA (1994) An endothelial cell receptor for plasminogen/tissue plasminogen activator (t-PA). II. Annexin II-mediated enhancement of t-PA-dependent plasminogen activation. *J Biol Chem* 269:21198–21203
- Hajjar KA, Guevara CA, Lev E et al (1996) Interaction of the fibrinolytic receptor, annexin II, with the endothelial cell surface. Essential role of endonexin repeat 2. *J Biol Chem* 271:21652–21659
- Mai J, Waisman DM, Sloane BF (2000) Cell surface complex of cathepsin B/annexin II tetramer in malignant progression. *Biochim Biophys Acta* 1477:215–230
- Vishwanatha JK, Chiang Y, Kumble KD et al (1993) Enhanced expression of annexin II in human pancreatic carcinoma cells and primary pancreatic cancers. *Carcinogenesis* 14:2575–2579
- Sharma MR, Koltowski L, Ownbey RT et al (2006) Angiogenesis-associated protein annexin II in breast cancer: selective expression in invasive breast cancer and contribution to tumor invasion and progression. *Exp Mol Pathol* 81:146–156
- Mohammad HS, Kurokohchi K, Yoneyama H et al (2008) Annexin A2 expression and phosphorylation are up-regulated in hepatocellular carcinoma. *Int J Oncol* 33:1157–1163
- Inokuchi J, Narula N, Yee DS et al (2009) Annexin A2 positively contributes to the malignant phenotype and secretion of IL-6 in DU145 prostate cancer cells. *Int J Cancer* 124:68–74
- Diaz VM, Hurtado M, Thomson TM et al (2004) Specific interaction of tissue-type plasminogen activator (t-PA) with annexin II on the membrane of pancreatic cancer cells activates plasminogen and promotes invasion in vitro. *Gut* 53:993–1000
- Shiozawa Y, Havens AM, Jung Y et al (2008) Annexin II/annexin II receptor axis regulates adhesion, migration, homing, and growth of prostate cancer. *J Cell Biochem* 105:370–380
- Tressler RJ, Updyke TV, Yeatman T et al (1993) Extracellular annexin II is associated with divalent cation-dependent tumor cell-endothelial cell adhesion of metastatic RAW117 large-cell lymphoma cells. *J Cell Biochem* 53:265–276
- Gerke V, Moss SE (2002) Annexins: from structure to function. *Physiol Rev* 82:331–371
- Mussunoor S, Murray GI (2008) The role of annexins in tumour development and progression. *J Pathol* 216:131–140
- Yan X, Yin J, Yao H et al (2010) Increased expression of annexin A3 is a mechanism of platinum resistance in ovarian cancer. *Cancer Res* 70:1616–1624
- Kim A, Serada S, Enomoto T et al (2010) Targeting annexin A4 to counteract chemoresistance in clear cell carcinoma of the ovary. *Expert Opin Ther Targets* 14:963–971
- Song J, Shih Ie M, Chan DW et al (2009) Suppression of annexin A11 in ovarian cancer: implications in chemoresistance. *Neoplasia* 11:605–614
- Chuthapisith S, Bean BE, Cowley G et al (2009) Annexins in human breast cancer: possible predictors of pathological response to neoadjuvant chemotherapy. *Eur J Cancer* 45:1274–1281
- Huebner K, Cannizzaro LA, Frey AZ et al (1988) Chromosomal localization of the human genes for lipocortin I and lipocortin II. *Oncogene Res* 2:299–310
- Rhiem K, Klein A, Münch M et al (2003) Chromosomal region 15q21.1 is a frequent target of allelic imbalance in advanced breast carcinomas. *Int J Cancer* 106:74–77
- Waisman DM (1995) Annexin II tetramer: structure and function. *Mol Cell Biochem* 149:301–322
- Deora AB, Kreitzer G, Jacovina AT et al (2004) An annexin 2 phosphorylation switch mediates p11-dependent translocation of annexin 2 to the cell surface. *J Biol Chem* 279:43411–43418
- Bellagamba C, Hubaishy I, Bjorge JD et al (1997) Tyrosine phosphorylation of annexin II tetramer is stimulated by membrane binding. *J Biol Chem* 272:3195–3199
- McCready J, Sims JD, Chan D et al (2010) Secretion of extracellular hsp90alpha via exosomes increases cancer cell motility: a role for plasminogen activation. *BMC Cancer* 10:294
- Lei H, Romeo G, Kazlauskas A (2004) Heat shock protein 90 {alpha}-Dependent translocation of annexin II to the surface of endothelial cells modulates plasmin activity in the diabetic rat aorta. *Circ Res* 94:902–909
