MINI-REVIEW

Cancer Stem Cells and Stemness Markers in Oral Squamous Cell Carcinomas

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Abstract

Head and neck squamous cell carcinoma (HNSCC) is one of the world top ten most common cancers with its highest occurrence in the Indian subcontinent and different aggressive and etiological behavioural patterns. The scenario is only getting worst with the 5 year survival rates dropping to 50%, persistent treatment failures and frequent cases of relapse/recurrence. One of the major reasons for these failures is the presence of cancer stem cells (CSCs), a small population of cancer cells that are highly tumourigenic, capable of self-renewal and have the ability to differentiate into cells that constitute the bulk of tumours. Notably, recent evidence suggests that cancer stem cells are especially resistant to conventional therapy and are the "drivers" of local recurrence and metastatic spread. Specific markers for this population have been investigated in HNSCC in the hope of developing a deeper understanding of their role in oral cancer pathogenesis, elucidating novel biomarkers for early diagnosis and newer therapeutic strategies. This review covers the fundamental relevance of almost all the CSC biomarkers established to date with a special emphasis on their impact in the process of oral tumourigenesis and their potential role in improving the diagnosis, prognosis and treatment of OSCC patients.

Keywords: Oral squamous cell carcinoma - cancer stem cells - stemness markers - self-renewal and proliferation

Asian Pac J Cancer Prev, **15 (20)**, 8549-8556

Introduction

Head and Neck Squamous Cell Carcinoma (HNSCC) is the 8th and 13th most common malignancy in the world for males and females, respectively, with a predominance of oral squamous cell carcinomas (OSCC) (Warnakulasuriya, 2009; Scully and Bagan, 2009; Nicole et al., 2010). The highest incidence of OSCC is found in India due to the increased preponderance of lifestyle habits like chewing tobacco, betel quid and areca-nut, which are the most important risk factors (Facompre et al., 2012). According to The Gujarat Cancer & Research Institute Registry, the scenario is worst in Gujarat because 53.65% in males and 15.64% in females of all cancers are found to be Tobacco Related Cancers (TRCs) (National Cancer Registry Report, 2008). Despite the recent advances in first line treatments, the 5 year survival rate after treatment remains disappointingly low at about 15-50% for the past 3 decades (Carvalho et al., 2005; Prince and Ailles, 2008; Warnakulasuriya, 2009; McCullough et al., 2010). The resultant poor prognosis is owed to a low response rate to current therapeutic strategies, late stage diagnosis, high risk of primary site recurrence and aggressive metastases to loco-regional lymph nodes, strongly suggestive of an urge to improve the diagnostic capabilities and treatment efficacy.

Increasing experimental evidence supports the CSC model in OSCC, which is in favor of a small proportion of cells with the capability of sustaining tumour formation, tumour growth, self-renewal & differentiation in a tumour type and context dependent manner. These CSCs have a probable role in resistance to therapy, establishment of metastasis and recurrence which is allusive of the fact that targeted elimination of these CSCs can be a new conceptual framework for oral cancer treatment (Prince et al., 2007; Satpute et al., 2013).

Cancer Stem Cells

CSCs are a small subpopulation of cancer cells that have a unique ability of self-renewal and are potent to differentiate into progenitor cells. The fundamental characteristics which segregate CSCs from other stem cells are - The ability to initiate and regenerate the tumour, representing a phenocopy of the original tumour, from a limited number of cells. In addition, these cells exhibit *in vivo* self-renewal capability and demonstrate a unique capacity to differentiate into various lineages, allowing them to give rise to a heterogeneous progeny (Chen, 2009).

