HHS Public Access

Author manuscript

Nat Rev Cancer. Author manuscript; available in PMC 2015 March 23.

Published in final edited form as:

Nat Rev Cancer. 2015 February ; 15(2): 96–109. doi:10.1038/nrc3893.

S100 proteins in cancer

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Abstract

In humans, the S100 protein family is composed of 21 members that exhibit a high degree of structural similarity, but are not functionally interchangeable. This family of proteins modulates cellular responses by functioning both as intracellular Ca^{2+} sensors and as extracellular factors. Dysregulated expression of multiple members of the S100 family is a common feature of human cancers, with each type of cancer showing a unique S100 protein profile or signature. Emerging *in vivo* evidence indicates that the biology of most S100 proteins is complex and multifactorial, and that these proteins actively contribute to tumorigenic processes such as cell proliferation, metastasis, angiogenesis and immune evasion. Drug discovery efforts have identified leads for inhibiting several S100 family members, and two of the identified inhibitors have progressed to clinical trials in patients with cancer. This Review highlights new findings regarding the role of S100 family members in cancer diagnosis and treatment, the contribution of S100 signalling to tumour biology, and the discovery and development of S100 inhibitors for treating cancer.

The term S100 was first used in 1965 to denote a mixture of the two founding family members, S100A1 and S100B[1]. This term alludes to the solubility of these approximately 10,000 Da proteins in 100% saturated ammonium sulphate. Although S100 family members exhibit a high degree of sequence and structural similarity, they are not functionally interchangeable and they participate in a wide range of biological processes such as proliferation, migration and/or invasion, inflammation and differentiation[2–4]. The structure and function of the S100 proteins are regulated by Ca^{2+} binding, which allows them to act as Ca^{2+} sensors that can translate fluctuations in intracellular Ca^{2+} levels into a cellular

Competing interests statement

The authors declare no competing interests.

FURTHER INFORMATION

RCSB Protein Data Bank: http://www.rcsb.org/pdb

S100 gene family: http://www.genenames.org/genefamilies/S100

SUPPLEMENTARY INFORMATION

See online article: S1 (table) | S2 (table) | S3 (table)

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response[5,6]. Individual family members show unique affinities for divalent metal ions, oligomerization properties, post-translational modifications and spatiotemporal expression patterns. Intracellular S100 proteins bind to and regulate the activity of many targets; in some cases, multiple S100 family members may regulate one target[2–4]. Several S100 proteins are present in the extracellular space where they can participate in local intercellular communication (autocrine and paracrine), enter the systemic circulation and coordinate biological events over long distances. S100 proteins lack a signal peptide for secretion via the conventional Golgimediated pathway, and whether extracellular S100 proteins are actively secreted from living cells or passively released is still debated[2,4]. Extracellular S100 proteins interact with a variety of cell-surface receptors including receptor for advanced glycosylation end products (RAGE; also known as AGER), G protein-coupled receptors, Toll-like receptor 4 (TLR4), scavenger receptors, fibroblast growth factor receptor 1 (FGFR1), CD166 antigen (also known as ALCAM), interleukin-10 receptor (IL-10R), extracellular matrix metalloproteinase inducer (EMMPRIN; also known as basigin) and the bioactive sphingolipid ceramide 1-phosphate[4,7–10]. The functional diversity of S100 proteins and the unique repertoire of family members expressed in cells and tissues enable individual cells to generate unique and adaptive responses to changes in intracellular Ca^{2+} levels and the extracellular environment.

There are 21 S100 proteins — which are exclusively found in vertebrates — encoded in the human genome[11]. As new family members were discovered, the S100 nomenclature evolved with the consequence that numerous aliases exist for some S100 proteins[4,12]. Four family members are dispersed throughout the genome: *S100B* on chromosome 21, *S100G* on the X chromosome, *S100P* on chromosome 4 and *S100Z* on chromosome 5. The remaining 17 family members (*S100A1–S100A14*, the *S100A7* genes and *S100A16*) are encoded in two tandem clusters within a 2 Mb region on chromosome 1q21 that is referred to as the epidermal differentiation complex (EDC). The EDC also contains genes encoding the S100-fused type proteins (SFTPs) trichohyalin (TCHH), TCHH-like 1 (TCHHL1), repetin (RPTN), hornerin (HRNR), filaggrin (FGL), FGL2 and cornulin (CRNN)[13]. SFTPs contain a full-length S100 protein domain fused in-frame to multiple tandem repeats composed of one or two sequences, for which the function is not well characterized. The five genomic loci that encode S100 proteins are highly conserved, but there are differences among species that affect the extrapolation of results from preclinical studies to human cancers. For example, the mouse genome lacks genes encoding S100A12 and S100P, and the protein encoded by the single mouse *S100a7* locus (*S100a7a*) differs substantially from the proteins encoded by the three human *S100A7* loci (*S100A7*, *S100A7A* and *S100A7L2*)[11]. Furthermore, although 1q21–25 is a hotspot for chromosomal alterations, mutations and/or translocations in *S100* genes are rare. The only reported event involving chromosomal deletions of S100 family members is oral cancer (in which there is a deletion of *S100A1–S100A16*)[14]. Of the four *S100A14* polymorphisms reported in oesophageal squamous cell carcinoma, only the mutation 461G>A is associated with increased cancer susceptibility due to diminished binding of S100A14 to p53 (REFS 14,15). *S100A2* polymorphisms have been reported in non-small-cell lung cancer (NSCLC), but they are not associated with altered S100A2 expression or function[16].

Nonetheless, dysregulation of S100 protein expression is a common occurrence in many human cancers. *In vivo* studies have shown that altered expression of ten family members contributes to the growth, metastasis, angiogenesis and immune evasion of numerous tumours (TABLE 1). Inhibitors directly targeting two family members, S100B and S100A9, are in clinical trials for melanoma and prostate cancer, respectively. This Review focuses on new advances regarding the role of S100 proteins in cancer diagnosis and treatment, the contribution of S100 signalling to cancer cell biology and the development of new S100 protein inhibitors for treating cancer.

Conformation and structure

The S100 proteins are typically symmetric dimers with each S100 subunit containing four α-helices[4]. Although there are several reports of *in vitro* heterodimerization among family members, and mixtures of S100A1 homodimers, S100B homodimers, and S100A1–S100B heterodimers can be isolated from brain[17], only the S100A8–S100A9 heterodimer has been documented to have physiologically relevant functions *in vivo*[18–20]. Each of the two S100 subunits contains two Ca^{2+}-binding domains: a carboxyterminal canonical EF-hand (a helix–loop–helix domain) composed of 12 amino acids, and an amino-terminal 'pseudo' or 'S100' EF-hand that is unique to S100 proteins and is composed of 14 amino acids[4]. These motifs are connected by a 'hinge' region (loop 2), which consists of 10–12 residues and is crucial for target interactions. In the absence of target, the Ca^{2+}-binding affinity of most S100 proteins is low, but when bound to target, the Ca^{2+}-binding affinity increases by 5–300-fold[21–23]. This biochemical coupling can be understood in terms of structural transitions, as upon binding Ca^{2+}, S100 proteins undergo a substantial conformational rearrangement that reorients helix 3 to expose a hydrophobic cleft that is required for target binding (FIG. 1). Recent studies have suggested that in the absence of a target, Ca^{2+}-bound S100 proteins exist as an equilibrium population of dynamic conformers with an overall weak Ca^{2+}-binding affinity, and that target binding narrows the distribution to favour S100 sub-states with high affinities for Ca^{2+} ions[24]. As a consequence, target binding is typically Ca^{2+}-dependent. Importantly, the three-dimensional structures of S100–target complexes have revealed that individual family members exhibit distinct modes of target recognition owing, in part, to differences in surface geometries, hydrophobic residue distribution and charge density[25]. In addition, some family members undergo a variety of post-translational modifications, such as oxidative modification and sumoylation, which can modulate S100–target complex formation and/or intracellular localization[26–29]. Structural and biochemical considerations are particularly relevant to the development of therapies targeting S100 family members. Importantly, the 3D structures of S100 proteins permit an extensive analysis of target selectivity, which can be exploited for drug discovery, as described later in this Review.

