# Annexin A2 and cancer: A systematic review

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**Abstract.** Annexin A2 is a 36-kDa protein interfering with multiple cellular processes especially in cancer progression. The present review aimed to show the relations between Annexin A2 and cancer. A systematic search for studies investigating cancer and Annexin A2 expression was conducted using PubMed. Acute lymphoblastic leukaemia, acute promyelocytic leukaemia, clear cell renal cell carcinoma, breast, cervical, colorectal, endometrial, gastric cancer, glioblastoma, hepatocellular carcinoma, lung, multiple myeloma, oesophageal squamous cell carcinoma, ovarian cancer, pancreatic duct adenocarcinoma, prostate cancer and urothelial carcinoma were evaluated. Annexin A2 expression correlates with resistance to treatment, binding to the bone marrow, histological grade and type, TNM-stage and shortened overall survival. The regulation of Annexin A2 is of interest due to its potential as target for a more individualized cancer management.

**Contents**



## 1. Introduction

In order to individualize treatment of cancer patients, there is a growing need to characterize different cancer subgroups. More knowledge on cancer subtypes could improve both prognosis and treatment.

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The protein Annexin A2 has been investigated as a prognostic marker because of its widespread presentation in several cancer forms. This study takes an overview of Annexin A2 and its presentation in various cancers including its prognostic values and potential as therapeutic target.

## 2. Background

Annexin A2 is part of the Annexin family consisting of up to 160 unique Annexin proteins (1). There are two criteria for being an Annexin protein. The first criterion is the ability to bind negatively charged phospholipid in a calcium-dependent manner. Second is the structural containment of an Annexin repeat, a segment of 70 amino acid residues. Annexin proteins comprise four or eight Annexin repeats and an α-helix disc (1). These folds allow Annexin to move intracellularly between lipofobic cytosol and lipophilic membrane compartment in a calcium-dependent manner (2).

Annexin proteins consist of three domains: a divergent $NH_2$-terminal, a C-terminal and a preserved domain making the core of the protein (1,3). For Annexin A2 the NH2-terminal acts as a binding site for S100A10 and tissue plasminogen activator (t-Pa). The core binds to calcium and the cell membrane. The C-terminal contains the binding site for F-actin (4), heparin (5) and plasminogen (6). The substrates for Annexin A2 reveal its function as an intercellular transport protein, interactor in cell division and migration and main interactor in plasmin production.

*Intracellular Annexin A2.* Free cytoplasmic Annexin A2 exists as a 36-kDa protein (1). Intracellularly, Annexin A2 is involved in exocytosis (7), endocytosis (7,8) and membrane trafficking through lipid micro-domains (9). Knockdown of the Annexin A2 gene, ANXA2, has been shown to diminish DNA synthesis and cell proliferation, suggesting that Annexin A2 is a factor in cell division (10). Furthermore, an interaction between Annexin A2 and CD44 has been shown to be essential for the formation of lipid rafts that interact with the cellular cytoskeleton (11). A complex of Annexin A2 and S100A10 directly binds F-actin at cholesterol-rich membrane passages, thereby interacting with the cytoskeleton (1). It has been shown that ANXA2 interacts in p53-mediated apoptosis (12) and prevents radiation-induced apoptosis, the latter by activating pro-survival signals such as nuclear factor κB (13). Annexin A2

also inhibits phospholipase A2 (PLA2) in an endogen manner, acting to inhibit PLA2 induced inflammation (14).

*Extracellular and membrane bound Annexin A2*. By binding directly or indirectly to phosphatidylserine on cells marked for apoptosis, Annexin A2 attends in the engulfment of cells (15). Membrane bound Annexin A2 contributes to fibrinolysis and has anticoagulation effects and involves binding to t-Pa and S100A10, hereby facilitating plasmin production (16,17). Furthermore, Annexin A2 seems to impact neo-angiogenesis which may explain its effect on solid tumours (17).

*Interactions of Annexin A2*

*S100A10*. S100A10 is part of the S100 protein family being calcium binding proteins of EF-hand type. The S100 family consist of 25 distinct isoforms weighing from 9 to 13 kDa (18). Twenty-two out of 25 S100 genes are located in the 1q21 chromosome region which is prone to genomic rearrangement. This indication of an unstable region supports how S100 proteins may be a relevant focus in cancer development (18).

The intracellular function of S100A10 includes calcium homeostasis, cell cycle regulation, phosphorylation, cell growth, migration and interactions with cytoskeleton components and regulation of transcriptional factors (18).

The extracellular function of S100 proteins is comparable to a cytokine-like behaviour by binding to cell surface receptors (18). S100A10 is seen to play a critical role in angiogenesis *in vivo*, suggesting its role in endothelial cell function (18).

S100A10 is unique in its way of being locked in a permanently open conformation (19). The binding of Annexin A2 is accommodated in the free hydrophobic space between Helix III and IV of the S100A10 dimer (19). In the cell membrane, Annexin A2 combines with S100A10 forming a 94 kDa heterotetramer of two Annexin A2 units and two 11-kDa S100A10 proteins. The Annexin A2-S100A10 heterotetramer is a key plasminogen receptor that on the cell surface mediates the formation of plasmin. S100A10 furthermore enhance the sensitivity of Annexin A2 to calcium, interfering with the calcium level needed to conduct Annexin A2 function (20). S100A10-Annexin A2 interaction seems to play a role in the cell-to-cell adhesion of breast cancer cells and multiple types of endothelium. Interaction has been shown by investigating the protein expression of Annexin A2 and S100A10 in tissue from breast cancer patients (21).

The plasminogen/plasmin system is interaction between coagulation factors and enzymes. Plasminogen is the inactive form of plasmin found in plasma and extra cellular matrix (ECM). Plasminogen is cleaved by plasminogen activators (tPa) through the hydrolysis of the Arg561-Val562 peptide bond to yield the serine protease, plasmin (22). Plasmin is an enzyme cleaving fibrin in the ECM by direct binding or by activating other proteases (23). Altogether plasminogen/plasmin/fibrin are important regulators of proteolysis of ECM, fibrin clot degradation, macrophage migration, tissue remodelling, invasion and angiogenesis (22). An overproduction of plasmin in the tumour microenvironment enhance the degradation of ECM, hereby facilitating tumour invasion (22). Annexin A2 has independent binding sites for both tPa and plasminogen. By assembling these proteins on the cell surface, Annexin A2 accelerates the production of plasmin (22). The binding site between Annexin A2 and tPa are mechanically blocked by lipoprotein(a) and homocysteine which both are arteriothrombotic agents (2). Annexin A2-S100A10 regulates 50-90% of the plasmin generation in several types of normal cells and cancer cells (24).

