Annexin-A1 - a blessing or a curse in cancer?

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Abstract

Annexin-A1 (ANXA1), a potent endogenous immunomodulatory protein has been implicated in multiple functions essential in cancer, including cell proliferation, apoptosis, chemosensitivity, metastasis, and invasion. ANXA1 expression is varied depending on tumor type, and there are contradicting reports on its role in the regulation of proliferation and tumor growth. Here, we summarize the differing reports on cell proliferation and metastasis and attempt to discuss the reasons behind these different effects. ANXA1 plays a role as a homeostatic protein which regulates essential transcription factors and microRNAs. A more coherent understanding of ANXA1 in cancer could present a more biologically meaningful and clinically relevant strategy.

Annexin A1 structure and function

Annexin A1 (ANXA1) is the first member of the Annexin superfamily to be discovered, and like the other members in the superfamily, it is a calcium-dependent phospholipid binding protein. This family of proteins is termed annexins as they annex to the phospholipid membrane of the cell [1]. ANXA1 consists of two distinct domains – N terminal and C terminal domain. The C-terminal domain is conserved across different annexins in the superfamily whereas the N-terminal is varied. The difference in the biological functions between the members of the superfamily is attributed to the difference in N-terminal domains as it is the site where most post-translational modifications occur [2]. In the absence of calcium, the N-terminus is hidden within the core C terminus, while the presence of calcium results in a conformational change to expose the N-terminus[3]. ANXA1 was discovered to mediate the anti-inflammatory effect of glucocorticoids where it inhibits the action of phospholipase A2, limiting the supply of arachidonic acids needed for the synthesis of prostaglandins, thus suppressing inflammation. Although initially discovered in the late 1970s for its role in inflammation, ANXA1 has also been found to play a role in tumorigenesis, with multiple functions in proliferation, differentiation, apoptosis, migration, and invasion (Figure 1) (reviewed in [4]).

Annexin A1 expression and use as a clinical biomarker in cancer

The process of tumorigenesis is complex and multi-factorial, where cells transform by acquiring several biological capabilities known as the hallmarks of cancer. This includes uncontrolled proliferation, ability to evade cell death, induction of angiogenesis, and metastasis and invasion [5, 6].

ANXA1 was initially thought to be useful as a diagnostic or prognostic biomarker due to its differential expression between normal and tumor tissue samples. However, the expression of ANXA1 can be upregulated or downregulated in different cancers, so its role may not be that simple, and may be cancer specific (see Clinician's Corner). In hairy cell leukemia, for example, ANXA1 is used as a clinical biomarker for diagnostic purposes using immunohistochemistry, distinguishing ANXA1+ hairy cell leukemia from splenic lymphoma with villous lymphocytes and variant hairy cell leukemia [7]. Similarly, ANXA1 was reported

to be a new immunohistological marker for cholangiocarcinioma, distinguishing this type of cancer from pancreatic ductal carcinoma [8, 9]. Through immunohistochemical staining of paraffin-embedded primary tumors, it was shown that ANXA1 is up-regulated in melanoma [10], hepatocellular carcinoma [11], pancreatic cancer [12], gastric cancer [13-15] and lung adenocarcinoma [16], which correlated with poor prognosis [11, 14, 16], reduced disease-free survival [14, 16, 17] and metastasis-free survival [11, 17] (Figure 2). Similar immunohistochemical studies report that ANXA1 is downregulated in head and neck squamous cell carcinoma [18]; nasopharyngeal carcinoma [19], esophageal carcinoma [20] and prostate carcinoma [20, 21], correlating with differentiation grade [19, 22] (Figure 2). The organs with reported ANXA1 loss in cancer focus around the head and neck region as well as the prostate, while most cancers of the gut and lungs overexpress ANXA1.

The expression of ANXA1 in breast cancer, however, proves to be more complicated with contradicting reports. One study reported no difference in ANXA1 expression levels between breast cancer and benign tissues, whereas others have reported a loss of ANXA1 expression in breast cancer tissues through immunohistochemistry [23, 24] and gene microarray analysis [25], which led to poorer overall survival [26]. However, when hormone receptor status was analyzed, it was found that ANXA1-negative tumors were mostly estrogen receptor positive (ER+) (which means that the tumor expresses the receptor for estrogen. This suggests that the tumor may receive signals from estrogen which could promote the growth and spread of the tumor, progesterone receptor (PR)-positive and HER2/neu negative, while ANXA1-expressing tumors were negative for estrogen, progesterone, and HER2/neu [24], and this was related to an unfavorable prognosis in these patients [27]. Taking into consideration receptor positivity, basal-like breast cancer and **triple negative breast cancers (TNBC)** (see Glossary) have been consistently reported to express higher levels of ANXA1 compared to other breast cancer subtypes using immunohistochemical analyses of patient samples [27-30] while ER+ breast cancer, which forms a majority of all breast cancer cases (84% for both Luminal A (which includes tumors that are ER+ and progesterone receptror (PR)+, but negative for HER2. Luminal A breast cancers are likely to benefit from hormone therapy and may also benefit from chemotherapy) and Luminal B (which includes tumors that are ER+, PR- and HER2+. Luminal B breast cancers are likely to benefit from chemotherapy and may benefit from hormone therapy and treatment targeted to HER2+ breast cancers) express very low levels of ANXA1