- Oliferenko S, Paiha K, Harder T et al (1999) Analysis of CD44-containing lipid rafts: recruitment of annexin II and stabilization by the actin cytoskeleton. *J Cell Biol* 146:843–854
- Schnitzer JE, Liu J, Oh P (1995) Endothelial caveolae have the molecular transport machinery for vesicle budding, docking, and fusion including VAMP, NSF, SNAP, annexins, and GTPases. *J Biol Chem* 270:14399–14404
- Kapoor M, Zhou Q, Otero F et al (2008) Evidence for annexin II-S100A10 complex and plasmin in mobilization of cytokine activity of human TrpRS. *J Biol Chem* 283:2070–2077
- Ohno Y, Izumi M, Kawamura T et al (2009) Annexin II represents metastatic potential in clear-cell renal cell carcinoma. *Br J Cancer* 101:287–294
- Duncan R, Carpenter B, Main LC et al (2008) Characterisation and protein expression profiling of annexins in colorectal cancer. *Br J Cancer* 98:426–433
- Domoto T, Miyama Y, Suzuki H et al (2007) Evaluation of S100A10, annexin II and B-FABP expression as markers for renal cell carcinoma. *Cancer Sci* 98:77–82
- Zheng L, Huang L, Mo G et al. (2009) Characterization of a new candidate target for pancreatic cancer immunotherapy: Tyrosine phosphorylation regulates cell surface translocation

- of annexin A2 and contributes to pancreatic cancer invasion. In: Proceedings of the 100th Annual Meeting of the American Association for Cancer Research Denver, Colorado, USA Abstract #: 713.
33. Reeves SA, Chavez-Kappel C, Davis R et al (1992) Developmental regulation of annexin II (Lipocortin 2) in human brain and expression in high grade glioma. *Cancer Res* 52:6871–6876
 34. Yeatman TJ, Updyke TV, Kaetzel MA et al (1993) Expression of annexins on the surfaces of non-metastatic and metastatic human and rodent tumor cells. *Clin Exp Metastasis* 11:37–44
 35. Chetcuti A, Margan SH, Russell P et al (2001) Loss of annexin II heavy and light chains in prostate cancer and its precursors. *Cancer Res* 61:6331–6334
 36. Liu JW, Shen JJ, Tanzillo-Swartz A et al (2003) Annexin II expression is reduced or lost in prostate cancer cells and its re-expression inhibits prostate cancer cell migration. *Oncogene* 22:1475–1485
 37. Xin W, Rhodes DR, Ingold C et al (2003) Dysregulation of the annexin family protein family is associated with prostate cancer progression. *Am J Pathol* 162:255–261
 38. Yee DS, Narula N, Ramzy I et al (2007) Reduced annexin II protein expression in high-grade prostatic intraepithelial neoplasia and prostate cancer. *Arch Pathol Lab Med* 131:902–908
 39. Banerjee A, Liu J, Yuan Y et al (2003) Expression of biomarkers modulating prostate cancer angiogenesis: differential expression of annexin II in prostate carcinomas from India and USA. *Mol Cancer* 2:34
 40. Wu W, Tang X, Hu W et al (2002) Identification and validation of metastasis-associated proteins in head and neck cancer cell lines by two-dimensional electrophoresis and mass spectrometry. *Clin Exp Metastasis* 19:319–326
 41. Pena-Alonso E, Rodrigo JP, Parra IC et al (2008) Annexin A2 localizes to the basal epithelial layer and is down-regulated in dysplasia and head and neck squamous cell carcinoma. *Cancer Lett* 263:89–98
 42. Karimi-Busheri F, Marcoux Y, Tredget EE et al (2002) Expression of a releasable form of annexin II by human keratinocytes. *J Cell Biochem* 86:737–747
 43. Davis RG, Vishwanatha JK (1995) Detection of secreted and intracellular annexin II by a radioimmunoassay. *J Immunol Methods* 188:91–95
 44. Zhao WQ, Chen GH, Chen H et al (2003) Secretion of annexin II via activation of insulin receptor and insulin-like growth factor receptor. *J Biol Chem* 278:4205–4215
 45. Ji NY, Park MY, Kang YH et al (2009) Evaluation of annexin II as a potential serum marker for hepatocellular carcinoma using a developed sandwich ELISA method. *Int J Mol Med* 24:765–771
