Amino Acids (2011) 41:797–807
DOI 10.1007/s00726-010-0747-4

REVIEW ARTICLE

# S100A11, a dual growth regulator of epidermal keratinocytes

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Received: 10 May 2010 / Accepted: 6 September 2010 / Published online: 25 September 2010


**Abstract** S100A11, a member of the family of S100 proteins, is a dimmer, each monomer of which has two EF-hands. Expression of S100A11 is ubiquitous in various tissues at different levels, with a high expression level in the skin. We have analyzed functions of S100A11 mainly in normal human keratinocytes (NHK) as a model cell system of human epithelial cells. High $Ca^{2+}$ and transforming growth factor-$\beta$ (TGF-$\beta$), two representative growth suppressors for NHK, need a common S100A11-mediated pathway in addition to unique pathways (NFAT1-mediated pathway for high $Ca^{2+}$ and Smad-mediated pathway for TGF-$\beta$) for exhibiting a growth inhibitory effect. S100A11 has another action point for growth suppression in NHK. Annexin A1 (ANXA1) complexed with S100A11 efficiently binds to and inhibits cytosolic phospholipase A2 (cPLA2), the activity of which is needed for the growth of NHK. On exposure of NHK to epidermal growth factor (EGF), ANXA1 is cleaved at 12Trp, and this truncated ANXA1 loses binding capacity to S100A11, resulting in maintenance of an active state of cPLA2. On the other hand, we found that S100A11 is actively secreted by NHK. Extracellular S100A11 acts on NHK to enhance the production of EGF family proteins, resulting in growth stimulation. These findings indicate that S100A11 plays a dual role in growth regulation, being suppressive in cells and being promotive from outside of cells.

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**Keywords** S100 protein · EF-hand · Cell growth · $Ca^{2+}$ · TGF-$\beta$ · EGF · Epithelial cell · Skin · Cancer · p21/WAF1 · RAGE

## Introduction

S100A11, also known as S100C and calgizzarin, is an EF hand-type $Ca^{2+}$-binding protein belonging to the S100 protein family. S100 proteins are named after their solubility in 100% saturated ammonium sulfate solution (Moore 1965). All S100 proteins are small with a molecular weight ranging from 9 to 14 kDa and have acidic properties. In humans, 16 family members are currently known to be clustered in a region of chromosome 1q21 (S100A1 to S100A16). Other S100 proteins such as S100B, S100G, S100P, and S100Z are encoded in other chromosomal regions (Leclerc et al. 2009a). Although the molecular structures of S100 proteins are similar, their expression profiles and cellular functions differ greatly depending on cell types and functional conditions. S100 proteins have been shown to be involved in pleiotropic cellular functions in various biological contexts, such as contact inhibition, epidermal differentiation, senescence, apoptosis, inflammation, and carcinogenesis.

In a study on immortalization of normal human fibroblasts, we identified S100A11 to be an essential mediator for contact inhibition of the growth of normal human fibroblasts (Sakaguchi et al. 2000). We also found similar mechanistic involvement of S100A11 in growth suppression of normal human keratinocytes (NHK) (Sakaguchi et al. 2003, 2004, 2005). Since NHK provide a rare opportunity for detailed mechanistic studies on the growth regulation of epithelial cells, we have analyzed functions of S100A11 in detail in NHK and found that S100A11 is a

dual regulator of the growth of NHK (Sakaguchi et al. 2008). In this review, we focus on intracellular and extracellular functions of S100A11.

## Structural features of S100A11 protein

### Structure of full-length S100A11

The three-dimensional structure of full-length S100A11, including details of the $Ca^{2+}$-binding sites, dimerization motif, and structural changes on binding with $Ca^{2+}$, has already been determined using NMR spectroscopy (Dempsey et al. 2003) and X-ray crystallography (Rety et al. 2000). As shown in Fig. 1a, the S100A11 monomer comprises two helix–loop–helix motifs called EF-hands. The N-terminal EF-hand is termed a 'pseudo' $Ca^{2+}$-binding site, whereas the C-terminal $Ca^{2+}$-binding site is a canonical EF-hand, which binds $Ca^{2+}$ through acidic side chains with higher affinity than that of the N-terminal one.

In a $Ca^{2+}$-free (apo) state, helices III and IV of S100A11 are close to anti-parallel, rather than parallel, forming a tight globular structure. On binding of $Ca^{2+}$ to the C-terminal EF-hand, helix III becomes nearly perpendicular to helix IV, resulting in exposure of a hydrophobic cleft of the protein. The exposed hydrophobic surface becomes a site

