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Biochimica et Biophysica Acta 1448 (1998) 190–199

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Calcium-binding protein S100A4 in health and disease

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Accepted 13 October 1998

Abstract

The S100 proteins contain two EF-hand motifs and are of generally unknown function. One of these proteins, S100A4, is an intracellular calcium-binding protein that is present in normal rodent and human cells. In cultured rodent mammary cells, S100A4 is expressed at a higher level in some metastatic epithelial cells than in non-metastatic counterparts. Similarly, in human breast cell lines, S100A4 is present at a higher level in cultured cells from the more malignant, than in those from the more benign tumours. Gene transfer experiments have shown that rodent or human S100A4 is able to induce metastatic capability in otherwise non-metastatic breast tumour cells. Furthermore, expression of rodent S100A4 transgenes can induce metastasis of benign tumours arising in transgenic model systems. Possible mechanisms for the metastasis-inducing effect of S100A4 and the relevance of these observations to human cancer are discussed. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: S100A4; p9Ka; Breast cancer; Metastasis

1. Nomenclature and early descriptions of S100A4

S1004 is known by a variety of names which refer to the protein, p9Ka [1,2], calvasculin [3], CAPL [4], or a cDNA, *mts*1 [5], pEL98 [6], 18A2 [7], 42A [8], fsp (fibroblast specific protein) [9] mainly from rodent and human cells. Many of the original cloned DNAs arose from differential screening experiments in which cDNAs to mRNA populations were compared in cultured cells before and following a variety of treatments. For example, S100A4 mRNA was reported to be up-regulated when quiescent BALB/c 3T3 mouse fibroblasts were stimulated to grow with serum [7], or when mRNA from cultured mouse fibroblasts was compared with mRNA from primary cultures of mouse embryo fibroblasts [6]. S100A4 mRNA was up-regulated in carcinogen- or oncogene-transformed fibroblasts [6]. More recently, S100A4 mRNA and protein have been shown to be increased in NIH 3T3 fibroblasts or in normal rat kidney cells transformed by v-Ki-*ras*, v-Ha-*ras* or v-*src* [10,11]. S100A4 mRNA is up-regulated when epithelial cells are induced to change morphology, for example when normal rat mammary cells [12] or benign mammary tumour cells [1] spontaneously convert to an elongated morphology in culture. Similarly, S100A4 has been identified as an up-regulated fibroblastic mRNA in a differential screen between murine tubulointestinal fibroblasts and isogenic proximal tubule cells of the kidney [9]. The up-regulation in these situations may be related to the epithelial/mesenchymal conversion described in fibros-

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PII: S0167-4889(98)00143-8

ing kidney, and S100A4 has been proposed to be an early event in such a conversion [13]. S100A4 mRNA is induced (together with S100A10 mRNA) [8], when rat pheochromocytoma cells are induced to elongate with nerve growth factor. Thus, elevated levels of S100A4 have been described in association with growth and morphological changes in cultured cells from a variety of origins. In rat sciatic nerve, S100A4 mRNA level increases in the distal portion following a crush injury [14], showing that S100A4 mRNA can also be modulated in vivo.

2. Distribution of S100A4 protein in normal tissues

S100A4 is widely distributed in normal tissues, usually showing a highly specific cell type distribution, and it has been necessary to use immunocytochemical techniques with highly specific antisera to obtain the precise distribution of S100A4. Cells which stain strongly for S100A4 in the rat include the proximal and distal tubules of the kidney, the ducts of the parotid salivary gland, which are involved in changing the composition of the saliva, the parietal cells of the stomach and the absorptive cells, but not the mucous-secreting cells, of the intestine [15,16]. A common feature of all these cells would seem to be their involvement in processes of ion transport. However, a more restricted pattern of expression has been reported in the kidney by others [9]. S100A4 seems to be generally produced at only a very low level in brain, but some peripheral neuronal cells stain weakly, and S100A4 mRNA increases in rat sciatic nerve 14-fold between days 2 and 23 postnatally [14]. S100A4 is associated with the smooth muscle of the gut, and the smooth muscle of arteries and other blood vessels [15]. In capillaries, however, which do not possess a smooth muscle wall, S100A4 is found in the endothelial cells [15,16]. In the immune system there is staining of particular cells in the spleen, marrow and lymph glands, and in the blood, occasional white cells are stained [15]. In humans, S100A4 has been found in bone marrow, spleen, lymphocytes and at a particularly high level in peritoneal macrophages [17]. S100A4 mRNA is not expressed in the mouse embryo until day 12.5, when it becomes detectable in fibroblastic cells in a variety of locations [9].

