Original paper

Close relationship between TAZhigh/SOX2high co-localization and metastasis in oral squamous cell carcinoma

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There is growing evidence which indicates that the development and the biological features of cancer such as the invasion, metastases and recurrence are related to the presence and behavior of the cancer stem cells (CSC). However, the regulatory mechanisms underlying CSCs-specific properties are poorly determined, the Hippo pathway has emerged as a fundamental regulator underlying CSCs stemness.
Immunohistochemical method was used to examine the immunoexpression of SOX2, TAZ and α-SMA in oral squamous cells carcinomas: with metastases – OSCC M+ (n = 42), and without metastases – OSCC M– (n = 44), and 17 control cases.
The immunoexpression of SOX2, TAZ and α-SMA was significantly increased in both group of OSCC in comparison to control groups. Moreover, significantly increased TAZ and α-SMA immunoexpression were found in OSCC M+ compared to OSCC M-. In OSCC M+ and OSCC M- groups there were statistically significant correlations between the immunoexpression of TAZ vs SOX2 ($r = 0.56$, $p < 0.001$; $r = 0.33$, $p < 0.03$ respectively), and TAZ vs α-SMA ($r = 0.64$, $p < 0.001$; $r = 0.67$, $p < 0.001$ respectively). Moreover, there was statistically significant association between $TAZ^{high}/SOX2^{high}$ coexistent immunoexpression and the presence of metastases ($p < 0.007$).
Our results may suggest that SOX2 and TAZ could potentially cooperate and contribute to process of metastasis, especially in cases with $TAZ^{high}/SOX2^{high}$ expression.

Key words: TAZ, SOX2, oral cancer, metastases, CSC.

Introduction

Oral cancer is one of the most common cancers in the world with poor prognosis and without specific biomarkers for disease [1, 2]. The global incidence of oral squamous cell carcinoma (OSCC) is approximately 300,000 new cases per year [3]. The high mortality is closely associated with the presence of metastases and high rate of recurrence [1, 2, 3]. There is growing evidence which indicates that oncogenically transformed stem cells – cancer stem cells (CSCs) may be associated with the biological features

of oral cancer such as the rapid growth, invasion, metastases, recurrence and resistance to treatment [4, 5]. Cancer stem cells are subpopulations of cells within tumors with common characteristics as normal stem cells such as capabilities of self-renewal and multi-lineage differentiation. CSCs are responsible for tumor growth and their heterogeneity and can be induced from differentiated cancer cells in the adaptation processes and cross-talks with the tumor microenvironment as well as a cellular and molecular adaptation in response to therapy [6]. Previous reports have pointed SOX2 as a substantial marker and a key regulator of CSC in head and neck squamous cell carcinoma (HNSCC) [7, 8]. SOX2 is a member of the SRY-related HMG-box (SOX) family of transcription factors, having an important role in various phases of normal embryonic development, and affects cell fate and differentiation [9]. SOX2 is involved in tumorigenesis and cancer progression by reprogramming of adult stem cells into induced pluripotent stem cells and maintains stemness [10]. However, the regulatory mechanisms underlying CSCs-specific properties are poorly determined. The Hippo pathway has emerged as a fundamental regulator underlying CSCs stemness in HNSCC [11]. TAZ (transcriptional coactivator with PDZ-binding motif) is a key effector in Hippo pathway, which regulate tissue homeostasis, the balance between proliferation and apoptosis through interaction with various transcription factors [12]. Literature data documented that TAZ is involved in tumorigenesis by induction of proliferation, migration and increased metastatic potential [13]. TAZ is required for the expansion of CSCs in various solid tumors including oral cancer cells, probably by induction of the EMT and mesenchymal markers [11, 13].

Although the immunoexpression of SOX2 and TAZ in various types of tumors have been extensively characterized, the precise role of the immunoexpression of SOX2 and TAZ and their associations with metastases are not clear. Therefore, the aim of our study was to evaluate the immunoexpression of SOX2, TAZ and α-SMA in patients with oral squamous cell carcinomas with metastases (OSCC M+), and without metastases (OSCC M–). Another purpose was to find a possible association between the immunoexpression of SOX2 and TAZ and the presence of metastases and immunoexpression of mesenchymal marker – α-SMA (α-smooth muscle actin).

Material and methods

Patients

The present retrospective study and experimental methods were approved by the university review board, and have been performed in accordance with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects. The study was conducted under the assumption that research findings would be kept anonymous.