*Human epididymis protein 4 (HE4)*. The binding site of HE4 to Annexin A2 is located after the 26th amino acid at the N-terminus (5). HE4 and Annexin A2 activates the extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) and focal adhesion kinase (FAK) pathways (25). The ERK/MAPK signalling pathway influences proliferation, migration and apoptosis (26). FAK is a regulator of cell signalling within the tumour microenvironment and controls cell movement, invasion and survival (27).

*Angiostatin (AS)*. Angiostatin is a 38-kDa internal fragment of plasminogen that interacts through the lysine binding domain on the C-terminus of Annexin A2 on the cell surface (28). This is also the binding site for plasminogen (22). By using AS to compete with plasminogen binding, ~40% of the binding to bovine arterial endothelium cells were blocked (29).

*Human procathepepsin*. Procathepepsin B is an enzyme hosted in lysosomes. Procathepepsin B can degrade ECM proteins such as laminin, fibronectin and collagen IV, hereby facilitating invasion of the tumours cells (30). Immunohistochemistry (IHC) showed recombinant procathepsin to interact with the Annexin A2-S100A10 heterotetramer (31). Likewise, it has been shown that procathepepsin and the Annexin A2 heterotetramer co-localize in two lines of human cancer cells, one of epithelial origin and one of mesenchymal origin. Procathepsin B can alone or in interaction with Annexin A2 activate other proteolytic proteins such as urokinase-type plasminogen activator and collagenase. These proteins also degrade ECM and facilitate invasion (30).

*Stromal derived factor 1 (SDF1/CXCL-12)*. Multiple myeloma, breast and prostate cancer are known to metastasize to the bone marrow (32) causing distinct pain and sometimes neural complications for the patients. *In vivo* and *in vitro* studies have showed how Annexin A2 and CXCL-12 co-localize in the bone marrow. Studies using whole bone marrow cells and investigating the migration of hematopoietic stem cells (HSC) demonstrated a synergistic effect of CXCL-12 bound to Annexin A2 (33). Expression of Annexin A2 in bone marrow stromal cells (BMSC) significantly increased the binding of prostate cancer (PC) cells to BMSC (34). Furthermore, studies demonstrated how CXCL-21 expression induced the migration of PC cells towards BMSC (34). Multiple myeloma cells also express Annexin A2. It was demonstrated how this Annexin A2 expression facilitated the adhesion of osteoblasts and stromal cells in the bone marrow (35).

*Functional regulation of Annexin A2*. The N-terminus of Annexin A2 is regulated by phosphorylation. Annexin A2 and S100A10 form their heterotetramer on the cell surface in response to changes in intracellular (ICL) calcium concentration (2). This presentation on the surface can be provoked by heat induced stress of the cell. By using small interfering

