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S100 PROTEINS: STRUCTURE, FUNCTIONS AND PATHOLOGY

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TABLE OF CONTENTS

1. ABSTRACT

S100 proteins regulate intracellular processes such as cell growth and motility, cell cycle regulation, transcription and differentiation. Twenty members have been identified so far, and altogether, S100 proteins represent the largest subgroup in the EF-hand Ca^{2+} -binding protein family. A unique feature of these proteins is that individual members are localized in specific cellular compartments from which some are able to relocate upon Ca^{2+} activation, transducing the Ca^{2+} signal in a temporal and spacial manner by interacting with different targets specific for each S100 protein. Some members are even secreted from cells exerting extracellular, cytokine-like activities partially via the surface receptor RAGE (receptor for advanced glycation endproducts) with paracrine effects e.g. on neurons, promoting their survival during development or after injury.

Another important aspect is that 14 bona fide S100 genes are found in a gene cluster on human chromosome 1q21 where a number of chromosomal abnormalities occur. This results in a deregulated expression of some S100 genes associated with neoplasias. Recently, S100 proteins have received increasing attention due to their close association with several human diseases including cardiomyopathy, neurodegenerative disorders and cancer. They have also been proven to be valuable in the diagnostic of these diseases, as predictive markers of improving clinical management, outcome and survival of patients and are considered having a potential as drug targets to improve therapies.

2. INTRODUCTION

Calcium (Ca^{2+}) functions as a messenger regulating a great variety of cellular processes in a spatial and temporal manner (1). The Ca^{2+} signalling network is composed of many molecular components including the large family of Ca^{2+}-binding proteins characterized by the EF-hand structural motif (2). S100 proteins represent the largest subgroup within this family and have received increasing attention in recent years due to their cell- and tissue- specific expression and their involvement in several human diseases such as rheumatoid arthritis, acute

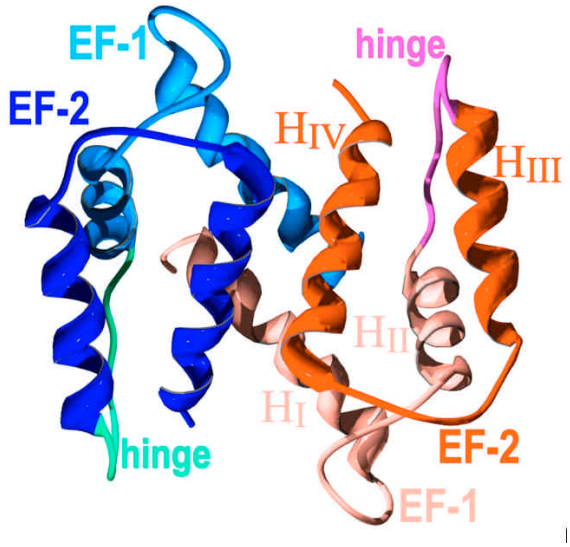

Figure 1. Dimer structure of S100 proteins. The monomers of the S100A3 dimer are depicted in red and blue, respectively. Each monomer consists of two EF-hands connected by a hinge region (84).

inflammatory lesions, cardiomyophathy, Alzheimer's disease, and cancer (3-8).

Another important aspect unique to the S100 protein family is that most S100 genes are located in a gene cluster on human chromosome 1q21 (9, 10) which is structurally conserved during evolution (11). Within this chromosomal region, several rearrangements which occurred during tumor development have been described (12). This might be linked to a deregulation of S100 gene expression in various tumor types and is associated with metastasis and tumor development (4, 7).

Another unique feature is that the individual members of S100 proteins are localized in specific cellular compartments from which some of them are able to relocate upon Ca^{2+} activation (6, 13-15, 44), transducing the Ca^{2+} signal in a temporal and spatial manner by interacting with different targets specific for each S100 protein. Furthermore, some S100 proteins are even secreted from cells to exert cytokine- and chemokine-like extracellular activities (3, 6, 13, 14). The individual members of S100 proteins seem to utilize distinct pathways (ER-Golgi route, tubulin or actin associated) for their secretion into the extracellular space (6, 14, 83).
The extracellular concentrations of S100 proteins seem to play a crucial role in their physiological response. For example, nanomolar concentrations of S100B have trophic effects on cells but pathological levels lead to glial activation, a prominent feature in Alzheimer's disease and apoptosis (16).

