

Review Article

The Annexin A2/S100A10 System in Health and Disease: Emerging Paradigms

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Since its discovery as a src kinase substrate more than three decades ago, appreciation for the physiologic functions of annexin A2 and its associated proteins has increased dramatically. With its binding partner S100A10 (p11), A2 forms a cell surface complex that regulates generation of the primary fibrinolytic protease, plasmin, and is dynamically regulated in settings of hemostasis and thrombosis. In addition, the complex is transcriptionally upregulated in hypoxia and promotes pathologic neoangiogenesis in the tissues such as the retina. Dysregulation of both A2 and p11 has been reported in examples of rodent and human cancer. Intracellularly, A2 plays a critical role in endosomal repair in postarthroplastic osteolysis, and intracellular p11 regulates serotonin receptor activity in psychiatric mood disorders. In human studies, the A2 system contributes to the coagulopathy of acute promyelocytic leukemia, and is a target of high-titer autoantibodies in patients with antiphospholipid syndrome, cerebral thrombosis, and possibly preeclampsia. Polymorphisms in the human *ANXA2* gene have been associated with stroke and avascular osteonecrosis of bone, two severe complications of sickle cell disease. Together, these new findings suggest that manipulation of the annexin A2/S100A10 system may offer promising new avenues for treatment of a spectrum of human disorders.

1. The Annexin A2/S100A10 System

1.1. Assembly of the (A2·p11)₂ Complex. Annexin A2 (A2) belongs to the annexin family of Ca²⁺-regulated phospholipid binding proteins, which are expressed in plants, animals, and protists throughout the phylogenetic tree [1]. A2 is a 36-kilodalton protein produced by endothelial cells, monocytes, macrophages, trophoblast cells, and some tumor cells and exists both free in the cytoplasm and in association with intracellular and plasma membrane surfaces [2, 3]. The human *ANXA2* gene consists of 13 exons distributed

over 40 kb of genomic DNA on chromosome 15 (15q21) [4]. Among mammalian species for which A2 has been sequenced, identity is approximately 98% at the amino acid level.

When A2 is membrane associated, the tightly packed, alpha-helical 33-kDa core domain forms a disk whose convex face is associated with membrane phospholipid and whose concave face is oriented away from the membrane. Membrane binding is mediated by at least two potential Ca²⁺-binding “annexin” repeats, features common to all annexin family proteins [3]. While the core domains of the annexin

proteins are relatively well conserved, the hydrophilic amino-terminal “tail” or “interaction” domains are highly variable and essentially unique to each family member.

Protein S100A10, also known as p11, is a well-described binding partner of A2 [7, 8]. As a member of the S100 family of proteins, p11 contains Ca^{2+} -binding helix-loop-helix motifs and confers increased phospholipid binding affinity on A2. Typical S100 proteins undergo a conformational change upon Ca^{2+} -binding that places helix III (HIII) in a perpendicular orientation relative to helix IV (HIV), thus forming a cleft that can accept associated target proteins [9]. This calcium activation rule, however, does not apply to p11, which has permanently assumed a “calcium-on” state, due to replacement of the bidentate E⁶⁵ by S⁷⁰, and the monodentate D⁵⁶ with C⁶¹ [10]. The published crystal structure of p11 in complex with the N-terminal 13 amino acids of A2 suggests that the basic unit of p11 structure is a noncovalently linked homodimer, each component of which can bind the A2 tail peptide to form a heterotetramer [10]. Upon binding, the A2 tail peptide assumes an α -helical conformation that presents key hydrophobic residues (V³, I⁶, L⁷, and L¹⁰) within a cleft formed by loop L2 and helix HIV of one monomer and helix HI of the other. The C-terminal region of p11, particularly its hydrophobic residues within the C-terminal extension (Y⁸⁵FVVHM⁹⁰), such as Y⁸⁵ and F⁸⁶, contributes critical contact points for binding to A2 [10].

1.2. The (A2·p11)₂ Complex and Fibrinolysis. The primary fibrinolytic protease, plasmin, is formed upon cleavage of plasminogen at a single peptide bond at position R⁵⁶⁰-V⁵⁶¹ by either of two serine proteases, tissue plasminogen activator (tPA), produced by vascular endothelial cells, or urokinase (uPA) [11–13]. tPA-dependent plasminogen activation is dramatically accelerated in the presence of fibrin, and to a lesser extent by cell surface fibrinolytic receptors. uPAR is expressed by monocyte/macrophages, tumor cells, and activated endothelial cells [14, 15], while the (A2·p11)₂ complex is expressed on both resting and activated endothelial cells [16, 17]. In addition, an interesting array of plasminogen-binding receptors, including α -enolase [18], TATA-box protein interacting protein (TIP49) [19], histone H2B [20], $\alpha_M\beta_2$ integrin [21], amphoterin [22], and Plg-R_{KT}, have been identified on many cell types [23].

On cell surfaces, the (A2·p11)₂ complex serves as an assembly site for plasminogen and tPA [16, 17, 25, 26]. Although it is clear that heterotetramer-mediated colocalization of activator and substrate accelerates plasmin generation, there are, interestingly, two main theories as to the exact site of interaction of plasminogen and tPA with components of the heterotetramer complex. While one group suggests p11 as the key ligand interaction site and annexin A2 as the molecule that anchors it to the plasma membrane [26], another proposes annexin A2, in complex with p11, as the ligand binding site [17]. A third group has suggested that, in the context of the cell surface and its proteolytic milieu, both annexin A2 and p11 may have

exposed lysine residues that are accessible to the lysine binding “kringle” domains of both tPA and plasminogen [27]. Detailed evidence for each view is outlined in the works cited above.