Dick and collaborators provided early evidence for CSCs using leukemia models which demonstrated that on transplanting human AML *CD*34+ *CD*38- cells into

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non-obese diabetic severe combined immuno-deficient (NOD/SCID) mice induces leukemia (Lapidot et al., 1994; Bonnet and Dick, 1997).The Clarke laboratory revealed the presence of CSCs in solid tumours (breast cancer) for the first time, using a *CD*44+ *CD*24- Lin- marker phenotype (Al-Hajj et al., 2003). Since then, CSCs have been isolated in hematopoietic malignancies and several solid tumours including breast, brain, prostate, lung, colon, pancreas, liver, melanoma, skin and head & neck (Visvader and Lindeman, 2008). Collectively, these studies strongly suggest that CSCs are highly tissue specific and establishment of a universal CSC marker is questionable (Visvader and Lindeman, 2008).

The resistance to current modalities of treatment such as chemotherapy and radiotherapy is owed to the CSC subpopulation's ability to orchestrate recurrence and facilitate metastasis, which has significant treatment implications (Bradletz et al., 2005; Davis et al., 2010; Sun and Wang, 2011). Hence, CSC hypothesis demands modifications in therapeutic applications & measurement of treatment success. Understanding the importance of the CSCs as prospective biomarkers and therapeutic targets, their isolation and characterization have been accomplished using various techniques. The isolation of CSCs using flow cytometry and anchorage-independent culture assay are widely used approaches (Bradletz et al., 2005). Techniques such as dye exclusion assays have been employed to isolate side populations from tumour tissues. The gold standard established for the quantification of these tests is a serial animal transplantation model wherein the identified cell must be able to recapitulate/reiterate the generation of a constantly growing tumour. The expression patterns of "stemness" genes in CSC populations were analyzed in several studies using reverse transcriptase polymerase chain reaction (RT-PCR) techniques. Several reports provide important insights and evidence of the role of CSCs in tumour initiation and progression, however there is currently no ideal assay for the identification of CSC (Bradletz et al., 2005; Davis et al., 2010).

This review aims to discuss the putative OSCC CSCs currently being explored and provide evidences for their potential role as probable novel diagnostic, prognostic markers or therapeutic targets.

Identification of OSCC Csc Markers

CD44

*CD*44 is the most familiar CSC marker that has previously been identified in various solid malignancies such as breast, CNS, colon, prostate and pancreas (Mishra and Verma, 2010). In both HNSCC cell lines and primary tissues, *CD*44+ subpopulation demonstrated its tumourigenic potential, tumour sphere formation and chemo-resistance. The positive population of these cells was also found to over express certain stemness markers like Bmi1 that maintains the undifferentiated state of the cell (Prince et al., 2007). *CD*44 expression individually negatively correlated with poor 5 year survival while its high levels along with *ALDH* and phosphorylated *STAT3* correlated with high-grade of HNSCC which is consistent with the previous findings in urothelial carcinoma (Chen et al., 2010; Keymoosi et al., 2014).

Since it is equally expressed in carcinoma and normal head and neck epithelium, the use of *CD*44 as a marker has been debatable. Inspite of this, we cannot refute that *CD*44 either alone or in combination can be considered 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 validated (Chikamatsu et al., 2011; Keymoosi et al., 2014).

CD133

*CD*133 (*prominin-1*) is a putative CSC marker that has been characterized in epithelial cells and in somatic stem cells from neural tissues, prostate, kidney, colorectal, liver, skin and lung (Chikamatsu et al., 2011)). In HNSCC & OSCC, *CD*133+ cells displayed increase in clonogenicity, EMT phenotype, tumour sphere formation, self-renewal, proliferation, differentiation, higher levels of stemness genes and tumourigenicity (Wu and Wu, 2009).

Higher levels of *CD*133, have been associated with *CD*44+ expression in HNSCC and with Bmi1 induced proliferation in laryngeal carcinomas (Zhang et al., 2010; Chen et al., 2011; Sun et al., 2012). In fact, positive correlation of *Oct*-4, *Nanog* with an increased expression status of *CD*133 depicted a poorer prognosis for oral cancer patients (Chiou et al., 2008).

Further investigation is mandatory to validate the inconsistency showing similar tumour-initiating behaviour between *CD*133+ and *CD*133- populations (Shmelkov et al., 2008; Zhang et al., 2010). Hence, *CD*133 might serve as a useful CSC marker in OSCC cases to identify patients that are resistant to conventional chemotherapy with paclitaxel.