[23, 24]. Thus, the pattern of ANXA1 expression in ER+ breast cancer cases may overpower the pattern of ANXA1 expression in TNBC cases (which only represent 12% of all breast cancer cases). In basal-like breast cancer and triple negative breast cancers, high expression of ANXA1 predicts for poorer overall survival, [27, 31]. By using a multivariable Cox model analysis, patients with ANXA1 positive tumors had worse breast cancer-specific survival (BCSS) than ANXA1 negative patients and ANXA1 was shown to be a significant independent predictor of survival in HER2+ patients and triple negative breast cancer patients (Clinician's Corner).

Summarizing the literature concerning ANXA1 expression and cancer is confusing, and the role of ANXA1 in cancer growth remains unclear. However, if one starts with the question: what is the effect of loss of ANXA1 in a normal (non-transformed) cell, the role of ANXA1 in transformation or tumorigenesis may become clearer. Quantitative proteomics and bioinformatic analysis of mammary gland epithelial cells from ANXA1-heterozygous (ANXA1(+/-)) and ANXA1-null (ANXA1(-/-)) mice revealed that ANXA1 plays a protective role in DNA damage, with an accumulation of DNA damage with slower recovery after heat-induced stress using COMET assay, showing a two-fold increase in mean tail moment in ANXA1-/- cells vs ANXA1+/- cells [32]. Yes-associated protein-1 (YAP-1) was the most downregulated protein in the oxidative stress pathway in ANXA1-/- cells, which when overexpressed with pdCNA-YAP1, reversed the lack of DNA damage response in ANXA1-/-, suggesting a regulatory role of YAP1. Further, quantitative phosphoproteomics and kinase analysis in ANXA1+/- and ANXA1-/- cells indicate that IκB kinase (IKK) is deactivated in ANXA1-deficient mammary cells, while other kinases such as P21-activated kinase (PAK1), Rho Associated Coiled-Coil Containing Protein Kinase 1 (ROCK1) and myotonic dystrophy kinase-related CDC42-binding kinase (MRCK) are activated in ANXA1-deficient mammary cells [33]. These results indicate that ANXA1-loss-dependent signaling may be involved in the initiation of cancer.

The studies above have focused on ANXA1 expression in tumors. However, ANXA1 can also be secreted and can be found in the serum of patients with lung cancer as compared to control patients [34]. The cognate receptors of ANXA1 are known as the formyl peptide receptors (FPRs), which are G-protein coupled receptors and ANXA1 associates with all three members of the FPR receptor family (FPR1,2,3), while is has the most affinity to FPR2 [35]. Apart from ANXA1, FPR2 binds to pro-resolving mediators such as lipoxin A4 and resolvins,

transducing their anti-inflammatory and pro-resolving functions. FPR2 can be present as a monomer or a homo-/hetero-dimer (with FPR1 or FPR3), and ANXA1 binding to these different FPR2 complexes can stimulate multiple signaling pathways, partly explaining how ANXA1 transduces different signaling pathways. Interestingly, FPR2 can also bind and be activated by pro-inflammatory mediators such as serum amyloid A, complicating matters further as the receptor for ANXA1 can play both anti-inflammatory and pro-inflammatory roles, depending on ligand and condition [36].

