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MINIREVIEW ARTICLE

Human S100A12: a novel key player in inflammation?

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Abstract S100A12 is a member of the S100 family of EF-hand calcium-binding proteins. Human S100A12 is predominantly expressed and secreted by neutrophil granulocytes and, therefore, has been assigned to the S100 protein subfamily of calgranulins or myeloid-related proteins. Intracellular S100A12 exists as an anti-parallel homodimer and upon calcium-dependent activation interacts with target proteins to regulate cellular functions. Extracellular S100A12 exists majorily as homodimer and hexamer, respectively, and shows cytokine-like characteristics. It is part of the innate immune response and linked to certain autoimmune reactions. Human S100A12 is markedly overexpressed in inflammatory compartments, and elevated serum levels of S100A12 are found in patients suffering from various inflammatory, neurodegenerative, metabolic, and neoplastic disorders. In this regard, interaction of calcium-activated S100A12 with the multiligand receptor for advanced glycation endproducts (RAGE) and its soluble form (sRAGE) plays a central pathogenetic role. Recent clinical evidence suggests a high potential of S100A12 as a sensitive and specific diagnostic marker of localized inflammatory processes.

Keywords Calcium-binding proteins · Canonical EF-hand · Copper-binding proteins · Damage-associated molecular pattern molecules · Inflammatory diseases · Pseudo EF-hand · Receptor for advanced glycation endproducts · Soluble RAGE

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Introduction

The S100 proteins represent the largest subgroup within the superfamily of EF-hand calcium-binding proteins. Currently, S100 proteins comprise a family of at least 21 low molecular weight (9–14 kDa) acidic proteins that are characterized by the presence of two calcium-binding EF-hand motifs and display unique properties (Marenholz et al. 2004). It is thought that S100 proteins majorily serve as calcium trigger or sensor proteins that, upon calcium-dependent activation, regulate the function and/or subcellular distribution of certain target proteins and peptides (Donato 2003; Ravasi et al. 2004; Santamaria-Kisiel et al. 2006). All S100 proteins, with the exception of S100G (calbindin D_{9k}), are organized as tight symmetric, anti-parallel homodimers (some as heterodimers) with the non-covalent interface between the two monomers being formed mostly by hydrophobic amino acid residues. Each monomer is composed of a C-terminal, classic EF-hand, common to all EF-hand proteins, and an N-terminal, pseudo EF-hand, that exclusively has been found in the N-termini of S100 and S100-like proteins (Zhou et al. 2006; Santamaria-Kisiel et al. 2006; Donato 2007). The C-terminal EF-hand motif contains the canonical calcium-binding loop with 12 highly conserved amino acid residues showing high affinity for calcium. The N-terminal pseudo EF-hand motif comprises a 14-amino acid consensus sequence that is specific for each S100 protein and binds calcium with markedly lower affinity. In each EF-hand motif the calcium-binding loop is flanked by two helices. Each S100 protein, excepting S100G, contains a central hinge region of variable length and both a C- and N-terminal variable domain (Scheme 1) (Zhou et al. 2006; Fritz and Heizmann 2006; Santamaria-Kisiel et al. 2006). Substantial evidence indicates that differences in the

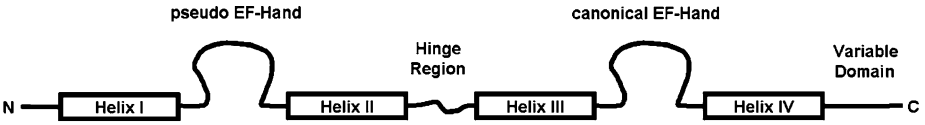
primary structure of the hinge region and the C-terminal variable domain contribute significantly to the specification of target recognition of individual S100 proteins. Upon calcium binding, almost all S100 proteins undergo a conformational change resulting in a concave hydrophobic surface in the C-terminal domain. As a consequence, the residues present in this groove create a target recognition site thus enabling selective interaction with a whole host of specific protein or peptide targets. Furthermore, oligomerization (formation of tetramers and hexamers) by some S100 proteins may provide another tier of functional regulation (Fritz and Heizmann 2006).