S100B and S100A12 have been found to trigger a cascade of signalling mechanisms by binding to the recently discovered surface receptor RAGE (receptor for advanced glycation endproduct)- a multiligand member of the immunoglobulin superfamily involved in inflammatory disorders (17, 18). Genetically manipulated mice (knock-out and transgenics for RAGE and S100 proteins) are now becoming available, which should advance our understanding of the intra- and extracellular activities of S100 proteins.

S100 proteins and/or specific antibodies were recently found to be reliable diagnostic markers for newly occurred melanoma metastasis (S100B; 19), hypoxic brain damage and to monitor the outcome after cardiac arrest (S100B; 20), acute myocardial infarction (S100A1; 21), amyotrophic lateral sclerosis (S100A6; 24), for the classification of astrocytomas and glioblastomas (22, 23), and as prognostic indicators for gastric cancer (S100A4; 25), laryngeal (S100A2; 26)- and esophageal (S100A4; 27) squamous-cell carcinomas and for breast cancer (28).

In this review we will focus on the most recent developments in the structure and functions of those S100 proteins which are closely associated with human diseases and which are of potential use in clinical diagnosis and as possible targets for therapeutic interventions.

3. THE FAMILY OF S100 PROTEINS

3.1. Protein structures and target interactions

S100 proteins are small acidic proteins with a size of 10-12 kDa and form homo- and heterodimers. Whereas a few years ago the structures of just three different S100 proteins were known (calbindin D_{9K}, S100A6, S100B) (29-32) several high-resolution structures are available nowadays. These comprise S100 proteins in the Ca^{2+}-free and Ca^{2+}-bound state, as well as in complex with target peptides. With the exception of calbindin D_{9K} all structures of S100 proteins revealed a tight homodimer whereby the dimerization plane is composed of strictly conserved hydrophobic residues, which are missing in the case of calbindin D_{9K}. Each S100 monomer consists of two helix-loop-helix Ca^{2+}-binding domains termed EF-hands (Figure 1). The N-terminal domain consisting of helices H_I and H_{II} connected by loop L_1 is different from the canonical EF-hand motif and is therefore called 'S100-specific' or 'pseudo EF-hand', whereas the C-terminal domain (H_{III}-L_3-H_{IV}) contains the canonical EF-hand motif. Upon Ca^{2+}-binding almost all S100 proteins undergo a conformational change exposing a previously covered hydrophobic patch. Since helices H_I and H_{IV} are involved in the dimer formation their relative position in the molecule is restricted. This leaves only helix H_{II} or H_{III} as candidates for a conformational change. The structures of S100A1, S100B and S100A6 in the Ca^{2+}-free and Ca^{2+}-bound state revealed that only the C-terminal canonical EF-hand (H_{III}–L_3-H_{IV}) undergoes a conformational change upon Ca^{2+}-binding (30, 32-38, 81, 82). The N-terminal pseudo EF-hand (H_I–L_1-H_{II}) shows no Ca^{2+} -dependent conformational change. This behavior is explained by the fact that the pseudo EF-hand is already in a conformation resembling the Ca^{2+}-bound state. The mechanism of this conformational stabilization was revealed by the crystal structure of S100A3, representing the first crystal structure of a S100 protein in the Ca^{2+}-free closed state (84). In Ca^{2+}-free S100A3 a water molecule is located exactly at the

