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Cancer stem cell markers in Oral Potentially Malignant Disorders – An insight

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Abstract

Oral cancer is usually preceded by asymptomatic clinical lesions collectively referred to as Oral Potentially Malignant Disorders (OPMDs). The progression of OPMD to Oral Squamous Cell Carcinoma (OSCC) is a multistep process that provides an opportunity for early cancer detection and interception. In the last few years, the cancer stem cell (CSC) hypothesis has attracted much attention concerning tumour initiation, progression, differentiation, treatment resistance, relapse, metastasis and aggressive behaviour of the tumour. The existence and association of CSCs in the prognosis of several malignancies, including OSCC have been reported extensively, but reports evaluating the prognostic value of CSCs in OPMDs are still scarce. Thus, the present paper aims to review the role of CSCs in OPMDs, the various CSC markers studied so far and their efficacy in predicting the malignant transformation in OPMDs.

Keywords: Cancer stem cells, Oral Potentially Malignant Disorders, Oral Cancer, Oral Epithelial Dysplasia, Podoplanin, ABCG2, Bmi1, ALDH1, CD44, Musashi 1

Introduction

Despite the tremendous advancements in the field of cancer prevention, detection and treatment, the overall prognosis of oral squamous cell carcinoma (OSCC) remains poor. This can be partly imparted to the lack of early detection of oral potentially malignant disorders (OPMDs). Virtually almost all the cases of OSCCs are preceded by clinically conspicuous but variable changes in the oral mucosa that are collectively referred to as

OPMDs.[1] The progression of OPMD to OSCC is a multistep process that provides an opportunity for early cancer detection and interception. In the last few years, the cancer stem cell (CSC) hypothesis has attracted much attention concerning tumour initiation, progression, differentiation, treatment resistance, relapse, metastasis and aggressive behaviour of the tumour. The hypothesis emphasizes that a small subset of cancer cells with stem cell-like characteristics (known as CSCs), possess unlimited proliferative potential and are responsible for tumour formation with phenotypically heterogeneous cell populations.[2] This CSC population is distinguished from other tumour cells by the expression pattern of stemness related markers.[3] The existence and association of CSCs in the prognosis of several malignancies, including OSCC have been reported extensively, but reports evaluating the prognostic value of CSCs in OPMDs are still scarce. Thus, the present paper aims to review the role of CSCs in OPMDs, the various CSC markers studied so far and their efficacy in predicting the malignant transformation in OPMDs.

OPMDs and Oral Epithelial Dysplasia

Oral cancer is usually preceded by asymptomatic clinical lesions collectively referred to as OPMDs.[1] OPMDs include leukoplakia, erythroplakia, reverse smoker's palate, erosive lichen planus, oral submucous fibrosis (OSMF), lupus erythematosus and actinic keratosis.[4] OPMD may histologically be characterised as hyperplasia, hyperkeratosis, oral epithelial dysplasia (OED) or OSCC.[5] OED is characterized by cytological and architectural alterations reflecting the loss of normal maturation and stratification pattern of surface epithelium.[5] Grading of OED is used to assess the probability of malignant transformation. The World Health Organisation 2017 grading of oral dysplasia graded OED as mild, moderate and severe.[6] The malignant Transformation Rate (MTR) of epithelial dysplasia ranges between 1.4% and 36%.[7] So far there is no specific well-established biomarker for cancer risk assessment in any of the OPMDs.

Cancer Stem Cells

The term “cancer stem cells” is defined by the American Association for Cancer Research Workshop on CSCs as a cell within a tumour that possesses the capacity to self-renew and to generate heterogeneous lineages of cancer cells that comprise the tumour.[2] In 1997, Bonnet et al. were the first to isolate CSCs in samples of acute myeloid leukaemia. In 2003 Al-Hajj et al. first identified and isolated a population of cancer stem cells from breast cancer. Since then subpopulation of cells has been identified in various solid tumours including breast, multiple myeloma, melanoma, prostate, HNSCC, colon and pancreatic cancers.[8] These CSCs can be identified using surface markers, determination of ALDH activity, ability to efflux vital dyes, and ability to form tumour spheres in vitro.[3]

