Published in final edited form as:
Curr Mol Med. 2013 January ; 13(1): 24–57.

Functions of S100 Proteins

R. Donato[*,1], **B.R. Cannon**[2], **G. Sorci**[1], **F. Riuzzi**[1], **K. Hsu**[3], **D.J. Weber**[2], and **C.L. Geczy**[3]

[1]Department of Experimental Medicine and Biochemical Sciences, University of Perugia, Via del Giochetto, 06122 Perugia, Italy

[2]Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, 108 N. Greene St., Baltimore, MD 21201, USA

[3] Inflammatory Diseases Research Unit, School of Medical Sciences, University of New South Wales, Sydney, New South Wales, Australia

Abstract

The S100 protein family consists of 24 members functionally distributed into three main subgroups: those that only exert intracellular regulatory effects, those with intracellular and extracellular functions and those which mainly exert extracellular regulatory effects. S100 proteins are only expressed in vertebrates and show cell-specific expression patterns. In some instances, a particular S100 protein can be induced in pathological circumstances in a cell type that does not express it in normal physiological conditions. Within cells, S100 proteins are involved in aspects of regulation of proliferation, differentiation, apoptosis, Ca^{2+} homeostasis, energy metabolism, inflammation and migration/invasion through interactions with a variety of target proteins including enzymes, cytoskeletal subunits, receptors, transcription factors and nucleic acids. Some S100 proteins are secreted or released and regulate cell functions in an autocrine and paracrine manner *via* activation of surface receptors (e.g. the receptor for advanced glycation end-products and toll-like receptor 4), G-protein-coupled receptors, scavenger receptors, or heparan sulfate proteoglycans and N-glycans. Extracellular S100A4 and S100B also interact with epidermal growth factor and basic fibroblast growth factor, respectively, thereby enhancing the activity of the corresponding receptors. Thus, extracellular S100 proteins exert regulatory activities on monocytes/macrophages/microglia, neutrophils, lymphocytes, mast cells, articular chondrocytes, endothelial and vascular smooth muscle cells, neurons, astrocytes, Schwann cells, epithelial cells, myoblasts and cardiomyocytes, thereby participating in innate and adaptive immune responses, cell migration and chemotaxis, tissue development and repair, and leukocyte and tumor cell invasion.

Keywords

S100 protein; calcium binding; calcium homeostasis; DAMPs; inflammation; cancer; tissue repair/regeneration; RAGE; TLRs; signaling pathways

[*]Address correspondence to this author at the Department of Experimental Medicine and Biochemical Sciences, University of Perugia, Via del Giochetto, 06122 Perugia, Italy; Tel: +39 075 5857453; Fax +39 075 5857451; donato@unipg.it.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

INTRODUCTION

Ca^{2+}-binding proteins have evolved from a common ancestor to regulate intracellular Ca^{2+} levels and numerous Ca^{2+}-signaling pathways [1,2]. Those that regulate Ca^{2+} levels are typically membrane proteins which have evolved to pump Ca^{2+} to the outside the cell, or towards intracellular Ca^{2+} stores to maintain low cytosolic free Ca^{2+} concentrations under resting conditions (~100 nM). This is a physiological requirement necessary to avoid Ca^{2+} precipitation and/or excess Ca^{2+} signal activity. For example, the Ca^{2+}-ATPase associated with the endoplasmic reticulum constitutes a Ca^{2+} reserve that can be released into the cytoplasm as necessary for specific cellular functions. Some other Ca^{2+}-binding proteins (e.g., calsequestrin and calretinin) are characterized by a low Ca^{2+}-binding affinity but high Ca^{2+}-binding capacity, due to their high levels and location within Ca^{2+} stores. Here they bind the ion, but make it available for release when required. Other Ca^{2+}-binding proteins, mostly cytoplasmic, either buffer Ca^{2+} during the course of Ca^{2+} transients as a result of their high Ca^{2+}-binding affinity (e.g., parvalbumin and S100G) or function as transducers of the Ca^{2+} signal. This latter group represents a large fraction of Ca^{2+}-binding proteins, which when bound to Ca^{2+}, interact with other protein targets to regulate a large number of cellular functions. Calmodulin, troponin-C and most S100 proteins are considered Ca^{2+}-signaling proteins and all have the conserved calcium-binding motif termed the EF-hand.

Contrary to calmodulin and troponin-C, whose activities are restricted to the intracellular milieu, several S100 proteins act as intracellular regulators and as extracellular signaling proteins and may be secreted and/or released to regulate activities of target cells in a paracrine and autocrine manner. Importantly, S100 proteins are expressed exclusively in vertebrates, and exhibit somewhat cell-specific distribution [3]. Of the 24 human S100 genes, 19 (S100 proteins, group A) are located within chromosome 1q21 [4]. Other gene locations include S100A11P, which maps to chromosome 7q22-q3, S100B, which maps to chromosome 21q22, S100G, which maps to chromo-some Xp22, S100P, which maps to chromosome 4p16, and S100Z, which maps to chromosome 5q13 [4].

Within cells, S100 proteins have been involved in the regulation of proliferation, differentiation, apoptosis, Ca^{2+} homeostasis, energy metabolism, inflammation and migration/invasion through interactions with a variety of target proteins including enzymes, cytoskeletal subunits, receptors, transcription factors and nucleic acids. Extracellular S100 proteins act in an autocrine and paracrine manner *via* activation of surface receptors, G-protein-coupled receptors, scavenger receptors, or heparan sulfate proteoglycans and N-glycans. As extracellular signals, S100 proteins have been shown to regulate cell proliferation, differentiation, survival and migration in normal and pathological conditions, inflammation and tissue repair, and/or to exert antimicrobial activity. Certain S100 proteins are also found in serum and other biological fluids during the course of pathological conditions and are used as disease markers.

Numerous S100 genes are induced in a somewhat cell-specific manner, by appropriate growth factors, cytokines and toll-like receptor (TLR) ligands. In these circumstances, they are generally secreted, and may function as extracellular alarmins or damage-associated molecular pattern factors that principally mediate functions of the innate and adaptive immune systems, stimulate cancer cell locomotion and/or participate in tissue repair [5-13]. Increased expression of certain S100 proteins may also enhance intracellular regulatory activities, as outlined below. Lastly, in particular cases, induction of expression of an otherwise repressed S100 gene may be functionally linked to the cell's response to an intervening event, particularly to stress. For example, S100B is not expressed in cardiomyocytes in normal physiological conditions, but is induced in cardiomyocytes surviving an infarct, and can limit the hypertrophic response by inhibiting expression of α-

actin and β-myosin [14]. S100A8 and S100A9 expression is upregulated in numerous cell types by oxidative stress, corticosteroids and by particular cytokines and growth factors [9]. However, information about the regulation of expression of most S100 proteins in normal and pathological conditions is fragmentary.

EF-HAND MOTIFS OF S100 PROTEINS

Since the E- and F-helices in the helix-loop-helix calcium-binding motif of parvalbumin were characterized by Kretsinger and colleagues [15], there have been over 650 crystal and nuclear magnetic resonance (NMR) structures of EF-hand Ca^{2+}-binding proteins deposited into the protein data bank. Typically, EF-hand Ca^{2+}-binding motifs are arranged in pairs of EF-hands held together by a very short anti-parallel β-strand and numerous hydrophobic interactions between the four helices. The canonical EF-hand has 12-residues with six or seven backbone or sidechain oxygen ligands utilizing residues in positions 1, 3, 5, 7, and 12 (bidentate) of the helix-loop-helix calcium-binding domain (Fig. **1**) [16]. S100 family proteins, on the other hand, are a unique set of EF-hand family members since one of the EF-hand motifs in the pair (termed the pseudo- or S100-hand) has 14 rather than 12 residues, and several of the ligands bind to Ca^{2+} include backbone carbonyl oxygen atoms rather than oxygen atoms from sidechain Asn, Asp, Gln, or Glu residues [17]. Likewise, the S100 proteins are dimeric, which does not allow for the movement of the exiting helix (helices 4, 4′) upon Ca^{2+}-binding as is found for other EF-hand proteins (i.e. calmodulin, troponin-C, etc.) [18]. Instead, it is the entering helix (helices 3, 3′), which rotates as much as 90 degrees upon binding Ca^{2+} to expose a hydrophobic patch as necessary for interacting with its specific protein targets (Fig. **2**) [19].

A question that has arisen is how can a cell have a large number of intracellular Ca^{2+}-binding proteins at high concentration without sequestering too much free Ca^{2+} ions, which is needed for signaling biological events, in the nM to very low μM range range (i.e. 100 nM to 2 μM)? Although the mechanistic details for this process are still being characterized, it is usually the case that, EF-hand binding proteins, including many S100 proteins (i.e. S100A1, S100B, and others), do not bind Ca^{2+} very tightly in the absence of their biological target (K_D >10 μM; Fig. **2**) [20]. It is the protein-target interaction itself that is necessary to allosterically regulate the complex, so that the EF-hand binding protein is then able to appreciably bind Ca^{2+} at physiologically relevant free Ca^{2+} ion concentrations inside the cell to signal for a functional response [20,21]. However S100A10 does not conform to other family members because it lacks a functional EF-hand Ca^{2+}-binding domain, so that its target-protein interactions are Ca^{2+}-independent [22,23]. It is also clear that some peptide targets show this trend, but generally they are not sufficiently intact to induce the same effects typical of the full-length proteins. Thus, target peptides derived from the ryanodine receptor (RyR) lower the dissociation constant of S100A1 by about a factor of 10; whereas, full-length RyR enabled S100A1 to interact with Ca^{2+} at 100 nM free Ca^{2+} concentration. This represents an over 100-fold lowering of the dissociation constant for Ca^{2+}-binding to S100A1 when compared to binding in the absence of target [24,25], and makes this S100 interaction physiologically relevant within the cytoplasm [26].

INTRACELLULAR FUNCTIONS

S100A1

S100A1 is abundantly expressed in skeletal muscle fibers, cardiomyocytes and certain neuronal populations [3]. Within these cells, it is found diffusely in the cytoplasm and associated with cytoskeletal components and mitochondria. The S100A1 promoter contains several negative regulatory motifs controlled by inhibitory transcription factors downstream of G-protein-coupled receptors and protein kinase C (PKC) [27] (Fig. **3A**). Accordingly,

chronic stimulation of cardiomyocytes with angiotensin II, endothelin 1, phenylephrine and PKC agonists, which cause hypertrophic growth, reduces S100A1 mRNA and protein levels [28] (Fig. **3A**). S100A1 interacts with the sarcoplasmic reticulum Ca^{2+}-ATPase and RyR2 in the heart, resulting in improved Ca^{2+} handling and contractile performance [29] (Fig. **3A**). It also targets the cardiac sarcomere and mitochondria, thereby reducing pre-contractile passive tension and enhancing oxidative energy generation [29] (Fig. **3A**). S100A1 deficiency results in abnormal sarcoplasmic reticulum Ca^{2+} content and fluxes, accelerated deterioration of cardiac performance and transition to heart failure [29,30] and S100A1 gene delivery rescues failing myocardium [31]. In skeletal myofibers S100A1 binds to RyR1 and potentiates its open probability and plays role in skeletal muscle excitation-contraction coupling [24,32] (Fig. **3A**). S100A1 also interacts with the giant sarcomeric kinase, titin, with potential improvement of sarcomeric compliance [30] (Fig. **3A**). It also stimulates membrane-bound guanylate cyclase in photoreceptors likely involved in dark adaptation (reviewed in [3]) and regulates energy metabolism by stimulating fructose-1,6-biphosphate aldolase and inhibiting phosphoglucomutase and glycogen phosphorylase [3].

S100A2

S100A2 expression is downregulated in many cancers and loss in nuclear expression is associated with poor prognosis [33]. Thus, S100A2 is a tumor-suppressing protein binding to p53 transactivation domain and potentiating p53 is a potential mechanism [34]. However, S100A2 is upregulated in some cancers and other functions are unclear [33].

S100A3—S100A3 is highly expressed in hair root cells and some astrocytomas. It is proposed to have a role in epithelial cell differentiation and Ca^{2+}-dependent hair cuticular barrier formation [35]. It may protect hair from oxidative damage due to very high Cys content [36].

S100A4—S100A4 expression is associated with outcome in patients witha number of tumor types by stimulating cell survival, motility, and invasion [37,38]. S100A4 interacts with cytoskeletal proteins such as nonmuscle myosin heavy chain (NMMHC) IIA, tropomyosin and actin, processes that can increase cell migration (Fig. **3B**). A direct role of S100A4 in metastatic progression is proposed on the basis of its interaction with NMMHC IIA [39]; phenothiazines inhibit this interaction by inducing S100A4 oligomerization [40] (Fig. **3B**). Deletion of *s100a4* also results in defective macrophage migration and macrophage responses to chemotactic stimuli due to altered NMMHC IIA dynamics [41]. Several other binding partners for S100A4 have been identified *in vitro*, including the tumor suppressor p53, S100A1, the GTP-binding septins, the matricellular biomolecule CCN3, the leukocyte common antigen-related transmembrane tyrosine phosphatase-interacting protein liprin 1, and the tumor suppressor methionine aminopeptidase 82MetAP29 (Fig. **3B**). However, the majority of these interactions have not been confirmed *in vivo*, and whether any of these binding proteins are involved in S100A4-induced metastasis is unknown. S100A4 is upregulated by heterotetrameric β-catenin/T-cell factor complex with resulting stimulation of tumor cell migration and invasiveness [42]. Accordingly, blockade of β-catenin downregulates S100A4 expression and reduces cell migration and invasion [43] (Fig. **3B**). Intracellular S100A4 expression has also been associated with transcriptional regulation of matrix metalloproteinases (MMPs) and E-cadherin and it is not known whether this is attributed to cytoplasmic or nuclear S100A4 (or both) and mechanisms remain unidentified. Apart from the functions mentioned above, the biological role of nuclear S100A4 remains uncharacterized.

S100A5—S100A5 is upregulated in bladder cancers [44] and recurrent grade I meningiomas [45], but its biological function is unknown.

S100A6—S100A6 is implicated in cell proliferation, cytoskeletal dynamics and tumorigenesis [46,47]. It interacts with calcyclin-binding protein/Siah-1-interacting protein, a component of ubiquitin ligase involved in ubiquitination of β-catenin. S100A6 also inhibits interactions between the heat shock proteins (Hsp70 and Hsp90) and Sgt1 or Hop, suggesting a potential role in cell responses to different stressors. In this respect, the presence of S100A6 favors apoptosis in some cells [48], but limits it in others [49]. S100A6 also interacts with caldesmon, calponin, tropomyosin and kinesin light chain although functional manifestations are still unclear.

S100A7—S100A7 promotes aggressive features in breast cancer by binding to c-Jun activation domain-binding protein 1 thereby stimulating Akt and NF-κB [50]. Proinflammatory cytokines upregulate S100A7 expression in human breast cancer [51]. However, the tumorigenic activity of S100A7 appears to be restricted to estrogen receptor α-negative breast cancers, because in estrogen receptor α-positive breast cancers the protein appears to reduce the activation of the β-catenin/T cell factor 4 pathway with consequent reduction of uncontrolled proliferation [52].