ALDH

Aldehyde dehydrogenase (*ALDH*) comprises of 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 carboxylic acid (Chen et al., 2009). *ALDH* expression was identified in solid malignancies such as breast, colon, liver, and lung tumours (Madjd et al., 2012). Later it was observed that *ALDH*+ cells maintained consistent behavior with OSCC CSCs holding a high capability of sphere formation, tumour formation, increased invasion, self-renewal and resistance to chemotherapeutics (Ginestier et al., 2007; Chen et al., 2009; Clay et al., 2010). In OSCC, increased levels of *ALDH* correlated with disease staging, radio-resistance and negative correlation with patient outcome (Chen et al., 2009).

Combination of *ALDH* with *CD*133 + and *CD*44+ markers facilitated isolation of highly tumourigenic sub-population, therefore exhibiting features of CSCs in OSCC (Chen et al., 2009). Interestingly, the knockdown of Snail decreased the expression of *ALDH* which inhibits cancer stem-like properties of *CD*44+ *CD*24- *ALDH*+ cells, thus exploring the therapeutic aspect along with the prognostic value of this marker (Chen et al., 2009).

c-Met

c-*Met* is a proto-oncogene that encodes for hepatocyte growth factor (HGF) tyrosine kinase receptor. Normally only stem cells and progenitor cells express *Met*, however, CSCs seize this ability (from the normal stem cells) associating its expression with metastasis and tumour invasion, decreased survival and angiogenesis in various neoplasms. In HNSCC, c-*Met*+ cells demonstrated self renewal and were able to generate heterogeneous tumours with more tumourigenic potential than by *CD*44+ marker. Also, c-*Met*+ /*CD*44+ combination yielded tumours in 80% of cases, while c-*Met*+/*ALDH*1+ displayed tumour formation in 66% cases (Sun and Wang, 2011). 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 give a clear depiction (Sun and Wang, 2011).

Side Populations (SPs)

Identification of CSCs is widely done by the side population approach which involves the elimination of Hoechst 33342. Hoechst 33342, a fluorescent DNA-binding dye, preferentially binds to A-T rich regions of tumourigenic cells. These SPs express high levels of the ATP-binding cassette (*ABC*) transporter superfamily (e.g. MDR1, MRP1, *ABC*B5, *ABC*G2) that facilitates the efflux of this dye and other drugs (Zhang et al., 2009).

In recent years, SP cells have been characterized in HNSCC as highly tumourigenic, metastatic and aggressive cells with stem-like phenotype. These HNSCC SP cells showed higher expression of stem cell related genes such as *Oct*-4, CK19, Bmi1 & *CD*44—and lower expression of genes such as involucrin & CK13 which are associated with a differentiation status (Tabor et al., 2011). Presence of *ABC*G2 is considered to be a marker for oral leukoplakia and high *ABC*B5 expression has been associated with OSCC progression and recurrence making them possible prognostic factor (Liu et al., 2012; Grimm et al., 2012).

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 interacting with other transcription factors (*STAT3*, HesX1, Zic3) and critical cell signaling molecules (TCF3, FGF2, LEFTY2).

Over expression of *Oct*-4 and *Nanog* genes, found in CSC-enriched subpopulation derived from HNSCC sphere formation colonies, positively correlated with treatment failure and stage while negatively correlated with differentiation status (Tsai et al., 2011; Vaiphei et al.,2014). *Oct*-4, individually was found to be competent enough to up regulate *ALDH*1+ in HNSCC cells while in combination with TRA1-60 (a tumour rejection antigen) were detectable as indicators of invasiveness. Furthermore, it was demonstrated that patients displaying a triple-positive expression of *Oct*-4, *Nanog* and *CD*133 had the worst survival prognosis in OSCC, indicating their usefulness as an invasiveness and predictive marker (Siu et al., 2012). *Sox2* has increased expression specifically in squamous cell carcinomas of the lung and esophagus, but not in the lung or esophageal adenocarcinomas (Bass et al., 2009), which suggests 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 found in the upstream of Akt in pre malignant lesions. It is a negative regulator of the cell cycle by repressing genes like p53 that promote proliferation and by activating genes like p21 (Bonner et al., 2006). *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 (Mao et al., 2004; Lu et al., 2006). The frequent loss of *Klf4* expression in gastric and colorectal cancers has led to its characterization as a tumour suppressor. Conversely overexpression of *Klf4* depicts the oncogenic feature of the gene which is observed in the skin, breast and OSCC (Marta et al., 2009).