Cancer Stem Cells in Oral Cancer

Studies proved that CSCs in HNSCC has the ability to tumour initiation, aggressiveness, metastasis, and therapy resistance.[9] HNSCC CSC was first described by Prince and colleagues in 2007 based on CD44 expression.[10] Various stem cell surface markers and stemness related markers have been studied tremendously in HNSCC like CD133, CD44, CD24, CD271, Octamer-binding transcription factor 4 (Oct-4), Sex determining region Y-box 2 (Sox-2), Nanog homeobox (NANOG), Aldehyde dehydrogenase-1 (ALDH1), ATP-binding cassette sub-family G member-2 (ABCG2), and B cell-specific Moloney murine leukaemia virus integration site 1 (Bmi-1).[3]

Cancer Stem Cells in OPMDs

OPMDs have the potential for regression as well as progression depending on cellular stress and tumorigenic environmental cues. As oral carcinogenesis is an intricate multistep process requiring genetic and epigenetic alterations in several genes and genomic instability, it usually provides a reasonable time frame to intercept cancer at the precancer stage.[11] Studies of premalignant adenomatous polyps of the colon and precancerous gastric lesions by Patel et al. in 2009 have shown that the presence of a CSC population in the precancer stage is an early indicator of malignant progression.[12] Since then, the identification of CSCs has been an area of research interest in OPMDs. A number of studies have been carried out to correlate the expression of CSC markers in OPMDs with OSCC development.

Cancer Stem Cell markers in OPMDs

Diverse cell surface and stemness markers have been used for the identification of cancer stem cells in human tumours using immunohistochemistry (IHC). Of them, only a few markers have been studied in OPMDs. Moreover, the role of these markers in the malignant transformation of OED and their role in carcinogenesis is also not clear. Hence this paper reviews the CSC markers studied in OPMDs so far. The various CSC markers and their relevance in OPMDs were tabulated in Table 1.

Table 1: List of CSC markers and their role in oral carcinogenesis

Sn.	CSC Marker	Marker type	Type of OPMDs studied	Role in carcinogenesis
1.	Podoplanin	Transmembrane sialoglycoprotein	OLP, OSMF, Leukoplakia	Tumour initiation, progression and metastasis
2.	ABCG2	ATP-binding cassette transporter protein	OLP, Leukoplakia, Erythroplakia	Maintains side population phenotype in CSCs
3.	Bmi1	Transcriptional repressor	OLP, Leukoplakia, Erythroplakia	Self-renewal of CSCs
4.	CD133	Transmembrane glycoprotein	OLP, OSMF, Leukoplakia	Tumour initiation, cellular adhesion, migration, angiogenesis, certain lymphocyte functions, and metastasis
5.	CD44	Type I transmembrane glycoprotein	OLP, Leukoplakia	Tumour progression, metastasis and proliferation
6.	ALDH1	Intracellular cytosolic iso-enzymes	OLP, Leukoplakia, Erythroplakia	Regulating self-renewal, differentiation, and tumour resistance of CSCs
7.	Musashi 1	RNA-binding protein	OED	Regulates stem cell pathways including Wnt and Notch signalling pathways
8.	SOX2	Transcription factor	Leukoplakia	CSC maintenance and regulation
9.	OCT4	Octamer transcription factor	OED	Regulator for pluripotency and self-renewal

Podoplanin

Podoplanin (PDPN), a transmembrane sialoglycoprotein, is a specific marker for lymphatic endothelial cells which in recent years has gained prominent notoriety for its role in tumour progression and metastasis. It is an extensively studied biomarker for predictive assessment of malignant transformation as well as biologic behaviour in various malignancies.[13] It is rarely expressed in normal epithelium while it is highly expressed in OSCC mainly in the invasive front leading to cell migration and metastasis. Its expression has been correlated with aggressiveness, cell migration, metastasis and poor prognosis.[14] Recent research has shown that CD44, a major hyaluronan receptor in CSCs, is associated with directional persistence of PDPN-positive epithelial cell motility and up-regulation of PDPN and CD44 co-expression was found in aggressive cancer cell lines.[13] Increased expression of PDPN has been demonstrated in oral lichen planus (OLP), leukoplakia and OSMF as well as in dysplastic oral epithelium.[15-18] It has been shown that podoplanin overexpression may occur in early oral tumorigenesis and it may serve as a biomarker indicating malignant transformation in OPMDs.[19,20]