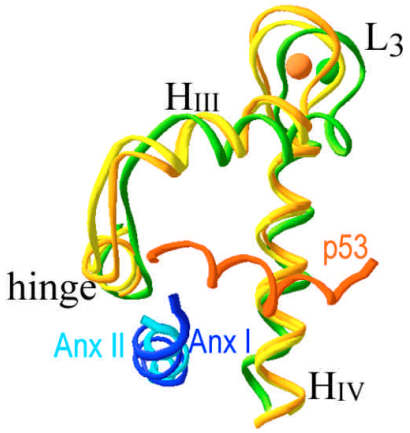

Figure 2. Target binding by S100 proteins. The binding modes of S100A10 (yellow) with an annexin II peptide (cyan), S100A11 (orange) with an annexin I (blue) peptide, and S100B (green) with a p53 peptide (red) are shown (41, 43, 81). The target peptides are bound between helix H_{III} and helix H_{IV} of the C-terminal canonical EF-hand. However the binding mode of the annexin peptides and the p53 peptide are rather different. The annexin peptides have further specific contacts with residues on helix H_I of S100A10 and S100A11 (not shown) turning these peptides about 90° in comparison to the p53 peptide in the binding pocket.

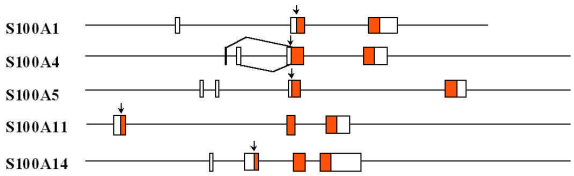

Figure 3. Generic S100 gene structure. A typical S100 gene (S100A1) is composed of three exons (boxes) with exon 1 being not translated (open boxes) and exons two and three containing the coding region (red boxes). Exeptions to this general rule are depicted below with straight lines in S100A4 indicating alternative splicing and arrow indicating the translational start.

position of the Ca^{2+} ion in the N-terminal EF-hand and adopts a very similar coordination.

The Ca^{2+} -dependent conformational change of S100 proteins was characterized by NMR and high-resolution X-ray studies. This conformational change of Ca^{2+}-bound S100 proteins is distinct from Ca^{2+} -dependent changes observed in other EF-hand proteins like calmodulin or troponin C. In the C-terminal EF-hand (canonical EF-hand) there is a large change in the position of helix H_{III} upon Ca^{2+}-binding. The interhelical angle between helices H_{III} and H_{IV} changes by 90° in S100B compared to the Ca^{2+}-free structure, opening the structure and exposing the residues required for target recognition and binding (33, 34, 36, 81). A similar change in conformation is observed for Ca^{2+}-bound S100A6, although the change in the interhelical angle is not as pronounced as in S100B. A further opening of the structure of Ca^{2+}-bound S100A6 might be triggered by the binding of the target molecule. The crystal structures of Ca^{2+}-bound S100A7 (39), S100A8 (40), S100A11 (41), and S100A12 (42) and S100A9 (88) confirmed the observations made for Ca^{2+}-bound S100B. All four structures revealed an open conformation suitable for target binding. A further interesting phenomenon was observed for S100A10, which is not able to bind Ca^{2+}. The crystal structure of S100A10 (43) showed that the Ca^{2+} free protein already is in an open conformation resembling the Ca^{2+} bound state. This enables S100A10 to interact with its target molecule annexin II in a Ca^{2+} -independent manner.

So far three different S100-target complexes have been characterized: S100B in complex with a peptide of the regulatory domain of p53, S100A10 with a peptide of annexin II, and S100A11 in complex with a peptide of annexin I (Figure 2; 41, 43, 81). All three peptides were located in a cavity formed by helices H_{III} and H_{IV} in the open conformation of the C-terminal canonical EF-hand. The binding of the target peptides with the protein matrix is accomplished by hydrophobic and ionic interactions. Furthermore the stoichiometry of the complex is two target peptides per S100 homodimer. However, the binding mode of the annexin peptides to S100A10 and S100A11 is strikingly different from that of the p53 peptide to S100B. In contrast to the p53 peptide the annexin peptides interact with both monomers whereby the required residues are located on the helices H_{III} and H_{IV} of one monomer and on helix H_I' of the second monomer. This different binding mode is evident from a structural alignment of the three protein-peptide complexes in Figure 2. The annexin peptides displayed in red and blue are rotated about 90° in comparison to the p53 peptide pointing to the second monomer of the S100 homodimer. Based on these observations one can suppose that there are further modes of target binding to other S100 proteins. Recently it was proposed (85) that the hexameric form of S100A12 might interact with three extracellular domains of the RAGE receptor important for intracellular signaling.