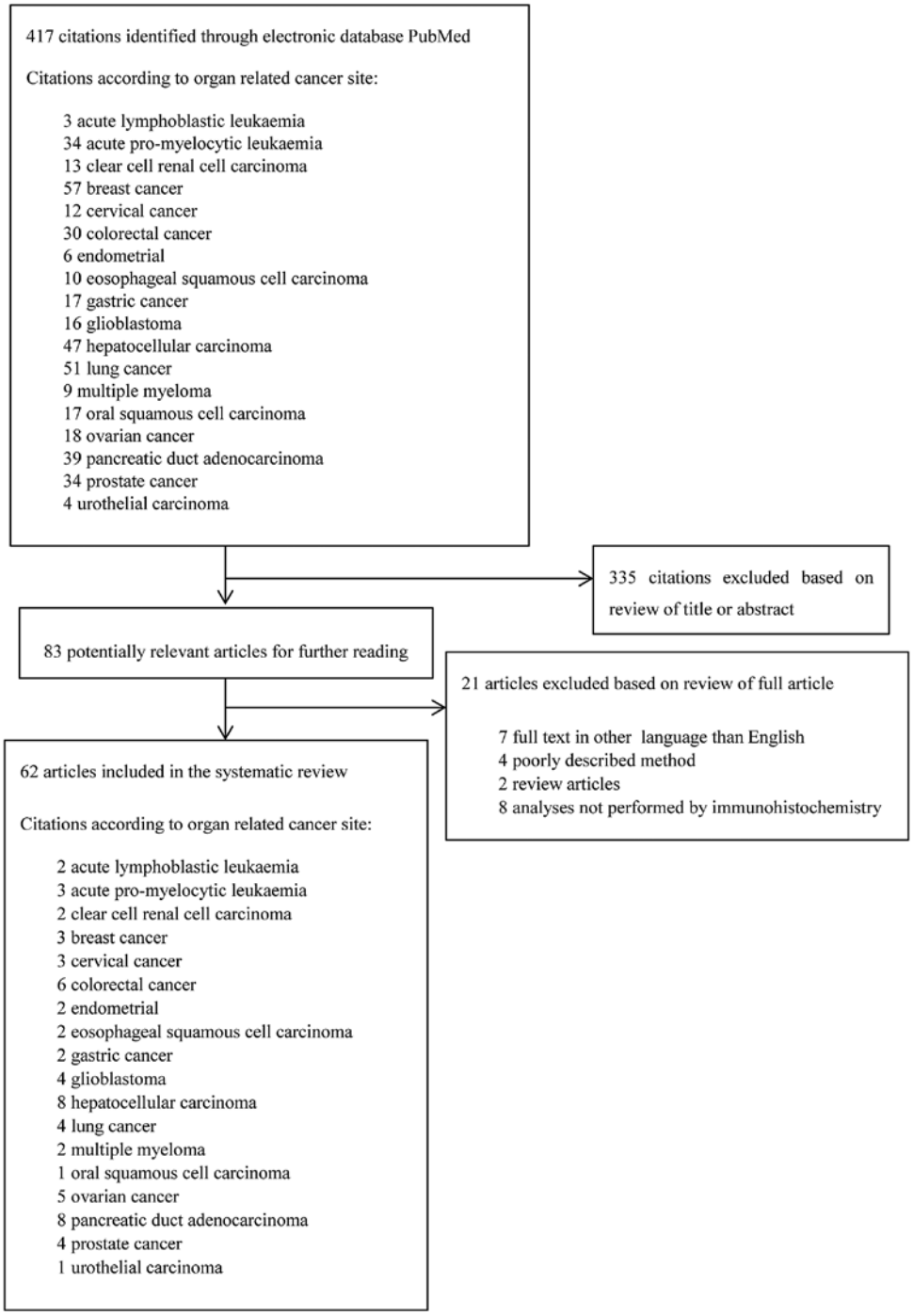


Figure 1. Flow diagram of the literature search in PubMed database. The keywords: 'the organ related cancer site' and 'Annexin A2' was used. A total of 62 articles published from 1990-2016 were included.

RNAs (siRNA), targeting S100A10, it was demonstrated that by reducing S100A10 expression, the heat stimulated translocation of Annexin A2 was markedly reduced (2). This shows how S100A10 is essential to the translocation of Annexin A2 caused by mild cellular stress (2).

Phosphorylation of Annexin A2 at residue 23 by src-like tyrosine kinases plays a role in the translocation of Annexin A2 to the cell surface (2). By using tyrosine kinase inhibitors, the heat stress induced expression of Annexin A2 on the cell surface was completely blocked demonstrating how tyrosine kinase phosphorylation of tyrosine 23 in Annexin A2 is essential to the translocation to the cell surface (2). Furthermore, protein kinase C (PKC) combined with calcium phosphorylates Annexin A2. This can be inhibited by Annexin A5 by interaction with PKC and diminishing its effect thereby reducing Annexin A2 translocation. Annexin A5 might have a therapeutic function in preventing Annexin A2s adverse function in cancer development.

*Method.* Initial search in PubMed database was made February 6, 2017 (Fig. 1). The following search criteria 'the organ related cancer site' and 'Annexin A2' was used. The search was combined with a screening for literature in reference sections of relevant studies. For preliminary screening of the articles,

all titles and abstracts were read. A total of 62 studies were included in the review and included studies were published from 1990-2016.

## 3. Overview of the publications

Annexin A2 is overexpressed in clear cell renal cell carcinoma, breast-, cervical-, colorectal-, endometrial-, gastric cancer, hepatocellular carcinoma, lung- and ovarian cancer, pancreatic duct adenocarcinoma, glioblastoma and urothelial carcinoma, acute lymphoblastic leukaemia, acute promyelocytic leukaemia and multiple myeloma (36-86).

Downregulation of Annexin A2 is reported in oesophageal squamous cell carcinoma (88,89).

Both upregulation and downregulation of Annexin A2 have been suggested as prognostic markers for patients diagnosed with oral squamous cell carcinoma (90) and prostate cancer (34,91-93). In Table I an overview of the relationship between Annexin A2 and different cancers is presented.

*Haematological cancers*

*Acute lymphoblastic leukaemia (ALL).* In patients with ALL, elevated levels of ANXA2 and increased amounts of phosphorylated Annexin A2 relate to resistance to glucocorticoid treatment (36). Annexin A2 is phosphorylated by Src-kinase, the reaction is facilitated by S100A10 (37). Elevated Annexin A2, S100A10 and Src-kinase activity may predict drug resistance in ALL patients (36,37). By inhibiting the Src-kinase, the ALL cells were sensitised to glucocorticoid treatment suggesting that Src-kinase inhibitors might be a supplement to treatment of glucocorticoid resistant ALL patients (36). Treatment of ALL cells with anti-Annexin A2 antibody and knockdown of S100A10 abrogate ALL adhesion to osteoblasts (37). This inhibition of ALL cell adhesion to osteoblasts indicates how Annexin A2 and S100A10 influence the binding and retention of ALL cells to the bone marrow (37). Additionally, long-term engraftment assays from mice showed reduced percentage of ALL cells in blood, spleen and bone marrow if treated with agents that disrupts the Annexin A2-S100A10 interaction (37). Furthermore, using mouse monoclonal antibodies against Annexin A2 increased the effect of treatment of ALL with dexamethasone and vincristine by disruption of the binding between ALL cells and osteoblasts (37).

*Acute promyelocytic leukaemia (APL).* Overexpression of Annexin A2 in APL cells is thought to be the mechanism behind haemorrhagic complications of APL patients (38,39). The t(15;17) translocation positive APL cells express Annexin A2 in a greater manner than other leukaemia cells (38). Annexin A2 facilitates the combining of t-Pa and plasminogen on the cell surface. APL cells with t(15;17) translocation had twice as effective t-Pa dependent plasmin generation. Annexin A2 overexpression might be the mechanism for haemorrhagic complications of APL patients (38,39). By treating APL cells with siRNA targeting Annexin A2, a decreased t-Pa mediated plasmin generation have been shown (39). Furthermore, APL cells treated with all-*trans* retinoic acid (ATRA) showed downregulation of Annexin A2. These findings indicate that treatment with siRNA targeting Annexin A2 or ATRA could resolve hyperfibrinolysis in APL (39,40).

*Multiple myeloma (MM).* Annexin A2 is expressed in MM cells in 8/8 patients (35). Annexin A2 has been shown to stimulate proliferation of MM cells and to support adhesion of MM cells to osteoblasts and stromal cells (35). Other studies, suggest that siRNA silencing of Annexin A2 can induce apoptosis in MM cell lines. Furthermore, by silencing Annexin A2 the invasive potential of MM cells were significantly diminished (74). This makes siRNA targeting Annexin A2 as a potential therapeutic focus to induce apoptosis of MM cells and interfere with the invasive potential (74).

