Archives of Oral Biology 79 (2017) 48–54

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/aob

Evidence of phenotypic stability after transduction of fluorescent proteins in two human tongue cancer cell lines

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ARTICLE INFO

Article history:
Received 10 May 2016
Received in revised form 1 March 2017
Accepted 2 March 2017

Keywords:
Human tongue cancer
Cell line
Lentiviral transduction
Stable cell labelling
Phenotypic stability

ABSTRACT

Objectives: This study investigated the phenotypic stability and biological properties of two human tongue cancer cell lines after transduction of fluorescent proteins.
Design: The human tongue cancer cell lines UM1 and UM2 were cultured with GFP and RFP lentiviral particles stock for 72 h. Cells with successful transduction of fluorescent proteins were selected in a medium containing G418 antibiotics for two weeks. The proliferation rates of parental and transduced cell lines were evaluated by their population doubling time (PDT). Transduction efficiency was assessed by fluorescence microscope and flow cytometry. The transduced cells in passage 1, 2, 10, 20 and 30 were collected to check the stability of fluorescent protein expression. Phenotypic stability of the transduced cells was detected by means of cell morphology, cell surface markers and cell function evaluating essay.
Results: The proliferation rates of the transduced cell lines showed no significant difference compared to their parental cells. Successful transduction with high efficiency (99% up) was demonstrated. High fluorescence expression on both transduced cells was detected until the thirtieth generation. UM1 and UM1-GFP displayed mesenchymal cell characteristics, while UM2 and UM2-RFP cell lines showed properties characteristic of epithelial.
Conclusions: Two human tongue cancer cell lines of epithelial and mesenchymal phenotype respectively, have been successfully labelled with green and red fluorescent proteins. The fluorescence maintained a high expression rate over thirty generations without influencing the original morphological phenotype and cadherin expression.

1. Introduction

Stable labelling of cancer cells constitutes a cornerstone for the investigation of tumour dormancy, proliferation, as well as the formation of micrometastases in animal models (Steinbauer et al., 2003). It is understood that during cancer progression the cancer cell phenotype frequently undergoes both epithelial to mesenchymal transition (EMT) and mesenchymal to epithelial transition (MET) (Jing, Han, Zhang, Liu, & Wei, 2011; Kalluri & Weinberg, 2009; Yang & Weinberg, 2008).

Previous in vitro fluorescence cell labelling using Calcein AM and fluorescent cytoplasmic microspheres provide only temporary labelling, as fluorescence was not transferred to the daughter cells (Guba et al., 2000; Steinbauer et al., 2003). Recently this shortcoming was overcome by using lentiviral vectors to transduce fluorescent proteins into the cell genome (Hechler, Nitsch, & Hendrix, 2006). This method enabled the investigation of mesenchymal and epithelial phenotypes of cancer cells during cancer progression in vivo (Tsuji, Ibaragi, & Hu, 2009; Tsuji et al., 2008). Whether the transduction of fluorescent proteins into the cell genome might affect the dynamic cell phenotype has not been investigated.

Both green (GFP) (Prasher, Eckenrode, Ward, Prendergast, & Cormier, 1992) and red (RFP) (Gross, Baird, Hoffman, Baldridge, & Tsien, 2000) fluorescent proteins delivered stable fluorescence expression patterns in daughter cells in vivo, offering thereby a

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http://dx.doi.org/10.1016/j.archoralbio.2017.03.002
0003-9969/

Table 1
Population doubling time (PDT) in hours of both parental and transduced tongue cancer cell lines UM 1 and UM 2.

Cell lines	Parental	Transduced	*P*-value
UM1	22.0 ± 0.6	21.9 ± 0.7	0.816
UM2	24.1 ± 0.5	23.7 ± 0.7	0.423
P-value	0.011	0.038	–

direct visualization possibility for cancer cells over a certain period (Chishima et al., 1997; Maeda et al., 2000; Peyruchaud et al., 2001).

In this study, two tongue cancer cell lines, one with a mesenchymal (named UM1) and one with an epithelial phenotype (named UM2), were labelled with GFP (UM1) and RFP (UM2) by lentiviral transduction, to investigate the stability of the fluorescence expression and the cell phenotype after several cell generations.

