EGYPTIAN DENTAL JOURNAL
www.eda-egypt.org
VOL. 69, 1049:1058, APRIL, 2023
PRINT ISSN 0070-9484 • ONLINE ISSN 2090-2360
ORAL MEDICINE, X-RAY, ORAL BIOLOGY AND ORAL PATHOLOGY
Submit Date : 16-12-2022 • Accept Date : 14-01-2023 • Available online: 01-04-2023 • DOI : 10.21608/edj.2023.181290.2380

SMARCB1 AS A POTENTIAL INDICATOR OF THE PROGNOSIS OF ORAL SQUAMOUS CELL CARCINOMA

Rehab Fouad Fathi* *and* Ismail Mohamed Shebl**

ABSTRACT

Background: Being the most common intraoral malignancy, studying of possible prognostic markers for squamous cell carcinoma (SCC) is of great benefit that can alter treatment and follow-up protocols proposed for different grades of the lesion. As a newly identified tumor suppressor gene, SMARCB1 protein level in different histological grades of SCC can be used as a predictor of prognosis. Similarly, levels of CD3 positive tumor infiltrating lymphocytes (TILs) in SCC can be used to distinguish tumor grades.

Aim: To assess the levels of SMARCB1 gene expression by immunohistochemical analysis of the gene protein and to evaluate the level of CD3 in different grades of SCC, and to correlate between their expression and tumor prognosis based on the histological grade.

Material & methods: Thirty three formalin fixed paraffin-embedded archival blocks of oral squamous cell carcinoma (OSCC) were used in this study; eleven cases from each group were tested (group 1 well-differentiated, group 2 moderately-differentiated and group 3 poorly-differentiated). Immunohistochemical study was performed on the specimens to evaluate the expression of the protein of SMARCB1 gene and CD3 positive tumor infiltrating lymphocytes (TILs). The area fraction of both genes' expression was calculated. The data was analyzed and expressed statistically.

Results: Group1 showed high CD3 gene expression and low SMARCB1 gene expression. Group2 to showed almost similar gene expression results as group 1. Group3 showed marked decrease in CD3 gene expression and high SMARCB1 gene expression.

Conclusion: Our findings revealed that high expression values of the protein of SMARCB1 gene may be used as an evidence for poor prognosis while presence of high numbers of CD3+ T cells is associated with good prognosis and increased survival in oral squamous cell carcinoma.

KEYWORDS: SCC, SMARCB1, CD3, TILs, OSCC.

* Lecturer of oral pathology, Oral pathology department, Faculty of Dentistry, Ain Shams University
** Lecturer of oral pathology, Histopathology Department, Faculty of oral and dental medicine, Misr International University.

INTRODUCTION

Oral cancer is considered the eighth most common cancer globally. Unfortunately, it is known for its poor prognosis, which is attributed to the late detection and possible metastasis at the time of diagnosis [1]. The most common oral malignancy, accounting for 90-95% of oral cancer incidents is oral squamous cell carcinoma (OSCC). The overall prognosis of OSCC has not been improved in the last three decades, showing high rates of morbidity and mortality, despite the recent improvements in the field of cancer management. In addition to the unreported cases, especially in under-developed countries, still the incidence of occurrence of this malignancy is expected to rise around 40% by the year 2024, according to Global Cancer Observatory[2,3]. These previous findings underline the need for better understanding of OSCC in terms of pathogenesis, and on the molecular level, in an attempt to increase patients' survival and decrease associated morbidity.

Starting with the presence of inflammatory cells, which is crucial in the microenvironment of different types of cancer. They can work in both directions, whether promoting cancer formation, or in the body's defense against cancer development. The cancer promoting mechanism can be explained by the ability of inflammatory cells to promote replication and endurance of cancer cells. They also have a role in the process of invasion and metastasis of malignant neoplasms (1, 4). The levels of inflammatory cells in certain malignancies can be considered as a forecaster of prognosis for such lesions. For example, in head and neck squamous cell carcinoma (HNSCC), elevated levels of tumor associated macrophages (TAM) indicated poor prognosis. On the other hand, the higher the number of tumor infiltrating lymphocytes (TIL), the better is the prognosis of HNSCC [4, 5].

In 2017, a systematic review by Ruiter et al.[6] evaluated the prognosis of HNSCC through assessing TILs. Meta-analysis was performed to calculate the association between TILs and different proteins, among which is CD3. This review showed that some studies reported a better survival rate with cases showing higher expression of CD3+ TILs. On the other hand, other studies showed no significant relation between high expression of CD3 positive cells and overall prognosis.

