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REVIEW

S100B protein in tissue development, repair and regeneration

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Abstract

The Ca^{2+}-binding protein of the EF-hand type, S100B, exerts both intracellular and extracellular regulatory activities. As an intracellular regulator, S100B is involved in the regulation of energy metabolism, transcription, protein phosphorylation, cell proliferation, survival, differentiation and motility, and Ca^{2+} homeostasis, by interacting with a wide array of proteins (*i.e.*, enzymes, enzyme substrates, cytoskeletal subunits, scaffold/adaptor proteins, transcription factors, ubiquitin E3 ligases, ion channels) in a restricted number of cell types. As an extracellular signal, S100B engages the pattern recognition receptor, receptor for advanced glycation end-products (RAGE), on immune cells as well as on neuronal, astrocytic and microglial cells, vascular smooth muscle cells, skeletal myoblasts and cardiomyocytes. However, RAGE may not be the sole receptor activated by S100B, the protein being able to enhance bFGF-FGFR1 signaling by interacting with FGFR1-bound bFGF in particular cell types. Moreover, extracellular effects of S100B vary depending on its local concentration. Increasing evidence suggests that at the concentration found in extracellular fluids in normal physiological conditions and locally upon acute tissue injury, which is up to a few nM levels, S100B exerts trophic effects in the central and peripheral nervous system and in skeletal muscle tissue thus participating in tissue homeostasis. The present commentary summarizes results implicating intracellular and extracellular S100B in tissue development, repair and regeneration.

Key words: S100B; Cell proliferation; Cell differentiation; Cell survival; Cell motility; Development; Tissue homeostasis; Tissue repair; Tissue regeneration

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INTRODUCTION

S100B belongs to a multigenic family of small (mol. wt. between 9 kDa and 14 kDa) Ca^{2+}-binding proteins of the EF-hand type comprising more than 20 members exclusively expressed in vertebrates[1,2]. Like other members of this protein family, S100B is expressed in a cell-specific manner; astrocytes, oligodendrocytes, neural progenitor cells, certain neuronal populations, ependymocytes, Schwann cells, enteric glial cells, melanocytes, kidney epithelial cells, adipocytes, chondrocytes, skin Langerhans cells, a subpopulation of lymphocytes, muscle satellite cells, skeletal myofibers, pituitary folliculo-stellate cells and Leydig cells in the testis are the cell types with the

highest expression of S100B. However, at least cardiomyocytes, which normally do not express the protein, do express S100B post-infarction, and in several cell types S100B expression is upregulated in pathological conditions.

Within cells S100B exists in the form of a homodimer, sometime as an S100B-S100A1 heterodimer, in which the two subunits are arranged in an antiparallel fashion[1,3]. Like the majority of S100 members, S100B is a Ca^{2+} sensor protein that becomes activated by Ca^{2+} on the occasion of Ca^{2+} transients. Ca^{2+} induces a relatively large conformational changes in S100B C-terminal half resulting in the exposure of a hydrophobic patch through which the protein interacts with a wide array of target proteins (*e.g.*, enzymes, enzyme substrates, cytoskeletal proteins, adaptor/scaffold proteins, transcription factors, ion channels and ubiquitin E3 ligases) thereby regulating their activities. Thus, S100B is involved in the regulation of energy metabolism, transcription, protein phosphorylation, cell proliferation, survival, differentiation and locomotion, and Ca^{2+} homeostasis.

However, S100B can also exert extracellular effects being secreted by certain cell types (*e.g.*, astrocytes and adipocytes) or passively released by several cell types upon tissue injury. In this latter context S100B can be viewed as a damage-associated molecular pattern (DAMP) or alarmin, *i.e.*, a danger signal capable of activating cells of the innate immune system[1,3-6]. Extracellular effects of S100B mostly have been studied in the context of the central nervous system likely because of its high abundance in the brain and the identification of neurons, astrocytes and microglia as its target cells. Indeed, extracellular S100B has long been implicated in the pathophysiology of Alzheimer's disease and neuroinflammation largely via engagement of the receptor for advanced glycation endproducts (RAGE). However, accumulating evidence suggests that effects of extracellular S100B are not restricted to the brain or to cells of the innate immune system, and that RAGE may not be the sole receptor transducing S100B effects. In the present commentary we shall discuss results implicating intracellular and extracellular S100B in tissue development, homeostasis, repair and regeneration.

S100B IN CELL PROLIFERATION AND DIFFERENTIATION

S100B is involved in cell proliferation, survival and differentiation both as an intracellular regulator and an extracellular signal. Within cells S100B binds to and activates Ndr (nuclear Dbf2-related)[7], a serine/threonine protein kinase implicated in the regulation of cell division and morphology[8]. Regulation of Ndr by S100B involves a conformational change in the catalytic domain triggered by Ca^{2+}/S100B binding to the junction region[9]. However, although S100B-dependent activation of Ndr in cell lines has been documented[7] and in non-dividing and dividing cells S100B localizes to centrosomes[10], which are Ndr targets[8], no evidence has been presented that S100B-dependent activation of Ndr results in stimulation of cell proliferation and/or changes in cell morphology.