*Urological cancers*

*Clear cell renal cell carcinoma (ccRCC).* Annexin A2 is expressed mainly in the membrane of ccRCC and the amount of Annexin A2 in ccRCC was higher compared to normal tissue (41). In primary ccRCC tumours, the expression of Annexin A2 was positively associated with a higher TNM-stage ($P<0.05$) (41,42), histological grade ($P<0.05$) (41), infiltration of the renal capsule ($P<0.01$) (41) and metastatic potential ($P<0.01$) (41,42). Furthermore, Annexin A2 overexpression was significantly correlated with shortened 5-year survival rate of ccRCC patients compared to patients with lower expression of Annexin A2 ($P<0.01$) (41).

*Urothelial cancer.* Higher Annexin A2 expression is reported in urothelial carcinoma (55%, 175/315) compared to overexpression in 17.5% (11/63) of the non-tumour mucosa samples ($P<0.01$) (87). The expression of Annexin A2 was associated with the depth of invasion, lymph node metastasis and distant metastasis ($P<0.05$). Furthermore, Annexin A2 expression is a significant independent prognostic factor for survival among urothelial carcinoma patients ($P=0.012$) (87).

*Prostate cancer (PC).* Annexin A2 is localized primarily in the membrane and faintly in the cytoplasm on PC cells (91). The expression level of Annexin A2 was significantly lower in the PC cases when compared to patients with benign prostate hyperplasia ($P<0.01$) (92,93). Lower Annexin A2 expression was negatively related to Gleason score 5-7, tumour stage, recurrence, lymph node metastasis and distant metastasis $P<0.01$ (92). Additionally, survival rate was significantly correlated to a downregulation of Annexin A2 expression ($P<0.01$) (92).

Annexin A2 expression seems to play a critical role in the homing and adhesion of PC cells to the bone marrow (34). Furthermore, it is demonstrated how Annexin A2 in bone marrow stromal cells could play a role in the resistance of PC cells to chemotherapy (34). Although low expression of Annexin A2 correlated to Gleason score 5-7, a strong and diffuse staining of Annexin A2 was seen in PC biopsies indicating an association between Annexin A2 and the most severe PC subtypes (91).

*Breast cancer.* No expression of Annexin A2 is found in normal or hyperplastic ductal epithelial cells of the human mammarian tissue. On the contrary, protein expression of Annexin A2 is found in breast cancer and ductal carcinoma *in situ* (CIS) (43). Correspondingly, Annexin A2 has been shown to be upregulated in HER-2 negative and herceptin resistant breast cancer cells (44). Annexin A2s ability to stimu-

Table I. Summary of the tissue protein expression of Annexin A2 in various cancer forms.

| | Cancer | Expression | Clinical manifestation | Therapeutic interest | Refs. |
|---|---|---|---|---|---|
| Haematological cancers | Acute lymphoblastic leukaemia | Upregulated | Resistant to glucocorticoid<br>Binding and retention of ALL cells in the bone marrow | Combining Annexin A2 inhibitors with dexamethasone and vincristine increased the effect.<br>Using agents to disrupt ANXA2-P11 interaction reduced amount of ALL cells in blood, spleens and bone marrow. | (36,37) |
| | Acute promyelocytic leukaemia | Upregulated | Haemorrhagic complications | Treatment with ATRA or siRNA targeting Annexin A2 could resolve hyperfibrinolysis in APL. | (38-40) |
| | Multiple myeloma | Upregulated | Proliferation<br>Adhesion to osteoblast and stromal cells<br>Invasive potential | siRNA targeting Annexin A2 could interfere with the malignant properties of MM cells | (35,73) |
| Urological cancers | Clear cell renal cell carcinoma | Upregulated | Higher TNM-stage<br>Histological grade<br>Infiltration of the renal capsule<br>Metastatic potential<br>Shortened 5-year survival rate | | (41,42) |
| | Urothelial cancer | Upregulated | Depth of invasion<br>Lymph node metastasis<br>Distant metastasis<br>Survival rate | | (86) |
| | Prostate cancer | Downregulated | Gleason score 5-7<br>Tumour stage<br>Recurrence<br>Lymph node metastasis<br>Distant metastasis<br>Survival rate | | (90-92) |
| | Prostate cancer | Upregulated | Gleason score 8<br>Homing and adhesion to bone-marrow<br>Resistance to chemotherapy | | (34,90) |
| Breast cancer | Breast cancer | Upregulated | Herceptin resistance<br>Neo-angiogenesis<br>Migration and invasion<br>Proliferation<br>Metastasis | Treatment with siRNA targeting Annexin A2 could diminish Herceptin resistance and cell proliferation<br>Anti-Annexin A2 antibodies disrupt neo-angiogenesis | (29,43,44) |
| Gynaecological cancers | Cervical cancer | Upregulated | Chemotherapy resistance | Treatment with anti-Annexin A2 antibodies or Annexin A2 ligands might decrease cervical cancer | (45-47) |

Table I. Continued.

| | Cancer | Expression | Clinical manifestation | Therapeutic interest | Refs. |
|---|---|---|---|---|---|
| | | | Advanced cancer stage<br>Decreased progression free-survival<br>HPV16 internalisation | caused by HPV16 | |
| | Endometrial cancer | Upregulated | Higher histological grade<br>Higher FIGO stage<br>Depth of invasion<br>Lymph node metastasis<br>Distant metastasis<br>Overall survival rate | | (54,55) |
| | Ovarian cancer | Upregulated | Poorly differentiated tumours<br>Histological grade<br>Ascites<br>Malignant tumour cells in peritoneal fluid<br>FIGO-stage<br>Invasion and migration<br>Metastasis<br>Disease progression<br>Death caused by OC<br>Reduced PFS<br>Shortened overall survival rate | siRNA targeting Annexin A2 decrease motility of OC cells<br>Treatment with Annexin A2 neutralizing antibodies reduced tumour burden *in vivo* and could possibly be transmitted to human OC patients<br><br>RNA nanoparticle harbouring Annexin A2 could be used to deliver doxorubicin directly into OC cells overcoming chemotherapy resistance | (22,74-77) |
| Gastroenterological cancers | Colorectal cancer | Upregulated | Tumour size<br>Higher TNM stage<br>Growth factor mediating<br>Poor prognosis<br>Recurrence | | (48-53) |
| | Colorectal cancer | Decreased in serum | Tumour size<br>TNM-stage<br>Tumour invasion<br>Lymph node metastasis<br>Distant metastasis | | (53) |
| | Esophagael squamous cell carcinoma | Downregulated | Lymph node metastasis<br>Depth of invasion<br>Poor differentiation | | (87,88) |