Together with two other S100 proteins (S100A8 and S100A9) S100A12 belongs to a S100 protein subfamily termed calgranulins or myeloid-related proteins (Odink et al. 1987; Dell'Angelica et al. 1994). The members of this subfamily are specifically linked to innate immune functions by their predominant expression in cells of myeloid origin. There is evidence that these phagocyte-specific S100 proteins are actively secreted via an alternative pathway bypassing the classical Golgi route (Rammes et al. 1997). This mode of secretion is typical for factors, which play a role in cell homeostasis as intracellular molecules but turn into proinflammatory danger or stress signals, so called damage-associated molecular pattern molecules or alarmins, after release to extracellular compartments due to cell damage, infections, auto-immune tissue destruction, or inflammation (Bianci 2007; Foell et al. 2007a, b).

Human S100A12 was first described by Guignard and colleagues in neutrophil granulocytes as a cytosolic protein, p6, which crossreacted with antibodies that were raised against S100A8 (Guignard et al. 1995). Homologous proteins were also found in other species, such as bovine, pig, and rabbit. Among natural targets of S100A12, the receptor for advanced glycation endproducts (RAGE) is of significant importance (Hofmann et al. 1999; Donato 2007). RAGE is a member of the immunoglobulin superfamily of cell surface proteins that interacts with a range of ligands, including advanced glycation endproducts (AGE), modified low density lipoproteins (LDL), amyloid fibrils, amphoterin (HMGB1), and various S100 proteins. A typical attribute of this multiligand or pattern-recognition receptor is that it is expressed at relatively low levels in homeostasis but in situations characterized by enhanced cellular activation or stress, the expression of RAGE is strikingly enhanced. The pathobiology observed in response to RAGE activation is enhanced by accumulation of its ligands at pathologic sites, e.g., in inflammatory compartments, initiating a vicious cycle comprising further upregulation of the receptor and sustained cell activation (Schmidt et al. 1999; Herold et al. 2007; Koyama et al. 2007; Logsdon et al. 2007). Thus, interaction of S100A12, also termed EN-RAGE (extracellular newly identified RAGE binding protein; common aliases of S100A12 are given in Table 1), with RAGE might play a key role in (pro)inflammatory reactions in various disease states.

Gene, mRNA and protein structure

The cytogenetic location of the *S100A12* gene is part of the tight *S100* gene cluster on human chromosome 1q21. Most of the known human *S100* genes are organized in this cluster that is part of the epidermal differentiation complex (EDC). The *S100A12* gene was mapped to 1q21.2–1q22 (1q21.3) and is located between the genes of *S100A8* and *S100A9* (Ravasi et al. 2004). The *S100A12* gene has a length of about 4.1 kbp. The mRNA size, exclusive of the polyadenylate stretch, is 466 bp (Acc No. NM_005621). The gene consists of three exons, which are divided by two introns of 900 and 400 bp. The first exon (48 nucleotides) is untranslated. It contains most of the 5′-UTR. A classical TATA box (TATAAA) is located at 30 nucleotides upstream of the transcription initiation site. Exon 2 contains a part of the 5′-UTR (20 nucleotides), exon 3 contains the 3′-UTR (122 nucleotides). The protein is encoded by sequences in exons 2 (138 nucleotides) and 3 (138 nucleotides), with the two EF hand motifs of the protein separately encoded by exons 2 and 3 (Acc Nos. X98288, X98289, X98290, D83657) (Wicki et al. 1996; Yamamura



Scheme 1 Overall S100 protein structure and protein sequence of the human S100A12. In the amino acid sequence (Acc No. NM_005621) of the mature S100A12 (without N-terminal methionine) the calcium-binding loops (*bold*), the hinge region (*underlined*) and the sequence of calcitermin (*frame*) are indicated. Human S100A12 structures have been analyzed (X-ray) and are available with the following protein data bank ID: 1E8A (human, Ca^{2+}, dimeric), 1GQM (human, Ca^{2+}, hexameric), and 1ODB (human, Ca^{2+}/Cu^{2+}, hexameric). Protein info: molecular weight, 10,444 Da; 91 residues; isoelectric point, 6.2; A280 nm (1 mg/mL), 0.28 (Ca^{2+})