In HNSCC, *Klf4* over expression was correlated with a worse disease-free survival of patients while in tongue squamous carcinomas enforced *Klf4* expression demonstrated increased in-vitro migration abilities, multi-drug resistance and *in vivo* tumourigenicity. Moreover the *ALDH*+ SP cells of nasopharyngeal carcinoma showed higher expression of stemness genes *Oct*-4, Bmi1, *Sox2* and *Klf4*. Recent reports state that the transcription factors Notch1 and *Klf4* together confer 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 (Marta et al., 2009).

Bmi1

Bmi1 is considered to be a stemness related gene and an essential constituent of the polycomb repressive complex 1 which is a key epigenetic regulator. It regulates a number of biological processes, including X chromosome inactivation, carcinogenesis, stem cell renewal also promotes cellular proliferation by modifying the chromatin and histone structures and influences central tumour suppressors Rb and p53 (Chen et al., 2011).

Insights into the role of Bmi1 in HNSCC was exemplified by reports stating that Bmi1 over expression in an *ALDH*1+ subpopulation increased tumour formation, tumour size, soft agar colony formation, migration, local invasion, distant metastasis to lungs and radio resistance. In addition, its elevated co expression with Snail, *ALDH* and embryonic stem cells was correlated with poor overall survival and high-grade, poorly differentiated HNSCC[44]. This suggests that presence of Bmi1 can be used as a predictive marker of cancerous transformation and progression of oral leukoplakia lesions (Liu et al., 2012).

Surprisingly in tongue squamous cell carcinoma, negative Bmi1 expression was associated with high

recurrence. This discrepancy of tongue SCC could possibly be due to the varying patho-physiologies and etiologies in HNSCC (Chiou et al., 2011). Despite this inconsistency, Bmi1 plays a considerable role in HNSCC & OSCC tumourigenesis but its suitability as a CSC marker is yet to be defined.

Lgr5/GPR49 (G-protein coupled receptor 49)

*Lgr*5, a seven-transmembrane-domain receptor protein, has been identified as a marker for adult stem cells in intestine, stomach, and hair follicle. *Lgr*5+ cells were identified to fuel stem cell activity through erroneous activation of Wnt signaling pathway, leading to cytoplasmic β-*catenin* accumulation which has been associated with tumourigenesis (Haegebarth and Clevers, 2009).

Hence, it has been established as a 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 associated 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 (Haegebarth and Clevers, 2009; Rahden et al., 2011).

This implies that Lrg5 is a tumour suppressor gene whose main role is delimiting stem cell expansion in their respective niches. Given that, *Lgr*5 as a candidate marker driving towards better prognostic and therapeutic implications in OSCC requires further investigation into its behavioral and expression patterns (Yamamoto et al., 2003).

CD117 (c-KIT)

*CD*117, 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 *CD*117+ stem cells and other mesenchymal stem cells (Radisky and LaBarge, 2008; Chikamatsu et al., 2011). *CD*117 was not identified as a CSC marker in OSCC, until recently when presence of *CD*117 was found in more than half of OSCC cell lines and primary cultured cells. In addition, data regarding OSCC reactivity to *CD*117 are few and contradictory. While one study suggests that *CD*117+ expression was observed in basal tongue SCC while other reports were contradictory to these findings suggesting that *CD*117+ expressions were limited to stromal spindle cells in OSCC (Yu et al., 1997; Yu and Stamenkovic, 1999). In any case, these cells were trypatase+, antivimentin+ and infrequently for CSC marker antibodies like *CD*44 & *CD*133.