Table I. Continued.

| | Cancer | Expression | Clinical manifestation | Therapeutic interest | Refs. |
|---|---|---|---|---|---|
| Gastroenterological cancers | Gastric cancer | Upregulated | Tumour size<br>Histological type<br>Depth of invasion<br>Vessel invasion<br>Lymph node metastasis<br>Distant metastasis<br>TNM-stage<br>Shortened 5-year survival rate | | (56,57) |
| | Hepatocellular carcinoma | Upregulated | Tumour size<br>Intra- and extrahepatic metastasis<br>Portal vein thrombosis<br>TNM-stage<br>Metastasis<br>Poor prognosis<br>Shortened 5-year survival rate<br>Progression free-survival | By facilitating degradation of Annexin A2, UBAP2 might be of therapeutic interest in countering the adverse effect of Annexin A2 among HCC patients. | (61-68) |
| | Oral squamous cell carcinoma | Upregulated | Tumour size<br>Tumour recurrence | | (89) |
| | Oral squamous cell carcinoma | Downregulated | Histological grade | | (89) |
| | Pancreatic duct adenocarcinoma | Upregulated | Cell motility and viability<br>Histological grade<br>Poor prognosis<br>Distant metastasis<br>Shortened progression free-survival<br>Shortened overall survival rate<br>Resistance to adjuvant chemotherapy with gemcitabine | Anti-Annexin A2 antibodies, microRNA-206 and shRNA harbouring Annexin A2 has shown to decrease the adverse outcomes from PDA patients. | (78-85) |
| Neurological cancers | Glioblastoma | Upregulated | Neo-angiogenesis<br>Tumour stages<br>Three year survival | | (58-60,95) |
| Lung cancer | Non-small cell lung cancer | Upregulated | Tumour diameter<br>Pathological grade<br>pN-status<br>pT-status<br>Pleural invasion<br>Advanced clinical stage<br>Shortened overall survival | Annexin A2 is expressed in NSCLC cell lines resistant to chemotherapy. Targeting Annexin A2 might diminish multi-drug resistant tumours. Sh-RNA plasmids toward Annexin A2 might inhibit the effect of Annexin A2 in development of advanced clinical stage tumours of NSCLC. | (69-72) |

late the production of plasmin combined with the functional role of plasmin, indicates the possible role of Annexin A2 in angiogenesis and metastasis of breast cancer cells (43,94). Annexin A2 has been shown to maintain constitutive activation of the EGFR-pathway leading to cell proliferation, migration and viability (94). Annexin A2 downregulation by siRNA increased apoptosis and decreased cell viability and migration by inhibiting the Annexin A2 induced, constitutively active EGFR-pathway (44). Furthermore, it was shown that anti-Annexin A2 antibodies inhibited neo-angiogenesis by inducing apoptotic cell death of endothelial cells (94). This suggests that siRNA against Annexin A2 could be of therapeutic value in HER-2 negative, herceptin-resistant cancer cells.

*Gynaecological cancers*

*Cervical cancer.* Abnormal expression of Annexin A2 and S100A proteins has been reported to induce resistance to cisplatin-based chemotherapy among cervical cancer patients. IHC analysis showed increased Annexin A2 expression in cervical tumour stromal cells after chemotherapy treatment. In addition to this, Annexin A2 tumour expression was significantly higher in the group of tumours not responding to chemotherapy treatment, indicating that Annexin A2 upregulation may play a role in resistance to chemotherapy. Furthermore, Annexin A2 expression in stromal cells of cervical cancer patient is an independent prognostic factor for decreased progression free-survival (46,47). Annexin A2 was shown to be positively correlated with advanced cancer (47) indicating how expression of Annexin A2 relates to higher cancer stages.

Human papilloviruses (HPV) are sexually transmitted viruses that causally associate with the development of cervical cancers. The most common, HPV16, is an obligatory intracellular virus that must gain entry into host cells to survive (48). This HPV16 internalisation has been demonstrated to be partly facilitated by the Annexin A2-S100A10 heterotetramer. By inhibiting Annexin A2 in an endogenous manner or with anti-Annexin A2 antibodies, the HPV16 internalisation was significantly decreased.

*Endometrial cancer.* Annexin A2 is expressed in both membrane and the cytoplasm of endometrial cancer cells in 95.2% of the endometrial carcinomas compared to 55.6% of the normal endometrium (P<0.05) (55). *In vitro* studies suggest Annexin A2 may play a role in the promotion of metastasis in that endometrial cancer. Knockdown of Annexin A2 resulted in the absence of lung and hematogenous metastasis (56), implying Annexin A2 to play a role in the development of distant metastasis among patients with endometrial cancer. For 91.7% (22/24) of endometrial carcinoma patients in stage III-IV, a high expression of Annexin A2 was found. The expression was significantly higher than for patients in stage I-II with 55% (33/60) (P<0.05). Overexpression of Annexin A2 is correlated with shorter overall survival (P<0.05) (55). Together, this suggests that Annexin A2 could be a potential therapeutic focus in order to avoid spread of endometrial tumours and to predict recurrence and overall survival.

