



Cancer Stem Cells-Role in Oral Squamous Carcinoma - A Review of Literature

Deepthi Sogasu¹, Devaraj Ezhilarasan^{*2}, Smiline Girija AS³

¹Saveetha dental college and hospitals, Saveetha institute of medical and technical sciences, Saveetha University, Chennai-600077, Tamil Nadu, India

²Department of Pharmacology, Saveetha dental college and hospitals, Saveetha institute of medical and technical science, Saveetha University, Chennai 600077, Tamil Nadu, India

³Department of Microbiology, Saveetha dental college and hospitals, Saveetha institute of medical and technical sciences, Saveetha University, Chennai-600077, Tamil Nadu, India

Article History:

Received on: 23 Jul 2020
Revised on: 23 Aug 2020
Accepted on: 02 Sep 2020

Keywords:

Cancer cells,
tumorigenicity,
metastasis,
stem cells,
cancer stem cells,
oral cancer,
oral squamous cells

ABSTRACT

The cancer stem cells (CSC) are responsible for the growth of cancerous tumours. The renewal of cancer stem cells is considered synonymous with that of normal cells. The cancer cells are considered a progeny of specific and designated stem cells. The identification of these cancer stem cells has proven to be more difficult than anticipated due to the specific niches they are present in, within the tumour. Oral squamous carcinoma is the Sixth most common cancer constituting up to 2–4% of all malignancies worldwide and with poor prognosis. Tumour size and extent of lymph node metastasis are the most important predictors. The CSC works on the mechanism of bulk tumour cell formation and treatment resistance. But there are increased studies under this topic as it has been found that specifically targeting these cells can curb cancer. This review compiles information about the role of CSC in oral squamous cell carcinomas, the applications of CSC, and their use in the development of novel treatment options for oral cancer.



*Corresponding Author

Name: Devaraj Ezhilarasan
Phone: 9944008201
Email: ezhild@gmail.com

ISSN: 0975-7538

DOI: <https://doi.org/10.26452/ijrps.v11iSPL3.2906>

Production and Hosted by

IJRPS | <https://ijrps.com>

© 2020 | All rights reserved.

INTRODUCTION

Stem cells refer to those cells having the unique ability to divide and differentiate into a variety of cells and form different organs. Cancer stem cells are similar to that of the normal physiologic stem cells by the fact that they can divide and form huge numbers of cells and differentiate. They form 1-2% of

the tumour in cancer conditions. They have the properties of mutagenicity and tumorigenicity. On the surface of these cells, there are specific tumorigenic and non-tumorigenic surface markers that mediate their functions. The various hallmarks of cancer include activation of invasion and metastasis, enabling replicative immortality, induction of angiogenesis, and resistance to cell death. Cancer stem cells are known to showcase such properties. Oral squamous cell carcinomas are one of the most common heterogeneous cancers arising from the mucosal lining of the oral cavity. Oral cancer constitutes about 30% of cancers in India (Ezhilarasan *et al.*, 2017).

The origin of these cancers is due to poor lifestyle habits such as smoking, betel quid chewing, and excess alcohol consumption. The approved treatment for OSCC as of 2006 is Cetuximab which is a targeted molecular therapy. It selectively acts on the epidermal growth factor receptor. It is the only approved molecular therapy for the treatment of

OSCC. Cancer stem cells are found in the tumorous part of the body during the cancerous condition. The tumours can be CSC positive or CSC negative. If the tumour is CSC positive, the prognosis for that stage is relatively lower compared to the treatment of a CSC negative tumor. Thus, the CSCs play a major role in predicting the course of treatment of the OSCC and the prognosis. The CSC is essentially cancer stimulators. Thus specific targeted therapy against the surface markers is highly necessary.

In a recent study, they were able to isolate CSC and CSC like cells from OSCC cell lines. It was suggested that CSC like cells possessed the reduced ability of cell proliferation. They were also able to identify the CSC marker CD133 which is responsible for uncontrolled proliferation. There was minimal to no trace of Ki67, which is a marker that suggests reduced drug sensitivity. In a recent 2019 study, Syringic acid (SA), known to possess antioxidant, hepatoprotective, and anti-cancer effects. This traditional medicine was tested to check the efficiency of apoptosis on the oral squamous cancer cells through the mitochondrial pathway. The results of this study were positive and suggest a novel treatment method for OSCC (Ezhilarasan and Abijeth, 2020).