The 86 cases of OSCC (53 men, 33 women), and 19 controls (normal mucosa; 10 men, 9 women), were sourced from archival tissue blocks of the Department of Pathomorphology, Medical University of Lodz, Poland. All tissue sections taken from postoperative material were routinely fixed in formalin, processed, embedded in paraffin, and stained with haematoxylin and eosin. Histopathological diagnoses were established according to the current WHO standards [14]. The main criteria used for selection of cases were as follows: 1) an anatomical placement of lesions – the floor of the mouth; 2) primary surgical resection of OSCC lesions (without receiving prior immuno-, radio-, or chemotherapy). All cases of OSCC were divided into two groups: OSCC M– (without metastases, n = 44), and OSCC M+ (with metastases to regional lymph nodes or/and with distant metastases, n = 42). Cases of OSCC were graded according to the WHO classification (15) (for OSCC M–: G1 n = 7, G2 n = 37, G3 n = 0, and for OSCC M+: G1 n = 1, G2 n = 33, G3 n = 8). The age range for the OSCC M– group was from 37 to 82 years (the mean ±SD = 63.77 ±11.80), for the OSCC M+ group was from 46 to 88 (the mean ±SD = 68.21 ±10.62), and for controls from 15 to 74 (the mean ±SD = 47,05 ±18,79).

Immunohistochemistry

Immunohistochemical staining was carried out according to a standard method. 3-μm tissue sections were deparaffinized in xylene and rehydrated through a graded alcohol series. Heating in a microwave oven in a solution of target retrieval solution pH 9.0 (TRS High pH; Dako), for 30 minutes was used for antigen retrieval. Endogenous peroxidase activity was quenched with 0,3% hydrogen peroxide in methanol for 30 minutes. The sections were washed with TBS and incubated all night with polyclonal rabbit primary antibodies against: SOX2 (ThermoFisher Scientific, USA, dilution 1:300, Catalog number PA1-094), and TAZ (Abcam, UK, dilution 1:400, Catalog number ab84927). The sections for α-SMA staining were incubated 30 minutes with monoclonal mouse primary antibodies against actin (Dako; clone: 1A4, RTU) After washing, an adequate EnVision-HRP detection system (Dako, Carpinteria, CA, USA) was used. 3,3'-diaminobenzidine was used as the chromogen. After counterstaining with Mayer's hematoxylin, the slides were washed, dehydrated, cleared in xylene and coverslipped. The negative controls for immunohistochemical staining were prepared with

Table I. The immunoreactivity of TAZ, SOX2 and α-SMA in oral squamous cell carcinomas with metastases (OSCC M+), in oral squamous cell carcinomas without metastases (OSCC M–) and control subjects

Groups	TAZ immunoreactivity (mean score)	SOX2 immunoreactivity (mean score)	α-SMA (mean score)
OSCC M+ (n = 42)	5.07 ±1.50	3.85 ±2.10	3.05 ±2.38
OSCC M– (n = 44)	3.86 ±1.35	3.47 ±1.40	1.65 ±2.11
Control (n = 19)	1.36 ±1.11	0.84 ±1.01	0.31 ±0.47
OSCC M– vs. OSCC M+	$p < 0.001$	$p = 0.33$ ns	$p < 0.007$
OSCC M+ vs. control	$p < 0001.$	$p < 0.001$	$p < 0.001$
OSCC M– vs. control	$p < 0.001$	$p < 0.001$	$p < 0.009$

Data are presented as a mean ± SD.

NS - not significant

the primary antibodies replaced by the antibody diluent.

Double-staining immunohistochemistry

To evaluate the $TAZ^{high}/SOX2^{high}$ coexistent immunoexpression, we performed sequential chromogenic immunohistochemical double staining with two HRP substrates – DAB and Magenta (Dako Omnis; DM857), using EnVision FLEX System (Dako).

Evaluation of SOX2, TAZ and α-SMA immunoexpression

The sections were independently examined and scored by two pathologists, who were blinded to the clinical features. The immunoexpression of TAZ, SOX2 and α-SMA was scored by addition of the signal intensity (on a scale of 0-3: 0 – no staining; 1 – weak staining; 2 – moderate staining; 3 – high staining), and the percentage of positively stained tumor cells. The extent of positivity was scored as 0 when the percentage of positive cells was < 10%; 1 when it was 11-24%; 2 when it was 25-49%; 3 when it was 50-74%; and 4 when it was 75-100%. The mean grade was calculated by averaging grades assigned by the two pathologists and approximating the arithmetical mean to the nearest unity. If disagreement occurred (intensity score discrepancy > 1, or percentage >10%), the slides were re-evaluated together to obtain a consensus diagnosis. For χ^2 test (TAZ and SOX2), the immunoreactivity was categorized into three subgroup based on final scores: 0 – negative; 1-4 – low expression, 5-7 – high expression.