*Ovarian cancer (OC).* Annexin A2 is expressed in the membrane (77%) and the cytoplasm (82.6%) of serous OC cells as well as the surrounding stromal cells (58.5%) (75). Annexin A2 seems to play a role in regulating cell proliferation of OC cell lines (76). A significant increase in Annexin A2 expression in FIGO-stage IV compared to stage II and III is reported (P=0.001 and P=0.005, respectively) (75). Annexin A2 expression is related to histological grade (P=0.002) (76). Furthermore, high expression of Annexin A2 was significantly related to presence of ascites (P<0.001) and malignant tumour cells in peritoneal fluid (P<0.001) (76). Downregulation of Annexin A2 in OC cells significantly reduced the ability of invasion and migration (P<0.05). By analysing metastasis from OC patients it was revealed that there was a high number of lung metastatic nodules in the high Annexin A2 expression group, whereas almost no lung metastasis were found in the low expression group (25). High stromal expression is significantly associated with reduced progression-free survival (PFS) (P=0.014) and reduced overall survival (OS) (75). Patients with high stromal Annexin A2 had a 1.8-fold increased risk of disease progression (P=0.0014) and a 1.6-fold increased risk of disease related death (P=0.046) (75). Combined with S100A10, Annexin A2 expression predicts adverse outcomes for OC patients. For patients with high expression of stromal Annexin A2 and cytoplasmic S100A10 the 5-year survival rate was 11.1% compared to 50% for the patients with low stromal Annexin A2 and cytoplasmic S100A10 (75).

siRNA targeting Annexin A2 significantly decreased motility (P=0.0069) and invasion (P=0.0047) in OC cell lines. *In vivo* studies in mice showed how treatment with Annexin A2 neutralizing antibodies significantly reduced the tumour burden (77). This makes siRNA targeting Annexin A2 and Annexin A2 neutralizing antibodies a potential therapeutic focus of OC treatment. Another study showed that RNA-nanoparticles harbouring Annexin A2 can be used to deliver doxorubicin into the OC cells. Knowledge of this mechanism may help overcome chemotherapy resistant OC and to minimize the adverse effect of chemotherapy to healthy tissue (78).

*Gastroenterological cancers*

*Colorectal cancer (CRC).* Annexin A2 is highly expressed in CRC cell lines, both on mRNA level and as protein (49). The expression of Annexin A2 is shown to induce significant changes on the microstructure of the cells (50). Upregulated Annexin A2 promotes proliferation, migration and invasion of CRC cells *in vitro* caused by the changes in microstructure (49). In studies investigating Annexin A2 using IHC, high expression of Annexin A2 was significantly correlated with tumour size (P=0.03), poorly differentiated tumours (P=0.01), depth of invasion (P=0.02) and TNM-stage (P=0.02) (51). Annexin A2 was shown to be an independent factor for poor prognosis in patients with CRC (51). Annexin A2 in the cell membrane is a characteristic for tumours with high invasiveness. This ability to invade tissue shows how Annexin A2 could affect lymph node metastasis (52,53). Annexin A2 has also been shown to be important for the effect of progastrins and gastrins, hereby partially mediating the effect of growth factors on colon cancer cells (95). Furthermore, Annexin A2 could be used to predict recurrence of CRC. The 5-year recurrence rate was 69.4% in the high expression group compared to 35.9% in the low expression group among patients with stage I-II disease (53). On the other hand, the serum Annexin A2 level is significantly

lower in patients with CRC compared with healthy controls (P<0.001) (54). Low serum Annexin A2 levels were related to increased tumour size (P=0.003), higher TNM-stage (P=0.004), tumour invasion (P=0.005), lymph node metastasis (P=0.003) and distant metastasis (P=0.005) (54). This makes Annexin A2 levels in serum of interest to classify colon cancer patients.

*Oesophageal squamous cell carcinoma (ESCC) and oral squamous cell carcinoma (OSCC).* Annexin A2 is downregulated on both mRNA and protein level in ESCC cells (88,89). Annexin A2 was found in 9.1% (2/22) of the ESCC samples compared to 90.9% (20/22) of the controls using qRT-PCR and western blot analysis. The expression of Annexin A2 was confirmed by IHC. Annexin A2 in ESCC cells is found mainly in the cell membrane. Low Annexin A2 expression is correlated with lymph node metastasis (P<0.05), depth of invasion (P<0.05) and poor differentiation (P<0.05) (88).

Annexin A2 is expressed in the cell membrane of normal epithelial cells of the oral cavity. Annexin A2 was expressed in 82% (87/106) of the OSCC cases (90). Annexin A2 is associated with histological grade (P=0.02). Low expression of Annexin A2 is seen in poorly differentiated tumours compared to well differentiated tumours (90). On the contrary, a higher expression of Annexin A2 correlated to tumour size (P=0.003) and tumour recurrence (P=0.04) (90).

There are few studies investigating the expression of Annexin A2 and its relation to ESCC and OSCC. The studies reviewed have at low number of participants, 22 and 106, respectively, and it is therefore difficult to indicate a clear correlation between the expression of Annexin A2 and ESCC and OSCC.

*Gastric cancer.* Annexin A2 is found predominantly on the cell membrane of gastric cancer cells. Annexin A2 is found in non-tumour mucosa and in human gastric cancer cases in 19.6 and 40.1% of patients, respectively (57,58). The expression of Annexin A2 correlates with tumour size (57), histological type (57), depth of invasion (57), vessel invasion (57), lymph node metastasis, distant metastasis and TNM-stage (P<0.05) (57,58). Furthermore, for cancer stages I, II and III, the 5-year survival rate of the patients with high expression of Annexin A2 were significantly lower compared with 5-year survival for the patients with low expression (57).

*Hepatocellular carcinoma (HCC).* Annexin A2 expression is localized to the cell membrane and cytoplasm of HCC cells (62,63). Annexin A2 expression was found in 73.8% (62/84) of the HCC tissues compared to 35.6% (21/59) of the benign liver disease (BLD) tissue (P<0.001) (64). Tumours with a high expression of Annexin A2 were larger in size compared to low expression tumours (P=0.016) (63,65). Annexin A2 expression significantly correlated with intrahepatic metastasis (P=0.02), portal vein thrombosis (P=0.003) and higher TNM-stage (P=0.024) (64,66). Survival analysis revealed how the high expression group had a poorer prognosis and a shortened 5-year survival rate (63,64). Furthermore, Annexin A2 seems to regulate actin remodelling thereby facilitating invasion and metastasis of HCC cells making high expression of Annexin A2 a marker for metastatic potential of HCC cells (67,68). By comparing serum levels of Annexin A2 from HCC patients and BLD patients, it was revealed that Annexin A2 was significantly elevated even at early stage HCC (P=0.0024 and P=0.0048, respectively) (69). Monitoring Annexin A2 expression levels in combination with alfa fetoprotein may contribute to a higher sensitivity and specificity in the clinical practice of diagnosing HCC (69). Ubiquitin associated protein 2 (UBAP2) has been shown to make a complex with Annexin A2, marking it for degradation. This makes UBAP2 a potential therapeutic focus for patients with HCC to counter the adverse effects of Annexin A2 (63).