Studies of the genetic makeup of different cancers have explored an unusually elevated frequentness of genetic mutations specifically in genes encoding certain subunits of the SWI/SNF (switch/sucrose non-fermentable) chromatin-remodeling complexes, with approximately 25% of all cancers harboring abnormalities in one or more of these genes [7]. The SWI/SNF is a complex that shares in chromatin remodeling, playing a role in controlling DNA availability for different cellular processes, such as DNA transcription and repair. Moreover, it is involved in the regulation of different pathways including the sucrose fermentation pathway [8]. Such roles take place by the actions of different protein subunits of this complex, including SMARCB1 (SWI/SNF-related matrix-associated actin dependent regulator of chromatin subfamily B member 1). SMARCB1 is a core subunit of the previously mentioned complex, also known as integrase interactor 1 (INI-1), which takes part in regulating different cellular processes. This takes place by their development at sites of promoters and enhancers of functioning genes, which possess important roles of tumor suppression and accordingly considered as tumor suppressor genes [9]. SMARCB1 is present in both normal and neoplastic cells, exerting its antitumor action as a member of a chromatin remodeling complex called (SWI/SNF) [10].

Studies made on mice showed that abnormalities in the expression of SMARCB1/INI1 were associated with early lethality during the embryonic life. Mice lacking SMARCB1/INI1 go dead between 3.5 and 5.5 days of their embryonic life[11]. SMARCB1/INI1

heterozygous-deficient mice and those with reduced expression of SMARCB1/INI1 developed, within the range of 11 weeks of embryonic life, different types of aggressive malignancies such as rhabdoid-like tumors and T-cell lymphomas[12,13]. When evaluated in relation to other tumor suppressor genes, this deadliness is considered to be more rapid. For instance, it takes around 20 weeks for cancer development in case of inactivation of P53, 38 and 60 weeks in case of p19Arf and p16Ink4a loss respectively. The rapid onset of cancer following inactivation of SMARCB1/ INI1 proves with no doubt its role as a tumor suppressor gene [14].

SMARCB1 also shows an adverse impact on the expression of another tumor suppressor gene, which is the cyclin-dependent kinase inhibitor p16 (INK4A). It also represses retinoblastoma (RB) target genes. Abnormalities in the expression of SMARCB1 gene was found in different tumors such as malignant rhabdoid tumor, medullary carcinoma and other malignancies [9, 10, 15-17].

Relating this tumor suppressor gene to the prognosis of different histological grades oral squamous cell carcinoma is not widely examined. In the light of this, we studied the expression of SMARCB1 in different histological grades of OSCC, which can be reflected on the clinical outcome of the neoplasm. In addition, studying the intensity of CD3+ TILs was performed and assessing if there is a relation with the expression of SMARCB1, to help understand the link between inflammation and the tumor suppressor gene introduced.

MATERIAL AND METHODS

Case selection

Archival blocks were acquired from the oral pathology departments of both, the faculty of Dentistry of Ain Shams University, and Misr International University, Egypt. The total number was thirty three blocks, all were formalin fixed and embedded in paraffin. The histopathological diagnosis of each case was included in the data of each paraffin block. Each case was then coded and patients' names were not revealed for ethical purpose. Eleven cases were identified as well-differentiated OSCC, eleven cases were diagnosed as moderately-differentiated OSCC and eleven cases were diagnosed as poorly-differentiated OSCC. To confirm the diagnosis, 5μm thick sections were sliced and mounted on glass slides. Staining with hematoxylin and eosin was then performed and slides were examined by light microscope.

Immunohistochemical procedures

From all specimens, four μm sections were cut then mounted on positively charged glass slides. Xylene was used to deparaffinize the sections, which were then rehydrated in graded ethyl alcohol. Before staining, sections were inserted in citrate buffer solution of pH 4.8 then placed in the microwave oven. The universal kit (Lab Vision) was used for immunostaining. Peroxidase-anti-peroxidase method of immunostaining using the streptavidin biotin system was carried out. To block the endogenous peroxidase activity, 3% hydrogen peroxide was added to the sections. Immune-staining of the sections was then made using the concentrated primary monoclonal antibody against CD3 and SMARCB1, and then incubated overnight at room temperature after rinsing with PBS (phosphate buffered saline) solution. Link antibody was then used to cover the sections, afterward the streptavidin biotin labeling antibody; after rinsing with PBS; DAB chromogen was applied to the sections followed by counter stain then dehydration was performed in graded alcohol, followed by the use of xylene for the sections to be cleared. Finally, the sections were mounted. Immunohistochemical staining was carried out in Ain Shams University Specialized Hospital.