3. S100A4 protein

S100A4 is a polypeptide of 100 amino acids, excluding the initiating N-terminal methionine (Fig. 1). The molecular mass of the natural protein, determined by electrospray ionisation mass spectrometry, 11 646 Da, differs from that of the recombinant protein (11 594 Da), reflecting the former's N-terminal acetylation; however, natural S100A4 is otherwise unmodified [18]. S100A4 has two EF-hand structures

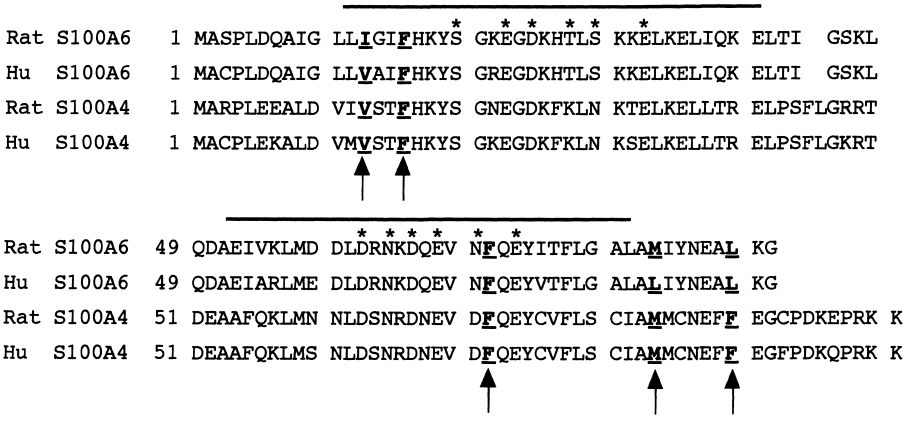

Fig. 1. The primary structures of rat and human S100A4 and a comparison with rat and human S100A6 (calcyclin). The amino acid sequences of rat and human S100A4 and S100A6 are shown as one-letter amino acid code and are aligned with one another. The helix–loop–helix regions of the two EF hands are shown as solid lines above the sequence with the calcium ligands of the calcium-binding loop marked with asterisks. Amino acid residues important for the dimer interface of S100A6 [22] are shown in bold and underlined above the vertical arrows. Amino acids in the equivalent positions of S100A4 are similarly indicated.

(Fig. 1), which conform to the variant forms of S100 proteins, separated by a short spacer. The C-terminal tail region is longer than for some other S100 proteins (Fig. 1). S100A4 has been purified from natural sources [19] and also produced in recombinant form [20]. Upon reverse phase HPLC, natural S100A4 behaves as a highly hydrophobic protein, being eluted by a high acetonitrile concentration [19]. However, purification is more conveniently carried out by a two-step procedure of ion exchange chromatography followed by chromatography on phenyl–Sepharose. S100A4 is eluted from the phenyl–Sepharose by removing calcium ions with EGTA [20]. There is evidence that S100A4 forms non-disulfide dimers, at least in vitro [3,18]. Although these observations need to be interpreted with caution, the conservation in S100A4 of four out of five amino acids thought to be important contacts in the dimer interface in dimeric S100A6 (calcyclin) [21,22] strongly suggests that S100A4 can also exist in a homodimeric form through hydrophobic, and not disulfide, interactions (Fig. 1).