ATP-binding cassette sub-family G member-2 (ABCG2)

ABCG2 is a member of the ATP-binding cassette transporter protein superfamily that produces multiple drug-resistant cancers. It is also known as a molecular determinant that maintains the side population phenotype in stem cells.[21] It has been isolated from several primary tumour types and cancer cell lines, including OSCC.[22] ABCG2 expression is also found in OPMDs like leukoplakia, OLP and erythroplakia.[23-25] The expression of ABCG2 was reported to be associated with a 4.09 fold increased risk of malignant transformation in OPMDs.[23] Also, the co-expression of Podoplanin and ABCG2 in oral lichen planus was crucially linked with a risk of OSCC transformation by Shi *et al*.[24] Thus it is believed that ABCG2 may act as a potential biomarker to predict the malignant transformation of OPMDs.

B cell-specific Moloney murine leukaemia virus integration site 1 (Bmi1)

Bmi 1 oncogene is a member of the polycomb-group gene family and a transcriptional repressor. Bmi 1 was first recognized in B-cell lymphoma.[26] BMI-1 was involved in the self-renewal, differentiation and tumour initiation of CSCs in various solid tumours including OSCC.[27,28] Overexpression of Bmi 1 in OLP[23,29], leukoplakia[30] and oral erythroplakia[31] were associated with malignant transformation suggesting that it may be valuable predictors for evaluating the risk of oral cancer.

Cluster of Differentiation 133 (CD133)

CD133, also known as Prominin-1 and AC133, was first described as a cell surface marker on hematopoietic stem cells and early progenitor cells in the bone marrow.[32] CD133 has been used extensively as a marker for the identification of CSCs derived from primary solid cancers including OSCC and has been demonstrated to be a prognostic marker of poor survival.[33] A gradual increase in the expression of CD133 was observed from normal oral mucosa to dysplasia to carcinoma.[34] CD133 expression was studied in OPMDs like OLP [35], leukoplakia [36] and OSMF [37]. It was found to be significantly associated with malignant progression in a longitudinal case-control study of patients with OLP [35] and leukoplakia [36]. These findings suggest that CD133 may serve as a candidate biomarker for risk assessment of oral potentially malignant lesions progressing to OSCC.

Cluster of Differentiation 44 (CD44)

CD44 is a type I transmembrane glycoprotein expressed in several cell types of mesenchymal and neuroectodermal origin [38]. CD44 functions as a major adhesion molecule

and in the cellular internalization of hyaluronic acid (HA) [39]. HA binds the CD44 ligand-binding domain inducing conformational changes that allow binding of adaptor proteins or cytoskeletal elements to intracellular domains that in turn activate various signalling pathways leading to cell proliferation, adhesion, migration, and invasion [40,41]. CD44 bound to HA has been proven to participate in various tumour biological activities, including tumour progression, metastasis and proliferation [42]. Though most of the shreds of evidence showed a negative role for CD44 in tumour progression, some disputing reports found a positive prognostic value for CD44 in cancers of the head and neck, particularly in oral cancer [42,43]. CD44 expression has been studied in OPMDs like leukoplakia and OLP with increasing expression in increasing grades of OED.[36, 44].

