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Review

CURRENT CANCER STEM CELL BIOMARKERS IN TONGUE SQUAMOUS CELL CARCINOMA

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Abstract: Tongue squamous cell carcinoma (TSCC) is known to be the most malignant cancer type amongst other oral cancers with increasing incidence and mortality rates in the past five years. Since the life expectancy for TSCC patients is limited and the current chemo-radiotherapy treatments are not curative, novel biomarkers are urgently needed. As many other solid tumors, TSCC has a heterogeneous cancer cell population, which includes a small subpopulation identified as cancer stem cells (CSCs) that are considered as the driving force for tumor initiation, development, spread, recurrence, and resistance to chemo-radiotherapy. Although the underlying molecular mechanisms of how CSCs are involved in the carcinogenesis are not completely understood, scientists and clinicians aim to utilize those cells as therapeutic tools in fight against different cancer types including TSCC. Here, we reviewed and summarized important findings and the most current literature to shed light on the potential of cancer stem cells markers in TSCC. Possible functions of CSCs biomarkers in TSCC pathogenesis during cancer initiation, progression, invasion or metastasis are also summarized.

Key words: Tongue squamous cell carcinoma, cancer stem cell, biomarker.

Özet: Dil skuamöz hücreli karsinomu (DSHK) son 5 yılda artan insidans ve mortalite oranları ile birlikte diğer ağız içi kanserleri arasında en kötü huylu kanser tipi olarak bilinmektedir. DSHK hastalarının yaşam süreleri sınırlı olduğundan ve mevcut kemo-radyoterapi yaklaşımlarının başarılı olmamasından dolayı acilen yeni biyolojik belirteçlere ihtiyaç duyulmaktadır. Diğer solid (katı) tümörlerde olduğu gibi, DSHK'da da, tümörün başlaması, gelişmesi, yayılması, nüksetmesi ve kemo-radyoterapiye direnç göstermesi noktasında itici bir güç olarak kabul edilen "kanser kök hücreleri" (KKH) olarak tanımlanan küçük bir alt popülasyonu da kapsayan heterojen bir hücre popülasyonuna sahiptir. KKH'nin kanser oluşum sürecinde nasıl bir katkı yaptıklarının altında yatan moleküler mekanizmalar tamamen anlaşılamamış olmasına rağmen, bilim insanları ve klinik tedavi uzmanları DSHK da dâhil olmak üzere kansere karşı mücadelede bu hücreleri tedavi edici araçlar olarak kullanmayı amaçlamaktadır. Burada, DSHK'daki potansiyel kanser kök hücrelerinin belirteçlerine ışık tutmak için önemli bulguları ve en güncel literatürü gözden geçirdik ve derledik. Ayrıca, DSHK kanser başlangıcı, ilerlemesi, invazyonu ya da metastazı sırasında KKH biyo-belirteçlerinin olası fonksiyonları özetlenmiştir.

Introduction

Oral cancer is the sixth most frequent cancer type worldwide with an annual global incidence of 300.000 cases and 130.000 deaths (Brockton *et al.* 2017, T.W. Chen *et al.* 2017 Daigo *et al.* 2018, Iqbal *et al.* 2017, Yu & Li 2016). Tongue squamous cell carcinoma (TSCC) is a very aggressive disease and is the most common cancer type of the oral cavity making up almost 40% of all oral carcinomas (Cui *et al.* 2017). TSCC is also known to be the most malignant cancer type amongst other oral squamous cell carcinomas and causes speech, mastication and deglutition problems (J. Chen & Li 2017, Zhang *et al.* 2017). Although overall cancer incidence and mortality rates reduced in the past five years, incidence and mortality rates of TSCC increased significantly within the same period (Siegel *et al.* 2016). In addition, contrary to improvements in multimodal diagnosis and treatment techniques in the past few decades, the 5-year survival rate for TSCC stayed almost unchanged, which makes it one of the most lethal cancer types seen in head and neck region (Xing *et al.* 2013). Therefore, novel early diagnostic and prognostic markers are urgently needed for TSCC since the life expectancy for patients is very limited and the current chemo-radiotherapy treatments mostly fail to give positive clinical outcomes (Alam *et al.* 2017 Boldrup *et al.* 2015).

As in many other solid tumors, TSCC has a heterogeneous cancer cell population (Weng *et al.* 2017) which includes a small subpopulation identified as cancer stem cells (CSCs). These stem cells are thought to be responsible for tumor initiation, development, spread,

recurrence, and resistance to chemo-radiotherapy. Therefore, their identification, isolation, and characterization are of paramount importance to enhance the success of treatment of TSCC through development of CSCs targeted therapy techniques.

Here, we reviewed and summarized important findings and the most current literature to shed light on the potential CSCs markers in TSCC. Possible functions of these CSCs biomarkers in TSCC pathogenesis during cancer initiation, progression, invasion or metastasis were summarized.

Presence of CSCs in TSCC

CSCs were initially identified in leukemia through isolation of a subpopulation with tumor initiating potential, which suggested that tumors are comprised of hierarchically organized heterogeneous cell populations (Vlashi & Pajonk 2015). Further studies conducted with pediatric brain tumors demonstrated the presence of CSCs for the first time in solid tumors (Hemmati *et al.* 2003).

CSCs were shown to have essentially similar properties as normal stem cells in terms of their self-renewal, unlimited proliferation, and differentiation potentials. CSCs and normal stem cells utilize similar signaling pathways (Dawood *et al.* 2014) and both have the ability to activate anti-apoptotic signals and have increased telomerase activity (D'Arcangelo *et al.* 2015).

CSCs are often the main source of genetic abnormalities for malignant transformation (Li *et al.* 2015) and unlike normal stem cells, they initiate and propagate tumor when they are transplanted into immunodeficient model organisms and take an active role in metastasis, recurrence, and resistance of tumor to chemo-radiotherapy. As a result of these features, CSCs are referred as "tumor-initiating cells" or "tumorigenic cells". Surface markers such as CD133, CD24 and CD44 are typically used for isolation and identification of CSCs (Dawood *et al.* 2014).

In the last decade, researchers demonstrated the presence of CSCs in TSCC through isolation and characterization of specific cell populations expressing certain surface markers such as CD133 and CD44 (Sun *et al.* 2012). CD133 and CD44 expressing cells were demonstrated to have increased tumorigenic potential both *in vitro* and *in vivo* (Saleem *et al.* 2014, Wang *et al.* 2016). Another recent study identified two different cancer stem cell subpopulations, p63+/NANOG+/SOX2+/SALL4+/pSTAT3+/OCT4- and p63-/NANOG-/SOX2-/SALL4-/pSTAT3-/OCT4+ in moderately differentiated TSCC (Baillie *et al.* 2016). Besides, ALDH1 positive cells exhibited increased proliferation and differentiation capacity compared to ALDH1 negative cells in Tca8113 cell line, which confirmed the presence of cell clusters showing CSCs properties in human TSCC (Zou *et al.* 2012).

However, further efforts are needed for elucidation of genetic and epigenetic circuits modulating the stem cell features of CSCs to help understanding the molecular basis of carcinogenesis.

CSCs Surface Markers

CD44

CD44 and its variants are among the well-described and studied biomarkers in TSCC (Kunishi *et al.* 1997, Sato *et al.* 2000). CD44, located on chromosome 11, encodes for a conserved transmembrane glycoprotein which is necessary for the maintenance of tissue integrity. It has been shown to be deregulated in several cancers including TSCC (Lindquist *et al.* 2012, Yanamoto *et al.* 2014). CD44 has important roles during carcinogenesis and metastasis in TSCC and is involved in several carcinogenic processes like proliferation, migration, cell-cell interactions, and apoptosis in TSCC cells (Saleem *et al.* 2014, Wu *et al.* 2017). Its expression shows significant correlation with several other critical biomarkers such as CD24 (Baillie *et al.* 2017), E-cadherin (Bánkfalvi *et al.* 2002), MMP-9 (Kosunen *et al.* 2007), ABCG2 (Patel *et al.* 2014), CD117 and CD133 (Mărgăritescu *et al.* 2011) during initiation of tumor formation and induction of metastasis. CD44 was also proposed as a stem cell marker for various types of cancer such as breast cancer and head and neck squamous carcinomas (Bourguignon *et al.* 2017, Cruz Paula & Lopes 2017). Recent studies showed that CD44 expression is increased in tumor cells (Lindquist *et al.* 2012, Yanamoto *et al.* 2014) and some other studies demonstrated that CD44 expression has a negative correlation with tumorigenic potential of cancer cells (González-Moles *et al.* 2004, Sato *et al.* 2000).