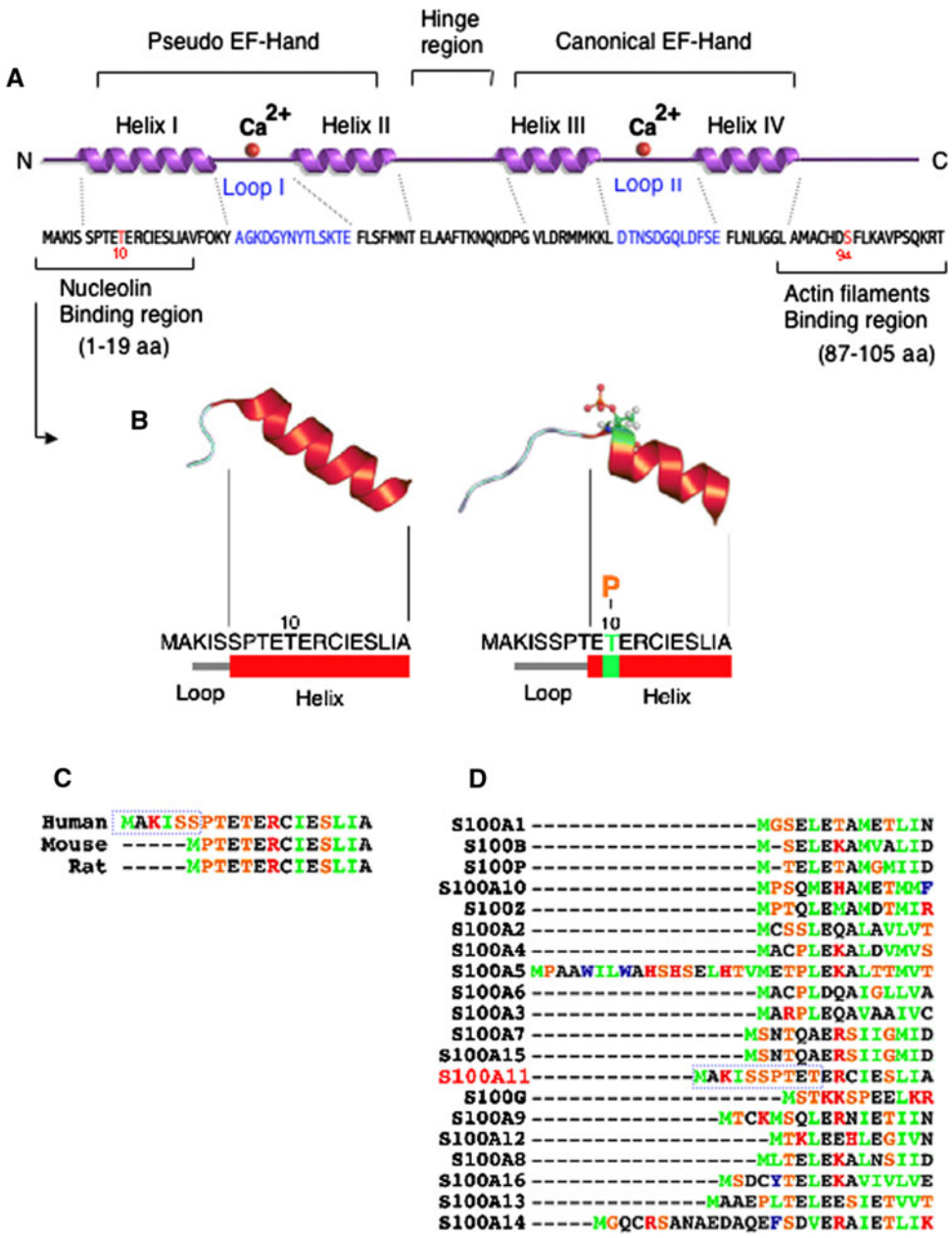


**Fig. 1** Structure of human S100A11 protein. **a** A simple diagram of the domain composition and amino acid sequence of human S100A11. S100A11 has two EF-hand motifs linked by a hinge region. Each EF-hand motif is composed of two α-helixes and a $Ca^{2+}$-binding loop. Two phosphorylation sites (Thr10 and Ser94) are indicated. **b** NMR-solution structures of the non-phosphorylated (PDB code IV4Z, *left*) and phosphorylated (IV50, *right*) N-terminal region (1–19 aa) of human S100A11 (Kouno et al. 2008). **c** Sequence alignment of the N-terminal regions of human, mouse and rat S100A11 proteins. The N-terminal 6 amino acids of human S100A11 is missing in S100A11 proteins of mouse and rat. **d** Sequence alignment of the N-terminal regions of different human S100 proteins. The N-terminal sequences of S100 proteins are not conserved

for protein–protein interaction (Rintala-Dempsey et al. 2008).

S100A11 forms a homodimer through interactions between helices I/I′ and IV/IV′, which are arranged individually in an anti-parallel fashion. Residue Cys13 within the helix I makes contact with Cys13′ of another monomer, and they are in a favorable position to form an intermolecular disulfide bridge without conformational change. Indeed, S100A11 dimers are cross-linked by a disulfide bond under non-reducing conditions (Todoroki et al. 1991; Sakaguchi et al. 2008).

### Structure and functional significance of the N-terminal region of S100A11

The N-terminal region of S100A11 is critical for interaction with nucleolin, which is a prerequisite for growth suppression of NHK by high $Ca^{2+}$ and transforming growth factor-$\beta$ (TGF-$\beta$) (Sakaguchi et al. 2003, 2004, 2005). We therefore examined the structure of an N-terminal peptide fragment (1–19 amino acids) in solution by CD and NMR spectroscopy (Kouno et al. 2008). In trifluoroethanol solution, the peptide adopts the $\alpha$-helical structure like the corresponding region of the full-length S100A11, helix I (Fig. 1b left). Phosphorylation at Thr10 disrupts the N-capping conformation of the $\alpha$-helical structure and leads to a tendency to perturb its surrounding structure (Fig. 1b right). This local structural change can reasonably explain why phosphorylation of the Thr10 residue that is initially buried in the interior of protein allows the region to be recognized by the binding partner nucleolin.

Notably, the first part (6 amino acids) of the N-terminal region of human S100A11 is missing in rodent S100A11 (Fig. 1c). Moreover, the sequence of the N-terminal 10-amino-acid region including Thr10 is not conserved among different animal species and S100 family proteins (Fig. 1d). These findings suggest that functions of S100A11 carried out by the N-terminal region are specific to human S100A11.

## Expression and intracellular localization of S100A11

### Regulation of S100A11 expression

Expression of S100A11 is ubiquitous in various tissues of different levels, being high in the skin and placenta, intermediate in the heart, spleen, kidney, liver, and lung, low in the skeletal muscle, colon, and thymus, and marginal in the brain (Inada et al. 1999; Sakaguchi et al. 2003).

In normal human fibroblasts, S100A11 was found to be down-regulated in the process of immortalization (Sakaguchi et al. 2000). By proteomic analysis using 2D-PAGE, Olsen et al. first reported that S100A11 was up-regulated by high $Ca^{2+}$ in NHK, suggesting an association of S100A11 function and epidermal differentiation in vitro (Olsen et al. 1995). We also found that exposure of NHK to high $Ca^{2+}$, a growth inhibitor, resulted in enhanced level of S100A11 (Sakaguchi et al. 2003).