EpCAM/ CD326

The epithelial cell adhesion molecule (EpCAM; *CD*326) 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 (Winter et al., 2003; Nübel et al., 2009).

Interestingly, expression of *EpCAM* has also been identified in pancreatic cancer (EpCAM- cells), hepatocellular and breast (both for EpCAM+ cells). In HNSCC, increased *EpCAM* expression was observed from hyperplasia to tumour giving clues about its role in oral carcinogenesis (Maetzel et al., 2009; Bernardina et al., 2009). Most studies did not find any association of this marker with clinic-pathological parameters but a study specifically on tongue SCC demonstrated a direct relationship between *EpCAM* expression with larger tumour size, nodal metastasis and tumour dedifferentiation.

Contradictorily, recent reports on OSCC associated decreased *EpCAM* expression with larger tumour size and presence of nodal metastasis. These adverse findings might be due to the diverse etiological factor, with areca quid increasing tumour necrosis factor-α production and therefore down-regulating *EpCAM* (Jeng et al., 2003). Inconsistent reports and conflicting associations of *EpCAM* expression in OSCC might be attributable to the heterogeneity of tumours; however, it is clear that EpCAM, is an additional marker for cancer initiating cells, thus having a great diagnostic and prognostic characteristics/potential (Bernardina et al., 2009).

Other Markers

Considerable efforts have been made to identify and characterize cancer stem cell and stemness markers in various tissue types. As a result there is a steady increase in the number of such markers which cannot be clearly categorized into earlier section either due to less frequency or lesser specificity. These markers are therefore listed herewith as other markers (Table 1).

Discussion

Cancer stem cells, a subpopulation of tumourigenic cells, and 'Stemness' markers are responsible for regulating the tumourigenesis, proliferation, aggressiveness, chemo-radioresistance, recurrence and metastasis of OSCC. Cellular heterogeneity in terms of altered pathophysiology and differential expression of various stem cell markers (*CD*44, *CD*133 and *ALDH*1) and transcription factors e.g. *Oct*4, *Sox2*, *Nanog* play a significant role in clonal proliferation. Isolation of CSCs from tumour specimen based on their cell surface markers followed by spheroid culture (oro-spheres) will be useful in enrichment and establishment of cell lines which may provide in-vitro model systems that mimic the functional characteristics of stem cells in the tumour microenvironment and their probable response to therapy.

Majority of research work in OSCC stem cell and stemness markers have been carried out in Non-Asian population where smoking is major etiological factor but a study on these markers also needs to be accomplished in Asian population where tobacco chewing is the major lifestyle habit. This can be achieved by using Next