Expression in cancer

Cancers exhibit a distinctive S100 protein profile that can be both stage-specific and subtype-specific. In gliomas, S100B expression positively correlates with proneuronal, neuronal and classic — but not mesenchymal — subtypes, whereas S100A8 and S100A9 expression positively correlate with mesenchymal subtypes[30]. Despite the availability of

protein signatures from breast[31], head and neck[32], prostate[33], melanoma[34] and colorectal[35,36] cancers, comprehensive analyses of S100 protein expression have not been carried out. Nonetheless, discernible trends and notable exceptions emerge when the S100 protein expression profiles in human cancers are compared (see Supplementary information S1 (table)). Dysregulation of multiple S100 family members occurs in most cancers and typically involves upregulation. One family member, S100P, is upregulated in all cancers that have been examined and the remaining S100 family members are upregulated in most but not all cancers. Two exceptions are head and neck, and ocular cancers, in which 11 out of 13, and 6 out of 12 dysregulated S100 proteins are downregulated, respectively (see Supplementary information S1 (table) and references therein). The contradictory expression profiles reported for some family members may be attributable to cancer subtype, disease stage, cellular distribution, or issues associated with S100 protein and/or mRNA detection. For example, S100A11 expression is increased in NSCLC, but is decreased in small-cell lung cancer[37]. S100A7 is expressed in pre-invasive, well-differentiated and early-stage oral squamous cell carcinomas, but not in non-invasive, poorly differentiated, late-stage tumours[38]. For S100A7, it is important to note that studies carried out before the development of paralogue-specific PCR primers and antibodies probably report on multiple loci[39]. As the S100A7 paralogues are differentially expressed and regulated, and exhibit diverse functions, it is important to discriminate between them in order to understand their distinct functional roles in tumorigenesis. In addition, only a limited number of commercial S100 antibodies have been tested for cross-reactivity against multiple family members[40], and the generic term anti-S100 indicates potential cross-reactivity with multiple family members.

S100 protein expression profiles can be used to facilitate diagnosis and/or prognosis, inform on treatment options and monitor patient response to therapy (see Supplementary information S2, S3 (tables) and references therein). S100B expression in the primary tumour has been used as a diagnostic marker for malignant melanoma in human and veterinary medicine since the 1980s. S100P levels have diagnostic utility in a variety of human cancers as S100P can be detected in primary tumours (breast, colorectal, pancreatic and ovarian cancer), metastatic lesions (lung cancer), serum (breast and colorectal cancer), saliva (head and neck cancer), bile (cancer of the bile duct) and faeces (colorectal cancer) (see Supplementary information S2 (table) and references therein). The combined expression levels of S100A2 and S100A10 are used for prognosis in recurrent colon cancer after adjuvant 5-fluorouracil (5-FU) therapy and radical surgery[5] (also see Supplementary information S3 (table) and references therein). In addition, autoantibodies directed against S100A7 have been reported in ovarian cancer and may facilitate diagnosis[24](also see Supplementary information S2 (table) and references therein). It should be noted that the utility of S100 proteins as cancer biomarkers may be limited by their elevated expression in other pathologies, including cardiovascular, neurological and inflammatory diseases.

The expression of S100 family members in human cancers is controlled by a complex regulatory network, which includes epigenetic mechanisms and signal transduction pathways, including pathways that are activated in response to chemotherapeutic agents. Epigenetic changes regulate *S100P* expression in prostate, pancreatic and cervical cancer;

S100A4 expression in pancreatic, endometrial, gastric, breast, ovarian, renal and brain tumours; *S100A2* in prostate and breast cancer; *S100A6* in prostate and gastric cancer; and *S100A10* expression in pituitary cancer[41–45]. In colon cancer, seven S100 genes are direct targets of histone-lysine methyltransferase MLL2 (also known as KMT2B and KMT2D)[46]; however, coordinated regulation of S100 proteins within a given cancer is atypical. For example, in colon cancer, *S100A4* expression is controlled by WNT–β-catenin signalling[47], whereas *S100P* expression is regulated by activation of both the prostaglandin E2 (PGE2) receptor EP4 subtype (PTGER4) and the MEK–ERK–cAMP-responsive element-binding protein (CREB) pathway[48]. In addition, the regulatory mechanisms modulating S100 expression can be cancer type-specific. For instance, the expression of S100A8 and S100A9 is regulated by hypoxia-inducible factor 1 (HIF1) and PGE2–protein kinase A catalytic subunit (PKA-C)– CCAAT/enhancer-binding protein-β (CEBPβ) signalling in prostate cancer[49,50]; by nuclear factor-κB (NF-κB) signalling in liver cancer[51]; and by ultraviolet radiation, intrinsic ageing and photo-ageing in skin cancer[52]. Finally, the modulation of S100 expression is a common downstream event in S100 signalling cascades, resulting in feedback loops that can sustain and exacerbate tumour progression. This is exemplified by the expression of S100A8 and S100A9 in skin carcinogenesis, in which RAGE activation by extracellular S100A8 and S100A9 upregulates the expression of these ligands, resulting in a feedforward loop that promotes tumorigenesis[53].

S100 signalling in cancer biology

Ten S100 family members actively contribute to *in vivo* tumour growth, metastasis, angiogenesis and immune evasion (TABLE 1). Although S100 proteins can also act as tumour suppressors, examples are rare and cancer type-specific. S100A2 functions as a tumour suppressor in oral cancer and as a tumour promoter in lung cancer[174,178]. S100A7 acts as a tumour suppressor in oestrogen receptor-α (ERα)-positive breast cancer but promotes ERα-negative breast tumour growth[54]. The roles of S100 proteins have been most widely examined in breast cancer and melanoma. As cell lines do not mimic the complex pathology or the S100 protein signatures that are observed in tumours *in vivo*[55,56], our discussion of S100 signalling in melanoma and breast cancer focuses on *in vivo* studies.

S100 signalling in breast cancer

Overexpression of several S100 family members (such as S100A1, S100A4, S100A6, S100A7, S100A8, S100A9, S100A11, S100A14 and S100P) has been reported in breast cancer[57–65]. Although alterations in S100 protein expression levels have been correlated with aggressive disease, the mechanistic contribution of individual family members to disease progression has only been evaluated for S100A4, S100A7 and the heterodimer S100A8–S100A9.

S100A7 expression is not detected in epithelial cells in the normal breast. However, high levels of S100A7 are observed in ductal carcinoma *in situ*, as well as in a subset of invasive breast cancers[66]. With respect to invasive breast carcinomas, S100A7 overexpression is associated with aggressive, high-grade, ERα-negative lesions with lymphocytic infiltration, and is an independent prognostic indicator of poor outcome in patients with these tumours[67]. *In vitro* studies indicate that S100A7 promotes the survival of ERα-negative breast tumour

cells under conditions of anchorage-independent growth[68]. *In vivo*, S100A7 induces ductal hyperplasia in the mammary glands of transgenic mice[69] and enhances tumour growth in orthotopic breast cancer models[68,70,71].

In ERα-positive breast cancer cells, S100A7 inhibits proliferative capacity by mediating the degradation of β-catenin through a mechanism that involves glycogen synthase kinase 3β (GSK3β) and E-cadherin signalling[54]. However, in ERα-negative breast cancer cells, S100A7 activates several pro-survival pathways by interacting with the transcription cofactor COPS5 (also known as JAB1), including upregulation of the activator protein 1 (AP-1) and NF-κB pathways, increased phospho-AKT and downregulation of cyclin-dependent kinase inhibitor CDKN1B (also known as p27 or Kip1)[68,70]. In addition to mediating pro-survival effects, S100A7 enhances the invasive capabilities of ERα-negative breast cancer cells by augmenting epidermal growth factor receptor (EGFR) signalling and matrix metalloproteinase 9 (MMP9) secretion[72,73]. S100A7 is also secreted by tumour cells[69,74], and extracellular S100A7 may facilitate tumour angiogenesis[75] and the recruitment of tumour-associated macrophages[69] through interactions with RAGE on endothelial cells and macrophages, respectively (FIG. 2).