Assessment of immunohistochemical procedures

Six microscopic fields viewing highest immunopositive areas were chosen from each positive section. Each field was photomicrographed at a magnification of 40X using a digital camera (LEICA DFC295, Wetzlar, Germany) which was mounted on a light microscope (LEICA DM LS2, Wetzlar, Germany). Image analysis was then performed after they were transferred to the computer. The manual count of immunopositive cells was recorded. Imaging and image analysis were made in the research unit, Oral Pathology Department, Faculty of Dentistry, Ain Shams University, Egypt.

Statistical analysis

The area fraction expressed by CD3 and SMARCB1 was calculated. The data were expressed as mean ± standard deviation (S.D.) Data were coded and entered using the statistical package for the Social Sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA). Comparisons between quantitative variables were done using the Post hoc multiple-comparison test (Tukey Test) tests (18). Quantitative variables were correlated using Spearman correlation coefficient (19). P-values equal or less than 0.05 were considered as statistically significant.

RESULTS

The immunoexpression of CD3 in all groups is shown in figure 1. **Group 1** showed strong positive immunoexpression in the mesenchymal cells, mainly lymphocytes with no expression in the malignant epithelial cells (fig.1A). **Group 2** presented moderately positive nuclear and cytoplasmic expression in the mesenchymal cells, mainly lymphocytes with still no immunoexpression in the malignant epithelial cells (fig.1B). However, **Group 3** showed mild positive immunoexpression of CD3 in the mesenchymal cells, also with no expression in the malignant epithelial cells (fig.1C).

The immunoexpression of the protein of SMARCB1 gene in all groups was shown in (fig.2). **Group 1** showed weak positive expression in few mesenchymal cells, mainly lymphocytes with minimal expression in the malignant epithelial cells (fig.2A). **Group 2** presented moderate expression in the nuclei of few mesenchymal cells, mainly lymphocytes and a stronger reaction in those of the malignant epithelial cells with a faint cytoplasmic reaction (fig.2B). However, **Group 3** showed strong positive immunoexpression of the protein of SMARCB1 gene in the mesenchymal cells, specifically lymphocytes, as well as a clear nuclear and faint cytoplasmic reaction of the invading malignant epithelial cells (fig.2C).

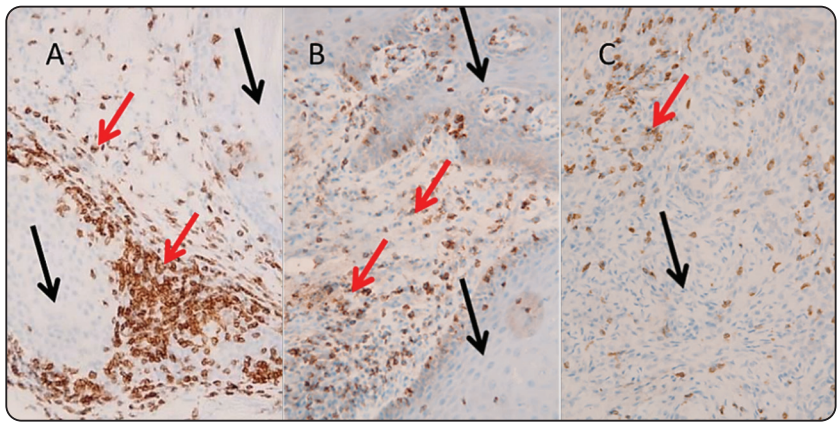

Fig. (1) Photomicrograph showing the immunoexpression pattern of CD3 ***a-Group 1***(well differentiated OSCC) with strong positive gene expression in mesenchymal cells (red arrows) and negative epithelial cells (black arrows); ***b- Group 2*** (moderately differentiated OSCC) with moderate positive gene expression in mesenchymal cells and negative epithelial cells; ***c- Group 3***(poorly differentiated OSCC) with mild positive gene expression in mesenchymal cells and negative epithelial cells. ***(org. mag. X400)***