S100B also interacts with the tumor suppressor, p53, inhibiting its phosphorylation and tetramerization , *i.e.*, its activation[11-14]. Also, S100B reduces p53 levels[15], and in turn, p53 upregulates S100B expression in melanoma cells[15]. In this scenario, p53 would reduce its own abundance by upregulating its inhibitor, S100B, which would result in uncontrolled proliferation[15] and reduced apoptosis[16] at least in melanoma cells (Figure 1A). However, phosphorylation of specific serine and/or threonine residues in p53 reduces the affinity of the S100B-p53 interaction by an order of magnitude, and is important for protecting p53 from S100B-dependent downregulation[17]. Thus, the S100B overall effect on p53 is likely to reflect a balance between inhibitory cues and intervening biochemical events (*e.g.*, p53 phosphorylation). However, conflicting conclusions have been reported regarding functional implications of S100B/p53 interactions[15,18,19], and it is not known whether these interactions are relevant for tumor progression in other cancers and in non-neoplastic cells. In addition, it has been suggested that by interacting with the ubiquitin E3 ligases, MDM2 (HDM2) and MDM4 (HDM4)[17,20], that are central negative regulators of p53[21], S100B may actually promote p53 activities[20], which adds another layer of complexity to S100B-p53 interactions (Figure 1A). We have shown that forced expression of S100B in neuronal PC12 cells has no effects on p53 levels or nuclear translocation, and it results in enhanced proliferation and reduced differentiation and oxidative stress-induced apoptosis via activation of a PI3K/Akt/$p21^{WAF1}$/cyclin D1/cdk4/Rb/E2F pathway in the absence of serum mitogens[22] (Figure 1B). S100B-dependent reduction of stress-induced apoptosis may also occur via interaction with and activation of the tetratricopeptide repeat protein, PP5, a member of the PPP family of serine/threonine phosphatases[23].

S100B is expressed in proliferating myoblast cell lines[24] and quiescent muscle satellite cells[25], the most relevant stem cell population in adult skeletal muscle tissue[26]. Increasing S100B levels in myoblast cell lines results in no effects on the proliferation rate of asynchronously proliferating myoblasts; however, S100B-overexpressing myoblasts are more resistant to basal and H_2O_2-induced apoptosis in an IκB kinase β (IKKβ)/nuclear factor κB (NF-κB)-mediated manner[27] (Figure 1C). Thus, increasing S100B levels in myoblasts results in augmented cell numbers in consequence of their increased survival rate in stress conditions. Moreover, S100B-overexpressing myoblasts are less prone to acquire mitotic quiescence and proliferate faster than control cells upon re-exposure to serum mitogens after quiescence[27]. Proliferation of muscle satellite cells and their resistance to death-inducing stimuli are critical for efficient muscle regeneration as well as for successful cell therapy of muscular dystrophy[26,28-30]. Thus, intracellular S100B may contribute to muscle regeneration by reducing apoptosis

