Induced Pluripotent Stem Cells (IPSCs) Line Derived from H103—Orals Squamous Cell Carcinoma (OSCC) Cells

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INDUCED PLURIPOTENT STEM CELLS (IPSCs) LINE DERIVED FROM H103 - ORAL SQUAMOUS CELL CARCINOMA (OSCC) CELLS

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INTRODUCTION

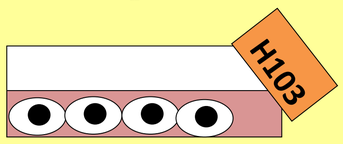
Cancer Cells can be reprogrammed into induced pluripotent stem cells (IPSCs) by forced expressions of pluripotency vectors (oct4, sox2, klf4 and c-Myc). Previous studies have shown the generated pluripotent stem cells from cancer cells achieved pluripotency capacity, but retain the genetic aberrations [1,2]. These properties facilitate cancer oncogenesis development study not achievable with current cancer models. To date, reprogramming of OSCC remained unexplored [3]. Hence, the urge to understand the cancer pathogenesis, prevention and treatment of OSCC leads to the proposed application of reprogramming technology. Derived OSCC-IPSCs may provide enormous application as a suitable oncogenesis model as well as cancer stem cell-targeted drug screening and toxicity testing.

OBJECTIVES

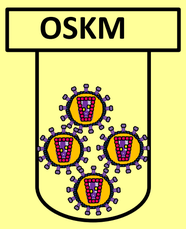
1. To reprogramme Oral Squamous Carcinoma Cell Lines into iPSCs derivatives.
2. To characterize its pluripotency properties for further downstream analysis.

METHODOLOGY

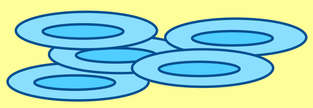
Step 1: Culturing of H103 Cells



Step 2: Generation of iPSCs

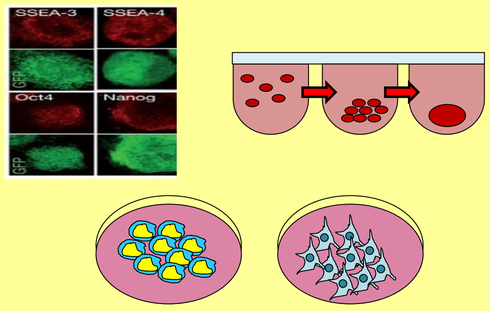


Yamanaka's Cocktail (**Oct4**, **Sox2**, **Klf4** and **c-Myc**)



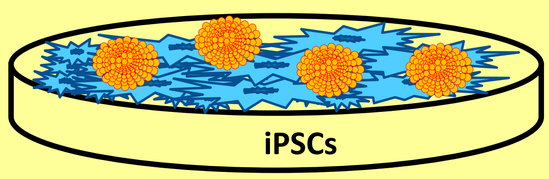
Integrating Viral Vector System used: **Retroviral-OSKM**

Step 3: Characterization of iPSCs Colonies



Characterization of morphology, pluripotency markers, embryoid bodies formation and directed differentiation capacity.

Step 4: Expand iPSCs Colonies



DISCUSSION

- **IPSC** cells were **successfully established** from parental H103 (OSCC) cancer cell line via retrovirus OSKM-based expression system.
- **Stable H103-IPSCs** exhibit **Embryonic Stem Cells (ESCs)** morphology and can be passaged *in-vitro* above passage 20 without change of properties indicative of self renewal **(Fig 1)**.
- Presence of **pluripotency expressions** were **confirmed** by means of immunofluorescence staining in H103-IPSCs **(Fig 2)**.
- **H103-IPSCs** formed **embryoid bodies'** with **compact structure** and **round borders.** Presence of **mesoderm marker (BRACHYURY)** in EB was **confirmed** by means of immunofluorescence staining **(Fig 3 & Fig 4)**.
- **H103-IPSCs** derived from H103 (OSCC) cancer cell line which originated from ectoderm-endoderm lineage, **was successfully** shown to posse **cross lineage differentiation capability into adipocytes and osteocytes stained positive for Oil-O-Red and Alizarin Red respectively (Fig 5).**
- In summary, **reprogramming** was **successful in H103 cell line** phenotypically exhibit **ESCs** like characteristics based on cell morphology, pluripotenct signals and differentiation capacity.
- Derived H103-IPSCs potentially used to develop **patient specific disease model** to **assess its cancer pathogenesis in future studies**.