Translocation of A2 to the outer leaflet of the plasma membrane of the endothelial cell is a key regulatory step governing vascular fibrinolysis [16, 17]. Although cell surface appearance of A2 has been linked to plasma membrane fusion of multivesicular bodies in NIH 3T3 fibroblasts [28], and as a consequence of membrane disruption upon exocytosis of secretory granules in chromaffin cells [29], it is not clear whether similar mechanisms apply to the endothelial cell. Endothelial cell translocation, which can occur within minutes, is initiated by several factors including heat stress, thrombin stimulation, and hypoxia [30–32] and is known to require the presence of adequate p11 as well as src kinase phosphorylation at Y²³. A2 was originally identified as a src kinase substrate [33], and translocation is driven by activation of pp60src [31].

1.3. Regulation at the Cell Surface. Translocation of A2 to the cell surface is dependent upon the abundance of p11. In the endothelial cell, p11 is stabilized by A2, which, upon binding, masks a critical “degron,” or polyubiquitination site on p11. In the absence of sufficient A2, p11 is polyubiquitinated and targeted to the proteasome for degradation [34]. In *AnxA2*^{-/-} mice, which demonstrate low to nondetectable p11 expression, treatment with bortezomib, a proteasome inhibitor, restored p11 expression, verifying its regulation via a proteasome-linked pathway *in vivo*. In nonendothelial cells, p11 may be stabilized by one or more of its other partner proteins, which include a number of transmembrane channels and membrane receptors, such as the tetrodotoxin-resistant sodium channel Na_v1.8 [35], the two predominate K⁺ channel TASK-1 [36], the acid-sensing ion channel ASIC1a [37], the transient receptor potential channels TRP5 and TRP6 [38], and the 5HT-1B serotonin receptor [39].

Protein kinase C-(PKC-) mediated phosphorylation of S¹¹ or S²⁵ residues on A2 appears to represent an additional regulatory pathway. Serine phosphorylation within the tail domain of A2 dissociates the heterotetramer complex, preventing further translocation to the cell surface by allowing polyubiquitination of p11 and its degradation in the proteasome [40, 41]. This event appears to be initiated by plasmin, which, once generated, signals activation of conventional PKC and thus limits its own generation. This mechanism appears to require cleavage of A2 by plasmin as well as activation of toll-like receptor 4.

In this paper, we summarize evidence from both animal models and human studies on the *in vivo* functions of the annexin A2/S100A10 system. The concept of the “annexinopathy” was first proposed in 1999 [42], and expanded in several subsequent reviews [43–47]. Here, we focus exclusively on the growing body of evidence that annexin A2 and its partner protein p11 contribute to human health and disease.

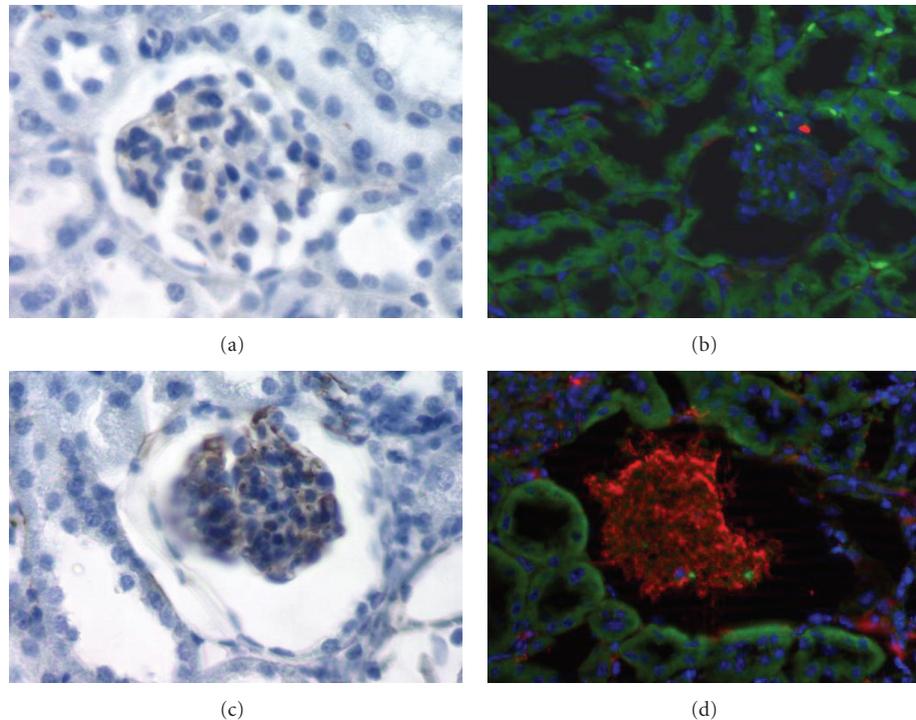


FIGURE 1: Fibrin accumulation in both *AnxA2*^{-/-} and hyperhomocysteinemic mice. Kidney sections from *AnxA2*^{+/+} mice on normal chow (a and b), from *AnxA2*^{-/-} mice on normal chow (c), or from *AnxA2*^{+/+} mice on a high methionine diet (d). Sections from heparin-treated, highly perfused animals were stained with a polyclonal rabbit antifibrin(ogen)-specific antibody using either immunoperoxidase plus diaminobenzidine (brown immunoreaction product) with hematoxylin counterstaining [5] (a and c), or biotinylated goat anti-rabbit IgG followed by Alexa 568-conjugated streptavidin (b and d); red staining represents Alexa 568, blue represents DAPI nuclear staining, and green is tissue autofluorescence [6].

2. In Vivo Animal Studies

2.1. Fibrin Balance and Models of Thrombosis. The *AnxA2*^{-/-} mouse has been highly informative in investigating the role of the annexin A2 system in vascular homeostasis *in vivo*. Although A2-deficient mice display normal development, fertility, and lifespan, fibrin accumulation is evident in both intravascular and extravascular locations within the lungs, spleen, small intestine, liver, and kidney (Figure 1) [5]. Microvascular endothelial cells isolated from *AnxA2*^{-/-} mice, moreover, lack the ability to support tPA-dependent plasmin generation *in vitro*, and arterial injury *in vivo* leads to an increased rate and severity of vascular occlusion in the *AnxA2*^{-/-} mouse. Recently, fibrinolysis was also assessed in p11-null mice, which also displayed increased vascular fibrin, reduced clearance of thrombi, and impaired neovascularization of Matrigel thrombi [48].