Table 1 Common aliases of S100A12

S100 Calcium-Binding Protein A12, Calgranulin C (CAGC)
p6, Calcium-Binding Protein in Amniotic Fluid 1 (CAAF1)
Neutrophil S100 protein, Calcitonin Gene-Related Peptide (CGRP)
Extracellular Newly identified RAGE Binding Protein (EN-RAGE)
Myeloid-Related Protein 6 (MRP6)
Corneal or Cornea-Associated Antigen (CO-Ag)

et al. 1996). The 276-bp open reading frame encodes a 92-amino acid polypeptide with a predicted molecular mass of 10,575 Da. The mature S100A12 consists of 91 amino acids (M_r 10,444 Da) missing the N-terminal methionine residue. The complete sequence of the human S100A12 protein is given in Scheme 1. Expression of S100 proteins has been observed exclusively in vertebrates, and not in those below *Chondrichthyes* (Kraemer et al. 2008). Human S100A12 has 70% sequence identity with both porcine and rabbit S100A12, and 66% with the bovine protein (Dell'Angelica et al. 1994; Yang et al. 1996; Hitomi et al. 1996; Nonato et al. 1997). Human S100A12 shares 40% identity with human S100A8 (calgranulin A; myeloid-related protein 8), and 46% identity with human S100A9 (calgranulin B; myeloid-related protein 14), respectively. In rodents, where S100A12 gene expression cannot be observed, S100A8 seems to be a functional homologue (Moroz et al. 2003a, b; Ravasi et al. 2004). Of interest, the C-terminal sequence of S100A12 (amino acids 77–91 of the mature protein) is equivalent to calcitermin, a 15-amino acid antimicrobial and antifungal peptide derived from human airway secretions (Cole et al. 2001). The crystal structure of calcium-bound S100A12 closely resembles structures of the entire calgranulin subfamily, presenting an anti-parallel homodimer of four-helix subunits (Moroz et al. 2001; Fritz and Heizmann 2006). However, there is no evidence that human S100A12 forms heterodimers with the other calgranulins (Vogl et al. 1999). At low millimolar calcium concentrations, human S100A12 can also form a hexamer consisting of three symmetrically positioned calcium-bound homodimers (Moroz et al. 2002). Furthermore, the structure of the human S100A12-copper complex was reported (Moroz et al. 2003a, b; Xie et al. 2007). In addition, total chemical synthesis of a functionally active human S100A12 using a neutralization solid-phase chemistry protocol was accomplished (Miranda et al. 2001).

Ion binding properties

Most of the S100 proteins have been shown in various conformational and functional states depending on intracellular or extracellular concentrations of calcium, zinc and copper. In both EF-hands, the calcium ion is coordinated in a pentagonal bipyramidal configuration. The residues involved in the binding are denoted by X, Y, Z, −Y, −X and −Z (Zhou et al. 2006). The allocation of these residues to the S100A12 structure is given in Table 2. The invariant glutamate or aspartate residues at axis −Z provide a carboxylate group for bidentate coordination of the calcium ion. Generally, the dimeric S100 proteins bind four calcium ions per dimer ($\sim 10^{-4}$ to 10^{-5} M (overall K_d)], with high affinity binding [$\sim 10^{-5}$ to 10^{-7} M (K_d)] at the C-terminal canonical EF-hand motifs and low affinity binding [$\sim 10^{-3}$ to 10^{-4} M (K_d)] at the N-terminal pseudo EF-hand motifs, respectively. In the hexamer structure observed for human S100A12 there is, in addition to the two calcium ions in the EF-hands, a further bound calcium ion per subunit. This results in additional six calcium ions per hexamer that are coordinated by residues from two adjacent dimers (Yang et al. 2002; Moroz et al. 2003a). Like many other EF-hand proteins, S100 proteins also bind magnesium ions into their EF-hand sites, but the affinities for magnesium are rather low [$K_d > 10^{-3}$ M] and selectivity for calcium over magnesium is very high [$\sim 10^4$]. S100A12 also binds zinc ions in a binding site formed by both subunits and closely located to the dimer's interface. Of importance, divalent copper ions may bind at the same site. Coordination of both zinc and copper ions is supported by the N-terminal residues His15 and Asp25 from one subunit and two appropriately positioned imidazoles of a His-Tyr-His-Thr-His motif comprising residues 85−89 in the C-terminus from the other subunit (Scheme 1) (Moroz et al. 2003a). These cations accessorily may regulate both intracellular and extracellular functions of S100A12. Studies on S100A12 monomers isolated from pig granulocytes