Statistical methods

Differences between groups were tested using unpaired Student's t-test preceeded by evaluation of normality and Levene's test. The Mann-Whitney U test was used where appropriate. Correlation coefficients were calculated using Spearman's method. The association between coexistent $TAZ^{high}/SOX2^{high}$ immunoexpression in OSCC M+ and OSCC M– group was estimated using χ^2 test. Results were considered statistically significant if $p < 0.05$.

Results

Our study revealed that SOX2 was expressed only in the cell nucleus. Immunoexpression of TAZ was observed in the cell nucleus and in part of cases in the cytoplasm as well, but only nuclear immunoexpression of TAZ was counted. In normal oral epithelium SOX2 and TAZ proteins were expressed in basal cell layer, and in OSCC cases predominantly on cancer cells. α-SMA was localized in the cytoplasm of basal cells of normal oral mucosa, vessels and cancer cells. The rates of SOX2 and TAZ positive immunoexpression in OSCC cases were 86.05% (74/86 cases), and 95.35 % (82/86 cases), respectively.

The semiquantitative data of the immunoexpression of SOX2, TAZ and α-SMA appear in Table I.

The nuclear immunoexpression of SOX2 in control group (Fig. 1A) was significantly lower in comparison to both group of OSCC. In OSCC M+ (Fig. 1B) SOX2 immunoexpression was slightly, but not significantly higher than in OSCC M– group (Fig. 1C).

The nuclear immunoexpression of TAZ was significantly increased in both group of OSCC in comparison to control group (Fig. 2A). Moreover, significantly increased TAZ immunoexpression was found in OSCC M+ (Fig. 2C) compared to OSCC M– (Fig. 2B).

The cytoplasmic immunoexpression of α-SMA was significantly increased in both group of OSCC in comparison to control group (Fig. 3A). We also found significantly higher immunoexpression of α-SMA in OSCC M+ (Fig. 3C) compared to OSCC M– (Fig. 3B).

In OSCC M+ and OSCC M- groups there were statistically significant correlations between the immunoexpression of TAZ and SOX2 ($r = 0.56$, $p < 0.001$; $r = 0.33$, $p < 0.03$ respectively). Moreover, in OSCC M+ and OSCC M– groups there were statistically significant correlations between the immunoexpression

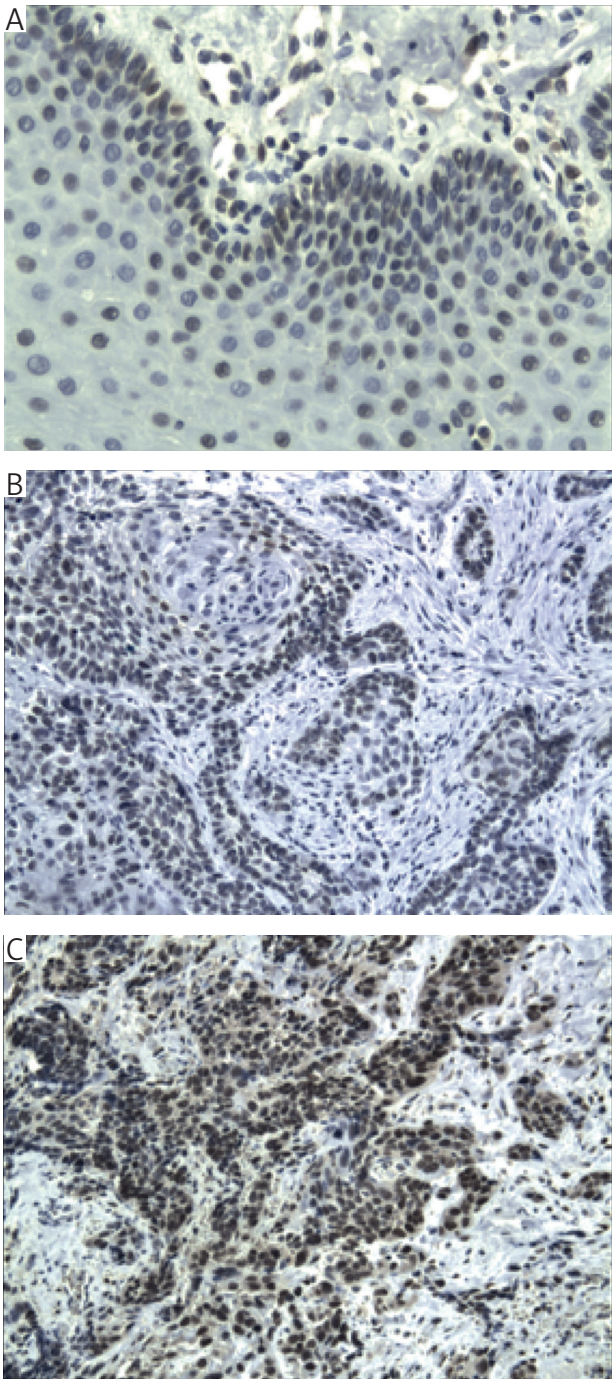

Fig. 1. Immunoexpression of SOX2 in: A) control; B) oral squamous cell carcinomas without metastases – OSCC M(–); C) oral squamous cell carcinomas with metastases – OSCC M(+). Immunohistochemistry. Total magnification 200× (control 400×)