Several studies have suggested that in early-stage breast cancer, expression of S100A4 in combination with expression of either hepatocyte growth factor receptor (HGFR; also known as MET) or osteopontin is a predictive indicator of metastatic disease and poor survival[58,59,76]. Consistent with these observations, engineered overexpression of S100A4 in non-metastatic rat mammary Rama 37 tumour cells induces a metastatic phenotype in orthotopic mammary tumours[77]. Although S100A4 overexpression in the mammary epithelium is not tumorigenic[78], it significantly enhances tumour metastasis in pre-existing tumorigenic backgrounds, such as those in the mouse mammary tumour virus (MMTV)-Neu transgenic[79] and GRS/A[78] mouse models of breast cancer. In both transgenic and orthotopic models, S100A4 expression has a negligible effect on tumour latency, suggesting that S100A4 specifically modulates tumour metastasis rather than tumour growth.

In breast cancer cells, S100A4 overexpression is also associated with increased migratory capacity. Accordingly, S100A4 is found in the pseudopodia of migrating cells[80,81], and Ca^{2+}-bound activated S100A4 localizes to the leading edge of breast cancer cells that are undergoing polarized migration[82]. Furthermore, the Ca^{2+}-dependent interaction of S100A4 with non-muscle myosin IIA regulates the formation and stability of lamellipodia to enhance chemotactic migration[83–85]. S100A4 also interacts with the Rhotekin–RHOA complex to promote membrane ruffling and invasion in EGF-stimulated breast cancer cells[86]. In addition to the interactions with myosin IIA and Rhotekin, S100A4 can bind several other cytoskeletal and adhesion proteins, including F-actin, non-muscle tropomyosin and liprin β1. However, the regulation of these putative S100A4 targets is not well characterized.

Not only does S100A4 drive metastasis when expressed in the tumour, but S100A4 expression in the host stroma also contributes to metastatic dissemination. The metastatic potential of orthotopic mammary tumours is significantly reduced in *S100a4*$^{-/-}$ mice compared with in *S100a4*$^{+/+}$ mice[87], and metastasis to the lungs is inhibited in MMTV-polyoma middle T antigen (MMTV-PyMT)–*S100a4*$^{-/-}$ transgenic mice[88,89]. Despite these

observations, the specific stromal cell types that contribute to S100A4-mediated tumour metastasis have not been well characterized. In the breast tumour microenvironment, the majority of S100A4$^+$ stromal cells originate from the bone marrow[90] and in MMTV-PyMT–*S100A4*$^{-/-}$ transgenic mice, the recruitment of CD45$^+$ leukocytes and CD3$^+$ T lymphocytes to tumours is significantly reduced[89], suggesting that stromal cell-derived S100A4 modulates the tumour immune response. In addition, recent studies indicate that non-bone-marrow-derived S100A4$^+$ cells, such as fibroblasts, are required for meta-static colonization of breast tumour cells to the lungs[87]. Currently, a molecular understanding of how stromal cell-derived S100A4 promotes metastasis is lacking and it remains to be determined whether this is mediated by intracellular and/or extracellular S100A4. Both macro-phages and fibroblasts can secrete S100A4 (REFS 91,92). S100A4 monoclonal antibodies significantly limit breast tumour invasion and metastasis *in vivo*, consistent with an extracellular function for S100A4 (REFS 93,94). Extracellular S100A4 has been shown to stimulate production of MMP13 in endothelial cells and may contribute to tumour angiogenesis[95,96], and recent studies suggest that extracellular S100A4 induces the expression and secretion of pro-inflammatory cytokines in tumour cells to elicit a pro-tumoural response in the microenvironment[97,98]. Although the cell-surface receptors responsible for S100A4 binding and its associated signal transduction pathways remain largely unknown, the identification of these molecules will provide the foundation for a mechanistic understanding of the signalling cascades that are crucial to the metastatic process.

Expression of both S100A8 and S100A9 is upregulated in invasive ductal carcinoma of the breast[63]. Notably, S100A9 upregulation is associated with basal type tumours, high-grade lesions, ERα- and progesterone receptor (PR)-negative status, and *HER2*- and EGFR-positive tumours[99]. The increased levels of S100A8 and S100A9 observed in breast tumour samples are due, in part, to the recruitment of S100A8- and S100A9-expressing myeloid-derived suppressor cells (MDSCs) to the tumour stroma[100,101]. S100A9 expression is down-regulated during the normal differentiation of myeloid precursors to macrophages and dendritic cells. However, in cancer, tumour-derived factors upregulate S100A9 expression in myeloid precursors, which inhibits macrophage and dendritic cell differentiation, and promotes MDSC accumulation[100]. The expression of S100A9 is strictly required for MDSC recruitment, as MDSC accumulation in the tumour is ablated in S100A9-null mice[100]. In addition, MDSC-secreted S100A8–S100A9 heterodimer binds to carboxylated N-glycans on RAGE on the MDSC cell surface[102]. Thus, S100A8 and S100A9 maintain an autocrine feedback loop that sustains MDSC recruitment and the maintenance of immune suppression within the tumour microenvironment.

S100A8 and S100A9 heterodimers and homodimers also have paracrine functions through interactions with RAGE and TLR4 on tumour cells[18,19,103]. In tumour cells, RAGE binding activates MAPK and NF-κB signalling pathways, and upregulates the expression of genes associated with tumour growth and invasion[18,103]. With respect to intracellular functions, in phagocytes, S100A8 and/or S100A9 bind arachidonic acid and activate NADPH oxidase through the direct interaction of S100A8 with cytochrome b_{558}, resulting in the formation of reactive oxygen species (ROS) and the activation of NF-κB signalling[20,104]. In addition, the S100A8–S100A9 complex is prone to oxidative modifications (such as nitrosylation,

glutathionylation and oxidation) by various forms of ROS[27,105,106], but how these modifications modulate the tumour-promoting functions of the complex has not been determined.

Extracellular S100A8 and/or S100A9 also contribute to the formation of the pre-metastatic niche. The release of tumour necrosis factor-α (TNFα), transforming growth factor-β (TGFβ) and vascular endothelial growth factor A (VEGFA) from the primary tumour promotes expression of S100A8 and/or S100A9 in pre-metastatic lung endothelium and lung-associated myeloid cells[107]. Furthermore, extracellular expression of S100A8 and S100A9 induces the expression of serum amyloid A3 (SAA3), which potentiates its own secretion via a TLR4-mediated NF-κB signalling cascade that recruits $CD11b^+$ myeloid cells to the pre-metastatic lung[108]. This produces a pro-inflammatory milieu that mobilizes circulating tumour cells and promotes pulmonary metastasis[108]. Signalling via extracellular S100A8 and S100A9 also supports the establishment of a pre-metastatic niche in the brain. The expansion of bone marrow-derived $CD11b^+GR1^+$ cells, which express high levels of S100A8 and S100A9, creates a local inflammatory environment that mediates the further recruitment of $CD11b^+GR1^+$ myeloid cells and tumour cells through TLR4 signalling to promote brain metastasis[109]. These observations indicate that for breast cancer, S100A8 and S100A9 are crucial factors for establishing the pre-metastatic niche at multiple organ sites. However, it is unknown whether the upregulation of S100A8 and S100A9 within each of these target organs occurs through similar signalling pathways or whether organ-selective factors mediate S100A8 and/or S100A9 expression, as well as the recruitment of bone marrow-derived cells. Lastly, the requirement for S100A8 and/or S100A9 in creating a permissive environment at other organ sites that are relevant to breast cancer metastasis (such as bone) requires further investigation.