Natural rat and recombinant rat S100A4 proteins bind calcium ions with similar affinities [19,20]. In one series of experiments, the K_d was found to be 38 μM with respect to calcium in minimal levels of other monovalent or divalent cations [20]. However, unexpectedly for an intracellular calcium-binding protein, potassium and magnesium ions were strongly antagonistic of calcium binding in vitro, raising the K_d more than sixfold and threefold, respectively, at near physiological concentrations of ions (Table 1). A lower affinity of S100A4 for calcium and no effect of magnesium ions, has been reported [18], a result that is entirely consistent with the high level of potassium ions used in their buffers. These effects of divalent and monovalent cations could be associated with the regulation of S100A4 activity in those cells which are involved in ion transport, in which case, the modulation of calcium binding by potassium might have physiological significance.

4. Gene structure and regulation

The transcribed *S100A4* gene consists of two coding exons, each of which contains DNA corresponding to the region around one of the EF-hand motifs, and one small non-coding 5′ exon. The intron which separates this small 5′ exon appears to be of regulatory significance, since in rats and mouse there is a region with homology to the T cell, CD3δ, enhancer [23,24]. In the mouse, footprinting analysis in vitro showed that a 16 bp region was protected using extracts from either high- or low-expressing S100A4 cell lines; however, footprinting in vivo showed protection only in the high-expressing cells and not in the low-expressing cells [23]. In the rat, a similar protection in high- and low-expressing cell lines is evident in vitro, and the same region is only protected in vivo in high-expressing cell lines. However, the precise region that is protected is not exactly the same in the two species [24]. More recently, Lukanidin's group, in an elegant series of experiments, have identified a sequence resembling an NFκB-related

Table 1
Calcium-binding parameters of S100A4 and calmodulin determined by flow dialysis[a]

Protein	Concentration of protein (μM)	Additions[b]	K_d (μM)
Calmodulin	4.4	None	7.4
S100A4			
pH 8.0	38.5	None	37.0 ± 0.5
pH 7.2	38.5	None	34 ± 0.5
pH 7.2	7.7	None	38 ± 0.6
pH 7.2	38.5	5 mM Mg^{2+}	97 ± 0.2
pH 7.2	38.5	100 mM K^+	161 ± 0.2
pH 7.2	38.5	100 mM Na^+	139 ± 0.6

[a]Ref. [20].
[b]Buffer = MOPS/Tris at pH and with additions indicated.

binding site which forms a complex with a 200 kDa protein [25]. In the mouse, this sequence is active in vitro in both high and low-expressing cells; however, in vivo the complex only forms in the high-expressing cells [25]. Although the NFκB-like site is different between mouse and human, the complex with the 200 kDa protein is also detected with the homologous human sequence [25].

In cultured mouse [26] and rat [24] cells, and also in cultured human lymphoma cells [27], the *S100A4* gene is methylated in low-expressing cells, but less methylated in high-expressing cells. A difference between the mouse and the rat *S100A4* genes in this regard is a potentially regulatory AP1 site in the first intron formed by methylation in the mouse [28]; however, this sequence is not present in the rat [29] and only partially conserved in the human *S100A4* gene intron sequences.

A further difference in the regulation of transcription between the rat and the mouse *S100A4* genes concerns the upstream regulatory regions. In the mouse, no active cis-acting upstream regulatory elements were identified in mammary tumour-derived cell lines [26]. However, an unidentified region within the upstream 1800 bp of the mouse *S100A4* gene has been reported to be active in mouse fibroblasts, but inactive in epithelial cells [9]. In the rat, a sequence, 1300 bp upstream of the start site of transcription, is related to the recognition sequence for GC-factor, an inhibitory transcription factor [30]. This sequence binds a factor which also recognises the GC-factor consensus sequence [29]. Binding of the inhibitory factor in low-expressing epithelial cells has been detected by DNAse footprinting in vitro and DNA footprinting in vivo (Fig. 2), and by gel mobility shift assays [29]. In a range of epithelial and epithelial-derived rat cells, which express S100A4 mRNA, there is a reduced level of a mRNA which hybridises

