Potential therapeutic effects of induced pluripotent stem cells on induced salivary gland cancer in experimental rats

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ABSTRACT

Salivary gland neoplasms exhibit complex histopathology in a variety of tumor types and treatment options depend largely on the stage of the cancer. Induced pluripotent stem cells (iPS) have been investigated for treating induced salivary gland cancer and for restoring salivary gland function. For our study in the vitro part, we re-programmed human skin fibroblasts to form iPS cells using a plasmid containing Oct4, Sox2, L-MYC and LIN28. In the vivo part, we used 30 white male albino rats divided into the following groups of 10: **group 1 (control)**: rats were injected with phosphate buffered saline (PBS), **group 2** induced squamous cell carcinoma (SCC): rat submandibular glands were injected with squamous carcinoma cells (SCC), **group 3** (induced SCC/iPS): SCC rats treated with 5 × 106 iPS cells. Submandibular glands from rats of all groups were examined histologically and real time PCR was performed for amylase, and COX I and COX II gene expression. We confirmed that submandibular gland specimens included tumor tissue before starting treatment with iPS. iPS treated cases exhibited regeneration of salivary glands, although minor degenerative and vascularization changes remained. The acinar cells regained their proper organization, but continued to exhibit abnormal activity including hyperchromatism. iPS cells may be useful for treating salivary gland carcinomas.

Introduction

Nearly 500,000 patients worldwide are treated yearly for head and neck cancer; of which 6% are salivary gland neoplasms being morphologically and clinically diverse and their treatments exhibit an unfavorable side effects, so a new treatment with no or limited side effects is needed. Induced pluripotent stem cells (iPS) is based on the report by Yamanaka (2009) that the use of four factors Oct4, Sox2, L-MYC and LIN28 could re-program human somatic cells to become pluripotent. Salivary gland stem cell therapy is an attractive approach to treat cancer patients.

Material and methods

❑**Squamous cell carcinoma animal model:** The rats were acclimatized for 4 days before starting the experiment. Hep2 laryngeal SCC passage 97 cell line was used. **Group 1(control**) was treated with 0.20 ml phosphate-buffered saline, For **group 2 (SCC),** submandibular salivary glands were injected with 60,000–95,000 cancer cells suspended in 0.20 ml PBS 3 times/week through an oblique ventral incision in the gland. For **group 3 (SCC/iPS**), SCC rats were inoculated with 5 × 106 iPS cells. The SCC group was sacrificed on week 7 after induction of cancer; 5 rats of the SCC/iPS group were sacrificed at week 4 and 5 were sacrificed at week 7 after iPS transplantation.

❑**iPS generation and implantation**: iPS were generated according to Okita et al. (2011). The fibroblasts collected from human volunteers Skin tissue 2 cm3, were re-programmed into iPS cells using an Amaxa 4D-Nucleofector with non-integrating plasmids containing Oct4, Sox2, L-MYC and LIN28. Reverse transcriptase-polymerase chain reaction (RT-PCR) for Oct4 and Sox2 genes expression was performed to confirm the transfection efficiency of induced iPS cells. Rats with induced salivary gland carcinoma were injected three times/week with 1 ml iPS cell suspension containing 60,000–95,000 cells/gland.

❑**Histopathology and quantification**: submandibular glands specimens embedded in paraffin and sectioned at 5 µm. Both mucous and serous acinar cells were counted manually in 5 different fields calculating the average number of acinar cell nuclei repeatedly for each tissue section for each group.

Immunohistochemistry: Oct4 was assessed by immunohistochemistry to confirm its expression in the SCC/iPS transplanted group and showed staining of more than 5% of the relevant nuclei was considered positive.

❑**Real time PCR**: Salivary gland tissue (30 mg) of each groups was lysed and total RNA for each sample was isolated using an RNA easy Mini Kit were the extracted RNA was quantified to express cox-1, cox-2 and amylase. Changes in the expression of each target gene were normalized to the mean critical threshold values of GAPDH as housekeeping gene using the ΔΔCt method.