Interestingly, mice with diet-induced hyperhomocysteinemia share this phenotypic feature with the *AnxA2*^{-/-} mouse (Figure 1) [6]. Homocysteine (HC) is a thiol-containing amino acid that is generated during the conversion of methionine to cysteine [50]. Elevated levels of circulating HC have been associated with both thrombotic and atherosclerotic vascular disease [51], although therapies that lower plasma HC have not been shown to reduce the

risk of recurrent cardiovascular disease [52]. Pretreatment, but not cotreatment, of endothelial cells with HC blocks their ability to bind tPA and inhibits endothelial cell-related, tPA-dependent plasminogen activation [53]. Incubation of purified A2 with HC, moreover, interferes with its ability to bind tPA [54]. When wild type mice were subjected to diet-induced hyperhomocysteinemia, fibrin accumulated in multiple tissues (Figure 1(d)), and extracted A2 failed to support tPA binding or tPA-dependent plasmin generation, revealing that HC-induced blockade of the cell surface A2 pathway can occur *in vivo*.

The potential clinical utility of recombinant annexin A2 protein (rA2) in ischemic stroke has emerged from thrombosis models in rats. Animals were treated with low-dose tPA with or without rA2 at 2 or 4 hours following the initiation of focal embolic stroke [55]. Those receiving both agents had a significantly lower infarct size and greater cerebral blood flow compared to animals treated with low-dose tPA alone. In similar experiments, in which animals underwent middle cerebral artery embolization with autologous clot, pretreatment with rA2 not only improved blood flow but also reduced infarct size compared to saline-treated controls [56]. These findings are significant in view of reported neurotoxicity and cerebral hemorrhage associated with the use of tPA in the treatment of thrombotic stroke in

humans [57–59]. Thus, rA2 or related agents may constitute a useful adjunct to tPA alone for the restoration of cerebral blood flow [60].

A2 or its analogs might also prove efficacious in the treatment of peripheral arterial occlusion. When carotid artery thrombosis was induced by adventitial application of FeCl₃, administration of recombinant full length, but not truncated, A2 was associated with improved cerebral blood flow and reduced thrombus size in comparison with untreated control animals [61]. This treatment had no effect on bleeding time, prothrombin time, or activated partial thromboplastin time, indicating that global clotting parameters remained intact. Thus, A2 or its analogs may constitute a useful adjuvant to conventional thrombolytic treatment by reducing the effective dose of tPA, thereby limiting its potential toxicity.

2.2. A Model of Hypercholesterolemic Atherosclerosis. The fibrinolytic system appears to modulate the development of plaque-like vascular lesions in mouse models of atherosclerosis in a complex fashion. Mice deficient in both plasminogen and apolipoprotein E (ApoE), for example, display an enhanced tendency toward atherosclerosis compared to those lacking ApoE alone, suggesting that plasminogen protects against lesion formation [62]. On the other hand, when macrophages overexpressed uPA in *ApoE*^{-/-} mice, plaque development was accelerated through a plasminogen-dependent pathway [63]. When ApoE deficiency is combined with global deficiency of either uPA or tPA, however, the predilection for early fatty streaks and advanced plaque development was similar to that seen in mice with isolated ApoE deficiency [64]. These data suggest that the fibrinolytic system acts at multiple levels in the regulation atherogenesis.

In order to determine whether blocking plasmin(ogen) binding to A2 on the surface of macrophages is an effective strategy to reduce the development of atherosclerosis, *ApoE*^{-/-} mice were crossed with *AnxA2*^{-/-} mice to generate double nulls (Figure 2). Following weaning, *ApoE*^{-/-}*AnxA2*^{-/-} mice were placed on a Western chow diet (30% fat) and sacrificed at 12 or 24 wks. Aortas were removed and evaluated for lesion development by *en face* Oil Red O staining and morphometry of histologic sections taken through the aortic root at the base of the heart. There was no difference in *en face* lesion area or lesion size in *ApoE*^{-/-}*AnxA2*^{+/+} mice as compared to *ApoE*^{-/-}*AnxA2*^{-/-} mice. Therefore, we conclude that the redundant nature of plasmin(ogen)-binding sites on macrophages renders targeting a single binding site ineffective in modulating lesion development in this model system.

2.3. Angiogenesis and Oxygen-Induced Retinopathy. Although embryonic vasculogenesis appears to be normal, *AnxA2*^{-/-} mice display diminished neovascularization in several *in vivo* assays, including Matrigel implant, corneal pocket, and oxygen-induced retinopathy (OIR) models [5]. Mice with diet-induced hyperhomocysteinemia also display impaired

corneal neovascularization, which can be corrected upon intravenous injection of recombinant annexin A2 [6]. Microvascular endothelial cells from *AnxA2*^{-/-} mice, as well as HC-treated human endothelial cells, moreover, migrate less efficiently in growth factor-enriched Matrigel. Together, these data suggest that absence of A2, or its modification by HC, leads to impairment of angiogenesis-related endothelial cell function.

Interestingly, annexin A2 is upregulated in OIR (Figure 3). In this model, newborn mouse pups are transitioned to room air after 5 days in a 75% oxygen environment, whereupon relative hypoxia initiates a robust vascular proliferative response in the retina [66]. The return to 21% oxygen also triggers A2 synthesis out of proportion to the increase in vascular endothelial cell abundance [32]. The retinal neovascular response is inhibited by about 50% in the *AnxA2*^{-/-} mouse. A2 expression is also increased in the endothelial cell under true hypoxia through the direct action of hypoxia-inducible factor-1 (HIF-1) with the A2 promoter. Electrophoretic mobility shift experiments, chromatin immunoprecipitation studies, and luciferase promoter reporter assays all indicate binding of HIF-1 α and HIF-1 β to a hypoxia-responsive element within the promoter region of the human A2 gene, leading to its activation.

