

## Expression profile and functional role of S100A14 in human cancer

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### ABSTRACT

**S100A14 is one of the new members of the multi-functional S100 protein family. Expression of S100A14 is highly heterogeneous among normal human tissues, suggesting that the regulation of S100A14 expression and its function may be tissue- and context-specific. Compared to the normal counterparts, S100A14 mRNA and protein levels have been found to be deregulated in several cancer types, indicating a functional link between S100A14 and malignancies. Accordingly, S100A14 is functionally linked with a number of key signaling molecules such as p53, p21, MMP1, MMP9, MMP13, RAGE, NF- $\kappa$ B, JunB, actin and HER2. Of interest, S100A14 seems to have seemingly opposite functions in malignancies arising from the gastrointestinal tract (tissues rich in epithelial components) compared to cancers in the other parts of the body (tissues rich in mesenchymal components). The underlying mechanism for these observations are currently unclear and may be related to the relative abundance and differences in the type of interaction partners (effector protein) in different cancer types and tissues. In addition, several studies indicate that the expression pattern of S100A14 has a potential to be clinically useful as prognostic biomarker in several cancer types. This review attempts to provide a comprehensive summary on the expression pattern and functional roles/related molecular pathways in different cancer types. Additionally, the prognostic potential of S100A14 in the management of human malignancies will be discussed.**

### INTRODUCTION

#### S100 proteins

Moore identified an unfractionated mixture of S100B and S100A1 from the bovine brain and called it 'S100' because the mixture was soluble in 100% saturated solution of ammonium sulphate [1]. The S100 proteins are low molecular weight acidic proteins that constitute the

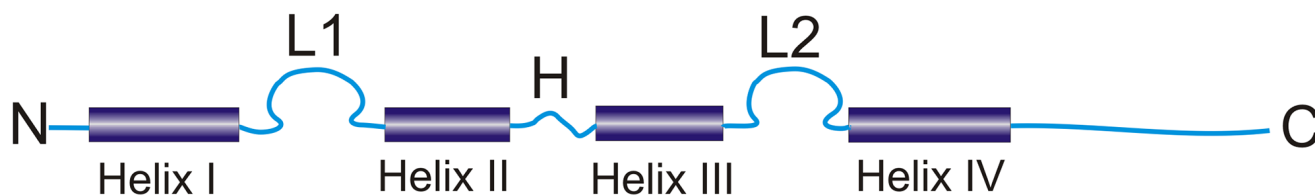
largest subfamily of calcium ( $\text{Ca}^{2+}$ )-binding protein of the EF-hand type. At least 25 closely related S100 proteins with a sequence homology of 25-65% are described. Out of 25 members, genes for 21 proteins are clustered at the chromosome locus 1q21, while the remaining 4 (*S100B*, *S100G*, *S100P* and *S100Z*) are dispersed throughout the genome [2-4]. These genes encode small (10-12 kDa) proteins with two characteristic EF-hands connected by a central hinge region [5]. The proteins are

expressed in a cell- and tissue-specific manner and are involved in several biological processes related to normal development and pathological conditions including malignancies [6–11]. S100 proteins have been reported to be involved in a number of intracellular and extracellular functions. The intracellular functions include regulation of protein phosphorylation, Ca<sup>2+</sup> homeostasis and activities of transcription factors; as well as the control of cell growth and differentiation, *etc* [6, 8, 12–14]. Some of the S100 members are secreted extracellularly and exert diverse biological functions in autocrine/paracrine manner via interaction with a variety of cell-surface receptors. Despite these diverse cellular functions, the S100 proteins do not possess any enzymatic activity to account for their functions. The ability of majority of the S100 proteins to interact with and to modulate the functions of effector proteins is one of the key mechanisms for their biological functions [15, 16]. Several members of the S100 family such as S100B [17–22], S100A4 [21, 23–25], S100A2 [26], S100A1 [27], S100A6 [28, 29], S100A9 [30] and S100P [31] have been shown to interact with the tumor suppressor protein p53 with variable effects on cellular functions. In addition, a number of S100 proteins can form homodimers/oligomers (for example: S100A4 [32], S100B [33] or heterodimers (for example: interactions between S100A8 and S100A9 [34], S100A4 and S100A1 [35], S100B and S100A6 [33], S100A14 and S100A16 [36], and this is considered to be important for their cellular functions. Similarly, interaction of extracellular S100 proteins with a number of cell-surface receptors like receptor for advanced glycation end products (RAGE), G protein-coupled receptors, scavenger receptors, CD166 antigen is key to several biological functions [37–40]. Interestingly, many of the S100 protein-mediated cellular functions such as cell growth, cell motility, signal transduction, transcription, apoptosis and cell survival are closely related to normal development and tumorigenesis [5, 8, 41]. In addition to these cancer related functions, observations such as occurrence of frequent structural and numerical aberrations in the chromosomal region 1q21 [42, 43] (where most of the *S100* gene members are located [2]) in human cancers; and altered mRNA and protein expression levels of several of the S100 members in different human malignancies [44–51] suggest that these proteins are closely related to human malignancies.

S100A14 (also known as Breast Cancer Membrane Protein 84) is one of the youngest members of the S100 protein family and has recently gained significant attention in cancer research. S100A14 was first identified in 2002 by analysing human lung cancer cell lines [3], and subsequently in 2003 as a membrane-associated protein in breast cancer cells [52]. S100A14 has been reported to be differentially expressed in various cancer types and has been suggested to be involved in key biological processes critical for cancerous phenotypes. However, the expression pattern and related cellular functions seem to be tissue-specific. This review will attempt to summarize the signaling pathways, expression patterns and functional roles of S100A14 in different human malignancies. In addition, the prognostic potential of S100A14 in the management of human malignancies will be discussed.

### Molecular structure of S100A14

The S100A14 gene is located on chromosome 1q21. In contrast to other S100 members which consist of 3 exons and 2 introns (except *S100A5*, consisting of 4 exons with exon 3 and 4 being the coding ones; *S100A4*, containing an additional alternatively spliced untranslated exon; and *S100A11*, with the coding sequence beginning already in the first exon), the *S100A14* contains 4 exons and 3 introns with exons 2-4 being the translated ones [3]. The *S100A14* gene encodes for a Ca<sup>2+</sup> binding protein (104 amino acids) with a predicted molecular weight of 11.6 kDa [3]. Similar to other S100 protein members, S100A14 protein monomer consists of two Ca<sup>2+</sup> binding motifs of the EF-hand type separated by a flexible hinge region (Figure 1). Each Ca<sup>2+</sup> binding motif consists of a Ca<sup>2+</sup> binding loop flanked by two  $\alpha$ -helices. Helices I and II flank the Ca<sup>2+</sup> binding loop in the N-terminal site whereas the Ca<sup>2+</sup> binding loop in the C-terminal site is flanked by III and IV helices (Figure 1). Helix IV in the C-terminal site is followed by a C-terminal extension [3]. The S100A14 amino acid sequence shares significant similarity (55-68%) and identity (30-38%) with other S100 members [3]. Nevertheless, the Ca<sup>2+</sup> binding loop at the N-terminal of the S100A14 protein contains 13-amino-acids loop which is in contrast to the 14-amino-acids loop characteristic of the S100 protein family. Moreover, the Ca<sup>2+</sup> binding loop at the C-terminal has been reported to carry mutations thus limiting the Ca<sup>2+</sup> binding ability of the S100A14 [3]. The functional



**Figure 1: Schematic illustration of the typical structure of the S100 protein (L1 and L2: calcium-binding loops, H: hinge region, N and C: N- and C-terminals) (modified from Donato R [8]).**

implications of limited Ca<sup>2+</sup> binding ability of S100A14 are currently unclear.

