Review Article

SOX2, OCT4 and NANOG: The core embryonic stem cell pluripotency regulators in oral carcinogenesis

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Abstract

Embryonic stem cells provide their major contribution to embryogenesis through formation of germ layers as they have pluripotency potential and capacity for self-renewal. Retention of pluripotency of these stem cells depends on expression/level of transcription factors, i.e., SOX2, OCT4 and NANOG. During organogenesis, the altered expression of the molecules also influences these stem cells to lose their pluripotency and turn toward the lineage selection. As the differentiation progresses, the maintenance of the somatic cells including the oral squamous cells also depends on differential expression of the transcription factors to some extent. Recently, many experimental and observational studies documented the significant contribution in carcinogenesis of various human cancers. In this review, we have attempted to summarize the evidences indicating about the putative role of these master pluripotency regulators in various phases of oral carcinogenesis i.e. initiation , progression and prognosis of oral squamous cell carcinoma.

Keywords: NANOG, OCT4, oral carcinogenesis, SOX2

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INTRODUCTION

Stem cells are defined as unspecialized cells which can differentiate into any cell type of an organism and also retain the capacity of self-renewal. Broadly, stem cells can be divided in five groups according to the degree of differentiation potential, i.e., (i) totipotent, (ii) pluripotent, (iii) multipotent, (iv) oligopotent and (v) unipotent stem cells.[1,2] Totipotent stem cells such as zygote and all cells in eight-cell stage morula possess the highest differentiation potential and capacity to form both embryo and extraembryonic structures including the placenta. Pluripotent stem cells retain the ability to differentiate into lineages of all three germ cell layers but cannot generate extraembryonic structures which include embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs).[3] ESCs are derived from the inner cell mass (ICM) of blastocysts of embryo whereas iPSCs are genetically reprogrammed and derived from the epiblast layer of implanted embryos. Once stem cells get restricted in a particular tissue, the potency of self-renewable ability decreases further in multipotent stem cells (MSCs) as they lack a high level of telomerase including hematopoietic stem cells and dental pulp stem cells.[4] Furthermore, oligopotent and unipotent stem cells exhibit more restricted lineages

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with narrowed differentiation capacity of a specific tissue which includes progenitor cells of postnatal development. The schematic representation of all types of stem cells with their properties is summarized in Figure 1. Pluripotent stem cells (ESCs) depend on various regulators including transcription factors for maintenance of their pluripotency. In this review, we have attempted to focus on journey of the transcription factors SOX2, OCT4 and NANOG as ESC pluripotency regulators, i.e., through embryogenesis to pathogenesis (oral carcinogenesis).

EMBRYONIC STEM CELLS IN EMBRYOGENESIS AND PHYSIOLOGY

In human embryo development, a blastocyst is composed of ICM and trophectoderm (TE). ICM cells eventually give rise to epiblasts and induce the fetus development, whereas TE becomes more specialized and forms extraembryonic supporting structures such as the placenta. ICM cells remain undifferentiated and retain their potency and called ESC. Human embryonic stem cells (hESCs) contribute to the germ layers: ectoderm, mesoderm and endoderm, each further giving rise to more differentiated cells and tissues of the fetus and, later on, the adult organism.[1,5] After formation of germ layers, hESCs become differentiated to a next generation of stem cells called MSCs. However, the pluripotent stem cells are thought to be present as undifferentiated cells in an organism and have the capacity for proliferation and differentiation into either a next-generation stem cells or specialized cells, respectively, under certain physiological conditions. The process of specialization/differentiation of stem cells is mainly controlled by various external (physical or chemical paracrine effect) or internal (autocrine/genetic influence) stimuli. This process also depends on the location of these stem cells in the body, i.e., the division and differentiation