### Cellular localization of S100A11

Cellular distribution of S100A11 differs depending on cell types and conditions. In general, S100A11 is localized in the cytoplasm, particularly in the peripheral region, where binding partners of S100A11, including microtubules (Broome and Eckert 2004), vimentin intermediate filaments (Bianchi et al. 2003), and actin filaments (Zhao et al. 2000; Sakaguchi et al. 2000), are abundant. This suggests that S100A11 plays some roles in cytoskeleton-mediated cellular events. S100A11 is also known to associate with ANXA1 in the cytoplasm (Naka et al. 1994; Seemann et al. 1997; Sakaguchi et al. 2007).

In growing normal human fibroblasts, S100A11 is mainly localized in the cytoplasm. When the growth of normal human fibroblasts was inhibited due to confluency, S100A11 was more preferentially found in nuclei (Sakaguchi et al. 2000). Accumulation of S100A11 took place in senescent normal human fibroblasts even in sparse conditions.

In NHK, S100A11 was shown to be a component of the cornified envelope, in which it binds to ANXA1 (Robinson et al. 1997). On exposure of NHK to $Ca^{2+}$, Broom et al. found that cytoplasmic S100A11 was transported to the periphery as a cornified envelope precursor and that the translocation depends on functional microtubules (Broome and Eckert 2004). It has not yet been determined whether S100A11 plays an essential role for formation of the cornified envelope in NHK.

On the other hand, S100A11 was preferentially localized in nuclei in NHK exposed to growth inhibitors such as high $Ca^{2+}$ (Sakaguchi et al. 2003) and TGF-$\beta$ (Sakaguchi et al. 2004) (Fig. 2a). In human squamous cell carcinomas that are resistant to growth inhibition by TGF-$\beta$, S100A11 remained in the cytoplasm even when exposed to TGF-$\beta$ (Sonegawa et al. 2007). In the normal human epidermis, nuclei of cells in the basal layer are consistently negative for S100A11 protein, whereas nuclei of cells in the suprabasal layers are positive (Fig. 2b), indicating that S100A11 accumulates in nuclei in non-growing cells in vivo as well as in vitro.

In nuclei of an immortalized keratinocyte line, HaCaT, exposed to damaging agents, Murzik et al. found that S100A11 is co-localized with DNA-dependent ATPase Rad54B at the foci of the DNA double-strand break repair site (Murzik et al. 2008). The interaction of S100A11 with

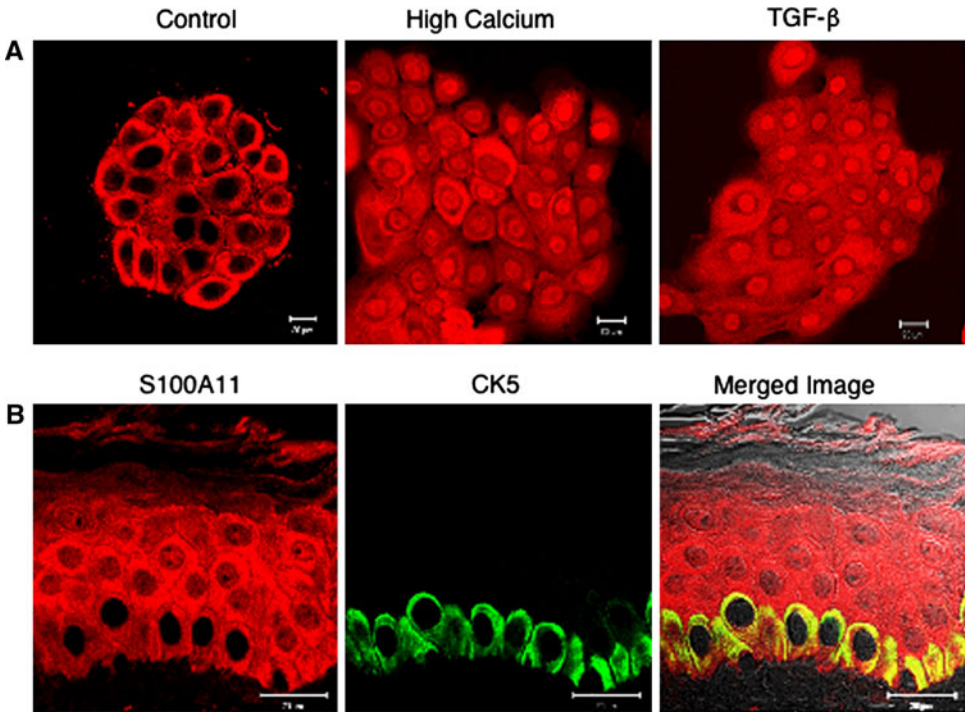


**Fig. 2** Immunohistochemistry of NHK in culture (**a**) and normal human skin (**b**) for S100A11. **a** S100A11 was translocated into nuclei in NHK exposed to high $Ca^{2+}$ or TGF-$\beta$. **b** Normal human skin was immunostained for S100A11 and cytokeratin K5. Nuclei of cells in the basal layer were specifically free from S100A11 protein. *Scale bars* 20 μm

Rad54B suggests a role of S100A11 in the DNA double-strand repair and cell cycle arrest.

On the other hand, it is known that some S100 family members, such as S100A1, S100A2, S100A4, S100A5, S100A6, S100A7, S100A8, S100A9, S100A12, S100A13, S100$\beta$, and S100P, are secreted into the extracellular space (Leclerc et al. 2009a). S100A11 was also detected in culture media of NHK (Sakaguchi et al. 2008) and chondrocytes (Cecil et al. 2005; Cecil and Terkeltaub 2008). Overall production and secreted amount of S100A11 in NHK was enhanced by treatment with growth factors such as EGF and IL-1F9 (Sakaguchi et al. 2008).