### Cellular signaling pathway of S100A14

The detailed cellular signaling pathways of S100A14 are not fully understood. Considering its diverse cellular functions and seemingly opposite roles in malignancies arising from the gastrointestinal tract (tissues rich in epithelial components) compared to cancers in the other parts of the body (tissues rich in mesenchymal components), it can be speculated that S100A14 is involved in a wide range of signaling pathways with a considerable variation in the outcome. Similar to many other S100 proteins, S100A14 has been shown to (i) interact with transmembrane receptors (such as RAGE, human epidermal growth factor receptor 2 (HER2 or ErbB2)) [53, 54] and other S100 protein members (for example S100A16) [36], and (ii) have a tight functional link with the tumor suppressor p53 in human cancers [10, 55] (Figure 2).

The extracellular S100A14 was shown to bind with RAGE leading to the activation of mitogen activated protein (MAP) kinase (extracellular signal-regulated kinase, ERK) and NF- $\kappa$ B signaling pathways, thereby promoting cell proliferation and survival in esophageal squamous cell carcinoma (ESCC) (Figure 2). However, higher concentration of extracellular S100A14 was found to induce cell apoptosis through mitochondria-mediated reactive oxygen species production, partly in a RAGE dependent manner [53]. Additionally, S100A14 was found to interact with HER2 in breast cancer cells. The interaction increased phosphorylation of HER2, thereby leading to the activation of down-stream signaling pathways (phosphorylation of AKT and ERK) contributing to breast cancer cell proliferation [54] (Figure 2).

We previously found a positive functional link between S100A14 and p53 contributing to the suppression of oral squamous cell carcinoma (OSCC) cell proliferation [10]. In contrary, a negative feedback loop between S100A14 and p53 was suggested to enhance motility and invasiveness of ESCC cells [55] (Figure 2).

### EXPRESSION PROFILE OF S100A14 IN NORMAL HUMAN TISSUES AND MALIGNANCIES

The expression profile of S100A14 in various normal human tissues and malignancies has been widely studied. Using Northern blot and Cancer profiling array, Pietas et al. showed that *S100A14* mRNA was expressed in a number of human normal tissues. Among them, tissues with epithelial-parenchymal phenotype such as colon, rectum, small intestine, stomach, thymus, thyroid, kidney and lungs were found to express higher levels of *S100A14*. In contrast, tissues with mesenchymal-stromal phenotype such as brain, white blood cells, muscles, spleen expressed

negligible amounts of *S100A14* mRNA [3]. In parallel, the protein and mRNA expression data from the Human protein atlas [56, 57] (image available from <https://www.proteinatlas.org/ENSG00000189334-S100A14/tissue>) and other mRNA databases, (GTEx) [58] and FANTOM5 [59, 60] revealed higher expression of S100A14 in various parts of oral-digestive tract and skin (Figure 3). Interestingly, anatomical sites with higher S100A14 expression include simple or stratified epithelium as a major component of their structures. The highly heterogeneous pattern of S100A14 expression among the normal tissues suggests that regulation of S100A14 expression and its functions may be complex and tissue- and context-specific. Compared to the normal counterparts, the expression of *S100A14* mRNA/protein has been found to be deregulated in several cancer types, indicating a functional link between S100A14 and malignancies. Below we describe the expression pattern of S100A14 in some of the cancer forms where S100A14 has been well investigated (Table 1).

