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The many faces of S100B protein: when an extracellular factor inactivates its own receptor and activates another one

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Summary

The Ca^{2+}-binding protein of the EF-hand type, S100B, is an intracellular regulator and an extracellular signal. Within cells S100B interacts with several proteins thereby regulating energy metabolism, Ca^{2+} homeostasis, protein phosphorylation and degradation, and cell locomotion, proliferation and differentiation. Once secreted/released, S100B exerts autocrine and paracrine effects on responsive cells by engaging the receptor for advanced glycation end products. However, recent evidence suggests that S100B might also activate basic fibroblast growth factor receptor 1 via prior binding to basic fibroblast growth factor.

Key words

S100B, proliferation, differentiation, RAGE, FGFR1, signaling pathways

Introduction

S100B protein belongs to the S100 family of Ca^{2+}-binding proteins of the EF-hand type exclusively expressed in vertebrates (Donato et al., 2009). S100B expression is restricted to a limited number of cell types where it is found diffusely in the cytoplasm and associated with intracellular membranes, microtubules and type III intermediate filaments. S100B is a Ca^{2+} sensor protein: upon binding of Ca^{2+}, S100B undergoes a conformational change resulting in the exposure to the solvent of a hydrophobic patch through which it interacts with target proteins thereby regulating their functions. Certain cell types (e.g. astrocytes and adipocytes) secrete S100B and all S100B-expressing cells passively release the protein in case of damage and/or necrosis. Once outside the cell, S100B exerts autocrine and paracrine effects mostly, though not exclusively, by engaging the receptor for advanced glycation end products (RAGE) on responsive cells (Donato, 2007). Therefore, S100B functions as an intracellular regulator as well as an extracellular signal.

Intracellular S100B

Within cells, S100B regulates enzyme activities, the state of assembly of cytoskeleton components, transcription factors, Ca^{2+} homeostasis, protein phosphorylation

and degradation, and cell locomotion, proliferation and differentiation (Donato et al., 2009). S100B has been long implicated in cell proliferation and differentiation with scant information about the underlying molecular mechanisms, though. We have investigated this latter issue using three cell systems, i.e. PC12 neuronal cells, astrocyte cell lines and myoblast cell lines.

We choose PC12 neuronal cells because they do not express S100B. PC12 cells that were forced to express S100B proliferated at a higher rate, compared to controls, and were unresponsive to the differentiating activity of nerve growth factor (NGF) (Arcuri et al., 2005). In PC12 cells S100B activated a PI3K/Akt/$p21^{WAF1}$/cyclin D1/cdk4/Rb/E2F pathway responsible for enhanced cell proliferation, and reduced apoptosis and sensitivity to NGF.

Astrocytes express relatively high amounts of S100B (Donato et al., 2009). Reducing S100B expression levels in astrocyte cell lines by transient transfection with S100B small interfering (si) RNA resulted in a modest decrease in cell proliferation, acquisition of a differentiated phenotype, and decreased migration (Brozzi et al., 2009). Contrary to control cells that exhibited stress fibers and little or no GFAP filaments, S100B siRNA-transfected astrocytes showed dissolution of stress fibers, collapse of F-actin onto the plasma membrane and GFAP filaments. S100B interacted with and activated Src kinase that in turn activated PI3K: this latter activated the RhoA/ROCK module thus promoting the formation of stress fibers (which are responsible for cell locomotion), and Akt thus inhibiting the GSK3β/Rac1 module (which is responsible to the formation of cell extensions). Thus, as long as S100B was expressed at relatively high levels astrocytes maintained a migratory capacity and did not differentiate. Combined with *in vivo* data (Raponi et al., 2007), our results suggest that reduction of S100B expression levels might be permissive for astrocyte precursor cells to differentiate, and astrocytes resume expressing the protein at completion of the differentiation program. The molecular mechanisms regulating the reduction of S100B expression at the beginning of astrocyte differentiation and resumption of S100B expression in differentiated astrocytes remain to be elucidated.