The expression of CD44 in TSCC samples was initially investigated in 1996, where 83 head and neck squamous cell carcinoma (including 12 TSCC specimens) samples were used for immunohistochemical analysis of CD44 variants (v5, v6, v7, v7-v8, and v10) in normal, dysplastic squamous epithelia, primary and metastatic squamous cell carcinoma samples. CD44 v7, v8, and v10 were reported to be significantly downregulated in primary tumor tissues and were not detectable in most of the metastasis-derived specimens. On the other hand, v5 and v6 of CD44 displayed no significant alteration between normal and tumor samples (Herold-Mende *et al.* 1996). However, in the study of Kunishi (1997), expression level of CD44v6 was found to be significantly reduced in tumor samples when compared to normal healthy mucosa. Decreased expression of CD44v6 was also associated with regional lymph node metastasis. Interestingly, although no significant correlation was found between the expression of CD44v6 and level of differentiation, poorly differentiated carcinoma samples tended to express reduced level of CD44v6 (Kunishi *et al.* 1997). In the following years, reduced expression of different CD44 variants was associated with different clinical parameters such as CD44H with late nodal metastases following interstitial brachytherapy (Masuda *et al.* 2000), CD44v9 with lymph node metastasis and poor survival of patients (Sato *et al.* 2000), CD44 v3, v4-5, and v6 with cell differentiation, tumor grade, and the pattern of neoplastic invasion (Fonseca *et al.* 2001), CD44v6 with high histological

grade (Cruz *et al.* 2009) and CD44 with cervical lymph node metastasis (Mostaan *et al.* 2011). CD44 expression was also demonstrated to be profoundly reduced in TSCC compared to other tumors within the oral cavity (Krump & Ehrmann 2013). Furthermore, loss of CD44 expression in normal epithelia of tongue was suggested as an early biomarker for tongue carcinogenesis (González-Moles *et al.* 2004) and its expression was demonstrated to be strongly reduced in tongue tumors in comparison to other sub-sites within the oral cavity (Krump & Ehrmann 2013).

The first findings about the elevated expression of CD44 in TSCC were obtained in the study of Järvinen *et al.* (2008) where the authors characterized genome-wide copy number and gene expression alterations on microarrays for 18 TSCC cell lines. They identified several high-level amplifications including 11p12-p13 region where CD44 gene is localized. In addition, high intensity CD44 staining was demonstrated as a strong indicator of poor prognosis (Lindquist *et al.* 2012) and was significantly associated with regional lymph node metastasis, pattern of invasion, depth of invasion, perineural invasion and local recurrence (Yanamoto *et al.* 2014).

CSCs were reported to be enriched in the highly invasive UM1 cell line which is strongly positive for CD44 expression but were not present in the less invasive UM2 cell line (Misuno *et al.* 2013). In a recent study, where 4-Nitroquinoline 1-oxide was used to induce tongue cancer in mice, CD44 expression along with CD133 was found to be slightly overexpressed in dysplasia group compared to normal rats (Lim *et al.* 2014). Furthermore, CD44 and CD133 expressions were found to be strongly overexpressed in 4-Nitroquinoline 1-oxide induced TSCC in addition to several other cancer stem cell markers including ALDH1, Nanog and OCT-4. These findings point out the importance of CD44 as well as other cancer stem cell markers in the multistep carcinogenesis process of TSCC (Lim *et al.* 2014).

In another recent study, a cell line called UM-SCC-103 was developed by isolating highly aggressive tumor cells from a pregnant woman diagnosed with TSCC. Sorted CD44+ cells from UM-SCC-103 were able to induce carcinogenesis, whereas, CD44- cells failed to produce the overall heterogeneity of the primary tumor. This study suggested that CD44 is an important putative marker for tongue CSCs (Owen *et al.* 2014).

Saleem *et al.* (2014) used biopsy specimens from TSCC and its neck nodules for development of primary cell cultures and examined the growth and sphere formation capacity of those cells. They found the lowest CD44 expression in hyperplastic tongue tissue and the highest expression in neck node positive TSCC specimens. Cells with increased CD44 expression showed much higher sphere-forming capabilities compared to the corresponding CD44- cells. Interestingly, CD44+ cells from hyperplastic or non-cancer tongue tissue did not produce spheres. Besides, primary cultures from metastatic TSCC specimens showed stronger CD44/CD24 expression and produced much more spheres in significantly shorter duration compared to those of node negative TSCC and normal tongue specimens (Saleem *et al.* 2014).

Besides, injection of CD44+ sorted and labeled TSCC CSCs into the tongue of nude mice resulted in formation of highly metastatic tumors which were larger in size compared to CD44- and unsorted SCC-9 cells. Tumor formation efficiency was 100% in CD44+ group, but CD44- group showed slightly reduced tumor formation efficiency. Wu *et al.* (2017) also demonstrated that CD44 expression in human clinical samples was significantly higher in metastatic tumors than in primary tumor samples.

The evidence presented here demonstrates that CD44 expression has the potential to suppress and induce cancer progression. Discrepancies reported in the literature might stem partially from differences in the cell lines used, antibody variability, culture conditions, and other experimental variability. However, the conflicting data about the potential of CD44 as a stem cell marker needs further studies and clarification for its successful utilization in therapeutic approaches against TSCC.

CD133

Cluster of differentiation (CD) 133 (also known as AC133; human homologue of mouse Prominin-1) is a highly conserved pentaspan transmembrane glycoprotein encoded by *PROM1* on chromosome 4 (4p15.32) (Yanagisawa *et al.* 2005). It consists of 865 amino acids and has a total weight of 120 kDa. Its expression has been associated especially with progenitor/stem cells and differentiation (Li 2013). Subsequently, CD133 has been identified as a promising CSCs surface marker in several cancer types including larynx, lung, brain, skin, colon carcinoma and TSCC (Li 2013, Major *et al.* 2013, Suer *et al.* 2014, Wang *et al.* 2016, X. Yu & Li 2016) and it is currently considered as a universal marker of tumor-initiating cells (Li *et al.* 2014, Shrivastava *et al.* 2015). CD133 positive (CD133+) cells promote tumorigenesis, invasion, metastasis, and they are significantly related with drug resistance and disease relapse (Major *et al.* 2013, Wang *et al.* 2016).

CD133+ cells were initially identified in TSCC Tca8113 cell line by Kang *et al.* who showed that a small portion of Tca8113 cell line were positive for CD133, and these CD133+ cells represented high proliferation capacity compared to corresponding CD133 negative (CD133-) control Tca8113 cells (Kang *et al.* 2010). This study pointed the importance of CD133 as a putative CSCs surface marker for tumor-initiating cells of human TSCC (Kang *et al.* 2010). In another study carried out with Tca-8113 cell line, it has been shown that proliferative capacity and differentiation potential of CD133+ cells were profoundly higher than those of CD133- cells *in vitro* (Wang *et al.* 2016).