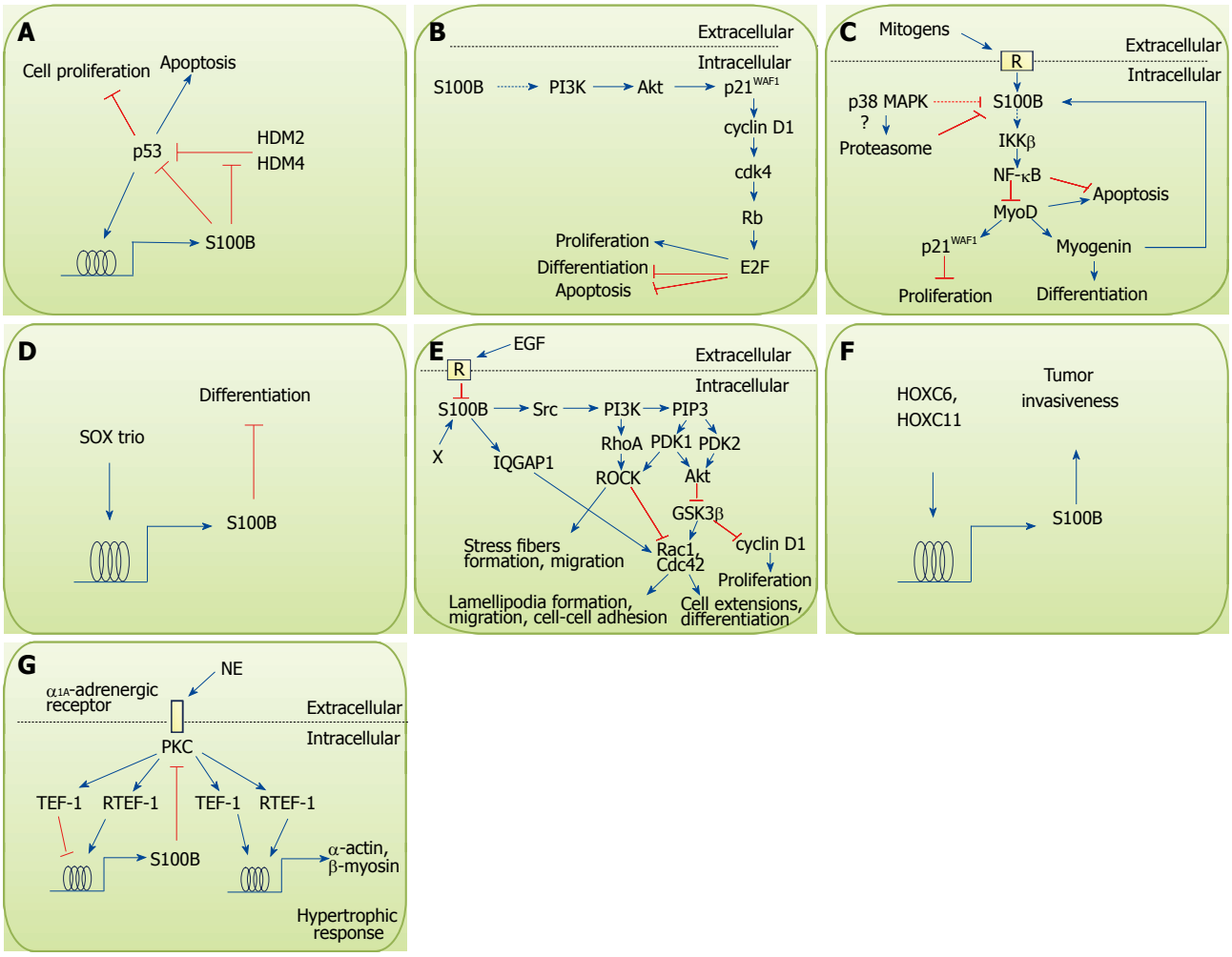

Figure 1 Effects of intracellular S100B on cell proliferation, differentiation, survival and motility. A: Schematics of S100B-p53 interactions in melanoma cells. p53 induces S100B that in turn blunts p53 inhibitory effects on proliferation and stimulatory effects on apoptosis. However, S100B interaction with the ubiquitin E3 ligases, HMD2 and HMD4, may inhibit HMD2/HMD4-dependent reduction of p53 levels; B: Expression of S100B in PC12 neuronal cells results in stimulation of proliferation and inhibition of NGF-induced differentiation and oxidative stress-induced apoptosis *via* activation of the PI3K/Akt pathway; C: Intracellular S100B protects myoblasts against oxidative stress-induced apoptosis and inhibits differentiation *via* activation of the IκB kinase β/ nuclear factor κB (NF-κB) pathway. Also, early after the transfer of myoblasts from growth medium to differentiation medium S100B becomes downregulated by the decrease in serum mitogens and activation of the promyogenic p38 MAPK, that likely stimulates S100B proteasomal degradation. However, S100B becomes re-expressed in differentiated myoblasts under the action of myogenin; D: S100B is induced in chondroblasts by the SOX trio and inhibits differentiation; E: S100B, induced in astrocytic progenitors by an unidentified mechanism (X), interacts with and activates a Src/PI3K pathway that stimulates RhoA/ROCK thereby promoting stress fiber formation and cell migration and Akt thereby inhibiting GSK3β resulting in stimulation of proliferation and inhibition of differentiation. Interaction of S100B with IQGAP1 results in activation of Rac1 responsible for lamellipodia formation during migration. The S100B/IQGAP1/Rac1 interaction may also results in an enhancement of cell-cell adhesion as observed in neurospheres (see text). EGF represses S100B expression during early phases of astrocyte differentiation, which appears to be permissive for astrocytic terminal differentiation. Whether such a mechanism also is operating in cerebellar granule cell progenitors remains to be determined; F: S100B, induced in breast cancer cells by HOXC6 and HOXC11, contributes to tumor invasiveness; G: S100B is induced in post-infarction cardiomyocytes by the norepinephrine/α1A-adrenergic receptor/PKC axis and inhibits hypertrophic response *via* inhibition of PKC

and stimulating the expansion of activated satellite cells (Figure 1C). However, excess expression of S100B in activated satellite cells may be detrimental because its mitogenic effect might interfere with the reconstitution of the satellite cell reserve pool which normally occurs during muscle regeneration and requires that a fraction of cells stop proliferating and enter a quiescent state[26,28,29], and because myoblast proliferation and differentiation are mutually exclusive[26]. Considering that S100B is expressed in high abundance in several cancers[2,3], enhanced expression of S100B in activated muscle satellite cells, from which embryonal rhabdomyosarcomas are thought to originate[31], may also contribute to rhabdomyosarcomagenesis. Preliminary results show that embryonal rhabdomyosarcoma cells do indeed express elevated S100B levels (Riuzzi F, Sorci G, and Donato R, unpublished results).

Collectively, these data suggest that intracellular S100B may intervene in the regulation of proliferation, survival and apoptosis by mechanisms that vary depending on the cell type, the context and, probably, the cell's normal or neoplastic condition. Further work is required to definitely establish the role of S100B in cell proliferation and survival in normal and neoplastic cells and the

molecular mechanism(s) behind S100B overexpression in many cancers.