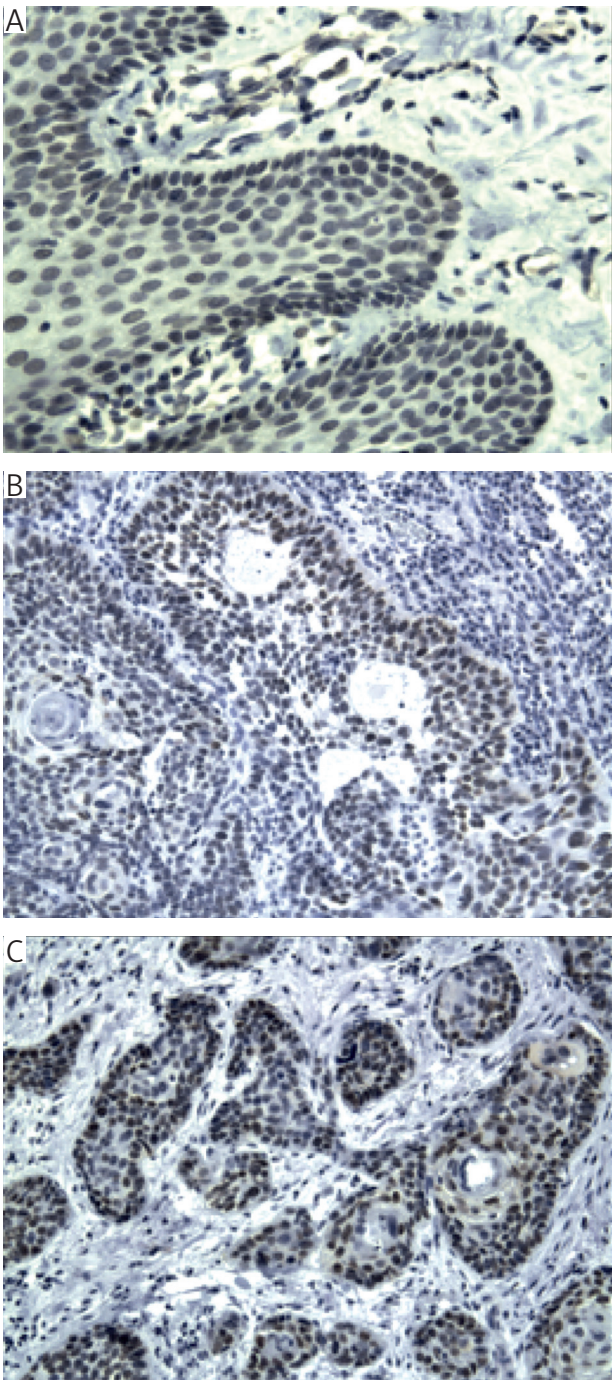

Fig. 2. Immunoexpression of TAZ in: A) control; B) oral squamous cell carcinomas without metastases – OSCC M–; C) oral squamous cell carcinomas with metastases – OSCC M+. Immunohistochemistry. Total magnification 200× (control 400×)

of TAZ and α-SMA ($r = 0.64$, $p < 0.001$; $r = 0.67$, $p < 0.001$ respectively) (Table II).

For further statistical analysis we selected cases only with a high immunoexpression of SOX2 and TAZ. The high immunoexpression of SOX2 was observed in 28 cases of OSCC (in 9 cases of OSCC M– and in 19 cases of OSCC M+). The high immunoexpression of TAZ was revealed in 44 cases of OSCC (in 14 cases of OSCC M- and in 30 cases of OSCC M+). The coexistent high immunoexpression of TAZ and SOX2 was observed in 19 cases of OSCC M+ and 8 cases of OSCC M– (Fig. 4 A-C). The association between coexistent $TAZ^{high}/SOX2^{high}$ immunoexpression in OSCC M+ and OSCC M– group was