3.2. Genomic organization

The structural organization of S100 genes is highly conserved both within an organism and also in different species. A typical S100 gene consists of three exons whereby the first exon carries exclusively 5' untranslated sequences. As illustrated in Figure 3 and taking S100A1 as an example, the second exon contains the ATG and codes for the N-terminal EF-hand. Finally, the third exon encodes the carboxy-terminal canonical EF-hand. Only a few genes like S100A4, S100A5 and the newly identified S100A14 are composed of 4 exons. In these genes, the first two exons can either be alternatively spliced (S100A4) (45) or non-coding (S100A5) (46), leaving the two exon splitting of the coding region intact. Interestingly, for both S100A11 (47) and S100A14 (86) this region encoding the corresponding proteins is split into three exons. Whether this reflects a functional or evolutionary close relationship between these two members of the S100 family remains to be seen. Nevertheless, on the protein level S100A14 is most closely related to S100A13, suggesting that these two proteins might be functionally

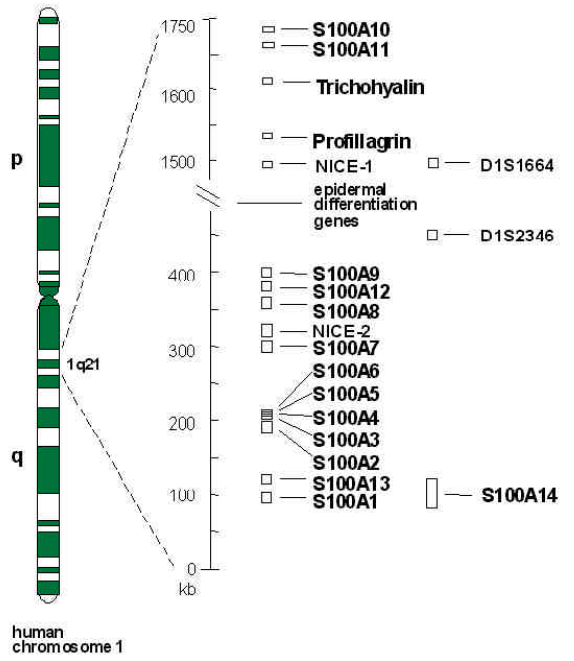

Figure 4. S100 gene cluster on human chromosome 1q21. Genes lying in the cluster region are indicated as well as two commonly used genomic markers (D1S1664 and D1S2346). P and q represent the long and the short arm of the chromosome, respectively.

related (86). So far, 14 bona fide S100 genes are found in a gene cluster on human chromosome 1q21 (Figure 4) which led to the introduction of the now widely accepted S100 nomenclature a few years ago (9). Four additional S100 genes are found on other human chromosomes and include the newly discovered S100Z (87) likely localized on chromosome 5 (the 3' terminal sequence of the S100Z cDNA is part of a human BAC clone on region 5q12-q13). Within the gene cluster, S100 genes are interrupted by epidermal differentiation genes as well as a gene of unknown function called NICE2 which lies between S100A7 and S100A8 (10). Hence, one can recognize at least 4 different subgroups of S100 genes located closely together (S100A1-S100A13-S100A14; S100A2 to S100A6; S100A8-S100A9-S100A12; S100A10-S100A11). This raises the question whether each gene is regulated by its own promoter elements or by as yet uncharacterized locus control elements as has been suggested for the epidermal differentiation genes. The evidence available today would rather suggest an individual regulatory mechanism for most S100 genes. Strikingly however, for some of these subgroups of genes related functions of their encoded proteins have been recognized (see below). Furthermore, a number of chromosomal abnormalities such as deletions, rearrangements or translocations in this region have been associated with neoplasias, suggesting that the expression of S100 genes might be altered in human cancer (7, 22, 23, 48).