 46. Yan GR, Ding W, Xu SH et al. (2010) Characterization of Phosphoproteins in Gastric Cancer Secretome. *OMICS*.
 47. Zhang J, Guo B, Zhang Y et al (2010) 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* 31:374–381
 48. Puisieux A, Ji J, Ozturk M (1996) Annexin II up-regulates cellular levels of p11 protein by a post-translational mechanisms. *Biochem J* 313:51–55
 49. Orend G, Chiquet-Ehrismann R (2006) Tenascin-C induced signaling in cancer. *Cancer Lett* 244:143–163
 50. Esposito I, Penzel R, Chaib-Haririche M et al (2006) Tenascin C and annexin II expression in the process of pancreatic carcinogenesis. *J Pathol* 208:673–685
 51. Chung C, Murphy-Ullrich J, Erickson H (1996) Mitogenesis, cell migration, and loss of focal adhesions induced by tenascin-C interacting with its cell surface receptor, annexin II. *Mol Biol Cell* 7:883–892
 52. Wilson KE, Bartlett JMS, Miller EP et al (1999) Regulation and function of the extracellular matrix protein tenascin-C in ovarian cancer cell lines. *Br J Cancer* 80:685–692
 53. Mai J, Finley RL Jr, Waisman DM et al (2000) Human procathepsin B interacts with the annexin II tetramer on the surface of tumor cells. *J Biol Chem* 275:12806–12812
 54. Cavallo-Medved D, Rudy D, Blum G et al (2009) Live-cell imaging demonstrates extracellular matrix degradation in association with active cathepsin B in caveolae of endothelial cells during tube formation. *Exp Cell Res* 315:1234–1246
 55. Lu G, Maeda H, Reddy SV et al (2006) Cloning and characterization of the annexin II receptor on human marrow stromal cells. *J Biol Chem* 281:30542–30550
 56. Wirl G, Schwartz-Albiez R (1990) Collagen-binding proteins of mammary epithelial cells are related to Ca²⁺(+)- and phospholipid-binding annexins. *J Cell Physiol* 144:511–522
 57. Das S, Shetty P, Valapala M et al (2010) Signal transducer and activator of transcription 6 (STAT6) is a novel interactor of annexin A2 in prostate cancer cells. *Biochemistry* 49:2216–2226
 58. Singh P, Wu H, Clark C et al (2007) Annexin II binds progastrin and gastrin-like peptides, and mediates growth factor effects of autocrine and exogenous gastrins on colon cancer and intestinal epithelial cells. *Oncogene* 26:425–440
 59. Singh P (2007) Role of annexin-II in GI cancers: interaction with gastrins/progastrins. *Cancer Lett* 252:19–35
 60. Semov A, Moreno MJ, Onichtchenko A et al (2005) Metastasis-associated protein S100A4 induces angiogenesis through interaction with annexin II and accelerated plasmin formation. *J Biol Chem* 280:20833–20841
 61. Nedjadi T, Kitteringham N, Campbell F et al (2009) S100A6 binds to annexin 2 in pancreatic cancer cells and promotes pancreatic cancer cell motility. *Br J Cancer* 101:1145–1154
 62. Kwaan HC (1992) The plasminogen-plasmin system in malignancy. *Cancer Metastasis Rev* 11:291–311
 63. Sharma MC, Sharma M (2007) The role of annexin II in angiogenesis and tumor progression: a potential therapeutic target. *Curr Pharm Des* 13:3568–3575
 64. Kassam G, Le BH, Choi KS et al (1998) The p11 subunit of the annexin II tetramer plays a key role in the stimulation of t-PA-dependent plasminogen activation. *Biochemistry* 37:16958–16966
 65. Hajjar KA, Jacovina AT, Chacko J (1994) An endothelial cell receptor for plasminogen/tissue plasminogen activator. I. Identity with annexin II. *J Biol Chem* 269:21191–21197
 66. Sharma M, Ownbey RT, Sharma MC (2010) Breast cancer cell surface annexin II induces cell migration and neoangiogenesis via tPA dependent plasmin generation. *Exp Mol Pathol* 88:278–286
 67. Coghlin C, Murray GI (2010) Current and emerging concepts in tumour metastasis. *J Pathol* 222:1–15
 68. Paciucci R, Tora M, Diaz VM et al (1998) The plasminogen activator system in pancreas cancer: role of t-PA in the invasive potential in vitro. *Oncogene* 16:625–633
 69. Roda O, Valero ML, Peiro S et al (2003) New insights into the tPA-Annexin A2 interaction. Is annexin A2 CYS8 the sole requirement for this association? *J Biol Chem* 278:5702–5709
 70. Zhang X, Zhou H, Wang J et al (2002) Arsenic trioxide, retinoic acid and Ara-c regulated the expression of annexin II on the surface of APL cells, a novel co-receptor for plasminogen/tissue plasminogen activator. *Thromb Res* 106:63–70