Annexin A1 in proliferation

Uncontrolled cellular proliferation is a hallmark of cancer that is crucial in the transformation of a healthy cell to become cancerous. The first reports on ANXA1 in cell growth and proliferation described an anti-proliferative function in A549 lung cancer cells [37] and RAW.264 macrophages, linked to the regulation of the MAPK/ERK pathway, with the disruption of the actin cytoskeleton and inhibition of cyclin D1 [38]. Interestingly, ANXA1 has also been reported to be phosphorylated by protein kinases and major growth factor receptors kinases by peptide mapping and immunological analyses [39-41]. ANXA1 is phosphorylated near its amino terminus by epidermal growth factor (EGF) receptor tyrosine kinase in A431 squamous carcinoma cells [40] and by hepatocyte-derived growth factor receptor kinase (HGFR) in A549 lung carcinoma cells [41]. The different kinases may phosphorylate different residues on ANXA1, such as Ser5 (Chak1), Ser27 (PKC) or Tyr21 (EGF-R, or Thr41 (PKC), Thr24 (PKC), Thr216 (PKA) to exert different effects on proliferation and other functions (Reviewed in [39]). Similarly, ANXA1 can be **SUMOylated** at K257 in the C-terminal calcium-binding repeat which can be enhanced with EGF stimulation, analyzed through the overexpression of SUMO-1 in LNCaP prostate cells [42]. These post-translational modifications may explain why the effects of ANXA1 on cellular proliferation appear to be divided and possibly cancer-specific, with some reports describing that ANXA1 increases proliferation (e.g. in Eca109 esophageal squamous carcinoma cells [43] and MIA-PaCa2 and BxPC3 pancreatic cancer cells [44] and MCF7 breast cancer cells [45]), while others report ANXA1 decreases proliferation and induces growth arrest (TCA-8113 and SCC-9 oral squamous cell carcinoma cells [46] and MIA-PaCa2 pancreatic cancer cells [47]. The most

interesting study where ANXA1 is shown to have both proliferative and anti-proliferative functions is described in MCF-7 breast cancer cells treated with estrogen [48]. Physiological levels of estrogen (1 nmol/L) induced proliferation, while high pregnancy levels of estrogen (100 nmol/L) induced growth arrest, and silencing ANXA1 reversed both proliferation and growth arrest. This may explain the disparate reports on ANXA1 either enhancing or suppressing cell growth, and ANXA1 may be a homeostatic protein which acts as a balance to regulate fast or slow cell growth.

A possible mechanism which has been proposed for the regulation of proliferation by ANXA1 is the regulation of microRNAs [49], specifically the downregulation of miR196a [50]. Interestingly, ANXA1 is a target of miR196a [51], which points towards a negative feedback loop where ANXA1 downregulates miR196a and in turn, miR196a inhibits ANXA1 expression [50, 52]. MiR196a is an oncogenic microRNA with functions in growth and development[53], a putative diagnostic biomarker for many cancers [54]including laryngeal [55], colorectal [56, 57] and glioma cancer [58], and promotes proliferation [50, 59, 60], targeting ANXA1 and other proteins involved in growth such as HOX proteins, which are a subset of homeodomain-containing transcription factors, proteins that are capable of binding to specific nucleotide sequences on the DNA where they either activate or repress genes [61]. Of course, ANXA1 may regulate other oncogenic or tumor suppressive microRNAs to exert its regulatory function.

Exogenous ANXA1 and its N-terminal peptides have also been examined to have functions in modulating cell proliferation through the FPR2 receptor, similarly disparate. ANXA1 N-terminal peptide Acetyl-2-26 (Ac2-26) stimulated proliferation by 1.5-fold in MCF-7 and MDA-MB231 breast cancer cells, which was blocked with a FPR2 antagonist [45]. In contrast, treatment of Ac-26 on Hep2 laryngeal squamous cell carcinoma slowed cell proliferation by 2-fold, which was partially rescued by a FPR2 antagonist BOC [62].

The *in vitro* functions of ANXA1 in proliferation described above have been examined *in vivo* using several mouse models of cancer. Once again, disparate results have been reported, yet using these different models, one can intricately dissect the importance of ANXA1 originating from the tumor or the stromal cells in the tumor microcirculation (Figure 3). In a DMBA (7,12-Dimethylbenz[a]anthracene) carcinogen-induced model of cancer (1st model), ANXA1 deficient mice exhibited significantly faster tumor growth, with a larger breast tumor size