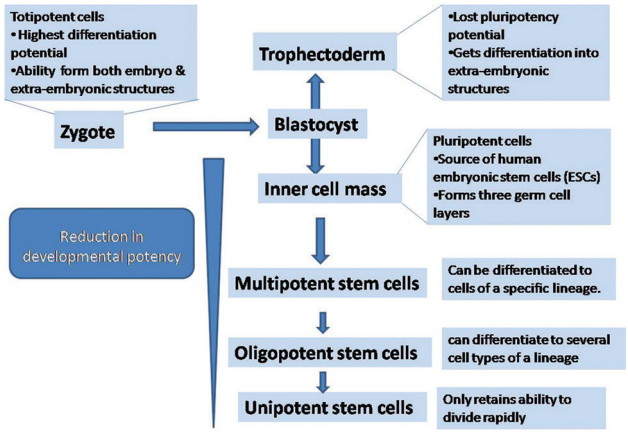

Figure 1: Schematic representation of developmental potency hierarchy of stem cells with their characteristic features

of stem cells is a continuous process in bone marrow, whereas in other organs, such as liver, kidney or pancreas, it only takes place in special physiological conditions.[1]

TRANSCRIPTION FACTORS AND PLURIPOTENCY OF EMBRYONIC STEM CELLS

Self-renewal and pluripotency of ESCs are maintained by a number of regulators including soluble extracellular signaling molecules, transcription factors, epigenetic factors (histone tail modifications and DNA methylation) and micro-RNA molecules.[6] Among all these molecules, several soluble signaling factors such as Noggin (an inhibitor of bone morphogenetic protein 4 signaling), basic fibroblast growth factor and few transforming growth factor-beta family members (NODAL and Crypto) are essential for maintenance of undifferentiated state of human ESCs. At epigenetic level, changes at various levels such as (i) transformation from "open" to "closed" chromatin environment, (ii) removal of activating mark (high level of K4 trimethylation) or repressing mark (high level of K27 trimethylation) on histone H3 of various developmental genes, (iii) expression of DNA methylation enzyme and (iv) loss of a distinct set of micro-RNAs propel the human ESCs toward differentiation.[6-8]

Transcription factors seem to play a critical role in maintaining the gene expression necessary for self-renewal and suppress the gene expression necessary for differentiation of ESCs. Among all, transcription factors trio OCT4, SOX2 and NANOG are important in maintaining these cells as alteration of the activity of these factors induces differentiation. These factors form the transcriptional core which preserves ESC self-renewal and pluripotency through autoregulatory, feedforward and feedback loops of regulation.[6]

OCT4 falls to class of octamer transcription factor which binds to an eight-base pair DNA sequence and consists a Pit/Oct/Unc family of homeodomain proteins. OCT4 is first expressed at four-cell stage development and continues to be expressed in ICM of blastocyst, germ cells and epiblast cells. In case of alteration of OCT4 level 50% from its normal expression, induction of differentiation in ESCs occurs. SOX2 is a sex-determining region-Y-related transcription factor which belongs to a family of DNA-binding protein. It shares a common DNA-binding sites with OCT4 and thought to have synergistic effect by formation of a heterodimer in regulation of their target genes. In addition, experimental studies support the potential of SOX2 in preservation of ESC stability by maintaining

OCT4 expression at appropriate levels. NANOG is another key transcription factor, a homeobox-containing protein act as one of the known target genes regulated by OCT4/SOX2. NANOG protein is expressed only in undifferentiated cells. Although the exact mechanism by which NANOG maintains pluripotency of undifferentiated cells is not clearly understood, it is thought to suppress the genes that lead to differentiation or activate other genes like OCT4 which helps in maintenance of undifferentiated state.[9] Heterogeneous expression of NANOG in embryogenesis, i.e., higher expression in ESCs and lower expression in primitive endodermal cells, is largely controlled by a activin/SMAD signaling pathway.[10] Furthermore, an interconnected regulatory circuit formed by OCT4/NANOG/SOX2 is also established as NANOG act as a direct target for OCT4/SOX2 binding, which maintains ESC self-renewal and pluripotency. In combination with these master transcription factors, other factors such as Klf4 (zinc finger transcription factor), LIN28 and C-Myc also contribute to preservation of self-renewal capability and pluripotency in ESCs.[11] Recently, Verneri *et al*. reported about the nuclear reorganization of transcription factors SOX2 and OCT4 that play a key role in differentiation and loss of pluripotency of ESCs by modifying the interaction of these molecules with their chromatin targets.[12]