 71. Bao H, Jiang M, Zhu M et al (2009) Overexpression of Annexin II affects the proliferation, apoptosis, invasion and production of proangiogenic factors in multiple myeloma. *Int J Hematol* 90:177–185
 72. Gillette JM, Chan DC, Nielsen-Preiss SM (2004) Annexin 2 expression is reduced in human osteosarcoma metastases. *J Cell Biochem* 92:820–832

73. Jung Y, Wang J, Song J et al (2007) Annexin II expressed by osteoblasts and endothelial cells regulates stem cell adhesion, homing, and engraftment following transplantation. *Blood* 110:82–90
74. Hayes MJ, Shao D, Bailly M et al (2006) Regulation of actin dynamics by annexin 2. *EMBO J* 25:1816–1826
75. Thiel C, Osborn M, Gerke V (1992) The tight association of the tyrosine kinase substrate annexin II with the submembranous cytoskeleton depends on intact p11- and Ca(2+)-binding sites. *J Cell Sci* 103:733–742
76. Pu Z, Wei Z, Juan T et al (2009) Annexin II promotes invasion and migration of human hepatocellular carcinoma cells in vitro via its interaction with HAb18G/CD147. *Cancer Sci* 101:387–395
77. Tatenhorst L, Rescher U, Gerke V et al (2006) Knockdown of annexin 2 decreases migration of human glioma cells in vitro. *Neuropathol Appl Neurobiol* 32:271–277
78. Hou Y, Yang L, Mou M et al (2008) Annexin A2 regulates the levels of plasmin, S100A10 and fascin in L5178Y cells. *Cancer Invest* 26:809–815
79. Chen J, He QY, Yuen AP et al (2004) Proteomics of buccal squamous cell carcinoma: the involvement of multiple pathways in tumorigenesis. *Proteomics* 4:2465–2475
80. Tomonaga T, Matsushita K, Yamaguchi S et al (2004) Identification of altered protein expression and post-translational modifications in primary colorectal cancer by using agarose two-dimensional gel electrophoresis. *Clin Cancer Res* 10:2007–2014
81. Takano S, Togawa A, Yoshitomi H et al (2008) Annexin II overexpression predicts rapid recurrence after surgery in pancreatic cancer patients undergoing gemcitabine-adjuvant chemotherapy. *Ann Surg Oncol* 15:3157–3168
82. Eustace AJ, Dowling P, Henry M et al. (2011) 2D-DIGE analysis of phospho-enriched fractions from dasatinib-treated melanoma cell lines. *J Proteomics*.
83. Laumonier Y, Syrovets T, Burysek L et al (2006) Identification of the annexin A2 heterotetramer as a receptor for the plasmin-induced signaling in human peripheral monocytes. *Blood* 107:3342–3349
84. He KL, Sui G, Xiong H et al. (2010) Feedback regulation of endothelial cell surface plasmin generation by PKC dependent phosphorylation of annexin A2. *J Biol Chem*.
85. Tsunozumi J, Yamamoto K, Higashi S et al (2008) Matrilysin (matrix metalloproteinase-7) cleaves membrane-bound annexin II and enhances binding of tissue-type plasminogen activator to cancer cell surfaces. *FEBS J* 275:4810–4823
86. Wang CY, Lin YS, Su WC et al (2009) Glycogen synthase kinase-3 and omi/HtrA2 induce annexin A2 cleavage followed by cell cycle inhibition and apoptosis. *Mol Biol Cell* 20:4153–4161
87. Wang H, Kachman MT, Schwartz DR et al (2004) Comprehensive proteome analysis of ovarian cancers using liquid phase separation, mass mapping and tandem mass spectrometry: a strategy for identification of candidate cancer biomarkers. *Proteomics* 4:2476–2495
88. Gagne JP, Ethier C, Gagne P et al (2007) Comparative proteome analysis of human epithelial ovarian cancer. *Proteome Sci* 5:16
89. Tchagang AB, Tewfik AH, DeRycke MS et al (2008) Early detection of ovarian cancer using group biomarkers. *Mol Cancer Ther* 7:27–37
90. Gunawardana CG, Kuk C, Smith CR et al (2009) Comprehensive analysis of conditioned media from ovarian cancer cell lines identifies novel candidate markers of epithelial ovarian cancer. *J Proteome Res* 8:4705–4713
91. Faca VM, Ventura AP, Fitzgibbon MP et al (2008) Proteomic analysis of ovarian cancer cells reveals dynamic processes of protein secretion and shedding of extra-cellular domains. *PLoS ONE* 3:e2425
92. Ween MP, Lokman NA, Hoffmann P et al. (2010) Transforming growth factor-beta-induced protein secreted by peritoneal cells increases the metastatic potential of ovarian cancer cells. *Int J Cancer*.