Table 2 EF-hand Ca^{2+} coordinating sequence of S100A12

	X		Y		Z		−Y		−X			−Z
Loop I	S_{18}	$V_{19}R_{20}$	K_{21}	G_{22}	H_{23}	$F_{24}D_{25}$	T_{26}	L_{27}	S_{28}	K_{29}	G_{30}	E_{31}
Loop II	D_{61}	A_{62}	N_{63}	Q_{64}	D_{65}	E_{66}	Q_{67}	V_{68}	D_{69}	F_{70}	Q_{71}	E_{72}

Calcium ions are coordinated via the sidechain carboxylates or the peptide carbonyls of the assigned residues and an additional bridged water molecule (according to Zhou et al. 2006 and Pietzsch 2008). Loop I, pseudo EF-hand; Loop II, canonical EF-hand

demonstrated a substantial regulation of the S100A12 calcium-binding affinity by zinc (Dell'Angelica et al. 1994). Upon copper binding human S100A12 dimers form close contacts possibly enabling changes in their target binding sites or formation of oligomers (Moroz et al. 2003a). From these date possible functions of S100A12 as either a copper chaperone for other copper modulated proteins or a regulator of copper homeostasis have been discussed (Moroz et al. 2003a). Furthermore, copper-mediated generation of reactive oxygen species like peroxide by S100A12 has been hypothesized as part of its function in host-parasite response (Moroz et al. 2003a; Xie et al. 2007). This is in part supported by preliminary experiments on copper-mediated protein and lipid oxidation in vitro first showing that S100A12 binding non-dialyzable copper can function as a prooxidant agent by supporting both copper reduction and redox-cycling leading to sustained generation of peroxide and hydroxyl radicals, respectively. As a consequence, copper-bound S100A12 oxidized ascorbate and both human LDL lipids and apolipoproteins in the presence of peroxide. These processes were substantially suppressed in the presence of copper-chelating agents, e.g., diethylenetriaminepentaacetic acid (DTPA). On the other hand, copper-free S100A12 can sequestrate copper ions thereby preventing ascorbate, lipids, and proteins from oxidation in a dose dependent-manner in vitro (J. Pietzsch, S. Hoppmann, unpublished results). The latter finding is comparable with data showing copper sequestration by S100B (Nishikawa et al. 1997; Shiraishi and Nishikimi 1998). However, the particular role of S100A12 zinc and copper binding in normal and disease states in vivo still has to be elucidated.

Putative functions and binding partners

Despite the large interest in the (patho-)physiological properties of human S100A12, the knowledge on its intracellular mammalian protein targets is still limited. Binding of human S100A12 to calcyclin-binding protein/Siah-1-interacting protein (CacyBP/SIP), has been identified as a regulatory component of a novel ubiquitinylation complex that influences, e.g., beta-catenin protein expression (Filipek et al. 2002). For bovine S100A12 other intracellular mammalian target proteins, e.g., annexin 5, aldolase, cytosolic $NADP^+$-dependent isocitrate dehydrogenase, and glyceraldehyde-3-phosphate dehydrogenase, showing either a strictly or weak calcium-dependent interaction with S100A12 have been described (Hatakeyama et al. 2004). The results suggested that in mammalians S100A12 may have a chaperone/anti-chaperone-like function. Among intracellular non-mammalian proteins paramyosin, a component of the thick filaments of muscle tissue of helminthes, has been identified as a specific S100A12 protein target. These findings provide one explanation for filariacidal and filariastatic activity of S100A12 (Akpek et al. 2002). The best known extracellular target protein of S100A12 is RAGE (Donato 2007). RAGE is the first cell surface receptor that is able to bind specifically several members of the S100 protein family, including S100A12, S100A1, S100B, and S100P (Hofmann et al. 1999; Arumugam et al. 2004; Dattilo et al. 2007; Donato 2007). The binding of other S100 proteins, including S100A4, S100A13, and the calgranulins S100A8 and S100A9, respectively, is still debated (Hsieh et al. 2004; Yammani et al. 2006; Gebhardt et al. 2008; Ghavami et al. 2008). Engagement of the extracellular domain of membrane RAGE by calcium-bound S100A12 activates intracellular signal cascades including MAP-kinase and NFκB, induces secretion of cytokines (e.g., tumor necrosis factor-α and interleukin-1β) and expression of adhesion molecules (e.g., intercellular adhesion molecule-1 and vascular cell adhesion molecule-1), and thereby mediates pro-inflammatory effects on lymphocytes, endothelial cells, neutrophil and mononuclear phagocytes (Yang et al. 2001). Human recombinant S100A12 has been reported to be chemotactic for neutrophils and monocytes in vitro and in vivo (Miranda et al. 2001; Rouleau et al. 2003). Of note, S100A12 also binds to the truncated soluble form of RAGE (sRAGE), consisting of only the extracellular ligand binding domain and lacking the cytosolic and transmembrane domains of full-length RAGE (Xie et al. 2007). Very recently, we first could demonstrate by small animal positron emission tomography studies that human S100A12 ligates with RAGE in a rat model in vivo (Hoppmann et al. 2008). In particular, organs and tissues showing distinctive expression of RAGE on mRNA and protein level, respectively, like lung and large blood vessels showed a remarkable interaction with S100A12. The in vivo kinetics of S100A12 was characterized by a longer residence time in the circulating blood as well as a more sustained tissue-associated temporary retention, particularly, in the lung and the whole vascular system, respectively, compared with other RAGE ligands among the S100 protein family like S100B and S100A1. Our data indicate that in vivo affinity of S100A12 to RAGE in peripheral organs (without brain) is higher than that for S100B and S100A1, respectively (Hoppmann et al. 2008). It is proposed that an extracellular calcium-bound S100A12 hexameric assembly might interact with up to four extracellular domains of RAGE, bringing them together into large multimeric assemblies (Xie et al. 2007). The affinity of calcium-bound S100A12 with RAGE (sRAGE) is $\sim$1,000-fold higher than that of the calcium-free form (90 nM versus 140 μM). Engagement of hexameric S100A12 by multimeric RAGE then creates a signaling platform that effectively further

