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STUDY OF CANCER STEM CELL MARKERS AS A RESOURCE TO PREDICT PROGNOSIS AND DIAGNOSIS IN ORAL SQUAMOUS CELL CARCINOMA

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ABSTRACT

Oral squamous cell carcinoma (OSCC) seems to be the most frequent oral tumor and perhaps the most widespread cancer worldwide. OSCC is a malignant occurrence within the squamous epithelial layer of the oral cavity. Comprises tumors found on the tongue, lip, gingival. Oral squamous cell carcinoma is a malignant cancer that shows up in the oral cavity's squamous epithelial layer. Consists of tumours located on the tongue, ear, and gingival. CSCs (Cancer stem cells) are extremely tumour-related to other carcinomas, and should be mainly accountable for the biological presence of cancer, including invasion, rapid growth, and metastasis. The whole analysis covers all the CSC biomarkers' important importance in the possible role in enhancing the diagnosis, prognosis and care of patients with OSCC.

Key words: Oral squamous cell carcinoma, Cancer Stem Cells, markers, carcinogenesis.

INTRODUCTION

The 5-year relative OSCC oral (squamous cell carcinoma) population has endured at 50%, virtually unchanged more than 50 years. Based on the running progress of the short concept of carcinogenesis, CSCs (the cancer stem cells) are based on a hierarchy of a mixed population of cancer cells and are functionally addressed as a subset of cells showing their stemming characteristics, that also involves abnormal cell growth, having caused peer-renewal of CSCs and the development of heterogeneous populations of cancer cells which are further down the graded ladder **[1,2].** CSCs can move easily, conquer but also in vitro **[3,4]**, the production of the much advanced disease is triggered by cancer stem cells with lesser cell types in immune-compromised xenograft mice compared to large figures of unordered melanoma cells1. For several years the concept of "stem cells" has important prospect of being able to create new human tissues in the laboratory and use them to replace those lost by injury or disease. Such stem cell concepts applied to Dentistry, as well as to general Medicine, could be greatly improved by researches defining the ability of stem cells to produce new tissues such as bone tissues and mucosa which helps to regenerate dental tissues including the whole teeth. Recently, however, the dental literature has begun to comprise references to "cancer stem cells" (CSCs) and these have quite a different concept. These are the cells that have the ability to stimulate the growth of oral cancers and allow tumours to attack therapy. This review will through some light on the difference between CSCs and normal stem cells, the ways by which they can isolated and studied to know how they have special properties and of most importance, how they spread cancer and how they could be the target for destruction. There is now good indication that CSCs exist in most tumours but this review will focus mainly on oral squamous cell carcinoma, which includes the great majority of malignant oral cancers.

Background of oral cancer Oral squamous cell carcinoma (OSCC) is the most commonly occurring oral malignancy and one of the most widely occurring cancers throughout the world **[5,6,7]**. OSCC is a malignancy that arises in the squamous epithelium lining the oral cavity and includes tumors found on the tongue, lip, gingival.

The risk factors for expansion of OSCC include tobacco exposure, alcohol drinking, and infection with oncogenic viruses such as HPV **[8]**. The tumour can occupy deeply into adjacent tissues of the tongue and of the alveolar crest **[6]**. Microscopically, OSCC usually shows flexible degrees of keratinization, cellular and nuclear pleomorphism, and mitotic activity. They are graded as well-, moderately- or poorly-differentiated (grades 1 to 3) according to WHO principles **[9,10]**. Including the size and site, the tumour shows histologic malignant grade, perineural spread at the invasive front, lymphovascular invasion and the thickness of the tumour, can OSCC patients, however, the main negative prognostic factor is the presence of lymph node metastasis, which occurs in 25 to 65% of cases **[3,8].** In cases of locally innovative OSCC, the treatment is multimodal, with either surgery followed by adjuvant radiation or chemo-radiation, as indicated by. Approximately half of all patients survive 5 years after stage of the disease at diagnosis.

