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Molecular imaging for assessment of mesenchymal stem cells mediated breast cancer therapy

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ABSTRACT

The tumor tropism of mesenchymal stem cells (MSCs) makes them an excellent delivery vehicle used in anticancer therapy. However, the exact mechanisms of MSCs involved in tumor microenvironment are still not well defined. Molecular imaging technologies with the versatility in monitoring the therapeutic effects, as well as basic molecular and cellular processes in real time, offer tangible options to better guide MSCs mediated cancer therapy. In this study, an in situ breast cancer model was developed with MDA-MB-231 cells carrying a reporter system encoding a double fusion (DF) reporter gene consisting of firefly luciferase (Fluc) and enhanced green fluorescent protein (eGFP). In mice breast cancer model, we injected human umbilical cord-derived MSCs (hUC-MSCs) armed with a triple fusion (TF) gene containing the herpes simplex virus truncated thymidine kinase (HSV-ttk), renilla luciferase (Rluc) and red fluorescent protein (RFP) into tumor on day 13, 18, 23 after MDA-MB-231 cells injection. Bioluminescence imaging of Fluc and Rluc provided the real time monitor of tumor cells and hUC-MSCs simultaneously. We found that tumors were significantly inhibited by hUC-MSCs administration, and this effect was enhanced by ganciclovir (GCV) application. To further demonstrate the effect of hUC-MSCs on tumor cells *in vivo*, we employed the near infrared (NIR) imaging and the results showed that hUC-MSCs could inhibit tumor angiogenesis and increased apoptosis to a certain degree. In conclusion, hUC-MSCs can inhibit breast cancer progression by inducing tumor cell death and suppressing angiogenesis. Moreover, molecular imaging is an invaluable tool in tracking cell delivery and tumor response to hUC-MSCs therapies as well as cellular and molecular processes in tumor.

1. Introduction

Mesenchymal stem cells (MSCs) are multipotent cells that can be derived from a variety of tissues or organs, including bone marrow, adipose, placenta and umbilical cord [1]. With their capacity of tumor-specific tropism, MSCs have been considered to be attractive vehicles for delivering therapeutic agents toward tumor sites [2–5]. Several therapeutic strategies based on the local production of biological agents in tumors by gene-manipulated MSCs have been developed and exhibit potent antitumor activity [6,7]. Engineered MSCs with herpes simplex virus thymidine kinase (HSV-ttk), interferons (IFNs), interleukins (ILs), apoptosis inducers (e.g. TRAIL) or oncolytic viruses have manifested selective tumor repression [8–11]. Compared to other vehicles and/or delivery platforms as therapeutic carriers, MSCs provide exciting new possibilities for drug delivery used in tumor-targeted therapy.

Over the last decade, a variety of imaging technologies is being investigated as tools for cancer diagnosis, monitoring of response to cancer therapies and predicting of tumor response to available therapies as well as developing new drugs prior to clinical translation [12]. The successful clinical application of MSCs based tumor therapies needs non-invasive imaging approaches to monitor tumor progression and treatment outcome in real time [11,12]. Molecular imaging provides the possibility to visualize and monitor

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cellular and molecular processes, such as angiogenesis and apoptosis, in the living subjects for assessing the effect of MSCs on tumor progression [13,14].

Here, we hypothesize that real time *in vivo* imaging technologies could offer tangible options for better guiding MSCs delivery and monitoring antitumor activity of MSCs therapy. To test this hypothesis, we developed mouse model to analyze the behavior and efficiency of human umbilical cord-derived MSCs (hUC-MSCs) as a cellular vehicle for breast cancer therapy. We introduced dual reporter genes renilla luciferase (Rluc) and firefly luciferase (Fluc) for bioluminescence imaging of tumor progression and hUC-MSCs survival simultaneously within the same animal. Moreover, near infrared (NIR) fluorescence imaging method was applied to assess angiogenesis and apoptosis of tumor after hUC-MSCs therapy.