Table 1. Other Important OSCC CSC Markers with their Functional Relevance

Markers	Findings	References
Musashi-1 (Msi-1)	-Msi-1,a RNA binding post transcriptional gene regulator, is associated with both stem cell and tumour biology and has recently been correlated with OSCC. -In OSCC, over expression is correlated with advanced stage of disease and poor differentiation of tumours. -Positive correlation is found between the expression of CD133 and Msi-1 genes in OSCC.	Ravindran and Devaraj, 2012; Aidan et al., 2013
CD97	-It is an EGF - 7 transmembrane surface protein which co-localizes within the basal cell layer of the oral squamous epithelium and its derivatives. -CD97+ β1--integrin-positive cells are highly expressed in BM cells, undifferentiated thyroid carcinoma and dedifferentiated (Grade 3) OSCC. -Their potential as an OSCC marker will be based on whether they express other known stem-like markers or possess higher proliferative potential.	Sabine et al., 2008)
Cripto-1	-Cripto-1, an extracellular GPI anchored signaling protein,is a key regulator of embryonic development and a marker of undifferentiated human ESC. -High expression of Cripto-1 is found in various malignancies including colon, gastric, cervix and pancreatic, modulating cancer proliferation, angiogenesis, migration and EMT. -In OSCC, it found to play a vital role in malignant transformation, progression and reprogramming into CSCs, thus having the potential of being identified as a putative marker.	Normanno et al., 2004; Yoon et al., 2011
Bone morphogenetic proteins (BMPs)	-BMPs play a diverse role in various biological processes by secreting pivotal morphogenetic signals through the BMP/SMAD pathway. -It regulates proliferation, differentiation, and apoptosis during development and plays a crucial role in adult tissue maintenance, remodeling, repair and deregulation, leading to malignant transformation. -In OSCC, BMP-4 induces EMT with acquisition of stem cell like behaviour in cell culture models,elevates the expression of CD44, ABCG2, Bmi-1, hTERT& Oct-4 and down-regulates E-cadherin expression. -Increased expression of BMP-2 led to increased proliferation and angiogenesis both in TSCC & OSCC and high levels of BMP-6 is found to be associated with bone invasiveness in OSCC. -Collectively, these findings suggest that BMPs can be implied as a transient therapeutic opportunity to interrupt OSCC in an early phase.	Gao et al., 2010; Qiao et al., 2011; Kejner et al., 2013
Chondroitin sulfate proteoglycan 4 (CSPG4)	-CSPG4 is a unique glycoprotein proteoglycan complex that has been implicated in melanoma, sarcoma and various carcinomas.	Campoli et al., 2010; Wang et al., 2010
CXCR4	-CXCR4 is a chemokine receptor found to play an important role in several cancers. -In OSCC, it promotes migration and invasion by regulating MMP-9 &13 via ERK signaling pathway. -Several studies state that this marker serves as an independent prognostic marker of aggressiveness, invasiveness and EMT both in OSCC & TSCC. -Recent report suggests its role in pre malignant transformation, thus indicating its role as a biomarker in early detection of cancer.	Meng et al., 2010; Albert et al., 2012
CD166 (ALCAM)	-Recently recognized as a potential membrane associated stem cell marker. -In HNSCC, over expression of CD166+ cells demonstrate a greater sphere formation ability in vitro, tumour formation ability in vivo and are positively correlated with poor patient outcome & higher tumour recurrence rates. -The consistency and clinic-pathological correlation of CD166 with oral carcinogenesis is higher than CD44 making it a valuable cell surface marker for the enrichment of HNSCC stem cells.	Yan et al., 2013
SLC2A13	-SLC2A13, a solute carrier protein family member, facilitates glucose transport -Its CSC behavioural patterns were witnessed in non-small cell lung and breast cancers -Consistent over expression of SLC2A13 is observed in sphere forming cells of primary cultures of OSCC samples	Bankovic et al., 2010; Massimo et al., 2012
Podoplanin	-It co-localizes with Nestin that is a protein expressed primarily in neural tissues. -In all SCCs, Podoplanin+ tumours demonstrated significantly better patient survival while its expression with ABCG2 facilitated predicting cancer progression in 90.9% of erythroplakic lesions. -Thus, it may be useful as a prognostic marker to monitor the development, progression and risk in HNSCC patients.	Shimada et al., 2009
Nestin	-Found to be over expressed in oral squamospheres and demonstrates simultaneous increased expression of ABCG2 -It shows inter-relationship between PARP-1, CAF-1/p60 -It is up-regulated in metastasizing samples of OSCC, hence depicting a positive correlation with the aggressiveness of oral tumourigenesis	Lim et al., 2011; Vincent- Chong et al., 2012
CD29/β1 integrin	-CD29 is an integrin unit associated with very late antigen receptors. -Combination of CD29high/CD44high cells can be used as markers to enrich CSCs in human SCC as they exhibit molecular characteristics of EMT, suggesting that CSC-associated pathways were involved in EMT -Studies on correlation of CSCs and the cells undergoing EMT may explain some aspects of tumour progression and drug resistance.	Geng et al., 2013