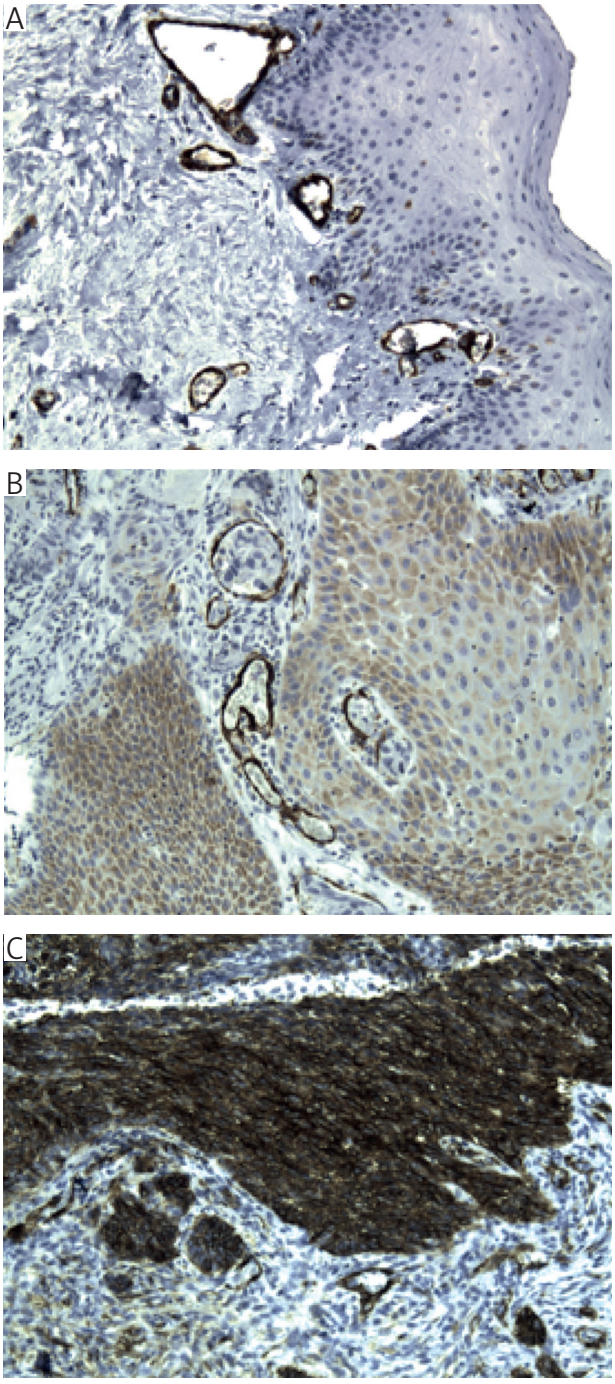

Fig. 3. Immunoexpression of α-SMA in: A) control; B) oral squamous cell carcinomas without metastases – OSCC M–; C) oral squamous cell carcinomas with metastases – OSCC M+. Immunohistochemistry. Total magnification 200× (control 400×)