CD133+ cells were also characterized with their relatively strong tumorigenic potential *in vivo* (Wang *et al.* 2016). In a 4-Nitroquinoline 1-oxide-induced rat tongue carcinogenesis model, CD133 expression during the progress of multistep carcinogenesis was slightly increased in the dysplasia group compared with normal rats, but the expression level was significantly increased in rats with TSCC along with other important CSCs markers. This study proposed CD133 as a crucial CSCs surface marker for identification of CSCs with a high potential of oral carcinogenesis (Lim *et al.* 2014).

In a recent clinical study conducted by Mascolo *et al.* (2012) the expression level of CD133 in the oral squamous cell carcinoma samples mostly comprised of TSCC was significantly higher compared to normal oral mucosa samples.

Taking all these findings into account, CD133 has been suggested to serve as an important CSCs surface marker for isolation, identification and characterization of CSCs in TSCC patients, which might help development of novel targeted therapeutic approaches to enhance the quality of life and survival of patients.

Stemness Related Markers in TSCC

Several transcription factors including the reprogramming so-called 'the Yamanaka Factors' have been shown to play critical roles in acquisition and maintenance of self-renewal and pluripotency characteristics in embryonic stem cells via their interaction with other transcription factors as well as important signaling molecules (Boyer *et al.* 2005, Loh *et al.* 2006, Patel *et al.* 2014). Interestingly, the gene expression profiling of CSCs and the rest of the tumor burden pointed the differential expression of those stemness-associated genes in CSCs (Chen 2009, Zhang *et al.* 2012).

SOX2 and OCT4

Qiao *et al.* (2014) investigated the expression status of stemness markers SOX2 and OCT4 in the tumorigenesis process of oral mucosa by immunohistochemistry using both rat and human samples. They demonstrated that individual expression and co-expressions of SOX2 and OCT4 were detected in normal oral mucosa, premalignant diseases, primary and metastatic sites of oral squamous cell carcinoma. However, co-expression of SOX2 and OCT4 in transforming oral mucosa cells were suggested to contribute to the malignant transformation of oral mucosa (Qiao *et al.* 2014). In another study performed in 2016, lentiviral transduction of Sox2 and Oct4 together into immortalized oral epithelial cells triggered formation of neoplasms in immunodeficient mice, but their individual introduction into immortalized oral epithelial cells did not result in tumor formation. Furthermore, injection of Cal27 cells, with simultaneous knockdown of Sox2 and Oct4, into immunodeficient mice caused reduced tumor size. Besides, positive expressions of OCT4 and SOX2 were preferentially located in the nucleus of tumor cells obtained from 51 TSCC patients (Jiang *et al.* 2017). These findings strongly indicate that both SOX2 and OCT4 might be required for reprogramming of cancer stem cells for induction of oral carcinogenesis (Cai *et al.* 2016).

Quantitative proteomic analysis of sphere-forming CSCs of highly invasive UM1 and low invasive UM2 cells both obtained from the same tongue squamous cell carcinoma patient demonstrated that UM2 cells did not possess sphere-forming CSCs. On the other hand, sphere-forming CSCs of UM1 cells were strongly positive for several stem cell factors including SOX2 and OCT4. Along with those markers, many proteins in cell cycle, metabolism, G protein signal transduction, translational elongation, development, and RNA splicing pathways were differentially expressed among the two cell phenotypes, which might be associated with elevated levels of stem cell factors like SOX2 and OCT4 (Misuno *et al.* 2013).

KLF4

Interestingly, another reprogramming factor, KLF4, has been found to play Janus-faced roles in oral cancer carcinogenesis, acting both as a tumor suppressor and as an oncogene. The expression of KLF4 was found to be lower in the poor-differentiated oral cancers compared to the well-differentiated cancers. KLF4 functions as a tumor suppressor *in vitro* and/or *in vivo* through suppressing cell proliferation, cell cycle progression, cell colony formation and by inducing apoptosis (W. Li *et al.* 2015). It was also shown to inhibit cellular proliferation and induce differentiation to help maintenance of epithelial homeostasis. In a recent study in which an inducible oral-specific mice model was utilized to knockout Klf4 specifically in the oral cavity, dysplastic lesions with increased cell proliferation and abnormal differentiation were reported in the tongue cells four months after induction (Abrigo *et al.* 2014). Utilization of 4-nitroquinoline 1-oxide along with Klf4 knockout in the oral cavity resulted in development of more severe dysplastic lesions in the oral cavity and a tendency for increased incidence of oral squamous cell carcinoma (Paparella *et al.* 2015). These findings support the potential of KLF4 as a tumor suppressor for TSCC, however, upregulation of KLF4 induced TSCC cell migration and invasion *in vitro*. In addition, although knockdown of KLF4 in TSCC cells increased cellular proliferation and colony formation, it significantly resulted in inhibition of cell migration and invasion. Contradictory to its tumor suppressor function, these recent findings assign an oncogenic role for KLF4 in TSCC. Therefore, further studies are necessary to clarify the roles of KLF4 as a CSCs marker during tongue carcinogenesis process.

NANOG

Overexpression of Nanog has been previously reported in several types of tumors including oral squamous cell carcinoma (Bourguignon *et al.* 2012, Ezeh *et al.* 2005, Meng *et al.* 2010, Tsai *et al.* 2011) and it has

been suggested to play critical roles during carcinogenesis, tumor-progression, and metastasis (Ratajczak *et al.* 2007, Trosko 2006). Nanog expression was found to be significantly higher in tongue tumor tissues than in corresponding normal oral mucosa tissues (Fu *et al.* 2016). Nanog expression together with ALDH1 and OCT4, which are other CSCs markers, were reported to significantly increase during multistep carcinogenesis in a 4-nitroquinoline 1-oxide-induced rat tongue carcinogenesis model (Lim *et al.* 2014). Besides, long-term exposure of the carcinogenic 4-methylnitrosamino-1-3-pyridyl-1-butanone resulted in promoted migration and invasion in a dose-dependent manner induced overexpression of several CSCs markers including Nanog (Nieh *et al.* 2015). Nanog expression together with Oct3/4 were also reported to be significantly upregulated in side population compared to remaining cell population and their expressions were found to be strongly correlated with development of delayed neck metastasis (Habu *et al.* 2015). Taking into account these findings, Nanog might be considered amongst important CSCs biomarkers for TSCC to be utilized for identification and characterization of CSCs present in TSCC tissues.

BMI1

Bmi1, which plays critical roles in the functioning and maintenance of both endogenous stem cells and CSCs (Tanaka *et al.* 2013), was demonstrated to be significantly overexpressed during tongue carcinogenesis starting from very early stages (He *et al.* 2015). A recent study reported that knockdown of Bmi1 in TSCC cell lines stimulated cell apoptosis and senescence along with suppression of cell proliferation and migration. Interestingly, its inhibition also reduced the colony formation efficiency and the proportion of CD44+/CD133+ sub-population with CSCs features (Li *et al.* 2014). Besides, Bmi1 positive (Bmi1+) lingual epithelial stem cells were reported as the origin for tongue cancer in 4-nitroquinoline-1-oxide induced tongue cancer models since certain Bmi1+ cells were found to proliferate constantly, which eventually resulted in the formation of tumors derived from single Bmi1+ cells. This *in vivo* model provided invaluable data about the presence of CSCs within TSCC specimens and pointed out the importance of Bmi1 as a critical CSCs marker (Tanaka *et al.* 2016).

NOTCH

A recent meta-analysis study explored the role of Notch signaling pathway in human oral squamous cell carcinoma to understand the role of Notch signaling in during oral carcinogenesis (Osathanon *et al.* 2016). The researchers utilized 13 Gene Expression Omnibus datasets including those carried out with TSCC samples and found that oral squamous cell carcinoma specimens showed significant overexpression of several genes in Notch signaling pathway including *JAG1*, *JAG2*, *ADAM17*, *NCSTN*, *PSEN1*, *NCOR2*, N*UMB*, *DVL3*, *HDAC1*, and *HDAC2*, pointing out the importance of Notch and its effectors for TSCC carcinogenesis (Osathanon *et al.* 2016). Notch is reported to be amplified and overexpressed in significant proportion of early stage TSCC tissue samples. Overexpression of Notch, which is characterized as inducer of stem cell maintenance in potential cancer-initiating cells, in TSCC cell lines caused enrichment of stem cell markers and formation of spheroids with CSCs features. Conversely, its inhibition suppressed spheroid forming capacity, transformation, survival and migration of TSCC cells, indicating an oncogenic role for Notch in TSCC through regulating stem cell characteristics (Upadhyay *et al.* 2016).