Normal stem cells

The general term “stem cells” includes several made is between (a) normal stem cells (SC), which are mainly responsible for the development and maintenance of all of the tissues of the body, and (b) their diseased matching part, called cancer stem cells (CSC) that have lost the close growth regulator that is a property of normal stem cells. As the embryonic growth proceeds, these totipotent stem cells become directed towards differentiation into the many distinct tissue types of the adult individual (e.g. stem cells for blood, bones, mucosa, etc.). The most primitive type of stem in development produces cells that retain the ability to produce all the different cell types of the adult body. They are therefore well-defined as “totipotent” and, remarkably for stem cells, they are temporary. As they do so they drop some of their progressive potential and become either “pluripotent”, that is, controlled to form only a certain type of tissues, or "unipotent", restricted to producing only a single tissue. Thus, every adult individual has several subtypes of stem cells. Every stem cell has different potential abilities depending on how they developed. Oral mucosal epithelia consists the epithelial stem cells which are typically unipotent and form only the type of epithelium typical of the region where they are found.

The general property that characterizes adult (somatic) stem cells is that they can be divided a stem cell and one cell that differentiates itself into a functional tissue cell. This normal "irregular" division pattern is important as it results in the maintenance of the same number of stem cells while also providing another cell for tissue function. However, when it is necessary to replace stem cells, such as those lost after wounding, stem cells can be divided "regularly" to form two stem cells and thus raise their number **[11]**.

Cancer stem cells

Current studies have unveiled and validated the pathophysiologic role of cancer stem cells (CSCs; also called tumour-initiating cells) in long-term nourishment of cancers **[12,13]**. CSCs are small subpopulations of tumour cells that share many molecular resemblances to embryonic and normal adult stem cells. CSCs have been isolated from various primary tumours and established cancer cell, including OSCC **[3,14]** .They play a vital role in tumourigenicity, metastasis, and reappearance and are thus considered to be the root of the cancer. Therefore, advancing our understanding in the molecular properties and signalling pathways unique to Oral cancer stem cells is crucial for developing novel targeted and effective therapies for OSCC.

Precancerous Stem Cells (PCSCs)

PCSCs also can generate from normal stem cells, theyhave conjointly self-renewal capability and adult cells and distinguish from mature cell **[15]**. They conjointly specific embryonic and adult stemness markers like CD133, organic compound dehydrogenase one, andOCT-4, which may be used to separate them from mature cells. Moreover, they can hide within the lesions' microenvironment **[16]**. These are looking on the microenvironment, these cells could make to primary CSCs for initiation of malignancy. many genetic and epigenetic factors which can conjointly contribute to the existing long method, like construction of various from of CSCs.

Chen et al. determined that in the alteration of pCSCs into CSCs in malignant neoplastic disease elicited in mice, specific markers and CD45 were upregulated. They conjointly found that PIWIL2 (a PIWI/AGO family cistron expressed in ESCs) will promote the proliferation of pCSCs**[17,18]**.

In study of 2008, connected the organic phenomenon pattern between ductal malignant neoplastic disease in place (DCIS) (a premalignant condition) and invasive ductal malignant neoplastic disease, found a variance within the expression pattern of 147 genes in these 2 lesions. Moreover, two genes, SULF1 and LOX, appeared to be related to the aggressive behaviour of the tumor and might act as biomarkers to expect the chance of DCIS progression **[18]**. Therefore, it can differentiate CSCs from pCSCs consistent with the subsequent criteria: first, metastatic tumor cells can form either a benignoramalignantlesion supported their micro-environment

condition whereas CSCs are liable for the recruit and development of a malignant lesion. Secondly, pCSCs are found in oral metastatic tumor lesions like oral leukoplakia.

Whereas CSCs are found in cancerous loci. The third part is that the epigenetic and genetic profile of CSCs and pCSCs**[19]**. Taken along, it appears that the identification of PCSCs in metastatic tumor lesions can provide with the ability to evaluate the risk of malignant transformation of precancerous lesions and prevent their progression in the early stages.

CSCs markers in oral carcinogenesis

Uses of Cancer Stem Cells in treatment, is believed that existence of CSCs may be the reason for the lack of effectiveness of conventional treatment methods **[20]**. In OSCC, where the first Open Science Journal Research Article and fundamental method of treatment involves surgical removal of the tumour, CSCs might have already metastasized at the time of surgery. They are probably responsible for tumourrepetition**[21]**. Cancer stem cells show more resistance to chemo- and radiotherapy than non-stem cells **[22]**. Targeted removal of these cells was considered to provide a new agenda for head and neck cancer treatment**[23]**.