## Involvement of S100A11 in growth inhibition of epidermal keratinocytes

### S100A11, an essential mediator of growth inhibitory signals in NHK

Normal human keratinocytes continuously proliferate in a culture medium with $Ca^{2+}$ less than 0.1 mM (low $Ca^{2+}$). An increase in the $Ca^{2+}$concentration to 1.2–2.0 mM (high $Ca^{2+}$) results in termination of cell growth and induction of terminal differentiation. This $Ca^{2+}$-mediated growth arrest and subsequent differentiation of NHK may reflect a physiological process in the skin, because the presence of an increasing gradient of $Ca^{2+}$ concentration was indicated from the basal layer to the cornified layer of the epidermis (Tu et al. 2004). We showed that on exposure of NHK to high $Ca^{2+}$, S100A11 was specifically phosphorylated at N-terminal Thr10 and C-terminal Ser94 (Fig. 1a) (Sakaguchi et al. 2003). Phosphorylation of S100A11 at Thr10 promoted binding to nucleolin via the N-terminal region of S100A11. The binding is a prerequisite for nuclear translocation of S100A11. In NHK, cytoplasmic S100A11 was partially co-localized with actin fibers. We found that S100A11 binds to actin filaments through the C-terminal region (Fig. 1a) and that phosphorylation at Ser94 reduced the affinity (unpublished data). The liberation of S100A11 from actin filaments may facilitate association with nucleolin and eventual nuclear translocation of S100A11. In nuclei, S100A11 liberated transcription factors Sp1/3 from nucleolin. The resulting free Sp1/3 transcriptionally activated p21/WAF1, a representative negative regulator of cell growth (Fig. 3). These results indicate that S100A11 is a key mediator of the $Ca^{2+}$-induced growth inhibition of NHK in culture and that it is probably involved in growth regulation in vivo as well.

TGF-$\beta$ is a representative growth inhibitor for epithelial cells, and resistance to TGF-$\beta$-induced growth suppression is often mechanistically linked to malignant conversion and/or progression of those cells. We showed that TGF-$\beta$-triggered signaling for growth suppression was mediated by S100A11 in a manner similar to that from high $Ca^{2+}$ (Sakaguchi et al. 2004). TGF-$\beta$ triggers PKC$\alpha$-mediated phosphorylation of cytoplasmic S100A11, driving it into nuclei, where it releases Sp1/Sp3 from its binding partner,

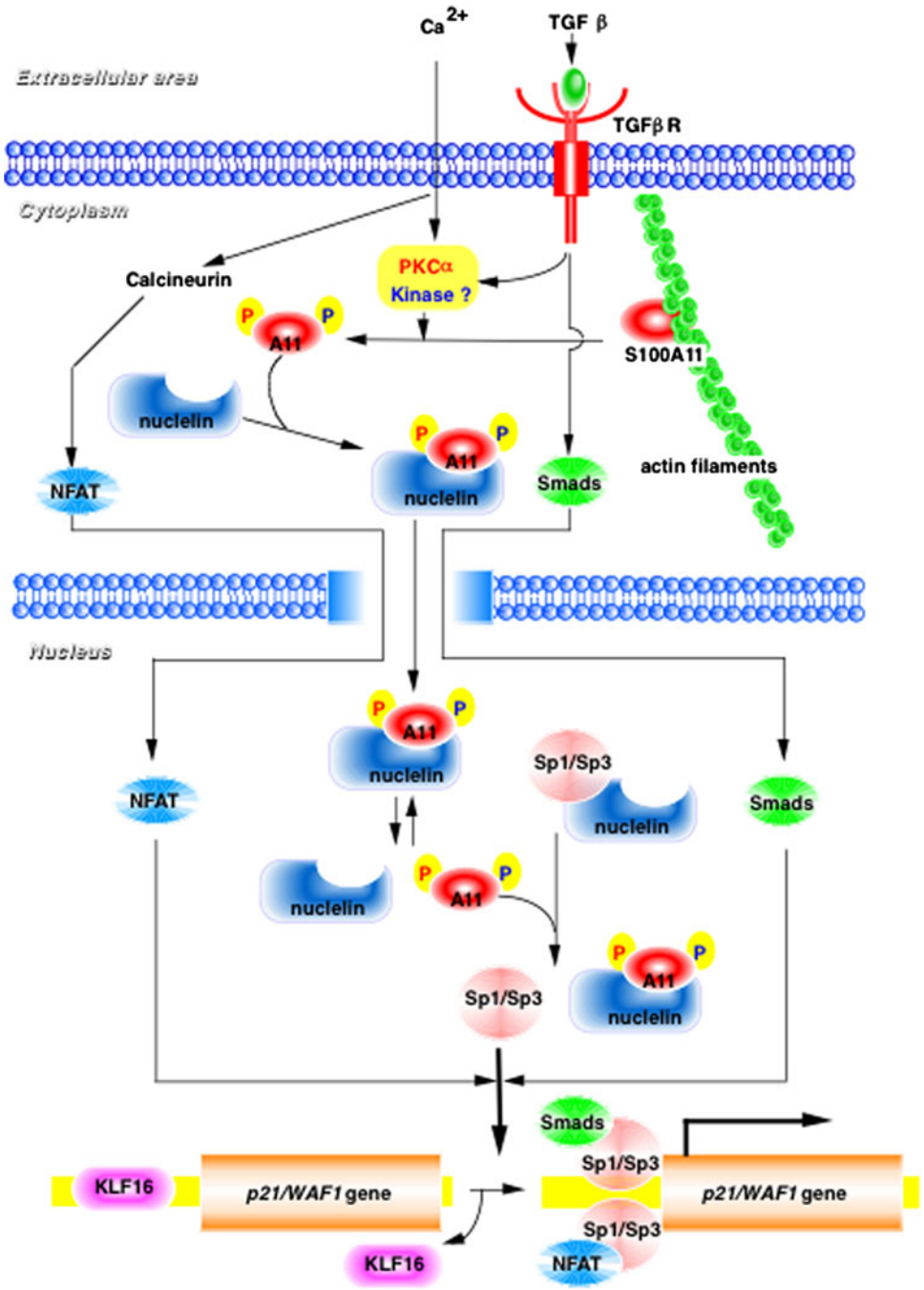


**Fig. 3** A schematic diagram of the signal transduction pathway mediated by S100A11 in NHK. High $Ca^{2+}$ and TGF-$\beta$ need a common S100A11-mediated pathway in addition to individual unique pathways (NFAT1-mediated pathway for high $Ca^{2+}$ and Smad-mediated pathway for TGF-$\beta$) for exhibiting inhibitory effects on the growth of NHK, and both pathways are indispensable for their growth inhibition