Although OIR-associated retinal neovascularization is impaired in the *AnxA2*^{-/-} mouse, it can be reestablished upon treatment of *AnxA2*^{-/-} mice with a subretinal injection of an A2-encoding adenovirus, which restores A2 expression [32]. In addition, neovascularization of the hyperoxia-treated *AnxA2*^{-/-} retina can be repaired upon treatment with the defibrinating agent anicrod, which depletes fibrinogen, thereby preventing fibrin formation. Together, these findings provide a link between fibrin accumulation and diminished neovascularization and imply that new therapeutic avenues for proliferative retinal vascular disorders, such as retinopathy of prematurity or diabetic retinopathy, could involve blockade of A2 in addition to inhibition of angiogenic growth factors.

2.4. Models of Cancer. In the central nervous system, glioblastomas, malignant tumors derived from glial cells, are usually highly aggressive and refractory to treatment due to the early development of widespread infiltrative loci [67]. Glioma-generated proteases, such as plasminogen activators and matrix metalloproteinases, contribute substantially to glioma cell invasion [67, 68]. High concentrations of annexin A2, similarly, are associated with the pseudopodia of invasive glioma cells [69], and knockdown of A2 reduces their migratory capacity *in vitro* [70]. In both mouse and rat *in vivo* models, stable knockdown of A2 expression in glioblastoma cells retarded overall tumor progression upon implantation of the cells into rodent brains; cellular invasion, proliferation, apoptosis, and angiogenesis were all inhibited [49]. Interestingly, when A2 expression was stably reduced by transfection of RNAi directed against A2 in rat GL261 glioma cells, tumor growth and invasiveness were reduced,