In addition to functions in the tumour microenvironment and the pre-metastatic niche, S100A8 and/or S100A9 also mediate chemoresistance and subsequent metastasis of breast cancer cells[101]. Chemotherapy induces the release of cytokines and chemokines, including TNFα, by the tumour stroma. Stromal cell-derived TNFα can then boost the expression and secretion of CXC-chemokine ligand 1 (CXCL1) and CXCL2 by breast tumour cells, resulting in the recruitment of $CD11b^+GR1^+$ myeloid cells and the release of S100A8 and S100A9 within the tumour microenvironment[101]. Activation of the pro-survival ERK1, ERK2 and ribosomal protein S6 kinase β1 (S6K1; also known as P70S6K) pathways by S100A8 and/or S100A9 facilitates the expansion of chemoresistant breast cancer cells both within the primary tumour and at distant metastatic sites[101], thus providing a survival advantage to breast cancer cells that are under chemotherapeutic stress. Clinical targeting of multiple aspects of the TNFα–CXCL1/CXCL2– S100A8/S100A9 signalling axis may provide a mechanism for limiting drug resistance and metastatic dissemination.

S100 signalling in melanoma

Malignant melanoma is a highly proliferative and heterogeneous cancer that is resistant to conventional chemotherapy[34]. In melanoma, unlike most cancers, mutations in *TP53* are rare. Instead, driver mutations that activate oncogenes (such as *BRAF* and *NRAS*) and inactivate cell cycle regulators (such as *CDKN2A* (which encodes p16) and *PTEN*) prevent

wild-type TP53 from activating downstream target genes and inducing cell cycle arrest and/or apoptosis. Twelve S100 family members are expressed in melanoma: four exhibit no change in expression (S100A8, S100A9, S100A10 and S100A11); one is downregulated (S100A2); and seven are upregulated (S100A1, S100A4, S100A6, S100A13, S100B and S100P; see Supplementary information S1 (table)). The considerable number of S100 family members that are expressed in melanoma is consistent with the localization of *S100A1–S100A16* genes to the EDC on human chromosome 1, and with previous observations that the skin expresses the largest number of S100 family members[110]. However, there is little information regarding the cellular distribution of S100 family members in melanoma. S100A4 is primarily expressed in stromal cells, whereas S100B, S100A6 (REFS 111, 112) and S100A10 (REF. 113) are expressed in tumour cells. Tumour and serum levels of S100B have been used as a diagnostic marker for melanoma for many years and more recently, urinary S100A7 levels have been reported as a potential diagnostic tool[114]. Decreased levels of serum S100B are associated with the dramatic initial clinical responses to targeted therapies for melanoma. Nonetheless, durable responses to these and other agents are rare owing to the rapid development of multifactorial resistance within individual tumours and patients. To date, increased expression of only one S100 family member, S100A13, has been associated with melanoma resistance to chemotherapy (specifically, resistance to the DNA-modifying agents dacarbazine and temozolomide; see Supplementary information S3 (table)). *In vivo* studies have confirmed that S100A4, S100A9 and S100B contribute to melanoma progression and may be therapeutic targets.

In melanoma, signalling via extracellular S100A4, S100A8 and S100A9 culminates in the expression of cytokines, chemokines, MMPs, and angiogenic and anti-apoptotic factors (FIG. 3). However, their molecular mechanisms of action are different. Extracellular S100A9, but not S100A8, binds the EMMPRIN receptor and requires the adaptor protein TNF receptor-associated factor 2 (TRAF2) to upregulate the expression of TNFα, IL-1, IL-6 and other factors[9]. Whether extracellular S100A8 homodimers and S100A8–S100A9 heterodimers also mediate cytokine expression has not been examined. Moreover, upregulation of S100A8 and S100A9 expression by these cytokines generates a feedforward mechanism that can drive tumour progression[9]. The interaction of stromal cell-derived S100A4 with RAGE on tumour cells also activates NF-κB-dependent gene expression[97]. The subsequent release of pro-inflammatory cytokines and paracrine factors by tumour cells stimulates endothelial cells and monocytes to promote angiogenesis and protumour immune responses, respectively[97]. On the basis of the well-established link between chronic inflammation and cancer[115], the role of S100B in chronic inflammation[116] and the presence of S100B in the systemic circulation of patients with melanoma, it is likely that signalling mediated by extracellular S100B also contributes to melanoma progression. Finally, melanoma-derived exosomes induce the expression of S100A8–S100A9 at pre-metastatic sites[117].

There is interest in identifying the mechanisms in melanoma that prevent wild-type TP53 from activating cell cycle arrest and/or apoptosis in response to DNA-damaging agents or UV radiation[118–120]. In melanoma cell lines, the regulation of growth and survival by intracellular S100B involves a feedback loop that inactivates TP53 (REFS 121–125). Wild-

type TP53 upregulates *S100B* expression by directly binding to the *S100B* promoter[121]. However, S100B downregulates TP53 levels and activity by directly binding to and dissociating the TP53 tetramer[126], and stimulating TP53 polyubiquitylation and degradation[122,126]. Additionally, S100B blocks covalent modification of TP53 (such as phosphory lation and acetylation)[127]. Notably, a reduction in S100B levels or activity by as little as twofold elevates TP53 and phospho-TP53 levels, reduces survival and restores UV sensitivity[125,128]. There are a number of additional TP53-binding proteins that directly compete with S100B and modulate the TP53-S100B interaction. For example, TP53 and S100B both bind MDM2 and MDM4, which may allow for synergy in TP53 downregulation[127]. As is typical for S100 proteins, the effects of S100B expression on melanoma cell growth are not limited to a single pathway. S100B binding to the p90 ribosomal S6 kinase (RSK) blocks ERK-dependent phosphorylation and results in cytoplasmic sequestration of RSK[124]. However, it is not known how the shift in the subcellular distribution of RSK affects cell growth. Ascertaining the pathways that regulate both anti-tumorigenic and protumorigenic melanoma responses will require a careful assessment of the number, relative abundance and cellular distribution of S100 family members and their target proteins. Nonetheless, the beneficial effects of S100B inhibition on multiple pathways that contribute to the melanoma cell phenotype make it an excellent target for drug discovery.

Other S100 family members

Although there is a considerable amount of literature on the contributions of S100A4, S100A7, S100A8, S100A9 and S100B to tumour growth and metastasis in a number of cancers (see TABLE 1 and references therein), the role of the other S100 family members in modulating tumorigenesis is less well defined. In murine models of cancer, S100A1, S100A2, S100A3, S100A6, S100A10, S100A11, S100A14 and S100P are all reported to affect tumour growth. However, with the exception of S100P, the contribution of these S100 family members to promoting a cancerous phenotype has only been examined in one or two model systems (TABLE 1) and the mechanistic basis for the observed effects on tumour progression has not been delineated. By contrast, S100P has been shown to enhance tumour growth in several cancers, including lung, prostate, colorectal and pancreatic cancer (TABLE 1). Although S100P enhances proliferation in all models that have been examined, transcriptional regulation of *S100P* expression is highly dependent on the type of cancer. In colon cancer, expression is mediated by PGE2–PTGER4 signalling, which activates CREB via the ERK–MEK pathway[48]; in prostate cancer by IL-6 (REF. 129); and in breast and cervical cancer by glucocorticoids[130]. Consistent with the observation that S100P enhances the migratory capabilities of tumour cells *in vitro*[131] and metastasis *in vivo*[132–134], the majority of S100P targets are cytoskeletal regulators. S100P–mediated activation of ezrin[135] (a membrane–cytoskeleton linker protein) and Ras GTPase-activating-like protein 1 (IQGAP1; a juxtamembrane scaffolding protein that links plasma membrane receptors with downstream signalling pathways)[136]provides a direct mechanism for the regulation of tumour cell migration by S100P. In addition, the binding of extracellular S100P to RAGE upregulates NF-κB activity to enhance cell survival[137]. Currently, extracellular S100P is thought to stimulate tumour cell proliferation through an autocrine signalling mechanism.

However, a complete examination of potential paracrine functions and the cell types involved has been limited by the absence of the *S100P* gene in the mouse genome.

Targeting S100 proteins

S100 family members are excellent targets for cancer treatment as mouse models suggest that genetic deletion has minimal effects on normal physiology. In addition to cancer, some members of the S100 family represent attractive targets for the treatment of other diseases. For example, S100B and S100A1 inhibitors may delay the progression of Alzheimer's disease[116,138]. However, S100A1 inhibitors may be contraindicated in patients with heart disease, as S100A1 delays the development of cardiomyopathy[139].