after 24 days of first palpation compared with wild-type mice [48]. Similarly, spontaneous tumors in ANXA1-deficient mice crossed with MMTV-PyMT mammary tumor mice grew significantly faster than their heterozygous littermates (2nd model) [63]. However, **orthotopic transplantation (OS)** of 4T1 mouse mammary cancer cells [64] or Lewis lung carcinoma (LLC) cells and B16 melanoma cells [65] resulted in significantly slower growth in ANXA1-deficient mice as compared to normal wildtype mice (3rd model). At 5 weeks post-injection of 4T1, B16 and LLC cells, tumors were 3-4x larger in WT mice compared to ANXA1-/- mice [64, 65]. Finally, after orthotopic transplantation of control or ANXA1-silenced 4T1 mouse mammary cancer cells in syngeneic BALB/c mice [30], or after orthotopic xenograft transplantation of parental, scrambled or ANXA1 CRISPR/Cas9 KO MIA PaCa-2 pancreatic cancer cells into SCID mice [47] no change in tumor growth was observed (4th model). The difference in the mouse models used may explain the differences observed, where the first two models are spontaneous cancer models in a complete ANXA1 deficient mouse, whereas the 3rd and 4th models are orthotopic transplantation models of cells expressing ANXA1 into ANXA1-deficient mice recipients. The last two models are orthotopic transplantation models of cells lacking ANXA1 into ANXA1 expressing mice. These results suggest that in general, ANXA1 inhibits tumor growth, as mice deficient in ANXA1 exhibit faster spontaneous tumor growth, and expression of ANXA1 in tumors but not the stromal cells reduces tumor growth, yet expression of ANXA1 in stromal cells but not tumor cells has no effect on tumor growth (Figure 3). Therefore, the expression of ANXA1 in tumor cells, but not stromal cells is important in the inhibition of tumor growth *in vivo.*

Annexin A1 and chemo sensitivity

ANXA1 has also been shown to affect the sensitivity of cancer cells to various chemotherapeutic drugs. Development of resistance to chemotherapeutic drugs is one of the major obstacles in treating patients with relapse. Indeed, clinically, high ANXA1 overexpression in rectal cancer patients was associated with advanced pre-treatment and post-treatment tumor and **nodal status (NodS)**, as well as poor therapeutic response and adverse outcomes in rectal cancer patients treated with neoadjuvant concurrent chemoradiation therapy [66]. Immunohistochemistry analysis of pre-treatment biopsies from 172 rectal cancer patients were correlated with clinicopathological features, therapeutic response and metastasis-free survival (MeFS). High expression of ANXA1 was associated with

advanced pre-treatment tumor status (T3, T4), advanced pre-treatment nodal status (N1, N2), advanced post-treatment tumor status (T3, T4), advanced post-treatment nodal status (N1, N2) and inferior tumor regression grading [66].

In recurrent ER+ breast cancer, ANXA1 expression is found to be positively associated with increased resistance to tamoxifen treatment and ANXA1-based patient stratification exhibited a significant association to time to progression (TTP) indicating that ANXA1 is an independent marker for tamoxifen therapy outcome [67]. Cisplatin-resistant A549 cells have a two-fold higher expression of ANXA1 as determined by PCR and western blotting, and silencing of ANXA1 with specific targeting compounds increases sensitivity to cisplatin treatment [26]. In addition, 5-fluorouracil-resistant SW480 colon cancer cells express 6.5-fold higher levels of ANXA1, as determined by PCR and overexpression of ANXA1 is shown to increase resistance while silencing ANXA1 renders these cells more sensitive to the chemotherapeutic drug, as examined with cell proliferation [68].

These results suggest that ANXA1 expression may play a critical role in chemotherapy resistance and provide a possible strategy to overcome resistance by modulating ANXA1 expression.

Annexin A1 in Metastasis

Metastasis is the ultimate fatal step in the progression of malignant carcinomas, primarily constitutes of processes involving intravasation, survival in circulation, extravasation into a distant organ, angiogenesis and proliferation [6]. While the proclivity of certain tumors for specific organs was noted more than a century ago [69], the identity and time of onset of the changes that endow tumor cells with these metastatic capabilities are largely unknown.

Beyond its roles as a key factor associated with tumorigenesis and clinical outcome, interest in the functional role of ANXA1 in metastasis arises from accumulating evidence of altered protein expression intracellularly in metastatic tumors. So far there has been no report on loss-of-function or dominant interfering mutations in ANXA1 with regards to cancer metastasis. The role of ANXA1 in metastasis has been discovered predominantly through

association studies, where intracellular ANXA1 levels were either increased or decreased to deduce a relationship associated with metastatic behaviors, such as migration, invasion, and **epithelial-mesenchymal transition (EMT).** Although ANXA1 expression has been correlated with the metastatic propensity of a variety of tumors, the reported correlations were seemingly contradictory. While positive associations of elevated intracellular ANXA1 levels with enhanced metastatic capacity has mostly been shown in triple negative basal-like breast cancer cell lines [28, 30, 63, 70], colorectal cancer [71] melanoma [10], gliobastoma [72], pancreatic cancer cell lines[47], and prostate cancer [73], a downregulation of ANXA1 has also been correlated with lymph node and distant metastasis of nasopharyngeal carcinoma cell lines [74, 75]as well as metastatic mouse and human mammary carcinoma cell lines [76].