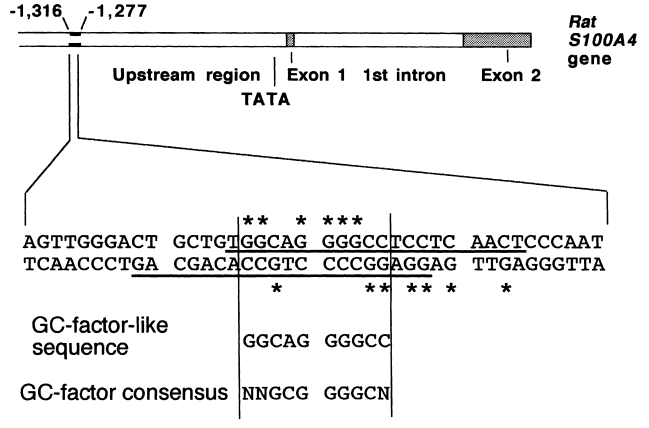

Fig. 2. A summary of footprinting experiments defining an upstream regulatory region of the rat *S100A4* gene. The location of the GC-factor like recognition sequence is shown diagrammatically relative to the TATA box, first and second exons of the *S100A4* gene. The sequence surrounding the GC-factor-like recognition sequence is shown in double-stranded form. Lines below each strand of sequence show the extent of the in vitro footprint, and the asterisks show protected G nucleotides arising from in vivo footprinting.

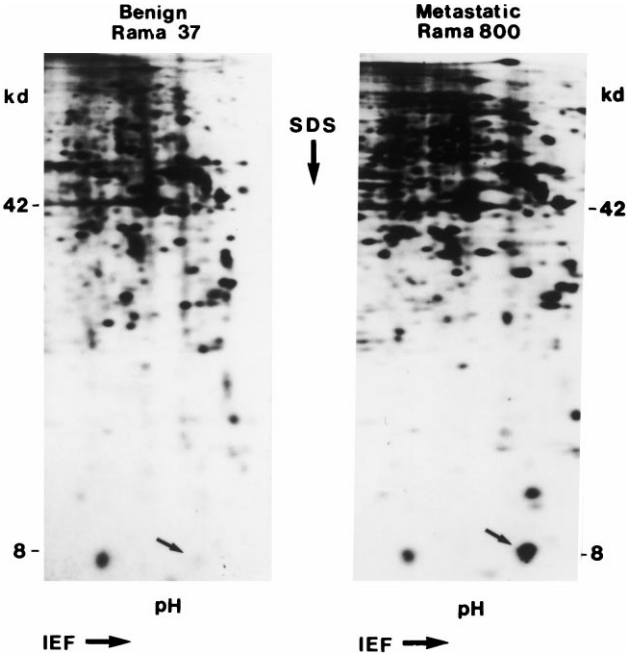

Fig. 3. Identification of S100A4 in Rama 800, a metastatic rat mammary epithelial cell line, but not in the benign rat mammary cell line, Rama 37. Cells were labelled with [^{35}S]methionine during culture and harvested into a denaturing buffer, and the extracts subjected to two-dimensional polyacrylamide gel electrophoresis using denaturing isoelectric focusing in the first dimension and SDS–polyacrylamide gel electrophoresis in the second dimension. Following staining, destaining and drying, the gels were subjected to autoradiography. The arrow on the right-hand panel points to the radioactive spot corresponding to S100A4 in the metastatic Rama 800 cells, and the arrow on the left-hand panel points to the corresponding position, where there is only very faint spot corresponding to S100A4 in the non-metastatic Rama 37 cells. IEF, isoelectric focusing; kd, molecular mass in kDa; SDS, sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Reprinted from The Stem Cells Handbook by C.S. Potter (1997) with permission of the publisher, Academic Press.

Table 2
Induction of metastasis in benign tumour-derived rat mammary epithelial cells by human S100A4

Cells[a]	Level of rat S100A4 mRNA (arbitrary units)[b]	Level of human S100A4 mRNA (arbitrary units)[b]	Incidence of tumours[c]	Incidence of metastases[d]
Untransfected/*neo* transfected recipient benign tumour cells	+[e]	−[e]	22/26[f]	0/20[f]
Recipient cells transfected with pSV2*neo* and human *S100A4* gene				
Pool 1	+	−	24/30[g]	0/30
Pool 2	+	+++	31/40[g]	20/30[h]