93. Sharma MR, Rothman V, Tuszynski GP et al (2006) 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* 81:136–145
94. Jacovina AT, Zhong F, Khazanov E et al (2001) Neuritogenesis and the nerve growth factor-induced differentiation of PC-12 cells requires annexin II-mediated plasmin generation. *J Biol Chem* 276:49350–49358
95. Tuszynski GP, Sharma MR, Rothman VL et al (2002) Angiostatin binds to tyrosine kinase substrate annexin II through the lysine-binding domain in endothelial cells. *Microvasc Res* 64:448–462
96. Braden AR, Kafka MT, Cunningham L et al (2009) Polymeric nanoparticles for sustained down-regulation of annexin A2 inhibit prostate tumor growth. *J Nanosci Nanotechnol* 9:2856–2865
97. Kesavan K, Ratliff J, Johnson EW et al (2010) Annexin A2 is a molecular target for TM601, a peptide with tumor-targeting and anti-angiogenic effects. *J Biol Chem* 285:4366–4374
98. Qi YJ, He QY, Ma YF et al (2008) Proteomic identification of malignant transformation-related proteins in esophageal squamous cell carcinoma. *J Cell Biochem* 104:1625–1635
99. Syed SP, Martin AM, Haupt HM et al (2007) Angiostatin receptor annexin II in vascular tumors including angiosarcoma. *Hum Pathol* 38:508–513
100. Emoto K, Yamada Y, Sawada H et al (2001) Annexin II overexpression correlates with stromal tenascin-C overexpression. *Cancer* 92:1419–1426
101. Nygaard SJ, Haugland HK, Kristoffersen EK et al (1998) Expression of annexin II in glioma cell lines and in brain tumor biopsies. *J Neurooncol* 38:11–18
102. Roseman BJ, Bollen A, Hsu J et al (1994) Annexin II marks astrocytic brain tumors of high histologic grade. *Oncol Res* 6:561–567
103. Zhong LP, Wei KJ, Yang X et al (2009) Increased expression of annexin A2 in oral squamous cell carcinoma. *Arch Oral Biol* 54:17–25
104. Hellman K, Alaiya AA, Becker S et al (2009) Differential tissue-specific protein markers of vaginal carcinoma. *Br J Cancer* 100:1303–1314
105. Bae SM, Lee CH, Cho YL et al (2005) Two-dimensional gel analysis of protein expression profile in squamous cervical cancer patients. *Gynecol Oncol* 99:26–35
106. Olwill SA, McGlynn H, Gilmore WS et al (2005) Annexin II cell surface and mRNA expression in human acute myeloid leukaemia cell lines. *Thromb Res* 115:109–114
107. Zhang F, Zhang L, Zhang B et al (2009) Anxa2 plays a critical role in enhanced invasiveness of the multidrug resistant human breast cancer cells. *J Proteome Res* 8:5041–5047
108. Rescher U, Ludwig C, Konietzko V et al (2008) Tyrosine phosphorylation of annexin A2 regulates Rho-mediated actin rearrangement and cell adhesion. *J Cell Sci* 121:2177–2185
109. Huang Y, Jin Y, Yan CH et al (2008) Involvement of annexin A2 in p53 induced apoptosis in lung cancer. *Mol Cell Biochem* 309:117–123
110. Masaki T, Tokuda M, Fujimura T et al (1994) Involvement of annexin I and annexin II in hepatocyte proliferation: can annexins I and II be markers for proliferative hepatocytes? *Hepatology* 20:425–435
111. Zimmermann U, Woenckhaus C, Pietschmann S et al (2004) Expression of annexin II in conventional renal cell carcinoma is correlated with Fuhrman grade and clinical outcome. *Virchows Arch* 445:368–374