A number of pharmacological approaches have been used to modulate S100 signalling in models of and in patients with cancer. Calcimycin (a calcium ionophore), niclosamide (an antihelminth drug) and sulindac (a non-steroidal anti-inflammatory drug) have all been identified as inhibitors of *S100A4* transcription[140,141]. However, the efficacy of this approach may be limited owing to the long half-life of S100 proteins, which could prevent the achievement of sufficiently low steady-state protein levels to elicit a therapeutic effect. Transcriptional modulators may also have clinically significant toxic off-target effects owing to their ability to regulate the expression of numerous proteins under the control of the same or related transcriptional regulatory assemblies. Although gene therapy has not been used to modulate the expression of S100 family members in patients with cancer, it has been used in preclinical animal models, in which it beneficially upregulates S100A1 expression in heart disease[142]. Other approaches to modulate S100 protein activity include S100A4- and S100P–neutralizing antibodies[93,94,143], and peptibodies (peptide–Fc fusion proteins) directed against S100A8 and S100A9 (REF. 144); both approaches reduce tumour growth in murine cancer models. Although the specificity of antibody-based therapies may reduce toxicity and off-target effects, their efficacy may be limited by their ability to target only extracellular S100 proteins. However, conformationally constrained inhibitory peptides directed against S100B, which are capable of penetrating cells, have been shown to reduce tumour growth in a melanoma xenograft model[145].

The most common strategies for inhibiting S100 proteins exploit small molecules that block the hydrophobic cleft required for the recognition of S100 targets, and for eliciting biological effects[5]. Examples include paquinimod (also known as ABR-215757) and tasquinimod (also known as ABR-215050), which are quinoline-3-carboxamide derivatives that block the interaction of S100A8 and S100A9 with RAGE and TLR4, respectively (REFS 19,146). Paquinimod exerts anti-inflammatory effects in a number of *in vivo* disease models, but has not been specifically tested in cancer models. However, tasquinimod improves progression-free survival in patients with metastatic castration-resistant prostate cancer, possibly by reducing the recruitment of MDSCs and inhibiting metastasis[147,148]. Cromolyn, an anti-histaminic drug, disrupts the S100P– RAGE interaction, reducing pancreatic tumour growth and increasing the effectiveness of the chemotherapeutic drug gemcitabine[149,150]. Cromolyn binds several other S100 family members (S100A1, S10012 and S100A13), but the biochemical and biological consequences of these interactions have not been examined. Amlexanox, another anti-inflammatory anti-allergic immunomodulatory

drug, interacts with several S100 proteins (S100A1, S100A4 and S100A13)[151–153]. Amlexanox inhibits S100A13 secretion, disrupts the interaction of S100A13 with fibroblast growth factor 1 (FGF1), and antagonizes the mitogenic and angiogenic effects of FGF1 (REF. 152). Phenothiazines also interact with multiple S100 family members. In the case of S100A4, phenothiazine-mediated S100A4 oligomerization results in the sequestration of S100A4 away from its protein targets[154,155]. It should be noted that all of these drugs were developed for other indications and their lack of selectivity within the S100 family is not surprising given the high degree of structural similarity between S100 proteins. Ongoing efforts to target S100 proteins are focused on improving selectivity and other pharmacological properties of S100 inhibitors such as affinity and biological half-life.

The large, shallow and relatively ‘featureless’ interfaces typified by most protein–protein interactions (PPIs) pose unique challenges for the development of high-affinity PPI inhibitors. In contrast to typical protein–protein interfaces, the relatively deep target-binding clefts of S100 proteins can readily accommodate small molecules that have been discovered through traditional high-throughput experimental screening and computer-aided drug design. These approaches have successfully identified new compounds that inhibit the interactions of S100P[149], S100A4 (REFS 156,157), S100A9 (REF. 156), S100A10 (REF 158) and S100B[123,159] with their respective targets. An examination of these S100–small-molecule complexes has revealed that most small molecules target one of three distinct pockets within S100 proteins[160] (FIG. 4). Site 1 is exposed by Ca^{2+}-mediated conformational rearrangements and involves residues from helices 3 and 4, and loop 2 (the hinge region), as occurs in the S100B– SEN205A interaction, for example[161] (FIG. 4a). Interactions at sites 2 and 3 involve residues from loop 2 and helix 4, and the C-terminal loop and helix 1, respectively. In some instances, multiple copies of the same compound occupy both sites 2 and 3 (as occurs in the S100B–pentamidine[162]and S100A4–trifluoperazine interactions[154]). However, compounds have also been identified that target only one of these sites (such as S100B–SBiX-inhibitor complexes[160]or the S100A13–amlexanox interaction[152]). A comparison of S100 protein–small-molecule structures reveals that the location and orientation of ligands bound in sites 2 and 3 are less well conserved than those bound in site 1. The diversity of binding positions observed at sites 2 and 3 probably reflects differences in the highly specific interactions that mediate the binding of various targets (such as H-bonds, hydrophobic interactions and other types of interactions) within the S100 protein family and thus provides opportunities for specific S100–small-molecule interactions.

Given that S100 proteins can accommodate small molecules at several sites, it is not unexpected that multiple mechanisms of inhibition are observed, including classic competitive binding between an inhibitor and physiological target to the same or overlapping sites on a S100 protein, such as amlexanox binding to S100A13 or SEN205A binding to S100B[152,161] (FIG. 4a). However, some inhibitors of S100 proteins may exhibit subtle allosteric effects as exemplified by pentamidine binding to S100B. Each S100B subunit binds two pentamidines, with one binding site adjacent to the target binding site and the second at the dimer interface[162]. Although the pentamidine binding sites exhibit minimal overlap with the S100B target-binding cleft, pentamidine disrupts the S100B–TP53 interaction and potently inhibits the growth of malignant melanoma cells[163], suggesting that inhibition occurs due to allosteric effects. More recently, structural, biophysical, and protein

dynamics studies have shown that the binding of small-molecule inhibitors and peptides can increase Ca^{2+}-binding affinity upon complex formation in a manner analogous to that associated with the binding of bona fide targets[24].

When considering how small molecules and peptides that bind at some distance from the S100 EF-hands may affect Ca^{2+}-binding affinities, it is important to take into account the structural features as well as dynamic properties of S100 proteins[24,164–166]. In the absence of a protein target, S100 proteins sample a large ensemble of conformational sub-states that exhibit a wide range of Ca^{2+} affinities but, overall, their apparent affinity is low. Target binding biases the distribution of sub-states towards those with high Ca^{2+} affinity. This model, termed the 'binding and functional folding' (BFF) model (FIG. 4c), provides a foundation for understanding how the biochemical activities of S100 proteins fit into overall cell physiology. The coupling of Ca^{2+} and target binding allows for high intracellular S100 protein concentrations (>1 μM) without substantial sequestration of free Ca^{2+} or disruption of Ca^{2+} oscillations, and thus cells remain highly responsive to changes in calcium and/or target availability. S100 inhibitor screens are now incorporating an examination of Ca^{2+} affinity and protein dynamics[24], which should allow for the identification of inhibitors with increased binding affinities and specificities.

Future directions

In the past decade, important advances regarding the expression, structure and signalling of S100 proteins have improved our understanding of normal cell physiology as well as the pathophysiology of cancer. The development of molecular probes — such as antibodies and small-molecule inhibitors — will be instrumental for deciphering the *in vivo* functions of specific S100 proteins, as well as distinguishing the contribution of intracellular and extracellular S100 proteins. Moreover, these probes may also have therapeutic potential for cancer or other diseases. Despite considerable progress in S100 protein biology, we currently have little information on how post-translational modifications or heterodimer formation affect S100 signalling. A mechanistic examination of both S100 protein biology and biochemistry will be required to define how each family member contributes to the proliferation, metastasis, angiogenesis and immune evasion of cancers and other diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors apologize to the numerous colleagues whose important contributions could not be included in this Review owing to space limitations. The authors thank S. C. Almo and J. M. Backer for critical reading of this Review. The authors' research is funded by grants from the New York State Department of Health, Health Research Science Board (H11R-040; to A.R.B.), the National Cancer Institute (US National Institutes of Health; CA100324 to A.R.B. and CA107331 to D.J.W.), the Albert Einstein Cancer Center (NCI, NIH; CA013330), the Marlene and Stewart Greenebaum Cancer Center (NCI, NIH; CA134274) and the Center for Biomolecular Therapeutics, University of Maryland School of Medicine, USA (to D.J.W. and D.B.Z.).

