Middle-East Journal of Scientific Research 16 (6): 775-781, 2013
ISSN 1990-9233

DOI: 10.5829/idosi.mejsr.2013.16.06.75160

S100A9 Expression in Oral Squamous Cell Carcinoma

[1]AzadehAndisheh Tadbir, [2]Mohammad Javad Ashraf and [3]Golshid Mehrabani

[1]Department of Oral and Maxillofacial Pathology, School of Dentistry,
ShirazUniversity of Medical Sciences, Shiraz, Iran
[2]Department of Pathology, School of Medicine,
ShirazUniversity of Medical Sciences, Shiraz, Iran
[3]School of Dentistry, International Branch,
Shiraz University of Medical Sciences, Shiraz, Iran

Abstract: S100A9 is a calcium binding protein which was over-expressed in different human cancers, presenting increased expression in neoplastic tumor cells as well as infiltrating immune cells. The aim of this study is to determine the S100A9 expression in oral squamous cell carcinoma (OSCC) and its relation with the clinicopathological features. In this study 60 cases of oral squamous cell carcinoma and 20 normal epithelium subjects were reviewed by IHC for S100A9 staining. Results revealed that S100A9 expression in OSCC was statistically higher than normal epithelium.S100A9 expression in inflammatory cells of stroma was seen in both of OSCC and normal epithelium and was statistically higher in OSCC than normal.The expression of S100A9 in squamous epithelial cells was associated with cellular differentiation, but was not related to the clinical stage and lymph node status. In conclusion, S100A9 expression plays an important role in the carcinogenesis and development of OSCC and the expression in tumor cells denotes to the histological differentiation.

Key words: S100A9 · Squamous Cell Carcinoma · Iran

INTRODUCTION

The S100 proteins are a multi-gene calcium-binding family, comprising more than 20 different proteins which are encoded by a separate gene and are expressed in a controlled tissue specific or cell type-specific manner [1, 2].

They are small, acidic proteins of 10-12 KDa and form the largest family of calcium binding proteins [3]. S100 proteins show a wide range of intracellular and extracellular functions [1].

They are in two forms within cells including homodimers or heterodimers and interact with several proteins in a Ca^{2+}-dependent manner [4]. They regulate many important cellular functions such as cytoskeleton organization, homeostasis, stress response, cell proliferation, cell motility and differentiation [3].

S100 A9 is a member of this family and was originally found in granulocytes and macrophages and has a role in myeloid cell differentiation [5]. This protein is also expressed in certain type of epithelial and carcinoma cells [6-8]. More recently, over-expression of S100 A9 was detected in different human cancers, presenting increased expression in neoplastic tumor cells as well as infiltrating immune cells (4). S100 A9 over-expression was also reported in oral squamous cell carcinoma (OSCC) [9].

However its distribution within the tissues and its association with clinicopathological features were not fully demonstrated. Therefore, in this study, we immunohistochemically studied the S100 A9 expression in OSCC and investigated its relation with clinicopathologic parameters.

MATERIALS AND METHODS

In this study, 60 cases of oral SCC (35 males and 25 females) with adequate tissue sample size that were registered in Khalili Hospital affiliated to Shiraz University

Corresponding Author: Azadeh Andisheh Tadbir, Department of Oral & Maxillofacial Pathology, School of Dentistry, Ghom Abad, Ghasrodasht Avenue, Shiraz University of Medical Sciences, Shiraz, Iran.
Tel: +98-0711-6263193-4, Fax: +98-0711-6270325.

of Medical Sciences and 20 normal tissues were enrolled. Demographic information such as age, gender and clinical data including grade, stage and lymph node metastasis were collected from medical records.

Immunohistochemistry (IHC) Method: Sections with 4 µ thickness were mounted on positive charged microscope slides. After dewaxing in xylene, sections were dehydrated in ethanol and rinsed in distilled water. Antigen retrieval was performed by DAKO Target Retreival solution (Dako Co., Carpinteria, CA, USA). The endogenous peroxidase was quenched by 3% H2O2. The peroxidase-labeled polymer conjugated to goat anti-mouse S100A9 method was used to detect antigen-antibody reaction (DAKO EnVision_ System; DAKO Corporation, Carpinteria, CA, USA).