Interestingly, the clustered organization of the human genes seems to be evolutionary conserved, at least in the mouse (11). In other species, S100 genes are less well characterized. Nevertheless, sequence comparisons using the publicly available genomes of yeast, Caenorhabditis elegans and Drosophila revealed no typical S100 genes in these invertebrate species. It will therefore be of interest to analyze more sequence information becoming available in the next years from a range of species to study the evolution of this protein family in more detail.

3.3. Biological function

S100 proteins generally are involved in a large number of cellular activities such as signal transduction, cell differentiation, regulation of cell motility, transcription and cell cycle progression (4). Such activities can be expected since S100 proteins are thought to modulate the activity of target proteins in a Ca^{2+}- (and possibly also in a Zn^{2+}- and Cu^{2+}-) dependent manner (5), thereby transferring the signal from the second messenger. Therefore, understanding the biological function of S100 proteins will crucially depend on the identification of these target proteins. During the last decade, a large number of such possible interactions have been described involving enzymes, cytoskeletal elements as well as transcription factors.

Apart from these intracellular functions, some S100 proteins like S100A8/A9, S100B, S100A4 and probably others can be secreted from cells and exhibit cytokine-like extracellular functions. These include chemotactic activities related to inflammation (S100A8/A9 and A12; 49, 50) neurotrophic activities (S100B; 51) as well as a recently described angiogenic effect (S100A4; 52). In all cases, the mechanisms of secretion as well as the nature of high affinity surface receptors remain largely unknown. One candidate receptor to mediate at least some of the described extracellular functions is the receptor RAGE which was shown to be activated upon binding of S100A12 and S100B (18). It is currently not known whether RAGE is a universal S100 receptor.

Nevertheless, most of the target protein interactions have extensively been characterized on the biochemical level using *in vitro* assay systems and been described in several comprehensive reviews recently (4-8). Despite this large amount of biochemical data, very little is known about the actual physiological function of S100 proteins. This can be ascribed to the fact that experiments using cell culture systems and especially whole organisms are still scarce. In the following section, we will therefore concentrate on the few animal experiments which have so far been conducted using S100 proteins.

3.4. Animal models

Generation of animal models will ultimately be required to study the physiological impact of S100 proteins. Since so far no S100 proteins have been detected in genetically tractable lower organisms, the model of choice for such studies will be the mouse. Two basically distinct genetic manipulations can be carried out in the mouse, namely the ectopic expression of a gene through pronuclear injection and the genetic inactivation via homologous recombination in embryonic stem cells.

Table1. Genetically engineered mouse models

S100 Gene	Model	Phenotype	References
S100B	Overexpression in brain	Hyperactivity, impaired hippocampal functioning	60, 90
S100B	Inactivation through homologous recombination	No obvious phenotype	91
S100A4	Overexpression in mammary epithelia	Induction of a metastasic phenotype in cooperation with a second oncogene	92, 93
S100A8	Inactivation through homologous recombination	Embryonic lethal at day 9.5 due to resorption	94
S100A1	Inactivation through retroviral insertion	Under investigation	Own unpublished results

Ectopic overexpression has been described for S100B and S100A4 (see Table 1 and references therein). In the case of S100B, enhanced expression in the brain led to hyperactivity associated with an impaired hippocampal functioning. Brains from S100B transgenic mice show a higher density of dendrites in the hippocampus postnatally compared to controls and a loss of dendrites by 1 year of age. In contrast to this mild phenotype, expression of S100A4 in oncogene bearing transgenic mice is capable of inducing metastasis of mammary tumors, suggesting that S100A4 has an important role in the acquisition of the metastatic phenotype during tumor progression. While stimulation of angiogenesis might play a role, the exact mechanisms of this function are still under investigation.

Inactivation through homologous recombination in mouse embryonic stem cells has been achieved for S100B and S100A8. While inactivation of S100B has no obvious consequences for life, S100A8 null mice die via early resorption of the mouse embryo suggesting a role for this protein in prevention of maternal rejection of the implanting embryo. Recently, our laboratory achieved inactivation of S100A1 through retroviral insertion. These mice are currently under investigation, although no obvious phenotype has been seen so far.