[a]Benign rat mammary tumour cells were co-transfected with pSV2*neo* and with DNA consisting of the entire human *S100A4* gene [34].
[b]The level of rat and human S100A4 mRNAs in the cells was determined by reverse transcription PCR specific for the rat and human S100A4 mRNAs. Levels represent intensities of bands following agarose gel electrophoresis of the PCR products.
[c]Number of tumours/number of sites inoculated.
[d]Number of animals with metastases/number of animals with tumours.
[e]Determined on pSV2*neo*-transfected benign rat mammary tumour cells.
[f]Determined on untransfected benign rat mammary tumour cells.
[g]Tumour incidence not significantly different from benign rat mammary tumour cells ($P > 0.4$; Fisher's exact test).
[h]Significantly more metastases than benign rat mammary tumour cells ($P < 0.0001$; Fisher's exact test).

to a GC-factor cloned cDNA compared with non-S100A4 expressing cells [29]. In these cells there is a correlation not only between the degree of protection in footprinting experiments and the level of this mRNA, but also an inverse correlation between the level of this GC-factor-hybridising mRNA and the level of S100A4 mRNA, suggesting that a reduced level of the GC factor-like activity might be responsible, in part, for the up-regulation of S100A4 mRNA in the high S100A4 expressing cells [29].

In the rat cells, transcription of the *S100A4* gene seems to be regulated by positive regulatory factors associated with the TATA box [29], with the first intron [24], a negative upstream regulatory element [29] and DNA methylation [31]. The rat *S100A4* gene does not contain an associated CpG island. Using bisulfite mapping techniques, methylcytosine is essentially absent from the upstream regulatory region in high and low expressing cell lines, whereas methylcytosine is detectable in the TATA box and first intron positive regulatory regions in low-expressing cell lines, but less so in high-expressing cell lines [31].

5. Association of S100A4 with processes leading to metastasis of cancer cells

The mRNAs for rat and mouse S100A4s are present at an abundant level in epithelial cell lines which possess metastatic properties in in vivo animal model systems, whereas related cell lines which are not metastatic in these systems contain only low or undetectable levels of S100A4 mRNA [5,32]. In the rat systems this extends to the protein, as detected by its characteristic migration position following two-dimensional gel electrophoresis (Fig. 3). A major step forward came with the demonstration that experimental elevation of S100A4 by transfection of a benign non-metastatic rat mammary cell line, Rama 37, with multiple copies of the rat *S100A4* gene in an expression vector induced, in some of the cells, the capability to metastasise from the mammary gland to the lungs and lymph nodes in syngeneic experimental rats, showing that S100A4 was able to cause metastasis in a suitable experimental system [33]. Similar experiments have shown that the human S100A4 has the same capability in the rat model system (Table 2) [34], and mouse S100A4 is able to induce malignant/invasive and metastatic properties tested in the immunodeficient nude mouse in the human breast carcinoma-derived cell line, MCF-7 [35]. This cell line is normally not metastatic in the immunodeficient nude mouse. These results taken together indicate that the induction of a detectable metastatic phenotype in cultured cells is a general property of rat, mouse and human S100A4 proteins, and that S100A4 can exert its effect on rodent or human cells. However, transfection of cul-