It is proven that there is significant downregulation of Mir 21 and Mir- 3 observed in the exosomes which have been isolated from the stem cell populations of OSCC tumors. This suggests that oral cancer cells have unique mi-RNA profiling. A remarkable study by discovered that the BMI1 inhibitor has therapeutic effects in cisplatin-resistant tumours. This is possible as it can reduce metastasis which is initiated by the circulating CSCs. The BMI1 inhibitor eventually leads to the necrotic cell death of CSCs. Thus, it was concluded that BMI 1 targeted inhibition is a novel treatment method for oral squamous cell carcinomas the authors of a review published recently suggested that the CSCs have the ability of epithelial-mesenchymal transition which accelerates the metastasis. They also suggested that the molecular-level understanding of CSC can help produce vaccines for cancer (Gurel, 2019).

This review is of importance currently due to the escalating number of patients succumbing to the harmful effects of cancer and in India, especially oral cancer. Finding a specific novel treatment and even possibly designing a vaccine against cancer can significantly reduce the frequency of the occurrence of cancer. A few challenges and difficulties faced by the other authors include the lack of single and specific biomarkers. The biomarkers for CSC are so many that it is not possible to target all of them at the same time. The treatment also can work only if a narrow-

targeted therapy is provided, so it is a daunting task to identify sensitive biomarkers for successful results correctly. This review also helps to understand CSCs, which provides an opportunity for creating unique treatment protocols. This research aims to help understand the role of cancer stem cells in oral squamous carcinomas.

MATERIALS AND METHODS

Cancer stem cells

The cancer stem cells (CSC) are specialized cells that function on the principle that tumor growth is analogous to the renewal of healthy tissues. Cancer stem cells from a small population of dedicated stem cells. Many tumors harbour CSCs in dedicated niches, thus making the identification of such cells very difficult. A recent study on gene-expression of cancer stem cell populations mediated a discovery of new prognostic biomarkers and pharmacological targets. Tumorigenic cancer stem cells have been observed to behave analogously to normal stem cells. The cancer stem cells possess the functional and phenotypic attributes similar to the normal physiologic cell from which they are derived. The main differentiating feature of the tumorigenic cancer stem cells and the physiologic stem cells are that they undergo a poorly regulated process of organogenesis. The importance of identification of the tumor subtypes is necessary as they possess the differential response to the anti-tumour drugs. This is possible via newer research avenues and advances made in the field of clinical oncology.

Oral squamous carcinoma

It is considered as the sixth most common cancer, accounting for up to 2–4% of all malignancies worldwide. Malignancy of oral cancers is relatively rare in the European expanse of the world. But in India, especially in Chennai, room for an exception must be anticipated. It is suggested that approximately 40% of malignant neoplasms in males are oral, and about 9% for the same among females. Among oral cancers, it is estimated that about 94.5% of them constitute oral squamous cell cancer. It is usually diagnosed with poor prognosis. The diagnosis and prognosis of oral squamous cell cancer can be made by careful analysis of the human metabolome. The results of the study suggest that the diagnostic potential for OSCC can be measured by evaluating the upregulation of L-carnitine, lysine, 2-methylcitric acid, putrescine; 8-hydroxyadenine; 17-estradiol; 5,6-dihydrouridine; and MTA (Sridharan et al., 2017).

A similar study suggested that saliva can be used

as a biomarker for the positive diagnosis of oral squamous cell carcinoma (Umamaheswari, 2014). Specific markers include MMP-9; Chemerin is used for early diagnosis of oral squamous cell carcinoma. The tumor size is proportionate to the extent of metastasis in the lymph node. They are one of the most important predictors of the extent of cancer. Oral squamous carcinomas are usually associated and accompanied by perineural invasions making the prognosis of the same worse. Poor clinical outcomes for oral squamous carcinoma is closely associated with the presence of multifocal and Extratem-poral PNI.

The tumor in oral squamous carcinoma is known to secrete exosomes surrounding the extracellular environment. This promotes the horizontal transfer of bioactive molecules via mechanisms involving microRNA. One of the important considerations to be looked at after tumor ablation is the functional and reconstruction of the dental structures with adequate rehabilitation. The problem of the hour is the lack of awareness among dental students about the methods diagnosis and prevention of oral cancer. This result is suggested by two different studies, conducted in India and Nepal (Prenit and Bandana, 2018).