EMT Markers

Accumulating evidence shows that epithelial mesenchymal transition (EMT), which is a significant physiological process where epithelial features are lost and mesenchymal properties are acquired (Iwatsuki *et al.* 2010, Thiery *et al.* 2009), is a crucial process playing critical roles during embryogenesis and carcinogenesis (Vered *et al.* 2010). It has been recently proposed that CSCs undergo an EMT for acquisition of migratory and invasive phenotype, which is necessary for progression of tumor (Pan *et al.* 2016).

In a recent study, treatment of Tca-8113 cells with Bone morphogenic protein 4 (BMP-4) was reported to promote EMT through acquisition of stem cell associated phenotypes (Qiao *et al.* 2011). BMP-4 significantly upregulated mesenchymal markers like Snail, Slug and Vimentin, and downregulated the epithelial marker E-cadherin along with overexpression of stemness gene which was not synchronous with the expression of EMT markers (Qiao *et al.* 2011). Another study investigating the expression profile of Bmi1, which is involved in self-renewal of stem cells, and that of ZEB1, a transcription factor that is associated with EMT, in TSCC cells and tongue specimens revealed that Bmi1 and ZEB1, both in mRNA and protein levels, were observed at the invasive front of TSCC cells, which were accompanied by the downregulation of epithelial markers and overexpression of mesenchymal markers both *in vitro* and *in vivo* (Kurihara *et al.* 2015). In addition, induced expression of Snail and Slug, two important EMT markers, resulted in a mesenchymal phenotype and morphology and induced cell invasion and stem cell related features in TSCC cells (Zheng *et al.* 2015).

Biomarkers in TSCC Prognosis

In addition to their tumor initiating and promoting properties, CSCs are significantly associated with cancer progression and clinical outcome and they are thought to be responsible for tumor development, metastasis, and recurrence as well as tumor initiation (Guzel *et al.* 2014). In addition, since CSCs and normal tissue stem cells share many similarities and CSCs are significantly involved in the malignant progress of tumors (Table 1), they are considered among the critical targets for future therapies.

Table 1. TSCC CSCs biomarkers associated with prognosis of disease.

CSCs Biomarker	Expression Level	Association	Reference
CD44	Low	Regional late nodal metastases	(Kunishi *et al.* 1997, Masuda *et al.* 2000)
		Poor survival of patients	(Sato *et al.* 2000)
		High histological grade	(Cruz *et al.* 2009)
		Cervical lymph node metastasis	(Mostaan *et al.* 2011)
		Lymph node metastasis	(Kunishi *et al.* 1997)
		5-year disease-free survival	(Dunkel *et al.* 2013)
	High	Poor prognosis	(Lindquist *et al.* 2012)
		Tumor local recurrence	(Yanamoto *et al.* 2014)
SOX2	High	Large tumor size, poorer overall, cancer-specific and disease-free survival of patients	(Du *et al.* 2011)
		Histological grade	(Jiang *et al.* 2017)
OCT4	High	Histological grade, lymph node metastasis	(Jiang *et al.* 2017)
		Vascular invasion, delayed neck metastasis	(Habu *et al.* 2015).
BMI1	High	Poor survival A positive node metastasis	(He *et al.* 2015)
		Cervical node metastasis and reduced overall survival	(Li *et al.* 2014)
	Low	Risk for recurrence	(Häyry *et al.* 2010)
CD147	High	Tumor diameter, clinical stage, poor overall survival	(Huang *et al.* 2009)
		Poor prognosis, a higher T stage	(Yu *et al.* 2015)
		Recurrence, node metastasis	(Huang *et al.* 2012)

The expressions of ALDH1, CD44, OCT4 and SOX2, as important cancer stem cell markers, were investigated in a recent clinical study by immunohistochemistry utilizing 66 TSCC tissue specimens (Huang *et al.* 2014). This study demonstrated that all of these CSCs markers were significantly overexpressed in TSCC samples, however, only the expression of SOX2 along with recurrence and distant metastasis were found as individual independent prognostic factors of overall survival in TSCC patients (Huang *et al.* 2014). Moreover, in another study, where tumor tissue samples from 82 histologically node-negative TSCC patients were evaluated with immunohistochemistry, SOX2 expression was significantly associated with large tumor size, and its increased expression was related with poorer overall, cancer-specific and disease-free survival of patients (Du *et al.* 2011). In a different study, paraffin embedded tissue specimens of 51 patients with TSCC were examined immunohistochemically, and OCT4 and SOX2 expressions were found to be significantly associated with histological grade of TSCC and poor overall survival of patients (Jiang *et al.* 2017). Moreover, OCT4 expression, but not SOX2, was also significantly correlated with lymph node metastasis. In the meantime, SOX2 was suggested as an independent prognostic factor for overall survival (Jiang *et al.* 2017). In addition, along with vascular invasion, expression of OCT4 was reported to be a potential predictor for identification of patients with high risk of delayed neck metastasis (Habu *et al.* 2015). Taking these findings into account, certain CSCs markers including SOX2 and OCT4 might be proposed as independent prognostic factors for patient survival in TSCC.

Apart from SOX2 and OCT4, deregulation of another important stem cell marker, Bmi1, was demonstrated to be a common event during TSCC progression. Its overexpression was significantly associated with poor

survival of TSCC patients. Late stage patients and patients with a positive node metastasis tended to have significantly higher Bmi1 expression compared to early stage patients and patients with a negative node metastasis, respectively (He *et al.* 2015). Its overexpression was also reported in a major fraction of tumor samples obtained from 52 patients with no prior history of chemotherapy or radiotherapy, and the overexpression was significantly associated with cervical node metastasis and reduced overall survival, revealing Bmi1 as an independent prognostic marker for TSCC patients (Li *et al.* 2014). On the other hand, an earlier study showed that negative or reduced Bmi1 expression was related to recurrence of patients and suggested negative Bmi1 expression as a biomarker for poor prognosis in TSCC patients (Häyry *et al.* 2010). The contradictory findings of the present studies question the power of Bmi1 as a prognostic marker and require further investigations to enlighten its roles in the progression of TSCC.

In several studies, reduced expression of different isoforms of CD44, as a widely investigated CSCs biomarkers in TSCC, was reported to be correlated with different clinical parameters including regional lymph node metastasis (Kunishi *et al.* 1997), late nodal metastases (Masuda *et al.* 2000), cervical lymph node metastasis (Mostaan *et al.* 2011), poor survival of patients (Sato *et al.* 2000), 5-year disease-free survival (Dunkel *et al.* 2013), and high histological grade (Cruz *et al.* 2009). However, increased expression of CD44 in TSCC samples, in accordance with its potential for contribution to CSCs features, was also associated with poor prognosis (Lindquist *et al.* 2012) and tumor local recurrence (Yanamoto *et al.* 2014). In the meanwhile, there are reports showing no statistically significant association between the expression of CD44 and different clinical or histological parameters (Krump & Ehrmann 2013, Yu *et al.* 2015). Thus, the exact role of CD44 for prediction of TSCC progression is not clear yet and further efforts are needed for clarification of its power for estimation of patients' prognosis in TSCC.

Expression of CD147, a potential oncogenic CSCs marker in TSCC, was significantly correlated with tumor diameter, clinical stages of the TSCC tumor samples and poor overall patient survival (Huang *et al.* 2009). CD147 expression level was also demonstrated to have significant association with poor prognosis, a higher T stage (Yu *et al.* 2015), recurrence, and node metastasis (Huang *et al.* 2012).