S100A8

Gene deletion of S100A8 in mice is embryonic lethal, suggesting an important non-redundant function [53]. It comprises some 20% of the neutrophil cytoplasm. It is found in the nucleus of some cells [54]. S100A8 is induced in macrophages, dendritic cells, microvascular endothelial cells but not endothelial cells from larger vessels, epithelial cells (e.g. keratinocytes) and fibroblasts by pro-inflammatory stimuli [9]. In murine macrophages, S100A8, but not S100A9, is induced by TLR agonists in an interleukin (IL)-10-dependent manner; some agonists require cAMP and/or PGE_2 generation for full expression. Our recent data indicates direct induction of S100A8 by oxidative stress in macrophages and this is amplified by the anti-inflammatory cytokine, IL-10. Mechanisms regulating S100A8 gene induction in macrophages are shown in Fig. (**4**). In murine keratinocytes, S100A8 is induced by oxidative stress whereas S100A9 is not, confirming discrete roles for these S100 proteins. In keratinocytes, S100A8 is entirely cell-associated and high nuclear expression is obvious [54].

S100A8 is implicated in myeloid cell differentiation [55] and inhibits differentiation-dependent telomerase activity in a keratinocyte cell line in a Ca^{2+}-dependent manner [56] (Fig. **3C**). S100A8 scavenges intracellular reactive oxygen species (ROS) generated by activated neutrophils and may stabilize nitric oxide (NO) in these cells [57] (Fig. **3C**). S100A8 reduces p38 mitogen-activated protein kinase (MAPK)-dependent phosphorylation of S100A9 in neutrophils in a Ca^{2+}-dependent manner, thereby regulating their transendothelial migration [58]. Thus, S100A8 stimulates keratinocyte differentiation *via* inhibition of telomerase activity and exerts anti-inflammatory effects.

S100A9

S100A9 abrogates S100A8-induced reduction in telomerase activity [56] (Fig. **3C**). S100A9 inhibits myeloid (dendritic cell and macrophage) differentiation and accumulation of myeloid-derived suppressor cells in pathological responses *via* intracellular ROS generation, thereby contributing to tumor growth [59] (Fig. **3C**). S100A9 may differentially modify the phenotypic states of myeloid cells: S100A9-deficient neutrophils produce reduced amounts of cytokines in response to TLR-4 stimulation, S100A9-deficient dendritic cells produce more cytokines after TLR stimulation, and macrophages rapidly loose S100A9 expression during maturation. S100A9 gene deletion compromises neutrophil responses to particular chemoattractants and some aspects of skeletal dynamics may be compromised, resulting in impaired transendothelial cell migration [60]. S100A9 is a p38 MAPK target [61],

phosphorylated after phagocyte activation. S100A9 reduces microtubule polymerization and F-actin cross-linking by the S100A8/S100A9 complex [58] and mediates Ca^{2+} signaling associated with inflammatory agonist-induced IP3-mediated Ca^{2+} release in neutrophils [62] (Fig. **3C**). S-glutathionylated S100A9 is involved in glutathione metabolism in activated neutrophils [63]. STAT3 mediates S100A9 expression in some cancer cells and expression correlates with growth suppression [64]. In MCF-7 breast tumor cells S100A9 is essential for oncostatin M-induced growth repression [64]. In esophageal squamous cell carcinoma, S100A9 is a p53 transcriptional target and mediates the p53 apoptosis pathway [65]. S100A9 mediates transformation and proliferation of human aortic smooth muscle cells [66]. Thus, S100A9 exerts particular effects in a cell-specific manner.

S100A8/S100A9

S100A8 and S100A9 form a heterocomplex. S100A8/S100A9 inhibits casein kinase I and II [67] suggesting a role in myeloid cell differentiation [55,68] (Fig. **3C**) and interacts with nuclear factors [69]. S100A8/S100A9 transports unsaturated fatty acids and arachidonic acid [70] and promotes NADPH oxidase activation in phagocytes by interaction with p67*phox* and Rac-2 [71]. S100A8/A9 is important in FcγR-1-mediated phagocytosis that requires depletion of intracellular Ca^{2+} stores for internalization; following phagocytosis of opsonized zymosan, S100A8/A9 acts as a cytoplasmic Ca^{2+} sensor that links Ca^{2+} influx to phagosomal ROS production [72]. S100A8 and S100A9 overexpression in HaCaT keratinocytes increases NADPH oxidase activity and enhances ROS levels (Fig. **3C**); in hepatocellular carcinoma cells, co-expression of the two proteins promotes malignant progression by induction of ROS, down-regulation of p38 MAPK signaling, and cell survival and resistance to tumor necrosis factor (TNF)-α-induced apoptosis [73]. The cytoplasmic S100A8/S100A9 complex translocates to the membrane following phagocyte activation and may promote formation and stabilization of microtubules and enhance tubulin polymerization in neutrophils [58]. Interactions with cytoskeletal components are Ca^{2+}-dependent and are important for migration, degranulation, phagocytosis of activated monocytes and neutrophils; the tetramer promotes microtubule polymerization and F-actin cross-linking [58,74] (Fig. **3C**). S100A8/S100A9 expression is associated with a macrophage subtype associated with low antimycobacterial activity [75]; S100A8/S100A9-expressing epithelial cells resist invasion by *P gingivalis, L. monocytogenes*, and *S. typhimurium* [76]. S100A8/S100A9 co-expression in ductal carcinomas of breast is associated with poor tumor differentiation, vessel invasion, and node metastasis [77] (Fig. **3C**): annexin 6 is involved in the Ca^{2+}-dependent expression of S100A8/S100A9 on surface of tumor cells, and the annexin 6/S100A8/S100A9 complex may mediate cell membrane-regulated events [78]. S100A8/S100A9 may mediate pathological differentiation of psoriatic keratinocytes; it interacts with keratin intermediate filaments and may modulate wound healing [79].

S100A10

S100A10 tethers certain membrane proteins (i.e., the small GTPase of the Rho family Cdc42, tetrodotoxin-resistant sodium channel Nav 1.8, background two-pore domain potassium channel TWIK-related acid sensitive K, acid-sensing ion channel ASIC1a, actin-binding protein AHNAK, tissue-type plasminogen activator [tPa], serotonin 1B receptor) to annexin 2 thereby assisting their traffic to the plasma membrane and/or their firm anchorage at certain membrane sites [80,81] (Fig. **5A**). A potential mechanism of S100A10/annexin 2/AHNAK ternary complex formation acting as a platform for membrane repair has been recently proposed [82]. S100A10 is downregulated in human and rodent depressive-like states and is implicated in the mechanism of action of antidepressant drugs and electroconvulsive seizures, in part due to its interaction with specific serotonin receptors [80,81] (Fig. **4A**). S100A10 is induced by neurotrophins [83].

S100A11

When phosphorylated by PKC-α, Ca^{2+}-bound S100A11 inhibits cell growth *via* binding to nucleolin, translocation to the nucleus, and activation of the cell cycle modulator $p21^{WAF1/CIP1}$ [84] (Fig. **5B**). S100A11 also binds Rad54B, a DNA-dependent ATPase involved in recombinational repair of DNA damage [85], and stimulates cell growth by enhancing the level of epidermal growth factor (EGF) family proteins [86].

S100A12

S100A12 is constitutively expressed in neutrophils and inducible in macrophages and smooth muscle cells. S100A12 [87] may modulate interactions between cytoskeletal elements and membranes [88] (Fig. **5C**). It inhibits aggregation of aldolase and GAPDH and may have Ca^{2+}-dependent chaperone/anti-chaperone-like functions [89] (Fig. **5C**). S100A12 expression in epithelial cells is associated with growth arrest [90]. S100A12 may play a role in vascular remodeling; it is expressed in human aortic aneurysms. Overexpression causes several vascular smooth muscle cell (VSCM) dysfunctions such as increased proMMP2 generation, increased phosphorylation and nuclear translocation of Smad2 and modulation of mitochondrial function [91] (Fig. **5C**). VSCMs from mice overexpressing S100A12 have increased NADPH oxidase-mediated generation of peroxide, possibly *via* interaction with Nox-1 [92] (Fig. **5C**). S100A12 potentiation of atherogenesis in Apo-$E^{-/-}$ mice, and increased vessel calcification *via* upregulation of multiple osteogenesis-related genes may occur through changes in ROS production [92]. On the other hand, S100A12 attenuated chemokine secretion in activated human airway SMC. TNF-α and interferon (IFN)-γ exposure enhanced CCL9, and CXCL10 mRNA and protein levels, and these were attenuated in S100A12 overexpressing cells, with the net effect of dampening allergic inflammation in the airway [93].

S100A13

S100A13 plays an important role in stress-induced release of fibroblast growth factor (FGF)-1 and IL-1α from several cell types including fibroblasts, osteoblasts and melanoma cells [94].

S100A14

S100A14 may function as a cancer suppressor affecting the p53 pathway [95] and modulating expression of matrix metalloproteinases, MMP1 and MMP9 [96]. However, recent studies show that S100A14 may play a dual role in tumor cells [97]; it acts as a negative regulator of p53 in cells expressing wild-type p53 likely decreasing p53 protein stability, which results in enhanced expression of MMP2 levels and consequent increase in tumor cell invasiveness; however, in tumor cells expressing mutant p53, S100A14 reduces MMP2 levels with consequent decrease in invasiveness.

S100A15

No intracellular role for S100A15 has been reported, the protein mainly acts as an extracellular factor [98].

S100A16

S100A16 is upregulated in several tumors [99]. It acts as a novel adipogenesis-promoting factor, having a negative impact on insulin sensitivity [100].

S100B

S100B is expressed in astrocytes, certain neuronal populations, Schwann cells, melanocytes, chondrocytes, adipocytes, skeletal myofibers and associated satellite cells, certain dendritic cell and lymphocyte populations and a few other cell types [7]. It acts as a stimulator of cell proliferation and migration and an inhibitor of apoptosis and differentiation [101-111] (Fig. **6**), which might have important implications during brain, cartilage and skeletal muscle development and regeneration/repair, activation of astrocytes in the course of brain damage and neurodegenerative processes, and of cardiomyocyte remodeling after infarction, as well as in melanomagenesis and gliomagenesis. In particular, downregulation of S100B expression in precursor cells in a defined temporal window appears to be permissive for cell differentiation [101-106,108-110]. Sex-determining region Y-type high mobility group box 5, 6 and 9 (the so-called SOX trio), NF-κB, EGF and the Th-1-derived cytokine IFN-γ, regulate S100B expression in several cell types [see 7 for review; also see 12]. However, cells that downregulate S100B expression at the onset of their differentiation resume S100B expression at completion of development [7,106], and in mature cells the protein regulate a large variety of key activities including maintenance of shape, transcription, protein degradation, Ca^{2+} homeostasis, energy metabolism and enzyme functions by interacting with a wide array of target proteins. Binding partners of S100B within cells are tubulin and the microtubule-associated τ protein, the actin-binding protein caldesmon, calponin, type III intermediate filament subunits, annexin 6 [7,112-119], membrane-bound guanylate cyclase, the small GTPase Rac1 and Cdc42 effector IQGAP1, Src kinase, the serine/threonine protein kinase Ndr, the tumor suppressor p53, intermediates upstream of IKKβ/NF-κB, the giant phosphoprotein AHNAK/desmoyokin, the E3 ligase hdm2, dopamine D2 receptor and the mitochondrial AAA ATPase, ATAD3A [7,120,121] (Fig. **6**). Thus, lack of S100B downregulation may maintain cell proliferation with potential beneficial effects during development and tissue regeneration, and detrimental effects during tumorigenesis. S100B also regulates Ca^{2+} homeostasis [122-125], but opposing results were reported in astrocytes and VSMCs [122,124] (Fig. **6**). Moreover, S100B binds to, and inhibits EAG1 potassium channels Ca^{2+}-dependently (Fig. **6**) raising the possibility that its negative effects on cell differentiation may be *via* this mechanism as well [125]. Chronically high S100B levels such as those obtained in S100B transgenic mice are proposed to be causally correlated with Parkinson's disease likely *via* downregulation of dopamine D2 receptor and G protein-coupled receptor kinase2 expression, increased dopamine synthesis and metabolism, and decreased serotonin levels [126] and/or S100B interaction with the third cytoplasmic loop of the dopamine D2 receptor and extracellular signal-regulated kinase (ERK)1/2-mediated inhibition of adenylyl cyclase activity in striatal neurons [127] (Fig. **6**). S100B is highly expressed in astrocytes [7] and to a lesser extent in certain neuronal populations [128,129], and its elevation in serum positively correlates with mood disorders [130] and schizophrenia [131]. Serum levels of S100B are of prognostic value in patients with cutaneous melanoma [105] and breast cancer [132]. Whether serum levels of S100B are an outcome predictor in severe traumatic brain injury is a matter of debate [133,134].

S100G

S100G is not a full member of the S100 sub-family and represents the only monomeric family member. Its major function is to act as cytosolic Ca^{2+} buffers in many tissues, resulting in modulation of Ca^{2+} adsorption [135]. A second designated function was on the interaction of S100G with phospholipids and may play a role for the transport of S100 proteins through membranes [136].

S100P

S100P interacts with ezrin/radixin/moesin thereby activating ezrin and promoting transendothelial migration of tumor cells [137]. S100P preferentially disperses NMMHC IIA fibers and subsequently reduces focal adhesion sites and cell adhesion thereby promoting cell migration and potentially metastasis [138]. S100P also interacts with the scaffolding protein IQGAP thereby reducing IQGAP ability to stimulate MAPK activity [139].

S100Z

S100Z is downregulated in several tumors [140], but no functional roles are reported.

SECRETION AND RELEASE OF S100 PROTEINS

Various S100 proteins are found in body fluids, including serum, urine, seminal plasma, saliva, sputum, cerebrospinal fluid and in feces and abscess fluid, principally associated with active disease states. Some, such as the S100A8/S100A9 complex and S100B are considered biomarkers for particular disease processes [74,105,141-147] (also see below). Secretion of some S100 proteins is stimulated by particular cell activators. For example, serotonin (5-HT_{1A}) receptor agonists, antidepressants, glutamate, adenosine, IL-1β, lysophosphatidic acid and changes in extracellular Ca^{2+} and K^{+} levels trigger release of S100B from astrocytes [7,148-150], or of S100A4 from human pulmonary artery smooth muscle cells [5,151]. The selective serotonin reuptake inhibitor, fluoexitine, stimulates S100B secretion from serotoninergic neurons thereby downregulating micro-RNA16 in noradrenergic neurons, which consequently acquire properties of serotoninergic neurons [152]. Metabolic/oxidative stress induces release from several cells, and activation of some cell types or adhesion of some cells can also promote secretion. For example, TLR-2 activation on epithelial cells by *Aspergillus* conidia promotes release of S100B that binds the receptor for advanced glycation end-products (RAGE) on neutrophils in a paracrine manner, and mediates its association with TLR-2, causing TLR-2 inhibition [12]. In bronchial epithelial cells S100B is upregulated in the early stages of fungal infection *via* MyD88-dependent activation of canonical NF-κB and downregulated *via* TLR-3/9-dependent activation of non-canonical NF-κB at late stages [12]. Human $CD8^{+}$ T cells and NK cells also express and secrete S100B following stimulation, thereby activating monocytes and neutrophils [153]. In addition to astrocytes [154], oligodendrocytes [155] and adipocytes [156] secrete S100B, and high S100B serum levels in schizophrenia are associated with insulin resistance [157].