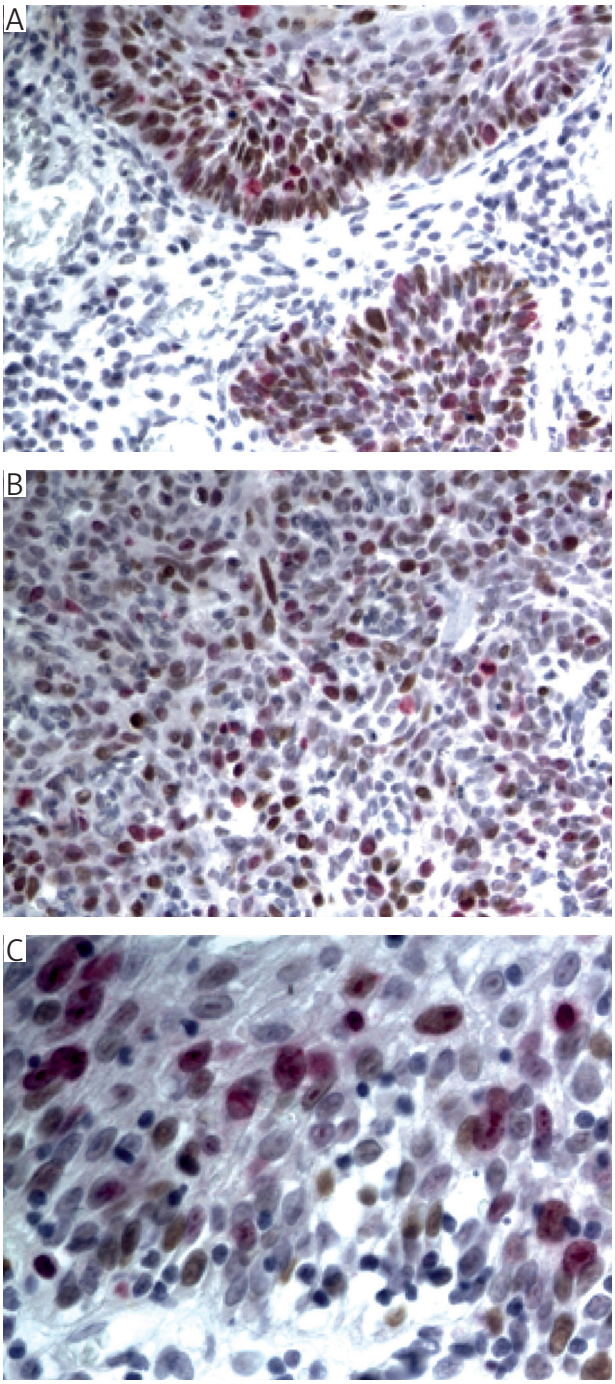

Fig. 4. Immunoexpression of TAZ and SOX2. Double-staining immunohistochemistry. Total magnification 200× (A, B), 400× (C). Nuclear co-localization of TAZ and SOX2 in cancer cells. The cancer cells with brown nuclei (DAB) are positive for TAZ, whereas cancer cells with red nuclei (Magenta) are positive for SOX2

estimated via Chi-square test. There was statistically significant association between $TAZ^{high}/SOX2^{high}$ co-existent immunoexpression and the presence of metastases ($p < 0.007$). In the control group all these correlations were weak and not significant (data not shown).

Discussion

Recent studies support the notion that metastasis is closely associated with the stem-like properties, suggesting crucial role CSCs in the metastasis process [4, 5, 6].

Table II. The correlations between the immunoexpression of TAZ, SOX2 and α-SMA in oral squamous cell carcinomas with metastasis OSCCM+, and oral squamous cell carcinomas without metastasis OSCCM–

Correlation between	OSCC M+ (n = 42)	OSCC M– (n = 44)
TAZ vs. SOX2	r = 0.56, p < 0.001	r = 0.33, p < 0.03
TAZ vs. α-SMA	r = 0.64, p < 0.001	r = 0.67, p < 0.001
SOX2 vs. α-SMA	r = –0.06, p = 0.7	r = –0.1, p = 0.93

Numerous studies documented that overexpression of SOX2 is associated with increased cancer aggressiveness, resistance to therapy and decreased survival rate in various cancer types [15, 16]. In our study the immunoexpression of SOX2 was significantly increased in OSCCs in comparison to control group. Low expression of SOX2 in normal mucosa seems to be in agreement with previous scanty findings. Qiao *et al*. also found significant higher SOX2 immunoexpression in oral cancer cells, compared with the normal oral mucosa [17]. Although our results showed lower immunoexpression of SOX2 in normal mucosa, other reports have not documented SOX2 immunoexpression in normal oral epithelium at all, and some reports concerning the other anatomical localization documented the opposite results. Yuan *et al.* revealed the higher expression of SOX2 in normal bronchial mucosa compared to bronchial dysplasia [18]. Li *et al*. demonstrated that expression of SOX2 in the normal gastric mucosa was higher than that in gastric cancer tissues [19].

Previous findings suggested that increased expression of SOX2 in cancer cells may be involved in lymph node metastasis. Neumann *et al*. demonstrated that higher expression of SOX2 was correlated with lymph node metastasis and distant metastasis in right-sided colon cancer [15]. Studies on oral cancer also demonstrated an association between SOX2 expression and lymph node metastasis [8, 20, 21]. However, there are few studies reporting conflicting results concerning immunoexpression of SOX2 and metastases. On the one hand, Ren *et al*. [20] showed correlation between increased immunoexpression of SOX2 and lymph node metastasis, and Michifuri *et al.* [21] indicated that SOX2 in OSCC has two staining patterns known as diffuse and peripheral, but only the diffuse pattern was significantly correlated with lymph node metastases. On the other hand, Fu *et al.* demonstrated association between increased SOX2 immunoexpression and the absence of lymph node metastases [8]. Similar results were showed by Züllig *et al.* who also documented a significant correlation between increased expression of SOX2 and the absence of lymph node metastasis, suggesting additionally that the expression of SOX2 can be an indicator of the absence of regional lymph nodes metastasis in oral cancer [22]. Moreover, it was demonstrated that no significant association existed between SOX2 expression and lymph node metastasis. Baghai Naini *et al.* documented similar expression of SOX2 protein and mRNA level in patients with and without lymph node metastasis [23]. In our study, immunoexpression of SOX2 was slightly, but not significantly higher in group of OSCC with metastasis in comparison to OSCC without metastasis, suggesting the lack of association between immunoexpression of SOX2 and metastases. Differences concerning the association between SOX2 and metastasis in various studies indicate that the role of SOX2 in process of metastasis is still not fully determined. Previous findings support hypothesis that SOX2 may not be sufficient for the induction of metastasis or may require another cooperative factors in this process. On the other hand, these discrepancies might be due to tumor heterogeneity, methods of patients stratification and methods of immunohistochemical evaluation.