Future Potential

Experiencing a recurrence after a successful therapy is quite frequent in various cancer types including TSCC. Besides, current conventional treatment options mostly fail to result in a positive clinical outcome in advanced cases, which ultimately results in cancer related death due to disease progression and related organ failure. Recent studies indicated that CSCs are critical contributors of tumor initiation, progression, metastasis, recurrence, and chemo-radioresistance. Although underlying molecular mechanisms of how CSCs contribute to cancer pathogenesis is not completely clarified and understood, scientists and clinicians aim to use those cells as therapeutic tools to fight against cancer. Unraveling both genetic and epigenetic machineries of CSCs is necessary to develop effective and successful therapeutic options.

One of the major challenges of CSCs research is the accurate identification and characterization of CSCs, which is needed for their specific targeting. Therefore, to develop a therapeutic approach aiming at preferentially killing CSCs, the initial goal needs to be the identification of true surface marker(s) and stemness genes in CSCs. To do so, further development of anti-CSCs agents will help overcoming the tumor chemoresistance or radioresistance.

Furthermore, early detection and accurate diagnosis are especially crucial for determination and application of effective cancer therapy options in the clinical decision making process. Detailed characterization of circulating tumor cells, especially CSCs, will give opportunity to predict the prognosis of the cancer and decide the personalized therapy strategies for each patient.

In conclusion, the area of CSCs research is in its infancy and current understanding of the CSCs biomarkers in tongue pathogenesis is quite limited. Therefore, further detailed investigations are essential for better understanding of the roles and functions of CSCs biomarkers in TSCC biology.

References

1. Abrigo, M., Alvarez, R., Paparella, M.L., Calb, D.E., Bal de Kier Joffe, E., Gutkind, J.S. & Raimondi, A.R. (2014). Impairing squamous differentiation by Klf4 deletion is sufficient to initiate tongue carcinoma development upon K-Ras activation in mice. *Carcinogenesis*, 35(3): 662-669.

2. Alam, M., Kashyap, T., Pramanik, K.K., Singh, A.K., Nagini, S. & Mishra, R. (2017). The elevated activation of NFκB and AP-1 is correlated with differential regulation of Bcl-2 and associated with oral squamous cell carcinoma progression and resistance. *Clinical Oral Investig*ations, 21(9): 2721-2731.

3. Baillie, R., Itinteang, T., Yu, H.H., Brasch, H.D., Davis, P.F. & Tan, S.T. (2016). Cancer stem cells in moderately differentiated oral tongue squamous cell carcinoma. *Journal of Clinical Pathology*, 69(8): 742-744.

4. Baillie, R., Tan, S.T. & Itinteang, T. (2017). Cancer Stem Cells in Oral Cavity Squamous Cell Carcinoma: A Review. *Frontiers in Oncology*, 7: 112.

5. Boldrup, L., Coates, P.J., Laurell, G., Wilms, T., Fahraeus, R. & Nylander, K. (2015). Downregulation of miRNA-424: a sign of field cancerisation in clinically normal tongue adjacent to squamous cell carcinoma. *British Journal of Cancer*, 112(11): 1760-1765.

6. Bourguignon, L.Y., Earle, C., Wong, G., Spevak, C.C. & Krueger, K. (2012). Stem cell marker (Nanog) and Stat-3 signaling promote MicroRNA-21 expression and chemoresistance in hyaluronan/CD44-activated head and neck squamous cell carcinoma cells. *Oncogene*, 31(2): 149-160.

7. Bourguignon, L.Y.W., Earle, C. & Shiina, M. (2017). Activation of Matrix Hyaluronan-Mediated CD44 Signaling, Epigenetic Regulation and Chemoresistance in Head and Neck Cancer Stem Cells. *International Journal of Molecular Science*, 18(9): 1849.

8. Boyer, L.A., Lee, T.I., Cole, M.F., Johnstone, S.E., Levine, S.S., Zucker, J.P., Guenther, M.G., Kumar, R.M., Murray, H.L., Jenner, R.G., Gifford, D.K., Melton, D.A., Jaenisch, R. & Young, R. A. (2005). Core transcriptional regulatory circuitry in human embryonic stem cells. *Cell*, 122(6): 947-956.

9. Brockton, N.T., Lohavanichbutr, P., Enwere, E.K., Upton, M.P., Kornaga, E.N., Nakoneshny, S.C., Bose, P., Chen, C. & Dort, J.C. (2017). Impact of tumoral carbonic anhydrase IX and Ki-67 expression on survival in oral squamous cell carcinoma patients. *Oncology Letters*, 14(5): 5434-5442.

10. Bánkfalvi, A., Krassort, M., Buchwalow, I.B., Végh, A., Felszeghy, E. & Piffkó, J. (2002). Gains and losses of adhesion molecules (CD44, E-cadherin, and beta-catenin) during oral carcinogenesis and tumour progression. *Journal of Pathology*, 198(3): 343-351.

11. Cai, J., He, B., Li,X., Sun, M., Lam, A.K., Qiao, B. & Qiu, W. (2016). Regulation of tumorigenesis in oral epithelial cells by defined reprogramming factors Oct4 and Sox2. *Oncology Reports*, 36(2): 651-658.

12. Chen, J. & Li, W. (2017). MicroRNA-149 targets specificity protein 1 to suppress human tongue squamous cell carcinoma cell proliferation and motility. *Oncology Letters*, 13(2): 851-856.

13. Chen, T. W., Lee, C.C., Liu, H., Wu, C.S., Pickering, C.R., Huang, P.J., Wang, J., Chang, I.Y., Yeh, Y.M., Chen, C.D., Li, H.P., Luo, J.D., Tan, B.C., Chan, T.E. H., Hsueh, C., Chu, L.J., Chen, Y.T., Zhang, B., Yang, C.Y., Wu, C.C., Hsu, C.W., See, L.C., Tang, P., Yu, J.S., Liao, W.C., Chiang, W.F., Rodriguez, H., Myers, J.N., Chang, K.P. & Chang, Y.S. (2017). APOBEC3A is an oral cancer prognostic biomarker in Taiwanese carriers of an APOBEC deletion polymorphism. *Nature Communications*, 8(1): 465.

14. Chen, Z.G. (2009). The cancer stem cell concept in progression of head and neck cancer. *Journal of Oncology*, 2009: 894064.

15. Cruz, M.C., Pereira, A.L., Lopes, F.F., Nonaka, C.F., Silva, R.R., Freitas, Rde. A., Souza, L.B. & Pinto, L.P. (2009). Immunohistochemical expression of E-cadherin and CD44v6 in squamous cell carcinomas of the lower lip and tongue. *Brazilian Dental Journal*, 20(1): 64-69.

16. Cui, X., Song, L., Bai, Y., Wang, Y., Wang, B. & Wang, W. (2017). Stromal interaction molecule 1 regulates growth, cell cycle, and apoptosis of human tongue squamous carcinoma cells. *Bioscience Reports*, 37(2): pii: BSR20160519.

17. D'Arcangelo, M., Todaro, M., Salvini, J., Benfante, A., Colorito, M.L., D'Incecco, A., Landi, L., Apuzzo, T., Rossi, E., Sani, S., Stassi, G. & Cappuzzo, F. (2015). Cancer Stem Cells Sensitivity Assay (STELLA) in Patients with Advanced Lung and Colorectal Cancer: A Feasibility Study. *PLoS One*, 10(5): e0125037.

18. DA Cruz Paula, A. & Lopes, C. (2017). Implications of Different Cancer Stem Cell Phenotypes in Breast Cancer. *Anticancer Research*, 37(5): 2173-2183.