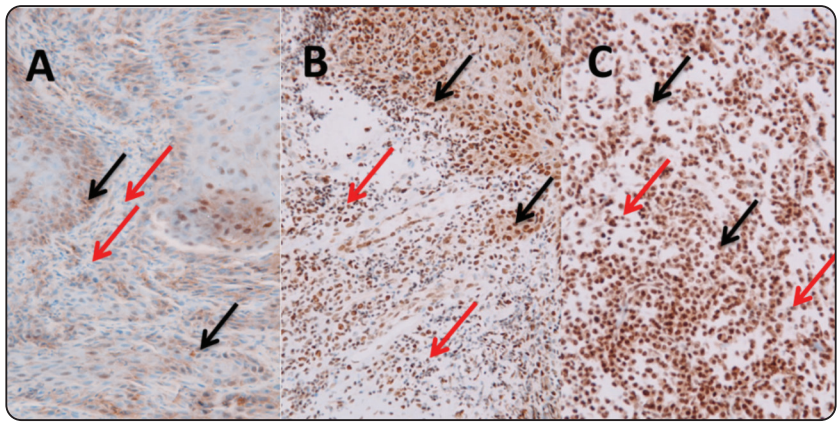

Fig. (2) Photomicrograph showing the immunoexpression pattern of the protein of SMARCB1gene ***a-Group 1***(well differentiated OSCC) with weak positive gene expression in mesenchymal cells (red arrows) and a positive nuclear reaction in few malignant epithelial cells (black arrows); ***b- Group 2*** (moderately differentiated OSCC) with positive expression in mesenchymal cells and malignant epithelial cells; ***c- Group 3***(poorly differentiated OSCC) with strong positive gene expression in mesenchymal cells as well as malignant epithelial cells . ***(org. mag. X400)***

Statistical results

Statistical findings indicated a significantly reduced expression of CD3 in group 3 than in group 1 and 2. By calculating the area fraction, the least expression was measured in group 3 followed by group 2. The highest expression was measured in group 1. The mean and standard deviation of all three groups are presented in table 1 showing a statistically significant difference on comparing the mean values for CD3 gene expression in the three groups. Boxplot for the three groups comparing the mean values of CD3 gene expression is shown in figure 3.

Post hoc multiple-comparison test (Tukey Test) results for comparison of mean values of CD3 gene expression for all studied groups are shown in table 2. This comparison revealed a statistically significant difference on comparing the mean values of CD3 gene expression between group 3 and both groups 1 and 2 as shown in table 2.

TABLE (1) The descriptive statistics of the mean values of CD3 gene expression of all studied groups.

		Group 1 : well differentiated SCC	Group 2: moderately differentiated SCC	Group 3: poorly differentiated SCC	P value
CD3	Median	3.24	3.17	0.99	0.003
	1st quartile	2.62	2.57	0.51	
	3rd quartile	3.56	3.69	1.44	

P value ≤ 0.05 is considered statistically significant.

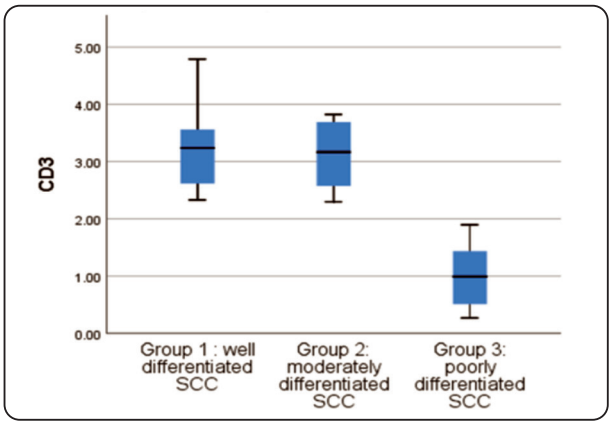

Fig. (3) Box plot with whiskers showing mean values of CD3 gene expression of all studied groups.

TABLE (2) Post hoc multiple-comparison test (Tukey Test) for comparison of the mean values of CD3 gene expression of all studied groups.

	P value
Group 3: poorly differentiated SCC VS Group 2: moderately differentiated SCC	0.015
Group 3: poorly differentiated SCC VS Group 1 : well differentiated SCC	0.007
Group 2: moderately differentiated SCC VS Group 1 : well differentiated SCC	1.000

**** The mean difference is significant at the 0.05 level.***

Statistical findings indicated a significantly increased expression of SMARCB1/INI1 in group 3 than in group 1 and 2. By calculating the area fraction, the least expression was measured in group 1 followed by group 2. The highest expression was measured in group 3. The mean and standard deviation of all three groups are presented in table 3 showing a statistically significant difference on comparing the mean values for SMARCB1/INI1 gene expression in the three groups. Boxplot for the three groups comparing the mean values of SMARCB1/INI1 gene expression is shown in figure 4.

Post hoc multiple-comparison test (Tukey Test) results for comparison of mean values of SMARCB1/INI1 gene expression for all studied groups are shown in table 4. This comparison revealed a statistically significant difference on comparing the mean values of SMARCB1/INI1 gene expression between group 3 and 1 as shown in table 4.