OCT4/NANOG/SOX2 IN NORMAL ORAL MUCOSA

Pluripotent transcription factors (NANOG/OCT4/SOX2) contribute in sustaining ESC pluripotency, but in the absence of NANOG, OCT4 or SOX2 can play a causal role in the determination of the mesodermal (ME) and neuroectodermal (NE) pathways, respectively. SOX2 has a contributory role in differentiation of NE lineage by binding with promoter region of Brachyury. Differentiation of NE lineages including the oral mucosa development requires the overexpression of SOX2 with repression of OCT4 [Figure 2].[13,14] Few human observational studies have reported about the expression of NANOG/OCT4/SOX2 in normal oral mucosa. Fu *et al*. observed the expression of these factors in normal Uvula epithelium in which NANOG showed very limited but both nuclear and cytoplasmic expression as compared to SOX2 and OCT4 which had only nuclear expression.[15] Qiao *et al*. observed individual expression of OCT4 and SOX2 in the basal cell layer of oral epithelium though co-expression of these factors was not evident in normal mucosa.[16] Michifuri *et al*. also observed the basal and suprabasal layer expression of SOX2, suggesting location of progenitors and stem cells in oral squamous epithelium.[17]

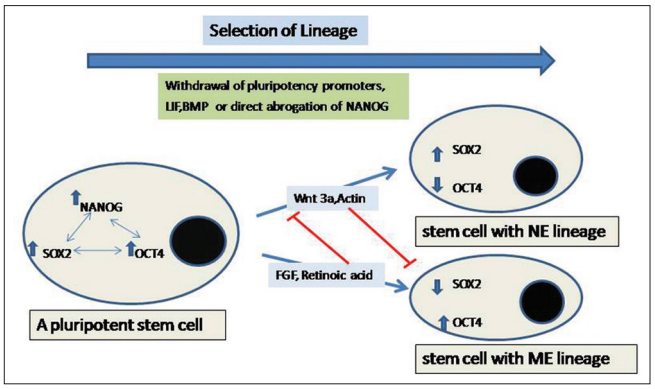

Figure 2: Influence of SOX2, OCT4 and NANOG in lineage selection of pluripotent stem cells. In lineage selection, ESCs lose their pluripotency to differentiate into NE or mesoendodermal lineage under the influence of differential expression of transcription factors, i.e., SOX2, OCT4 and NANOG. Other factors such as Wnt-3a, actin, fibroblast growth factor and retinoic acid have positive and negative control in the aforementioned selection process

OCT4/NANOG/SOX2 IN ORAL PREMALIGNANT AND MALIGNANT LESIONS

Cancer stem cell (CSC) hypothesis is a well-proven concept in various stages of oral carcinogenesis including tumor initiation, progression, microenvironment modulation and metastasis. According to the hypotheses, there may be various epithelial and nonepithelial origins of CSC in oral cavity. Epithelial source of CSCs responsible for oral carcinogenesis is thought to be basal layer-derive adult stem cell or progenitor which is capable of harboring the cumulative genetic damage or alteration caused by carcinogens. Other nonepithelial sources of CSCs could be mesenchymal stem cells (originated from blood, vessel wall, muscle or adipocytes), induction of stem cell-like properties in mutated keratinocyte by hematopoietic stem cells or dedifferentiation of mature cells.[18] As transcription factors NANOG, OCT4 and SOX2 play a crucial role in preservation of pluripotency and self-renewal capacity of ESCs and adult stem cells, recently these factors have drawn the attention of many researchers to endeavor their potential role in oral cancer initiation.

In altered or dysplastic mucosa

Fu *et al*. designed a comprehensive observational study with an aim to examine the possible association of OCT4, SOX2 and NANOG expression levels not only with the development but also prognosis of patients with oral squamous cell carcinoma (OSCC). Both OCT4 and SOX2 were expressed in normal tissue and corresponding tumor-adjacent normal (CTAN) tissue. However, immunoexpression of OCT4 was found to be higher in normal tissue, whereas SOX2 showed overexpression in CTAN tissue.[15] Another similar study