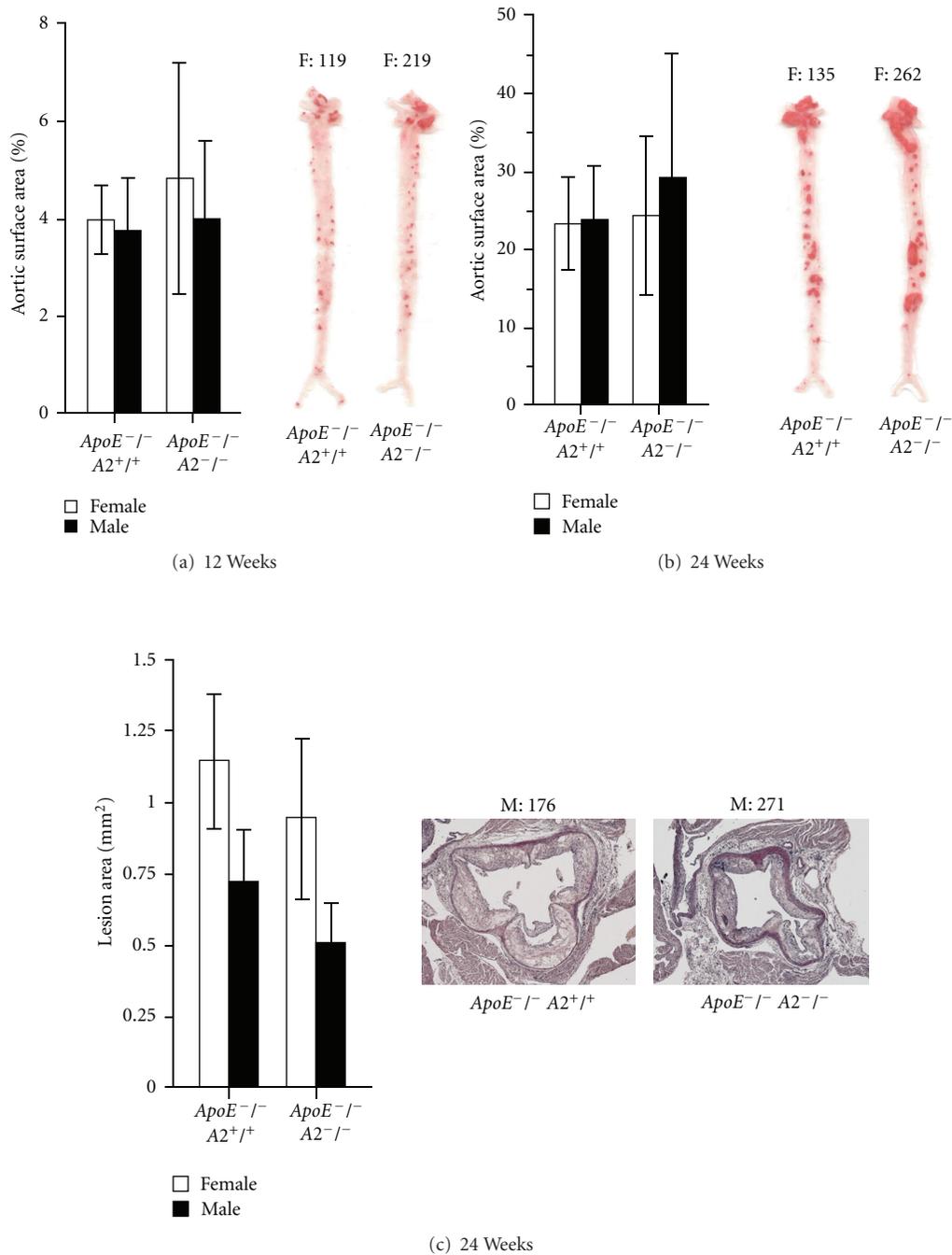


FIGURE 2: Diet-induced atherogenesis in $AnxA2^{+/+}$ and $AnxA2^{-/-}$ mice. $AnxA2^{-/-}$ mice on the C57Bl/6 background were backcrossed to $ApoE^{-/-}$ mice (Jackson Laboratories) to generate $AnxA2^{-/-} ApoE^{-/-}$ double knockout mice. At 3 weeks of age, both $AnxA2^{+/+} ApoE^{-/-}$ and $AnxA2^{-/-} ApoE^{-/-}$ mice male and female mice were placed on a western diet, composed of 1% (wt/wt) adjusted calories from fat and 0.15% (wt/wt) cholesterol (TD88137, Harland Tekland Laboratory) *ad libitum*, for 12 ($n = 14-20$ mice/group) or 24 weeks ($n = 17-23$ mice/group). At 12 or 24 weeks (a and b, resp.), the animals were perfused with PBS for 20 minutes, whereupon the entire aorta from the heart to 5–10 mm below the bifurcation of the iliac arteries was removed and fixed in 4% paraformaldehyde. The aorta was evaluated for lesion development by *en face* Oil red O staining, and morphometry of digital images of the stained aortas was performed using Adobe Photoshop 7.0 software [24]. For aortic root analyses, hearts removed at 24 weeks (c) were fixed in 3% PFA and paraffin embedded. Serial sections, 10 microns in thickness, obtained from the region of the proximal aorta to the level of the aortic leaflet, were stained with hematoxylin and eosin. Digital microscopic images were analyzed using image analysis software (NIH Image 1.63). Lesion size for each mouse was calculated as the average lesion size in 10–15 sections over a distance of 200 to 300 microns in the aortic root. Representative lesions are shown.

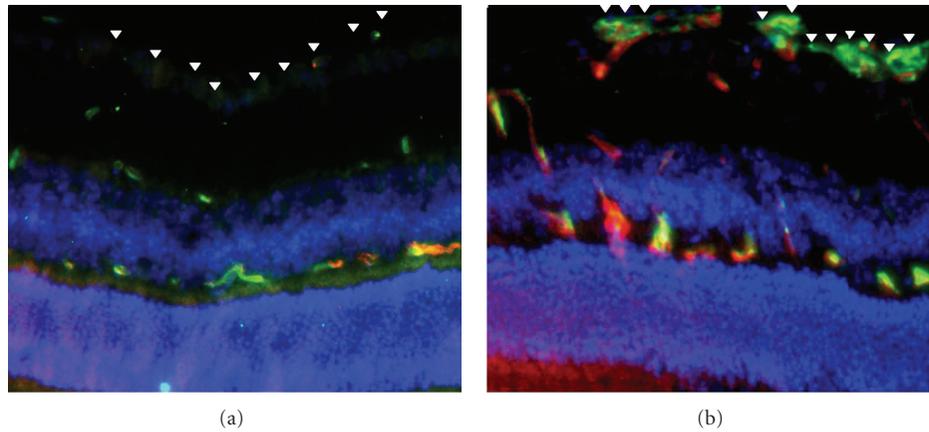


FIGURE 3: A2-expressing neovessels in oxygen-induced retinopathy in the mouse. Sections through retinas of wild type mice maintained in room air (a) or treated for five days with 75% oxygen to initiate oxygen-induced retinopathy (b) were stained with an endothelial cell-specific lectin (isolectin B4—green), 4',6-diamidino-2-phenylindole for nuclei (DAPI—blue), and anti-A2 (red) as described [32]. Note A2-positive neovascular tufts arrayed at the inner limiting membrane of the oxygen-treated retina (b, arrowheads) versus control (a, arrowheads).

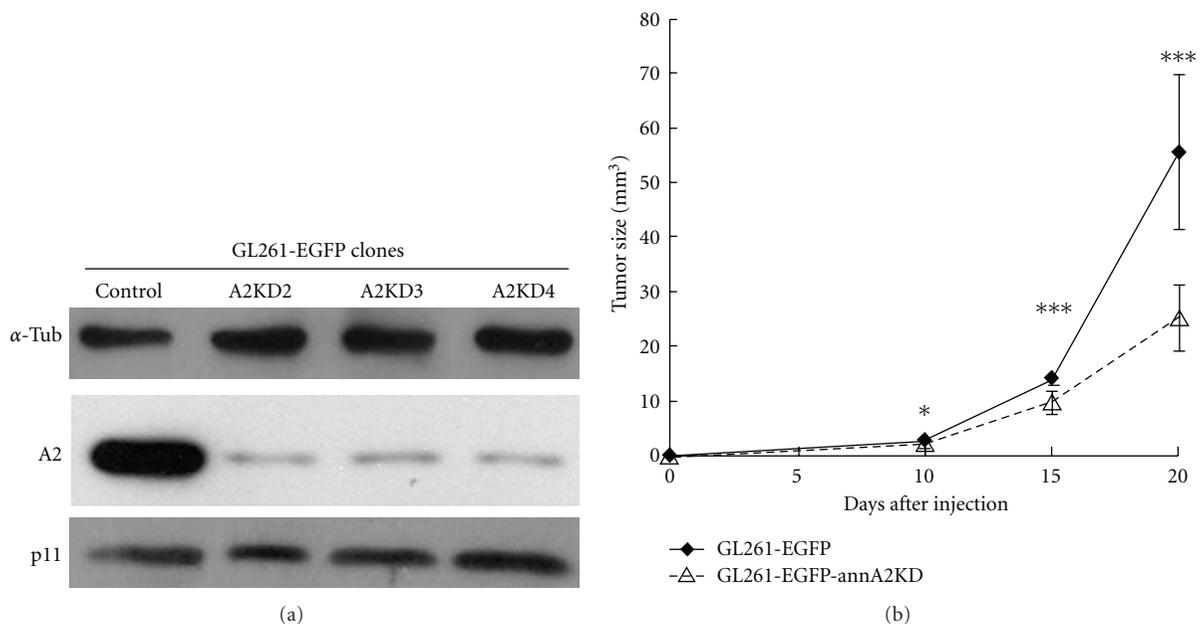


FIGURE 4: The glioblastoma-promoting effect of A2 is p11 independent. (a) When A2 expression in GL261 murine glioma cells was suppressed with siRNA, p11 expression remained intact. (b) Suppression of A2 expression significantly dampened the growth of intracranial gliomas derived from stereotactically implanted cells. Reproduced with permission [49].

even though p11 expression persisted; this result indicated that the contribution of A2 to tumor invasiveness was p11 independent (Figure 4). These data suggest that A2-directed treatment could offer a new therapeutic modality for human glioblastoma.