### Oral squamous cell carcinoma (OSCC)

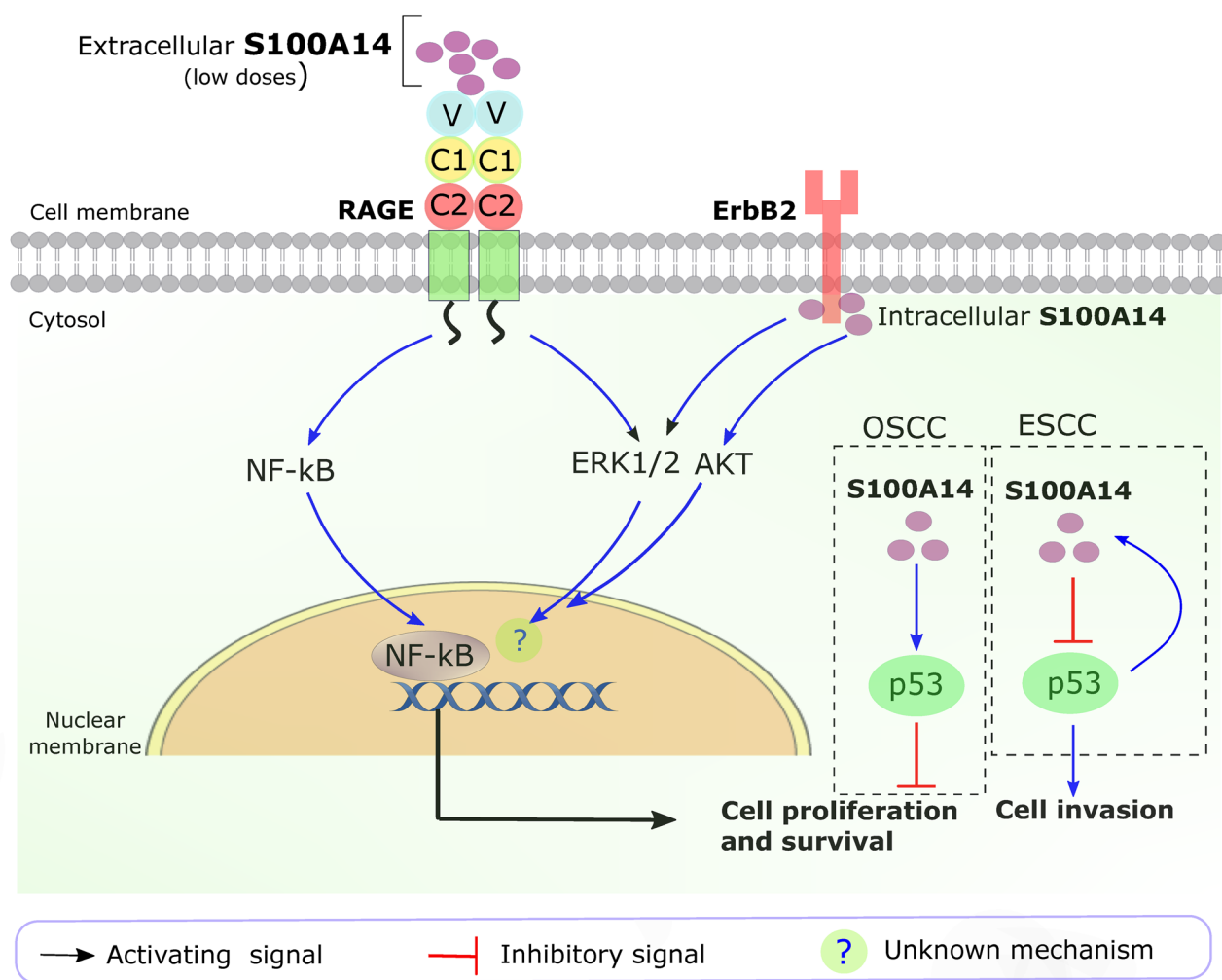
Oral cancer, a sub-group of head and neck cancers, refers to the malignancy arising from the mucous membranes and other tissues of oral cavity [61]. OSCC is the major type of oral cancer encompassing at least 90% of all oral malignancies [61–63]. Among the multiple types of genetic changes occurring in OSCCs, a frequent loss of chromosomal regions (including the 1q21) harboring S100 family gene members has been shown to be a common event [64, 65]. Chromosomal region harboring *S100A14* gene has been found to be frequently deleted in OSCC [64]. Accordingly, we previously reported downregulation of *S100A14* mRNA levels in OSCC specimens and OSCC-cell lines as compared to their corresponding normal controls [9, 10, 66]. Immunohistochemical analysis of normal human oral mucosa showed strong membranous expression of S100A14 protein in all suprabasal epithelial cells with low expression in the basal cells and almost no expression in the stromal compartment (Figure 4A) [9]. In contrast, OSCC specimens displayed a variable S100A14 expression pattern across different areas of the lesion. A gradual loss of S100A14 from the surface/tumor center (more differentiated areas) to the invading front/island (poorly differentiated areas) was observed in OSCC samples (Figure 4B), following the same pattern for decreased gradient of expression from surface to the basal cell layer as observed in the normal oral mucosa. Interestingly, a change in the sub-cellular localization, from predominantly membranous staining seen in the central areas of the OSCC to mixed membranous and cytoplasmic staining was found at the invading tumor islands (Figure 4B) [9]. In parallel to these findings, a gradual loss of *S100A14* mRNA and protein was found as well in an *in vitro* OSCC-progression model consisting of normal, dysplastic (pre-malignant) and cancerous oral keratinocyte cell lines

[9, 10]. Taken together, downregulation of S100A14 seems to be a common molecular alteration in OSCC. One of the mechanisms responsible for this finding could be the loss of chromosomal region 1q21 harboring *S100A14* gene. However, the heterogeneity of the protein expression in the same lesion and the common pattern for decreased gradient of protein expression observed in both normal and neoplastic mucosa might indicate a more complex, posttranslational regulation of S100A14 expression, governed by hierarchical cellular processes of differentiation. On the other hand, occurrence of hypermethylated CpG islands in the promoter region, a common occurrence for some of the S100 members [67–70], seems to be a less likely mechanism for *S100A14* downregulation in OSCC as no CpG islands have been reported in 5' upstream region and introns of *S100A14*

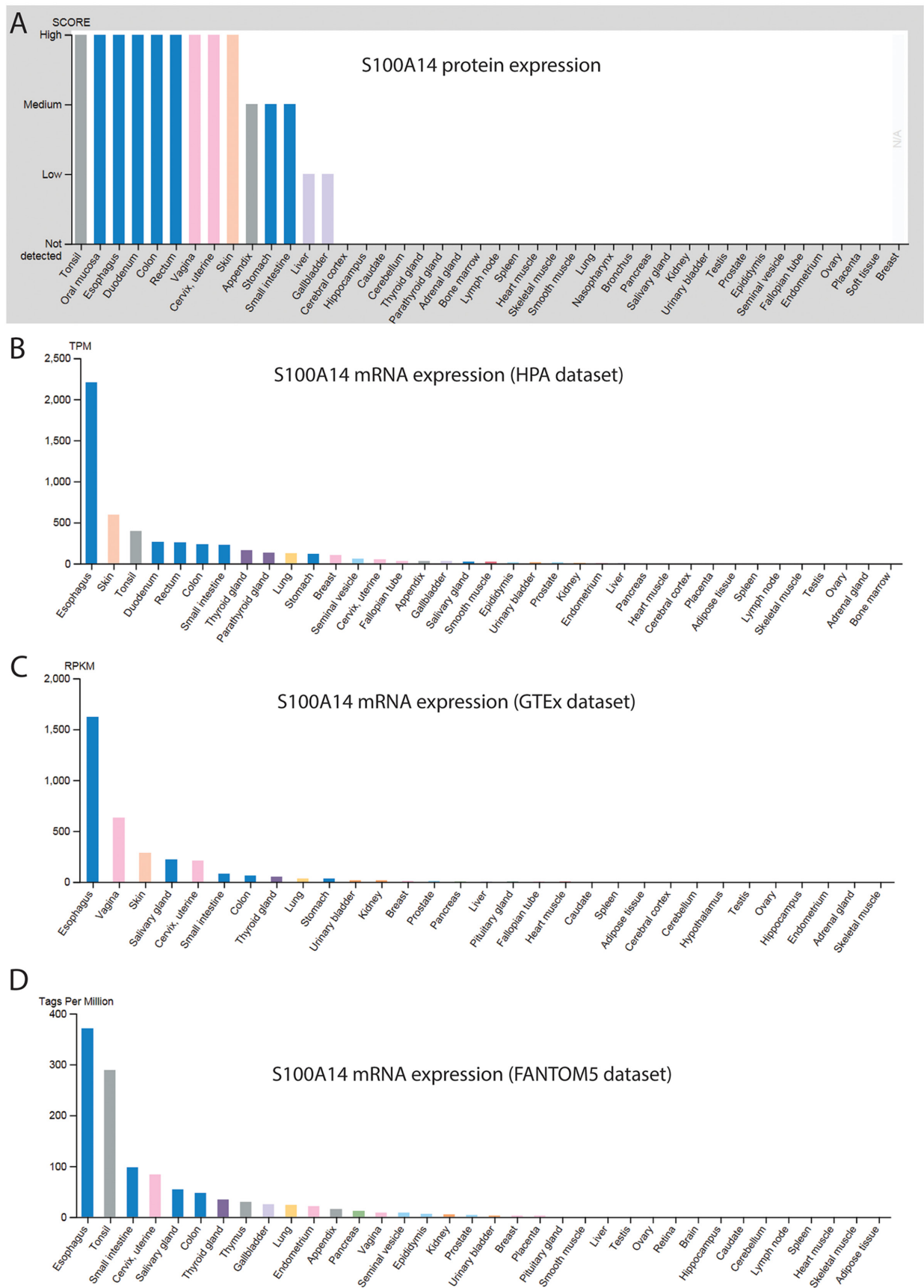
[3]. In support of this line of thinking are also findings from *in vitro* models of another epithelial malignancy which revealed that treatment with 5-aza-2'-deoxycytidine could not induce re-expression of *S100A14* mRNA in eight lung cancer cell lines [3].