The landmark studies that suggested ANXA1 is involved in metastasis have so far been investigated predominantly in breast cancer, with some studies in other malignancies. EMT is recognized as a hallmark of epithelial cancers and plays a pivotal role in tumor progression and metastasis. Depletion of ANXA1 in human MDA-MB-231 and mouse 4T1 basal-like breast cancer (BLBC) cell lines resulted in a morphological switch from a migratory, mesenchymal-like phenotype to a resting, epithelium-like phenotype and inhibition of spontaneous lung metastasis in mice [30]. Thus, ANXA1 could be a candidate positive regulator of the EMT-like phenotypic switch in metastasis. This reversal of the migratory phenotype was associated with an evident rearrangement of the actin cytoskeletal network (actin-rich ruffles were lost and long F-actin stress fibers were produced) [30]. Silencing of ANXA1 using either transient siRNA- or stable lentiviral shRNA-mediated knockdown, abrogated an EMT-like switch **via transforming growth factor β (TGF-β)** signaling, which subsequently induces Smad2 phosphorylation, Smad4 nuclear translocation, and stimulation of Smad3/Smad4 transcriptional activity, thereby allowing efficient cell migration and invasion through actin cytoskeletal reorganization [30]. Ectopic over-expression of ANXA1 in luminal-like breast cancer cell lines, MCF-7, T47D and BT474 cells that do not readily express ANXA1, facilitated a clear EMT-like phenotypic switch and enhanced TGF-β signaling by ~2fold via increase of Smad3/Smad4 transcriptional response [30]. High ANXA1 expression in TNBC has functional implications for strengthening migratory behavior. Silencing ANXA1 with shRNA in breast cancer cells suppressed NF-κB activity, which resulted in the direct inhibition of chemokine receptor CXCR4 expression [63]. ANXA1 regulates NF-κB activity directly by binding to

IKKγ/NEMO , determined using co-immunoprecipitation and stabilizes the NEMO–RIP1–IKK complex, which is vital for NF-κB phosphorylation and nuclear translocation [63]. Activation of NF-κB stimulates breast cancer cell invasion by regulating the expression of MMP9, chemokine receptor 4 (CXCR4) and urokinase plasminogen activator (uPA) expression [63]. Ectopic over-expression of intracellular ANXA1 promoted cell migration but inhibited immunofluorescence-analysed focal adhesion and stress fiber formation through activation of the extracellular signal-related kinase (ERK) and RhoA [77]. The process of tumor invasion is dependent not only on the motility of tumor cells but also on extracellular **matrix metalloproteases (MMPs)**, which belong to a family of zinc-dependent endopeptidases that function to degrade and remodel **extracellular matrix (ECM)**. Recent studies have shown that MMP2 and MMP9 are critical determinants of the invasive ability of tumor cells, and inhibition of MMP2/ MMP9-mediated invasion suppresses metastasis of tumor cells [78].

Although ANXA1 has been implicated in several steps involved in metastasis, the largely discordant *in vitro* findings relating to ANXA1 did not reveal a robust, causal connection with EMT and enrichment in metastatic phenotypes, which is contrary to conventional oncogenic (eg EGFR or HER2) or tumor suppressor genes (P53 or PTEN) in all types of cancer. The incoherence across studies, predominantly in breast cancer, may be explained by the inability of cell lines to capture the broad spectrum of inter- and intra-tumoural genotypic and phenotypic heterogeneity in tumor populations. The inconsistency in morphological appearance and the mostly varied EMT gene signature with stable loss of ANXA1 could also be explained by the existence other mechanisms coupled with ANXA1 that could take part in the induction of EMT. As mentioned above, ANXA1 is phosphorylated by multiple kinases, and these post-translational modifications can exert different functions depending on the context of the experiment and experimental set-up. Indeed, ANXA1 is reported to bind to and bundle actin filaments [79], colocalizing with F-actin in membrane ruffles during macrophage phagocytosis [80, 81]and migrating tips of metastatic breast cancer cells [30] and can influence actin polymerization through interaction with profilin [82]. While actin cytoskeletal remodeling may engender cell migration, and invasion, successful colonization of distant organs requires a more complex combination of metastatic effectors accompanied by the loss of epithelial markers, gain of mesenchymal markers, overall re-organization of cytoskeletal proteins and induction of signaling pathways that promote EMT.