Myoblast cell lines express moderate amounts of S100B (Donato et al., 2009). Overexpression of S100B in myoblasts resulted in reduction of differentiation (i.e., absence of expression of the muscle-specific transcription factors, MyoD and myogenin, and no myotube formation), whereas reduction of S100B expression levels by RNA interference resulted in enhanced differentiation (Tubaro et al., 2010). S100B activated the IKKβ/NF-κB module thereby inhibiting MyoD and, hence, myogenin expression. It is known that NF-κB inhibits MyoD expression in myoblasts (Guttridge et al., 2000; Bakkar et al., 2008). S100B is expressed in skeletal muscle satellite cells (Tubaro et al., 2010), i.e. the adult stem cells of muscle tissue that play a fundamental role in muscle regeneration (Chargé and Rudnicki, 2004); thus the protein might play a physiological role in the regulation of differentiation of activated satellite cells following muscle damage.

Extracellular S100B

Astrocytes secrete S100B constitutively, and S100B secretion can be stimulated or reduced by a number of factors/conditions (Donato et al., 2009). S100B has been long associated with promotion of neuronal survival and neurite extension at low,

physiological concentrations, and to cause neuronal death at high (i.e., submicromolar/micromolar) concentrations. All these effects require S100B interaction with and stimulation of RAGE (Huttunen et al., 2000). RAGE is a multiligand receptor of the immunoglobulin superfamily involved in the innate immune response; however, RAGE also plays a role in tissue development (Rauvala and Rouhiainen, 2010). Also, low S100B protects neuronal cells against β-amyloid toxicity via RAGE engagement (Businaro et al., 2006). However, high S100B concentrations cause excessive RAGE stimulation in neuronal cells resulting in overproduction of reactive oxygen species (ROS), the ultimate cause of apoptosis (Huttunen et al., 2000).

Extracellular S100B plays a role in neuroinflammation. Indeed, S100B was shown to activate microglia, the brain resident macrophages, albeit at high concentrations only. In the presence, but not absence of bacterial endotoxin or INF-γ, S100B upregulated inducible nitric oxide (NO) synthase and stimulated NO release (Petrova et al., 2000; Adami et al., 2001, 2004). However, S100B upregulates the expression of the pro-inflammatory enzyme, COX-2, in microglia and stimulates the release of IL-1β and TNF-α, in the absence of co-factors in a RAGE-dependent manner (Bianchi et al., 2007, 2010). Also, at non pro-inflammatory doses, S100B synergizes with non pro-inflammatory doses of IL-1β and TNF-α to upregulate COX-2 expression (Bianchi et al., 2010). Current work suggests that high S100B stimulates microglial chemotaxis via RAGE-dependent induction and secretion of certain chemokines (Bianchi R., Kastrisianaki E., Giambanco I. and Donato R., submitted for publication). The participation of S100B in neuroinflammation is supported by findings by other research groups (for review see Donato et al., 2009). In this context, S100B exhibits properties of a damage associated molecular pattern (DAMP) factor, signaling tissue damage and participating in the inflammatory response. However, S100B is not just a DAMP factor as at low concentrations it exerts trophic effects on neurons (Huttunen et al., 2000) and counteracts the pro-inflammatory activity of the neurotoxin, trimethyltin, on astrocytes and microglia (Reali et al., 2005).

Extracellular S100B also plays a role in myogenesis. S100B inhibits myoblast differentiation (Sorci et al., 2003) and stimulates the proliferation of myoblasts and reduces their apoptosis (Riuzzi et al., 2006). Surprisingly, these effects are independent of RAGE signaling despite myoblasts do express RAGE (Sorci et al., 2003). Incidentally, during the course of these studies we serendipitously found that RAGE, activated by another of its ligands, i.e. HMGB1 (amphoterin), transduces a promyogenic, anti-proliferative and anti-tumor effect (Sorci et al., 2004; Riuzzi et al., 2006, 2007). Recent work shows that under conditions that favor myoblast differentiation and fusion into myotubes S100B causes the formation of an FGFR1/bFGF/S100B/RAGE transcomplex thereby enhancing FGFR1's mitogenic and anti-myogenic signaling and reducing RAGE's promyogenic signaling (Riuzzi F., Sorci G. and Donato R., submitted for publication).

Conclusions

S100B is a multifaceted protein in that it exerts intracellular and extracellular regulatory effects and, once released into the extracellular space, it can affect neuronal, microglial and myogenic cells with different outcomes depending on its concentra-

tion. Although RAGE is the receptor transducing S100B effects in several cell types (Donato, 2007), S100B might activate at least another receptor (i.e. FGFR1) via prior binding to FGFR1-bound bFGF. Whether or not S100B alters the function of other receptors via interaction with their canonical ligands remains to be established.

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