nucleolin. Sp1 turns on the *p21/WAF1* gene to inhibit growth of NHK (Fig. 3). Both branches, the Smad-mediated pathway and the newly identified S100A11 pathway, are essential to stop the growth of NHK in response to TGF-$\beta$. Notably, the S100A11-mediated pathway was deteriorated in human squamous carcinoma cell lines that are resistant to TGF-$\beta$ (Sonegawa et al. 2007), indicating that this pathway is at least partly involved in conferring upon human epithelial cancers resistance to TGF-$\beta$.

Pardali et al. and our group reported that growth suppression of NHK by high $Ca^{2+}$ or TGF-$\beta$ was mediated by p21/WAF1 via Sp1/Sp3 (Pardali et al. 2000; Sakaguchi et al. 2003, 2004). Sp1/Sp3 has long been thought to be a ubiquitous transacting factor. However, accumulating evidence, including evidence obtained by ourselves, indicates that Sp1 site-dependent transcription is highly regulated by many different factors in a context-dependent manner. The amount of Sp1 protein in NHK remained unchanged before and after treatment with high $Ca^{2+}$ or TGF-$\beta$. Sp1 could bind to the p21/WAF1 promoter but only when the cells were treated with high $Ca^{2+}$ and TGF-$\beta$. Sp1 and Sp3 belong to the Sp/Krueppel-like factor (KLF) family, which

comprises at least 20 members. The members bind to transcriptional elements designated as Sp1 sites with varying affinities and modulate each other's activity in synergistic or antagonistic manners.

For TGF-$\beta$-triggered growth inhibition, both Smad- and S100A11-mediated pathways are indispensable. An emerging question is whether the S100A11-mediated pathway alone is sufficient in the case of growth inhibition by high $Ca^{2+}$. Santini et al. showed that the calcineurin-NFAT pathway is involved in up-regulation of p21/WAF1 in mouse keratinocytes exposed to high $Ca^{2+}$ (Santini et al. 2001). We studied roles of calcineurin and NFAT in NHK exposed to high $Ca^{2+}$ and found that (a) high $Ca^{2+}$ induced dephosphorylation of NFAT1 by calcineurin in NHK, (b) the NFAT1-mediated pathway as well as the S100A11-mediated pathway is indispensable for growth inhibition of NHK by high $Ca^{2+}$, (c) in growing NHK, an Sp/Krueppel-like factor, KLF16, binds to and suppresses the p21/WAF1 promoter, and (d) Sp1 complexed with either NFAT1 or Smad3, but not Sp1 alone, can expel KLF16 from the p21/WAF1 promoter and transcriptionally activates the *p21/WAF1* gene (Sakaguchi et al. 2005). Collectively, high $Ca^{2+}$ and TGF-$\beta$ have a common S100A11-mediated pathway in addition to unique pathways (NFAT1-mediated pathway for high $Ca^{2+}$ and Smad-mediated pathway for TGF-$\beta$) for exhibiting a growth inhibitory effect on NHK, and both pathways are indispensable for growth inhibition (Sakaguchi et al. 2005). It is known that many genes have Sp1/Sp3 binding sites on their promoters. Difference in binding partners of Sp1/Sp3 may be one of the factors to determine specificity of Sp1/Sp3 target genes in a given context.

### S100A11, a blocker of EGF-triggered growth stimulation signaling in NHK

Epidermal growth factor (EGF) has growth simulation activity toward many different types of cells. On binding of EGF to its specific receptors, several different intracellular signaling pathways are activated. Among those, activation of cytosolic phospholipase A2 (cPLA2), a rate-limiting enzyme of the arachidonic acid cascade, is one of the major pathways (Matsuzawa et al. 2009), since blocking of the pathway results in abrogation of EGF-triggered growth stimulation of NHK (Sakaguchi et al. 2007). Cytoplasmic ANXA1 is known to bind and inhibit cPLA2 (Kim et al. 1994, 2001a, b). ANXA1 is phosphorylated by the ligand-activated EGF receptor at 21Tyr, and phosphorylated ANXA1 becomes labile to tryptic cleavage at an N-terminal site(s) (Gerke and Moss 2002). We explored the functional significance of phosphorylation and N-terminal cleavage of ANXA1 in detail. In NHK exposed to EGF, ANXA1 was cleaved solely at 12Trp and the cleavage was specifically executed by cathepsin D (Sakaguchi et al. 2007). Mailliard et al. demonstrated that ANXA1 binds to S100A11 via its N-terminal site in a $Ca^{2+}$-dependent manner (Mailliard et al. 1996). We found that the cleavage of ANXA1 at an N-terminal site abrogated the binding capacity of ANXA1 to S100A11. ANXA1 bound to and inhibited cPLA2 more effectively when complexed with S100A11. In NHK treated with high $Ca^{2+}$, the S100A11 firmly bound to ANXA1, and the interaction inhibits N-terminal phosphorylation and eventual cleavage of ANXA1 even on exposure to EGF. The stable ANXA1-S100A11 complex more effectively inhibits activity of cPLA2, resulting in growth suppression (Figs. 4, 5).