Glossary

Sub-states	Closely related interconverting conformational states that can be sampled by a protein under a given set of conditions
GRS/A	An inbred mouse strain carrying the *Mtv2*^{a} allele, which controls the expression of endogenous mouse mammary tumour virus and the early development of hormone-induced mammary tumours
Lamellipodia	Transient cellular protrusions that form during cell migration
Chemotactic migration	Directional cell migration in response to soluble extracellular ligands
MMTV-polyoma middle T antigen	(MMTV-PyMT). A murine breast cancer model with expression of PyMT under the control of the mouse mammary tumour virus (MMTV) promoter

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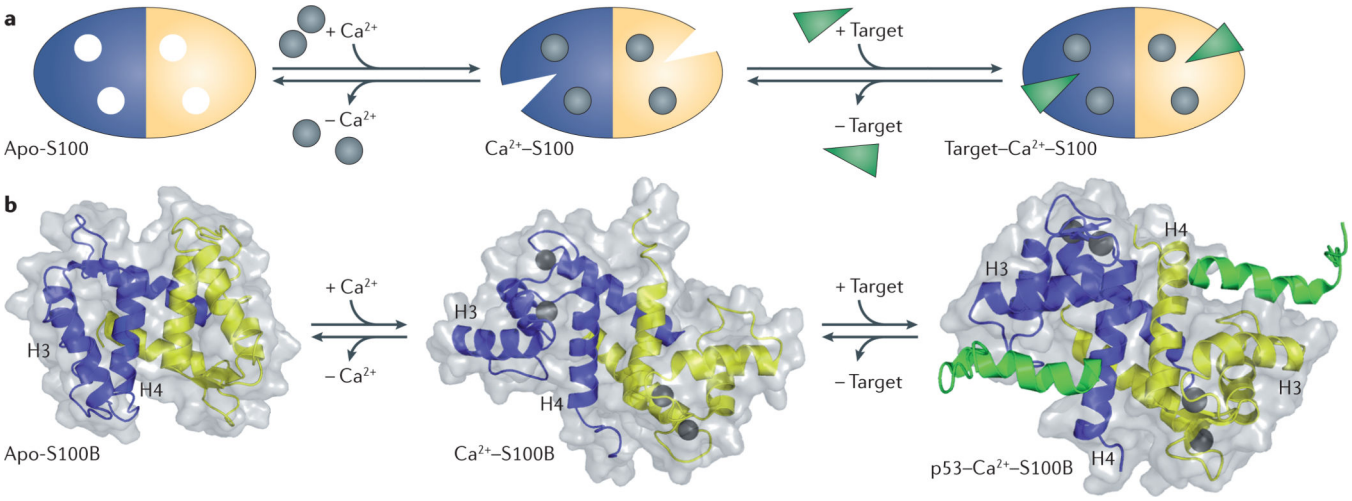

Figure 1. S100 protein structural organization

a | Apo-S100 protein shown with one blue subunit and one yellow subunit. S100 proteins are regulated by Ca^{2+} binding (grey circles), which allows them to act as Ca^{2+} sensors that can translate alterations in intracellular Ca^{2+} levels into a cellular response. Ca^{2+} binding induces a conformational rearrangement that exposes a hydrophobic cleft, allowing the S100 protein to bind its cellular targets (green) and elicit a physiological response. **b** | Ribbon and surface diagrams of apo–S100B, Ca^{2+}–S100B and the Ca^{2+}–S100B–p53 peptide complex. Individual subunits are shown in blue and yellow, the Ca^{2+} ions are shown as dark grey spheres, and the TP53 peptide is shown in green. The conformational rearrangement that occurs upon Ca^{2+} binding is referred to as the 'Ca^{2+} switch', and involves the reorientation of helix 3 (H3) and subsequent exposure of hydrophobic residues that participate in target protein binding.