S100 proteins lack a leader sequence and are not secreted *via* the classical Golgi pathway, and mechanisms remain somewhat unclear. S100A8/S100A9 may be passively released from necrotic myeloid cells, or actively secreted following translocation to the membrane, in a process requiring an intact microtubule network and PKC activation [58]. S100B also can be passively released from injured tissues [11,12]. S100 proteins may themselves have roles in nonclassical secretion. These proteins have diverse affinities to lipid structures that allow translocation across the plasma membrane following cell stress or activation. For example, S100A13-lipid interactions and formation of a multiprotein complex with FGF-1 (which also lacks a classical secretion sequence) and synaptotagmin peptides allows release, possibly mediated by N-type Ca^{2+} channel activity, or flip-flop to the extracellular compartment *via* annexin binding [158]. The heterotetrameric S100A10 and annexin 2 complex is associated with von Willebrand factor secretion from endothelial cells [159]. S100B associates with natural [160] and artificial [161] membranes; this, although not formally proven, might represent a mechanism of its secretion. In this respect, the interaction of S100G with phospholipid micelles has been proposed as a prototype of S100 protein interaction with membranes for subsequent passage through them [136]. Other mechanisms include vesicular S100A8/S100A9 release in neutrophil extracellular traps

(NETs), triggered by production of ROS from dying neutrophils. NETs are chromatin fibers (histones and DNA) bound with antimicrobial proteins that deliver high local concentrations to pathogens [162].

S100 RECEPTORS

The oligomeric forms of some S100 proteins, and their putative binding partners can determine function [163]. For example, S100A8 and S100A9 have functions that can be dependent or independent of the heterocomplex. In some circumstances, more highly oligomerized S100 proteins may more functionally efficient and in some cases high concentrations of S100 proteins are required for activation whereas other functions depend on very low amounts [5], indicating different receptor affinities, different extents of ligand-induced receptor oligomerization or requirements of coreceptors. Divalent cation binding can also determine functional outcomes. In addition, some S100 proteins are structurally altered and particular posttranslational modifications can promote changes in function. These include oxidation products of S100A8 and/or S100A9 generated by NO, oxygen-free radicals and hypohalous acids [164,165], and of S100B [166].

Receptors mediating extracellular functions of S100 proteins have been elusive and remain a matter of debate and there is evidence for multiple receptors. Both non-receptor- and receptor-mediated endocytosis is implicated. For example, exogenous S100A1 is internalized into neurons *via* multiple endocytic pathways and delivered to early/recycling endosomes, Golgi apparatus, late endosomes, and lysosomes [167]. Although many effects may be mediated by RAGE, others are not (Fig. **7**). Structural studies of some S100 proteins indicate at least three recognition sites within two distinct surfaces that may accommodate multiple binding partners [168] that result in complex interactions, or binding to specific ligands on different target cells. This is supported by reports that some S100 functions reside within the divergent hinge domains between the Ca^{2+}-binding regions and are mimicked by relevant peptides [169] whereas others require homo- or hetero-S100 complexes. Structural and binding data suggest that tetrameric/octameric S100B triggers RAGE by receptor dimerization [170]. Furthermore, some S100s, such as S100A6 preferentially bind the C2 domain of RAGE rather than the V domain that binds S100B and S100A12, indicating another layer of receptor complexity [171]. S100A12 binds RAGE *in vitro* with very low affinity but this increases >1000 fold when S100A12 is in the Ca^{2+}-[172] or Zn^{2+}-bound hexameric states [173]. Although S100A8/S100A9 are proposed to bind RAGE, particularly on tumor cells [174,175] (Fig. **7**), only S100A9 in the presence of Ca^{2+} and Zn^{2+} has a high affinity; S100A8 has virtually none, and binding of S100A8/A9 is relatively weak [176]. Also, RAGE ligation by S100B results in RAGE/TLR-2 association in neutrophils leading to inhibition of TLR-2-provoked responses [12], and ligation of S100B to bFGF-bFGF receptor (FGFR1) complex on myoblasts (Fig. **7**) results in recruitment of RAGE in a RAGE/ S100B/bFGF/FGFR1 tetracomplex that enhances bFGF/FGFR1 signaling and inactivates RAGE signaling [11]. Some outcomes of S100 protein-stimulated RAGE signaling are summarized in [5,177,178].

S100A12 was the first S100 protein for which RAGE was the designated receptor on myeloid cells [179] although RAGE participation is controversial [164] and other receptors including N-glycans (including glycosylated RAGE) [180], a G-protein-coupled receptor [169] and scavenger receptors [181,182] are implicated (Fig. **7**). Glycosylated RAGE can form higher-order multimeric complexes with S100A12 which are reduced by deglycosylation or by non-glycosylated soluble RAGE. Thus carboxylated N-glycans on RAGE may enhance binding potential and promote receptor clustering upon oligomeric S100A12 binding [178]. In addition to RAGE, receptors for S100A8 and or S100A9 include the scavenger receptor CD36 [183,184], and heparan sulfate proteoglycans and carboxylated

N-glycans [184] (Fig. **7**). In one study, TLR-4 is proposed as an S100A8 receptor and S100A9 inhibits binding [60] (Fig. **7**). The S100A8/A9 complex does not activate TLR-4 but enhances cytokine production by bone marrow cells stimulated with lipopolysaccharide (LPS) [60]. In contrast, another study comparing S100A8 and S100A9 binding to TLR-4/MD2 showed high affinity S100A9 binding that was enhanced by Ca^{2+} and Zn^{2+}; binding of the S100A8/S100A9 complex was 5-fold less. Moreover, binding sites in S100A9 for TLR-4/MD2 appear to be the same as those for RAGE [176]. Other examples include effects of S100A4 on neurite outgrowth that depend on binding to heparan sulphate proteoglycans and a putative Gαq-coupled receptor [185].

EXTRACELLULAR FUNCTIONS OF S100 PROTEINS

S100A1

S100A1 is found in the extracellular compartment after heart ischemia. It enhances Ca^{2+} influx in cultured ventricular cardiomyocytes [186], and Cav1 channel currents in a protein kinase A-dependent manner, prolonged action potentials, and amplified action potential-induced Ca^{2+} transients in neurons [167].

S100A2

S100A2 is chemotactic for eosinophils [187]. S100A2 may be involved in calcification of cartilage/bone [188].

S100A3

No extracellular function has been reported.

S100A4

S100A4 has effects on numerous cell types. S100A4 released by tumor or stroma cells triggers pro-metastatic cascades by modifying the cytoskeleton and focal adhesions of tumor cells, downregulating the pro-apoptotic Bax and the angiogenesis inhibitor thrombospondin-1 genes, and increasing production of MMPs by endothelial and tumor cells [189]. S100A4 induces tube formation in endothelial cells in a RAGE-independent pathway, possibly through interactions with annexin 2 and accelerated plasmin formation [190]. S100A4 has various effects on leukocyte migration. It stimulates cytokine production, particularly granulocyte colony-stimulating factor and eotaxin-2 from T lymphocytes [191], and may thereby influence allergic inflammation as it is expressed in normal myeloid cells [192]. It may also stimulate T cell infiltration into primary tumors [191]. S100A4 is released under the action of the chemokine RANTES (CCL5) *via* microparticle shedding from the plasma membrane of tumor and stromal cells, and in turn extracellular S100A4 cooperates with RANTES (CCL5) in promoting tumor progression [193]. Extracellular S100A4 may also regulate tumor progression by interacting with EGF receptor (EGFR) ligands, thereby enhancing EGFR/ErbB2 receptor signaling and cell proliferation [194] (Fig. **8A**). S100A4 may promote TCRγΔ T-cell mediated lysis [195] and negatively regulate matrix mineralization/calcification [196]. S100A4, induced in articular chondrocytes by IL-7, stimulates MMP-13 production and release in a RAGE-dependent manner [197,198] (Fig. **8B**). S100A4 may be cardioprotective by promoting smooth muscle cell motility and proliferation, and possibly mediating neointimal hyperplasia and arterial muscularization, and promoting cardiac myocyte growth, survival, and differentiation [199]. Oligomeric S100A4 forms (tetrameric or more) promote growth and survival of cultured neurons, possibly protective after injury and regulates astrocyte motility. ERK1/2 is activated in many of the S100A4-responsive cells and may modulate growth and survival responses of S100A4 [200].

S100A5

No extracellular function has been reported.

S100A6

S100A6 is expressed by many cells and tumors [46,47]. It modulates RAGE-dependent survival of neuroblastoma cells by triggering apoptosis and generation of ROS through c-Jun NH2 terminal protein kinase activation [171]. S100A6 may regulate secretory processes in some cells. It stimulates secretion of lactogen II by trophoblasts and insulin release from pancreatic islet cells. S100A6 may modulate allergic responses by inhibiting histamine release by mast cells [46,47].

S100A7

S100A7 (psoriasin) is overexpressed in inflammatory skin diseases and induced in keratinocytes by IL-17 and IL-22 [201] and by the TRL-5-ligand, flagellin [202]. It has roles in antimicrobial responses and innate immunity. S100A7 adheres to, and reduces *E. coli* survival; the hinge region (amino acids 35-80) is sufficient for full activity [203,204]. S100A7/RAGE and Zn^{2+} binding are required for chemotactic activity for lymphocytes, monocytes and granulocytes, and S100A7 acts synergistically with S100A15 [205]. Mice expressing elevated amounts of doxycycline-regulated mS100A7/A15 in skin keratinocytes have an exaggerated inflammatory response characterized by leukocyte infiltration and elevated levels of T helper 1 and T helper 17 proinflammatory cytokines, linked to the pathogenesis of psoriasis [205, 206]. S100A7 promotes α-secretase activity by promoting ADAM (a disintegrin and metalloproteinase)-10 production from primary cortico-hippocampal neuron cultures and in brain. In so doing, it may prevent generation of amyloidogenic peptides in Alzheimer's disease [207]. S100A7 stimulates ROS generation from neutrophils [206].

S100A8

S100A8 is implicated in regulation of inflammation. Deletion of the gene in mice indicates a non-redundant function that may involve an immunoregulatory role in maternal-fetal tolerance [53]. Murine S100A8 and its hinge domain are chemotactic for leukocytes at picomolar concentrations. These promote actin polymerization in monocytes and neutrophils, likely *via* a G-protein-coupled receptor [208-210]. Human S100A8 is chemotactic for neutrophils and activity may depend on its oxidation state [87]. Murine S100A8 provokes a mild and transient influx of neutrophils and monocytes when injected intradermally or intraperitoneally (i.p.), with kinetics similar to those elicited by a delayed-type hypersensitivity reaction [211]. The chemotactic properties of murine S100A8 are modified by oxidation, particularly by hypohalous acids generated from activated phagocytes [164,165] and these effects are obvious *in vitro* and following injection *in vivo.* S100A8 is also chemotactic for periodontal ligament cells [212]. Effects of S100A8 on leukocyte adhesion are controversial. It was reported to stimulate neutrophil adhesion to fibrinogen [209] whereas others found S100A8 suppressed expression of the high affinity β-2 integrin epitope on neutrophils induced by S100A9, thereby likely reducing leukocyte adhesion [213]. S100A8 is reported to induce TNF-α and IL-1β production from murine bone marrow cells *via* TLR-4 activation, and induction was negated by S100A9 [214]. However this may be a property only exhibited by neutrophils activated with S100A8 [60]. S100A8 activates FcγRI and FcγRIV on macrophages through the activation of TLR-4 [72,214], and upregulates and activates MMPs and aggrecanase enzymes from chondrocytes suggesting a role in pericellular matrix degradation [215]. However S100A8 can also inhibit MMP activity, suggesting an auto-regulatory mechanism [216].

The S100A8 gene is upregulated in several cell types by the anti-inflammatory agent corticosteroid, and in macrophages its induction by TLR agonists depends on the anti-inflammatory cytokine IL-10 [9,217,218]. S100A8 induction in some cells may be dependent on ROS generation. This is implicated in its induction in endotoxin-activated macrophages (K. Hsu and C.L. Geczy, in preparation) and is involved in S100A8 expression in keratinocytes provoked by UV irradiation [54]. Some of the factors regulating S100A8 gene induction in macrophages are depicted in (Fig. **4**).

S100A8 has anti-inflammatory effects by triggering oxidation-sensitive repulsion of neutrophils [219] and is an effective 2-electron oxidant scavenger and at high concentrations, may protect from oxidative damage in acute and chronic inflammatory lesions [220]. S100A8 is protective in an acute asthma model as it suppresses mast cell activation by allergen by reducing intracellular ROS required for signaling, thereby suppressing production of key mediators required for eosinophil migration and reducing symptoms of allergic inflammation [221] (Fig. **9A**). S100A8 is also readily S-nitrosylated by physiological NO donors and endogenous levels found in neutrophils suggest that it may regulate NO availability as it is a relatively stable adduct. S-nitrosylated S100A8 reduces mast cell activation and mast cell-mediated leukocyte adhesion and transmigration in the microcirculation *in vivo*, and shuttles NO to hemoglobin [57] (Fig. **9A**). Thus S100A8-SNO formed during an inflammatory episode may contribute to maintenance of vessel patency in the microcirculation. Moreover, overexpression of S100A8 accelerates healing in wound models [222] and an Ala-Cys_{42} S100A8 mutant promotes more effective wound healing than the native form [223]. Thus the oxidantscavenging capacity of S100A8, and the functional modifications generated, may have important roles in resolution of inflammation (Fig. **9A**).

S100A9

S100A9 affects leukocyte migration, adhesion and transmigration from blood vessels [209,224]. Murine S100A9 is chemotactic for alveolar and peritoneal macrophages [8] and human S100A9 is chemotactic for neutrophils [209]; it activates expression of high affinity β-2 integrin epitope on neutrophils [213] and promotes their adhesion to fibronectin, a property that may maintain these cells within the extravascular compartment [225]. S100A9 also promotes degranulation of secretory and specific/gelatinase granules from neutrophils [226] and enhances human neutrophil bactericidal activity [227]. One report indicates that S100A9 induces TNF-α, IL-1β, IL-6 and IL-8 in macrophages *via* NF-κB activation [228] whereas another claims that it principally activates neutrophils *via* TLR-4 [60] and S100A9 decreases phorbol-12-myristate-13-acetate-triggered peroxide production by BCG-activated macrophages [229]. In the presence of Zn^{2+} and Ca^{2+} S100A9 is a RAGE ligand and a TLR-4 ligand [177] and may contribute to the pathogenesis of autoimmune diseases. S100A9 is mitogenic for fibroblasts [230] and stimulates IL-8 release from epithelial cells [231]. S100A9 may mediate dystrophic calcification [220] and is incorporated into urinary calcium oxalate crystals [232].

S100A9 also has anti-inflammatory properties. S100A9 has neutrophil repelling activity that may suppress migration [233]. It also suppresses macrophage activation following uptake of apoptotic neutrophils by inhibiting NO, H_2O_2 and TNF-α production *in vitro* [234,235]. S100A9 is readily glutathionylated and this alters its pro-adhesive properties [63]. S100A9 can scavenge ROS, likely through methionine sulfoxide formation on several Met residues; oxidation or mutation of key Met residues negates fugetactic activity for neutrophils [233]. S100A9 may be protective in asthma. In a rat model, it decreased pulmonary resistance and increased compliance upon antigen challenge and significantly decreased the isometric

tension of isolated tracheal spirals [236]. S100A9 also inhibits MMP activity by chelating Zn^{2+} from the active site of the enzymes [216].