Intracellular S100B also functions as an inhibitor of differentiation. As mentioned above, expression of S100B in PC12 neuronal cells results in impaired NGF-induced differentiation via activation of a PI3K/Akt/$p21^{WAF1}$/cyclin D1/cdk4/Rb/E2F pathway[22] (Figure 1B). However, induction of S100B expression in NGF-differentiated PC12 neuronal cells does not reverse the differentiated phenotype[22]. Also, S100B is induced in early-stage chondroblast differentiation by the SOX trio and negatively regulates chondrocyte terminal differentiation via an as yet undetermined mechanism[32] (Figure 1D). Interestingly, S100B expression in astrocytic cells is developmentally regulated albeit with different characteristics depending on whether subventricular or cortical astrocytic cells are considered[33]. These studies[33] have established that during the time interval between post-natal days 2 and 8 ramified, differentiating (*i.e.*, GFAP filament-positive) astrocytes are S100B-negative. This suggests that during that time interval S100B may be downregulated and that the protein becomes re-expressed during the final phase(s) of astrocytic differentiation. S100B is expressed in radial glial precursors[34], in the ventricular zone of embryonic mouse cerebellum and progenitors of cerebellar granule cells[35], the protein being expressed in these latter cells as long as they are migrating. S100B interacts with the small GTPase Rac1 and Cdc42 effector, IQGAP1, at the polarized leading edge and areas of membrane ruffling in astrocytoma cell lines[36]. Hence, S100B has been proposed to regulate IQGAP1 activity in relation to cell migration (Figure 1E). In accordance with this view, reduction of S100B levels in astrocyte cell lines and primary astrocytes results in decreased proliferation and migration and acquisition of a differentiated phenotype (*i.e.*, stellation) consequent to reduced activity of a Src/PI3K/RhoA/ROCK pathway and increased activity of the GSK3β/Rac1 module[37] (Figure 1E). These results are consistent with the possibility that repression of S100B expression at certain phases of development of astrocytes and certain neuronal populations may be functionally linked to their differentiation. Thus, S100B may contribute to expand the population of progenitors of neural cells and confer migratory capacity on undifferentiated astrocytes and neuroblasts, and S100B expression has to be repressed for differentiation to take place. In this context, S100B may act to avoid premature differentiation besides promoting cell migration; however, deregulated S100B expression may contribute to gliomagenesis. Intriguingly, knockdown of S100B in the Müller cell line, MIO-M1, results in remarkably inhibited neurosphere formation and differentiation of these cells towards the astrocyte phenotype[37]. Because MIO-M1 neurospheres have been shown to be made of neural precursor cells differentiating towards a neuronal phenotype when cultivated in the presence of bFGF or retinoic acid[38], the results in[37] suggest that S100B may contribute to confer stem cell-like properties on MIO-M1 cells and to reduce their propensity to differentiate into astrocytes. The expression of S100B in the murine cerebellar ventricular zone including the embryonic cerebellar rhombic lip and in cells lining cerebral ventricles[33-35] adds to the possibility that intracellular S100B may contribute to confer pluripotency on precursors of neural cells. Incidentally, the studies in[36,37] highlight S100B's ability to regulate F-actin-based cytoskeleton in an indirect manner, *i.e.*, *via* stimulation of a Src/PI3K/RhoA/ROCK and an IQGAP1/Rac1 pathway, and reduction of the activity of the GSK3β/Rac1 module (Figure 1E), as opposed to the protein's direct effects on microtubule- and intermediate filament-based cytoskeleton[39-43].

On the other hand, HOXC6 and HOXC11, members of homeobox genes that encode transcription factors driving morphogenesis and cell differentiation during embryogenesis[44,45], have been reported to increase transcription of *s100b* in neuroblastoma cells[46] and this was interpreted as indicative of HOXC6 and HOXC11 stimulating differentiation of neuroblastoma cells into Schwann cells through the transcriptional activation of *s100b*. However, in the absence of data on the expression of additional markers such as myelin basic protein or GFAP, the expression of *s100b* may not be itself a proof of cell differentiation towards Schwann cells, oligodendrocytes or astrocytes[22,35,37]. Also, interactions of HOXC11 with the steroid receptor coactivator protein SRC-1, which is a strong predictor of reduced disease-free survival in breast cancer patients, induce the expression of S100B in resistant breast cancer cells[47] (Figure 1F). This latter study supports the notion that expression of S100B in proliferating and/or tumor cells may interfere with differentiation and/or is mechanistically linked to tumor progression. This study[47] also highlights the fact that S100B can be induced in precursors of certain cell types (breast cells, in the present case) and becomes repressed at completion of differentiation; differentiated breast cells do not express the protein whereas persistence of S100B in breast cell precursors may concur to tumor progression and invasion.