These results together with the findings of intranuclear function of S100A11 lead us to conclude that S100A11 has two arms for growth suppression of NHK, namely, being involved in the transcriptional activation of p21/WAF1 in the nuclei and in the inhibition of cPLA2 through the binding to ANXA1 in the cytoplasm. In some human squamous carcinoma cell lines, we showed that ANXA1 was constitutively cleaved and thus lost capacity to bind to S100A11 (Sakaguchi et al. 2007).

## Involvement of S100A11 in growth stimulation of NHK

### Secretion of S100A11

More than half of the 21 human S100 proteins have been shown to be secreted (Leclerc et al. 2009a). Intriguingly, however, all of the secreted S100 proteins lack the signal peptide. Such proteins are secreted via an endoplasmic reticulum (ER)/Golgi-independent pathway, i.e., unconventional or non-classical secretion (Prudovsky et al. 2008; Keller et al. 2008). Cecil et al. reported that S100A11 was markedly overexpressed and secreted into the surrounding matrix by human chondrocytes in osteoarthritis (Cecil et al. 2005, 2009). We showed that S100A11 was secreted from epithelial cells (Sakaguchi et al. 2008). Furthermore, S100A11 protein was identified in human serum after condensation using affinity chromatography (unpublished data), indicating that secretion of S100A11 takes place in vivo as well.

Several possible mechanisms of unconventional secretion have been proposed and partly verified (Blott and Griffiths 2002; Holt et al. 2006; Keller et al. 2008). S100A13 was shown to be co-secreted with fibroblast growth factor-1 (Landriscina et al. 2001; Prudovsky et al. 2002; Matsunaga and Ueda 2006). Both proteins lack the conventional signal sequence. An inhibitor of actin stress fiber formation suppressed secretion of FGF1 and hence secretion of S100A13. S100A13 is known to bind to the actin fiber. HSP70 and ANXA1, both of which lack the

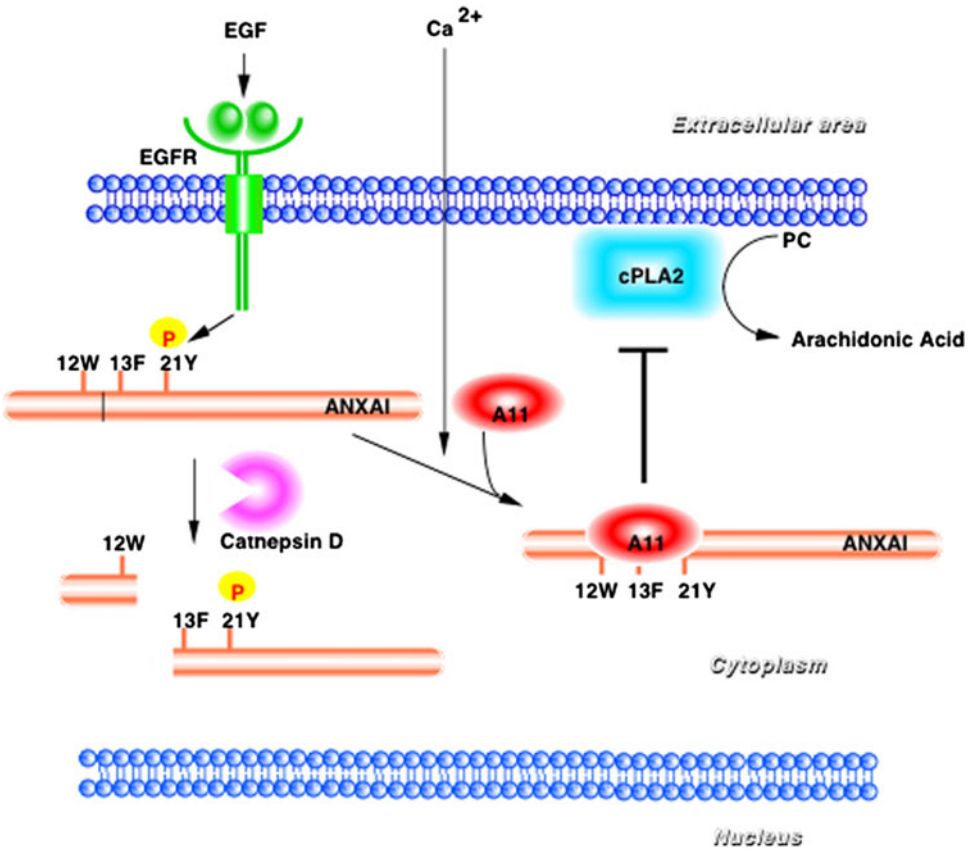


**Fig. 4** A schematic diagram of another growth-suppressive function of S100A11 in NHK. Complex formation of S100A11 and ANXA1 facilitates its binding to cPLA2 and suppression of cPLA2 activity, which is essential for the growth of NHK. Ligand-activated EGF receptor promotes cleavage of ANXA1, resulting in loss of binding capacity to S100A11 and thus leaving cPLA2 activity unhampered

signal peptide, were shown to be transported into lysosomal vesicles via ABC transporters (Danielsen et al. 2003; Wein et al. 2004; Mambula et al. 2007). The proteins were then exported to the outside of cells upon stimulation. EGF was shown to up-regulate ABC transporters (Chen et al. 2000; Garcia et al. 2006; Meyer zu Schwabedissen et al. 2006). S100A11 has a capacity to bind to actin fibers (Zhao et al. 2000) (Sakaguchi et al. 2000), and its secretion was enhanced by EGF (Sakaguchi et al. 2008). These findings suggest that S100A11 is secreted in a manner similar to that for ANXA1 and HSP70.