CSC Potential Diagnostic Implications

According to the fact diagnostic tests should be safe, easy, relatively non-invasive, cost-benefit and acceptable for patients and clinicians, taking tissue samples for diagnosis suggests could not be measured as asuitableoption.Ahuge number of tumour cells known as circulating tumour cells are being unconfined on a daily basis in to the bloodstream whose smallest portion is believed to be circulating CSCs **[23,24]**.

According to the standard metastasis cascade, including local invasion, intravasation, survival in the blood circulation, and colonization in new organ, is expected in order to complete the metastatic process **[25]**. Based on CSC theory, CSCs, as the most aggressive and qualified cells to form metastasis, will pass in the blood stream to achieve their metastasis mission. Their presence in blood is our privilegedchance to apply them in diagnostic implications. Therefore, tracing the variable cancer stem cells in blood pool using their specific markersobserved as a promisingmethod which has been exploited in a significant number of recent researches and the results have been promising.Yang et al. **[26]** in china in 2008 exposed that identification of $CD45^{-}CD90^{+}$ CSCs in both tumour tissues and blood circulation of patients with liver cancer could be used as a target for diagnosis and therapy of these patients. Linuma et al. **[27]** in Japan estimated the clinical importance of circulating tumour cells (CTCs) including CSCs as a prognostic factor in patients with oral cancer and colorectal cancer after healing surgery and found that detection of CEA/CK/CD133 mRNA in peripheral blood would be a useful for identification of patients who are at higher risk of repetition and poor prognosis. Pilati et al. **[28]** studied the prognostic value of supposed circulating cancer stem cells in patients undergoing hepatic resection for colorectal liver metastasis and resolved that $CD133^{+}$CTC may represent anappropriate prognostic marker to stratify the risk of these patients. Valladares et al **[29]** evaluated the adenocarcinoma-associated gene AGR2 and the intestinal stem cell marker LGR5 as biomarkers in colorectal cancer. Their conclusions indicated that assessment of AGR2 and LGR5 in peripheral blood might reflect the presence of CTCs and CSCs in colorectal cancer and increased AGR2 and LGR5 are associated with poor outcomes. Wang and his group studied the role of CTCs and CSCs of breast cancer patients as well as their clinical significance using flow cytometry concluding that flow cytometric detection of CTC and CTSC could be used to diagnose disease at early stage, which would be helpful for clinical therapy guidance or prognosis prediction **[29]**. Although these studies confirm the potential application of circulating cancer stem cell for diagnostic purposes, further studies are needed to more elucidate CCSC diagnostic potential and supportive evidences in this field needs to be broaden.

CSC Potential Prognostic Implications

Much more interests have been devoted to CSC prognostic and diagnostic implications associated to its therapeutic applications. While therapeutic application of CSC markers requires very demanding strong clinical trial processes to minimize undesirable off-target effects of such interventions, a clinically appropriate CSC marker would easily enter the clinical utilization cycle without going through exhausting time-consuming clinical trial processes, leading to a more interest in investigators.

Considering CSC as the supplier source of tumour cells in the tumour bulk and according to its responsibility in uncontrolledtumour growth, maintenance, metastasis, recurrence, resistance to treatment, and absolutelytumour biological behavior, evaluation of the presence and degree of this population rationally outcome in more clinical relevance than the other tumour proliferating or differentiated cells do. Generally, it is believed that a higher CSC proportion signifies a worse prognosis. For instance, in breast cancer, the most poorly differentiated tumours contain the highest burden of breast CSCs **[30]**. Likewise, elevated CD133 expression in colon cancer is also a marker of poor prognosis and is associated to liver metastasis **[31]**. In pancreatic cancer, high CD133 is an adverse prognostic factor for 5-year survival and is associated with lymph node invasion **[32]**. CD133 expression is also associated with poor clinical outcome in ovarian cancer **[33]**. Similarly, $CD133^{-}$positive non- small cell lung cancer (NSCLC) had worse prognosis **[34]**.

In addition, expression of ALDH, another CSC specific marker, is associated with poor prognosis in a number of tumours including breast, head and neck, prostate, colon and AML. ABC transporter, as latent CSC associated marker, has also been reported to be an indicator of poor prognosis in AML patients **[25]**.