Tissue sections were then incubated for 1 hour with the anti-S100A9 antibody (Abcam, UK) at a dilution of 1/100. The tissue sections were then visualized with 3, diaminobenzidine as a chromogen for 5 minutes and counterstained with Harris's hematoxylin. Slides were washed in tap water, dehydrated and mounted with glass cover slips. Positive controls were the sections of breast cancer tissue which were previously found to be positive for the S100A9. The negative controls consisted of duplicated sections of the same specimens in which the primary antibody had been excluded and replaced with PBS.

Immunohistochemical Analysis: S100A9 immunoreactivity was observed in the cytoplasm and nucleus. All immunopositive cells were counted in at least 10 high-power fields (×40 objective, ×10 eye-piece) chosen at random. The number of S100A-positive cell was given as a percentage for each case. Extent of immuno-staining (based on the percentage of positive cells) was scored as 0 (none), 1 point (1-9%), 2 points (10-50%), 3 points (51-75%) and 4 points (76-100%) [3]. To evaluate the extent of S100A9 expression in stroma of tumor, the number of S100A9- positive inflammatory cells in each tumor tissue was measured by averaging the cell counts of three fields (original magnification, 200×) in the area with the greatest number of positive cells at the site of deepest tumor invasion [11].

Statistical Analysis: Mann-Whitney test, Independent t-test, Chi-Square and Fisher tests were used for analysis the result. Significant level for tests was 0.05.

RESULTS

The expression of S100A9 in OSCC and normal epithelium illustrated in Figs 1& 2. OSCCs consistently showed diffuse and intense positive staining for S100A9. The staining of S100A9 was focal and weak in normal squamous epithelium and S100A9 immuno-reactivity was absent in the basal cells S100A9 expression in OSCC was statistically higher than normal epithelium (p=0.001).

S100A9 expression in inflammatory cells of stroma was seen in both of OSCC and normal epithelium and was statistically higher in OSCC than normal epithelium (p=0.4) (Table 1) (Figure 3).

Association between S100A9 expression in squamous epithelial cells and clinicopathological parameters in OSCC.

The relationship between S100A9 expression in squamous epithelial cells and clinico-pathological parameters in OSCC is shown in Table 2.

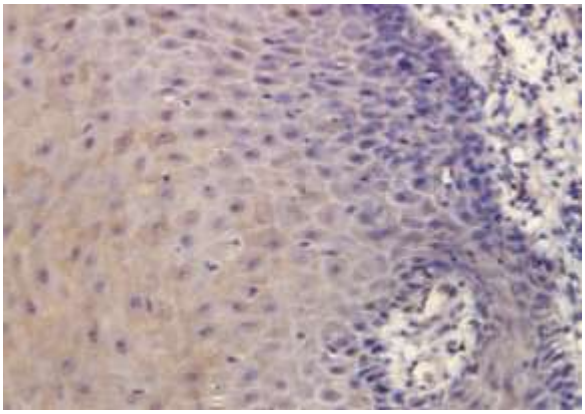

Fig. 1: Weak S100A9 expression in normal epithelium. No staining is seen in basal layer (×200).

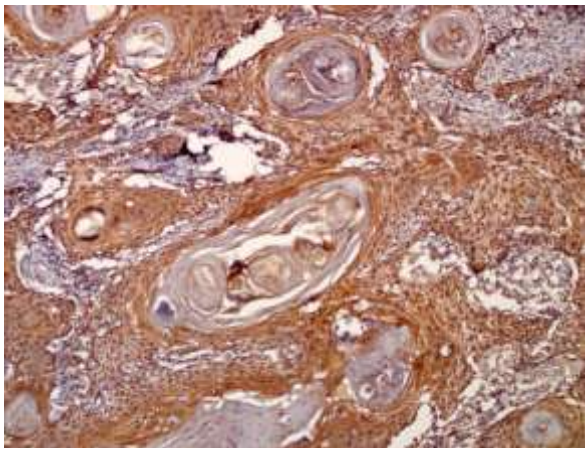

Fig. 2: Strong S100A9 expression in well differentiated squamous cell carcinoma (×200).