## Growth stimulation of epidermal keratinocytes by S100A11

Expression of S100A11 is often enhanced in different types of human cancers (Tanaka et al. 1995; Mori et al. 2004; Rehman et al. 2004; Sakaguchi et al. 2008; Salama et al. 2008). This is not readily reconciled with the notion described above that S100A11 transduces growth inhibitory signals. We therefore further examined the function of S100A11, focusing on secreted S100A11. Our findings are summarized as follows: (a) S100A11 was secreted into the culture medium from NHK and the secretion was accelerated by EGF, (b) recombinant S100A11 promoted growth of NHK cells by inducing EGF family genes, (c) EGF was induced through the following pathway: S100A11 binds to the receptor for advanced glycation endproducts (RAGE), thus activating Akt, and Akt in turn phosphorylates the cAMP response element-binding protein (CREB), resulting in activation of AP-1, which induces the EGF promoter, (d) production and secretion of S100A11 was markedly enhanced in all human squamous carcinoma cell lines examined, and (e) application of an antibody against S100A11 reduced the growth of human squamous carcinoma cell line. These results clearly show that S100A11 is secreted into the outer cell space and acts as an autocrine factor for stimulation of the growth of NHK.

## Cell surface receptor(s) for S100A11

Receptor for advanced glycation endproducts is a multiligand receptor that belongs to the immunoglobulin superfamily. RAGE was first identified as a receptor for advanced glycation endproducts (AGE), but it was soon found to bind diverse ligands, including amyloid $\beta$, HMGB1 (amphoterin), and some S100 family members (Hori et al. 1995; Yan et al. 1996; Kislinger et al. 1999; Hofmann et al. 1999; Rauvala and Rouhiainen 2007; Leclerc et al. 2009a, b). Different S100 family members that bind to RAGE seem to activate various cell signaling molecules, including Ras-mediated extracellular

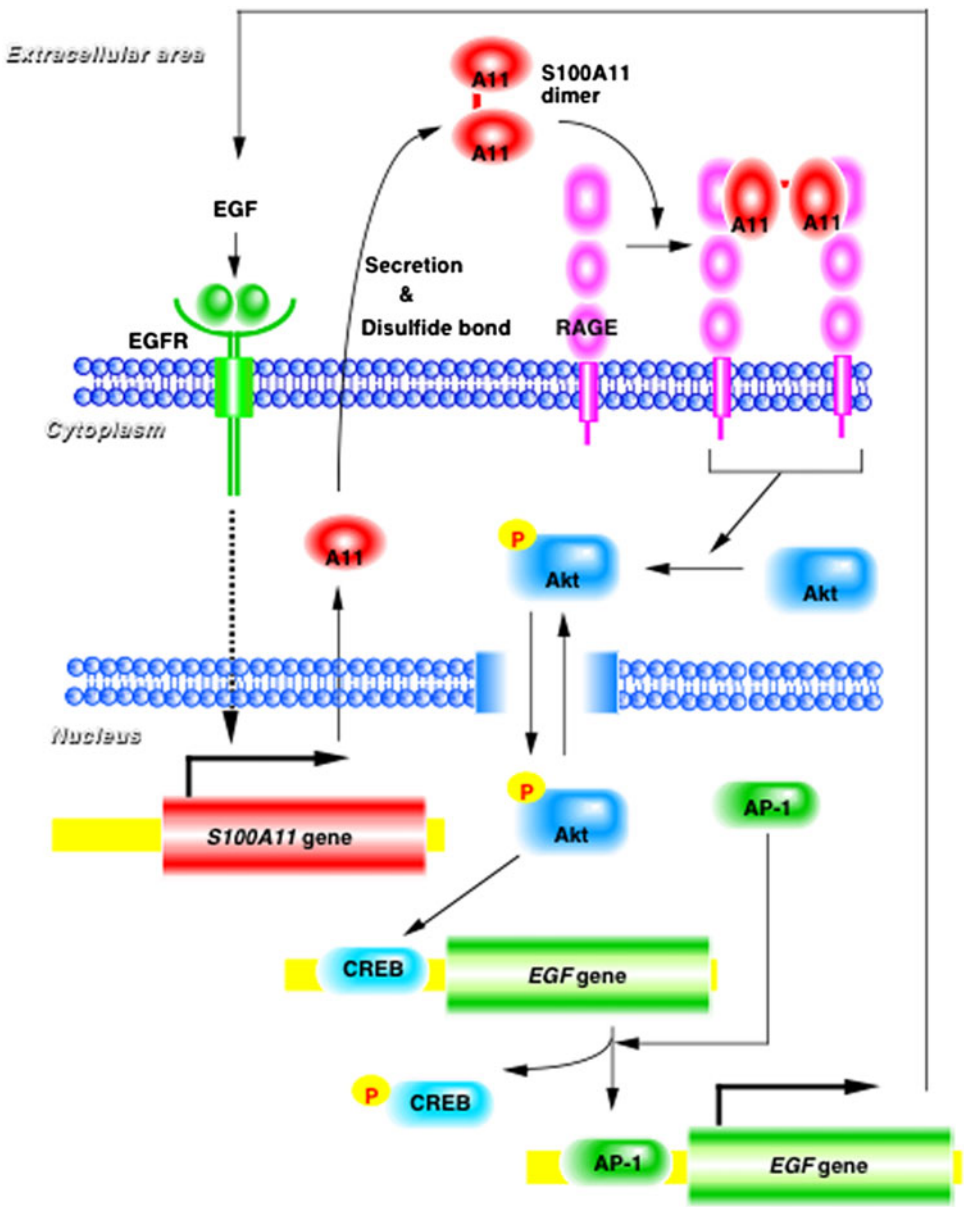


**Fig. 5** A schematic diagram of the function of secreted S100A11 in NHK. S100A11 binds to RAGE and induces expression of EGF family genes via the Akt signaling pathway. EGF family proteins promote cell proliferation and survival and also induce expression and secretion of S100A11