p63	-TP63 is a member of the p53 family of transcription factors -It is strongly expressed in the innermost basal layer of epithelial cells with high clonogenic and proliferative capacity and plays a vital role in altered stem cell regulatory pathways (p63) -It encodes for two main isoforms by alternative promoters (1) ΔNp63: involved in adult stem/progenitor cell regulation (2) TAp63:involved in regulating the apoptotic function -In HNSCC, ΔNp63 isoform leads to the up-regulation of keratins 6A and 14 and down-regulation of keratins 4 and 19. -A study showed that p63 directly binds to the CD44 promoter, but found that over expression of p63 specifically increased expression of CD44 lacking variant exon 2, indicating its role in the regulation of adhesion, metastasis and the cancer stem cell phenotype.	Boldrup et al., 2007; Soundarya et al., 2008
hTERT	-GRP78 plays a crucial role both in stem cell and cancer by mediating tumour proliferation, metastasis and conferring resistance to treatment -It is overexpressed in many malignancies including lung, breast, stomach, prostate, colon, liver cancer and multiple phenotypes of HNSCCs -ALDH1+ cells in head and neck – Cancer Initiating cells (HN-CIC) showed increased GRP78 anchored at the plasma membrane (memGRP78+), exerting cancer stemness properties of self-renewal, differentiation and radio-resistance -Co-expression of either CD133 or Cripto-1 with memGRP78 in comparison to parental HNSCCs confirmed the above findings Clinically, co-expression of GRP78 and Nanog predicted the worse survival prognosis of HNSCC patients -Thus, memGRP78 should be a novel surface marker for isolation of HN-CICs and targeting GRP78 signaling might be a potential therapeutic strategy for HNSCC through eliminating HN-CICs.	Meng Ju et al., 2010
GRP78	-GRP78 plays a crucial role both in stem cell and cancer by mediating tumour proliferation, metastasis and conferring resistance to treatment -It is overexpressed in many malignancies including lung, breast, stomach, prostate, colon, liver cancer and multiple phenotypes of HNSCCs -ALDH1+ cells in head and neck – Cancer Initiating cells (HN-CIC) showed increased GRP78 anchored at the plasma membrane (memGRP78+), exerting cancer stemness properties of self-renewal, differentiation and radio-resistance -Co-expression of either CD133 or Cripto-1 with memGRP78 in comparison to parental HNSCCs confirmed the above findings -Clinically, co-expression of GRP78 and Nanog predicted the worse survival prognosis of HNSCC patients -Thus, memGRP78 should be a novel surface marker for isolation of HN-CICs and targeting GRP78 signaling might be a potential therapeutic strategy for HNSCC through eliminating HN-CICs.	Hiroshi et al., 2013
p75	-p75 is a low affinity neurotrophic receptor that binds to NGF and plays a critical role in protecting stem cells from apoptosis and affect cell growth -The presence of this progenitor stem cell marker was observed and characterized from explants cultures of oral epithelial cells along with other similar markers like p63, ABCG2 & CD29	Sen et al., 2011
HMGA2	-HMGA2 is a transcriptional factor initiating mesenchymal tumour formation -In OSCC, HMGA2+ has sphere initiation and colony formation capabilities, leading to the repression of CD24 (adhesion molecule) which is associated with other cancers but has no direct relevance with head and neck cancers -It is associated with decreased disease free survival, thus showing potential of being a good tumour progression marker.	Hiroshi et al., 2013

Generation Sequencing and microarray platforms to establish a tumour specific gene expression, miRNA and methylome profiles in OSCC (Poage et al., 2012; Huang et al., 2013). Comparing the gene expression profile of OSCC stem cell with the tumour cells as controls may provide a differential expression pattern which may be useful in establishing the signature expression profile for OSCC stem cells. These set of genes may be utilized as novel diagnostic or prognostic markers and potential therapeutic target for better management of OSCC patients in the future. The epigenetic modulations such as promoter methylation, histone modification and miRNA dynamics also needs to be explored as potential confounders in oral carcinogenesis.

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