S100B is induced in post-infarction cardiomyocytes under the action of norepinephrine and phenylephrine *via* protein kinase C activation thereby limiting the hypertrophic response through the inhibition of the expression of the fetal proteins, skeletal α-actin and β-myosin heavy chain[48-50] (Figure 1G). Accordingly, norepinephrine-induced cardiac hypertrophy is inhibited in S100B transgenic mice[51]. Thus, S100B, which is not expressed in cardiomyocytes in normal physiological conditions, participates in the regulation of cardiomyocyte remodeling after infarction. These results appear in line with the notion that S100B is expressed in cells exhibiting properties of immature cells (post-infarction cardiomyocytes, in the present case). However, similarly to the majority of neuronal cells, in which S100B becomes stably repressed before differentiation, and differently from astrocytes (see above) and myoblasts (see below), in which a transient downregulation of S100B occurs at the beginning of dif-

ferentiation, full maturation of cardiomyocytes is accompanied by stable repression of S100B expression.

Intracellular S100B modulates the differentiation of myoblasts, the precursors of skeletal myofibers. Indeed, overexpression of S100B in myoblasts blocks myogenic differentiation *via* IKKβ/NF-κB-mediated inhibition of expression of the muscle-specific transcription factor, MyoD, and the MyoD-downstream effectors myogenin and $p21^{WAF1}$, and conversely, reduction of S100B expression in myoblasts by siRNA techniques results in reduced NF-κB activity and enhanced myogenic differentiation[25] (Figure 1C). It is known that NF-κB is a negative regulator of myogenic differentiation *via* inhibition of expression and/or reduction of stability of MyoD[52-54]. Also, S100B binds to, and inhibits EAG1 potassium channels Ca^{2+}-dependently[55]. Because these channels have been reported to play a role in myoblast fusion into myotubes[56] it is possible that S100B may negatively affect myoblast differentiation *via* inhibition of EAG1 potassium channels as well. Moreover, compared with young subjects, muscle satellite cells from aged human subjects, which are known to be proliferation and differentiation defective[29,57], express higher levels of S100B and knockdown of S100B in aged satellite cells rescues their myogenic potential in part[58]. Notably, despite their high S100B levels, aged muscle satellite cells show a low proliferation rate and a remarkably reduced ability to secrete S100B and bFGF[58]. However, treatment of aged satellite cells with S100B or bFGF rescues their proliferative potential in part[58]. These results suggest that physiological levels of S100B in activated satellite cells and the satellite cells' ability to secrete the protein concur to optimize the expansion of activated satellite cells required for satellite cell homeostasis, the maintenance of optimal muscular mass and/or efficient skeletal muscle regeneration after acute injury. In this context it is noteworthy that aged human satellite cells also exhibit altered expression of RAGE[58] shown to exert promyogenic effects[59-61] and to be required for S100B secretion[62]. Because transient transfection of aged satellite cells with full-length RAGE rescues their myogenin potential in part[58], one may speculate that the combination of enhanced S100B expression and expression of an altered form of RAGE may contribute significantly to their reduced myogenic potential, hence to sarcopenia. The recent demonstration that levels of bFGF are high and are responsible for disrupted satellite cell quiescence in aged skeletal muscle in homeostatic conditions[63] lend support to the possibility that excess S100B in aged satellite cells, potentially caused by high bFGF[1,3] may ultimately lead to defective muscle regenerative capacity as observed in sarcopenia. Interestingly, levels of S100B decrease in non-fused myoblasts early after their transfer to differentiation medium and S100B becomes re-expressed in differentiating (*i.e.*, myogenin-positive) myocytes[25,27], which supports the notion that S100B levels have to decrease transiently in certain cell types for they to differentiate. Both differentiation cues (namely the activation of the promyogenic p38 MAPK) and reduction of mitogens appear to determine the transient downregulation of S100B in myoblasts in differentiation medium *via* transcriptional and post-translational (proteasome-dependent) mechanisms[27] (Figure 1C). Collectively, these results suggest that S100B in myoblasts contributes to reduce their premature differentiation which would be detrimental to skeletal muscle regeneration after acute injury, and that levels of S100B should be kept within a certain range of abundance in order to avoid excessive expansion of activated satellite cells leading to defective reconstitution of the damaged tissue and the pool of quiescent satellite cells.

Whereas EGF has been reported to reduce S100B expression in developing astrocytes[33] (Figure 1E), the extracellular stimuli and intracellular mechanisms causing transient or stable downregulation of S100B expression during cell differentiation are not completely defined. Also, because mature astrocytes, chondrocytes, myocytes (*i.e.*, differentiated myoblasts), skeletal myofibers and certain neuronal populations in the adult brain express S100B[3,25,64], mechanisms should exist that cause re-expression of the protein at later developmental stages without determining cell de-differentiation[22,27]. In the case of skeletal muscle cells, the muscle-specific transcription factor, myogenin, that is essential for myogenic differentiation[26], has been implicated in the re-expression of S100B in myocytes[27] (Figure 1C). Overall, these observations suggest that functions of S100B may be different in developing and mature cells and that S100B may regulate different signaling pathways and functions depending on the cell type and the cell's status. Future work should dissect the molecular mechanism(s) responsible for the regulation of S100B expression in immature (proliferating) and fully differentiated cells.