TABLE (3) The descriptive statistics of the mean values of SMARCB1/INI1 gene expression of all studied groups.

		Group 1 : well differentiated SCC	Group 2: moderately differentiated SCC	Group 3: poorly differentiated SCC	P value
INI-1	Median	0.26	1.38	3.37	0.001
	1st quartile	0.20	1.31	2.25	
	3rd quartile	0.34	1.93	4.17	

P value ≤ 0.05 is considered statistically significant.

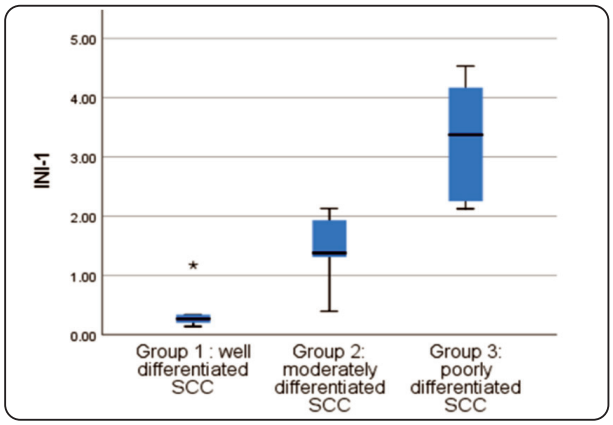

Fig. (4) Box plot with whiskers showing mean values of SMARCB1/INI1 gene expression of all studied groups.

TABLE (4) Post hoc multiple-comparison test (Tukey Test) for comparison of the mean values of SMARCB1/INI1 gene expression of all studied groups.

	P value
Group 1 : well differentiated SCC VS Group 2: moderately differentiated SCC	0.175
Group 1 : well differentiated SCC VS Group 3: poorly differentiated SCC	<0.001
Group 2: moderately differentiated SCC VS Group 3: poorly differentiated SCC	0.175

**** The mean difference is significant at the 0.05 level.***

DISCUSSION

In our present work, we studied the expression of SMARCB1/INI1 gene in different grades of previously diagnosed OSCC by evaluating the immunohistochemical expression of the gene protein. In addition, we investigated the intensity of CD3+ TILs to assess the relation between the expression of SMARCB1 and the degree of inflammation, aiming to understand the link between the tumor suppressor gene, the grade of the neoplasm and the immune response.

The tumor immune microenvironment (TIME) plays a serious part in the identification and removal of tumor cells, in addition to the creation of an unfavorable immunosuppressive microenvironment[20,21]. Immune surveillance is an essential process that counteracts carcinogenesis and retains homeostasis [22, 23]. Increasing evidence indicates that the composition of immune cell infiltrates may be a possible prognostic marker in OSCC [24-26].

INI1 (integrase interactor 1), which is another name for SMARCB1, is one of the core subunit proteins in the SWI/SNF. This complex is considered an ATP-dependent chromatin remodeling complex, encoded at chromosomal position 22q11.2 [27]. Chromatin remodeling elements are a group of proteins that redesign the way DNA architecture is bundled for the sake of facilitating the access to the condensed genomic DNA by the transcription machinery; therefore regulating gene expression. Chromatin's main purpose is thus to collect long DNA molecules into more compact, yet complicated structures [28]. In addition, nucleosomes play a main role in controlling gene expression as they prevent the binding of transcription factors, which are the key proteins in control of activating or inactivating the expression of specific genes. Thus, mutations in the chromatin remodeling complexes, as SWI/SNF genes, lead to failure in DNA repair mechanisms, to eventually lead to cancer occurrence [7].

SWI/SNF complexes were first recognized in oncogenesis after discovering that SMARCB1 is inactivated by a series of mutations in nearly all cases of rhabdoid tumor, typically developing in children <3 years of age, with a noticeably poor prognosis [29]. Previous studies have also suggested that SMARCB1/INI1 may have the ability to suppress tumor progression by activating the p16INK4a and retinoblastoma tumor suppressors to down regulate cell cycle progression from G0/G1 to the S-phase [30]. It was later proved that SMARCB1/INI1 signals via the p16INK4a-Rb-E2F pathway

regulate chromosomal stability, implying a new role in tumor suppression for this chromatin-remodeling protein [31].

Our study investigated the level of gene expression of CD3 and SMARCB1/INI1 in thirty three cases of OSCC. The three degrees of differentiation were tested to determine the relation between the expression of the genes and the prognosis of the malignancy.