*Pancreatic duct adenocarcinoma (PDA).* Annexin A2 is localized in the cytoplasm of normal pancreatic epithelial cells. In late stage pancreatic intraepithelial neoplasia (PanIN) and invasive PDA, Annexin A2 is relocated to the outer luminal surface (79). The expression of Annexin A2 gives PDA tumour cells the ability to grow into the liver (79). Annexin A2 has been shown to co-localize with S100A6 on the cell membrane of PDA tumours. Annexin A2 combined with S100A6 contributes to PDA cell motility (81). Annexin A2 is also significantly associated with histopathological grading (P=0.029) (82). Annexin A2 promotes secretion of the class 3 Semaphorin Sema3D. It was demonstrated how Sema3D is enriched in metastatic tumours of PDA. Furthermore, Sema3D is expressed in primary PDA from patients with a poor prognosis and patients who died from widely metastatic disease (79). This indicates how Annexin A2, through Sema3D, promotes metastasis in human PDA and could predict a poor prognosis for PDA patients (79). Another mechanism by which Annexin A2 promotes invasion of PDA cells is mediated by Tyrosine 23 phosphorylation of Annexin A2 (80). Annexin A2 can interact with the p50 subunit of NF-κB independently of calcium. By interfering with NF-κB Annexin A2 helps to induce cellular viability of PDA (83). An *in vivo* study on mice showed how expression of Annexin A2 significantly correlated with a shortened survival rate (P=0.001) (80). Attributing to this, patients with high expression of Annexin A2 showed a significantly shortened PFS and OS compared to low expression patients (P=0.008 and P=0.033, respectively) (84). Treatment with anti-Annexin A2 antibodies prolonged the survival rate of mice compared with mice treated with isotype control IgG (P=0.02). Furthermore, microRNA-206 has shown the ability to reduce Annexin A2 plasmin production and thereby inhibiting PDA cell invasion (86).

Takano *et al* (84) examined the relevance of Annexin A2 in drug-resistant PDA and found high expression of Annexin A2 as an independent factor for recurrence in patients undergoing gemcitabine adjuvant chemotherapy. Knocking down Annexin A2 by shRNA significantly increased the cytotoxic effect of gemcitabine by the following downregulation of the NF-κB pathway (83).

These studies of anti-Annexin A2 antibodies, microRNA-206 and shRNA targeting Annexin A2 altogether indicate Annexin A2 to be of therapeutic relevance in patients with PDA (85).

*Neurological cancers*

*Glioblastoma.* The majority of human glioblastoma cases expressed Annexin A2 (59,60). Twenty-five out of 30 cases

Table II. Summary of the clinical manifestations of overexpression of Annexin A2 and the organ related cancer site.

| Clinical manifestation | Organ related cancer site |
|---|---|
| Resistance to treatment | Acute lymphoblastic leukaemia, breast, cervical, pancreatic duct adenocarcinoma, prostate cancer |
| Binding to the bone marrow | Acute lymphoblastic leukaemia, multiple myeloma, prostate cancer |
| pT-stage | Clear cell renal cell carcinoma, colorectal, esophageal squamous cell carcinoma (downregulation), endometrial, gastric, hepatocellular carcinoma, non-small cell lung, ovarian, oral squamous cell carcinoma, urothelial cancer |
| pN-stage | Clear cell renal cell carcinoma, colorectal, esophageal squamous cell carcinoma (downregulation), endometrial, gastric, non-small cell lung cancer, ovarian, pancreatic duct adenocarcinoma, prostate (downregulation), urothelial cancer |
| pM-stage | Clear cell renal cell carcinoma, breast, colorectal, endometrial, gastric, hepatocellular carcinoma, ovarian, pancreatic duct adenocarcinoma, prostate (downregulation), urothelial cancer |
| Histological grade and type | Clear cell renal cell carcinoma, cervical, endometrial, gastric, non-small cell lung, ovarian, oral squamous cell carcinoma (downregulation), pancreatic duct adenocarcinoma, prostate cancer |
| Shortened survival | Clear cell renal cell carcinoma, endometrial, gastric, glioblastoma, hepatocellular carcinoma, non-small cell lung, ovarian, pancreatic duct adenocarcinoma, prostate cancer (downregulation) |

were Annexin A2 positive, with 11/30 cases strongly positive, 14/30 cases weakly positive and the remaining 5 cases negative (60). Annexin A2 expression is not detectable in normal glia cells (96). Furthermore, Annexin A2 is overexpressed in the cases with a higher grade tumour, pathological grade II-IV, compared to low grade tumours without infiltration (61). The 3-year OS rate of the Annexin A2 positive group was significantly lower than survival of the Annexin A2 negative group, 31.5 and 51.8%, respectively (59). Annexin A2 expression was shown to correlate with vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF), $P<0.05$. Annexin A2's effect on glioblastoma cells through VEGF and PDGF may represent an important anti-angiogenesis therapeutic target in the treatment of glioma (60). A significant lower cell proliferation was shown after knockdown of Annexin A2 by using a wound closure rate (59). Knockdown of Annexin A2 reduced the invasiveness of glioma cells (59).

*Lung cancer*

*Non-small cell lung cancer (NSCLC).* Annexin A2 is shown in cytoplasm and cell membranes in lung adenocarcinoma cells lines (70). Annexin A2 expression is significantly correlated with tumour diameter ($P=0.003$), pathological grade ($P=0.014$), pT status ($P<0.001$) and pleural invasion (71). High expression of Annexin A2 was associated with lymph node metastasis in comparison with tumours with low Annexin A2 expression ($P<0.001$) (72). Annexin A2 overexpression correlates to advanced clinical stage ($P<0.001$) and patients with high Annexin A2 expression have shorter survival compared to patients with low expression ($P<0.001$) (71,72).