### Esophageal squamous cell carcinoma (ESCC)

The expression levels of both *S100A14* mRNA and protein were reported to be significantly downregulated in ESCC compared to the normal adjacent epithelial tissue [71–73]. Using S100A14 immunohistochemistry, Chen et al. found a predominantly membranous staining in the keratinocytes in normal esophageal epithelium. However, a mixed staining pattern (plasma membranous



**Figure 2: Key S100A14 signaling pathways in human cancers.** Extracellular S100A14 (low amount) binds with RAGE and activates ERK1/2 and NF-κB signaling pathways, leading to ESCC cell proliferation and survival. Intracellular S100A14 binds to the intracellular domain of ERbB2, leading to the activation of ERK1/2 and AKT pathways in breast cancer cells. In OSCC, S100A14 positively affects the function of p53. In ESCC, p53 positively regulates the transcription of *S100A14*, whereas S100A14 negatively regulates the expression and function of p53. OSCC, oral squamous cell carcinoma; ESCC, esophageal squamous cell carcinoma; ?, unknown mechanisms downstream of ERK1/2 and AKT pathways.



**Figure 3:** Protein (A) and mRNA (B-D) data demonstrating differential expression of S100A14 in various human normal tissues.

**Table 1: Deregulation of S100A14 in human cancers**

Cancer type	Source	mRNA/protein	Up/downregulation (cancer versus normal)	Ref.
OSCC	Cultured cells/Tissue	mRNA and protein	Down	[9, 10, 66]
Gastrointestinal cancer				
Esophageal sq. cell carcinoma	Tissue	mRNA and protein	Down	[71, 73]
Small intestinal adenocarcinoma	Tissue	Protein	Down	[76]
Colorectal carcinoma	Tissue	mRNA and protein	Down	[3, 77]
Ovarian cancer				
Epithelial ovarian cancer	Cultured cells/Tissue	mRNA and protein	Up	[3, 78]
Serious ovarian cancer	Tissue/Serum	Protein	Up	[79]
Cervical cancer	Tissue	Protein	Up	[93]
Breast cancer	Tissue	mRNA and Protein	Up	[3, 81]
Breast cancer	Tissue	Protein	Up	[54]
Lung adenocarcinoma	Tissue/Serum	Protein	Up	[83]
Lung adenocarcinoma	Tissue	Protein	Up	[84, 85]
Hepatocellular carcinoma	Tissue	Protein	Up	[82]
Uterine cancer	Tissue	mRNA	Up	[3]
Renal cancer	Tissue	mRNA	Down	[3]
Bladder cancer	Tissue	mRNA	Up	[80]

Down: downregulation; Up: upregulation.

and cytoplasmic) was reported in ESCC specimens. Interestingly, similar to our previous observation in OSCC, a stronger S100A14 expression was reported in more differentiated (keratinized) areas as compared to the less differentiated (invading areas) of ESCC [71, 73], suggesting again a functional link between S100A14 and keratinocyte differentiation.

## Other gastrointestinal carcinomas

### Gastric cancer

The expression of S100A14 in gastric carcinoma appears to be variable as compared to the normal control. Zhu et al. investigated the expression of S100A14 in a large cohort of gastric cancer specimens (n=485) with pair-wised matched control gastric mucosa (n=289). Nevertheless, the authors did not find any significant difference in S100A14 expression between gastric cancers and matched controls [74]. However, Zhang et al. reported a differential expression of S100A14 in gastric carcinoma as compared to controls (S100A14 was upregulated in some gastric carcinomas, while downregulated in others) [75].