Our results showed a decrease in the level of CD3 gene expression in the poorly differentiated group, with a mild increase in the gene expression in moderately differentiated group. Yet, a strong positive immune reaction was clear in the mesenchymal cells of the well differentiated group. As expected, malignant epithelial cells showed no expression for CD3 gene. These findings are in accordance with previous studies, for example Raísa Sales de Sá et al. (2021) who studied tongue squamous cell carcinoma, proved that elevated expression of CD3+ T cells are predictive of improved overall survival and indicative of immunologically active inflammatory mature cells with better prognosis [32].

Surprisingly, our results showed a marked increase in the level of SMARCB1/INI1 in poorly differentiated OSCC compared to well and moderately differentiated groups. These results are in accordance with previous studies that proved atypical expression of the SMARCB1/INI1 protein in a variety of tumors [33]. Yet, several studies on different types of sarcomas raised contradicting results. Forrest S. et al. (2020) concluded that various destructive pediatric cancers carry alterations in SMARCB1, including epithelioid sarcoma, chordoma and rhabdoid tumors. They proved that SMARCB1 deletions or aberrant mutations are associated with loss of INI1 protein expression and could be utilized as a prognostic marker [34]. Agaimy A. et al. (2017) analyzed 39 SMARCB1-deficient sinonasal carcinomas collected from multiple medical centers. They confirmed immunohistochemical loss of SMARCB1 (INI1) expression for all 39 tumors. Their results presented complete loss of nuclear SMARCB1 (INI1) expression in all tumors; yet, strong reactivity was recorded in the background inflammatory, stromal and/or epithelial cells [35].

Previous investigations studied the mutations that may occur in SMARCB1 in different neoplasms, using different techniques, like combinations of fluorescence in situ hybridization, array comparative genomic hybridization and quantitative polymerase chain reaction [36]. These techniques proved that mutations and changes of SWI/SNF expression are highly non-specific and do not necessarily confirm a poor prognosis [7]. Furthermore, several studies on genome sequencing declared that SMARCB1 was not without help (37, 38); it is currently evident that at least nine genes encoding subunits of the SWI/SNF complexes are frequently mutated in cancer across a wide variety of tumor types [39, 40]. This raises the curiosity about the exact mechanism by which mutations in different genes, including the SWI/SNF subunits, can promote development of cancer. Much of this data has recently developed yet clinical linkages between chromatin remodeling genes and immunotherapy remain debatable.

To our concern, the gene was expressed in the nuclei of the inflammatory cells in the three grades of OSCC. Apparently, the main difference among the three grades was the intensity of the immunoexpression in the malignant epithelial cells. The intensity of the immunoexpression was obviously more in the poorly differentiated group. Yet, the reaction was not only nuclear but a rather faint stain of the cytoplasm was also obvious in all the moderately and poorly differentiated cases of this study. Clearly, the role of SWI/SNF genes in malignancy is not yet completely understood and further studies are mandatory to uncover their linkage to different types of malignancies and whether they can be useful as immunotherapeutic markers.

RECOMMENDATIONS

We recommend further studies on SMARCB1 genes using more advanced screening assays. Alterations in other components of the SWI/SNF complex should be highlighted and the dysregulation that occurs in different neoplasms of different biological behaviours require combined studying techniques to determine whether the loss or alteration in the components of this complex could be used as a prognostic aid.

CONCLUSIONS

Our findings revealed that high expression values of SMARCB1 may be used as an evidence for poor prognosis while presence of high numbers of CD3+ T cells is associated with good prognosis and increased survival in oral squamous cell carcinoma. Still not much is known about the exact alterations that can occur in SWI/SNF complex group of genes and their role in tumor development when compared to other genes that have been studied for decades. Accordingly, recent research studies should focus on realizing the prognostic and the likely therapeutic suggestion of mutations in genes encoding SWI/SNF subunits.