Short hairpin plasmid mediated RNA (shRNA) interference is a way of post-translation gene silencing (70). A plasmid expressing a shRNA targeting Annexin A2 could effectively inhibit the expression of Annexin A2. This implies that the shRNA plasmid against Annexin A2 could be of therapeutic interest to decrease the proliferation and invasion capability of NSCLC cells (70).

Another study using immunoprecipitation and flow cytometry showed Annexin A2 expression and phosphorylation in cell lines with resistance properties to doxorubicin, vinca alkaloids and epipodophyllotoxins indicating a relation of Annexin A2 to prediction of multi-drug resistance (73).

## 4. Discussion

The mechanism for Annexin A2 influence in different tumour types varies and thus the general role of Annexin A2 in malignant tumours remains unclear. Table II summarizes the clinical manifestations correlated to overexpression of Annexin A2 and the cancers in which they are represent. In general it seems that upregulation is the predominant phenomenon which can be correlated to an adverse clinical outcome for the patients.

Annexin collaborate with different proteins such as plasminogen, S100A10 and HE4. It might be the complex interaction between these agents and Annexin A2 that play a part in its malignant potential. Activation by phosphorylation seems to play a role in carcinogenesis and to some extent Annexin A2 seems to be regulated by Annexin A5. This accentuates the need to investigate the expression patterns of different Annexins within the different cancer forms. The ongoing regulation and collaboration of Annexin A2 might be the foundation for its malignant potential and might be the focus for further investigation on targeting treatment toward Annexins and plasminogen, S100 proteins and HE4.

Although it is hypothesised how Annexin A2 mediated activation of proteases leads to tumour cell proliferation and invasion, the fact that Annexin A2 is downregulated in some malignant tumours, poses some inconsistencies in the theory of Annexin A2 relation in carcinogenesis.

By making this overview on Annexin A2 expression in different cancers there were some contradicting studies on the topic (90-92,97). For OSCC and prostate cancer, a down-regulation of Annexin A2 was demonstrated. Differences between demographic data (age, sex and time of data collection) could influence the outcome of the results in both studies investigating OSCC and prostate cancer. The influence is more noticeable when the number of patients is low, and larger studies to confirm results are needed. In the OSCC study by Rodrigo Tapia *et al* (90) the non-malignant tissue from patients undergoing non-cancerous surgery was investigated. In another study by Zhong *et al* (97) the non-malignant tissue is not taken from healthy controls but taken from the cancer patients as biopsies of epithelial tissue 2 cm from the cancer. This difference could be debated because it is not well known which kind of non-malignant tissue is best standard for comparison.

Yee *et al* (91) suggests that Annexin A2 expression distinguish benign illnesses of the prostate gland, like basal cell hyperplasia and prostate atrophy, from high grade intraepithelial neoplasia and prostate cancer. Conflicting data on the Annexin A2 expression in tumours with Gleason score 8-10 has been published. Ding *et al* (92) did not confirm upregulation of Annexin A2 in Gleason score 8-10 tumours. The contradictive data could be caused by a different scoring of expression levels in combination with the slightly different laboratory protocol. Although both studies used the same Annexin A2 mouse antibody different kit lot number may result in staining differences.

The dichotomic expression of Annexin A2 in different cancers emphasizes the need to characterize the individual protein expression profiles according to the different cancers. That said, it might not be of interest to make a comparison of Annexin A2 expression among different cancers but more relevant to have a broader knowledge of Annexin A2 profiling within the different cancer subtypes.

All the studies enrolled in the present review were using IHC which gives them some limitations. The staining level, antibody concentration and cut-off values could be different among studies. When comparing the staining level of Annexin A2 the individual pathologist makes the classification of the tumours. No international scoring system for Annexin A2 exists. In most of the studies in this review, two independent pathologists made the scoring resulting in a consensus score. If Annexin A2 should be used in clinical routine setting then the quality of staining should be monitored by attending an assurance program.

This review has some limitations. Even though we sought to run through the literature of Annexin A2 and cancer, not all human tumour types are included. Secondly, within the cancer forms it is not discussed on behalf of the individual histological cancers subtypes. All histological subtypes are included and might therefore give a less specific correlation. Thirdly, publications bias cannot be completely ruled out because of the tendency to include papers with positive results compared to negative results. This bias is elaborated by discussing the papers giving a negative result.

Looking at the possibility of implementing IHC staining for Annexin A2 in the common practice at the hospital, the actual use of IHC and the possibility of elaborating the IHC panel are evaluated. IHC is a supplement to the histopathological description. Today most tumours are already undergoing IHC staining with other antibodies. Having to stain for Annexin A2 would only be an extension to the IHC panel that are used today.

Overall there is a limited amount of research on the topics concerning the Annexin A2 expression and recurrence rates of different cancers or the possible correlation between upregulated Annexin A2 and primary chemoresistance. Also there is a need for more profound research on the pathways and mechanisms of Annexin A2 in tumour genesis. This should be of interest because of the potential of Annexin A2 as a therapeutic agent in the treatment of different cancers. There are only a limited number of studies concerning the use of anti-Annexin A2 antibodies on human cancers tissue but the results on mice are promising (98). The ability of siRNAs to interfere with Annexin A2 expression might be of interest in the treatment of malignant diseases because of its potential to diminish treatment resistance and hyperfibrinolytic complications (39,44). There is a growing need to individualize cancer treatment and it could be of interest to have more knowledge on the use on anti-Annexin A2 antibodies and siRNAs on human cancer cells.

## 6. Conclusion

Overall, the present study reviews the ability of Annexin A2 expression in various cancer cells to predict adverse outcome. Expression of Annexin A2 is correlated to advanced stages and metastatic disease. Annexin A2 is demonstrated to predict reduced OS, shortened PFS and resistance to present treatment regimens. Although an overexpression of Annexin A2 is correlated to adverse outcome of the patient, a lowered expression of Annexin A2 is also correlated to poor prognosis in a few cancer types.

The present review sought to make an outline on Annexin A2s influence on cancer and make a basis for further investigation hereby contributing to knowledge of Annexin A2 resulting in consensus of a possible clinical use of this biomarker.

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