Importantly, S100A9 may also regulate the magnitude of the acquired immune response by modulating levels of the co-stimulatory molecule, B7, expressed on antigen-presenting cells. Costimulation through the B7/CD28 pathway is important for activation of lymphocytes by alloantigens. S100A9 may modulate tolerogenic dendritic cell-T cell activation by reducing B7 levels and subsequent T-cell priming [237], and its over-expression inhibits dendritic cell differentiation [59].

A C-terminal peptide reduces spreading and phagocytosis of adherent macrophages induced by proteinase-activated receptor-1 agonists [234] and this peptide, like S100A9, suppresses macrophage activation following ingestion of apoptotic neutrophils [235]. S100A9 C-terminal peptide also modulates primary afferent nociceptive signals by inhibiting activation of N-type voltage operated Ca^{2+} channels and may reduce pain responses in inflammation [238].

S100A8/S100A9

The S100A8/S100A9 complex is also known as calprotectin. Heterodimerization with S100A8 stabilizes S100A9, causing elongation of its C-terminal α-helix, and tetramers form and expose two high affinity Zn^{2+}-binding sites at the S100A8/S100A9 subunit interface, which may be important functional sites for functions of calprotectin [239,240]. Anti-microbial properties, particularly to fungi and *Staphylococcus aureus*, are chiefly mediated by chelation of Zn^{2+} and Mn^{2+} [9,240,241], and when produced by IL-1β-activated keratinocytes, S100A8/S100A9 contributes to the anti-invasive properties of skin [9]. However S100A8/S100A9 increases *Mycobacterium tuberculosis* growth *in vitro*, suggesting a role in immunopathogenesis of tuberculosis [242]. It also promotes HIV-1 transcriptional activity and viral replication in infected $CD4^+$ T-lymphocytes [243], and upregulation of these S100 genes in monocytes by dsRNA may potentiate this effect [217]. In the aging prostate, amyloid deposition of S100A8/S100A9 associated with bacterial infection and macrophage activation may contribute to processes that increase risk of malignancy [244].

Human S100A8/S100A9 is chemotactic for neutrophils [209] and influences migration of other cell types, including myeloid-derived suppressor cells [245] and some tumor cells [174], and may facilitate tumor cell invasion [246,247] (Fig. **9B**). S100A8/S100A9 may activate proinflammatory cytokine production by human monocytes and macrophages *via* the NF-κB and p38 MAPK pathways [228] and promotes tumor development *via* RAGE-mediated production of inflammatory mediators [248] (Fig. **9A**). Furthermore, low concentrations of S100A8/S100A9 promote growth of some tumor cells through RAGE signaling and NF-κB activation [249], and binding to carboxylated glycans on RAGE promotes proliferation of colon cancer cells by this pathway [250]. Binding of the complex to RAGE on prostate cancer cells activates the MAPK pathway [174]. S100A8/S100A9-activated melanoma cells over-express MMPs -2, -9 and -14 [245]. On the other hand, S100A8/S100A9 inhibits MMPs by sequestering Zn^{2+} from their active sites [216].

S100A8/S100A9 potentiates the TLR-4 response of bone marrow cells to LPS, but does not directly activate TLR-4 [214]; it may induce NO production by macrophages [251]. On the other hand, S100A8/S100A9 may suppress acute inflammation by binding and modulating activities of pro-inflammatory cytokines [252]. S100A8 and S100A9 inhibit the spontaneous and stimulated oxidative burst of neutrophils, possibly mediated by P1 adenosine receptors [253]. The complex may also regulate lymphocyte functions. It stimulates $CD8^+$ T lymphocytes from individuals with lupus erythematosus to produce IL-17, suggesting a role

in development of autoreactive lymphocytes [254] although it inhibits immunoglobulin synthesis [255]. S100A8/S100A9 is elevated in psoriatic lesions; the complex induces a number of cytokines and chemokines in normal human keratinocytes, and at low concentrations stimulates keratinocyte growth [256]. S100A8/S100A9 may stimulate pro-inflammatory properties of endothelial cells, possibly mediated by RAGE and potentiates their activation by advanced glycation end-products [257]. S100A8/S100A9 delivers arachidonic acid to endothelium *via* CD36-mediated uptake [183] and stabilizes and protects leukotriene A(4) from nonenzymatic hydrolysis, possibly increasing availability of bioactive leukotrienes [258]. It also causes loss of contacts in microvascular endothelial cells *in vitro*, triggering apoptosis *via* caspase-dependent and independent mechanisms and is suggested to promote endothelial cell damage in vasculitis and inflammatory disease [259]. It also dissociates pancreatic acinar cell-cell contacts in a Ca^{2+}-dependent manner [224].

Extracellular S100A8/A9 can affect cell growth and have cytotoxic/apoptotic effects. It inhibits proliferation and differentiation of C2C12 myoblasts and induces caspase-3-dependent apoptosis [260]. At relatively high concentrations, it inhibits growth of a variety of normal cell types (macrophages, bone marrow cells, lymphocytes, fibroblasts) (Fig. **9A**), and has apoptosis-inducing activity to numerous tumor cell lines [261]. In some cells S100A8/S100A9 can reduce mitochondrial membrane potential, causing Smac/Diablo and Omi/HtrA2 (without cytochrome C) release and inhibition of mitochondrial fission machinery, Drp 1, inducing cell death by altering the balance between pro- and anti-apoptotic proteins [262]. S100A8/S100A9 may also promote autophagy-like death; apoptosis and autophagy may involve translocation of BNIP3, a BH3 only pro-apoptotic Bcl2 family member, to mitochondria, and cross-talk between mitochondria and lysozomes and generation of ROS [263]. Zn^{2+} chelation may be another mechanism [9,216,264]. However, *s100a9*$^{-/-}$ mice, which also lack S100A8 [265], show reduced formation of spontaneous prostate cancer and in EL-4 lymphoma model tumor growth inhibition was observed in *s100a9*$^{-/-}$ mice [266]; S100A9 is proposed to act as a tumor promoting factor *via* interaction with TLR-4.

A role for S100A8/A9 in the pathophysiology of atherosclerosis is proposed. The proteins are abundant in foam cells in atherosclerotic lesions [220] and because urokinase plasminogen activator upregulates S100A8/A9 expression in, and secretion from macrophages [267], the S100A8/A9 released may promote endothelial apoptosis. This could facilitate entry of monocytes and lipids into the artery wall [257]. S100A8 and S100A9 influence cardiomyocyte contractility by causing RAGE-dependent decreases in Ca^{2+} flux [268] and S100A9 in calcifying microvesicles from plaque may promote dystrophic calcification [232]. However studies in Apo-E low-density lipoprotein (LDL)-receptor-deficient, S100A9-deficient bone marrow chimeras question the role of S100A9 in atherogenesis [68].

S100A10

Heterotetrameric complexes of S100A10 with annexin 2 serve as extracellular binding partners for pathogens and host proteins. The promyelocytic leukemia-retinoic acid receptor α oncoprotein increases the expression of cell surface S100A10 which binds tPA and plasminogen *via* C-terminal lysine residues, promoting tPA-dependent plasmin production that can contribute to fibrinolysis as seen in the clinical hemorrhagic phenotype of acute promyelocytic leukemia [269]. Plasmin binds S100A10 at a distinct site; the S100A10-plasmin complex stimulates plasmin autoproteolysis thereby providing a highly localized transient pulse of plasmin activity at the cell surface [270]. Besides regulating fibrinolysis, S100A10 also plays an important role in angiogenesis *in vivo*, pointing to a critical role in endothelial cell function [271]. S100A10 mediates macrophage recruitment in response to

inflammatory stimuli by activating pro-MMP-9 that in turn, promotes plasmin-dependent invasion *in vivo* [272]. As to its function as plasminogen receptor, S100A10 is essential for the migration of tumor-promoting macrophages into tumor sites *in vivo* [273]. The tumor promoting properties of S100A10 might be reduced by the Rho GTPase-activating protein, DLC1, that competes with S100A10 for annexin 2 decreasing the steady-state level of S100A10 expression in a dose-dependent manner by displacing it from annexin 2 and making it accessible to ubiquitin-dependent degradation [274]. Interaction of S100A10 and a viral NS protein is essential for intracellular trafficking of nonenveloped bluetongue virus [275].

S100A11

S100A11 localizes in the cytosol of luteal cells in the mouse ovary, and oviductal epithelial cells, and suppresses fertilization through its action on cumulus cells [276]. S100A11 is induced/released by chondrocytes cultured with IL-1β, TNF-α, and CXCL8 [277]. It promotes hypertrophic chondrocyte differentiation, and stimulates RAGE-dependent type X collagen and IL-8 production by reticular chondrocytes. Transamidation generates covalently-bonded S100A11 homodimers that can signal through RAGE and activate the p38 MAPK pathway to accelerate chondrocyte hypertrophy and matrix catabolism that may promote osteoarthritis progression [278].

S100A12

There is no S10012 in rodent genomes [279,280]. It is constitutively expressed in neutrophils; TNF-α, IL-6 and endotoxin induce the gene in monocytes/macrophages, LPS in smooth muscle cells [281]. S100A12 inhibits growth and motility of filarial parasites by binding paramyosin [282]. Low amounts can immobilize microfilariae and high concentrations kill them, possibly by interruption of helminthic contractile elements. The C-terminal peptide (calcitermin) is antimicrobial and antifungal; unlike calprotectin, Zn^{2+} enhances activity [283].

Low concentrations of S100A12 and its hinge domain are chemotactic for monocytes and mast cells; a G-protein-coupled receptor is implicated [169] (Fig. **10**). Higher levels activate mast cells and potentiate IgE-mediated activation in a RAGE-independent manner. S100A12 induced pro-inflammatory cytokine production from mast cells, particularly IL-6 and IL-8, chemokines important for neutrophil, monocyte and lymphocyte recruitment, and caused TNF-α release from pre-formed stores [284] (Fig. **10**). On the other hand, overexpression of S100A12 in smooth muscle cells in lung resulted in S100A12 release with less production of some chemokines and reduced symptoms of acute asthma upon antigen challenge [93].

Bovine S100A12 stimulates RAGE-dependent TNF-α and IL-1β production from murine BV-2 microglial cells, IL-2 from lymphocytes and intercellular adhesion molecule-1 and vascular adhesion molecule expression on endothelial cells [179] (Fig. **10**). However S100A12 does not induce cytokine production by human monocytes or macrophages [285]. S100A12 enhances Mac-1 integrin affinity and L-selectin shedding from neutrophils and modulates neutrophil release from bone marrow [286]. S100A12 stimulates neurite outgrowth of rat hippocampal neurons *via* RAGE ligation and activation of phospholipase C, PKC, CAM-kinase II and MAPK pathways [287] (Fig. **10**). S100A12 binds to RAGE in the form of a hexamer [172]. S100A12 strongly inhibits MMP-3 and -9 by chelating Zn^{2+} from their active sites and complexes containing Zn^{2+} colocalize with these MMPs in atherosclerotic lesions, supporting this role *in vivo* [285]. Its overexpression in smooth muscle cells in mice leads to aortic aneurysms, linked to leukocyte influx, increased IL-6 in response to LPS, and increases of latent MMP-2 levels [91]. Overexpression of S100A12 in vascular smooth muscle cells in mice promotes atherosclerotic plaque remodeling and

nodular calcification, possibly by influencing osteoblastic genes in a feedback mechanism involving RAGE [92]. Cu^{2+} sequestration by S100A12 may modulate redox [288].

S100A13

S100A13 promotes it own intracellular translocation, possibly *via* RAGE binding on endothelial cells [289]. S100A13 is involved in the non-classical secretion of FGF-1 [94,158]; the complex may contribute to angiogenesis [290].

S100A14

S100A14 stimulates proliferation and apoptosis in an esophageal squamous cell carcinoma cell line *via* RAGE engagement at low and high doses, respectively [291].

S100A15

S100A15 is expressed in keratinocytes in inflamed skin and can be induced by LPS, IL-1β and Th-1 cytokines [292]. It is chemotactic for monocytes and granulocytes, possibly *via* a G-protein-coupled receptor and acts synergistically with S100A7 in leukocyte recruitment *in vitro* and *in vivo* [201]. Human S100A15 has antimicrobial activity against *E. coli* [293].

S100A16

No extracellular function has been reported.

S100B

S100B secreted or released from astrocytes has different (trophic and toxic) effects on neurons, astrocytes and microglia depending on the concentration [7,294,295]. Up to a few nanomolar amounts S100B behaves like a neurotrophin protecting neuronal cells against neurotoxic stimuli through stimulation of ERK1/2 and NF-κB-mediated upregulation of the anti-apoptotic Bcl-2, whereas at micromolar doses it kills neurons through excess ERK1/2 stimulation and ROS production and/or potentiation of neurotoxic effects of β-amyloid, *via* RAGE engagement in both cases [7,294-297] (Fig. **11A, B**). Others have shown that at high doses S100B still is pro-survival towards neurons *via* RAGE-dependent activation of phosphatidylinositol 3-kinase/Akt and NF-κB [171]. S100B stimulates astrocyte proliferation at low doses and promotes inflammatory activities in astrocytes at high doses [7,294-297] (Fig. **11A, B**). Whereas at low doses S100B attenuates microglia activation *via* the STAT3 pathway [298,299], at high doses it activates microglia as evidenced by NF-κB- and AP-1-dependent the stimulation of cytokine expression and release and cyclo-oxygenase-2 expression [7,294,295,300-304] and stimulates microglia migration *via* NF-κB- and AP-1-dependent upregulation of chemokine expression and chemokine release and upregulation of chemokine receptors [305], in a RAGE-dependent manner (Fig. **11A, B**). After permanent middle cerebral artery occlusion in S100B transgenic mice infarct volumes are significantly increased during the first days post-infarct and astrogliosis is enhanced compared with controls [306]. Moreover, S100B transgenic mice show increased susceptibility to perinatal hypoxia-ischemia [307], and overexpression of S100B accelerates Alzheimer disease-like pathology with enhanced astrogliosis and microgliosis [308]. Collectively, these results support the possibility that following accumulation in the extracellular space S100B might contribute significantly to neuroinflammation. However, intraventricular S100B infusion induces neurogenesis within the hippocampus, which has been associated with an enhanced cognitive function following experimental traumatic brain injury [309,310], and S100B is released by *in vitro* trauma and reduces delayed neuronal injury [311,312]. S100B is upregulated in experimental autoimmune encephalomyelitis, a model of multiple sclerosis, and activates RAGE in $CD4^+$ T cells that infiltrate the central nervous system, pointing to a pathogenic role of S100B-RAGE interactions in multiple

sclerosis [313]. The different effects exerted by S100B at low and high doses on nervous cells likely depend on the level of RAGE expression, different intensities of RAGE activation, the duration of RAGE stimulation and/or different extents of S100B-induced upregulation of RAGE expression in neurons, astrocytes and microglia.