2. Materials and methods

2.1. Fluorescent protein transduction

Two tongue cancer cell lines UM1 and UM2 donation from Dr. David Wong, School of Dentistry, University of California Los Angeles, USA, originated from Nakayama, Okayama University Dental School, Okayama, Japan (Nakayama, Sasaki, Mese, Alcalde, & Matsumura, 1998–1999) were seeded into two wells of a 24-well plate and maintained in a 0.5 ml standard medium (DMEM/F-12, a mixture of Dulbecco's Modified Eagle Medium and Ham's F-12 medium at 1:1, Sigma, New York, NY, USA). A GFP lentiviral particles stock (LVP300, GenTarget, Inc., San Diego, America) in 5 μl was added into the UM1-seeded well. The same volume of a RFP lentiviral particles stock (LVP299, GenTarget Inc.) was added into the UM2-seeded well. The cell and lentiviral particle mixtures were then cultured in an incubator at 37 °C/5%CO_2. Fluorescent images of the transduced cell lines UM1-GFP and UM2-RFP were captured under a fluorescent microscope (Nikon, Tokyo, Japan) daily to assess the transduction status. Seventy-two hours later the UM1-GFP and UM2-RFP cell lines were trypsinized with 0.5% trypsin-ethylenediaminetetraacetic acid (trypsin-EDTA, Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) and transferred into a 25 cm^2 flask. GFP or RFP positive cancer cells were cultured in a medium containing G418 (Sigma, New York, NY, USA) for two weeks.

2.2. Population doubling time (PDT) calculation

The proliferation rates of all four cancer cell lines (UM1, UM1-GFP, UM2, UM2-RFP) were assessed by their population doubling time (PDT). The cells were harvested in their logarithmic growth phase using a 0.25% trypsin solution (Sigma, New York, NY, USA). A single cell suspension was then prepared and kept in a 50 ml centrifuge tube (Sigma, New York, USA). A 10 μm cell suspension was aspirated and placed on a hemocytometer to determine the cell concentration. The cells were then seeded onto a six well plate in triplicate with 1×10^5 cells per well. Thereafter the cells were harvested with 0.25% trypsin after being cultured in a standard medium for 48 h. The final concentrations of the harvested cells were determined with the above mentioned method. PDT was calculated according to the formula (Gruber et al., 2012):

$$PDT = (T - T_0) \log 2 / (\log N - \log N_0)$$

where $(T - T_0)$ represents the culture period in hours, N the final population in the end point of the experiment, and N_0 the initial number of seeded cells in one well. The mean PDT value was calculated based on the triplicated test arrangement. PTD calculation results were compared by independent-samples *t*-test with IBM SPSS software (SPSS version 20, Chicago, IL, USA). The significance level was set at $p < 0.05$.

2.3. Transduction efficiency and stability

2.3.1. Qualitative evaluation under fluorescent microscope

To qualitatively evaluate the transduction efficiency, UM1-GFP and UM2-RFP cell lines were observed under a total internal reflection fluorescence (TIRF) microscope (Nikon, Tokyo, Japan). Fluorescent images were obtained with Bandpass Emission (FGP (R)-BP) or FITC-Texas Red filter combination for UM1-GFP and UM2-RFP, respectively.

2.3.2. Quantitative evaluation with flow cytometry

The transduction efficiency was quantitatively assessed with flow cytometry (BD LSR Fortessa® Analyzer, Becton Dickinson Biosciences, Franklin Lakes, New Jersey, USA). UM1-GFP and UM2-RFP were collected at passage 1, 2, 10, 20 and 30 and a standard flow cytometry analysis was carried out on the collected cells.

UM1-GFP and UM2-RFP were rinsed with 1 X Phosphate buffered saline solution (PBS, Sigma, New York, USA) before performing trypsinization. Single cell suspensions were obtained and collected in a 15 ml centrifuge tube after being treated with of trypsin-EDTA (Sigma, New York, USA) for 3 min. The cell suspensions were then centrifuged at 1000 revolutions per minute

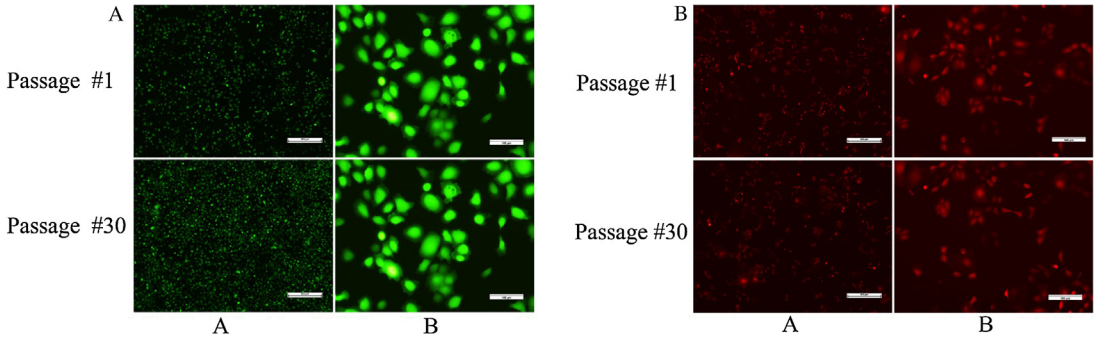

Fig. 1. a) and b): Fluorescent microscopy of UM1-GFP and UM2-RFP tongue cancer cell lines. After continuous culture for 3 months, the thirtieth passages of both UM1-GFP and UM2-RFP transduced daughter cells showed distinct and stable fluorescent signals under the fluorescent microscope, under low (A = 40×) and high (B = 200×) magnification.