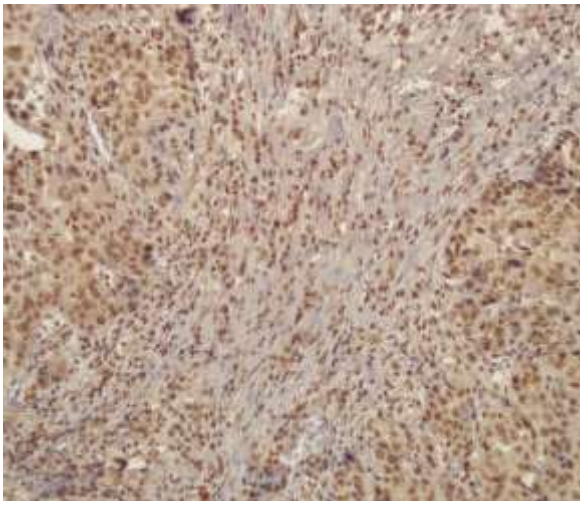

Fig. 3: S100A9 expression in endothelial cells and stromal cells in the stroma of squamous cell carcinoma (×200).

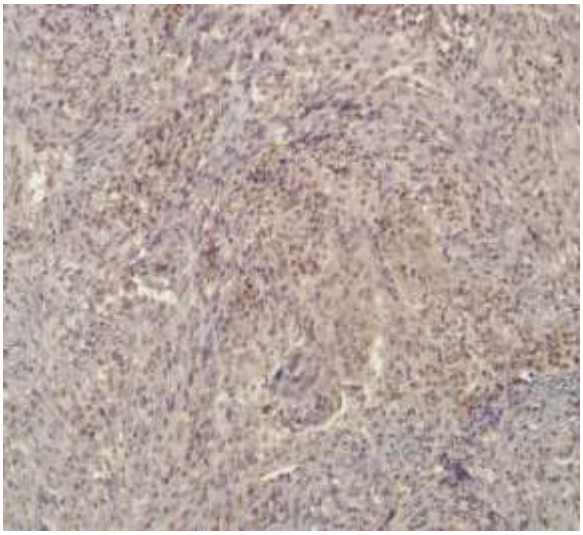

Fig. 4: Weak S100A9 expression in poorly differentiated squamous cell carcinoma (×200).

Table 1: Expression of S100A9 in OSCC and normal epithelium

	Score of S100 A9		Stromal expression
Tissue	Cases	Mean rank	Mean
Normal epithelium	20	14	8.25 ± 7.6
OSCC	60	49.3	12.2 ± 7.9

Table 2: Expression of S100 A9 in oral SCC according to clinicopathologic features

	Epithelial expression					
		Score				Stromal expression
Clinicopathologic features	Cases	1	2	3	4	Mean
Stage						
I	14	4	5	4	1	11 ± 6.3
II	14	3	5	4	2	10.2 ± 8.3
III	21	4	6	4	4	15 ± 9
IV	11	3	4	1	3	11.2 ± 7.9
Differentiation						
Well	20	0	0	7	13	13.9 ± 8.7
Moderate	20	0	14	6	0	11 ± 7.7
Poor	20	15	5	0	0	11.8 ± 7.4
Lymphatic Metastasis						
Present	32	7	10	5	10	10.6 ± 7.3
absent	28	7	10	8	3	13.7 ± 8.2

Table 2: Expression of S100A9 in OSCC and normal epithelium

	Score of S100 A9		Stromal expression
Tissue	Cases	Mean rank	Mean
Normal epithelium	20	14	8.25 ± 7.6
OSCC	60	49.3	12.2 ± 7.9

The expression of S100A9 in squamous epithelial cells was associated with cellular differentiation ($p<0.001$), but was not related to the clinical stage and lymph node status, (respectively $p=0.4$ and $p=0.3$).

The expressions of S100A9 in squamous epithelial cells in SCC were gradually increasing from poorly and moderately to well-differentiated tumors (Figure 2&4).

The expression of S100A9 in inflammatory cells of stroma was not associated with cellular differentiation, the clinical stage and lymph node status (respectively p=0.5, p=0.4 and p=0.1).

DISCUSSION

Human cancer is a chronic disease caused by proliferation of transformed cells which contains genetic and epigenetic alterations [11]. However, cancer tissue contains other cells besides cancer cells including epithelial cells, fibroblast, endothelial cells and immune cells [12]. In this complex tumor microenvironment, different stages of tumor development and progression are regulated by inflammatory mediators [13].