Extracellular S100B also regulates cell proliferation, survival and differentiation. Several factors/conditions regulate either positively or negatively S100B secretion by astrocytes, among which are interleukin-1β, extracellular levels of Ca^{2+} and K^{+}, inhibitors of gap junctions, antioxidants, lipopolysaccharide, apomorphine and certain antipsychotic drugs[65-69]. At the low nM concentration found in the brain extracellular space in normal physiological conditions[3], S100B exerts pro-survival effects on neurons[70-73], stimulate astrocyte proliferation[74] and reduce microglial reactivity[75,76], *via* RAGE engagement in most cases (Figure 2). However, at low nM levels S100B synergizes with proinflammatory cytokines to activate microglia[77] suggesting that S100B may switch from antiinflammatory to proinflammatory at early phases of neuroinflammation (*i.e.*, in the presence of low levels of inflammatory cytokines). Yet, attenuation of microglia activity by low concentrations of S100B may contribute to local tumor immunosuppression[76].

S100B has been implicated in the activity of antidepressants. The selective serotonin reuptake inhibitor, fluoxetine, increases S100B content in the hippocampus[78] and stimulates S100B secretion from astrocytes[79] and serotoninergic neurons[80]. It has been shown that

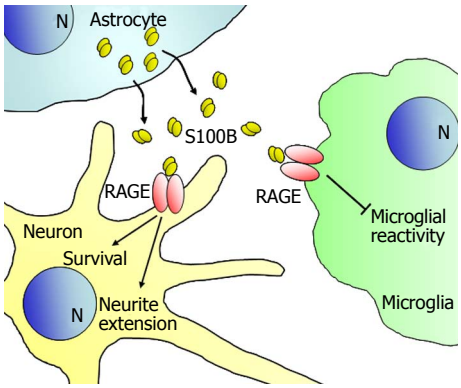

Figure 2 Schematics of effects of low concentrations of S100B on neuronal and microglial cells. By engaging receptor for advanced glycation end-products, S100B exerts trophic effects on neurons and reduces microglia reactivity. RAGE: Receptor for advanced glycation end-products.

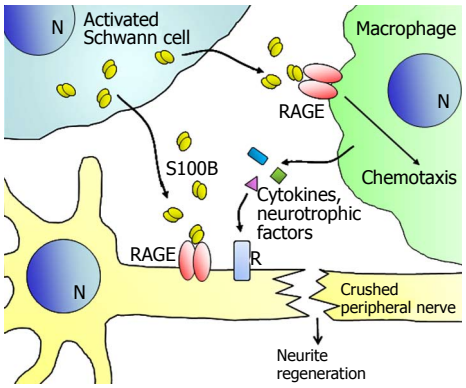

Figure 3 Schematics of effects of low concentrations of S100B on axonal regeneration of crushed peripheral nerves. S100B secreted/released from activated Schwann cells stimulates recruitment of Schwann cells and macrophages to the injury site and release of cytokine and trophic factors leading to axonal regeneration, in a receptor for advanced glycation end-products -dependent manner. RAGE: Receptor for advanced glycation end-products.

secreted S100B downregulates microRNA-16 in noradrenergic neurons, which consequently acquire properties of serotoninergic neurons[80]. Although no information is available regarding the mechanism whereby fluoxetine induces serotoninergic neurons to express and secrete S100B, the mechanism whereby secreted S100B reduces microRNA-16 levels in noradrenergic neurons or the S100B-serotoninergic neuron relationships in S100B-null or transgenic mice, these results point to an important role of extracellular S100B in fluoxetine-dependent neurogenesis and neuronal plasticity[81,82].

Serum levels of S100B increase remarkably following an intense physical exercise[83,84], the source of the protein reasonably being skeletal myofibers in these circumstances. Indeed, intense physical exercise is associated with reversible skeletal muscle tissue damage and release of intracellular proteins[26], and the local concentration of S100B may be even higher than in serum thus allowing paracrine S100B effects on activated muscle stem (satellite) cells. In fact, at picomolar to low nanomolar concentrations S100B inhibits myoblast differentiation and stimulates myoblast proliferation[85-87] raising the possibility that the protein may participate in the process of skeletal muscle regeneration by expanding the myoblast population (see below).