In a xenograft model in which highly invasive and metastatic breast cancer cells were implanted into nude mice, both tumor growth and vascular density were blocked by administration of anti-A2 monoclonal antibody [71]. The tumor cells employed in this experiment expressed abundant

A2, strongly supported tPA binding and tPA-dependent plasmin generation and exhibited plasmin- and A2-dependent cellular matrix invasion [72, 73]. These studies suggest that A2 may contribute to aggressive breast cancer cellular invasion and tumor angiogenesis through production of localized protease activity.

In a third *in vivo* model, growth of Lewis lung and T241 sarcoma tumors implanted into p11-deficient mice was markedly reduced compared to wild type controls [74]. Impaired tumor growth was correlated with diminished

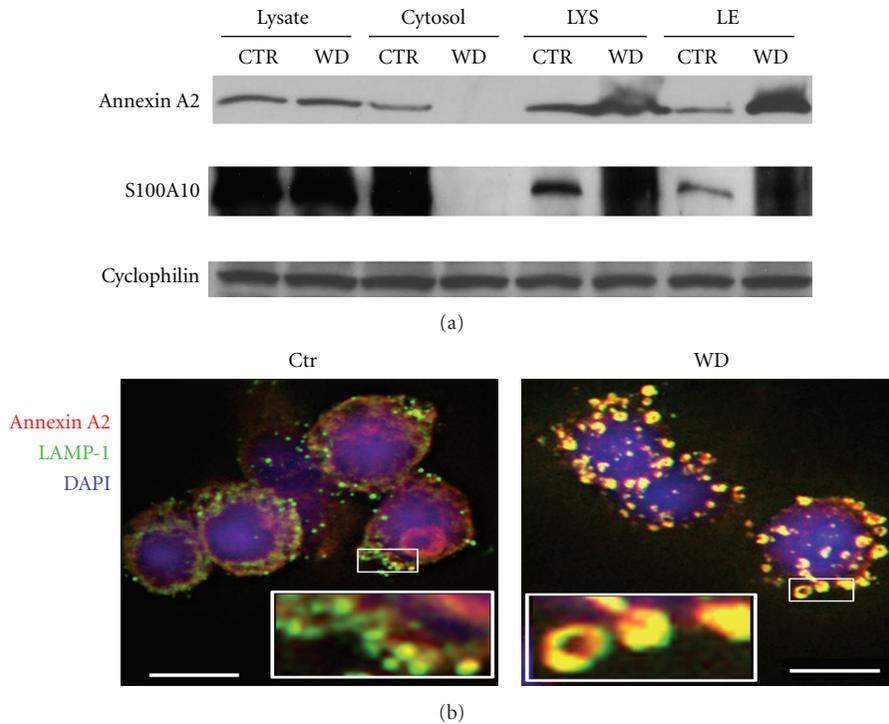


FIGURE 5: Intracellular A2 redistributes to lysosomal and late endosomal membranes in dendritic cells upon endocytosis of wear debris particles (WDP). (a) Western blot analysis of A2 and p11 localization in late endosomes (LE) and lysosomes (LYS) isolated from WDP-treated dendritic cells. (b) Confocal analysis of untreated and 12 h WDP-treated dendritic cells stained with anti-LAMP1 (green) and anti-A2 (red) revealed well-delineated LAMP⁺ endosomal compartments with cytosolic distribution of A2 in control cells. Cells cultured with WDP for 12 h (b) demonstrated an extensive increase in the number and size of the endosomal compartments with the subsequent loss of the cytosolic distribution of A2. Bar corresponds to 10 μ m. Reproduced with permission [65].

macrophage density within the tumors, and clodronate-mediated depletion of macrophages in wild type mice led to a similar reduction in tumor size. This study recapitulates the finding that thioglycollate-induced macrophage invasion into the peritoneum, and macrophage invasion of subcutaneous Matrigel plugs, were also impaired in the p11 knockout mouse [75]. A related study reveals that soluble (A2·p11)₂ tetramer activates human and murine monocyte-derived macrophages, that this activation requires toll-like receptor 4 (TLR-4), and that the tetramer modulates cytokine production in the macrophage [76]. Thus, tumor infiltration by macrophages may in part be due to (A2·p11)₂ tetramer signaling.

2.5. Aseptic Osteolysis. Arthroplastic surgery has revolutionized the treatment of arthritis and related rheumatologic disorders. Nevertheless, aseptic osteolysis, due to generation of wear debris particles (WDP), is an emerging problem that leads to failure of 10–30% of all joint replacements [77]. Recent investigations have thus focused on the mechanism by which WDP induce joint inflammation. Alkane polymers, 8–12 carbon atoms in length and derived from the breakdown of WDP, bind directly to toll-like receptors 1 and 2 and activate the downstream signaling pathway [78]. In addition, endocytosed WDP can induce endosomal membrane damage and disruption in phagocytic cells, and this process is

associated with dramatic recruitment of cytoplasmic annexin A2 to the endosomal membrane (Figure 5). In the absence of A2, endosomal disruption leads to leakage of lysosomal cathepsins and H⁺ ions into the cytosol with subsequent activation of the NLRP inflammasome and an accelerated inflammatory response [65].