REFERENCES

1. Goertzen C., Mahdi H., Laliberte C., Meirson T., Eymael D., Gil-Henn H. and . Magalhaes M.: Oral inflammation promotes oral squamous cell carcinoma invasion. Oncotarget. 2018; 9(49): 29047-29063.
2. Elaiwy O, El Ansari W, AlKhalil M and Ammar A.: Epidemiology and pathology of oral squamous cell carcinoma in a multi-ethnic population: Retrospective study of 154 cases over 7 years in Qatar. Ann Med Surg. 2020; 60:195-200.
3. Ali K.: Oral cancer – the fight must go on against all odds. EBD. 2022; 23(1): 4-5.
4. Ljokjel B., Haave H., Lybak S., Vintermyr K., Helgeland L. and Aarstad J.: Tumor Infiltration Levels of CD3, Foxp3 (+) Lymphocytes and CD68 Macrophages at Diagnosis Predict 5-Year Disease-Specific Survival in Patients with Oropharynx Squamous Cell Carcinoma. Cancers (Basel). 2022; 14(1508):1-15.
5. Linette P. and Carreno BM.: Tumor-Infiltrating Lymphocytes in the Checkpoint Inhibitor Era. Curr Hematol Malig Rep Hum. 2019;
6. Ruiter EJ De., Ooft L., Devriese A. and Willems M.: The prognostic role of tumor in fi ltrating T-lymphocytes in squamous cell carcinoma of the head and neck : A systematic review and meta-analysis. Oncoimmunology. 2017; 6(11):1-10.
7. Priya Mittal 1, Charles W M Roberts: The SWI/SNF complex in cancer - biology, biomarkers and therapy Nat Rev Clin Oncol. 2020 Jul; 17(7): 435-448.
8. Padilla-benavides T., Reyes-gutierrez P. and Imbalzano N.: Regulation of the mammalian SWI / SNF family of chromatin remodeling enzymes by phosphorylation during myogenesis. Biology (Basel). 2020; 9(7): 152.
9. Lee VH, Tsang RK, Wing A, Lo I, Chan S, Chung JC, Chi-Chung Tong, To-Wai Leung and Dora Lai-Wan Kwong.: SMARCB1 (INI-1)-deficient sinonasal carcinoma: A systematic review and pooled analysis of treatment outcomes. Cancers (Basel). 2022; 14(3285):1-15.
10. Neves-Silva R., Almeida Y., Silveira A., Colturato N., Duarte A., Ferrisse M., Silva V., Vanzolin F., Bufalino A., Ribeiro-Silva A. and León E.: SMARCB1 (INI-1) and NUT immunoexpression in a large series of head and neck carcinomas in a Brazilian reference center. Head Neck. 2020; 42(3): 374-84.
11. Guidi CJ, Sands AT, Zambrowicz BP et al. Disruption of Ini1 leads to periimplantation lethality and tumorigenesis in mice. Mol Cell Biol. 2001; 21: 3598-603.
12. Roberts CW and Orkin SH.: The SWI/SNF complex–chromatin and cancer. Nat Rev Cancer. 2004; 4: 133-42.
13. Roberts CW, Leroux MM, Fleming MD. and Stuart H Orkin.: Highly penetrant, rapid tumorigenesis through conditional inversion of the tumor suppressor gene Snf5. Cancer Cell. 2002; 2: 415-25.
14. Reisman D, Glaros S. and Thompson EA.: The SWI/SNF complex and cancer. Oncogene. 2009; 28: 1653-68.
15. Kim KH. and Roberts CW.: Mechanisms by which SMARCB1 loss drives rhabdoid tumor growth. Cancer Genet. 2014; 207: 365-72.
16. Cooper GW. and Hong AL.: SMARCB1-Deficient Cancers : Novel molecular insights and therapeutic vulnerabilities. Cancers (Basel). 2022; 14(3645):1-19.
17. Masliah-Planchon J., Bièche I., Guinebretière M., Bourdeaut F. and Delattre O.: SWI/SNF chromatin remodeling and human malignancies. Annu Rev Pathol Mech Dis. 2015; 10:145-71.