also showed increased expression of SOX2 in normal tissue adjacent to OSCC.[19] These results support the hypothesis that cells with elevated SOX2 expression may harbor early molecular changes and eventually contribute to field carcinogenesis to develop preinvasive lesions. Although both the studies observed dissimilarity in expression of OCT4, NANOG showed a consistent weaker expression which indicates about controversial aspect of synergistic contribution of these transcription factors. A similar observation was reported in few clinical studies in leukoplakia, an oral potentially malignant disorder which not only advocates the potential role of SOX2 in oral carcinogenesis[20] but also provides hypothesis of its contribution in expansion of stem cell population from basal cell layer to suprabasal layer in dysplastic lesions. However, a recent study suggested that NANOG expression in oral potentially malignant disorders could be used as an early predictor of malignant transformation risk.[21] Both cytoplasmic and nuclear NANOG expressions were observed in oral epithelial dysplastic lesions where normal adjacent epithelium deprived for the same. In addition, positive immunoreactivity of NANOG in oral dysplasias is also correlated with increased risk of their progression to oral cancer. Similarly, Qiao *et al*. observed consistent co-expression of OCT4 and SOX2 from oral precancerous lesions, epithelial noncancer tissues adjacent to oral cancer and oral carcinoma samples, which suggests about synergistic contribution of these transcription factors in tumor initiation possibly through restoration of pluripotency in residing stem cells and ultimately their transformation to CSCs [Table 1].[4,16]

In oral squamous cell carcinoma

In vitro experimental studies have been advocating the contributory role of pluripotent ESCs markers, i.e., OCT4 and SOX2 in carcinogenesis.[22,23] Cai *et al*. investigated the roles of these factors in the reprogramming of oral CSCs. They hypothesized that somatic cells could be reprogrammed to tumor-initiating cells through double transduction of OCT4 and SOX2, where OCT4 plays a role of derivation as SOX2 plays the role of stem cell property. They also observed that tumor initiation could not be possible in the absence of OCT4 expression and neoplastic cells lost their self-renewal capacity without SOX2 expression.[24] On reviewing the results of clinical studies on expression of these master molecules in OSCC, we have noticed unanimous overexpression of individual markers in tissue samples. However, controversial observations have emerged out when altered expressions of SOX2/OCT4/NANOG have correlated with prognosis of the oral cancer patients. A small number of studies have denied any correlation between expression of these molecules and various prognostic factors of OSCC,[19,20] although few researchers concur that SOX2, predominantly studied transcription factor, was found to be consistently related to early tumorigenesis or only lymph node metastasis irrespective its influence on other clinicopathologic factors affecting the prognosis of the tumor. Although majority clinical studies supported the putative role of these stem cell markers in worsening the survival outcomes of oral cancer patients,[17,25-30] few studies have reported about contrasting results, i.e., higher expression of these molecules was correlated either with negative lymph node metastasis or better prognosis of OSCC patients [Table 2].[15,31]

POTENTIAL THERAPEUTIC IMPLICATION OF OCT4/NANOG/SOX2 IN ORAL CARCINOMA

In OSCC, although surgical resection with neck dissection is continued to be the mainstay in the conventional treatment paradigm along with escalating contribution from radiotherapy and chemotherapy, the prognosis of this tumor is still not considered as favorable, due to tumor recurrence or treatment resistance. As CSCs are

Table 1: Details of clinical observational studies on transcription factors in oral potential malignant disorders

Author	Transcription factors studied	Sample	Observation	Inference
Qiao B *et al*., 2014[16]	OCT4 SOX2	Oral leukoplakia (*n*=12) Lichen planus (*n*=8)	OCT4 (14/20) and SOX2 (18/20) were positively detected, respectively. Twelve cases of premalignant lesion showed co-expression of OCT4 and SOX2 immuno-positivity	Co-expression of OCT4 and SOX2 may contribute to malignant transformation of oral mucosa
de Vicente *et al*., 2019[20]	SOX2	Oral epithelial dysplasia (*n*=55)	Nuclear SOX2 expression was detected in 7% of cases when a cutoff of 10% stained nuclei was used (SOX2 >10) and in 29% of cases when any positive nucleus was observed	SOX2 protein expression was found to significantly increase with the grade of dysplasia
de Vicente *et al*., 2019[21]	NANOG	Oral epithelial dysplasia (*n*=55)	Nuclear and cytoplasmic expression of NANOG detected in 3.6% and 16.4% of oral dysplasia cases as compared to the negative expression in normal adjacent epithelia	NANOG expression emerges as an early predictor of oral cancer risk in patients with OPMD

OPMD: Oral potentially malignant disorder

Table 2: Details of clinical observational studies on transcription factors in oral squamous cell carcinoma