2.6. Neuropsychiatric Disease. A large body of work has focused on the role of p11 in neuropsychiatric function. p11 binds to both the serotonin 1B and serotonin 4 receptors, suggesting a role for p11 in regulation of mood [39]. p11-deleted mice show depression-like behavior, characterized by increased immobility in the tail suspension test, increased thigmotaxis, and decreased responsiveness to a sucrose reward. In wild type mice, adenovirus-mediated deletion of p11 specifically within the nucleus accumbens (NA) resulted in depressive behavior, indistinguishable from that seen in mice with global p11 deficiency; exogenous administration of p11 within the NA of p11-deleted mice restored normal behavior [79]. These data correlate with findings in human depression, in which p11 protein levels were reduced in the NA. Furthermore, reduced p11 mRNA levels in peripheral blood mononuclear cells may serve as a potential biomarker for patients at high risk of suicide [80]. These studies raise the possibility that some forms of human depression may be reversible by augmentation of p11 expression [81]. Indeed,

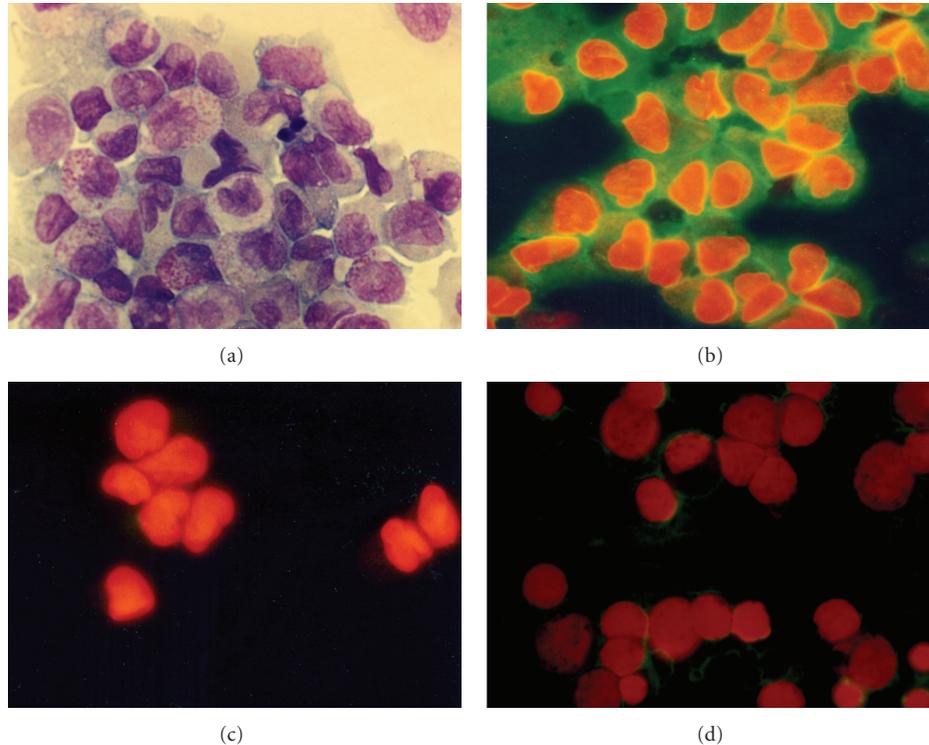


FIGURE 6: Expression of A2 is specifically elevated in acute promyelocytic leukemia cells. (a) Wright-Giemsa stain of human acute promyelocytic leukemia (APL) cells (M3). (b) APL cells stained with anti-A2 antibody (green fluorescence) and counterstained with ethidium bromide to visualize nuclei. (c) APL cells stained with nonimmune IgG and ethidium bromide. (d) Undifferentiated leukemia cells (M0) stained with anti-A2 antibody and ethidium bromide.

commonly used anti-inflammatory drugs that attenuate the antidepressive effects of serotonin reuptake inhibitors may do so by inhibiting the effects of interferony, a known inducer of p11 [82, 83].

p11 also potentiates nociception. The tetrodotoxin-resistant sodium channel ($\text{Na}_v1.8/\text{SNS}$), whose expression is restricted to sensory neurons, is the major pain perception receptor and is expressed in 85% of neurons emerging from the dorsal root ganglia. p11 binds to the amino terminus of the $\text{Na}_v1.8$ protein and promotes its translocation to the plasma membrane to produce functional channels [35]. Deletion of p11 specifically in primary nociceptor sensory neurons was achieved using $\text{Na}_v1.8$ promoter-directed Cre recombinase and led to a loss of tetrodotoxin-resistant sodium current density, and severe compromise of noxious coding in sensory neurons from the dorsal root horn [84]. Thus, directed p11 targeting may prove useful in the treatment of refractory pain disorders.

3. Lessons from the Clinic

3.1. Acute Promyelocytic Leukemia. At diagnosis, acute promyelocytic leukemia (APL) is commonly associated with life-threatening hemorrhage [85]. In APL, clonal expansion

of immature promyelocytes harboring a balanced chromosomal translocation ($t(15; 17)(q22-24; q12-21)$) gives rise to the transcriptionally active promyelocytic leukemia-retinoic acid receptor α (PML-RAR α) fusion protein. APL frequently responds to differentiation therapy with all-trans retinoic acid (ATRA), which triggers degradation of PML-RAR α [86]. While disseminated intravascular coagulation promotes coagulopathy in APL, consumption of the plasmin inhibitor α_2 -antiplasmin and development of a hyperfibrinolytic state due to excessive plasmin generation is also a primary driver.

High-level expression of annexin A2 occurs specifically in APL blast cells (Figure 6). A2 was detected in blast cells recovered from 6 of 6 APL patients, all of whom had evidence of hyperfibrinolysis, as evidenced by elevated circulating fibrin degradation products and D-dimer and depletion of plasma fibrinogen [87]. NB4 cells, which carry the $t(15; 17)$ translocation and express the PML-RAR α fusion protein, displayed steady state A2 mRNA levels that were approximately 10-fold higher than those found on leukemia cells that lacked the fusion protein. Treatment of NB4 cells with the retinoic acid receptor ligand, all-trans retinoic acid (ATRA), attenuated A2 expression in a time frame associated with clinical resolution of bleeding [87]. Intracranial bleeding, an unusually frequent problem in APL, may be due to the relatively high level expression of the A2

system on cerebral microvascular endothelial cells compared to those of other vascular beds [85]. Elevated expression of p11 in NB4 cells was also recently demonstrated and shown to respond to treatment with ATRA [88].