### Small intestine adenocarcinoma (SIAC)

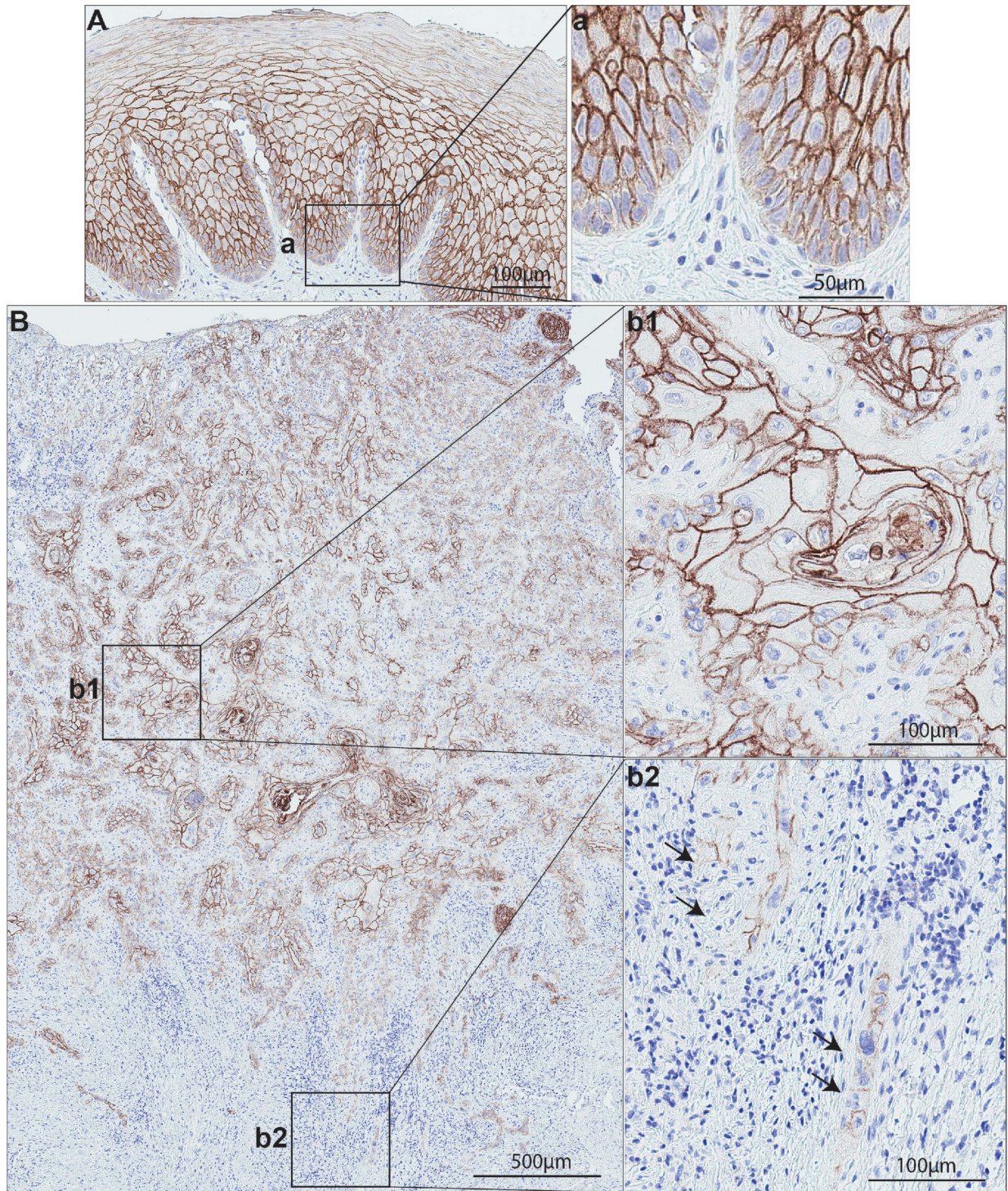
Using immunohistochemistry, Kim et al. reported a downregulated expression of S100A14 in SIACs as compared to normal intestinal epithelium. S100A14 was mostly expressed in cell membrane of normal small intestinal mucosal epithelial cells. The expression was pronounced on the surface epithelial cells and reduced in the crypts, with no expression in Brunner's glands. Out of 175 SIACs, loss of S100A14 was reported in 73% of the cases. The loss of expression was common mainly in the ulceroinfiltrative and polyploid tumors compared to flat tumors. In addition, the loss was more frequent in distal (ileum or jejunum) tumors of the intestine than proximal (duodenum) ones [76].

### Colorectal cancer (CRC)

Downregulation of S100A14 is reported in majority of the CRC samples, however, a significant proportion of the CRC samples has also been reported to express higher levels of S100A14. Using RT-PCR, Wang et al. reported both downregulation (52.5%) and upregulation (47.5%) of *S100A14* mRNA in primary colorectal cancer (n=40) as compared to their matched normal mucosa.

In line with the mRNA data from RT-PCR, S100A14 immunohistochemistry showed downregulation or loss of S100A14 expression in majority (56.5%) of colorectal cancer. Membranous S100A14 staining was prominent

in the well-differentiated areas in the tumor. The author reported a substantial expression of S100A14 in different human colorectal cancer cell lines (SW840, SW620, LoVo and HT29) [77].



**Figure 4: S100A14 expression on normal human oral mucosa and oral cancer specimens.** (A) Representative normal human oral mucosa specimen showed strong, predominantly membranous S100A14 expression in the epithelial compartment. (B) Representative oral cancer lesion showing a gradient of S100A14 expression: central area (b1) showed a strong, membranous staining in contrast to a very weak, staining in the invading front area (b2).

## Ovarian cancers

Overall, S100A14 has been reported to be overexpressed in ovarian cancers as compared to the normal control tissues. Using immunohistochemistry in formalin-fixed paraffin embedded specimens of ovarian cancers (n=71), borderline ovarian tumors (n=10), mucinous cystadenomas (n=10) and normal ovary tissues (n=13), Cho et al. reported a gradual over-expression of S100A14 from normal tissues to benign, borderline and ovarian carcinomas [78]. In parallel, Qian et al. reported an upregulation of S100A14 in serous ovarian carcinomas compared to normal controls [79]. Both authors found predominant cytoplasmic S100A14 staining in carcinoma cells. In addition, over-expression of *S100A14* mRNA and protein in ovarian cancer cell lines as compared to human ovarian surface epithelial cells has been reported [78].

## Bladder cancer

Yao et al. reported an upregulation of *S100A14* mRNA in 4-hydroxybutyl(butyl)nitrosamine-induced mouse and human bladder cancers compared to their corresponding controls [80].

## Breast cancers

S100A14 has been reported to be upregulated in breast cancers. Tanaka et al. performed immunohistochemical analysis of S100A14 in archival specimens of primary tumors

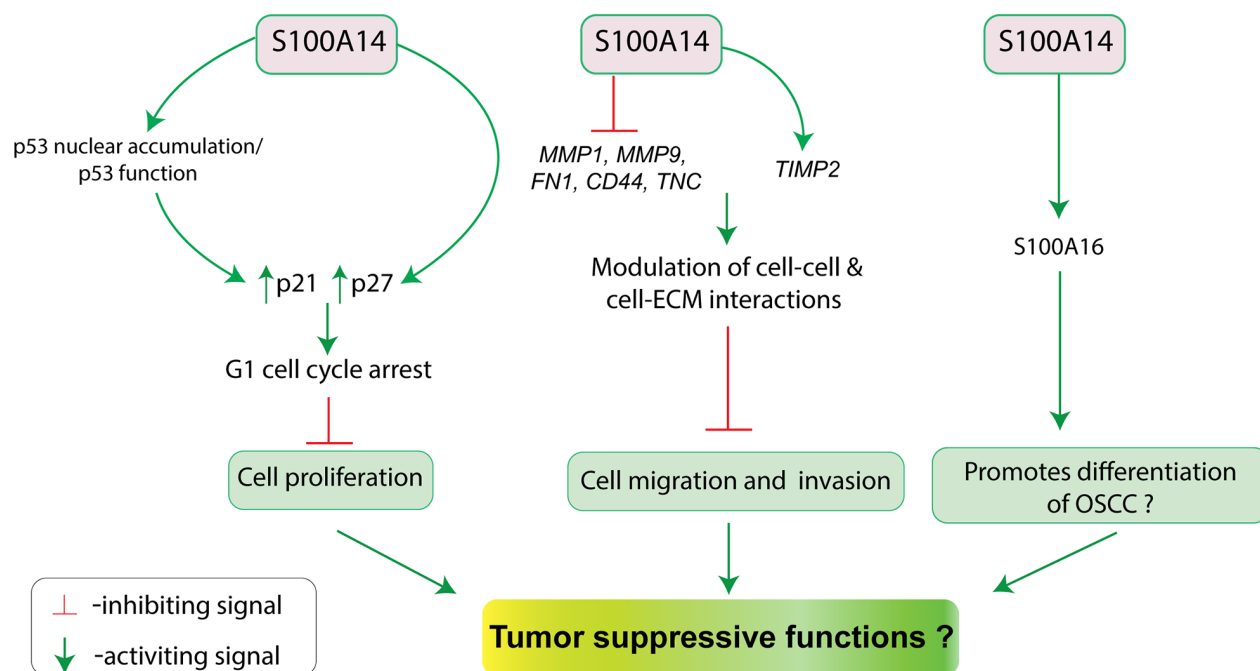
of 167 breast cancer patients and found that 53% of cases expressed strong, predominantly membranous S100A14, while the control normal tissue showed no or faint staining [81]. Similarly, Xu et al. found strong membranous S100A14 staining in breast cancer specimens (n=74) compared to their matched control specimens. In addition to the tissue specimens, the assessment of cell lines suggests a predominant plasma membrane (inner side) localization of S100A14 [54]. Interestingly, Tanaka et al. showed that S100A14 expression was polarized to the lateral and the intercellular junctions but not to the basal or the apical region, indicating that S100A14 potentially binds to membrane-associated proteins [81].