Author	Transcription factors studied	Methodology	Sample	Observation and inference
Chiou S *et al*., 2008[25]	OCT-4, NANOG	IHC	OSCC (*n*=52)	Elevated expression of OCT-4 and NANOG was observed to be positively associated with tumor progression and worse prognosis of oral cancer. On comparison of expression of OCT4 and NANOG with prognosis of OSCC patients, NANOG was found to be a better predictor for worse prognosis as compared to OCT4
Freier K *et al*., 2010[27]	SOX2	FISH, qRT-PCR and IHC	frozen tumor samples of OSCC (*n*=40)	This study demonstrated gene copy number gain and consecutive increased protein expression of SOX2 in OSCC. In addition , the peculiar chromosomal location (3q) of SOX2 makes it a potential target proto-oncogene which could contribute to the initiation and progression of OSCC
Du L *et al*., 2011[26]	SOX2	IHC	Tongue squamous cell carcinoma (*n*=82)	SOX2 expression was significantly associated with large tumor size. Multivariate analysis of this study demonstrated that SOX2-positive expression was an independent prognostic indicator of unfavorable survival outcome
Michifuri Y *et al*., 2012[17]	SOX2	IHC	OSCC (*n*=80)	Two different staining patterns of SOX2 were observed, i.e., diffuse and peripheral. Diffuse staining pattern of SOX2 was significantly correlated with lymph node metastasis
Zullig L *et al*., 2013[31]	SOX2	IHC	T1/T2 oral SCC (*n*=120)	A significant correlation between high SOX2 expression and negative lymph node status was observed, and the authors suggested that SOX2 could be a potential predictive marker in early SCC of the oral cavity
Tsai L *et al*., 2014[30]	OCT4	Real-time RT-PCR analysis	Not Mentioned	The level of OCT4 expression was higher in recurrent and metastatic OSCC specimens but lower in primary OSCC specimens
Huang C *et al*., 2014[28]	OCT4 and SOX2	IHC	Tongue squamous cell carcinoma (*n*=66)	Overexpression of SOX2 and OCT4 was noticed. In addition, SOX2 emerged as an independent prognostic factor of poor survival outcome of carcinoma patients
Fu *et al*., 2016[15]	OCT4, SOX2 and NANOG	IHC	OSCC (*n*=436)	High SOX2 and OCT4 expression was significantly associated with a better prognosis for patients with OSCC
de Vicente *et al*., 2019[20]	SOX2	IHC	OSCC (*n*=125)	No correlation of expression of SOX2 with clinicopathologic prognostic factor was found. SOX2 was thought to be associated with early oral tumorigenesis rather than in tumor progression
Pradhan *et al*., 2019[29]	SOX2	IHC	0SCC (*n*=60)	A high expression of SOX2 was associated with early stage of the tumor
Baghai Naini F *et al.* 2019.,[19]	OCT4, SOX2 and NANOG	Quantitative reverse transcription PCR	OSCC (*n*=30)	No significant association between expression of OCT4, SOX2 and NANOG and clinical or pathological data of carcinoma patients

IHC: Immunohistochemistry, FISH: Fluorescence *in situ* hybridization, qRT-PCR: Real-time quantitative polymerase chain reaction, OSCC: Oral squamous cell carcinoma

thought to be a subpopulation of primary tumors which shows well-documented role in tumor relapse and initiation of metastasis, could possibly have special implications in targeted oncotherapy, mainly in head-and-neck carcinoma.[32] In experimental studies, pluripotent stem cell regulators, i.e., SOX2, OCT4 and NANOG have been observed to associated with treatment resistance in prostate cancer through thyroid signaling pathway.[9] In addition, OCT4 with or without expression with NANOG was observed to show higher expression in cisplatin-resistant solid cancers including OSCC, though few studies have been conducted to explore the potential of these molecules in therapeutic approach of OSCC.[33,34] These observations indicate about the possible cross-talk between cancer stemness and chemoresistance. Hence, these meager but growing evidences of their potential contribution as novel therapeutic approach create an opportunity of further clinical research in this context to expand diagnostic and prognostic utility of SOX2, OCT4 and NANOG in OSCC.