In a second clinical study, a cohort of 26 patients were studied prospectively and found to have enhanced fibrinolysis at diagnosis, despite normal tPA levels and increased PAI-1 [89]. APL cells harvested from these subjects expressed 3-fold higher levels of A2, and their rate of tPA-dependent plasmin generation was similarly elevated over that seen in the presence of M1, M2, M4, or acute lymphoblastic leukemia cells. Both elevated A2 expression in blast cells and hyperfibrinolytic hemorrhage corrected in 23 patients upon treatment with differentiation therapy, consisting of all-trans retinoic acid (ATRA) or ATRA plus arsenic trioxide. This study confirms the role of the A2 system in fibrinolytic bleeding in patients with APL.

3.2. Solid Tumors. Expression levels of annexin A2 have been examined in a variety of human malignancies. In some, such as renal cell [90–92], gastric [93], prostate [94], pancreatic [95], breast [72] carcinoma, and osteogenic sarcoma [96], increased expression levels appear to correlate with higher histologic grade and/or development of distant metastases. In human glioblastoma, A2 expression correlates with histologic grade and CNS dissemination [49, 97–99]. In human breast cancer, A2 appears to be associated with the surface of invasive, malignant cells, but not normal ductal or acinar epithelial cells, and expression correlated with neoangiogenic activity [73]. Proteomic profiling of colorectal cancer, moreover, revealed differentially increased expression of A2 in tumors that had progressed to lymph node metastases versus localized tumors [100]. Primary multiple myeloma cells harvested from a cohort of patients displayed 10-fold higher cell surface A2 expression than that observed on normal plasma cells; silencing of A2 in related cell lines suppressed expression of proangiogenic genes [101]. A2 and a related A2-binding “receptor” has been reported to promote myeloma cell adhesion and growth in the bone marrow [102]. On the other hand, A2 expression in oral squamous cell carcinoma [103] or sinonasal adenocarcinoma [104] was found to be inversely related to histopathologic grade. These studies suggest that expression levels of A2 may have prognostic value in malignancy, but would need to be validated for each specific tumor.

Expression of p11 in distinct tumor types has been less extensively studied. High levels of both p11 and A2 were found in 100% of anaplastic thyroid carcinomas, and correlated with their aggressive behavior [105]. In a comprehensive study of S100 gene expression in over 300 primary breast cancers, both p11 (S100A10) and S100A11 were selectively upregulated in basal versus nonbasal breast cancer subtypes, but did not predict overall survival [106]. Among 62 cases of human esophageal squamous cell carcinoma, 11 of 12 S100 genes, including p11, were downregulated, based on reverse transcription-polymerase chain reaction assays [107]. p11 transcripts have also been reported to be increased in both renal cell and gastric carcinomas

[92, 108, 109]. Further studies may define p11 expression as a viable biomarker or prognostic indicator in selected tumors.

3.3. Antiphospholipid Syndrome. Antiphospholipid syndrome (APS) is a major cause of acquired thrombophilia [110]. It is characterized by thrombosis and recurrent fetal loss in association with circulating antiphospholipid antibodies. The latter are distinct, often coexisting antibodies directed against either β 2-glycoprotein I or other intravascular proteins, which may be found in complex with anionic membrane phospholipids [111]. A2 has been identified as a prominent target of autoantibodies arising specifically in patients with APS with severe thrombosis and/or pregnancy morbidity [112, 113]. *In vitro*, patient-derived antiannexin A2 antibodies blocked endothelial surface tPA-dependent plasmin generation, and also “activated” cultured endothelial cells, inciting them to express elevated levels of the prothrombotic agent, tissue factor [112]. Other groups have noted that A2 can serve as a binding site for β (2)-glycoprotein I [114] in APS and can initiate A2-dependent endothelial cell activation [115]. Together, these data implicate A2 in the pathogenesis of APS-associated thrombosis through several possible mechanisms [116].

3.4. Cerebral Thrombosis. Cerebral venous thrombosis is a rare disorder of unknown etiology that mainly affects children and young adults [117]. Among a cohort of 40 consecutive patients studied 2 to 6 months following the index thrombotic event, 12.5% were found to have high titer anti-A2 antibodies compared to 2.1% in healthy subjects [118]. Thus, anti-A2 may define a new subset of individuals with immune-mediated thrombosis, play a role in the pathogenesis of this disorder, and/or offer novel therapeutic targets.

3.5. Preeclampsia and Pregnancy Loss. Both A2 and p11 are expressed on the brush border of the placental syncytiotrophoblast [119]. In 60 patients with preeclampsia, A2 mRNA and protein levels in placenta were significantly reduced compared to those in placentas from 30 matched normal pregnancy controls [120]. High titer anti-A2 antibodies, moreover, were detected more frequently in sera from subjects in the pre-eclamptic group and associated with increased placental vascular thrombosis. Impaired local fibrinolytic function due to blunted A2 expression may contribute to the pathogenesis of pre-eclampsia and maternal and perinatal infant morbidity.

3.6. Sickle Hemoglobinopathy. Sickle hemoglobinopathy arises from a point mutation within the 6th codon of the human β -globin chain [121]. Polymerization of abnormal hemoglobin under deoxygenating conditions induces erythrocyte shape change and non deformability, which leads to vascular occlusion, impaired vasodilatation, distal ischemia, and endothelial cell activation with adhesion of leukocytes. In children, sickle cell disease is complicated by stroke in 6–8% of patients. A recent analysis of

108 single nucleotide polymorphisms in 39 candidate genes revealed that variations in the annexin A2 (*ANXA2*) gene, among several others, were associated with increased risk of stroke [122]. A second independent study linked *ANXA2* polymorphisms to increased risk for stroke in sickle cell disease [123], while additional *ANXA2* SNPs have been associated with avascular necrosis of bone (osteonecrosis) in sickle hemoglobinopathy [124]. These data suggest that annexin A2 may represent a significant modifier gene that shapes the clinical expression and natural history of sickle cell disease.

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