S100B IN TISSUE REGENERATION

Since the discovery that a protein factor purified from brain and endowed with neurite extension activity was a disulfide cross-linked form of S100B[88] and the demonstration that S100B is found in the brain extracellular space[89] and is actively secreted by astrocytes[90], a mess of information has been provided over time on the protective and trophic role of S100B on neurons[70-73,91-98](Figure 2). S100B is found expressed in Schwann cells in uninjured peripheral nerves as well as in activated Schwann cells during the degeneration period of crushed nerves, *i.e.*, up to day 7 post-injury, and in normal Schwann cells reappearing during the regeneration period, *i.e.*, after day 7 post-injury, in the zone of the crush and proximal and distal to it[99]. In similar conditions, RAGE becomes expressed in axons and in infiltrating mononuclear phagocytes and reduction of RAGE expression and/or activity results in suppression of anatomical regeneration and functional recovery[100,101]. Upon acute peripheral nerve injury, S100B released from Schwann cells in damaged nerves activates RAGE in infiltrating macrophages[100,101] and in activated Schwann cells[102]; infiltrating macrophages exert beneficial effects by clearing cell debris and dead neutrophils and releasing cytokines and trophic factors, whereas activated Schwann cells release cytokines and neurotrophic factors shown to be crucial for the repair of injured nerves (Figure 3). S100B-activated RAGE promotes Schwann cell migration during the course of repair of injured peripheral nerves through the induction of thioredoxin interacting protein and activation of p38 MAPK, CREB and NF-κB[102]. S100B also stimulates proliferation and differentiation of neural progenitor cells from the subventricular zone of the adult mouse brain *via* RAGE activation[103]. These results complement the long-standing notion that S100B stimulates neuronal cell survival and differentiation *via* RAGE engagement[71,72].

S100B also is expressed in skeletal myofibers[25,64] from which it is massively released early upon acute injury with declining release during the regeneration phase[104] (Figure 4A). Released S100B stimulates myoblast proliferation and concomitantly activates the myogenic differentiation program *via* RAGE engagement early after injury (Figure 4B), *i.e.*, at a time when myoblast density and the level of released bFGF are low[104], thereby contributing to the timely and limited expansion of the myoblast population required for efficient muscle regeneration. Indeed, acutely injured *Rage*$^{-/-}$ muscles show delayed regeneration[61]. However, persistence of extracellular S100B in the damaged tissue is likely to prolong the myoblast proliferation phase at the expense of differentiation and reconstitution of the pool of quiescent satellite cells *via* enhancement of bFGF/FGFR1 signaling and blockade of RAGE

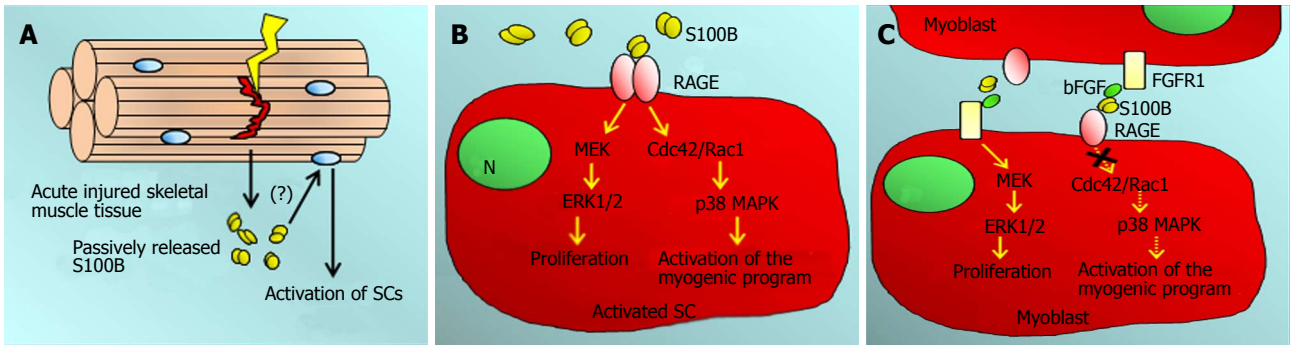

Figure 4 Effects of S100B in skeletal muscle regeneration. A: S100B is passively released from acutely injured skeletal muscle tissue early after injury. Whether S100B activates quiescent muscle satellite cells (SCs) is not known (?); B: Released S100B may stimulate myoblast proliferation and simultaneously activate the myogenic program *via* receptor for advanced glycation end-products (RAGE) engagement, during the next few days post-injury (early regeneration phase); C: However, during the intermediate regeneration phase (*i.e.*, from day 3 to day 7 post-injury, in coincidence with the peak of released bFGF and the myoblast proliferation phase), S100B may enhance bFGF-FGFR1 mitogenic signaling thereby contributing to expand the myoblast population while simultaneously inactivating its canonical receptor, RAGE. RAGE: Receptor for advanced glycation end-products.

signaling[87,104] (Figure 4C). The switch of S100B from a RAGE-activating factor to a bFGF/FGFR1 activating factor depends on the S100B concentration, the presence of bFGF and myoblast density[87,104]. Current findings indicate that neutralization of released S100B in acutely injured wild-type skeletal muscles results in defective regeneration as a consequence of reduced expansion of the population of activated satellite cells, reduced infiltration of the injured tissue with macrophages and delayed transition of macrophages from the M1 (proinflammatory) to the M2 (antiinflammatory) phase (Riuzzi F, Sorci G, Beccafico S and Donato R, in preparation). These results indicate that released S100B participates in the regeneration of acutely injured muscles by stimulating myoblast proliferation, macrophage infiltration and macrophage transition from a proinflammatory phenotype to an antiinflammatory phenotype. Our ongoing results also show that these effects of S100B are strictly RAGE-dependent, because neutralization of released S100B in acutely injured *Rage*$^{-/-}$ muscles does not change the muscle regeneration pattern described in[61]. However, one may anticipate that chronic release of S100B from skeletal myofibers in, *e.g.*, muscular dystrophies and chronic inflammatory muscle diseases may translate into high local S100B concentrations amplifying or perpetuating muscle damage, a situation reminiscent of what occurs in the brain where low S100B levels are beneficial whereas chronically high S100B levels are detrimental, *via* RAGE engagement in both cases[1,3,71,72].