19. Daigo, K., Takano, A., Thang, P.M., Yoshitake, Y., Shinohara, M., Tohnai, I., Murakami, Y., Maegawa, J. & Daigo, Y. (2018). Characterization of KIF11 as a novel prognostic biomarker and therapeutic target for oral cancer. *International Journal of Oncology*, 52(1): 155-165.

20. Dawood, S., Austin, L. & Cristofanilli, M. (2014). Cancer stem cells: implications for cancer therapy. *Oncology (Williston Park)*, 28(12): 1101-1107, 1110.

21. Du, L., Yang, Y., Xiao, X., Wang, C., Zhang, X., Wang, L., Zhang, X., Li, W., Zheng, G., Wang, S. & Dong, Z. (2011). Sox2 nuclear expression is closely associated with poor prognosis in patients with histologically node-negative oral tongue squamous cell carcinoma. *Oral Oncology*, 47(8): 709-713.

22. Dunkel, J., Vaittinen, S., Grénman, R., Kinnunen, I. & Irjala, H. (2013). Prognostic markers in stage I oral cavity squamous cell carcinoma. *Laryngoscope*, 123(10): 2435-2441.

23. Ezeh, U.I., Turek, P.J., Reijo, R.A. & Clark, A.T. (2005). Human embryonic stem cell genes OCT4, NANOG, STELLAR, and GDF3 are expressed in both seminoma and breast carcinoma. *Cancer*, 104(10): 2255-2265.

24. Fonseca, I., Pereira, T., Rosa-Santos, J. & Soares, J. (2001). Expression of CD44 isoforms in squamous cell carcinoma of the border of the tongue: A correlation with histological grade, pattern of stromal invasion, and cell differentiation. *Journal of Surgical Oncology*, 76(2): 115-120.

25. Fu, T.Y., Hsieh, I.C., Cheng, J.T., Tsai, M.H., Hou, Y.Y., Lee, J.H., Liou, H.H., Huang, S.F., Chen, H.C., Yen, L.M., Tseng, H.H. & Ger, L.P. (2016). Association of OCT4, SOX2, and NANOG expression with oral squamous cell carcinoma progression. *Journal of Oral Pathology & Medicine*, 45(2): 89-95.

26. González-Moles, M. A., Bravo, M., Ruiz-Avila, I., Esteban, F., Bascones-Martínez, A. & González-Moles, S. (2004). Adhesion molecule CD44 expression in non-tumour epithelium adjacent to tongue cancer. *Oral Oncology*, 40(3): 281-286.

27. Guzel, E., Karatas, O.F., Duz, M.B., Solak, M., Ittmann, M. & Ozen, M. (2014). Differential Expression of Stem Cell Markers and ABCG2 in Recurrent Prostate Cancer. *Prostate*, 74(15): 1498-1505.

28. Habu, N., Imanishi, Y., Kameyama, K., Shimoda, M., Tokumaru, Y., Sakamoto, K., Fujii, R., Shitegomi, S., Otsuka, K., Sato, Y., Watanabe, Y., Ozawa, H., Tomita, T., Fujii, M. & Ogawa, K. (2015). Expression of Oct3/4 and Nanog in the head and neck squamous carcinoma cells and its clinical implications for delayed neck metastasis in stage I/II oral tongue squamous cell carcinoma. *BMC Cancer*, 15: 730.

29. He, Q., Liu, Z., Zhao, T., Zhao, L., Zhou, X. & Wang, A. (2015). Bmi1 drives stem-like properties and is associated with migration, invasion, and poor prognosis in tongue

squamous cell carcinoma. *International Journal of Biological Sciences*, 11(1): 1-10.

30. Hemmati, H.D., Nakano, I., Lazareff, J.A., Masterman-Smith, M., Geschwind, D.H., Bronner-Fraser, M. & Kornblum, H.I. (2003). Cancerous stem cells can arise from pediatric brain tumors. *Proceedings of the National Academy of Science of the USA*, 100(25): 15178-15183.

31. Herold-Mende, C., Seiter, S., Born, A.I., Patzelt, E., Schupp, M., Zöller, J., Bosch, F.X. & Zöller, M. (1996). Expression of CD44 splice variants in squamous epithelia and squamous cell carcinomas of the head and neck. The *Journal of Pathology*, 179(1): 66-73.

32. Huang, C., Sun, Z., Sun, Y., Chen, X., Zhu, X., Fan, C., Liu, B., Zhao, Y. & Zhang, W. (2012). Association of increased ligand cyclophilin A and receptor CD147 with hypoxia, angiogenesis, metastasis and prognosis of tongue squamous cell carcinoma. *Histopathology*, 60(5): 793-803.

33. Huang, C.F., Xu, X.R., Wu, T.F., Sun, Z.J. & Zhang, W.F. (2014). Correlation of ALDH1, CD44, OCT4 and SOX2 in tongue squamous cell carcinoma and their association with disease progression and prognosis. *Journal of Oral Pathology & Medicine*, 43(7): 492-498.

34. Huang, Z., Huang, H., Li, H., Chen, W. & Pan, C. (2009). EMMPRIN expression in tongue squamous cell carcinoma. *Journal of Oral Pathology & Medicine*, 38(6): 518-523.

35. Häyry, V., Mäkinen, L.K., Atula, T., Sariola, H., Mäkitie, A., Leivo, I., Keski-Säntti, H., Lundin, J., Haglund, C. & Hagström, J. (2010). Bmi-1 expression predicts prognosis in squamous cell carcinoma of the tongue. *British Journal of Cancer*, 102(5): 892-897.

36. Iqbal, M.S., Chaw, C., Kovarik, J., Aslam, S., Jackson, A., Kelly, J., Dobrowsky, W. & Kelly, C. (2017). Primary Concurrent Chemoradiation in Head and Neck Cancers with Weekly Cisplatin Chemotherapy: Analysis of Compliance, Toxicity and Survival. *International Archives of Otorhinolaryngology*, 21(2): 171-177.

37. Iwatsuki, M., Mimori, K., Yokobori, T., Ishi, H., Beppu, T., Nakamori, S., Baba, H. & Mori, M. (2010). Epithelial-mesenchymal transition in cancer development and its clinical significance. *Cancer Science*, 101(2): 293-299.

38. Jiang, X.D., Luo, G., Wang, X. H., Chen, L.L., Ke, X. & Li, Y. (2017). [Expression of Oct4 and Sox2 and their clinical significance in tongue squamous cell carcinoma]. *Zhonghua Kou Qiang Yi Xue Za Zhi*, 52(1): 27-33.

39. Järvinen, A.K., Autio, R., Kilpinen, S., Saarela, M., Leivo, I., Grénman, R., Mäkitie, A.A. & Monni, O. (2008). High-resolution copy number and gene expression microarray analyses of head and neck squamous cell carcinoma cell lines of tongue and larynx. *Genes Chromosomes Cancer*, 47(6): 500-509.

40. Kang, F.W., Wang, K., Wu, M., Wang, Z.L., Zhu, Y. & Min, R. (2010). [Biological characteristics of CD133+ subpopulation in tongue squamous cell carcinoma Tca8113 cell line]. *Hua Xi Kou Qiang Yi Xue Za Zhi*, 28(5): 560-564.

41. Kosunen, A., Pirinen, R., Ropponen, K., Pukkila, M., Kellokoski, J., Virtaniemi, J., Sironen, R., Juhola, M., Kumpulainen, E., Johansson, R., Nuutinen J. & Kosma, V. M. (2007). CD44 expression and its relationship with MMP-9, clinicopathological factors and survival in oral squamous cell carcinoma. *Oral Oncology*, 43(1): 51-59.

42. Krump, M. & Ehrmann, J. (2013). Differences in CD44s expression in HNSCC tumours of different areas within the oral cavity. *Biomedical Papers of the Medical Faculty of the University Palacky Olomouc Czech Republic*, 157(4): 280-283.