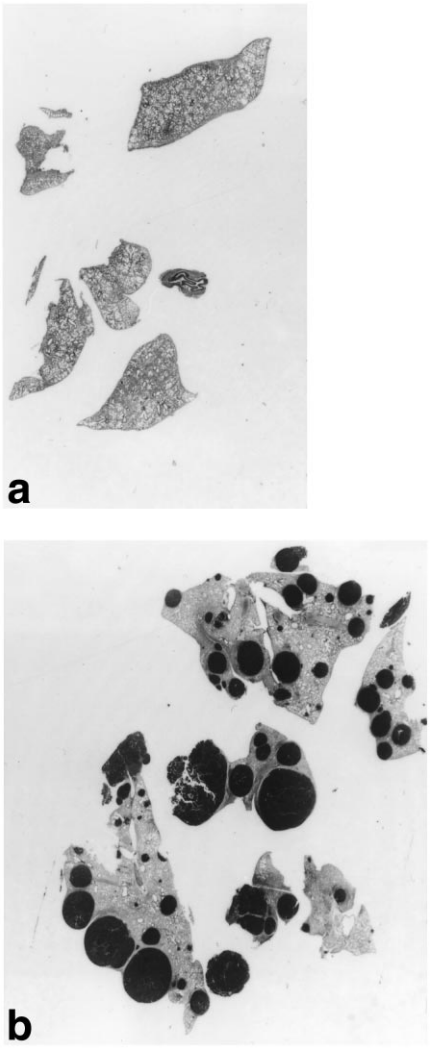

Fig. 4. One effect of S100A4 on metastasis of *neu*-induced mammary tumours in transgenic mice. Stained histological sections are from the lungs of a female transgenic mouse which had inherited the *neu* transgene (a) and from a female transgenic mouse which had inherited both the *neu* and *S100A4* transgenes (b). Both animals had primary tumours in the mammary glands. Panel (b) reprinted from Ref. [39] with permission of the publisher, Stockton Press.

tured cells with an expressed gene and the transfer of the cells into the mammary glands of host animals is not a complete model of cancer, since spread of the cells in this model might be a consequence of the injection procedure.

In order to investigate the metastasis-inducing properties of S100A4 in cells in a normal tissue environment, transgenic mice have been produced which contain multiple additional copies of the *S100A4* gene regulated by the natural rat promoter of the *S100A4* gene [36]. The mice contain elevated levels of immunocytochemically determined rat S100A4 in their tissues. These mice develop normally, display no detectable phenotype and have a normal life span. They do not show a higher incidence of tumour formation than non-transgenic mice of the same strain. The distribution of the rat S100A4 mRNA in the tissues of the transgenic mice was precisely that found in the normal rat even though it was being expressed in mouse cells [36]. This is not unexpected, since the rat *S100A4* transgene was expressed from its own promoter in these transgenic mice. The result shows that the *S100A4* transgene contains all the signals required to direct its own expression to normal tissues, and that these rat signals are functional in the mouse cells. These transgenic mouse strains provide a unique opportunity to examine the comparative tissue distribution of the rat and mouse mRNAs for S100A4 in the same animal environment. Using specific probes in Northern blotting experiments, it was possible to show clearly that there is a different distribution between rat and mouse S100A4 mRNAs. The mouse mRNA is present at very high level in tissues of the immune system, spleen, thymus and lymph node, whereas the rat S100A4 mRNA is present at its highest level in lung. The distribution of S100A4 in rat and mouse cells is surprisingly different in two species as closely related as rat and mouse [36].

Since S100A4 is not able to induce tumour formation on its own, the *S100A4* transgenic mice were mated with mice bearing the oncogene, *neu*, which is the activated rodent homologue of the human c-*erb*B-2 oncogene. Human c-*erb*B-2 is evident on the surface membranes of cells in 20–30% of human breast cancers and is prognostic of a poor outcome of the disease [37]. In the transgenic mice, the *neu* oncogene is under the control of the mouse mammary tumour virus long terminal repeat and expression of the *neu* transgene becomes activated after the mice have undergone multiple pregnancies, resulting in stochastic mammary neoplasia between 7 and 14 months of age [38]. In transgenic mice bearing *neu*-induced tumours, Neu protein is expressed at a high level on the membranes of tumour cells, but not on the normal mammary cells. When the *neu* transgenic

mice were mated with the *S100A4* transgenic mice, offspring which inherited the *S100A4* transgene yielded no tumours as expected [39]. Offspring which inherited the *neu* oncogene succumbed to stochastic mammary neoplasias after multiple pregnancies, with 50% of the mice yielding tumours at about 14.5 months; however, there were no observable growing metastases in the lungs (Fig. 4) or in other tissues examined [39]. Mice which inherited both the *S100A4* and *neu* transgenes yielded stochastic mammary tumours after multiple pregnancies, with 50% of the mice yielding tumours at 11.4 months. In 10 out of 17 mice examined, metastases were present in the lungs, constituting up to 24% of the area of histological sections of the lungs (Fig. 4), and the tumour tissue was invasive [39].