Since S100 proteins can form homo- and also heterodimers and usually more than one S100 protein is found to be expressed in a given cell type, functional redundancy or compensatory mechanisms might explain the lack of phenotype observed in these animal models. Clearly, more animal models inactivating single S100 proteins as well as combinations thereof are needed before the physiological role of individual S100 proteins can be definitely clarified.

4. ASSOCIATIONS WITH HUMAN DISEASES AND DIAGNOSTICS

Each member of the S100 protein family shows a characteristic and unique expression pattern in normal cells which is deregulated in a wide range of human diseases (Table 2). This has made individual S100 proteins valuable diagnostic markers for various human diseases (Table 3).

S100B for example interacts with other Alzheimer's diseas-associated proteins such as presenilin (PS1 and PS2) and with the amyloid precursor protein (APP). These interactions are altered by the phosphorylation state of tau and the overexpression of S100B, strongly indicating that S100B plays an important role in pathways associated with neurodegeneration (53-59). Animals, which overexpress S100B, showed dementia-like cognitive deficits which will now permit further analysis of the structual and physiological changes in this animal model (60). Its interaction partner, the microtubule-associated tau protein, is individually affected in AD. S100B is synthesized by astrocytes, oligodendrocytes and Schwann cells and represents about 0.2% of the total brain proteins. Brain injury causes a selective leakage of S100B into the cerebrospinal fluid and then into the blood, where measurements of S100B levels were found to be good indicators for the assessment of patients with cerebral ischemia due to stroke (73, 74). Similarly, blood levels of S100B can be used to monitor malignant melanomas (19) and pediatric patients undergoing corrective cardiac surgery (75). However, the commercially available S100B diagnostic kit must be more carefully tested for its possible crossreactivity with other S100 proteins e.g. S100A5 (46), S100A6 (76) co-expressed in the brain.

S100A1 displays a specific and high expression level in the human myocardium and is considered to be an important regulator of heart function. Reduced levels measured in the left ventricles of patients with end stage heart failure possibly contribute to a comprised contractility (61). This is an agreement with the reported interactions of S100A1 with SR proteins, regulating Ca^{2+} -induced Ca^{2+} release (62), and with SERCA 2a, phospholamban (63) and titin (64), modulating Ca^{2+} homeostasis and contractile performance (65, 66). In chronic pulmonary hypertension (67) S100A1 levels increased specifically in the hypertrophied right ventricle indicating an adaptive response to pressure overload by upregulating S100A1 which then would affect Ca^{2+} homeostasis and heart physiology. Therefore an S100A1 gene transfer to the heart *in vivo* might provide a new therapeutic approach to correct the altered Ca^{2+} signaling pathways that cause abnormal myocardial contractility. A similar approach was reported recently (68) where defective cardiac muscle relaxation has been corrected by parvalbumin gene delivery. S100A1 was found to be an early marker of heart damage possibly because of its mainly cytosolic localization in cardiomyocytes in contrast to troponin, which is firmly attached to the thin filament (21).

S100A4 has been implicated in invasion and metastasis. The prognostic significance of its selective expression in various cancers has been investigated

Table 2. Association of S100 proteins with human diseases

Proteins	Diseases Associations	References
S100B	Alzheimer`s disease	95
	Down syndrome	3
S100A1	Cardiomyopathy	61, 96
S100A2	Cancer, Tumorsuppression	69, 97, 98
S100A4	Cancer, Metastasis	28, 52, 99
S100A6	Cancer	100, 101
	Amyotrophic lateral sclerosis	24, 102
S100A7	Cancer	103
	Psoriasis	104
S100A8/A9	Inflammation	119
	Wound healing	71
S100A11	Cancer	120, 121
	Ocular diseases	105
S100A12	Mooren`s ulcer (autoimmune disease)	106
	Inflammation	117
S100P	Cancer	122