43. Kunishi, M., Kayada, Y. & Yoshiga, K. (1997). Down-regulated expression of CD44 variant 6 in oral squamous cell carcinomas and its relationship to regional lymph node metastasis. *International Journal of Oral & Maxillofacial Surgery*, 26(4): 280-283.

44. Kurihara, K., Isobe, T., Yamamoto, G., Tanaka, Y., Katakura, A. & Tachikawa, T. (2015). Expression of BMI1 and ZEB1 in epithelial-mesenchymal transition of tongue squamous cell carcinoma. *Oncology Reports*, 34(2): 771-778.

45. Li, Q., Rycaj, K., Chen, X. & Tang, D.G. (2015). Cancer stem cells and cell size: A causal link? *Seminars in Cancer Biology*, 35, 191-199.

46. Li, W., Liu, M., Su, Y., Zhou, X., Liu, Y. & Zhang, X. (2015). The Janus-faced roles of Krüppel-like factor 4 in oral squamous cell carcinoma cells. *Oncotarget*, 6(42): 44480-44494.

47. Li, Z. (2013). CD133: a stem cell biomarker and beyond. *Experimental Hematology & Oncology*, 2(1): 17.

48. Li, Z., Wang, Y., Yuan, C., Zhu, Y., Qiu, J., Zhang, W., Qi, B., Wu, H., Ye, J., Jiang, H., Yang, J., Cheng, J. (2014). Oncogenic roles of Bmi1 and its therapeutic inhibition by histone deacetylase inhibitor in tongue cancer. *Laboratory Investigations*, 94(12): 1431-1445.

49. Lim, W., Choi, H., Kim, J., Kim, S., Jeon, S., Ni, K., Song, S.Y., Oh, H.K., Im, Y., Lee, G., Lee, J.Y., Moon, Y.L., You, J.W. & Kim, O. (2014). Expression of cancer stem cell marker during 4-nitroquinoline 1-oxide-induced rat tongue carcinogenesis. *Journal of Molecular Histology*, 45(6): 653-663.

50. Lindquist, D., Ahrlund-Richter, A., Tarján, M., Tot, T. & Dalianis, T. (2012). Intense CD44 expression is a negative prognostic factor in tonsillar and base of tongue cancer. *Anticancer Research*, 32(1): 153-161.

51. Loh, Y.H., Wu, Q., Chew, J.L., Vega, V.B., Zhang, W., Chen, X., Bourgue, G., George, J., Leong, B., Liu, J., Wong, K.Y., Sung, K.W., Lee, C.W., Zhao, X.D., Chiu, K.P., Lipovih, L., Kuznetsov, V.A., Robson, P., Stanton, L.W., Wei, C.L., Ruan, Y. & Ng, H.H. (2006). The Oct4 and Nanog transcription network regulates pluripotency in mouse embryonic stem cells. *Nature Genetics*, 38(4): 431-440.

52. Major, A.G., Pitty, L.P. & Farah, C.S. (2013). Cancer stem cell markers in head and neck squamous cell carcinoma. *Stem Cells International*, 2013: 319489.

53. Mascolo, M., Ilardi, G., Romano, M.F., Celetti, A., Siano, M., Romano, S., Luise, C., Merolla, F., Rocco, A., Vecchione, M.L., De Rosa, G. & Staibano, S. (2012). Overexpression of chromatin assembly factor-1 p60, poly(ADP-ribose) polymerase 1 and nestin predicts metastasizing behaviour of oral cancer. *Histopathology*, 61(6): 1089-1105.

54. Masuda, M., Kuratomi, Y., Shiratsuchi, H., Nakashima, T., Naonobu, K. & Komiyama, S. (2000). Decreased CD44H expression in early-stage tongue carcinoma associates with late nodal metastases following interstitial brachytherapy. *Head & Neck*, 22(7): 662-665.

55. Meng, H.M., Zheng, P., Wang, X.Y., Liu, C., Sui, H.M., Wu, S.J., Zhou, J., Ding, Y.Q. & Li, J. (2010). Over-expression of Nanog predicts tumor progression and poor prognosis in colorectal cancer. *Cancer Biology & Therapy*, 9(4): 295-302.

56. Misuno, K., Liu, X., Feng, S. & Hu, S. (2013). Quantitative proteomic analysis of sphere-forming stem-like oral cancer cells. *Stem Cell Research & Therapy*, 4(6): 156.

57. Mostaan, L.V., Khorsandi, M.T., Sharifian, S.M., Shandiz, F.H., Mirashrafi, F., Sabzari, H., Badiee, R., Borghei, H. & Yazdani, N. (2011). Correlation between E-cadherin and CD44 adhesion molecules expression and cervical lymph node metastasis in oral tongue SCC: Predictive significance or not. *Pathology Research & Practice*, 207(7): 448-451.

58. Mărgăritescu, C., Pirici, D., Simionescu, C. & Stepan, A. (2011). The utility of CD44, CD117 and CD133 in identification of cancer stem cells (CSC) in oral squamous cell carcinomas (OSCC). *Romanian Journal of Morphology & Embryology*, 52(3 Suppl): 985-993.

59. Nieh, S., Jao, S.W., Yang, C.Y., Lin, Y.S., Tseng, Y.H., Liu, C.L., Lee, T.Y., Liu, T.Y., Chu, Y.H. & Chen, S.F. (2015). Regulation of tumor progression via the Snail-RKIP signaling pathway by nicotine exposure in head and neck squamous cell carcinoma. *Head & Neck*, 37(12): 1712-1721.

60. Osathanon, T., Nowwarote, N. & Pavasant, P. (2016). Expression and influence of Notch signaling in oral squamous cell carcinoma. *Journal of Oral Science*, 58(2): 283-294.

61. Owen, J.H., Hauff, S.J., Tang, A.L., Graham, M.P., Czerwinski, M.J., Kaddoura, M., Papagerakis, S., Bradford, C.R., Carey, T.E. & Prince, M.E. (2014). UM-SCC-103: a unique tongue cancer cell line that recapitulates the tumorigenic stem cell population of the primary tumor. *Annals of Otology, Rhinology& Laryngology*, 123(9): 662-672.

62. Pan, Y., Guo, X., Yang, Z., Chen, S., Lei, Y., Lin, M., Wang, L., Feng, C. & Ke, Z. (2016). AEG-1 activates Wnt/PCP signaling to promote metastasis in tongue squamous cell carcinoma. *Oncotarget*, 7(2): 2093-2104.

63. Paparella, M.L., Abrigo, M., Bal de Kier Joffe, E. & Raimondi, A.R. (2015). Oral-specific ablation of Klf4 disrupts epithelial terminal differentiation and increases premalignant lesions and carcinomas upon chemical carcinogenesis. *Journal of Oral Pathology & Medicine*, 44(10): 801-809.

64. Patel, S.S., Shah, K.A., Shah, M.J., Kothari, K.C. & Rawal, R.M. (2014). Cancer stem cells and stemness markers in oral squamous cell carcinomas. *Asian Pacific Journal of Cancer Prevention*, 15(20): 8549-8556.

65. Qiao, B., He, B., Cai, J. & Yang, W. (2014). The expression profile of Oct4 and Sox2 in the carcinogenesis of oral mucosa. *International Journal of Clinical & Experimental Pathology*, 7(1): 28-37.

66. Qiao, B., Johnson, N.W., Chen, X., Li, R., Tao, Q. & Gao, J. (2011). Disclosure of a stem cell phenotype in an oral squamous cell carcinoma cell line induced by BMP-4 via an epithelial-mesenchymal transition. *Oncology Reports*, 26(2): 455-461.

67. Ratajczak, M.Z., Machalinski, B., Wojakowski, W., Ratajczak, J. & Kucia, M. (2007). A hypothesis for an embryonic origin of pluripotent Oct-4(+) stem cells in adult bone marrow and other tissues. *Leukemia*, 21(5): 860-867.