Similar results have been obtained by combining an independent mouse model system containing murine *S100A4* transgenes, and mating these mice with the GS/A mouse strain which has a high incidence of spontaneous mammary tumours [40]. These results taken together clearly show that S100A4 is not itself able to induce tumorigenesis, and its metastasis-inducing capability becomes evident in cells which have otherwise acquired tumorigenic potential.

The causative relationship between S100A4 and metastasis has been further established in human metastatic osteosarcoma cells, which express high levels of S100A4. These cells have been transfected with a hammerhead ribozyme directed against S100A4 transcripts. The effect of the ribozyme was to reduce the capacity of the cells to give skeletal metastases following cardiac injection into nude mice [41]. In a similar series of experiments, Lewis lung carcinoma cells were engineered to contain an inducible expression plasmid encoding antisense S100A4 mRNA. Activation of the antisense construct reduced the motile and invasive behaviour of the cells, and reduced their ability to colonise the lungs when the cells were injected into the tail vein of the recipient mice [42]. These experiments show that it is possible to reverse the effects of S100A4. Colonisation of the lungs/skeletal muscle represents only one part of the metastatic cascade, thus, on face value, these experiments suggest that S100A4 affects the later stages of metastasis, survival in the circulation and/or the establishment of colonies at the secondary site.

6. Mode of action of S100A4

Several lines of evidence suggest an involvement of S100A4 in processes of cell motility. S100A4 has been immunofluorescently localised to the cytoskeleton when cultured cells are stained with antibodies to S100A4 [17,20,33], and this staining seems to be identical to the pattern of staining obtained using phalloidin, which interacts with actin-containing filaments in the cell [17,20,33]. In HL-60 promyelocytic leukaemia cells treated with phorbol 12-myristate 13 acetate (PMA), dimethylsulfoxide (DMSO) or all-*trans* retinoic acid, the induction of S100A4 by PMA and DMSO coincides with the acquisition of cell motility [43]. In a group of untransformed and corresponding transformed mouse cells, the level of S100A4, or its mRNA, correlates with the motility of the cells on a fibronectin/colloidal gold substratum [43]. In a variant v-*src* transformed cell line of low S100A4 level, and low cell motility, raising the level of S100A4 by transfecting the cells with additional copies of a mouse S100A4 cDNA, not only raised the S100A4 level, but also increased the cells' motility [43]. Non-metastatic mouse mammary adenocarcinoma cells transfected with the *S100A4* gene and expressing S100A4 protein showed altered morphology, and increased motility in a Boyden Chamber assay [44]. However, the cells did not show increased invasion through Matrigel in vitro, nor increased metastasis in vivo in these experiments [44].

By analogy with other calcium-binding proteins, binding partners have been sought for S100A4. In common with other members of the S100A family, many binding partners have been identified, and it is not yet clear which particular interactions of S100A4 mediate its metastasis-inducing properties. Some correlations have been reported which have been interpreted as involvement of S100A4 in the depolymerisation of tubulin [45] and in the sequestration of p53 by S100A4 [46,47]; however, there is as yet little evidence of direct interactions to support these hypotheses. Direct calcium-dependent interactions in vitro have been reported between S100A4 and several separate cytoskeletal components, F-actin [48], amino acid residues 39–107 of non-muscle tropomyosin [49], and non-muscle myosin molecules [50–53] under a variety of interacting conditions. In the case of non-muscle myosin, deletion mapping experi-

ments have identified the S100A4 binding region close to the C-terminus of the myosin heavy chain [53]. S100A4 might act by destabilising myosin filaments [52], and it has been shown that S100A4 inhibits the actin-activated myosin ATPase of non-muscle myosin in a calcium dependent manner, but not the same activity of non-muscle myosin heavy meromyosin, suggesting that it is the binding of S100A4 to the C-terminal region of the myosin heavy chains that influences ATPase activity [52].