S100B null mice exhibit enhanced spatial and fear memories as well as enhanced long-term potentiation (LTP) in the hippocampal CA1 region, and perfusion of hippocampal slices with S100B reverses the levels of LTP to those of the wild-type slices [314]. This suggests that extracellular S100B might play a role as a regulator of synaptic plasticity, although the molecular mechanism underlying this activity remains to be elucidated. S100B appears to have an important role in the mechanism of action of the antidepressant, fluoxetine. Fluoxetine-treated serotoninergic neurons secrete S100B which acts on noradrenergic neurons thereby reducing the otherwise elevated microRNA-16, which blocks serotonin transporter translation therein [152]. Thus, secreted S100B induces noradrenergic neurons to acquire properties of serotoninergic neurons, thus mediating effects of fluoxetine and representing a potent endogenous antidepressant. However, no information is available concerning the mechanism whereby fluoxetine induces serotoninergic neurons to express and secrete S100B or the mechanism whereby S100B reduces microRNA-16 levels in noradrenergic neurons. Overall, whereas S100B was localized to *in vitro* cultured serotoninergic neurons [152], no evidence has been provided that that serotoninergic neurons do express S100B following *in vivo* treatment with fluoxetine. RAGE ligation might be implicated because S100B reduces microRNA-16 levels in monocytes in a RAGE-dependent manner [315]. While it is not known whether S100B null or RAGE null mice are refractory to fluoxetine, the enhanced spatial and fear memories reported in S100B null mice [314] might be consistent with the proposed role of S100B as a transducer of fluoxetine antidepressant action. The neurotoxin 1-methyl-4-phenyl 1,2,3,6 tetrahydropyridine (MPTP), which causes neurological and pathological changes comparable to those observed in Parkinson's disease [316], increases S100B expression in astrocytes *in vivo* [317], and the culture medium of MPTP-treated astrocytes reduces PC12 neuronal cell viability, an effect that can be counteracted by an S100B neutralizing antibody [318]. Reduced PC12 neuronal cell viability under these conditions has been thus attributed to enhanced release of S100B by astrocytes and S100B-induced cell death. However, treatment of MPTP-treated mice with the antiepileptic drug, zonisamide, which improves Parkinson's disease symptoms, has been shown to result in enhanced S100B expression in astrocytes and suggested to ameliorate clinical signs of the disease *via* augmented secretion of the neurotrophic S100B [319]. Thus, it seems that at the levels found in the brain extracellular space in normal physiological conditions, S100B is trophic to neural cells. However, in a background of inflammation and/or neurodegenerative disorders, which is accompanied by enhanced expression levels of S100B and the S100B receptor, RAGE, in neural and inflammatory cells [7,320-322], S100B acts synergistically with proinflammatory cytokines and, at higher concentrations, behaves itself as a cytokine, amplifying and perpetuating inflammation and causing oxidative damage to neurons.

Outside the central nervous system, S100B RAGE-dependently promotes Schwann cell migration during the course of repair of injured peripheral nerves through the induction of the expression of thioredoxin interacting protein and the consequent activation of p38 MAPK, CREB and NF-κB in Schwann cells [323] (Fig. **11C**). Indeed, reduction of RAGE activity in acutely damaged peripheral nerves results in suppression of anatomical regeneration and functional recovery [324,325], supporting a role of RAGE in neurite outgrowth. Also, S100B released from injured skeletal muscle tissue is a potent stimulator of myoblast proliferation and inhibitor of myoblast differentiation *via* the enhancement of bFGF/FGFR1 signaling and blockade of RAGE's promyogenic signaling provided bFGF is present [11,326,327] (Fig. **11D**). However, S100B effects on myoblasts are strongly

dependent on cell density because in low-density myoblast cultures, and at early stage of low-density myoblast differentiation, it engages RAGE but not bFGF/FGFR1, thereby simultaneously stimulating proliferation and activating the myogenic program [328]. Thus, released S100B might contribute to the regeneration of injured skeletal muscle tissue by expanding the myoblast population at the site of injury and preventing precocious myoblast differentiation. Moreover, upon forming complexes with TLR-2 ligands, (low) S100B inhibits TLR-2 activation *via* RAGE, through a paracrine epithelial cell/neutrophil circuit that restrains pathogen-induced inflammation; however, upon binding to nucleic acids, S100B activates intracellular TLR-3/9 eventually resolving danger-induced inflammation *via* transcriptional inhibition of S100B [12]. Yet, at high doses, S100B engages RAGE on monocytes, promoting activation [329] and upregulation of microRNA-16 [315]. In activation of RAGE in autoimmune diabetes [330], S100B increases cyclooxygenase 2 expression in human pancreatic islets [331], and activates oxidant stress and inflammatory pathways in VSMCs [332] (Fig. **11E**). S100B engages RAGE in endothelial cells thereby activating NF-κB transcriptional activity, increasing expression of vascular cell adhesion molecule-1 and inducing monocyte chemoattractant protein-1 and RAGE transcripts and eliminating sodium nitroprusside-potentiated vasodilatation in response to acetylcholine in endothelial dysfunction in type II diabetic (Leprdb) mice; also, S100B enhances the interaction of RAGE with the leukocyte β2-integrin Mac-1 thus potentially increasing leukocyte adhesion to endothelial cells (for review see [7]). At high doses S100B enhances eosinophil survival in a RAGE-dependent manner, an event that might contribute to the role of eosinophils in wound repair and the clearance of damaged cells [333].

S100B also stimulates RAGE-mediated proliferation of T cells directed to the acetylcholine receptor and upregulation of cyclo-oxygenase 2 expression in splenocytes, implicating the S100B/RAGE axis in the pathogenesis of myasthenia gravis [334]. S100B is expressed in, and secreted by $CD8^+$ T lymphocytes and NK cells [153], thus potentially participating in the innate immune response, as outlined above, and in the adaptive immune response *via* RAGE engagement. The disparate effects of S100B [12,334], i.e. resolution and exacerbation of inflammation by the S100B/RAGE axis, respectively, are likely to depend on different types of responding cells coming into play in two conditions and differences in S100B doses and levels of RAGE expression. For example, for TLR-2 signaling, there is no role associated with myasthenia gravis whereas this has a critical role in inflammation caused by fungal infections. Thus, effects of the activity of the S100B/RAGE axis are likely to be strongly context-dependent. As mentioned earlier, S100B is induced by catecholamines in cardiomyocytes surviving an infarct, thereby modulating left ventricular remodeling [14,335]. However, S100B can be released by injured cardiomyocytes and at relatively high doses it causes RAGE-dependent cardiomyocyte apoptosis [336] (Fig. **11F**) and contributes to scar formation in infarcted myocardium by stimulating VEGF secretion by cardiomyocytes, again in a RAGE-dependent manner [337]. S100B-RAGE interactions have important roles in vascular smooth muscle cell proliferation in diabetes [338] and in neovascular macular disease [166].

S100G

No extracellular function has been reported for S100G.

S100P

S100P can mediate tumor growth, drug resistance and metastasis through RAGE binding on cancer cells [339].

S100Z

No extracellular function has been reported for S100Z.

S100 PROTEINS AS DISEASE MARKERS

In acute inflammation, there are generally elevated systemic levels of S100A8, S100A9 and/or S100A12, likely released from extravasating neutrophils and activated macrophages that dominate these lesions and these proteins are suggested as non-specific markers of phagocyte activation [340]. Importantly, S100A8/A9 and S100A12 are not disease-specific, and would likely not discriminate one particular condition if there were an underlying second pathology. Incorporation of S100A8/A9 and S100A12 levels with other particular disease markers may increase effective use for diagnosis. However there are limitations concerning the diagnostic use of these markers, as some results are conflicting [336], and for some applications, more robust assessment, with large patient cohorts is required. High circulating levels of S100A8/A9 and S100A12 are found in patients suffering from numerous infections or acute and chronic inflammatory disorders, various types of tumors, obesity, and Alzheimer's disease [262,341,342]. Secretions, including tears, saliva, sputum, cyst fluid, cerebrospinal fluid, synovial fluid, urine and feces may contain these proteins in particular circumstances. Concentrations of S100 proteins frequently correlate with disease course and/or severity, and may correlate with clinical scores and decline following therapy. Conditions associated with elevated S100A8/A9 and S100A12 levels in the circulation and secretions, and the use of these S100 proteins as clinical markers of inflammation are reviewed in [143,340].

Among the most recent applications is the measurement of S100A8/A9 or S100A12 in the circulation and/or feces from patients with gastrointestinal inflammation. The usefulness of a noninvasive fecal test is particularly useful for pediatric cases and levels may be used as markers for differential diagnosis, monitoring activity, or disease relapse in inflammatory bowel disease (IBD) [341,344]. Similarly, in cardiovascular disease, increased plasma levels of S100A8/A9 may predict cardiovascular events in humans [345]. When measured with myeloperoxidase and C-reactive protein, elevated levels predict all-cause mortality from acute myocardial infarction with moderate accuracy [346]. Others showed that the risk of a recurrent cardiovascular event increased with each increasing S100A8/S100A9; patients with the highest levels had a 2.0-fold increased risk [347]. However, in patients with stable coronary artery disease, S100A8/S100A9 levels did not associate with any other cardiovascular disease risk factor, and serum levels did not differ from normal subjects [348]. S100A8/S100A9 levels are also elevated in patients with inflammatory arthritides [143], and may be a predictive marker of clinical improvement following treatment of patients in the early phase of rheumatoid arthritis [349]. High amounts of S100A8, S100A9 and S100A12 in synovial fluid distinguished rheumatoid arthritis from miscellaneous inflammatory arthritides with high accuracy [350] and S100A8/S100A9 may be a prognostic biomarker for erosive disease in patients with rheumatoid arthritis [351].

S100A12 may be a useful marker of some lung disorders. We found that elevated sputum levels may be useful to assess eosinophilic asthma [284]. High concentrations of MMP-7, ICAM-1, IL-8, VCAM-1, and S100A12 predicted poor overall survival of patients with idiopathic pulmonary fibrosis [352] and S100A12 is useful as an early marker of acute, early-stage lung injury in patients with sepsis [353,354].

The use of other S100 proteins as markers is only emerging, particularly from proteome studies and gene arrays. Some that are elevated in certain cancers may be useful. For example, S100A9 is proposed as a good marker for identifying myeloid-derived suppressor cells in the circulation of patients with certain cancers [355]; overexpression of S100A9 is associated with a poor prognosis in non-small cell lung cancer patients and may identify patients at high risk, even at an early pathological stage [356]. High expression of S100A11 in pancreatic adenocarcinoma might be a significant tumor marker and an unfavorable

predictor for prognosis of patients who have undergone surgical resection [357]. S100A4 acts as a metastasis inducer; high levels in primary colon, rectal, and gastric tumors are prognostic for metastasis and correlate with reduced patient survival [358], elevated amounts of nuclear S100A7 is associated with poor prognosis in head and neck cancer [359] and S100A7 is associated with the worst prognosis in estrogen receptor α-negative invasive breast cancers [50].

S100B is normally found in pM amounts in human serum [for review see Refs. 141,360], saliva [361,362], urine [363,364], amniotic fluid [365] and milk [366]. Serum levels of S100B are elevated after central nervous system injury such as stroke, subarachnoid hemorrhage and brain trauma, generally peaking 2-3 days after injury, and correlate positively with patient outcome [141-144,367-370]. However, in accordance with the notion that S100B may be released from non-nervous cells as well [7], elevated serum S100B levels are also found in several non-neurological clinical conditions such as cardiac ischemia, cardiomyopathy and extracerebral infections, and in patients who have undergone cardiothoracic surgical interventions or had a trauma without brain injury [371-378]. Schizophrenia [131,157,379,380], depressive/bipolar disorders [381,382] and obesity [383] also are associated with high serum S100B levels, but there is uncertainty as to whether nervous or non-nervous cells are the source of S100B in these conditions. High S100B levels are also found in the cerebrospinal fluid of patients with multiple sclerosis during the acute phase of exacerbation [384] and in bacterial meningitis [385]. Moreover, elevated S100B levels in amniotic fluid correlate positively with the occurrence of central nervous system malformations and damage [386] and are found in trisomy 21-complicated pregnancies [387,388]. Lastly, as mentioned earlier serum levels of S100B are of prognostic value in patients with cutaneous melanoma [105] and breast cancer [132].

THERAPEUTIC CONSIDERATIONS FOR THE S100 PROTEIN FAMILY

In addition to the use of S100 proteins as diagnostic markers, there is evidence that deregulation of S100 proteins can contribute to numerous disease states including cancer. With this in mind, regulating S100-dependent biology is becoming more widespread. The most straightforward means to achieve this goal in the clinic is to inhibit S100 proteins directly with small molecule inhibitors. For this approach, typically a combination of computer aided drug design (CADD), high-throughput screening, structural biology, medicinal chemistry, and *in vivo* biology and drug testing approaches are employed. Structure-based design is particularly useful since target specificity issues can be considered, and many high-resolution 3D structures and dynamic data for S100 complexes are available [20,389-396]. Having structural and dynamic information at atomic resolution is very helpful because of the somewhat difficult hurdle of inhibiting protein-protein interactions involving S100s [392-395,397,398]. One promising approach for this is to use CADD, NMR, X-ray crystallography and medicinal chemistry approaches to identify and then simultaneously block adjacent small molecule binding sites with a single engineered molecule [399,400]. As with any drug-design program, there is also a need to obtain physiological data at an early stage in the process to help determine whether a compound or series of compounds induce off-target effects and/or cause other unanticipated toxicities. This is particularly important for S100 inhibitors since there are over 20 structurally similar proteins, and they regulate many physiological pathways in a cell-specific manner [20,401].

Small molecule inhibitors have been reported for numerous S100 family members, some of which are in human clinical trials. For example, the anti-allergic drug cromolyn disrupts the S100P-RAGE interaction and reduces pancreatic tumor formation in animal models [402]. Cromolyn also binds other S100 family members (S100A1, S100A12 and S100A13), but its effects on the interaction of these proteins have not yet been fully investigated. Inhibitors of

the S100A10-annexin A2 interaction are also available with clinical uses predicted for angiogenesis and cancer metastasis [403]. With the goal of treating several cognitive disorders, small molecules that block S100A1 are also being pursued with drugs such as pentamidine, propranolol, and others S100A1 inhibitors (termed SA1iXs); although, for safety purposes, it will be important to consider the role S100A1 has in normal muscle contraction [396]. For S100A4, several phenothiazines including trifluoperazine and prochlorperazine were shown to disrupt the S100A4/myosin-IIA interaction by sequestering S100A4 *via* small molecule-induced oligomerization [40,404]. It is clear that these and/or other S100A4 inhibitors can be developed further and may have promise for several metastatic cancers [40,405]. In another study, S100A4 was shown to bind to anti-allergic drugs and a modified version of azaxanthone is being tested clinical trials for treatment of metastatic disease [405,406]. Likewise, a role for S100A4 has been suggested for the regulation of MMP and tissue inhibitors of MMPs activities, so in addition to cancer, pathological consequences of elevated S100A4 may also be important to block in patients with arthritis [407]. For S100B, a series of inhibitors were discovered (termed SBiXs) for treating malignant melanoma [107,408]. This work has included repurposing drugs such as pentamidine [408] and chlorpromazine [409,410] and modified versions of these SBiXs for melanoma treatment. Currently, the goal of this work is to use structure-based drug design approaches to engineer improved and highly specific S100B inhibitors, which have higher efficacy and fewer side effects than those currently being tested. Thus SBiXs that restore p53 function by specifically inhibiting S100B, as found for S100B siRNA, is a major goal [107,408]. In this regard, human and veterinary clinical trials are underway with two such SBiXs as potential melanoma therapeutics. However, as with all biological targets, it is often necessary to consider how populations of cells become resistant to drug treatment. In the case of blocking the S100B-p53 protein-protein interaction, this is necessary because a negative feedback loop is initiated when p53 levels are restored since the gene for S100B itself is up regulated by p53 [411]. Thus, cells that survive the initial SBiX treatment predictably have extremely high levels of the S100B protein expressed and require higher secondary doses of drug to induce apoptosis [unpublished data, Weber D.J. *et al.*].