References:

1. Kim, J. and Zaret, K. S., 2015. Reprogramming of human cancer cells to pluripotency for models of cancer progression. *The EMBO Journal,* 34**,** pp.739-747.
2. Lee, D. F., et al., 2015. Modeling Familial Cancer with Induced Pluripotent Stem Cells. *Cell,* 161**,** pp.240-254.
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RESULTS

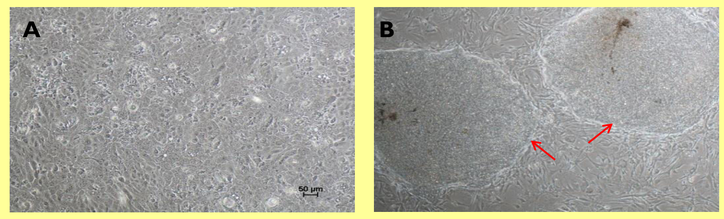

Figure 1: Reprogrammed H103 OSCC-Grade 1. (A) Morphology of H103 parental cancer cells, magnification:10x. (B) Representative image of an established H103-IPSC cell colonies at Passage 15 on MEF feeder layer. Distinct morphologies observed between parental cancer cells and the IPSCs derivatives. Red arrow indicates the clear borders of derived H103-IPSCs, magnification: 4x.

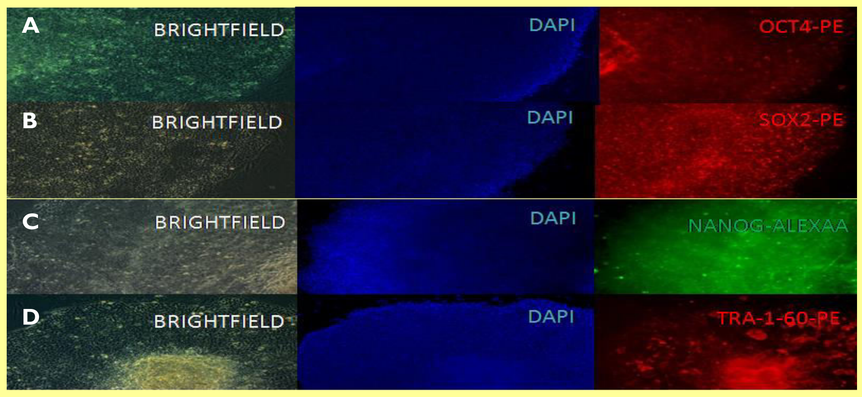

Figure 2: Expression of Pluripotent Markers in H103-IPSC. (A-D) H103-IPSCs colonies expressed markers common to pluripotent cells including OCT4, SOX2, NANOG, and TRA-1-60. Magnification: 10x

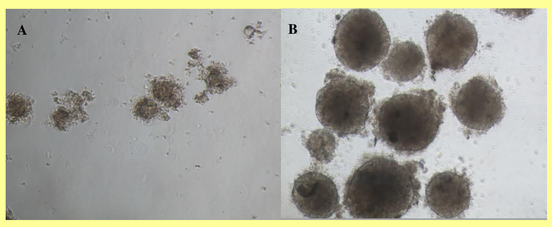

Figure 3: Embryoid Bodies (EBs) formation. Representative images of (A) parental H103-EB cells and (B) H103-IPSC-EB cultured in suspension for 8days. EBs from parental H103 was lack of compact structure and round borders unlike in H103-IPSC. Nikon inverted microscope, original magnification: 10x.

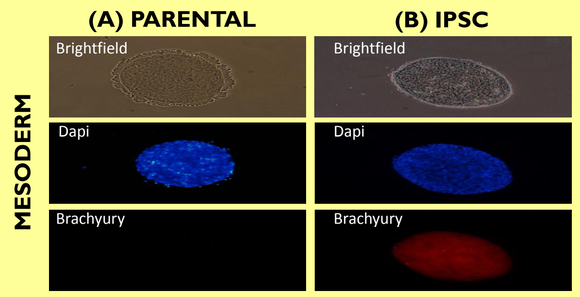

Figure 4: Immunofluorescence (IF) staining of parental H103-EB and H103-IPSCs-EB.(A&B)Representative images of parental H103-EB cells and H103-IPSCs-EB stained for embryoid bodies mesoderm marker (BRACYURY). EB mesoderm expression was not detected in parental H103 but expressed in H103-IPSCs derived embryoid bodies. Nikon inverted microscope, original magnification: 10x.

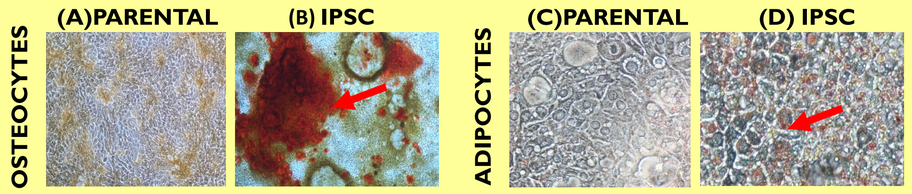

Figure 5: Differentiations into Osteocytes and Adipocytes. (A) Control parental H103 cells showing negative for the Alizarin red staining (B) H103-IPSCs cells showing positive for the Alizarin red staining indicating. Calcium deposits indicated by their red colour. (Red arrow) (C) Negative staining for Oil-O-Red in control parental H103 cells. (D) Adipogenesis induced lipid droplets observed in red colour after Oil-O-Red staining. (Red arrow) indicates the tiny lipid droplets.Nikon inverted microscope, original magnification: (A & B) 10x, (C & D) 40x.