Oral squamous carcinoma - normal propagation

A significant correlation has been established with RNF 8, and the predictive features of the tumor such as tumor thickness, ECS and nodal stage, radiore-sistance is expressed with causes spread of OSCC. About 30% of OSCC is RNF 8 positive. An OSCC tumor that is RNF 8 negative results in a much better prognosis. Recently, a novel DNA damage response protein has been discovered against RNF 8. It integrates protein phosphorylation, ubiquitylation signalling and plays a major role in cellular response by inducing genotoxic stress. Another reason for the propagation of oral squamous cancer is oxidative stress due to the reactive oxygen species. Oxidative stress potentiates the metastasis of cancer. This is similar to the effect of ROS in chronic liver disease, too (Ezhilarasan, 2018).

Application of cancer stem cells

The presence of CSCs in the tumor will accelerate cancer progression and metastasis and cause a low proliferation rate and high drug resistance, thus evading treatment. The cancer stem cells being a minor constituent of the tumor, can differentiate into bulk tumor cells. The cancer stem cells can also metastasize and cause alteration of adjacent stromal cells. This eventually causes the evasion of conventional treatment therapies. Thus, leading to a poorer prognosis. The cancer stem cells possess fea-

tures allowing its migration, invasion, and metastasis. Such features of the cancer stem cells interfere with the treatment process and eventually become treatment-resistant cells. One of the studies suggests that cancer stem cells can potentially be managed by bioenergetic signalling pathways involving fatty acid metabolism, glutamine metabolism, and the AKT-mTOR pathway (Chae and Kim, 2018).

The cancer stem cells are very dangerous and difficult to identify because they notoriously imitate the physiologic stem cells. One such case is that the cancer stem cells use the same signalling pathways that are found in normal stem cells which are the Wnt, Notch, and Hedgehog (Hh) pathway (Marimuthu, 2018). The main applications of cancer stem cells depend on two critical properties of the establishment and recurrence of cancerous tumors. Novel treatment using target therapy to treat cancers mainly works on the principle of intervening tumor progression and targeted inhibition of the cancer stem cells. Their self-renewal capacity and potential to differentiate is unlimited. They form heterogeneous populations of cancer cells. Novel therapeutic targets can be made for treatment by the prevention of tumor progression. The severity of cancer can be determined by evaluating the composition of CSCs within a tumor. The more stem cells, the larger is the tumor, and the poorer is the prognosis.

Current treatment options

The CSCs have components of the Renin-Angiotensin System, which combine with cancer stem cells which can be applied as a part of future cancer treatment. Octamer binding transcription 4 (OCT 4) and histone modification methods can be used to regulate the embryogenesis and pluripotency of CSC's. The "Two - hit" therapy involves the metabolic inhibitors which inhibit CSC propagation. Vitamin C is used as a target inhibitor of the glycolysis pathway, thus affecting CSC's (Satheesh et al., 2020). It has been suggested in a review article that Vitamin C is a powerful antioxidant in physiologic oral tissues.

It has been noted that the pro-oxidant activity of Vitamin C is activated in pathological oral tissues. This brings a conflict on the extensive use of Vitamin C as a target inhibitor. According to a study performed by (Francesco, 2019) suggest that TPP derivatives are considered a "powerful" candidate to block CSCs (Francesco, 2019). A meta-analysis for the novel treatment for oral tongue squamous cell cancer suggests that prognosis is possible through certain indicators such as occult node positivity, expression of E- cadherin and assessment of MMP9 at ITF. These markers are especially useful for high-

risk patients requiring invasive treatment strategies.

Another prognostic marker for oral tongue squamous cell cancer is based on the extent of p53 expression. It is associated with tumor depth and aggressiveness. It has been proven that a patient who has underlying conditions such as diabetes mellitus, can aggravate the metastasis of their tongue squamous cell carcinoma. Thus, hyperglycemia potentiates propagation and metastasis of tongue squamous cell carcinoma. It can thus be useful to reduce the blood glucose level to prevent spread and metastasis of cancer. This can be achieved using natural bioactive compounds such as those from *Caralluma Fimbriata* (Ashwini et al., 2017). Natural extracts of neem are also known to have anti-cariogenic properties against some oral cancers.