S100 A9 is a member of S100 family, which in previous studies, was proved to be expressed in normal, inflammatory and neoplastic squamous epithelium [14, 15] and in granulocyte and monocytes in various inflammatory conditions [11]. In this study, we found that S100 A9 was specifically located in inflammatory cells infiltrated in OSCC tissues as well as, tumor cells and normal squamous cells.

Recent studies have showed that adenomatous malignancies exhibited a strong up-regulation of S100 A9 which was associated with tumor growth, progression, invasion and metastasis [16-18]. However, S100 A9 expression in SCC demonstrated conflicting results. S100 A9 was up-regulated in oral tongue cancer tissues [9] and cervical SCC [10], when compared to adjacent normal tissues.

In contrast, decreased expression of S100 A9 was seen in laryngeal and esophageal squamous cell carcinoma compared with their paired normal tissues [19, 20]. In the present study which is identical to studies of He *et al.* [9] and Zhu *et al*. [10], we found that S100 A9 was up-regulated in OSCC. The relationship between S100 A9 expressions, clinicopathological features and patient prognosis in different cancer types was debated.

Overexpression of S100 A9 in lung cancer and invasive ductal carcinoma of the breast has been shown to be related with cancer development and progression [21, 22]. In bladder tumors, S100 A9 expression was not associated with tumor invasion, stage and poor survival [23].

Arai *et al*. [2001] showed that increased S100 A9 expression in pulmonary adenocarcinoma was correlated with poor differentiation [24]. In thyroid carcinoma, S100 A9 and S100 A8 expression in cancer cells played an important role in tumor dedifferentiation [23]. Kong *et al*. [20] reported that S100 A9 was down regulated in poorly differentiated esophageal SCC.

Zhu *et al*. [2013] revealed that S100 A9 expression was associated with histological differentiation but not with clinical stage and nodal metastasis [10]. Our results also showed that S100 A9 expression was significantly higher in well differentiated tumors in comparison with moderately and poorly differentiated ones.

These findings suggest that S100 A9 is associated with regulation of cell differentiation and proliferation in oral SCC. It has been shown that, the reorganization of cytoskeleton filaments is regulated by some members of S100 protein family [25].

Also it is known that S100 A9 is involved in the regulation of the assembly or disassembly of cytokeratins via Ca^{2+}-dependent manner in epithelial cells [26]. Generally, cytoskeleton filaments organization determines cell shape and cell differentiation [27].

So S100 A9 influence on the cell differentiation might be through changes in cytoskeleton organization. The tumor microenvironment and stromal compartment are recognized to have a role in cancer [10]. Most studies have been done in cancer, having focused on S100 A9 expression in tumor tissue, while only few studies assessed S100 protein expression in inflammatory cells infiltrating cancer [28-31].

Ang *et al*. [2010] revealed that high S100 A9 cell count was correlated with tumor size but not associated with patient survival [28]. Fan *et al*. [2012] showed that high S100 A9 cell count in gastric tissues was negatively correlated with advanced stages, tumor invasion and lymph node metastasis [11].

Our results indicated that the positive stromal S100 A9 immunoreactivity was observed in most OSCCs, but there was no association between S100 A9 expression in stromal cells of OSCC and clinico-pathological parameters. High expression of S100 A9 in the inflammatory cells in OSCC may exhibit that S100 A9 plays an important role in development.

Absence of association of S100 A9 expression with clinico-pathological features and patient's prognosis among cancer patients can be due to complexity of the cancer microenvironment. Immune mediators like S100 A9 may play both tumor promoting and anti-tumor roles based on the type of cancer [32, 33]. It was shown that anti-tumor immunity and tumor promoting inflammation co-exit at various points along the path of tumor progression and that micro-environment and

environmental conditions are responsible for the balance between the two [34, 35] since direct *in vivo* models to determine the effects of these phenomenon on cancer progression are lacking further studies are needed.

CONCLUSION

In conclusion, S100A9 expression plays an important role in the carcinogenesis and development of OSCC and the expression in tumor cells denotes to the histological differentiation.

ACKNOWLEDGMENTS

This paper has been extract from Mrs. Golshid Mehrabani's DDS thesis which was conducted under supervision of Dr. Azadeh Andisheh Tadbir and Dr. Mohammad Javad Ashraf. The study was approved, registered with ID Grant # 8591019 and supported by the Vice-Chancellery of International Branch of Shiraz University of Medical Sciences.

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