In vivo studies investigating the roles of ANXA1 on mouse models of metastasis have mostly shown that ANXA1 enhances metastasis. Intracardiac injection of nu/nu athymic mice with MCF7 cells overexpressing ANXA1 metastasized more to the lungs [48]. Similarly, spontaneous metastasis in ANXA1-deficient MMTV-PyMT mammary cancer mice was lower compared to their heterozygous littermates [63]. ANXA1-deficient mice exhibited lower metastasis after orthotopic transplantation of 4T1 mouse mammary cancer cells [64] or Lewis lung carcinoma (LLC) cells and B16 melanoma cells [65]. Likewise, lung metastasis in orthotopic transplantation of 4T1 mouse mammary cancer cells silenced for ANXA1 using shRNA into syngeneic BALB/c mice was reduced compared to control mice [30]. Finally, liver metastasis was also lower in MIA-PaCa2 pancreatic cancer cells silenced for ANXA1 using crispr/Cas9 orthotopically transplanted into SCID mice compared to control or scramble cells [47]. All these models show that silencing ANXA1 reduces the risk of metastasis and suggests that ANXA1 expression in both the stroma and the tumor is essential in promoting and enhancing tumor metastasis (Figure 2).

Concluding Remarks and Future Perspectives

Owing to discrete and distinct variations of individual traits in different cancer cells, and the various post-translational modifications of ANXA1, the temporal and spatial dynamic actions of ANXA1 may lead to different outcomes in tumorigenesis and metastasis. In most cases, mouse models of cancer have shown that ANXA1 is important in suppressing tumor growth yet promotes tumor metastasis.

Despite increasing studies postulating the roles of ANXA1 in cancer, consensus thus far is that ANXA1 in cancer cells might only be a partial functional mediator of tumorigenesis and metastasis and certainly does not qualify as a tissue-specific mediator for predicting the occurrence of metastasis or cancer in general. Nevertheless, the functional requirement of ANXA1 in the tumor stroma and microenvironment for tumor progression and metastasis is preeminent, as highlighted by evidence from *in vivo* studies demonstrating impaired tumor growth [63, 75] and metastasis [63] in ANXA1-null mice tumor models. Therefore, therapeutic avenues focusing particularly on ANXA1 should not rely solely on intracellular ANXA1 in

cancer cells. A more coherent understanding of ANXA1 in the tumor microenvironment, and therapeutic exploitation of FPR ligands with careful consideration of timing and systematic approach for target selectivity could present a more biologically meaningful and clinically relevant strategy.

Is ANXA1 is a blessing or a curse in cancer? This can depend on the type of cancer and the cancer grade. In the transition from premalignant to malignant stages, the loss of ANXA1 may render cells more susceptible to DNA damage and oxidative stress. The expression of ANXA1 may add protection against transformation of healthy cells as it protects against DNA damage induced by stress and impaired oxidative damage [32]. The loss of ANXA1 in cancer leads to slower recovery from these stresses, resulting in the accumulation of DNA damage (Box 1). At later stages of cancer, high ANXA1 expression can promote migration ability and metastatic behavior (Box 2). Thus, the timing of use of ANXA1 as a clinical prognostic biomarker must be taken into consideration. Future research should be focused on the roles of ANXA1 in the tumor microenvironment, namely in tumor associated macrophages (recently shown to enhance pro-tumorigenic alternative macrophage polarization) [64], cancer-associated fibroblasts or tumor infiltrating lymphocytes, and if it plays any roles in the immunosuppressive mechanisms responsible for development of cancer and responses to immune checkpoint inhibitors. Whether ANXA1 acts as a homeostatic protein, which may function to resolve inflammation: enhancing proliferation in low-proliferating cells and reducing proliferation in highly proliferating cells, balancing the “Yin and Yang” in the tumor microenvironment is yet to be evaluated– to some cancers, it may be the Angel of Life, and yet to others, Death (Figure 3).

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Box1: Blessings of ANXA1

Discovered in the late 1970s as an anti-inflammatory mediator in the suppressive effect of glucocorticoids, Annexin-A1 (ANXA1)'s role as an immunomodulatory protein has since been cemented with studies consistently reporting its role in both innate and adaptive immunity (Figure 1). With the wide usage of glucocorticoids as anti-inflammatory and immunosuppressive drugs in the treatment of a widespread of diseases, i.e., autoimmune diseases, allergies and asthma, ANXA1 proves to be beneficial, as it plays a necessary and critical role in the clinical effectiveness of glucocorticoids. Importantly, ANXA1 serves as a blessing under normal physiological situations, in conferring an added protection against transformation and death of healthy cells as it protects against DNA damage induced by stress and impaired oxidative damage [32]. The absence of ANXA1 leads to slower recovery from these stresses, resulting in the accumulation of DNA damage. In the context of cancer, ANXA1 can also be beneficial in diagnosis and thus, leading to the ability to provide more suitable treatments, particularly in hairy cell leukemia [7]. The consistently high expression of ANXA1 in triple negative breast cancer and basal-like breast cancer compared to other breast cancer subtypes, not only serves as a potential diagnostic marker but also a prognostic marker for poorer survival [27]. With a highly predictive diagnostic and prognostic marker, clinicians may be better able to strategize a suitable treatment regime. ANXA1 plays a protective anti-tumorigenic role *in vivo* since the absence of ANXA1 promotes thespontaneous tumor growth. Deeper delving shows that the presence of ANXA1 in tumor cells may play a beneficial role in regulating and thus slowing down tumor growth *in vivo*. This may lead to the conclusion that the presence of ANXA1 in tumor cells itself is a blessing, leading to greater self-preservation.