Another therapeutic strategy is to control S100 expression. It is well established that the genes for several S100 proteins are associated with cell differentiation, malignant transformation, and cell cycle growth (i.e. S100B, S100A6, and others), and that their expression levels are varied according to various growth- and growth-inhibitory conditions, often in a cell cycle dependent manner [17]. Thus, treatments with general cell growth-inhibitors, such as the topoisomerase II inhibitor VP-16 or phorbol 12-myristate 13-acetate often have varying effects on levels of specific S100 proteins and in a cell-specific manner [412]. Therefore, to regulating specific S100s in particular pathological cell types, screens for inhibitors that down-regulate the activation of a known promoter is typically used. For example, in a screen monitoring S100A4 expression, calcimycin was identified as a down-regulator of the S100A4 promoter [413,414]. Similarly expression of S100B in melanoma is high and problematic, and it was found that one regulator of S100B expression is the HOXC11 protein. Thus, treatment with the Src/Abl inhibitor, dasatinib, reduced the HOXC11-SRC-1 interaction and prevented recruitment of HOXC11 to the S100B promoter, so the mRNA and protein levels for S100B were both reduced, as was the growth and migratory properties of drug-treated MeWo melanoma cells. Thus, profiling patients for HOXC11 and S100B levels has the potential to increase the efficacy of dasatinib treatment in melanoma [415]. It is also noteworthy that the effect of specific drugs can be tissue-specific, particularly for regulation of the S100A2 gene, which is thought to suppress growth in some cell types (i.e. breast) and accelerate growth in others (i.e. skin). In keratinocytes, S100A2 mRNA levels were 5-fold induced after organ culture with its induction blocked by the drug PD153035, a specific inhibitor of EGF receptor tyrosine kinase activity. These and other studies in immortalized keratinocytes demonstrated that EGF receptor activation

selectively upregulates S100A2, which is now thought to be an important mediator of regenerative epidermal hyperplasia [416].

Modulating S100 proteins is not limited to activities within the cell. For example, one major source of extracellular S100B are astrocytes, which sense, integrate, and respond to stimuli generated by neurons or neural injury, and this biology can involve gap junction (GJ) communication resulting in neurite growth, glial proliferation, and neuronal survival, at low levels of S100B. Interestingly, blocking GJ communication with specific inhibitors was found to stimulate S100B secretion in astrocyte cultures and acute hippocampal slices with one GJ blocker, carbenoxolone, shown to induce a fast and persistent increase in S100B secretion. Physiologically, a local GJ closure associated with release of low levels of S100B is postulated to be a mechanism necessary to limit the extension of lesion and increase the chances of neuronal cell survival [417]. However, elevated S100B levels within the brain demonstrate pathological effects because of its specific localization and selective overexpression in Alzheimer's disease [7]. Such an effect is thought to result from S100B-dependent activation of NO synthase to produce excessive NO [300-302,420-422]. Such a scenario can lead to astrocytic and neuronal cell death as found for astrocyte-neuron co-cultures [421]. Consistent with this hypothesis, block of this pathological effect was observed using specific NO synthase inhibitors and or *via* specific S100B antibodies, representing yet another therapeutic strategy to combat elevated S100B found in neurodegenerative cells associated with Alzheimer's and Parkinson's disease [422]. Clearly, though, such extracellular effects of S100s are not limited to the brain since S100s are involved in a in a wide array of important biological processes, such as angiogenesis, cell differentiation, and tumor growth. For example, a direct inhibitor of the S100A13-FGF1 complex, amlexanox, was shown to block the release of S100A13 and FGF1 resulting in the blocking of the angiogenic and mitogenic activities of FGF1 [423].

Another long-range goal is to regulate gene products *via* gene therapy approaches and or by up- or down-regulating specific micro-inhibitor RNA constructs (mIRs). For example, when an S100 is thought to be important for tumor suppression (i.e. S100A2 in breast) or when it is under-expressed or missing altogether (i.e. S100A1 in myopathies), gene therapy approaches have been considered. In one case, a conditionally replicative adenovirus (Ad/SA) for S100A2 was examined for its anti-tumor activity *in vitro* and *in vivo* for non-small-cell lung carcinoma [424]. In two EGFR-activated tumor xenograft animal models, Ad/SA exhibited potent anti-tumor activity, whereas cetuximab, an EGFR-targeting anticancer drug, was active transiently or ineffective. Combined treatment with cetuximab or cisplatin plus Ad/SA resulted in enhanced anti-tumor activity. Collectively, these results demonstrated that the S100A2 promoter-driven adenovirus could be a potent inhibitor of cancers such as non-small-cell lung carcinoma [424]. Likewise, knockout studies indicate that S100A1 is important for normal function of both heart and skeletal muscle, so gene therapy approaches have also been considered by many groups for the introduction of S100A1 for the treatment of myopathies lacking sufficient levels of S100A1 [425]. Likewise, using pharmacological and behavioral data, miR-16 was found to contribute positively to the therapeutic action of selective serotonin reuptake inhibitors (SSRI) *via* S100B for treating depression and anxiety [152], so there is also therapeutic potential for using mIRs and/or regulating their production specifically as yet another way to regulate S100 function in the future.

CONCLUDING REMARKS

With the exception of S100G which is a Ca^{2+}-modulator protein implicated in the buffering of cytosolic Ca^{2+}, all other members of the S100 protein family are Ca^{2+}-sensor proteins involved in the regulation of several functions including cell proliferation and

differentiation, tumor development, growth and metastasis, Ca^{2+} homeostasis, cell motility, apoptosis, redox, energy and glutathione metabolism, the inflammatory response, and transcription. However, these activities are not shared by all S100 members, each exhibiting cell-specific expression patterns and a peculiar set of intracellular target proteins which points to non-redundant functions of individual S100 protein members. In contrast to the universal Ca^{2+}-sensor protein, calmodulin, which only acts intracellularly, certain S100 proteins are secreted (either constitutively or following specific stimuli) or released following tissue injury. Extracellular S100 proteins can act as growth factors involved in the regulation of tissue development and regeneration/repair, or as damage-associated molecular pattern factors (alarmins) involved in the pathogenesis of inflammatory (including autoimmune) and infectious diseases, allergy, tumorigenesis and metastasis, and microbial killing. Extracellular effects of S100 proteins are transduced by a battery of receptors such as RAGE, TLR-4, heparan sulfate proteoglycans and carboxylated N-glycans, G-protein-coupled receptors or scavenger receptors. Certain S100 proteins may also activate cell surface receptors in an indirect manner, i.e. by interacting with canonical receptor ligands to potentiate their signaling. S100 proteins are only expressed in vertebrates, and their expression and/or activities appear to be mechanistically linked to the refinement or fine tuning of cell-specific gene expression and responses to external stimuli. Intracellular and extracellular functions of S100 proteins are beginning to be described in detail, making these proteins less enigmatic than in the past. Cardiac function, tissue repair/regeneration, inflammation (including neuroinflammation), infection and cell growth and differentiation, are processes in which certain S100 proteins are active players. However, regulation of their expression requires more investigation; overexpression of particular S100s is associated with tumorigenesis, chronic inflammation and neurodegeneration, and elucidation of mechanisms controlling this may help identify molecular determinants of deranged cell/tissue functions and indicate new ways to target therapies. Also, the identification of highly specific inhibitors of individual S100 proteins and their usage in *in vivo* studies may establish functions of members of this protein family and represent therapeutic tools.

Acknowledgments

We wish to thank the reviewers for criticism, comments and suggestions. Supported by Ministero dell'Istruzione, dell'Università e della Ricerca - Italy (PRIN 2007LNKSYS, 2007AWZTHH_004, PRIN_2009 WBFZYM_002), Associazione per la Ricerca sul Cancro (Project 6021), Association Française contre les Myopathies (Project 12992) and Fondazione Cassa di Risparmio di Perugia (Project 2009.020.0021), NIH GM58888, NIH CA107331, and The National Health and Medical Research Council of Australia.

ABBREVIATIONS

RyR	Ryanodine receptor
TLR	Toll-like receptor
NMR	Nuclear magnetic resonance
PKC	Protein kinase C
NMMHC IIA	Nonmuscle myosin heavy chain IIA
MMPs	Matrix metalloproteinases
IL	Interleukin
ROS	Reactive oxygen species
NO	Nitric oxide
p38 MAPK	p38 mitogen-activated protein kinase

TNF-α	Tumor necrosis factor-α
tPa	Tissue plasminogen activator
EGF	Epidermal growth factor
VSMCs	Vascular smooth muscle cells
IFN-γ	Interferon-γ
FGF	Fibroblast growth factor
IL	Interleukin
ERK1/2	Extracellular signal-regulated kinase 1/2
RAGE	receptor for advanced glycation end products
NETs	neutrophil extracellular traps
FGFR1	bFGF receptor 1
EGFR	epidermal growth factor receptor
LPS	Lipopolysaccharide
LTP	Long-term potentiation
MPTP	1-Methyl-4-phenyl 1,2,3,6 tetrahydropyridine
SBiXs	S100B inhibitors
GJ	Gap junction
mIRs	Micro-inhibitor RNA constructs

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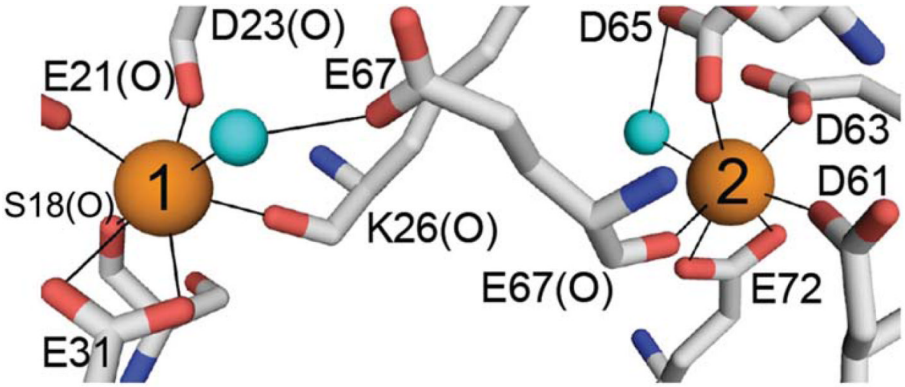
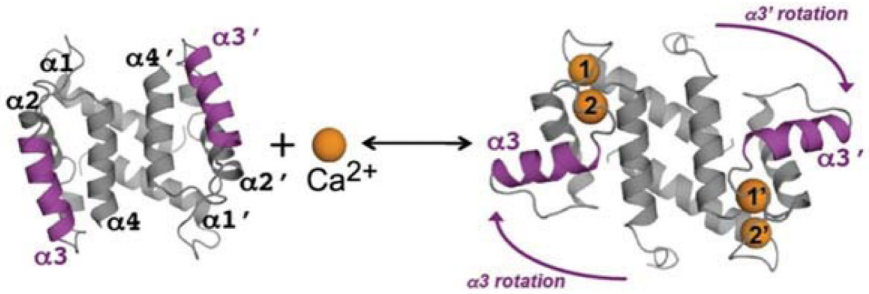


Position	1	2	3	4	5	6	7	8	9	10	11	12
Ligand Coordinate[a]	X		Y		Z		-Y		-X			-Z
Canonical EF-hand[b]	D	Basic	D/N	G	D/N	G	*Any*	Hyd	O	Hyd	Acidic	E
S100 *Canonical EF-hand*	D		N		D		E(O)		H_2O			E
S100 *Pseudo EF-hand*	S		E		D		K(O)		H_2O			E
S100B (EF1)[c] *Pseudo EF-hand*	S18(O)	G19	E21(O)	G22	D23(O)	K24	K26(O)	L27	H_2O	K28 K29	S30	E31
S100B (EF2) *Canonical EF-hand*	D61	S62	D63	G64	D65	G66	E67(O)	C68	H_2O	F69	Q70	E72

Fig. (1).
Coordinating residues for the canonical (site 2) and S100 EF-hand (site 1) for the S100 protein, S100B. In the table below the residues typically at each position of the EF-hand are illustrated. It should be noted that the S100 EF-hand has 14 rather than 12 residues.



Protein	Kd, μM (EF-1, EF-2)[a]	α3 rotation[b] (Ca^{2+}, apo)	Target (Kd, μM)	Target Reference	α3 rotation[c] (Target, Ca^{2+})
S100A1	–, 27	27°	$TRTK^{Pep}$ 24 μM	2	4°
			RyR^{Pep} 8 μM	3	-11°
S100A2	–, 470	33°			
S100A3	–, 4000				
S100A4 (MTSI)	–, 2.6	52°	TFP 55 μM[d]	4	1°
			PCP 56 μM[d]	4	3°
S100A5	160, 0.2	46°			
S100A6 (Calcyclin)	–, 3	38°	$Siah1^{Pep}$ 5 μM	5	-5°
S100A7 (Psoriasin)	–, 1				
S100A10 (p11)	No Ca^{2+}-binding				
S100A11 (Calgizzarin)	–, 500	25°			
S100A12	–, 50	35°			
S100A13	400, 8	30°	IL1A 3 μM	6	-16°
			FGF1 2 μM	7	0°
			Amlexanox 20 nM	8	-22°
S100A16	x, 430	6°			
S100B	>350, 56	61°	$p53^{Pep}$ <24 μM	9	-7°
			$TRTK^{Pep}$ 3 μM	10	-3°
			Pentamidine 53 μM	11	1°
			Ndr^{Pep} 20 μM	12	-1°
			S44 20 μM	13	-3°
			SBi132 80 μM	14	1°
			SBi279 2 mM	14	1°
			SBi523 120 μM	14	3°
			SC0067 700 μM	15	4°
			SC0322 55 μM	15	-3°
S100G (Calbindin)	0.004, 0.004[f]	-4°			
S100P	800, 2	16°			

Fig. (2).
Ribbon diagram illustrating the rotation of helix 3 upon the addition of calcium and a table listing the degree of movement upon calcium and target protein and/or drug binding. The dissociation constants are from [8].

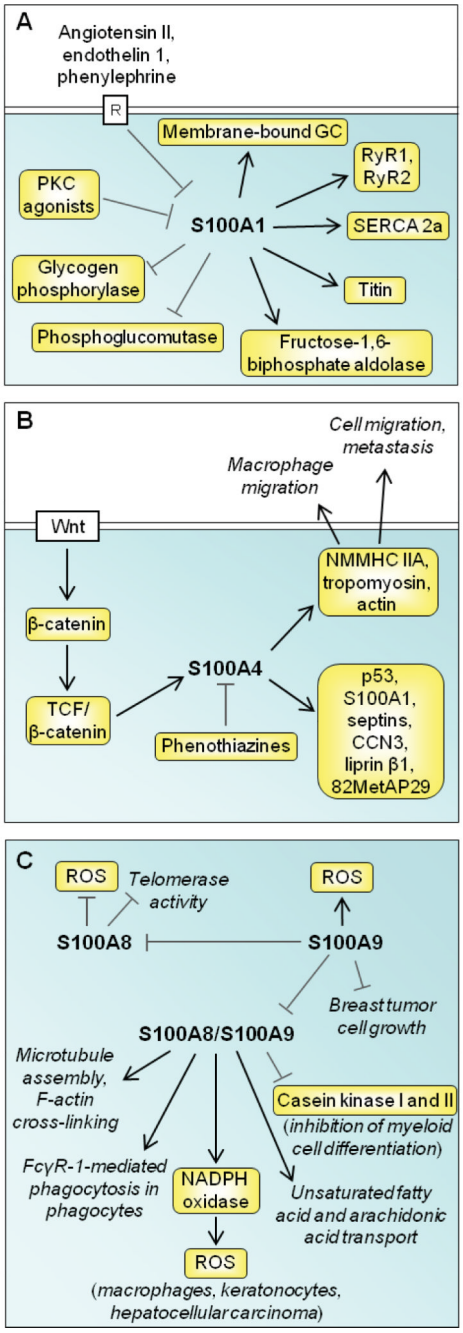


Fig. (3).