amplifies the intracellular signal transduction (Moroz et al. 2003a; Xie et al. 2007). These observations are consistent with the in vivo findings discussed above. On the other hand, there is evidence that S100A12 also might interact with non-RAGE cell surface binding sites. In this line, S100A12 has been shown to activate mast cells, which do not express RAGE, thus underscoring the notion that RAGE is possibly not the sole S100A12 receptor (Yang et al. 2007). Very recently, a G-protein-coupled mechanism has been proposed to be responsible of non-RAGE-mediated effects both in mast cells and monocytes (Yan et al. 2008). Additionally, S100A12 in a calcium-dependent manner binds the anti-allergic drugs/mast cell stabilizers amlexanox, cromolyn, tranilast, and olopatadine (Shishibori et al. 1999; Kishimoto et al. 2006).

Tissue and subcellular location

Constitutive gene expression of S100A12 in human tissue is almost completely restricted to neutrophil granulocytes. It was found that monocytes express S100A12, but at a lower extent than neutrophils (Guignard et al. 1995). At an early stage of differentiation, expression of S100A12 was also observed both in epithelial and dendritic cells (Hitomi et al. 1996, 1998). Under normal conditions expression of S100A12 can be observed in tissues and organs where neutrophils and monocytes/macrophages belong to the most abundant cell types, like spleen and lung. Intracellularly, in the absence of calcium, S100A12 was found predominantly in the cytosol, whereas the addition of calcium induces translocation to membrane and cytoskeletal components, respectively (Vogl et al. 1999; Foell et al. 2004a, 2007).