CONCLUSION

Based on the phenomenon of consistent overexpression of SOX2, OCT4 and to some extent NANOG, in oral potentially malignant disorders and OSCC patients, the role of these molecules in cancer initiation is undeniable. Although there are an ample amount of evidences that support their association with other aspects of carcinogenesis including cancer progression and metastasis or in predicting the prognosis of OSCC patients, controversial observations raise a concern on clinical application of these potent markers in cancer diagnosis, therapy or as survival predictors. Elucidation of exact underlying molecular mechanism causing their overexpression in OSCC through additional clinical or experimental studies is necessary to prove them as novel promising candidates in diagnostic, prognostic or interventional approaches for oral cancer patients.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Zakrzewski W, Dobrzy M, Szymonowicz M, Rybak Z. Stem cells: Past, present, and future. Stem Cell Res Ther 2019;5:1-22.
2. Gawlik-Rzemieniewska N, Bednarek I. The role of NANOG transcriptional factor in the development of malignant phenotype of cancer cells. Cancer Biol Ther 2016;17:1-10.
3. Díez Villanueva P, Sanz-Ruiz R, Núñez García A, Fernández Santos ME, Sánchez PL, Fernández-Avilés F. Functional multipotency of stem cells: What do we need from them in the heart? Stem Cells Int 2012;2012:817364.
4. Bissels U, Diener Y, Eckardt D, Bosio A. Characterization and classifi cation of stem cells. In: Regenerative Medicine – From Protocol to Patient. Cham, Switzerland: Springer International Publishing; 2016. p. 1-25.
5. Zhao W, Ji X, Zhang F, Li L, Ma L. Embryonic stem cell markers. Molecules 2012;17:6196-236.
6. Fritsch MK, Singer DB. Embryonic stem cell biology. Adv Pediatr 2008;55:43-77.
7. Ginis I, Luo Y, Miura T, Thies S, Brandenberger R, Gerecht-Nir S, *et al*. Differences between human and mouse embryonic stem cells. Dev Biol 2004;269:360-80.
8. Kimura H, Tada M, Nakatsuji N, Tada T. Histone code modifications on pluripotential nuclei of reprogrammed somatic cells. Mol Cell Biol 2004;24:5710-20.
9. Hepburn AC, Steele RE, Veeratterapillay R, Wilson L, Kounatidou EE, Barnard A, *et al*. The induction of core pluripotency master regulators in cancers defines poor clinical outcomes and treatment resistance. Oncogene 2019;38:4412-24.
10. Xu J, Zheng Z, Du X, Shi B, Wang J, Gao D, *et al*. A cytokine screen using CRISPR-Cas9 knock-in reporter pig iPS cells reveals that Activin A regulates NANOG. Stem Cell Res Ther 2020;11:67.
11. Kashyap V, Rezende NC, Scotland KB, Shaffer SM, Persson JL, Gudas LJ, *et al*. Regulation of stem cell pluripotency and differentiation involves a mutual regulatory circuit of the NANOG, OCT4, and SOX2 pluripotency transcription factors with polycomb repressive complexes and stem cell microRNAs. Stem Cells Dev 2009;18:1093-108.
12. Verneri P, Vazquez Echegaray C, Oses C, Stortz M, Guberman A, Levi V. Dynamical reorganization of the pluripotency transcription factors Oct4 and Sox2 during early differentiation of embryonic stem cells. Sci Rep 2020;10:5195.
13. Loebel DA, Watson CM, De Young RA, Tam PP. Lineage choice and differentiation in mouse embryos and embryonic stem cells. Dev Biol 2003;264:1-4.
14. Thomson M, Liu SJ, Zou L, Smith Z, Meissner A, Ramanathan S, *et al*. Pluripotency circuit members mediate germ layer fate choice of embryonic stem cells. Cell 2017;145:875-89.
15. Fu TY, Hsieh IC, Cheng JT, Tsai MH, Hou YY, Lee JH, *et al*. Association of OCT4, SOX2, and NANOG expression with oral squamous cell carcinoma progression. J Oral Pathol Med 2016;45:89-95.