CD44 isananother proposed cancer stem cell marker and its prognostic value has also been shown in several studies. Mima et al. **[35]** showed that the over-expression of CD44 was in relation with poor prognosis in the hepatocellular carcinoma patients, including reduced disease-free and overall survival.

Mulder et al. **[36]** also showed that CD44 has prognostic value self-regulating of Dukes' stage in colorectal cancer patients and it may predict the tendency to metastasis after curative surgery. CD44 expression was also associated with decreased overall survival in pancreatic cancer **[37]**.Based on the presented of evidences, patients with higher CSC marker's expression mostly tend to have a poorer prognosis.

Identification of OSCCPremalignant and Malignant CSC Markers

The proliferation of mucosa from normal to mild, moderate, and severe dysplasia and then to an OSCC is a multivari4 Int J Cancer Manag. 2019; 12(10):e96139**[38]**. Ghazi N et al.**[39]** ate process, which includes structural and functional changes of cells.There is high risk that the lesions that show higher expression of ALDH1 could transform into primary malignant lesions. It can be valuable for the clinician to evaluate the progression risk of premalignant lesions on the way to cancer, and preventive approaches can be performed. CSCs have been identified by using specific markers in various studies. Identifying a reliable CSC marker that is associated with cancer treatment is important.

ALDH1

ALDH1 is an isoform of the ALDH enzyme family, it acts as a detoxifying enzyme that oxidized aldehydes. It also oxidises retinol (vitamin A) to retinoic acid (RA), the efficient form of this vitamin **[40]**. The overexpression of ALDH1 has been found in the lower epithelial layers of oral premalignant lesions and has been correlated with the degree of cellular dysplasia **[41,42]**.There is a high risk that the lesions that show higher expression of ALDH1 could transform into primary malignant lesions. High ALDH expression has also been linked with a decreased overall 5-year survival of patients **[43,44,45].** One reason for this might be that cells with the higher expression of ALDH1 are more capable of metabolizing chemotherapeutic agents and free radicals produced following radiotherapy **[46,47]**.

CD44

CD44 is the most familiar CSC marker that has previously been identified in several solid malignancies such as breast, CNS, colon, prostate and pancreas **[48]**.

In OSCC primary tissues, CD44+ subpopulation established its tumourigenic potential, tumour sphere growth and chemo-resistance. The positive population of these cells was also found to over express positive stemness markers like Bmi1 that maintains the undifferentiated state of the cell **[49]**. CD44 expression individually negatively correlated with poor 5 year survival while its high levels along with ALDH and phosphorylated STAT3 correlated with high-grade of OSCC which is reliable with the previous findings in urothelial carcinoma **[49,50]**. Since it is equally expressed in carcinoma and normal head and neck epithelium, the use of CD44 as a marker has been controversial. Inspite of this, we cannot contest that CD44 either alone or in combination can be measured to have the properties of a cancer stem cell marker and being a tumour initiator in OSCC but its role and consistency needs to be confirmed **[51]**.

c-Met

c-Met may be a proto-oncogene that encodes for hepatocyte protein (HGF) tyrosine kinase receptor. Normally only stem cells and progenitor cells express Met, however, CSCs seize this capability (from the conventional stem cells) associating its expression with metastasis and tumourinvasion, reduced survival and angiogenesis in various neoplasms. In OSCC, c-Met+ cells demonstrated self-renewal and were ready to create heterogeneous tumours with more tumourigenic potential than by CD44+ marker. Also, c-Met+ /CD44+ combination yielded tumours in 80% of cases, while c-Met+/ALDH1+ displayed tumour formation in 66% cases **[50]**. Thus c-Met has been proposed as a potent CSC marker in OSCC but further investigation with a greater number of samples and a comparison of c-Met+ with other CSC and stemness markers could provide a clear description.

CD133

CD133 (prominin-1) is a reputed CSC marker that has been characterized in epithelial cells and in somatic stem cells from neural tissues, prostate, kidney, colorectal, liver, skin and lung **[52]**. In HNSCC & OSCC, CD133+ cells presented increase in clonogenicity, EMT phenotype, tumour sphere formation, self-renewal, proliferation, differentiation, higher levels of stemness genes and tumourigenicity **[53]**. Higher levels of CD133, have been connected with CD44+ expression in HNSCC and with Bmi1 induced proliferation in laryngeal carcinomas **[48,54,55]**.