Aldehyde Dehydrogenase 1 (ALDH1)

Aldehyde dehydrogenase comprises a family of intracellular cytosolic iso-enzymes that are mostly found in the liver. Their known functions include the conversion of retinol to retinoic acid in early stem cell differentiation and catalyzing the oxidation of toxic intracellular aldehyde metabolites, similar to those formed during alcohol metabolism and chemotherapeutics, into a carboxylic acid.[45] ALDH1 plays a vital role as a marker of CSCs by regulating the self-renewal, differentiation, and tumour resistance of CSCs.[46] It was observed that ALDH+ cells maintained consistent behaviour with OSCC CSCs holding a high capability of sphere formation, tumour formation, increased invasion, self-renewal and resistance to chemotherapeutics.[45,47,48] In OSCC, increased levels of ALDH correlated with disease staging, radio-resistance and negative correlation with patient outcome.[45] ALDH expression was strongly associated with a worse malignant prognosis of patients with leukoplakia[49] and erythroplakia.[50] ALDH1 expression was also significantly associated with a 6.71-fold increased risk of malignant transformation of OLP.[51] As ALDH1 expression was high in samples of oral dysplasia compared to the normal buccal mucosa,[49,51] it can be a promising biomarker for malignant transformation of potentially malignant disorders with dysplasia.

Musashi 1

Musashi-1 belongs to a family of RNA-binding proteins which activates the translation of target mRNAs. It regulates the stem cell pathways including Wnt and Notch signalling pathways. Initially, Musashi-1 expression was found in neural, gastric, intestinal, and hair follicle stem cells in where they maintain the stem cell characteristics.[53] Its expression was also observed in several tumours including colorectal adenoma, glioma, retinoblastoma, hepatoma and cervical carcinoma.[54-57] The expression of Musashi-1 was found to be increased in OSCC compared to severe dysplasia and also higher in mild-moderate dysplasia compared with normal oral epithelium suggesting its role in the progression of oral cancer.[58,59]

Sex determining region Y-box 2 (SOX2)

SOX2 belongs to a group of related transcription factors located in the sex-determining region on the Y-chromosome.[60] It regulates embryonic development and stem cell maintenance to homeostasis in adult tissues.[61] SOX2 amplification has previously been found in several cancer types including OSCC.[62,63] Studies demonstrated that SOX2 amplifications are common in OSCC and was significantly associated with the pathological grade.[64] In addition, a significant difference in SOX2 staining was demonstrated between OSCC, oral epithelial dysplasia and normal oral mucosa.[65] It was hypothesized that cells with elevated SOX2 expression may harbour early molecular changes

and eventually contribute to field carcinogenesis to develop preinvasive lesions.[66] Also, studies in leukoplakia showed the potential role of SOX2 in oral carcinogenesis and demonstrated its contribution to the expansion of the stem cell population from basal cell layer to suprabasal layer in dysplastic lesions.[67,68] Thus, detection of SOX2 amplifications in the early stages of the disease may be crucial for early disease detection and a more accurate prognosis.

Octamer-binding transcription factor 4 (OCT4)

OCT4 belongs to the class of octamer transcription factor which binds to an eight-base pair DNA sequence and consists of a Pit/Oct/Unc family of homeodomain proteins. OCT4 is considered to be very crucial in the maintenance and pluripotency of embryonic stem cells.[69] OCT4 expression is increased in OPMDs with OED and also in OSCC.[70-72] However, whether the molecular mechanisms accounting for OCT4 is associated with a favourable prognosis in OSCC is still unknown due to contradictory reports by various studies.[73-75]

Conclusion

It is widely accepted that CSCs contribute crucially to the metastasis, tumorigenicity, and recurrence of various malignancies including OSCC. For this reason, they are regarded as the origin of cancer. Also, it is well known that OSCCs are usually preceded by OPMDs and the progression of OSCC requires sequential steps which provides an ideal opportunity for early cancer detection and interception. Hence, it is necessary to increase knowledge of the molecular features and signalling paths specific to the oral CSCs and their markers to develop new targeted and efficient treatments for head and neck cancer. Thus, a comprehensive assessment of cancer stem cell markers concerning the progression of OPMDs to oral cancer is performed in this review. From this review, it is understood that no single marker can be efficiently used to identify the CSCs in OPMDs. However, a combination of different markers will be very helpful in this aspect. Large cohort studies with long term follow-up are the need of the hour.

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