Results

❑**iPS characterization:** RT-PCR of the Oct4 and Sox2 genes expression confirmed reprogramming of iPS through successive passages, morphological characterization of pluripotency included small cell size and single nucleus, which confirmed the typical features of iPS cells and, immunostaining of Oct4/iPS was strong in transplanted salivary glands, which confirmed colonization by iPS **(fig.1)**

❑**Histopathology:** Microscopic examination of the control group revealed normal acinar structures **(fig.2).** SSC group exhibited degeneration of acini with replacement by connective tissue. Malignancy was evident owing to hyperchromatism, individual cell keratinization and cellular pleomorphism, were keratin pearls and abnormal mitotic figures were observed in most specimens. The connective tissue stroma included significant inflammatory cell infiltration **(fig.3**). iPS group sacrificed at 4 weeks after transplantation exhibited nearly normal acinar architecture, but some cells still exhibited malignant hyperchromatic architecture and minor degenerative changes such as vacuolization. The iPS group that was sacrificed at 7 weeks after transplantation exhibited normal acinar cells with normal organization and maintenance of abnormal activity such as hyperchromatism. An increased number of ducts, dilation of interlobular ducts and chronic inflammatory cell remained between acini , in some areas **(fig.4).**

❑**RT-PCR:** The mRNA expression of the amylase gene was significantly decreased in the SCC group compared to the control group. mRNA expression was significantly increased in the SCC/iPS group compared to the SCC group . The mRNA expression of the COX-1 gene was significantly less for the SCC and SCC/iPS groups than for the control group. The expression of the COX-2 gene was significantly greater in the control group compared to the SCC and SCC/iPS groups **(fig.5)**.

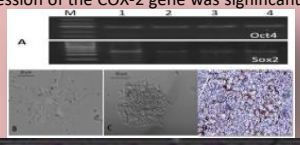

Fig.1

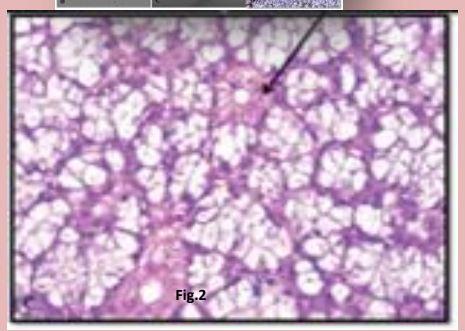

Fig.2

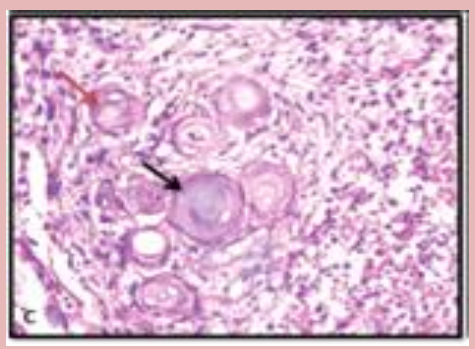

Fig.3

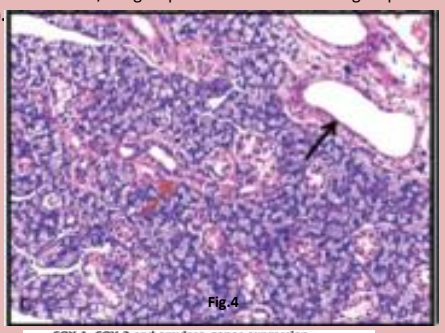

Fig.4

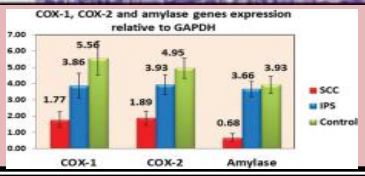

Fig.5

Discussion

The use of iPS cells is promising for many fields of study. It may be possible to correct genetic defects using iPS cells. For cancer, iPS cells enable investigation of epigenetic events and also a means to generate cells that will enable better drug screening and more targeted therapies with limited toxicity. Hep2 SCC cell line exhibited greater tumor formation than other cell lines. Salivary gland carcinomas are life threatening owing to their aggressive attributes. Traditional treatment modalities including surgery, chemotherapy and ionizing radiation add to the destructive side effects.