Box 2: The curse of ANXA1

An arsenal of proteins which generally play homeostatic roles in normal cells are ironically involved in the process of tumorigenesis and metastasis of cancer. ANXA1 being one of them comes as no surprise, but what exactly causes this switch remains unclear. The initial discovery of ANXA1, which began as an anti-inflammatory protein in the late 1970's has led to extensive research that reaches into many aspects of cell function (Figure 1), and it is

often overexpressed or downregulated in many types of cancer (Figure 2). Further evaluation on ANXA1 in malignancies have revealed that this protein could impart diverse roles in cancer progression at different levels. ANXA1 might act as a tumor suppressor in the early stages of cancer, due to the possibility that the loss of ANXA1 could render a normal cell genetically unstable, increasing its chance to gain an insidious advantage of unlimited proliferative capacity [32]. The initial 'blessing' of having ANXA1 as a tumor suppressor in the early stages may prove to be a double-edged sword, as the protein confers a more invasive and metastatic phenotype in advanced stages of cancer. Since ANXA1 has been shown to have a nuclear, cytoplasmic and membrane-associated presence, the localization of ANXA1 in different compartments of the cell could be one of the critical determinants in assessing invasiveness and aggressiveness of cancer[36]. Membrane-associated ANXA1 has been documented to be proteolytically-cleaved by some proteases, hence becoming accessible to its cognate receptors, the formyl-peptide receptors to stimulate downstream oncogenic pathways [72]. Dysregulation of many fundamental mechanisms that modulate various cellular activities such as proliferation, differentiation, epithelial-mesenchymal transition, and invasion has all been linked to cancer progression. The post-translational modifications of different residues on ANXA1 may also be another explanation for the various functions of ANXA1 in cancer [39]. Indeed, the originally envisaged simplistic view of ANXA1 stymying tumorigenesis by limiting cellular proliferation and promoting apoptosis has now evolved into an increasingly complex biological process. While establishing homeostasis requires two opposing factors to regulate each other, the often contradictory effects of ANXA1 on tumorigenesis and metastasis may suggest that there are caveats that concern the validity of the reported results, or that ANXA1 is the balance which acts to maintain homeostasis.

Box3: Clinician's Corner

ANXA1 is used as a specific and selective marker for hairy cell leukemia [7], cholangiocarcinoma [8, 9] and is indicated as a potential serum biomarker for lung cancer [34].

Through immunohistochemistry, ANXA1 is reported to be up-regulated in melanoma (88%, 54/61 biopsies) [10]; hepatocellular carcinoma (55/90 (61%) of tumors larger than 5cm, 35% (30/85) in grade I/II tumors vs (80% (60/75) grade III/IV tumors) [11]; pancreatic cancer

(30/42 (71%) vs matched control pancreatic tissues 7/38 (18%))[12]; 76 /135 (56%) cases of gastric cancer [14] and 41/96 (43%) lung adenocarcinoma [16], 9/14 (64%) [27], basal like/triple negative breast cancer, which correlated with poor prognosis[11, 14, 16, 31], reduced disease free survival [14, 16, 17, 31]and metastasis-free survival [11, 17, 31].

Similar immunohistochemical studies report that ANXA1 is downregulated in 22/ 32 (68%) head and neck squamous cell carcinoma patients [18] ; nasopharyngeal carcinoma (20/20 (100%) [19]esophageal carcinoma (14/14 (100%)) and prostate carcinoma (5/5(100%)) [20], 30/30 (100%) [22], Gleason score 6 (7/40(17.5)) vs Gleason scores 7-8 (22/27(81.5%)) and Gleason scores 9-10 (9/10 (90%)) and high grade prostatic intraepithelial neoplasia (38/50(76%))[21], which correlated with differentiation grade [19, 22] and inversely correlated with tumor grade[21].

Therefore, the expression of ANXA1 in cancer could be tissue specific, with ANXA1 overexpressed in many cancers of the gut, while downregulated in most cancers of the head and neck. Figure 2 lists the expression of ANXA1 in all cancer types.