## Hepatocellular carcinoma

Similar to breast, ovarian and bladder cancers, overexpression of S100A14 has been reported in hepatocellular carcinoma. Using immunohistochemistry on specimens of hepatocellular carcinoma (n=120) and their corresponding paratumor normal liver tissue (n=14), Zaho and co-workers found a higher S100A14 expression in carcinoma compared to the controls. Interestingly, a nuclear or cytoplasmic localization of S100A14 was reported in carcinoma cells [82].

## Lung adenocarcinoma

S100A14 has been reported to be over-expressed in lung adenocarcinomas as compared to the normal control tissues. A predominantly membranous staining was commonly found in the carcinoma cells [83–85].



**Figure 5: Schematic illustration for the putative tumor suppressive functions (inhibition of cell proliferation, migration and invasion; promotion of differentiation?) of S100A14 in oral cancer.**



## FUNCTIONAL ROLE OF S100A14 IN HUMAN CANCERS

S100A14 has been suggested to be involved in a number of biological pathways (cell proliferation and apoptosis, cell migration and invasion, differentiation, *etc.*) related to carcinogenesis. However, in parallel to the differential expression pattern and sub-cellular localization in different cancer types, the S100A14-mediated functions seem to be specific with respect to cancer types. In some tumor types, S100A14 seems to function as a tumor suppressor protein while in others it seems to promote tumorigenesis. Below we will summarize the functional roles and related molecular pathways of S100A14 in major cancer types.

### Oral cancer

Based on the progressive downregulation of *S100A14* mRNA and protein from normal oral mucosa to OSCC, we previously hypothesized that S100A14 might be related to tumor suppressive functions in OSCC (Figure 5). Subsequent studies showed that S100A14 might indeed function as a tumor suppressor by inhibiting OSCC cell proliferation and/or invasion [9, 10].

Retroviral-mediated over-expression of S100A14 in OSCC cell lines (CaLH3 and OSCC1) harboring wild type p53 led to reduced cell proliferation and G1-cell cycle arrest with concomitant upregulation of the cell cycle inhibitor p21 and p27 proteins. As the tumor suppressor protein p53 is one of the key regulators of p21 expression [86], a possible link between p53 and S100A14-mediated upregulation of p21 was examined by using shRNA-mediated knock-down of p53. Partial suppression of p21 expression with p53 knock-down suggested that S100A14 over-expression mediated upregulation of p21 and associated G1-phase cell cycle arrest might, at least, be partly dependent on the activity of the WT p53 in CaLH3 and OSCC cells. Additionally, S100A14 was found to enhance nuclear accumulation of p53 in OSCC cells. Taken together, S100A14 was found to inhibit OSCC cell proliferation by inducing G1-cell cycle arrest, partly, through p21-p53 axis [10].

Significant downregulation of S100A14 particularly at the invasive tumor islands of OSCC led us to examine the role of S100A14 in the regulation of invasive phenotype of OSCC cells. Experimentally, retroviral-mediated over-expression of S100A14 resulted in significant decrease of the migratory and invasive phenotype whereas siRNA mediated knock-down led to opposite effects in OSCC cells. The reduction in the invasive potential of OSCC cells was associated with the concomitant downregulation of matrix metalloproteinase (MMP) like *MMP1* and *MMP9* mRNAs, and suppression of *MMP9* gelatinolytic activity [9]. These observations suggest that S100A14 might contribute to less aggressive

OSCC behavior by suppressing the invasion potential of OSCC cells.

Although not yet experimentally proven, the finding that S100A14 expression was significantly lost at the invading front/island (poorly differentiated areas) of OSCC as compared to the tumor surface/tumor center (more differentiated areas), suggests a functional link between S100A14 and cellular differentiation. Indeed, there are several observations which indicate a role for S100A14 in cellular differentiation. Firstly, genes encoding several S100 proteins including S100A14 are clustered in the epidermal differentiation complex on chromosome 1q21 and many of the S100 members have been reported to be involved in cellular differentiation and differentiation-related pathologies [87, 88]. Secondly, S100A14 has been shown to promote differentiation of ESCC and gastric cancer cells [73, 74]. Thirdly, S100A14 was found to interact with and regulate the expression of S100A16 (another member of S100 protein family), which suppresses the OSCC progression by promoting OSCC cell differentiation [89]. Hence, future studies looking for the expression of differentiation markers in OSCC cells with modulation of S100A14 expression might reveal a link between S100A14 and OSCC differentiation.

### ESCC

Sequencing analysis of *S100A14* gene and untranslated regions has shown that genetic variants can occur in *S100A14* gene and that might influence S100A14-mediated cellular functions and susceptibility for ESCC development. Further, in-depth functional studies have linked S100A14 to the regulation of differentiation, proliferation, apoptosis and invasive potential of ESCC cells.

Chen et al. reported that a S100A14 gene (461G>A) variant in 5' untranslated region contributed to reduced expression of S100A14 in ESCC, probably due to reduced transcriptional activity mediated by p53. Additionally, the genetic variant 461G>A was found to be associated with susceptibility to ESCC in a Chinese population with smoking habit [72]. In contrary, another study by Zhao et al. in Chinese population showed that the same genetic variant (rs11548103G>A) can have a protective effect in ESCC carcinogenesis [90]. These results warrant further studies including a sufficiently large number of samples and with detailed clinicopathological information to clarify the association between S100A14 genetic variant and ESCC carcinogenesis.