Also, cell/tissue identity appears to profoundly condition S100B's extracellular effects. For example, whereas at concentrations $\leqslant$ 50 nmol/L S100B exerts trophic effects on neuronal and astrocytic cells and skeletal myoblasts[3,105,106], at doses $\geqslant$ 50 nmol/L the protein causes RAGE-dependent cardiomyocyte apoptosis[107]. However, a short-term (1 h) treatment of cardiomyocytes with S100B (100 nmol/L) (a condition insufficient to cause apoptosis) results in a RAGE-dependent secretion of vascular endothelial growth factor (VEGF) which in turn induces myofibroblast proliferation[108]. By this mechanism S100B might contribute to post-infarction scar formation, a kind of tissue reparative process. Whether S100B also causes VEGF-dependent post-infarction neoangiogenesis remains to be investigated. Intriguingly, whereas S100B is induced in the heart of diabetic mice as well, S100B mRNA and protein expression levels decrease in diabetes post-infarction by a mechanism that remains to be identified, and deletion of *s100b* has a deleterious effect on cardiac function in this condition partly attributed to increased ventricular dilation associated with increased AGE formation and reduced GLUT4 expression, *i.e.*, reduced cardiac glucose metabolism[109]. Whether these changes are due to reduced intracellular or extracellular effects of S100B is not known. Yet, these results point to a protective role of S100B in post-infarction heart.

S100B IN RESOLUTION OF INFLAMMATION

The role of extracellular S100B as a DAMP involved in inflammation is an accepted notion (see Refs.[1,3-6,106] for pertinent literature). However, for S100B to sustain inflammation *via* activation of macrophages/microglia it has to be present at relatively high concentration at injury sites[1,3-6,106,110], as it reasonably occurs during the course of chronic tissue damage as a result of a continuous release of the protein from injured cells, cell necrosis and/or defective clearance. However, recent evidence points to a novel role of S100B in resolution of inflammation in *Aspergillus fumigatus* infection in lung[111]. TLR2 activation on bronchial epithelial cells by the fungus results in upregulation of expression and release of S100B, that paracrinally binds to RAGE on polymorphonuclear neutrophils and mediates its association with TLR2 for subsequent inhibition. In addition, S100B upon binding to nucleic acids in bronchial epithelial cells, also activates an intracellular TLR3/TLR9/TRIF-dependent pathway leading to repression of *s100b* transcription. The transcriptional repression of *s100b* by the sequential action of down-

stream MyD88- and TRIF-dependent NF-κB signaling pathways[111] thus provides the molecular basis for a braking circuit in infection whereby the endogenous danger protects the host against pathogen-induced inflammation and a nucleic acid-sensing mechanism resolves danger-induced chronic inflammation. Whether this is a general mechanism of action of the S100B/RAGE axis in the course of infections remains to be determined. However, high local S100B concentrations exacerbates *Aspergillus fumigatus*-induced pulmonary inflammation[111] likely *via* sustained stimulation of RAGE signaling. Interestingly, the *S100B* (+427C/T) polymorphism results in S100B overexpression which associates with susceptibility to invasive aspergillosis in patients undergoing hematopoietic stem cell transplantation whenever the recipients show *RAGE* (-374T/A) polymorphism resulting in RAGE overexpression[112].

CONCLUDING REMARKS

During the last decade there has been a burst of interest in S100B functions[3,6,106] following the seminal demonstration that S100B engages RAGE in immune cells and behaves like a DAMP[113]. Evidence has been provided shortly after that both the neurotrophic and neurotoxic effects of low and high S100B levels, respectively, on neuronal cells[1,3], are mediated by RAGE engagement[71]. However, S100B mostly has been viewed as a DAMP involved in the inflammatory response and S100B often has been used as a generic RAGE activator in the context of the inflammatory response[3,6,113].

Yet, a large body of information indicates that S100B protein is involved in cell proliferation, survival, motility and differentiation by acting as an intracellular regulator and an extracellular signal in normal physiological conditions and during the acute phase of tissue damage. In so doing, S100B may play a role in tissue development and repair after acute injury, through the refinement or fine tuning of enzyme activities, the dynamics of the cytoskeleton and cell-specific gene expression, and responses to external stimuli. Moreover, S100B exerts anti-infection effects in the bronchial epithelium where a tight regulation of its expression and release is mechanistically linked to the resolution of inflammation after fungal infection. Future work should assess the molecular mechanism(s) regulating S100B expression in developing and mature cells and during tissue repair/regeneration.

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