Table 3. S100 proteins in clinical diagnostics

Proteins	Diagnostic Markers	References
S100B	Tumor marker for newly occurred melanoma metastasis	19
	Sensitive marker of hypoxic brain damage and outcome after cardiac arrest	
	Marker for traumatic brain damage: serum level might be used to select patients for CT scanning	20
	Increased/decreased levels in schizophrenic patients; controversial results	
	Indicator of infarction volume and prognosis in ischemic stroke	73
	Down Syndrome and Alzheimer disease	107-113
	Temporal lobe epilepsy	
S100A1	Marker for acute myocardial ischemia	21
S100A2 to S100A6	Differential expression was exploited for the immunohistochemical classification of tumors in the brain	22-28
	Tumor prognosis and clinical management	80
	Marker to amyotrophic lateral sclerosis	6, 114
S100A8/A9	Inflammatory disorders including rheumatoid arthritis and chronic bronchitis	115
	Markers in the human gingival cerevicular fluid of periodontal diseases	116
S100A12	Mooren`s ulcer	117, 118
	Acute and chronic inflammatory diseases	

(Figure 5). Identification of predictive markers of cancer is of major importance to improve clinical management, therapeutic outcome and survival of patients. In gastric cancer the inverse expression of S100A4 in relation to E-cadherin (a tumor supressor) was found to be a powerful aid in the histological typing and in evaluating the metastatic potential/prognosis of patients with this type of cancer (25). In esophageal squamous cell carcinoma, a cancer with poor patient survival, the increased expression of S100A4 was correlated with the progression of this tumor and a poor prognosis (Figure 5A, B; 27). The presence of S100A4 and other prognostic factors were also compared in primary breast carcinomas (28). The results suggested that the tumor variable most tightly correlated with patient mortality was S100A4. Recently it was demonstrated (52) that S100A4 could act as an angiogenic factor and might induce tumor progression via an extracellular route stimulating angiogenesis. Inhibiting the process of tumor angiogenesis might be possible by either blocking S100A4 secretion or its extracellular function (89).

A prognostic significance of **S100A2** in laryngeal squamous-cell carcinoma has also been found (**Figure** 5C, D; 26) allowing discrimination of high and low risk patients in the lymph-node negative subgroup and a better adjusted therapy. S100A2 expression together with the methyl-p-hydoxyphenylacetate-esterase status allows discrimination of high- and low risk patients in the lymph node-negative subgroup. These results are of direct clinical relevance in that an aggressive initial treatment of the patients with S100A2-negative tumors would avoid under-treatment and a much less aggressive treatment would be beneficial for patients with S100A2-positive tumors. S100A2 and some other S100 proteins not only can be further developed into biomarkers of various types of cancer but also have a potential as drug targets for more subtle chemotherapies.

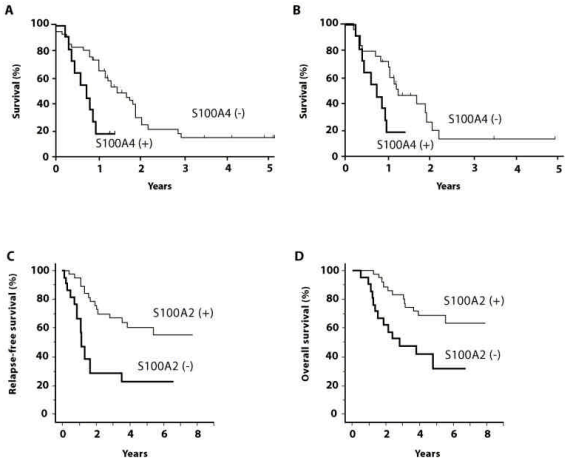

Figure 5. Overall survival curves of 52 patients with esophageal SCC (A) and patients with deep invasion of primary cancer (T3, T4), (B) according to the S100A4 expression status. Modified from (27). Survival rate according to S100A2 tumor immunostaining in 62 laryngeal cancer patients: (C) relapse-free survival (31 patients had locoregional recurrences); (D) overall survival (25 patients had died). Modified from (26).

S100A2 was also identified by DNA array technology to be expressed differently in normal human bronchial epithelial cells versus tumorigenic cells (69) and thus may be useful as a marker for early stages of lung carcinogenesis.