In fact, positive correlation of Oct-4, Nanog with an increased expression status of CD133 depicted a poorer prognosis for oral cancer patients **[16]**. Further investigation is mandatory to validate the inconsistency showing similar tumour-initiating behaviour between CD133+ and CD133- populations **[54,56]**. Hence, CD133 might serve as a useful CSC marker in OSCC cases to categorize patients that are resistant to conventional chemotherapy with paclitaxel.

Stemness Markers

Oct-4, Sox2 &Nanog Transcription factors Oct-4, Nanog and Sox2 play vital roles in the maintenance of pluripotency and self-renewal of embryonic stem cells by relating with other transcription factors (STAT3, HesX1, Zic3) and critical cell signalling molecules (TCF3, FGF2, LEFTY2). Over expression of Oct-4 and Nanog genes, found in CSC-enriched subpopulation derived from OSCC sphere formation colonies, positively associated with treatment failure and stage while negatively correlated with differentiation status **[57,58]**. Oct-4, individually was initiate to be competent enough to up regulate ALDH1+ in OSCC cells while in combination with TRA1-60 (a tumour rejection antigen) were detectable as indicators of invasiveness. Furthermore, it was established that patients displaying a triple-positive expression of Oct-4, Nanog and CD133 had the worst survival prognosis in OSCC, indicating their value as an invasiveness and predictive marker **[59]**. Sox2 has increased expression specifically in squamous cell carcinomas of the lung and esophagus, but not in the lung or esophageal adenocarcinomas **[60]**, which proposes its importance as a lineage specific stem cell marker for squamous cell carcinoma. Collectively, these data indicate that cells that exhibit stem-like features in cancer express the transcriptional factors Oct-4, Sox2 and Nanog.

Klf4

Krüppel-like factor 4 (Klf4), a zinc finger transcription factor, is start in the upstream of Akt in pre malignant lesions. It is a negative regulator of the cell cycle by suppressing genes like p53 that encourage proliferation and by activating genes like p21 **[61]**. Klf4 has recently been recognized as a “pluripotency gene” that is involved in the reprogramming of somatic cells into a stem cell-like state, maintaining the self-renewal capability of cells, regulating growth and differentiation **[62,63]**. The frequent loss of Klf4 expression in gastric and colorectal cancers has led to its characterization as a tumour suppressor.

Equally overexpression of Klf4 represents the oncogenic feature of the gene which is observed in the skin, breast and OSCC **[64]**. In HNSCC, Klf4 over expression was correlated with a worse disease-free survival of patients while in tongue squamous carcinomas executed Klf4 expression demonstrated increased in-vitro migration abilities, multidrug resistance and in vivo tumourigenicity. Moreover, the ALDH+ SP cells of nasopharyngeal carcinoma showed higher appearance of stemness genes Oct-4, Bmi1, Sox2 and Klf4. Recent reports state that the transcription factors Notch1 and Klf4 together deliberate stem cell properties, suggesting a functional relationship wherein each gene can act to promote or suppress tumourigenesis. Collectively these data support the notion that Klf4 is potentially a reliable marker of OSCC **[65]**.

Lgr5/GPR49 (G-protein coupled receptor 49)

Lgr5, a seven-transmembrane-domain receptor protein, has been recognized as a marker for adult stem cells in intestine, stomach, and hair follicle. Lgr5+ cells were identified to fuel stem cell activity through erroneous activation of Wntsignalling pathway, leading to cytoplasmic b-catenin build-up which has been associated with tumourigenesis**[66]**. Hence, it has been well-known CSC marker that is down-regulated in colorectal cancer (CRC) and is up-regulated in esophageal adenocarcinoma (EAC), basal cell carcinomas (BCCa) of the face and cancers of the ovary & liver. Recent reports have correlate this marker with head and neck carcinoma as it has been detected in the oral tissue of mice as well as in the side populations of HNSCC cell lines **[67].** This implies that Lrg5 is a tumour suppressor gene whose main role is bounding stem cell expansion in their respective niches. Given that, Lgr5 as a candidate marker driving towards better prognostic and therapeutic inferences in OSCC requires further investigation into its behavioural and expression patterns **[68].**

CD117 (c-KIT)

CD117, a proto-oncogene, is a cytokeratin receptor that is characterized as stem cell marker for hematopoietic stem and progenitor cells, ovarian cancer initiating cells from primary human tumours, cardiac CD117+ stem cells and other mesenchymal stem cells **[69,70]**.CD117 was not identified as a CSC marker in OSCC, until recently when presence of CD117 was found in more than half of OSCC cell lines and primary cultured cells.