TAZ is a transcriptional coactivator and major effector of an evolutionarily and functionally conserved Hippo pathway that controls cell proliferation and apoptosis [24]. Recent findings showed that TAZ is an essential modulator for CSCs self-renewal and maintenance, associated with metastatic dissemination and recurrence of cancer [11]. Overexpression of TAZ was associated with aggressive features and poor prognosis in a various types of human cancer [25, 26]. Recent data revealed that TAZ is aberrantly overexpressed in human oral cancer as well [11]. In concordance with previous studies, we also indicated that TAZ was poorly expressed in normal oral epithelium but was increased in OSCCs. Moreover, we indicated that TAZ immunoexpression was significantly higher in OSCC with metastasis than in group of OSCC without metastasis and was correlated with immunoexpression of α-SMA. Previous studies have shown that TAZ can significantly modify the activity of epithelial to mesenchymal transition (EMT), a major cellular process responsible for metastatic spreading [13, 26, 27]. Bartucci *et al.* revealed that TAZ overexpression in patient-derived breast cancer stem cell induced cell transformation and migratory function [28]. In experimental study, Lei *et al*. revealed that TAZ promotes epithelial-mesenchymal transition by inducing EMT-associated factors such as Snail and FoxC2 [29]. Li *et al.* documented that Slug, Twist and Snail – main transcription factors of EMT, were

significantly upregulated upon TAZ overexpression, suggesting that TAZ might be capable of promoting EMT in oral cancer cells [11]. In this context, higher TAZ immunoexpression in group of OSCC with metastasis and their association with α-SMA – marker of mesenchymal transition, seems to be coherent and may suggest that TAZ is important factor that participates in the spreading of cancer cells.

Li *et al*. revealed a previously unknown relationship between TAZ and SOX2 expression, suggesting that TAZ enhances CSCs self-renewal and maintenance by direct transcriptional activation of SOX2 in HNSCC [11]. Li *et al.* showed that TAZ knockdown significantly reduced expression of SOX2, whereas its ectopic overexpression markedly increased SOX2 abundance in HNSCC cells. Authors observed positive correlations between TAZ and SOX2 expression at both mRNA and protein levels, and significant associations between TAZ/SOX2 abundance and nodal metastasis.

Presence of the statistically significant correlation between the immunoexpression of SOX2 and TAZ in OSCC M+, the co-localization and statistical association between $TAZ^{high}/SOX2^{high}$ coexistent immunoexpression seems be in agreement with previous findings and point out involvement of these proteins in process of metastasis.

Interestingly, significant differences immunoexpression of SOX2 and TAZ were observed in individual cases of OSCCs. In both group of OSCC, there were cases negative for SOX2 and TAZ immunoexpresion or one of this proteins. Moreover, in group of OSCC without metastasis we also observed cases with the highest intensity and the percentage of positively stained cancer cells. Although, in our study SOX2 and TAZ were expressed uniformly within tumor, in cases with $TAZ^{high}/SOX2^{high}$, both proteins were distributed in the same part of tumors. We assume that an correlations between immunoexpression of TAZ and SOX2 as well as similar tissue localization may indicate to close relationship between studied proteins.

The present study revealed overexpression of SOX2 and TAZ in OSCCs. Our results may suggest that SOX2 and TAZ could potentially cooperate and contribute to process of metastasis, especially in cases with $SOX2^{high}/TAZ^{high}$ expression. Further studies concerning the immunoexpression of SOX2 and TAZ are needed to better understand their presumptive role in oral cancer metastases.

The authors declare no conflict of interest.