In parallel to the observation that more differentiated ESCC expressed higher expression of S100A14 in clinical specimens, Chen et al. reported increased expression of S100A14 in 12-O-tetra-decanoylphorbol-13-acetate and Ca<sup>2+</sup>-induced more differentiated ESCC cells [73]. Further, S100A14 expression was found to regulate the expression of terminal differentiation markers and induce G1-phase cell cycle arrest. Additionally, the authors suggested that JunB

could transcriptionally regulate S100A14 expression and this regulation might be important in S100A14-mediated ESCC differentiation [73]. On the other hand, the effect of exogenous treatment of S100A14 seems to display different cellular functions based on the amount of S100A14. Low amount of S100A14 activated ERK1/2 and NF- $\kappa$ B signaling thereby stimulating ESCC cell proliferation or survival via RAGE signaling. However, higher amount resulted in increased production of reactive oxygen species in a RAGE-dependent manner [53].

In addition to these cellular functions, Chen et al. have linked S100A14 with ESCC invasion. The authors showed that overexpression of S100A14 promoted motility and invasiveness of ESCC, at least partly through upregulating the expression and activity of MMP2 in a p53-dependent manner. Moreover, based on the observation that expression of S100A14 is dependent on the p53 regulation and that S100A14 decreases the expression of p53, the authors suggested a negative feedback loop between S100A14 and p53 [55]. In contrary, our previous work suggested a positive mutual functional regulation between S100A14 and p53 in OSCC cells [10]. A positive mutual regulation has also been suggested for S100A2 and p53 [26, 91]. Nevertheless, the discrepancy in the nature of mutual regulation between S100 protein members and p53 between oral and esophageal epithelial cells needs further investigation.

### Other gastrointestinal carcinomas

Expression analyses have found a correlation between loss/downregulation of S100A14 in gastrointestinal cancers and disease aggressiveness as well as poor patient outcomes [74, 76, 77]. In parallel with these observations, S100A14 has been reported to regulate differentiation and migratory/invasive potential of gastric cancer cells [74, 75]. Zhu et al. found that S100A14 was positively correlated with more differentiated phenotype of gastric cancer cells with concomitant regulation of E-cadherin and PGII expression. Additionally, expression of S100A14 negatively regulated the migratory/invasive potential of gastric carcinoma cells *in vitro* and *in vivo*. The authors, using a series experiments, further suggested that the reduced invasive potential was related to the concomitant activation and stabilization of FAK, and downregulation of MMPs (MMP2, MMP9 and MMP11), probably mediated by the inhibition of store-operated Ca<sup>2+</sup> influx in the gastric carcinoma cells [74].

### Ovarian cancer

Ovarian cancer specimens with positivity or upregulation of S100A14 have been correlated with advanced stage, poor tumor grade, tumor recurrence, resistance to platinum-based therapy and shorter survival probabilities [78, 79, 92]. By using lentiviral-mediated expression and knock-down strategies, Cho and coauthors have investigated the role of S100A14 in the malignant

phenotype of epithelial ovarian cancer cells. The authors reported increased cell proliferation, colony and sphere formation abilities, and migration and invasion of epithelial ovarian cancer cells upon S100A14 overexpression. The opposite results have been shown with the knock-down of S100A14. In line with these *in vitro* results, S100A14 was found to increase the growth of subcutaneous tumor xenograft in mice. Investigation of the underlying signaling pathway revealed that S100A14 might contribute to epithelial ovarian cancer progression via PI3K/Akt pathway [78].

### Cervical cancer

Wang et al. found a positive correlation between higher expression of S100A14 with poor prognosis in cervical cancer specimens. To investigate the biological roles of S100A14 in cervical cancer progression, the authors used lentiviral-mediated modulation of the endogenous S100A14 in cervical cancer cells. The modulation of S100A14 correlated positively with increased proliferation, migration and invasion of cervical cancer cells *in vitro*. The increased cell proliferation was associated with reduced proportion of cells in G1- and increased proportion in G2/M- phase of cell cycle. Further, S100A14 was shown to enhance epithelial mesenchymal transition of cervical cancer cells as evidenced by the upregulation of N-cadherin and Vimentin, and downregulation of E-cadherin [93].

### Breast cancer

In breast cancer tissues and cell lines, S100A14 has been found to co-localize with HER2 [54] and actin proteins [81]. Immunoprecipitation studies have shown that S100A14 indeed interacts with HER2 [54] and actin [81]. The interaction between S100A14 and HER2 seems to be Ca<sup>2+</sup>-dependent as treatment with 1mM Egtazic acid was found to inhibit the interaction [54]. Nevertheless, interaction with actin seems to be independent of Ca<sup>2+</sup> as increasing the concentration of Ca<sup>2+</sup> did not increase immunoprecipitation [81]. The latter is in line with our previous study where increasing Ca<sup>2+</sup> concentration did not enhance interaction. One of the possible explanation for this observation could be the fact that S100A14 is a poor Ca<sup>2+</sup> binder with semi-open conformation even in apo-state and this conformation might not necessarily change even after binding Ca<sup>2+</sup> ions [94].

These interactions have been suggested to have a functional significance in breast cancer cells. For example, Tanaka et al. suggested that S100A14 may promote cancer invasion by binding to the actin, thereby activating cell movement [81]. Similarly, Xu et al. examined the functional significance of S100A14 and HER2 by silencing S100A14, and found a subsequent reduction in HER2 phosphorylation and downstream signaling with

concomitant reduction in the HER2-stimulated breast cancer cell proliferation [54]. Furthermore, several other studies have supported the role of S100A14 in breast cancer progression [95, 96]. These observations indicate that one of the ways that S100A14 executes its diverse functions in breast cancer cells could be through molecular interaction with other cellular proteins involved in key biological pathways.

### Hepatocellular carcinoma

Zhao et al. investigated the functional roles of S100A14 in hepatocellular carcinoma cells by using S100A14 over-expression or knock-down strategies [82]. A decrease in cell proliferation and invasion was found with knock-down of S100A14 in hepatocellular carcinoma cells *in vitro* [82, 97]. Opposite effects were reported with S100A14 over-expression. In parallel with these *in vitro* data, smaller subcutaneous tumor xenografts and significantly reduced number of lung metastases were found in severe combined immunodeficient mice with knock-down of S100A14. Although no molecular mechanisms have been suggested, these results indicate that S100A14 might promote hepatocellular carcinoma [82].

### Lung adenocarcinoma

Biological functions of S100A14 in lung adenocarcinoma have not been well investigated as compared to oral cancer or ESCC. Ken et al. used siRNA technique to downregulate the expression of S100A14 in LC-2/ad cells and showed that downregulation of S100A14 was correlated with less migratory (scratch assay) and less invasive (Matrigel invasion assay) phenotype [84]. This observation is in line with association of high S100A14 expression in lung adenocarcinoma specimens with lymph node metastasis, intratumoral vascular invasion, intratumoral lymphatic invasion, pleural invasion [83, 84].