Figure 1. Key events in the history of ANXA1.

Figure 2. **Differential expression of ANXA1 in cancer.**
ANXA1 expression is reduced in some cancers (left, green) and increased in others (right, red). In some cancer types, reports are conflicting (red font)

Figure 3. ANXA1 expression in the tumor or stromal/immune cells in the microenvironment.

(A) Spontaneous mammary cancer mice deficient in ANXA1 (B) Xenograft of mouse tumor cells into ANXA1 deficient syngeneic mice. (C) Xenograft of mouse tumor cells silenced for

ANXA1 into wildtype syngeneic mice. (D) Xenograft of human cancer cells silenced for ANXA1 into immunodeficient mice.

Figure 4. The balancing art of ANXA1 in cancer.

Figure legend ANXA1 plays protective anti-tumorigenic roles on one hand, and on the other, can enhance metastasis and invasion in later stages of cancer.

Glossary

Epithelial-mesenchymal transition (EMT): The polarized epithelial cell can change to a mesenchymal cell phenotype via multiple biological processes. This transition causes a series of biochemical changes to the cells, enhancing the migratory capacity, invasiveness, elevated resistance to apoptosis and increased extracellular matrix components such as N-cadherin and fibronectin. Once the EMT process is complete, the underlying basement membrane which interacted with the epithelial cell before the transition will degrade, and the newly formed mesenchymal cell migrates away.

Extracellular matrix (ECM): In general, the extracellular matrix (ECM) is a non-cellular component of extracellular molecules secreted by support cells which provides structural and biochemical support to the surrounding cells. Due to the evolution of independent cell lineages, the composition of ECM varies between multicellular the structures. The ECM is composed of proteoglycans (PGs) and fibrous proteins.

Matrix metalloproteinases (MMPs): MMPs also known as matrixins and are calcium-dependent zinc-containing endopeptidases. MMPs are the main group of enzymes responsible for the collagen degradation as well as the degradation of the extracellular matrix (ECM).

Nodal status (NodS): Lymph node status shows whether or not the lymph nodes in the underarm area (axillary nodes) contain cancer: Lymph node-negative means the lymph nodes do not contain cancer, while Lymph node-positive means the lymph nodes contain cancer. Prognosis is poorer when cancer has spread to the lymph nodes (lymph node-positive). The number of positive nodes guides treatment and helps predict the chances for long-term survival.

SUMOylation: SUMOylation is a post-translational modification process which occurs via Small Ubiquitin-like Modifier (or SUMO) proteins. Like ubiquitination, SUMOylation is directed by an enzymatic cascade. SUMOylation involves a cascade of three enzymes: the E1-activating complex SAE1/SAE2, the E2-conjugating enzyme UBC9 and one of the several E3 ligases (such as PIAS superfamily or RANBP2). However, in contrast to ubiquitin, SUMO is not used to tag proteins for degradation but affect a protein's structure and subcellular localization.

Transforming growth factor-β (TGFβ) pathway: Transforming growth factor-β (TGF-β) is a multifunctional polypeptide which plays an essential role in tumor proliferation,

differentiation, angiogenesis, and other functions. TGF-β pathway includes the canonical SMAD pathway signaling and DAXX pathway which induces apoptosis, or programmed cell death, in human lymphocytes and hepatocytes. Like a double-edged sword, TGF-β acts as a tumor suppressor in the early stage of tumor progression in normal epithelial cells. But In advanced cancers, TGF-β enhances cell growth, invasion and metastasis.

Triple negative breast cancers (TNBC): TNBC refers to the type of breast cancer that does not express the genes for estrogen receptor (ER-), progesterone receptor (PR-) and HER2-. This type of breast cancer is not supported by the hormones estrogen, progesterone which makes it more difficult to treat. Generally, triple-negative cancers often require combination chemotherapy and target therapies.

Tumor microenvironment (TME): The TME is created by malignant and non-transformed cells such as stromal cells and immune cells. Here, the Intercellular communication is driven by cytokines, chemokines, growth factors, and inflammatory and matrix remodeling enzymes which can form a complex and dynamic network. Many types of immune cells are also involved such as, lymphocytes (B and T), NK and NKT cells, tumor-associated macrophages (TAMs), dendritic cells, Tumor-associated neutrophils and myeloid derived suppressor cells (MDSC). Hence, targeting the cells or mediators of their communication could provide other treatment options.

Orthotopic implantation (OS): OS is commonly used to generate more relevant murine models of cancer, where the tumor cells are injected in the native organ which they originated from.

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