In addition, data regarding OSCC reactivity to CD117 are few and contradictory. While one study suggests that CD117+ expression was observed in basal tongue SCC while other reports were contradictory to these findings suggesting that CD117+ expressions were limited to stromal spindle cells in OSCC **[71]**. In any case, these cells were trypatase+, antivimentin+ and infrequently for CSC marker antibodies like CD44 & CD133. EpCAM/ CD326 the epithelial cell adhesion molecule (EpCAM; CD326) is a transmembrane glycoprotein that is expressed by the epithelium of healthy individuals, except by squamous epithelium, hepatocytes and keratinocytes. Several biological functions of EpCAM have been described: EpCAM is able to abrogate E-cadherin mediated cell–cell adhesion, rearrange the cytoskeleton of the cell, increase cell motility, proliferation and metastasis. Recently, EpCAM has also been identified as a signal transducer and an intramembranous proteolysis regulator, stating its unambiguous role as an oncogene (86).

BMI1

B cell-specific Moloney murine leukaemia virus integration site 1 protein (BMI1) is a member of polycomb group proteins encoded by the BMI1 gene. This protein acts by the remodelling of chromatins and modification of histones and serves a vital role in the cell cycle their regulation **[72]**. It is also believed that role of these genesare important in the maintenance and self-renewal property of mainly embryonic and adult stem cells **[73,74]**. The unpattern expression of these genes has been associated with many solid malignancies including HNSCCs **[75]**. BMI1 expression is significantly higher in oral leukoplakia and OSCC compared to the normal oral mucosa and there is also a significant difference in the expression pattern of BMI1 in mild dysplastic lesions compared to moderate and severe ones **[76]**. BMI1+ leukoplakias have a higher risk of conversion into a primarytumour**[77]**. The role of BMI1 in HNSCC seems to be a material in dispute, asking for further studies. However, we believe that BMI1 upregulation is significantly connected with the aggressiveproperties of the tumour cells and also the overall survival of patients **[78,79,80].** The higher expression of BMI1 has been detected in invasive cells, which was escorted by the upregulation of vimentin and downregulation of E-cadherin, linking this marker to the EMT process **[81,82,83].**

BMI1+ cells are also believed to resist chemotherapeutic agents **[85,86]**. Thismight be due to the high expression of "AP-1", a transcription factor linked to tumour metastasis and chemoresistance.

Simultaneous Targeting of Different Cancers

Cancer as one disease provides another possibleprospect. Beside screening possibility of several cancers consuming their shared CSC marker, such marker might be used to target several cancers, at the same time. In addition to reduced treatment costs of such therapeutic potential, it also has the potential to be used for preventive resolutions. Although the treatment of numerous cancers with one drug may seem determined, a closer look at nature may make it more realistic. Nearly all dietaries with anti-tumoureffects, spread over their anti-tumour effects in more than one tumour. eventually, turmeric anti-cancer effects have been described in several cancers such as hepatic cancer **[77]**, breast cancer **[78]**, head and neck carcinoma **[79]**, colon cancer **[80]**, lung cancer **[81]**, prostate cancer **[82]**, ovarian cancer **[83]** and pancreatic cancer **[85,86,87]**. As a result, the presence of an anti-cancer drug which is appropriate to more than one cancer is not an impossible achievement.

CONCLUSION

In conclusion, our meta-analysis revealed the value of ALDH1, CD133 and CD44s as 3 significant clinical indicators for patients with Oral Cancer. ALDH1 overexpression was related to lymph invasion. CD133 overexpression was significantly correlated with tumour differentiation grade. CD44s overexpression was associated with chemotherapy resistance. Moreover, ALDH1, CD117, CD133 and CD44s were associated with worse prognosis. Combined detection of ALDH1, CD117, CD133 and CD44s expression may be a powerful tool in clinical practice for predicting prognosis of patients with OSCC. However, because of certain limitations, further well-designed studies with large samples, standard cohorts, and long-term follow-up are required for confirmation.

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