Cancer stem cells in all cancers

Cancer stem cells can be found in tumors of various types of cancers. They have been isolated and identified in myeloid leukaemia and more commonly in solid tumors of brain and breast cancer. Tumorigenic cancer stem cells have been isolated in Glioblastoma multiforme and medulloblastoma. The further detailed study concluded that the cancer stem cells had been restricted to the CD133+ subpopulation. Some of the most accepted theories suggest that the CSC arise as a result of epigenetic and genetic alterations to these resident tissue stem cells. Self-renewal and differentiation capabilities reside within the subpopulation of tumor cells, termed cancer stem cells (CSCs). The remaining tumor cell population cannot initiate tumor development or support continued tumor growth and proliferation. Cancer is metastatic primarily due to the ability of such CSCs to proliferate. The main principle that governs the functions of CSCs is that the cancers are dysregulated tissue clones with continual, distinct subsets of cells.

Cancer stem cells in oral squamous carcinoma

Cancer stem cells are of many subpopulations in the OSCC tumors. They show signalling pathways that are very similar to physiologic stem cells - Wnt, Notch, and Hedgehog (Hh) pathway. The cancer stem cells have the property of chemoresistance and radioresistance, allowing it to evade treatment. Even if treatment in oral squamous cell carcinoma. The recurrence is a more dangerous and aggressive form of cancer that can be fatal. The recurrence rate is 32.7% of the cases. The recurrence time ranges from 2-96 months, with an average of 14 months. Thus, it is necessary first to identify and then inhibit these cancer stem cells.

To identify such stem cells, it is necessary to know the various biomarkers of the cancer stem cells. Thus, in a similar review, where the markers have been isolated by flow cytometry. The CD-24 marker is known to promote tumor growth and facilitate angiogenesis. CD-29 is another biomarker which helps in tumor invasion, migration and metastasis of CSC (De Moraes, 2017). CD-44 is closely associated with the general characteristics of cancer stem cells. CD-98 promotes the tumor generation and at high levels destroys DNA repair genes. CD-133 can demonstrate the properties of cancer stem cells, especially of oral squamous carcinoma. A translational regulator, Musashi - 1, is considered as a marker of oral squamous cancer stem cells. It is known to be closely related to CD 133, indicating their utilitarian role in oral carcinogenesis (Jayanthi et al., 2020).

Another common biomarker specific to oral squamous carcinoma is Nestin. Nestin aids in neovascularization and angiogenesis. It is considered as an early biomarker for oral squamous carcinoma. Prognosis of OSCC can be predicted by the CSC proportion, tumor size and stage by examining the primary patient tumor. For inhibiting the biomarkers, it has been found that SOX 2 is a CSC regulator, so by inhibiting SOX 2, we can control the proliferation of CSCs also. It has also been suggested that the upregulation of BAX and PARP cleavage causes the simultaneous downregulation of Bcl-xl. This principle can be applied for long term treatment of CSCs, where the majority of cells undergo apoptosis. *Acacia catechu* has been known to increase the expression of the bax and Bcl-x gene and induce anti-cancer properties in SCC-25 cells (Ezhilarasan et al., 2017).

Novel treatment options

The main reason for seeking novel treatment options apart from the conventional cancer treatment options is that conventional methods such as chemotherapy, radiation and surgical intervention are unspecific. These methods subject the adjacent healthy cells to unwanted trauma. Hence, the necessity for newer specific treatment options arises. QKI is known to be a novel CSC inhibitor. It impairs multiple oral cancer stem cell properties via partial repression of SOX 2. Quinacrine based on gold hybrid nanoparticles has been used to inhibit DNA repair in cancer stem cells. Various stem cell markers such as Oct 4, SOX 2, Nestin, CD 44, can be suppressed by all-trans-retinoic acid.

NK cell function has been observed to increase in function when cultured with CSC of oral squamous carcinoma. This principle can thus be used to prepare novel treatment. NK cells can be repeatedly

transplanted to the site of the tumor, which causes the specific elimination of the oral squamous cancer cells. Histone modification which alters calcium regulation can control the signalling pathway of the CSC, which can be used as a novel treatment. TPP derivatives are considered powerful target inhibitors that block CSCs. Another novel treatment option to block the CSC is an iron chelator target therapy. The iron chelator is combined with chemotherapy which suppresses the stemness of the cancer stem cells. It can be used especially for oral squamous cell carcinoma.

In silico and in-vitro trials prove that coumarin derivatives help in an intrinsic pathway mediated apoptosis. This was tested for human stomach cancer cells, but this can also be tested against human oral cancer cells (Perumalsamy, 2018). The newer drugs can also be modified and made into nanoparticles and liposomes. This allows for better drug delivery and allows for efficient drug action. Among nanoparticles, it has been proven that selenium and zinc oxide nanoparticles are good chemotherapeutic agents (Menon, 2018). Target therapy to enhance pro-apoptotic agents is also considered as a novel treatment method for OSCC.