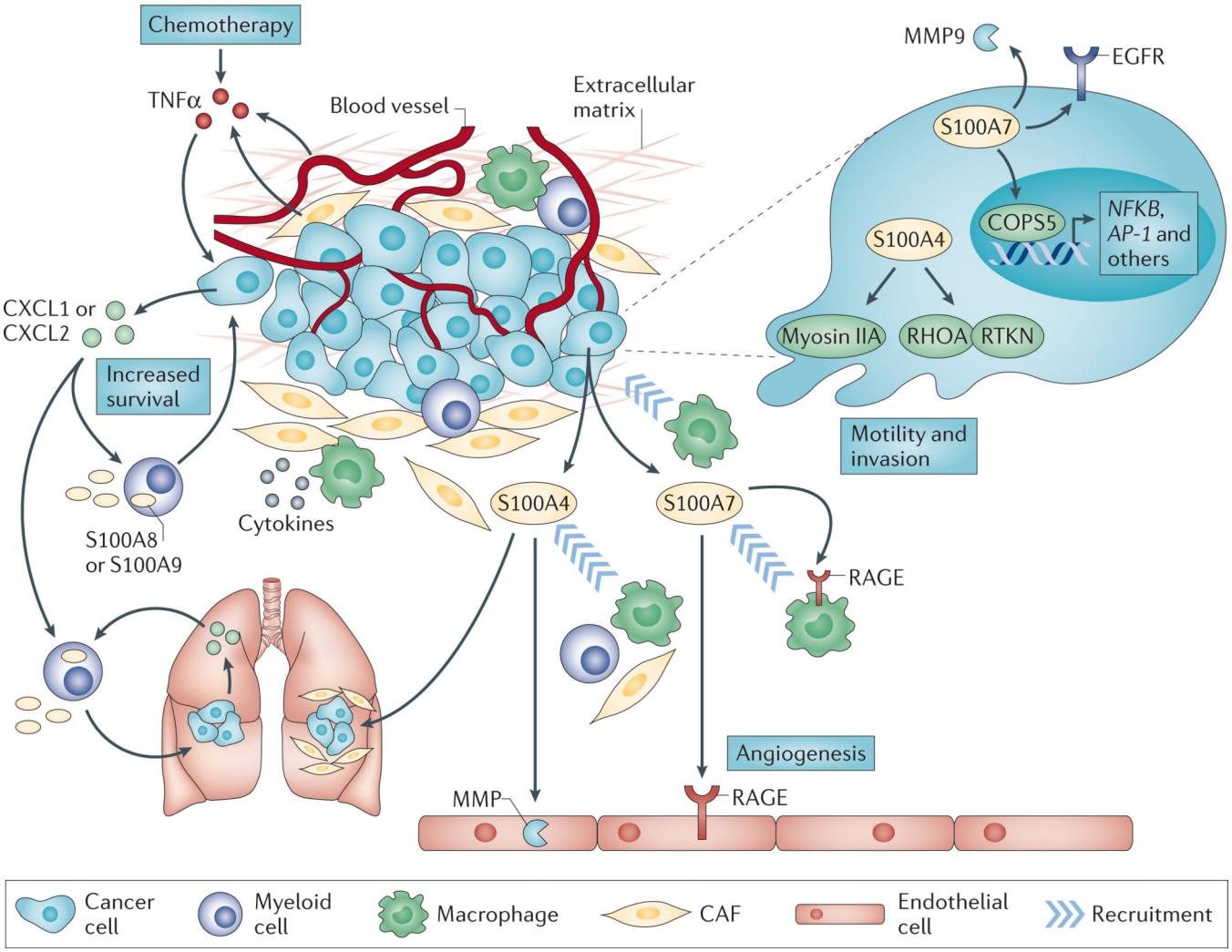

Figure 2. S100 signalling in breast cancer

Intracellular and extracellular S100A4 and S100A7, and extracellular S100A8 and S100A9 mediate breast tumour progression and metastasis. In cells that do not express oestrogen receptor-α(ERα), intracellular S100A7 enhances cell survival and invasion by upregulating nuclear factor-κB (NF-κB; which is encoded by *NFKB*) and epidermal growth factor receptor (EGFR) signalling. Tumour cell-derived extracellular S100A7 binds receptor for advanced glycosylation end products (RAGE) on macrophages to mediate their recruitment to the tumour microenvironment. In addition, extracellular S100A7 also binds RAGE on endothelial cells to promote angiogenesis. S100A4 expression in tumour cells enhances cell migration and invasion through interactions with cytoskeletal effectors such as myosin IIA and Rhotekin (RTKN). Stromal cell-derived S100A4 mediates the recruitment of myeloid cells to the tumour microenvironment and is required for metastatic colonization in the lung. Whether these responses are elicited by extracellular or intracellular S100A4 is unknown; however, extracellular S100A4 stimulates matrix metalloproteinase (MMP) production in endothelial cells. Within the tumour microenvironment, S100A8 and S100A9 have both autocrine and paracrine functions that sustain myeloid recruitment and/or immune

suppression and NF-κB signalling, respectively. The release of cytokines by the primary tumour also promotes the recruitment of myeloid cells expressing S100A8 or S100A9 to the pre-metastatic lung to maintain a pro-inflammatory milieu that promotes tumour metastasis. Lastly, chemotherapy induces the release of tumour necrosis factor-α (TNFα) from endothelial and other stromal cells, which results in a CXC-chemokine ligand 1 (CXCL1)- or CXCL2-induced S100A8 or S100A9 signalling axis between tumour cells and myeloid cells that facilitates the survival of chemoresistant tumour cells both within the primary tumour and at metastatic sites. AP-1, activator protein 1; CAF, cancer-associated fibroblast.

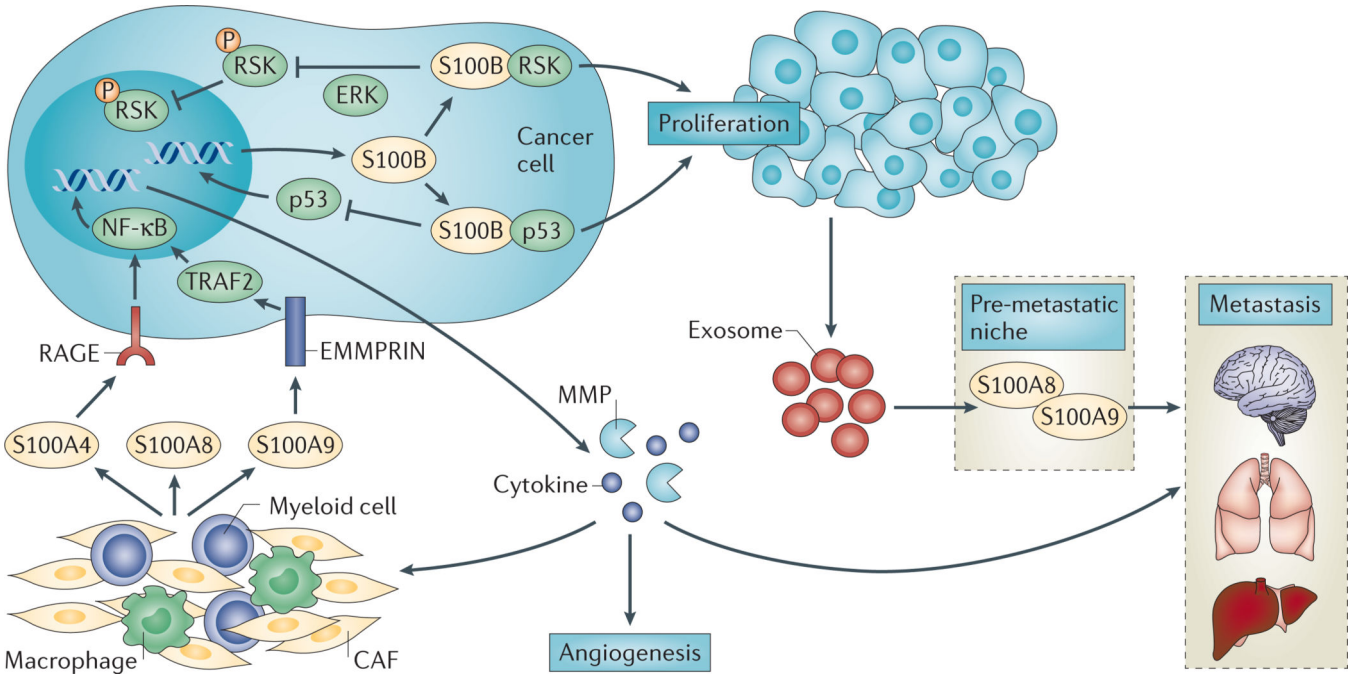

Figure 3. S100 signalling in melanoma

Intracellular and extracellular S100A4, S100A8 and S100A9 contribute to melanoma proliferation and metastasis. Stroma-derived extracellular S100A4 and S100A9 — by signalling through receptor for advanced glycosylation end products (RAGE) and extracellular matrix metalloproteinase inducer (EMMPRIN), respectively — activate nuclear factor-κB (NF-κB)-mediated tumour cell expression of cytokines and matrix metalloproteinases (MMPs) that promote tumour cell invasion and metastasis. Extracellular S100A8 and S100A8–S100A9 heterodimers are also released from stromal cells, but their contribution to melanoma biology and their molecular mechanisms of action have not been elucidated. Melanoma-derived exosomes promote S100A8 and S100A9 expression at metastatic niches. In tumour cells, wild-type TP53 upregulates S100B expression, which promotes proliferation via p90 ribosomal S6 kinase (RSK) and a negative feedback loop involving TP53. S100B binding to TP53 blocks oligomerization and stimulates TP53 polyubiquitylation and degradation, which decreases TP53 transcriptional activity and downregulates the expression of pro-apoptotic genes. S100B binding to RSK blocks ERK-dependent phosphorylation and cytoplasmic sequestration of phospho-RSK. CAF, cancer-associated fibroblast; TRAF2, tumour necrosis factor receptor-associated factor 2.