Disease associations and diagnostic relevance

Furthermore, S100A12 is known to be secreted into the extracellular compartment (Yang et al. 1996; Donato 2003). Extracellular human S100A12 is part of the innate immune response against microorganisms and, particularly, parasites like helminthes; and also is involved in autoimmune reactions, e.g., in progressive ulcerative keratitis of the cornea (Marti et al. 1996; Gottsch et al. 1997, 1999; Gottsch and Liu 1998). However, after S100A12 is released from granulocytes in response to cell stress it turns into a danger signal, which shares characteristics of cytokines and chemokines (Foell et al. 2007a). S100A12 demonstrates chemotactic activity and may attract circulating and bone marrow leucocytes in inflammatory conditions (Hofmann et al. 1999; Yang et al. 2001; Rouleau et al. 2003). Human S100A12 is markedly overexpressed in inflammatory conditions and high concentrations of S100A12 are found in various inflammatory compartments, e.g., synovial fluid of inflamed joints, suprabasal epidermal layers of psoriatic skin lesions, the colonic mucosa of inflamed bowel, atherosclerotic lesions, and bronchoalveolar lavage fluids of inflamed lungs, and in the circulating blood (Mirmohammadsadegh et al. 2000; Foell et al. 2003a, b; Eckert et al. 2004; Burke et al. 2004; Heizmann et al. 2007; Leach et al. 2007; Lorenz et al. 2008). Serum concentrations of S100A12 correlate well with disease activity. In this regard, S100A12 quantification is supposed to become relevant for diagnostic use in various inflammatory disease states, such as chronic active inflammatory bowel disease (Crohn's disease and ulcerative colitis), acute vasculitis syndromes (Kawasaki Disease), juvenile idiopathic arthritis, rheumatoid and psoriatic arthritis, respiratory distress syndrome, glomerulonephritis, and asthma (Foell et al. 2003b, c, 2004b; Komatsuda et al. 2006; McMorran et al. 2007; Yang et al. 2007; Lorenz et al. 2008). S100A12 also has been implicated in neurodegenerative diseases, diabetes mellitus type 2, atherosclerosis-related inflammation, and, moreover, in tumorigenic processes (Niini et al. 2002; Burke et al. 2004; Kosaki et al. 2004; Shepherd et al. 2006; Heizmann et al. 2007). Very recently, S100A12 has been demonstrated to be a sensitive marker of subclinical chronic inflammation in peritoneal dialysis patients (Uchiyama-Tanaka et al. 2008). Elevated S100A12 serum concentrations have also been found in severe bacterial infections and in patients with acute infectious exacerbations of cystic fibrosis (Foell et al. 2003d; Kim et al. 2006; Buhimschi et al. 2007). Fecal S100A12 has been proposed to be a suitable non-invasive marker of disease activity of inflammatory bowel disease (IBD) (de Jong et al. 2006). Furthermore, fecal S100A12 was shown to distinguish chronic IBD from non-organic disease including irritable bowel syndrome (IBS) or healthy individuals, with high sensitivity and specificity (Kaiser et al. 2007). Determination of fecal S100A12 in children also provided a non-invasive test with exceptional specificity in distinguishing children with IBD from children without IBD (Sidler et al. 2008). Under these several conditions, the diagnostic capacity of S100A12 seems to be superior to many conventional parameters of inflammation due to its close correlation to local inflammatory processes that involve activation of granulocytes and monocytes. However, sensitivity and specificity, particularly, of serum S100A12 in several inflammatory pathologies as well as its specific advantages compared to other diagnostic tests including the measurement of laboratory parameters, e.g., high-sensitivity C-reactive protein (hs-CRP), immunoglobulins, and erythrocyte sedimentation rate (ESR), or, on the other hand, medical procedures such as bowel endoscopy or the

ultrasonographic measurement of carotid intima-media thickness still have to be ascertained. Of importance, inverse regulation of both sRAGE and its proinflammatory ligand S100A12 seems to be a relevant molecular mechanism promoting systemic inflammation, e.g., in diabetes mellitus type 2, Kawasaki Disease, rheumatoid arthritis, and juvenile idiopathic arthritis (Pullerits et al. 2005; Basta et al. 2006; Wittkowski et al. 2007). In pathologies where tissue or serum sRAGE expression does not correspond with increased S100A12 levels a lack of antagonist regulation by sRAGE could allow an increased RAGE activation via binding of S100A12. Thus the diagnostic value of S100A12 measurement in serum or inflammatory compartments might be further strengthened by the estimation of sRAGE levels in parallel. Moreover, in other (pro)inflammatory disorders characterized by decreased sRAGE levels, e.g., Sjögren's syndrome, hypertension, and non-diabetic coronary artery disease, but also in certain neoplasias, a potential contribution of S100A12 to the disease process should be considered (Falcone et al. 2005; Geroldi et al. 2005; Geroldi et al. 2006; Santilli et al. 2007; Tesarova et al. 2007; Stewart et al. 2008).