18. Chan YH (2003a): Biostatistics102: Quantitative Data – Parametric & Non-parametric Tests. Singapore Med J. 44(8): 391-396.
19. Chan YH (2003b): Biostatistics 104: Correlational Analysis. Singapore Med J. 44(12): 614-619.
20. Munn H. and Bronte V.: Immune suppressive mechanisms in the tumor micro-environment. Curr. Opin. Immunol. 2016; 39: 1-6.
21. Hadler-Olsen, E. and Wirsing M.: Tissue-infiltrating immune cells as prognostic markers in oral squamous cell carcinoma: a systematic review and meta-analysis. Br. J. Cancer. 2019; 120: 714-727.
22. Kim R., Emi M. and Tanabe K.: Cancer immunoediting: from immune surveillance to immune escape. Cancer Immunother. 2007; 121(1): 1-14.
23. Ferris L.: Immunology and immunotherapy of head and neck cancer. J. Clin. Oncol. 2015; 33: 3293-3304.
24. Almangush A., Mäkitie A., Triantafyllou A., de Bree R., Strojan P., Rinaldo A., Hernandez-Prera C., Suarez C., Kowalski P., Ferlito A. and Leivo I.: Staging and grading of oral squamous cell carcinoma: an update. Oral Oncol. 2020; 107:104799.
25. Perri, F., Ionna, F., Longo, F., Della Vittoria Scarpati, G., De Angelis, C., Ottaiano, A., Botti G., Caponigro F.: Immune response against head and neck cancer: biological mechanisms and implication on therapy. Transl. Oncol. 2020; 13: 262-274.
26. Zhou C., Diao P., Wu Y., Wei Z., Jiang L., Zhang W., Li Z., Jinhai Ye, Song X., Wu H., Jiang H., Wang Y. and Cheng J.: Development and validation of a seven-immune-feature-based prognostic score for oral squamous cell carcinoma after curative resection. Int. J. Cancer. 2020; 146: 1152-1163.
27. Roberts CW and Biegel JA.: The role of SMARCB1/INI1 in development of rhabdoid tumor. Cancer Biol Ther. 2009; 8: 412- 416.
28. Krishnamurthy N., Kato S., Lippman S. and Kurzrock R.: Chromatin remodeling (SWI/SNF) complexes, cancer, and response to immunotherapy J Immunother Cancer. 2022; 10(9): e004669.
29. Finetti A., Grabovska Y., Bailey S. and Williamson D.: Translational genomics of malignant rhabdoid tumours: current impact and future possibilities. Semin. Cancer Biol. 2020; 61: 20-41.
30. Imbalzano N. and Jones N.: Snf5 tumor suppressor couples chromatin remodeling, checkpoint control, and chromosomal stability. Cancer Cell. 2005; 7: 294-295.
31. Vries RG, Bezrookove V, Zuijderduijn L., Kia S., Houweling A., Oruetxebarria I., Raap A. and Verrijzer P.: Cancer-associated mutations in chromatin remodeler hSNF5 promote chromosomal instability by compromising the mitotic checkpoint. Genes Dev. 2005; 19: 665-70.
32. Raísa Sales de Sá1, Marisol Miranda Galvis, Bruno Augusto Linhares Almeida Mariz , Amanda Almeida Leite, Luciana Schultz , Oslei Paes Almeida, Alan Roger Santos-Silva, Clovis Antonio Lopes Pinto, Pablo Agustin Vargas , Kenneth John Gollob and Luiz Paulo Kowalski: Increased Tumor Immune Microenvironment CD3+ and CD20+ Lymphocytes Predict a Better Prognosis in Oral Tongue Squamous Cell Carcinoma Front Cell Dev Biol . 2021; 18: (8):622161.
33. Kenichi Kohashi and Yoshinao Oda: Oncogenic roles of SMARCB1/INI1 and its deficient tumors. Cancer Sci. 2017; 108(4): 547-552.
34. Suzanne J Forrest, Alyaa Al-Ibraheemi, Duong Doan, Abigail Ward, Catherine M Clinton, Juan Putra, R Seth Pinches, Cigall Kadoch, Susan N Chi, Steven G DuBois, Patrick J Leavey, Nicole R LeBoeuf, Elizabeth Mullen, Natalie Collins, Alanna J Church and Katherine A. Janeway: Genomic and Immunologic Characterization of INI1-Deficient Pediatric Cancers. Clin Cancer Res. 2020; 15; 26(12): 2882-2890.
35. Agaimy A. and Hartmann A.: SMARCB1 (INI-1)-deficient sinonasal carcinoma: a series of 39 cases expanding the morphological and clinicopathological spectrum of a recently described entity. Am J Surg Pathol. 2017; 41(4): 458-471.
36. Lisa M. Sullivan, Andrew L. Folpe, Bruce R. Pawel, Alexander R. Judkins and Jaclyn A. Biegel: Epithelioid sarcoma is associated with a high percentage of SMARCB1 deletions. Mod Pathol. 2013; 26(3): 385-392.
37. Wiegand, K. C. et al. ARID1A mutations in endometriosis-associated ovarian carcinomas. N. Engl. J. Med. 2010; 363: 1532-1543.
38. Jones S., Wang T., Shih I., Mao T., Nakayama K., Roden R., Glas R., Slamon D., Diaz L. Jr, Vogelstein B., Kinzler K., Velculescu V. and Papadopoulos N.: Frequent mutations of chromatin remodeling gene ARID1A in ovarian clear cell carcinoma. Science. 2010; 330: 228-231.
39. Shain H. and Pollack R.: The spectrum of SWI/SNF mutations, ubiquitous in human cancers. PLoS One. 2013; 8: e55119.
40. Kadoch, C., Hargreaves D., Hodges C., Elias L., Ho L., Ranish J. and Crabtree G.: Proteomic and bioinformatic analysis of mammalian SWI/SNF complexes identifies extensive roles in human malignancy. Nat. Genet. 2013; 45: 592-60.