16. Qiao B, He B, Cai J, Yang W. The expression profile of Oct4 and Sox2 in the carcinogenesis of oral mucosa. Int J Clin Exp Pathol 2014;7:28-37.
17. Michifuri Y, Hirohashi Y, Torigoe T, Miyazaki A, Kobayashi J, Sasaki T, *et al*. High expression of ALDH1 and SOX2 diffuse staining pattern of oral squamous cell carcinomas correlates to lymph node metastasis. Pathol Int 2012;62:684-9.
18. Moharil RB, Dive A, Khandekar S, Bodhade A. Cancer stem cells: An insight. J Oral Maxillofac Pathol 2017;21:463.
19. Baghai Naini F, Aminishakib P, Abdollahi A, Hodjat M, Mohammadpour H, Kardouni Khoozestani N. Relative expression of OCT4, SOX2 and NANOG in oral squamous cell carcinoma versus adjacent non- tumor tissue. Asian Pac J Cancer Prev 2019;20:1649-54.
20. de Vicente JC, Donate-Pérez Del Molino P, Rodrigo JP, Allonca E, Hermida-Prado F, Granda-Díaz R, *et al*. SOX2 expression is an independent predictor of oral cancer progression. J Clin Med 2019;8:1-14.
21. de Vicente JC, Rodríguez-Santamarta T, Rodrigo JP, Allonca E, Vallina A, Singhania A, *et al*. The emerging role of NANOG as an early cancer risk biomarker in patients with oral potentially malignant disorders. J Clin Med 2019;8:1-16.
22. Kopp JL, Ormsbee BD, Michelle Desler AR. Small increases in the level of Sox2 trigger the differentiation of. Stem Cells 2008;26:903-11.
23. Niwa H, Miyazaki J, Smith AG. Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. Nat Genet 2000;24:372-6.
24. Cai J, He B, Li X, Sun M, Lam AK, Qiao B, *et al*. Regulation of tumorigenesis in oral epithelial cells by defined reprogramming factors Oct4 and Sox2 . Oncol Rep 2016;36:651-8.
25. Chiou SH, Yu CC, Huang CY, Lin SC, Liu CJ, Tsai TH, *et al*. Positive correlations of Oct-4 and Nanog in oral cancer stem-like cells and high-grade oral squamous cell carcinoma. Clin Cancer Res 2008;14:4085-95.
26. Du L, Yang Y, Xiao X, Wang C, Zhang X, Wang L, *et al*. Sox2 nuclear expression is closely associated with poor prognosis in patients with histologically node-negative oral tongue squamous cell carcinoma. Oral Oncol 2011;47:709-13.
27. Freier K, Knoepfle K, Flechtenmacher C, Pungs S, Devens F, Toedt G, *et al*. Recurrent copy number gain of transcription factor SOX2 and corresponding high protein expression in oral squamous cell carcinoma. Genes Chromosomes Cancer 2010;49:9-16.
28. Huang CF, Xu XR, Wu TF, Sun ZJ, Zhang WF. Correlation of ALDH1, CD44, OCT4 and SOX2 in tongue squamous cell carcinoma and their association with disease progression and prognosis. J Oral Pathol Med 2014;43:492-8.
29. Pradhan S, Guddattu V, Solomon MC. Association of the co-expression of SOX2 and Podoplanin in the progression of oral squamous cell carcinomas – An immunohistochemical study. J Appl Oral Sci 2019;27:e20180348.
30. Tsai LL, Hu FW, Lee SS, Yu CH, Yu CC, Chang YC. Oct4 mediates tumor initiating properties in oral squamous cell carcinomas through the regulation of epithelial-mesenchymal transition. PLoS One 2014;9:e87207.
31. Züllig L, Roessle M, Weber C, Graf N, Haerle SK, Jochum W, *et al*. High sex determining region Y-box 2 expression is a negative predictor of occult lymph node metastasis in early squamous cell carcinomas of the oral cavity. Eur J Cancer 2013;49:1915-22.
32. Elkashty OA, Ashry R, Tran SD. Head and neck cancer management and cancer stem cells implication. Saudi Dent J 2019;31:395-416.
33. Villodre ES, Kipper FC, Pereira MB, Lenz G. Roles of OCT4 in tumorigenesis, cancer therapy resistance and prognosis. Cancer Treat Rev 2016;51:1-9.
34. Tsai LL, Yu CC, Chang YC, Yu CH, Chou MY. Markedly increased Oct4 and Nanog expression correlates with cisplatin resistance in oral squamous cell carcinoma. J Oral Pathol Med 2011;40:621-8.