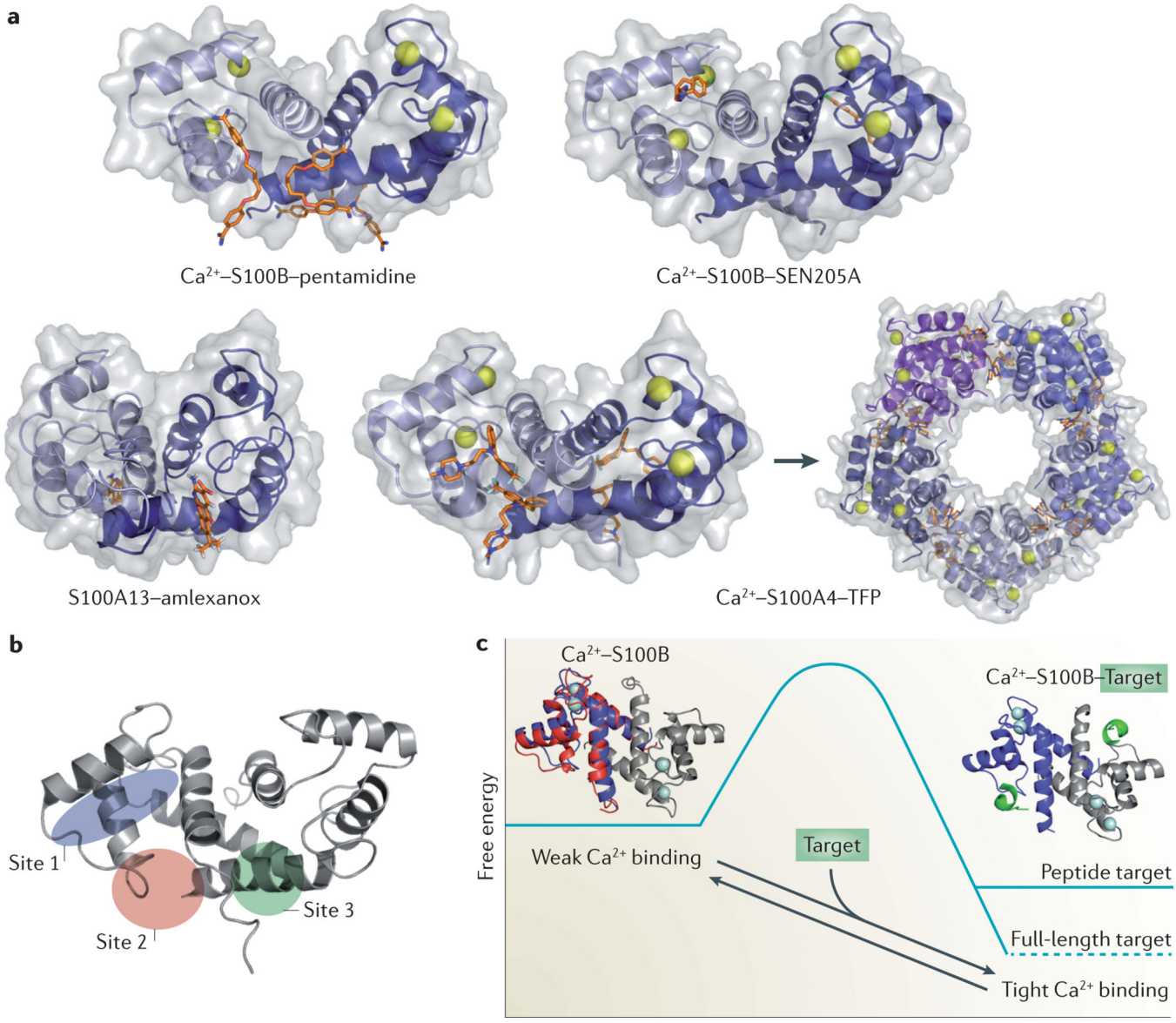

Figure 4. S100 protein–inhibitor complexes

a | Ribbon and surface diagrams of the Ca^{2+}–S100B–pentamidine complex (RCSB Protein Data bank (PDB) identifier: 3CR4), the Ca^{2+}–S100B–SEN205A complex (PDB identifier: 3HCM), the Ca^{2+}–S100A13–amlexanox complex (PDB identifier: 2KOT) and the Ca^{2+}–S100A4–trifluoperazine (TFP) complex (PDB identifier: 3KO0). Individual S100 subunits are shown in light and dark blue, the Ca^{2+} ions are shown as grey spheres and the inhibitors as orange sticks. Small-molecule inhibitors can bind S100 proteins in distinct orientations. TFP binding induces the assembly of five Ca^{2+}–S100A4–TFP dimers into a pentameric ring. **b** | Ribbon diagram of Ca^{2+}–S100B showing three small-molecule binding sites. Site 1 involves residues from helices 3 and 4, and loop 2 (the ‘hinge’ region); site 2 involves residues from loop 2 and helix 4; and site 3 involves residues from the carboxy-terminal loop and helix 1. **c** | The ‘binding and functional folding’ (BFF) model for S100 protein–protein interactions (PPIs). In the absence of a molecular target, Ca^{2+}-bound S100 proteins

(that is, Ca^{2+}–S100B) sample a large ensemble of dynamic sub-states with a range of Ca^{2+}-binding affinities (red and blue ribbon diagrams) that result in a low net apparent affinity for Ca^{2+}. Target binding induces a mini-folding event that stabilizes these dynamic features and biases the ensemble towards those sub-states (blue ribbon diagram), with high affinity for Ca^{2+} and a lower (that is, more favourable) global free energy (a complete description of the model can be found in Liriano *et al*[24] and Markowitz *et al*[167]). Typically, complexes with full-length targets exhibit lower free energies than complexes involving target-derived peptides. This property allows for high intracellular S100 protein concentrations (>1 μM) without substantial sequestration of free Ca^{2+} or disruption of Ca^{2+} oscillations, but provides a highly responsive system that is poised to regulate cellular processes upon target binding. Drugs that also enhance S100 protein Ca^{2+} occupancy can be identified and/or better engineered by monitoring changes in the structure and dynamic properties of the S100 protein upon drug and/or target binding. Part **c** reprinted from *J. Mol. Biol* **423**, Liriano, M. A. *et al*. Target binding to S100B reduces dynamic properties and increases Ca^{2+}-binding affinity for wild type and EF-hand mutant proteins, 365–385 © (2012), with permission from Elsevier.

Table 1

In vivo cancer phenotypes of S100 family members

Cancer	Family member	Model systems	Phenotype	Refs
Breast	S100A1	Xenograft	↑ Growth	168
	S100A4	Xenograft and GEMM	↑ Metastasis	77–79,87–89,169,170
	S100A7	Xenograft and GEMM	↑ ↓ Growth	54,69,71
	S100A8	Xenograft	↑ Metastasis	103
Osteosarcoma	S100A4	Xenograft	↑ Metastasis	171–173
Oral	S100A2	Xenograft	↓ Growth	174
	S100A7	Xenograft	↓ Proliferation and ↓ metastasis	38
Head and neck	S100A4	Xenograft	↑ ↓ Growth and ↑ metastasis	175–177
Lung	S100A2	Xenograft	↔ Growth and ↑ metastasis	178
	S100A4	Xenograft	↑ Metastasis	179
	S100A9	GEMM	↓ Inflammation	180
	S100A10	Xenograft	↑ Growth and ↑ immune evasion	181
	S100A11	Xenograft	↑ Growth	37
	S100P	Xenograft	↑ Angiogenesis and ↑ metastasis	132
Prostate	S100A3	Xenograft	↑ Growth	182
	S100A4	Xenograft and GEMM	↑ Growth, ↑ metastasis and ↑ angiogenesis	183–185
	S100A9	GEMM	↑ Growth, ↑ metastasis and ↑ angiogenesis	19,148
	S100A8 and S100A9	Xenograft	↔ ↓ Growth, ↔ metastasis and ↓ inflammation	186
	S100P	Xenograft	↑ Growth	187
Colorectal	S100A4	Xenograft	↑ Metastasis	188
	S100A8 and S100A9	Xenograft	↑ Growth and ↑ metastasis	18
	S100P	Xenograft	↑ Growth and ↑ metastasis	133,134
Brain	S100A4	Xenograft	↑ Metastasis	189
	S100A9	Xenograft	↑ Growth	190
	S100B	Xenograft	↑ Growth, ↑ angiogenesis and ↑ inflammation	30
Gastric	S100A4	Xenograft	↑ Growth	191
	S100A6	Xenograft	↑ Growth and ↓ metastasis	192
Bladder	S100A4	Xenograft	↑ Metastasis	193
Lymphoma	S100A9	Xenograft	↑ Growth and ↑ immune evasion	19,100
Pancreas	S100A4	Xenograft	↑ Growth and ↑ angiogenesis	94
	S100P	Xenograft	↑ Growth and ↑ metastasis	143,194
Melanoma	S100A4	Xenograft	↑ Growth and ↑ angiogenesis	94
	S100A9	Xenograft	↑ Metastasis	9
	S100B	Xenograft and GEMM	↑ Growth	123,145
Renal	S100A4	Xenograft	↑ Growth and ↑ metastasis	195
Liver	S100A14	Xenograft	↑ Growth and ↑ metastasis	196
Thyroid	S100A4	Xenograft	↑ Growth and ↑ metastasis	197

Cancer	Family member	Model systems	Phenotype	Refs
	S100A8	Xenograft	↑ Growth and ↑ metastasis	198
	S100A11	Xenograft	↑ Growth	199
Thymus	S100A8 and S100A9	Xenograft	↑ Growth and ↑ immune evasion	144

↓, decreased; ↑, increased; ↔, no change; GEMM, genetically engineered mouse model.