## In vitro Establishment and Characterization of Oral Squamous Cell Carcinoma Derived Cancer Stem-Like Cells

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Abstract: Evolving evidence proposes the existence of a highly tumorigenic subpopulation of undifferentiated, self-renewing cancer stem cells, responsible for exhibiting resistance to conventional anti-cancer therapy, recurrence, metastasis and heterogeneous tumor formation. Importantly, the mechanisms exploited by cancer stem cells to resist chemotherapy are very less understood. Oral squamous cell carcinoma (OSCC) is one of the most regularly diagnosed cancer types in India and is associated commonly with alcohol and tobacco use. Therefore, the isolation and in vitro characterization of cancer stem-like cells from patients with OSCC is a critical step to advance the understanding of the chemoresistance processes and for designing therapeutic strategies. With this, the present study aimed to establish and characterize cancer stem-like cells in vitro from OSCC. The primary cultures of cancer stem-like cell lines were established from the tissue biopsies of patients with clinical evidence of an ulceroproliferative lesion and histopathological confirmation of OSCC. The viability of cells assessed by trypan blue exclusion assay showed more than 95% at passage 1 (P1), P2 and P3. Replication rate was performed by plating cells in 12-well plate and counting them at various time points of culture. Cells had a more marked proliferative activity and the average doubling time was less than 20 hrs. After being cultured for 10 to 14 days, cancer stem-like cells gradually aggregated and formed sphere-like bodies. More spheroid bodies were observed when cultured in DMEM/F-12 under low serum conditions. Interestingly, cells with higher proliferative activity had a tendency to form more sphere-like bodies. Expression of specific markers, including membrane proteins or cell enzymes, such as CD24, CD29, CD44, CD133, and aldehyde dehydrogenase 1 (ALDH1) is being explored for further characterization of cancer stem-like cells. To summarize the findings, the establishment of OSCC derived cancer stem-like cells may provide scope for better understanding the cause for recurrence and metastasis in oral epithelial malignancies. Particularly, identification and characterization studies on cancer stem-like cells in Indian population seem to be lacking thus provoking the need for such studies in a population where alcohol consumption and tobacco chewing are major risk habits.

Keywords: cancer stem-like cells, characterization, in vitro, oral squamous cell carcinoma

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