Schematic representation of proposed intracellular effects of S100A1, S100A4, and S100A8/S100A9. (**A**) S100A1 expression is negatively controlled by transcription factors downstream of G-protein-coupled receptors and PKC. S100A1 regulates energy metabolism and Ca^{2+} efflux from Ca^{2+} stores, stimulates striated muscle contraction, and activates a membrane-bound form of guanylate cyclase (GC) in photoreceptors in relation to dark adaptation. (**B**) S100A4 is induced by a Wnt/APC/GSK3/β-catenin/TCF pathway and targets several intracellular factors including NMMHC IIA, tropomyosin and actin with ensuing stimulation of cell migration and metastasis. Phenothiazines blocks intracellular S100A4 interactions. (**C**) S100A8 reduces telomerase activity and ROS production under the

negative control of S100A9. S100A9 promotes ROS production, reduces breast cancer cell growth and negatively regulates S100A8/S100A9 heterotetramer complex activities as shown.

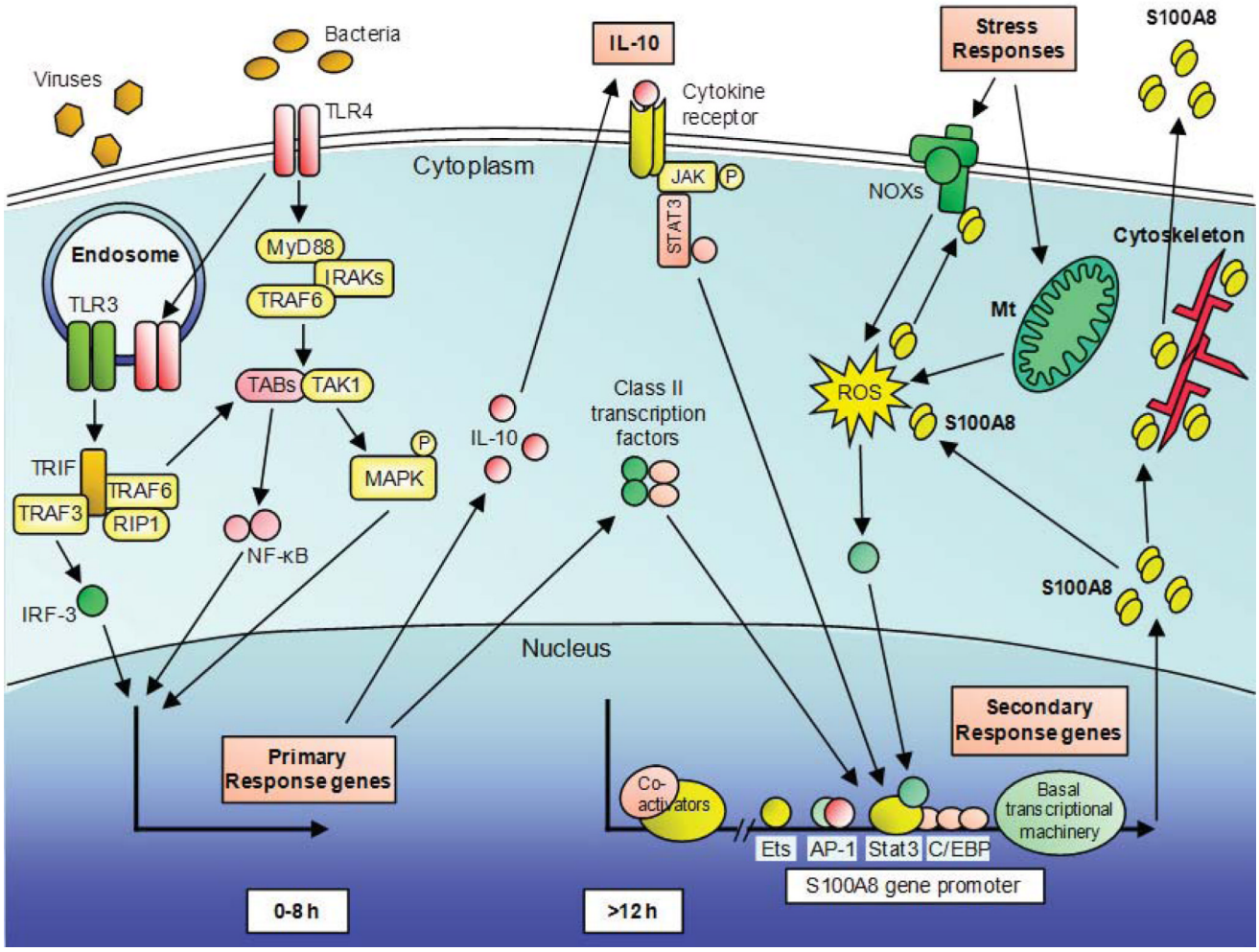


Fig. (4).

Schematic representation of *S100A8* induction in macrophages. LPS from bacteria is recognized by the surface receptor TLR4, activating MyD88-dependent and independent pathways. IRAKs and TRAF6 are recruited to MyD88 and subsequently activates a complex of TAK1 and TABs resulting phosphorylation of IκB and nuclear translocation of NF-κB. Simultaneously, TAK1 activates MAP kinase cascades leading to activation of AP-1. For the MyD88-independent pathway, TLR4 translocates to the endosome together with TRAM. In addition, TLR3 in the endosome, recognizes viral dsRNA. TLR3 and TLR4 activate TRIF-dependent signaling and subsequently activate NF-κB and IRF3. TLR-3 and TLR-4 activation triggers S100A8 gene induction in macrophages, but requires other factors. Induction is a late event that relies on *de novo* synthesized proteins, particularly IL-10, and class II transcription factors e.g. C/EBPs. AP-1 and Stat-3 bind to the *S100A8* promoter. S100A8 is considered a stress response gene, and intracellular ROS generation either *via* NOXs or mitochondria (Mt) may be essential for induction. Intracellular S100A8, together with S100A9, can interact with components of the cytoskeleton and may mediate their rearrangements and dynamics. S100A8 and S100A9 directly bind to components of the NOX complex and mediate its activity. On the other hand, S100A8 is a potent oxidant scavenger and oxidative modifications of S100A8 can change its functions. S100A8 is actively secreted *via* a non-classical pathway which requires a functional microtubule network to exert its extracellular functions.

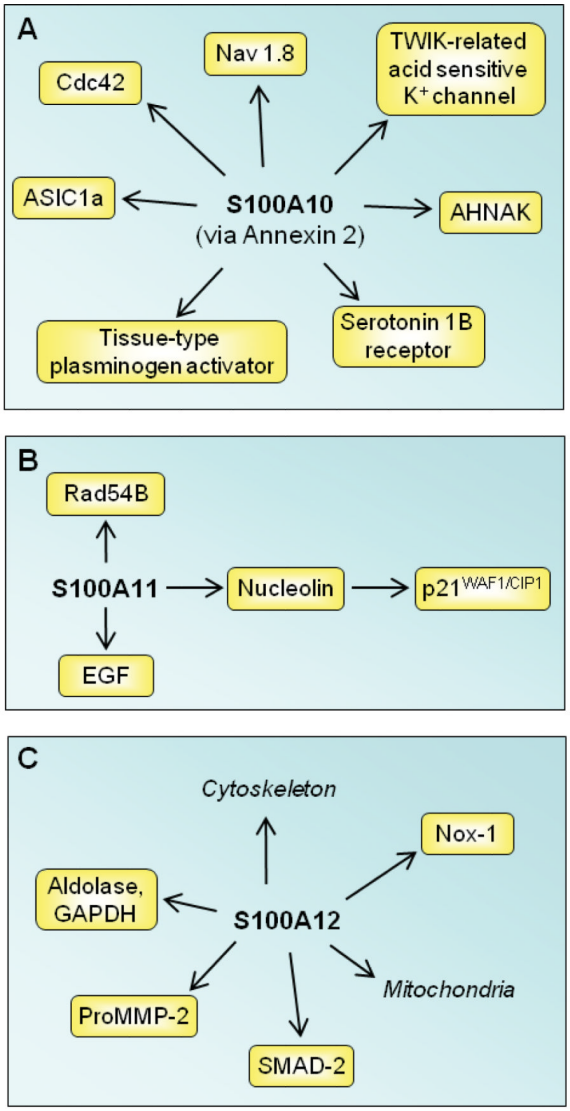


Fig. (5).

Schematic representation of proposed intracellular effects of S100A10, S100A11 and S100A12. (**A**) S100A10 is implicated in the mechanism of action of antidepressant drugs *via* interaction with serotonin 1B receptor. By binding annexin 2, S100A10 assists the traffic of several membrane proteins to plasma membranes. (**B**) S100A11 participates in the regulation of cell cycle by several mechanism as shown. (**C**) S100A12 regulates cytoskeleton-membrane interactions and has Ca^{2+}-dependent chaperone/anti-chaperone-like functions.

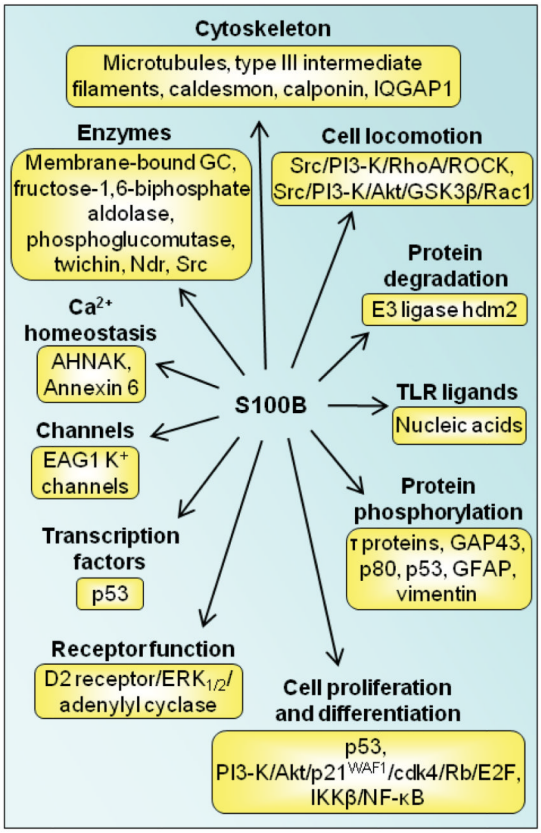


Fig. (6).

Schematic representation of proposed intracellular effects of S100B. S100B interacts with several intracellular proteins as shown thereby regulating protein phosphorylation, enzyme activities, the state of assembly of certain cytoskeleton components, the transcription factor p53, protein degradation, cell proliferation, locomotion and differentiation, dark adaptation of photoreceptors, Ca^{2+} homeostasis and the innate inflammatory response.

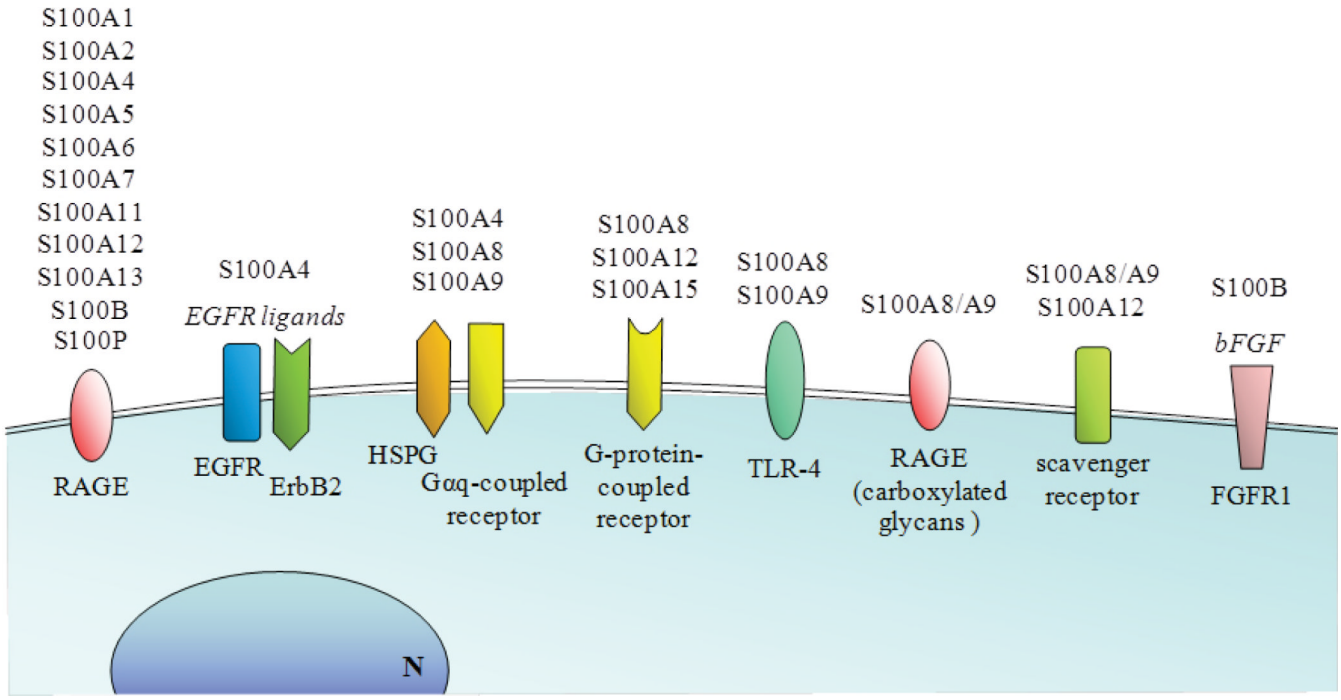


Fig. (7).

Schematic representation of the receptors involved in the transduction of S100 protein signaling. RAGE is an established receptor for several S100 protein members in a variety of cell types. By interacting with EGFR ligands and bFGF, S100A4 and S100B can also activate EGFR and FGFR1, respectively. By interacting with heparan sulfate proteoglycans (HSPG) S100A4, S100A8 and S100A9 also activate $G_{\alpha q}$ receptors. S100A8, S100A12 and S100A15 can activate G-protein-coupled receptors. S100A8 and/or and S100A9 interact with TLR-4 in phagocytes, and as a heterocomplex S100A8/S100A9 can bind to carboxymethylated RAGE. S100A8/S100A9 and S100A12 also activate scavenger receptors.