However, the currently used diagnostic assays for measurement of S100A12 concentration are for research purposes only. Most of the enzyme-immuno assays applied used antibodies, recombinant S100A12 as standard, and preanalytical and test protocols that were in-house developments of several groups (Foell et al. 2004c; Kosaki et al. 2004; Basta et al. 2006; Larsen et al. 2007; Leach et al. 2007) (Table 3). So far, no commercially available routine tests have been validated in clinical studies. Normal or reference serum values are still debated (Larsen et al. 2007). Larsen and colleagues systematically studied the influence of blood sampling, calcium, heparin, and sample storage on S100A12 levels using a polyclonal enzyme linked immunosorbent assay (Larsen et al. 2007). These authors suggested that the serum S100A12 reference interval in a normal population, in which also persons with acute and chronic diseases are represented, should be 35–1,570 μg/L without considering gender specific differences. Gender specific reference intervals of serum S100A12 were also reported (women 49–1,343 μg/L; men 27–1,746 μg/L) (Larsen et al. 2007). Normal values of healthy control subjects of selected age and gender are expected to be lower. This seems to be consistent with data obtained by monoclonal sandwich enzyme linked immunosorbent assays showing mean serum S100A12 levels of healthy control subjects ranging from 10.7 to 75.0 μg/L (Foell et al. 2003a; Kosaki et al. 2004; Ye et al. 2004; Uchiyama-Tanaka et al. 2008). However, when comparing values obtained by the different protocols, influencing factors like the binding properties of the used antibodies or the recombinant S100A12 have to be considered (Larsen et al. 2007). Other methods, like mass spectrometry, possibly underestimate serum concentrations of S100A12 by resulting, e.g., in mean levels below 10 μg/L for healthy control subjects (Liao et al. 2004). When comparing the reported values for healthy control subjects and several disease states, the mean serum S100A12 concentration varied more than 200-fold, but majorily were represented by the suggested reference range (Foell et al. 2003a; Kosaki et al. 2004; Basta et al. 2006; Wittkowski et al. 2007; Larsen et al. 2007; Uchiyama-Tanaka et al. 2008). Reference values for S100A12 in fecal or synovial fluid specimen still have not been reported. Of note, both fecal and synovial fluid specimen obtained from patients suffering from, e.g., IBD or various inflammatory arthritides, exceeded substantially the serum S100A12 reference interval (Foell et al. 2003a; Kaiser et al. 2007). High local S100A12 levels at the site of inflammation seem to be responsible for the strongly correlating serum levels. This is in turn the prerequisite for using serum S100A12 concentrations as reliable marker for monitoring the effects of a local or systemic anti-inflammatory therapy.

Very recently, an implication of RAGE signaling has been shown to be involved in inflammatory reactions during tumor promotion, particularly, in the skin (Gebhardt et al. 2008). Gebhardt and colleagues state that RAGE activation by its ligands may trigger a positive regulatory loop that maintains an inflammatory microenvironment required for the promotion of tumor development (Gebhardt et al. 2008). These findings could encourage the hypothesis that S100A12 interacting with RAGE might also be implicated in tumorigenic processes, in particular,

Table 3 ELISA/Antibodies/siRNA/shRNA

The table lists a representative selection of commercially available tests and reagents that currently are in research use aiming at S100A12 characterization and quantification (according to Pietzsch 2008)

MBL International: http://www.mblintl.com	S100A12/EN-RAGE ELISA Kit, CircuLex
Abcam: http://www.abcam.com	Anti-human S100A12, rabbit polyclonal
	Anti-human S100A12 [19F5], mouse monoclonal
Santa Cruz Biotechnology: http://www.scbt.com	Anti-human Calgranulin C, goat polyclonal
	Anti-human Calgranulin C, rabbit polyclonal
	20–25 nt siRNA against Calgranulin C
R&D Systems: http://www.rndsystems.com	Anti-human EN_RAGE, goat IgG
Invitrogen: http://www.invitrogen.com	S100A12 StealthTM Select RNAi
OriGene: http://www.origene.com	HuSH 29mer shRNA constructs against S100A12

in epithelial tissues where chronic and persistent inflammation is supposed to be in close association both to cancer development and to predispose to carcinogenesis, e.g., in the skin, the gut or the lung. In this line, determination of S100A12 levels both in serum and inflammatory compartments should also be considered of high potential as a diagnostic indicator of epithelial cancer risk.

Conclusion

As for other human S100 proteins, the exact role for S100A12 in metabolic homeostasis and disease has not yet been fully defined. However, findings on the involvement of S100A12 in both intracellular and extracellular processes, including chaperone/anti-chaperone-like regulation of cellular homeostasis, multifunctional response of the innate immune system, and the cytokine-like interaction with RAGE involved in various inflammatory situations predestine S100A12 to be one of the most versatile members of the S100 calcium-binding protein family. From a clinical point of view, the potential usefulness and capacity of S100A12 as both a diagnostic marker of systemic and local inflammatory processes that involve phagocyte activation and a sensitive parameter for monitoring response to treatment have to be emphasized.

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