S100A8 and S100A9 tend to form homo- and heterodimers and are predominantly expressed by cells of the myelomonocytic lineage. The quantity of S100A8/A9 heterocomplexes is higher in neutrophiles than in monocytes. Except for some epithelial cells, S100A8/A9 expression is low in healthy people, whereas in inflammation, specific cell populations release homo- or hetero complexes depending on the phase and/or type of inflammation. Tests have been developed to detect S100A8 and S100A9 in body fluids of patients with rheumatoid arthritis (77, 78) for the discrimination of active and non-active osteoarthritis from rheumatoid arthritis (79). Also they are associated with chronic inflammatory diseases in-cluding bowel disease and chronic periodontitis (70). Both proteins are also involved in wound repair by reorganization of the keratin cytoskeleton in the injured epidermis (71). In addition, some of the therapeutic effects of retinoids in inflammatory and hyperproliferative skin diseases might be associated with repression of S100A8 function. S100A8 was recently identified in cervico-vaginal secretions stimulating HIV replication (72).

It was proposed that blocking of S100A8 action could help to reduce the risk of sexual transmission and maternal-infant transmission of HIV.

By immunohistochemistry using specific antibodies against S100 proteins, it was possible to differentiate pilocytic astrocytomas from WHO grade II-IV astrocytic tumors and to distinguish between low (WHO grade I and II), high (WHO grade III and IV) grade astrocytic tumors and WHO grade II from WHO grade III astrocytic tumors (22, 23) This results in a better identification of these tumors and improvement of the prognostic accuracy.

5. CONCLUSION AND PERSPECTIVES

The S100 protein family constitutes the largest subgroup of the EF-hand family of Ca^{2+}-binding proteins with 20 members discovered to date, and the very recent discovery of S100A14 and S100Z indicates that their number might further increase. S100 proteins have been implicated in pleiotropic Ca^{2+}-dependent cellular events, with specific functions for each of the family members. However, some S100 proteins have also physiologically relevant Zn^{2+} affinities. S100A2 and S100A3, for example, have very low affinities for Ca^{2+} but high affinities for Zn^{2+}, suggesting that Zn^{2+} rather than Ca^{2+} controls their biological activities. In order to understand how the biological functions of S100 proteins are regulated by Zn^{2+} and Ca^{2+} it will be necessary to persue the determination of the three-dimensional structures of the Zn^{2+} loaded S100 proteins and to characterize both the distinct mode of Zn^{2+}-binding in the presence of Ca^{2+}, and the Zn^{2+}-dependent interaction with target proteins.

In resting cells, S100 proteins are localized in specific cellular compartments from which some of them relocate upon cellular stimulation and even are secreted exerting extracellular, cytokine-like activities. This suggests that translocation might be a temporal and spatial determinant of their interactions with different partner proteins. Interestingly, our recent experiments suggest that different S100 proteins utilize distinct translocation pathways (tubulin-or actin- dependent, or the classical Golgi-ER pathway) which might lead them to certain subcellular compartments in order to perform their physiological tasks in the same cellular environment. Further studies are needed to unravel the mechanisms involved in the translocation and secretion of S100 proteins. This will also give valuable information about the pathways of other components such as cytokines which have intra- and extracellular activities similar to those of S100 proteins. It has been found that some S100 proteins (after secretion) can have paracrine effects on neighbouring cells and that the extracellular concentrations play a crucial role in the physiological response. Nanomolar levels in the case of S100B have trophic effects on cells but micromolar levels of this protein have been implicated in glial activation (a prominent feature in Alzheimer's disease). The discovery of the surface receptor RAGE for two S100 proteins (S100B and S10012) shed more light on the extracellular functions of these two proteins. However, the mechanism of their secretion, their mode of interaction with RAGE and the question if other S100 protein members act via RAGE or other surface receptors still remains to be investigated. This could be done using animal models with inactivated single S100 proteins or deletion mutants of RAGE. Future research activities will also focus on the deregulated expression of S100 genes which is a hallmark of a wide range of human diseases, and the

application of S100 proteins and antibodies in clinical diagnostics, to further evaluate their prognostic significance to improve clinical management. These proteins are also considered in some cases as drug targets for the inhibition of their intra- and extracellular pathological activities.

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