signal-regulated kinase 1/2 (ERK1/2), Akt, stress-activated protein kinase/c-Jun-NH2-terminal kinase (SAPK/JNK), p38 MAP kinase, and Cdc42/Rac, and to exert distinct biological effects (Lander et al. 1997; Taguchi et al. 2000; Yeh et al. 2001; Leclerc et al. 2007; Donato 2007). Ghavami et al. showed that heterodimeric S100A8 and S100A9 bound to RAGE and stimulated growth of human cancer cell lines at low concentrations (Ghavami et al. 2008). In NHK, we demonstrated that S100A8/A9 induced cytokines such as IL-8, IL-1F9, and TNFα but neither induced EGF nor activated Akt in NHK (Nukui et al. 2008). S100A11, which also binds to RAGE, activates Akt and eventually induces EGF in NHK (Sakaguchi et al. 2008) as described above. Cecil et al. showed that exogenous S100A11 induced production of type X collagen and CXCL8/IL8 through activation of p38 MAPK via RAGE in articular chondrocytes in culture (Cecil et al. 2005; Cecil and Terkeltaub 2008). The multiple actions of RAGE-binding S100 proteins may be due to the fact that each S100 protein binds to different extracellular domains of RAGE (Leclerc et al. 2007, 2009a; Ghavami et al. 2008). The extracellular part of RAGE comprises one variable-like V-domain and two constant-like C-domains. S100A1, S100A2, S100A5, S100A6, S100A12, and S100B bind to the V-domain, S100A12 binds to the C1-domain, and S100A6 binds to the C2-domain (Leclerc et al. 2009a).

Receptor for advanced glycation endproducts has only a short cytoplasmic domain, on which RAGE-triggered signaling solely depends. The cytoplasmic domain lacks tyrosine kinase activity and any motifs known for receptor

signaling. Hudson et al. identified Diaphanous-1 (Dia-1) to be a binding partner to the RAGE cytoplasmic domain by a yeast two-hybrid assay. Dia-1 is known to control activation of Rac-1 and Cdc42, resulting in promotion of cellular migration (Hudson et al. 2008).

Other receptors for S100A11

An alternative possibility for multiple functions of S100A11 is the presence of new/additional receptors. It has been reported that AGE can bind not only to RAGE but also to galectin-3, FEEL-1, FEEL-2, CD36, SR-A, SR-BI, and LOX-1 (Pricci et al. 2000; Ohgami et al. 2001a, b; Jono et al. 2002; Tamura et al. 2003; Horiuchi et al. 2005). Among those, CD36, SR-A, SR-BI, and LOX-1 act as scavengers for AGE. Cecil et al. showed that CD36 functions as a receptor also for S100A11 and that overexpression of CD36 in human chondrocytes blocks RAGE-dependent p38/MAPK phosphorylation and hypertrophy by S100A11 (Cecil et al. 2009). Further intensive investigation is needed to understand such an interaction network of a multiple ligand-receptor system.

## Concluding remarks

S100A11, a member of the family of S100 proteins, is a monomer that comprises two helix–loop–helix motifs called EF-hands. Expression of S100A11 is ubiquitous in various tissues of different levels, being high in the skin and placenta, intermediate in the heart, spleen, kidney, liver, and lung, low in the skeletal muscle, colon, and thymus, and marginal in the brain. We analyzed functions of S100A11 mainly in NHK as a model cell system of human epithelial cells and found that S100A11 is an essential dual regulator of the growth of NHK.

At first, we demonstrated that S100A11 is a key mediator for growth inhibition of NHK triggered by high Ca2 + or TGF-$\beta$. On exposure of NHK cells to either agent, S100A11 is phosphorylated by PKC$\alpha$ and transferred to nuclei, where it induces p21/WAF1 through activation of Sp1/Sp3. High $Ca^{2+}$ activated NFAT1 through calcineurin-dependent dephosphorylation. A signal triggered by TGF-$\beta$ activates the well-characterized Smad-mediated pathway. Sp1 complexed with NFAT1 or with Smad3, but not Sp1 alone, expels a silencer factor, of the p21/WAF1 promoter, KLF16, and transcriptionally activates the *p21/WAF1* gene. Thus, high $Ca^{2+}$ and TGF-$\beta$ have a common S100A11-mediated pathway in addition to unique pathways (NFAT1-mediated pathway for high $Ca^{2+}$ and Smad-mediated pathway for TGF-$\beta$) for exhibiting a growth inhibitory effect.

S100A11 has another action point for growth suppression in NHK. Growth of NHK depends on arachidonic acids. ANXA1 complexed with S100A11 efficiently binds to and inhibits cPLA2, a rate-limiting enzyme of the arachidonic acid cascade. On exposure of NHK to EGF, ANXA1 is cleaved at 12Trp, and this truncated ANXA1 loses binding capacity to S100A11, resulting in maintaining cPLA2 in an active state. In squamous cancer cells, this pathway was shown to be constitutively activated. The mechanistic intersection by S100A11 may be a promising target for establishing new measures against human cancer and other cell growth disorders.

On the other hand, we found that S100A11 is actively secreted by NHK. Extracellular S100A11 acts on NHK to enhance the production of epidermal growth factor family proteins, resulting in growth stimulation. RAGE, NFkB, Akt, CREB, and AP-1 are involved in the signal transduction. Production and secretion of S100A11 are markedly enhanced in human squamous cancer cells. These findings indicate that S100A11 plays a dual role in growth regulation, being suppressive in cells and being promotive from the outside of cells.

As examined in a case of S100A11, a single S100 protein has pleiotropic functions in a single type of cell. Considering that there are 21 members of the S100 protein family, further effort to understand the aspects of complicated functions of S100 proteins is clearly needed. We have prepared nearly all of the human S100 proteins for a comprehensive study in this direction.

**Acknowledgments** This review partly depends on our studies, which were supported by grants from the Ministry of Health, Labor and Welfare (Research for Intractable Diseases) (N. Huh), from the Ministry of Education, Culture, Sports, Science, and Technology of Japan [Grant-in-Aid for Young Scientists (A)] (M. Sakaguchi), and from Takeda Science Foundation (M. Sakaguchi). We particularly thank our co-workers, Dr. Masayoshi Namba, Dr. Hidenori Yamada, and Dr. Keiichi Kawano.

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