S100A4 binds to a region of the myosin heavy chain which can be phosphorylated in vitro by protein kinase C [53]. This is a potentially interesting observation because there have been reports, over a number of years, of interactions of other S100 proteins being associated with the modulation of protein kinase activity and more specifically of protein kinase C (PKC) activity. Thus, S100A10 interacts with the cytoskeletal p36, a substrate for viral tyrosine kinases [54], an interaction which it has been suggested inhibits the phosphorylation of p36. S100A1, in dimeric form, interacts with the giant myosin-associated twitchin kinases of *aplysia* [55] and *Caenorhabditis elegans* [56], and stimulates in a calcium-dependent manner their kinase activity up to over 1000-fold [57], by relieving the twitchin kinase auto-inhibitory activity [55]. The related S100b (ββ) protein has been reported to interact with the microtubule associated protein, τ and to inhibit its phosphorylation by calmodulin-dependent protein kinase II [58]. S100b/β has been reported to inhibit protein kinase C-mediated phosphorylation of the nervous-system growth-associated protein (GAP)-43 [59,60], PKC-dependent phosphorylation of p53 [61] and of derivative peptides [62]. Thus it is possible that, as suggested before [58], the S100 proteins, including S100A4, might be involved in the modulation of specific phosphorylation events in the cell.

7. S100A4 in human tumours

Since rodent and human S100A4 proteins are able to induce metastasis in a variety of model systems, it is possible that S100A4 protein might be associated with breast cancer in humans. S100A4 mRNA is present at a high level in malignant breast cancer-derived cell lines, such as MDA-MB-231 and SK-Br-3, than cell lines derived from benign breast tumours, or SV40 virus-transformed normal human breast epithelial cells (Lloyd and Barraclough, unpublished data).

S100A4 mRNA is present at a higher level in malignant breast cancer specimens than in benign breast lesions, and in the malignant specimens there was a statistically significant inverse correlation between the level of S100A4 mRNA detected by Northern blotting and immunocytochemically detected oestrogen receptor level (Lloyd and Barraclough, unpublished data). A similar approach has suggested that a high level of mRNA for S100A4 in infiltrating ductal carcinoma of the breast is associated with spread to the local lymph nodes [63]. Using a Western blotting approach on breast tumour specimens, the level of S100A4 protein was found to correlate with the level of activity of urokinase-like plasminogen activator, but not with another marker of malignancy, cathepsin D [64]. S100A4 mRNA has also been detected using Northern blotting, in melanocytic lesions, but its presence failed to correlate with indicators of tumour progression [65]. However, since all these results rely on Northern or Western blotting techniques, which fail to distinguish the cellular source of the mRNA, it is not clear from these experiments which cells contain the S100A4. This is an important point because S100A4 is present in blood vessels [15] and in fibroblastic cells [9], and both these cell types are present in normal tissues and their tumours.

A preliminary immunocytochemical approach, in which a small panel of tumours was stained using antibodies to five S100 proteins, suggested that specific antibodies to S100A4 stained the cancer cells in breast specimens, and variable expression was detected in colon and ovarian cancer specimens [66]. In contrast, experiments aimed specifically at detecting S100 proteins in normal skin and its lesions, including malignant melanomas, using highly specific antibodies, found the staining for S100A4 to be weak and thus uninformative [67]. In contrast, in colonic lesions, immunocytochemically detected S100A4 was not found in adenoma specimens, but was detectable in 44% of foci of carcinoma within adenoma specimens, and 94% of colon carcinoma specimens stained immunocytochemically for S100A4. Furthermore, and more interestingly, all the carcinoma cells in 14 metastases of the liver were immunocytochemically

stained positive for S100A4 [16], a result that strongly supports a role for S100A4 in the metastasis of natural cancers.

So far, the limited data on human tumour specimens suggests that there is a relationship between the level of S100A4 and the metastasis/malignancy of cancer cells; however, in order to establish any prognostic significance, it will be necessary to use archival specimens for which sufficient time has elapsed so that intratumoral levels of S100A4 can be correlated with the outcome of the disease. For breast cancer this requires both a large patient base and follow-up periods of 10 years or more.

Although it has been possible to establish a causal link between S100A4 and metastasis of cancer cells, it will be more difficult to demonstrate the causal mechanisms involved. Current knowledge points to cell motility as the most likely target of overexpressed S100A4, and more detailed examination of the molecules involved in this process will undoubtedly shed light upon the mechanism of action of S100A4 in normal and cancer cells.

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