68. Saleem, S., Jamshed, A., Faisal, S., Hussain, R., Tahseen, M., Loya, A. & Sutton, C. (2014). Patterns of cancer cell sphere formation in primary cultures of human oral tongue squamous cell carcinoma and neck nodes. *Cancer Cell International*, 14(1): 542.

69. Sato, S., Miyauchi, M., Takekoshi, T., Zhao, M., Kudo, Y., Ogawa, I., Kitagawa, S., Fujita, M. & Takata, T. (2000). Reduced expression of CD44 variant 9 is related to lymph node metastasis and poor survival in squamous cell carcinoma of tongue. *Oral Oncology*, 36(6): 545-549.

70. Shrivastava, S., Steele, R., Sowadski, M., Crawford, S.E., Varvares, M. & Ray, R.B. (2015). Identification of molecular signature of head and neck cancer stem-like cells. *Scientific Reports*, 5: 7819.

71. Siegel, R.L., Miller, K.D. & Jemal, A. (2016). Cancer statistics, 2016. *CA Cancer Journal for Clinicians*, 66(1): 7-30.

72. Suer, I., Karatas, O.F., Yuceturk, B., Yilmaz, M., Guven, G., Oz, B., Cansız, H. & Ozen, M. (2014). Characterization of Stem-Like Cells Directly Isolated from Freshly Resected Laryngeal Squamous Cell Carcinoma Specimens. *Current Stem Cell Research & Therapy*, 9(4): 347-353.

73. Sun, Y., Han, J., Lu, Y., Yang, X. & Fan, M. (2012). Biological characteristics of a cell subpopulation in tongue squamous cell carcinoma. *Oral Diseases*, 18(2): 169-177.

74. Tanaka, T., Atsumi, N., Nakamura, N., Yanai, H., Komai, Y., Omachi, T., Tanaka, K., Ishigaki, K., Saiga, K., Ohsugi, H., Tokuyama, Y., Imahashi, Y., Hisha, H., Yoshida, N., Kumano, K., Okazaki, K. & Ueno, H. (2016). Bmi1-positive cells in the lingual epithelium could serve as cancer stem cells in tongue cancer. *Scientific Reports*, 6: 39386.

75. Tanaka, T., Komai, Y., Tokuyama, Y., Yanai, H., Ohe, S., Okazaki, K. & Ueno, H. (2013). Identification of stem cells that maintain and regenerate lingual keratinized epithelial cells. *Nature Cell Biology*, 15(5): 511-518.

76. Thiery, J.P., Acloque, H., Huang, R.Y. & Nieto, M.A. (2009). Epithelial-mesenchymal transitions in development and disease. *Cell*, 139(5): 871-890.

77. Trosko, J.E. (2006). From adult stem cells to cancer stem cells: Oct-4 Gene, cell-cell communication, and hormones during tumor promotion. *Annals of the NEW YORK Academy of Sciences*, 1089: 36-58.

78. Tsai, L.L., Yu, C.C., Chang, Y.C., Yu, C.H. & Chou, M.Y. (2011). Markedly increased Oct4 and Nanog expression correlates with cisplatin resistance in oral squamous cell carcinoma. *Journal of Oral Pathology & Medicine*, 40(8): 621-628.

79. Upadhyay, P., Nair, S., Kaur, E., Aich, J., Dani, P., Sethunath, V., Gardi, N., Chandrani, P., Godbole, M., Sonawane, K., Prasad, R., Kannan, S., Agarwal, B., Kane,

S., Gupta, S., Dutt, S. & Dutt, A. (2016). Notch pathway activation is essential for maintenance of stem-like cells in early tongue cancer. *Oncotarget*, 7(31): 50437-50449.

80. Vered, M., Dayan, D., Yahalom, R., Dobriyan, A., Barshack, I., Bello, I.O., Kantola, S. & Salo, T. (2010). Cancer-associated fibroblasts and epithelial-mesenchymal transition in metastatic oral tongue squamous cell carcinoma. *International Journal of Cancer*, 127(6): 1356-1362.

81. Vlashi, E. & Pajonk, F. (2015). Cancer stem cells, cancer cell plasticity and radiation therapy. *Seminars in Cancer Biology*, 31: 28-35.

82. Wang, K., Zhou, X.K., Wu, M., Kang, F.W., Wang, Z.L. & Zhu, Y. (2016). Role of CD133(+) cells in tongue squamous carcinomas: Characteristics of 'stemness' in vivo and in vitro. *Oncology Letters*, 12(2): 863-870.

83. Weng, J., Zhang, H., Wang, C., Liang, J., Chen, G., Li, W., Tang, H. & Hou, J. (2017). miR-373-3p Targets DKK1 to Promote EMT-Induced Metastasis via the Wnt/β-Catenin Pathway in Tongue Squamous Cell Carcinoma. *Biomed Research International*, 2017: 6010926.

84. Wu, T.F., Chen, L., Bu, L.L., Gao, J., Zhang, W.F. & Jia, J. (2017). CD44+ cancer cell-induced metastasis: A feasible neck metastasis model. *European Journal of Pharmaceutical Sciences*, 101: 243-250.

85. Xing, Y., Qi, J., Deng, S., Wang, C., Zhang, L. & Chen, J. (2013). Small interfering RNA targeting ILK inhibits metastasis in human tongue cancer cells through repression of epithelial-to-mesenchymal transition. *Experimental Cell Research*, 319(13): 2058-2072.

86. Yanagisawa, A., Endo, C., Okawa, K., Shitara, S., Kugoh, H., Kakitani, M., Oshimura, M. & Tomizuka, K. (2005). Generation of chromosome-specific monoclonal antibodies using in vitro-differentiated transchromosomic mouse embryonic stem cells. *Stem Cells*, 23(10): 1479-1488.

87. Yanamoto, S., Yamada, S., Takahashi, H., Naruse, T., Matsushita, Y., Ikeda, H., Shiraishi, T., Seki, S., Fujita, S., Ikeda, T., Asahina, I. & Umeda, M. (2014). Expression of the cancer stem cell markers CD44v6 and ABCG2 in tongue cancer: effect of neoadjuvant chemotherapy on local recurrence. *International Journal of Oncology*, 44(4): 1153-1162.

88. Yu, X. & Li, Z. (2016). MicroRNA expression and its implications for diagnosis and therapy of tongue squamous cell carcinoma. *Journal of Cellular and Molecular Medicine*, 20(1): 10-16.

89. Yu, Y.H., Morales, J., Feng, L., Lee, J.J., El-Naggar, A.K. & Vigneswaran, N. (2015). CD147 and Ki-67 overexpression confers poor prognosis in squamous cell carcinoma of oral tongue: a tissue microarray study. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology*, 119(5): 553-565.

90. Zhang, H. X., Liu, O.S., Deng, C., He, Y., Feng, Y.Q., Ma, J.A., Hu, C.H. & Tang, Z.G. (2017). Genome-wide gene expression profiling of tongue squamous cell carcinoma by RNA-seq. *Clinical Oral Investigations*, 22(1): 209-216.

91. Zhang, Z., Filho, M.S. & Nör, J.E. (2012). The biology of head and neck cancer stem cells. *Oral Oncology*, 48(1): 1-9.

92. Zheng, M., Jiang, Y.P., Chen, W., Li, K.D., Liu, X., Gao, S.Y., Feng, H., Wang, S.S., Jiang, J., Ma, X.R., Cen, X., Tang, Y.J., Chen, Y., Lin, Y.F., Tang, Y.L. & Liang, X.H. (2015). Snail and Slug collaborate on EMT and tumor metastasis through miR-101-mediated EZH2 axis in oral tongue squamous cell carcinoma. *Oncotarget*, 6: 6797-6810.

93. Zou, B., Sun, S., Qi, X. & Ji, P. (2012). Aldehyde dehydrogenase activity is a cancer stem cell marker of tongue squamous cell carcinoma. *Molecular Medicine Reports*, 5(4): 1116-1120.