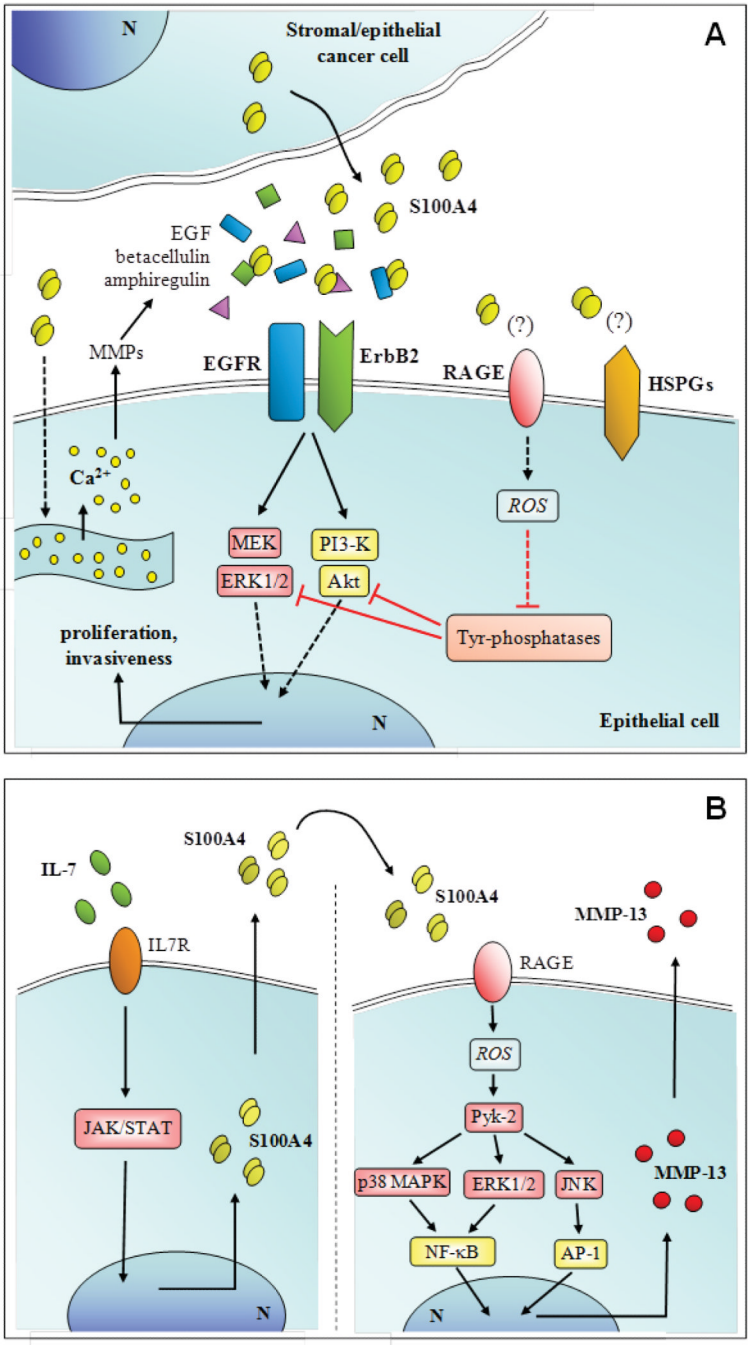


Fig. (8).

Schematic representation of proposed extracellular effects of S100A4 on epithelial tumor cells (**A**) and articular chondrocytes (**B**).

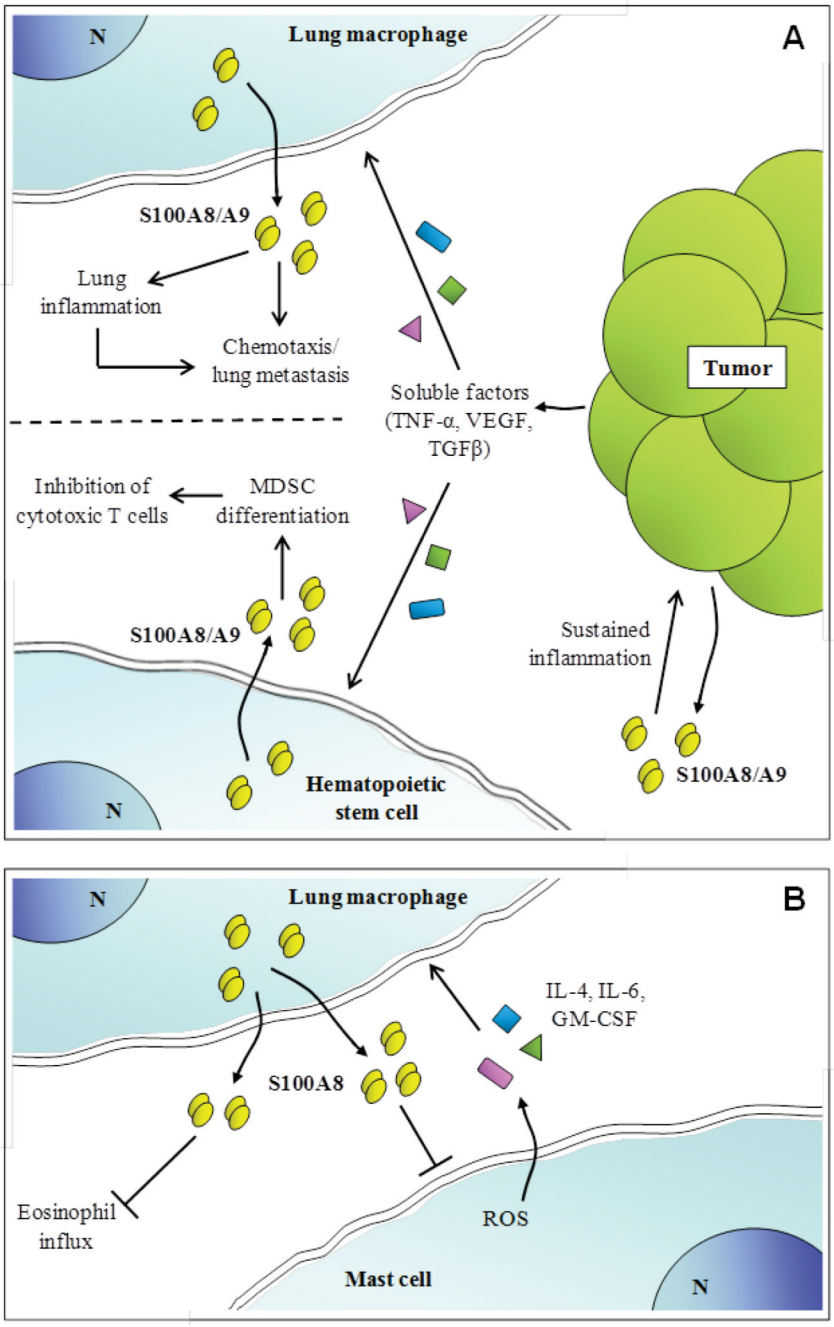


Fig. (9).

Schematic representation of proposed extracellular effects of S100A8/S100A9. (**A**) Activated by TNF-α, TGF-β and VEGF secreted by distant tumors, lung macrophages release S100A8/S100A9 that promotes local inflammation and attract metastatic cells thus promoting tumor cell homing in the lung. Moreover, S100A8/S100A9 might sustain inflammation in the original tumor site. (**B**) It is also suggested that S100A8/S100A9 released by hematopoietic stem cells under the action of TNF-α, TGF-β and VEGF might inhibit cytotoxic T cells thereby contributing to tumor growth by several mechanisms.

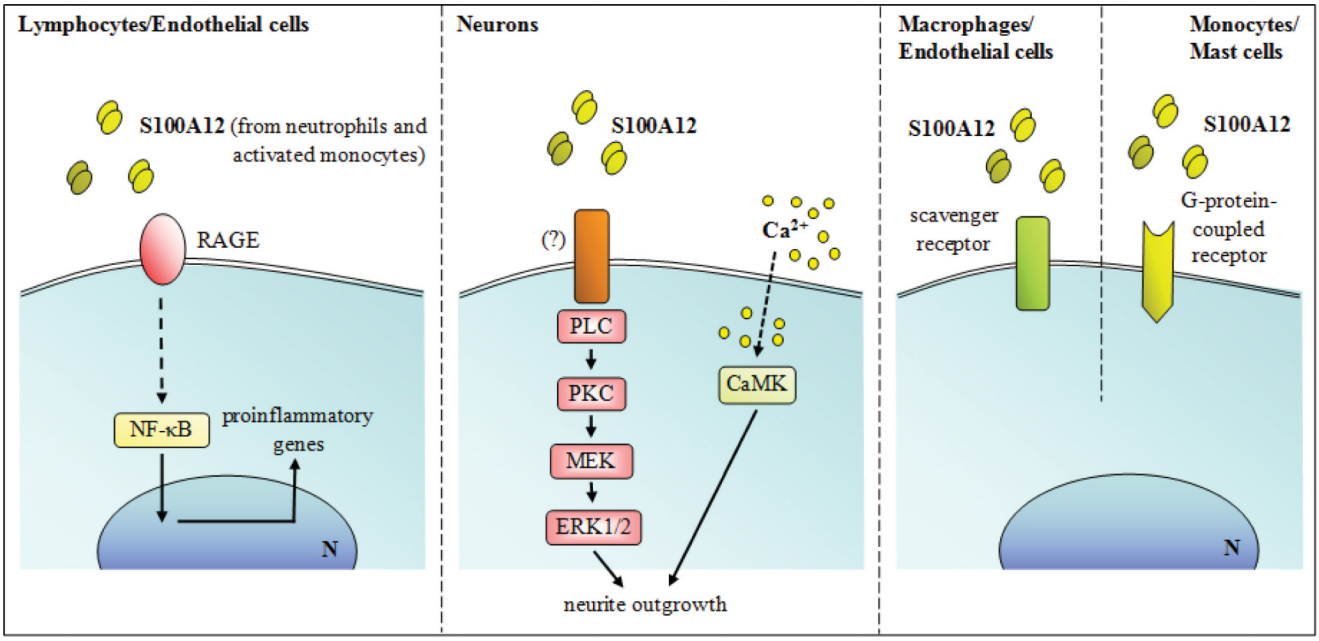


Fig. (10).
Schematic representation of proposed extracellular effects of S100A12 on lymphocytes, endothelial cells, neurons, and macrophages.

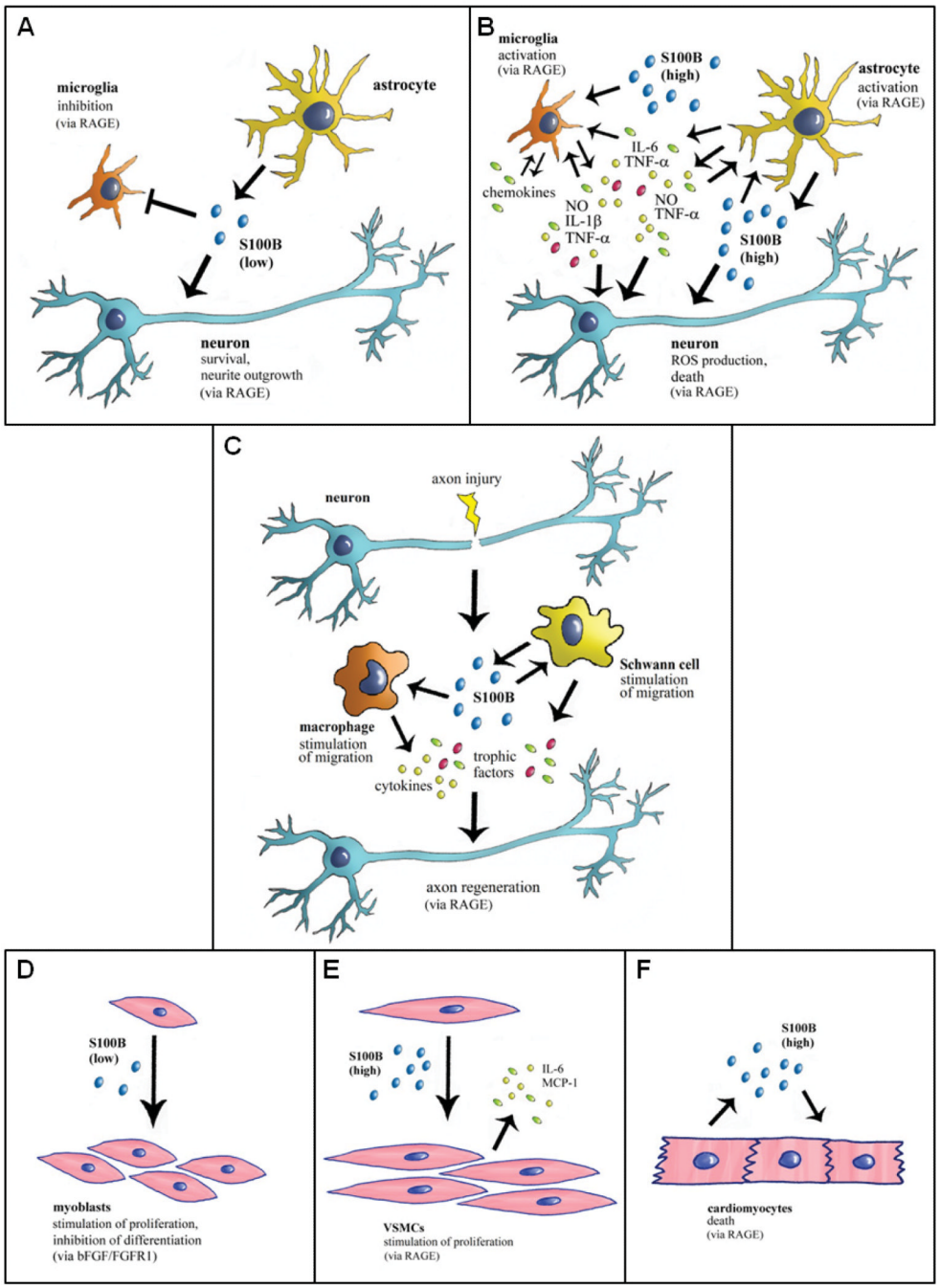


Fig. (11).

Schematic representation of proposed extracellular effects of S100B on neurons microglia, astrocytes, myoblasts, VSMCs, cardiomyocytes, and peripheral nerves. (**A**) In normal physiological conditions S100B secreted by astrocytes exerts trophic effects on neurons and modulates microglial activity by engaging RAGE. (**B**) When present in the brain extracellular space at high concentrations, S100B activates microglia and astrocytes thus participating in the inflammatory response and is toxic to neurons by excessively stimulating RAGE. (**C**) In acute peripheral nerve injury S100B released from activated Schwann cells promotes macrophages and Schwann cell migration and the release of trophic factors via

RAGE engagement thereby participating in peripheral nerve regeneration. (**D**) At subnanomolar-nanomolar doses S100B stimulates myoblast proliferation and inhibits myoblast differentiation by enhancing bFGF/FGFR1 signaling. However, in low-density myoblast cultures and at the very beginning of skeletal muscle regeneration, S100B engages RAGE in activated muscle satellite cells thereby stimulating proliferation and activating the myogenic program. (**E**) At high doses S100B stimulates VSMC proliferation and secretion of IL-6 and MCP-1 from VSMCs via RAGE engagement thus potentially participating in atherogenesis. (**F**) At high doses S100B causes cardiomyocyte apoptosis via RAGE engagement.