CONCLUSION

One of the main limitations of the study is that there is a variety of biomarkers for CSCs. It is extremely difficult to individualize one specific biomarker for treatment to fulfil the criteria of narrow range therapy to inhibit cancer stem cells. There is a risk of reoccurrence of cancer due to the progressive action of CSC and its metastasis. Thus, further research for specific target therapies is required. Understanding the mechanism of CSC will help develop novel treatment methods for cancer. Vaccine development based on principles of CSCs is underway and may eradicate cancer shortly. The study suggests that there is a significant role of cancer stem cells in oral squamous carcinoma.

Funding Support

The authors declare that they have no funding support for this study.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

REFERENCES

- Ashwini, S., Ezhilarasan, D., Anitha, R. 2017. Cytotoxic Effect of Caralluma fimbriata Against Human Colon Cancer Cells. *Pharmacognosy Journal*, 9(2):204–207.
- Chae, Y. C., Kim, J. H. 2018. Cancer stem cell metabolism: a target for cancer therapy. *BMB Reports*, 51(7):319–326.
- De Moraes, F. P. P. 2017. Expression of stem cell markers in oral cavity and oropharynx squamous cell carcinoma. *Oral surgery, oral medicine, oral pathology and oral radiology*, 123:113–122.
- Ezhilarasan, D. 2018. Oxidative stress is a bane in chronic liver diseases: Clinical and experimental perspective', Arab Journal of gastroenterology: the official publication of the Pan-Arab Association of. *Gastroenterology*, 19(2):56–64.
- Ezhilarasan, D., Abijeth, B. 2020. Syringic acid induces apoptosis in human oral squamous carcinoma cells through mitochondrial pathway. *Journal of Oral and Maxillofacial Pathology*, 24(1):40–40.
- Ezhilarasan, D., Lakshmi, T., Vijayaragavan, R., Bhullar, S., Rajendran, R. 2017. Acacia catechu ethanolic bark extract induces apoptosis in human oral squamous carcinoma cells. *Journal of Advanced Pharmaceutical Technology & Research*, 8(4):143–143.
- Francesco, E. M. D. 2019. Dodecyl-TPP Targets Mitochondria and Potently Eradicates Cancer Stem Cells (CSCs): Synergy With FDA-Approved Drugs and Natural Compounds (Vitamin C and Berberine). *Frontiers in oncology*, 9.
- Gurel, C. 2019. Cancer and Cancer Stem Cells: New Molecular Perspectives. *Critical reviews in oncogenesis*, (1):24–24.
- Jayanthi, P., Varun, B. R., Ramani, P. 2020. Cancer stem cells: A comprehensive review on identification and therapeutic implications. *Journal of Oral and Maxillofacial Pathology*, 24(1):190–190.
- Marimuthu, M. 2018. Canonical Wnt pathway gene expression and their clinical correlation in oral squamous cell carcinoma. *Indian Journal of dental research: official publication of Indian Society for Dental Research*, 29(3):291–297.
- Menon, S. 2018. Selenium nanoparticles: A potent chemotherapeutic agent and an elucidation of its mechanism. *Colloids and surfaces. B, Biointerfaces*, 170:280–292.
- Perumalsamy, H. 2018. In silico and in vitro analysis of coumarin derivative induced anti-cancer effects by undergoing intrinsic pathway-mediated apoptosis in human stomach cancer. *Phytomedicine: International Journal of phytotherapy and phytopharmacology*, 46:119–130.
- Prent, P. K., Bandana, K. 2018. Oral cancer aware-

- ness among undergraduate dental students in Nepal. *EC Dent Sci*, 17:2175–2178.
- Satheesh, N. J., Samuel, S. M., Büsselberg, D. 2020. Combination Therapy with Vitamin C Could Eradicate Cancer Stem Cells. *Biomolecules*, 10(1):1–20.
- Sridharan, G., Ramani, P., Patankar, S. 2017. Serum metabolomics in oral leukoplakia and oral squamous cell carcinoma. *Journal of Cancer Research and Therapeutics*, 13(3):556–561.
- Umamaheswari, T. N. 2014. Salivary biomarkers in oral leukoplakia-a review. *International journal of pharma and biosciences*, 6(5):3014–3018.