## S100A14 AS A POTENTIAL PROGNOSTIC MARKER AND THERAPEUTIC TARGET IN HUMAN CANCERS

Several studies have used immunohistochemistry to examine the prognostic potential of S100A14 in different human cancers. It is interesting to note that higher or positive S100A14 expression in the carcinomas of gastrointestinal tract was found to be correlated with better patient survival. In contrary, the higher expression of S100A14 in carcinomas from other organs such as ovary, breast, cervix, lung and liver seem to predict poorer survival. S100A14 expression pattern and correlation with clinicopathological and prognostic parameters in different cancer types is summarized in Table 2.

Results from the *in vitro* and *in vivo* functional studies in several cancer types indicate that S100A14 might be useful as a therapeutic target for the treatment of malignancies. Malignancies where S100A14 functions as a tumor promotor [95, 97], strategies aimed to downregulate the S100A14 expression might be employed. On the other hand, overexpression strategies might be useful in malignancies [9, 10, 73, 74] where S100A14 functions as a tumor suppressor. Indeed, the differentiation promoting properties of S100A14 in ESCC and CRC are of immense interest for differentiation therapy. The differentiation therapy, forcing the proliferative tumor cells towards more differentiated and mature phenotype, has been realized long before and some of the drugs, such as retinoic acid and histone deacetylase inhibitors are currently in clinical use in the treatment of human malignancies [98]. Further molecular characterization of S100A14 mediated tumor suppressive functions might contribute to the better understanding of cancer biology and provide opportunity for S100A14 based therapeutics.

## CONCLUSIONS

S100A14 is differentially expressed in various normal human tissues and its deregulated expression seems to be a common event in human carcinogenesis. Functionally, S100A14 has been linked to the regulation of a number of cellular functions related to carcinogenesis, such as cell proliferation and apoptosis, tumor cell migration and invasion, and keratinocyte differentiation. It is, however, interesting to note that S100A14 seems to have a tumor suppressive functions in cancers arising from the oro-gastrointestinal tract (tissues rich in epithelial components) compared to cancers in the other parts of the body (tissues rich in mesenchymal components) where it is related to the tumor promotive functions. Although the underlying mechanism for this observation are currently unclear, it can be speculated that S100A14-mediated functions are dependent on the relative abundance and the type of the interaction partners (effector protein) thereby activating different downstream signaling pathways leading to the opposite functions.

Expression pattern of the S100A14 in different cancer types has a potential to be clinically useful as prognostic biomarker in the management of human cancers. Nevertheless, studies involving a larger and well-characterized patient cohorts and with subsequent validations are clearly warranted.

## Abbreviations

BCMP84: Breast Cancer Membrane Protein 84  
Ca<sup>2+</sup>: Calcium  
CRC: Colorectal cancer  
ERK: Extracellular signal-Regulated Kinase

**Table 2: Association of S100A14 protein expression with clinicopathological and prognostic variables in human cancers**

Cancer type	<i>n</i>	Association with clinical/prognostic variables	Ref.
Gastrointestinal cancer			
Esophageal sq. cell carcinoma	110	low S100A14 correlated with poor differentiation and higher clinical stage	[73]
Gastric cancer	485	high S100A14 positively correlated with Lauren classification and differentiation and better OS <sup>a</sup> , but negatively correlated with tumor depth, lymph node status and distant metastasis	[74]
Gastric cancer	79	high S100A14 positively correlated with differentiation and survival	[75]
Small intestinal adenocarcinoma	175	-ve S100A14 correlated with lymph node metastasis and advanced disease stage	[76]
Colorectal carcinoma	115	-ve S100A14 correlated with poor differentiation, distance metastasis and shorter OS <sup>b</sup>	[77]
Ovarian cancer			
Epithelial ovarian cancer	71*	+ve S100A14 correlated with advanced stage and poor tumor grade, shorter DFS and OS <sup>c</sup>	[78]
Serious ovarian cancer	127	high S100A14 correlated with tumor recurrence and lymph node metastasis, shorter OS <sup>c</sup>	[92]
Serious ovarian cancer	125	high S100A14 correlated with resistance to platinum based therapy	[79]
Cervical cancer	98	high S100A14 correlated with FIGO stage and Lymph node metastasis	[93]
Breast cancer	167	high S100A14 correlated with ER-negative, HER-2 positive and shorter OS <sup>c</sup>	[81]
Breast cancer	148	high S100A14 correlated with poorer DFS and OS <sup>c</sup>	[99]
Lung adenocarcinoma	208	high S100A14 correlated with poor differentiation, metastasis, stage <sup>d</sup>	[83]
Lung adenocarcinoma	166	+ve S100A14 correlated with poor differentiation, node metastasis, stage, tumor size, vascular and lymphatic invasion, shorter survival <sup>e</sup>	[84]
Lung adenocarcinoma	62	+ve S100A14 correlated with well/moderate differentiation	[85]
Hepatocellular carcinoma	120	+ S100A14 correlated with multiple tumor nodes, high Edmondson-Steiner grade, vascular invasion, shorter DFS and OS <sup>e</sup>	[82]

*n*: sample size; Ref.: references; OS: overall survival; DFS: disease free survival; +ve: positive; -ve: negative; MVA: multivariate analysis

<sup>a</sup> No univariate and multivariate cox analysis was presented.

<sup>b</sup> Only multivariate cox analysis results presented.

<sup>c</sup> OS: significant correlation both with the univariate and multivariate cox analysis.

<sup>d</sup> The authors did not find any significant association of S100A14 expression with survival in their own IHC data. However, they have reported, using Kaplan Meier plotter online tool, that high S100A14 expression significantly correlated with reduced overall and post progression survival.

<sup>e</sup> Significant correlation only with univariate and not with multivariate cox analysis.

\* Only 65 cases were used for survival analysis.

ESCC: Esophageal Squamous Cell Carcinoma  
HER2: Human Epidermal Growth Factor Receptor 2  
MAP: Mitogen Activated Protein  
MMP: Matrix Metalloproteinase  
OSCC: Oral Squamous Cell Carcinoma  
RAGE: Receptor for Advanced Glycation End Products  
SIAC: Small Intestine Adenocarcinoma.

### Author contributions

SB, SS, DEC and DS wrote the papers; DS designed the figures; SB, SS, DEC and DS reviewed the manuscript.

### Ethics statement

The use of normal oral mucosa and oral cancer specimens was approved by the Committee for Medical and Health Research Ethics in Norway. All of the specimens were collected after informed patient consent.

### CONFLICTS OF INTEREST

The authors declare that they have no competing interest.

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