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References

1. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015; 136: E359-E386.
2. Schmidt Jensen J, Jakobsen KK, Mirian C, et al. The Copenhagen Oral Cavity Squamous Cell Carcinoma database: protocol and report on establishing a comprehensive oral cavity cancer database. Clin Epidemiol 2019; 19: 733-741.
3. Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. Oral Oncol 2009; 45: 309-316.
4. Rodini CO, Lopes NM, Lara VS, Mackenzie IC. Oral cancer stem cells – properties and consequences. J Appl Oral Sci 2017; 25: 708-715.
5. Kaveh K, Nathan L, Reigh-Yi L. The role of cancer stem cells in head and neck squamous cell carcinoma and its clinical implications. In: Dmitry Bulgin E (ed.). New Aspects in Molecular and Cellular Mechanisms of Human Carcinogenesis. Saint Louis, MO, InTech 2016; 97-113.
6. Ayob AZ, Ramasamy TS. Cancer stem cells as key drivers of tumour progression. J Biomed Sci 2018; 25: 20.
7. Chou MY, Hu FW, Yu CH, Yu CC. Sox2 expression involvement in the oncogenicity and radiochemoresistance of oral cancer stem cells. Oral Oncol 2015; 51: 31-39.
8. Fu TY, Hsieh IC, Cheng JT, et al. Association of OCT4, SOX2, and NANOG expression with oral squamous cell carcinoma progression. J Oral Pathol Med 2016; 45: 89-95.
9. Kamachi Y, Uchikawa M, Kondoh H. Pairing SOX off: with partners in the regulation of embryonic development. Trends Genet 2008; 16: 182-187.
10. Kim J, Chu J, Shen X, Wang J, Orkin S. An extended transcriptional network for pluripotency of embryonic stem cells. Cell 2008; 132: 1049-1110.
11. Li Z, Wang Y, Zhu Y, et al. The Hippo transducer TAZ promotes epithelial to mesenchymal transition and cancer stem cell maintenance in oral cancer. Mol Oncol 2015; 9: 1091-1105.
12. Santucci M, Vignudelli T, Ferrari S, et al. The Hippo pathway and YAP/TAZ-TEAD protein-protein interaction as targets for regenerative medicine and cancer treatment. J Med Chem 2015; 58: 4857-4873.
13. Zhang H, Liu CY, Zha ZY, et al. TEAD transcription factors mediate the function of TAZ in cell growth and epithelial-mesenchymal transition. J Biol Chem 2009; 284: 13355-13362.
14. Barnes L, Everson JW, Reichart P, et al. World Health Organization Classification of Tumours. Pathology and Genetics Head and Neck Tumours. IARC Press Lyon 2005; 168-176.
15. Neumann J, Bahr F, Horst D, et al. SOX2 expression correlates with lymph-node metastases and distant spread in right-sided colon cancer. BMC Cancer 2011; 11: 518.
16. Yang F, Gao Y, Geng J, et al. Elevated expression of SOX2 and FGFR1 in correlation with poor prognosis in patients with small cell lung cancer. Int J Clin Exp Pathol 2013; 6: 2846-2854.
17. Qiao B, He B, Cai J, Yang W. The expression profile of Oct4 and Sox2 in the carcinogenesis of oral mucosa. Int J Clin Exp Pathol 2013; 7: 28-37.
18. Yuan P, Kadara H, Behrens C, et al. Sex determining region Y-Box 2 (SOX2) is a potential cell-lineage gene highly expressed in the pathogenesis of squamous cell carcinomas of the lung. PLoS One 2010; 9; 5: 9112.
19. Li XL, Eishi Y, Bai YQ, et al. Expression of the SRY-related HMG box protein SOX2 in human gastric carcinoma. Int J Oncol 2014; 24: 257-263.
20. Ren ZH, Zhang CP, Ji T. Expression of SOX2 in oral squamous cell carcinoma and the association with lymph node metastasis. Oncol Lett 2016; 11: 1973-1979.
21. Michifuri Y, Hirohashi Y, Torigoe T, et al. High expression of ALDH1 and SOX2 diffuse staining pattern of oral squamous cell carcinomas correlates to lymph node metastasis. Pathol Int 2012; 62: 684-689.
22. Züllig L, Roessle M, Weber C, et al. High sex determining region Y-box 2 expression is a negative predictor of occult lymph

node metastasis in early squamous cell carcinomas of the oral cavity. Eur J Cancer 2013; 49: 1915-1922.
23. Baghai Naini F, Aminishakib P, Abdollahi A, et al. Relative Expression of OCT4, SOX2 and NANOG in Oral Squamous Cell Carcinoma Versus Adjacent Non-Tumor Tissue. Asian Pac J Cancer 2019; 20: 1649-1654.
24. Zhao B, Tumaneng K, Guan KL. The Hippo pathway in organ size control, tissue regeneration and stem cell self-renewal. Nat Cell Biol 2011; 13: 877-883.
25. Harvey KF, Zhang X, Thomas DM. The Hippo pathway and human cancer. Nat Rev Cancer 2013; 13: 246-257.
26. Khosravi A, Jafari SM, Asadi J. Knockdown of TAZ decrease the cancer stem properties of ESCC cell line YM-1 by modulation of Nanog, OCT-4 and SOX2. Gene 2020; 145207.
27. Zhang H, Liu CY, Zha ZY, et al. TEAD transcription factors mediate the function of TAZ in cell growth and epithelial-mesenchymal transition. J Biol Chem 2009; 284: 13355-13362.
28. Bartucci M, Dattilo R, Moriconi C, et al. TAZ is required for metastatic activity and chemoresistance of breast cancer stem cells. Oncogene 2015; 34: 681-690.
29. Lei QY, Zhang H, Zhao B, et al. TAZ promotes cell proliferation and epithelial-mesenchymal transition and is inhibited by the hippo pathway. Mol Cell Biol 2008; 28: 2426-2436.

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