2. Materials and methods

2.1. Cell culture

Human breast cancer cell line MDA-MB-231 was purchased from ATCC (Manassas, VA, USA) and human umbilical cord mesenchymal stem cells (hUC-MSCs) were isolated and cultured as described [15,16]. MDA-MB-231 cells were grown in DMEM medium supplemented with 10% fetal bovine serum (FBS), 1% penicillin–streptomycin solution (Gibco, Rockville, MD), and 1% MEM non-essential amino acid solution (Gibco). hUC-MSCs were cultured with DMEM/F12 medium (Gibco) containing 10% FBS, 1% penicillin–streptomycin solution (Gibco), 10 ng/ml human recombinant epidermal growth factor (EGF; Gibco). For tracking transplanted cells *in vivo*, MDA-MB-231 cells were transduced with a self-inactivating lentiviral vector carrying an ubiquitin promoter driving firefly luciferase and enhanced green fluorescence protein (Fluc-eGFP) double fusion (DF) reporter gene. In addition, hUC-MSCs were labeled with a self-inactivating lentiviral vector carrying an EF1α promoter driving renilla luciferase (Rluc), red fluorescence protein (RFP), and herpes simplex virus truncated thymidine kinase (HSV-ttk) (Rluc-RFP-HSV-ttk) triple fusion (TF) reporter gene as described previously [9,11].

2.2. Tumor model

8–12 weeks old female Nu/Nu Nude mice (Laboratory Animal Center, the Academy of Military Medical Sciences, Beijing, China) were housed under standard laboratory conditions. All experimental procedures were conducted in conformity with institutional guidelines for The Care and Use of Laboratory Animals in Nankai University, Tianjin, China, and conformed to the National Institutes of Health (NIH) Guide for Care and Use of Laboratory Animals. Mice were injected with 1×10^6 MDA-MB-231 (Fluc-eGFP) cells into the fourth pair of mammary fat pad (day 0). After 13 days later, an intratumor injection of 1×10^6 MSC-TF cells was given on day 13, 18, 23. Twenty-four hours after hUC-MSC-TF cells injection, mice of the group were intraperitoneally injected with GCV (30 mg/kg) or PBS on days 14–17, 19–22, 24–27. Intratumor injection with PBS and ganciclovir (GCV; Keyi Pharmaceutic, Wuhan, Hubei, China) application for tumor bearing mice severed as control. Tumor development was evaluated by BLI of Fluc and hUC-MSC-TF cells were tracked by BLI of Rluc. At the end of experiment (day 30), all mice were euthanized and tumors were harvested for further analysis.

2.3. Optical imaging

Optical imaging was performed using IVIS 200 Imaging System (Xenogen Corporation, Hopkinton, MA). BLI of the fate of transplanted cells in living mice was done as described previously [17]. Imaging of Fluc and Rluc expression was used for assessing tumor development and hUC-MSC-TF cells' fate respectively. D-Luciferin (150 mg/kg; Biosynth International, Naperville, IL) was intraperitoneally injected into mice for evaluating Fluc expression, and each mouse was imaged for 1 s to 3 min. Coelenterazine (2.5 mg/kg; NanoLight Technology, Pinetop, AZ) was intravenously into mice for assessing Rluc expression. After injection of coelenterazine, mice were imaged for 2 min, immediately.

To monitor tumor angiogenesis and apoptosis status after hUC-MSC-TF cells administration, NIR fluorescence imaging of integrin $\alpha v\beta_3$ and annexin V was carried out with reagents IntegriSense™ 750 and Annexin-Vivo™ 750 (PerkinElmer, Waltham, MA). For IntegriSense™ 750 imaging, fluorescence signal was measured at excitation 755 nm (emission 775 nm) 24 h post-injection; similarly, fluorescence signal was obtained 2 h after Annexin-Vivo™ 750 imaging agent administration at excitation 755 nm (emission 772 nm) following the manufactures' recommendations.

2.4. Cell viability and bystander effect

To determine the effects of GCV on hUC-MSC-TF (Rluc-RFP-HSV-ttk), cells were seeded in 96-well plates (2×10^3/well) and treated with different doses of ganciclovir (GCV; Keyi Pharmaceutics, Wuhan, Hubei, China) (0–40 μg/ml) 24 h after plating. Cell viability was assessed at 48 h post GCV treatment by Cell Counting Kit-8 (CCK-8) (Dojindo). To investigate the time courses survival of hUC-MSC-TF cells after GCV treatment (40 μg/ml) over time, CCK-8 assay was carried out at day 1, day 2 and day 3. To assess bystander effect, different proportions hUC-MSC-TF cell were mixed with MDA-MB-231 (Fluc-eGFP) cells in 24-well plates and treated with GCV (40 μg/ml) after plating. After 2 days, the survival of MDA-MB-231 cells was evaluated by Fluc expression using IVIS 200 Imaging System (Xenogen Corporation). Moreover, to

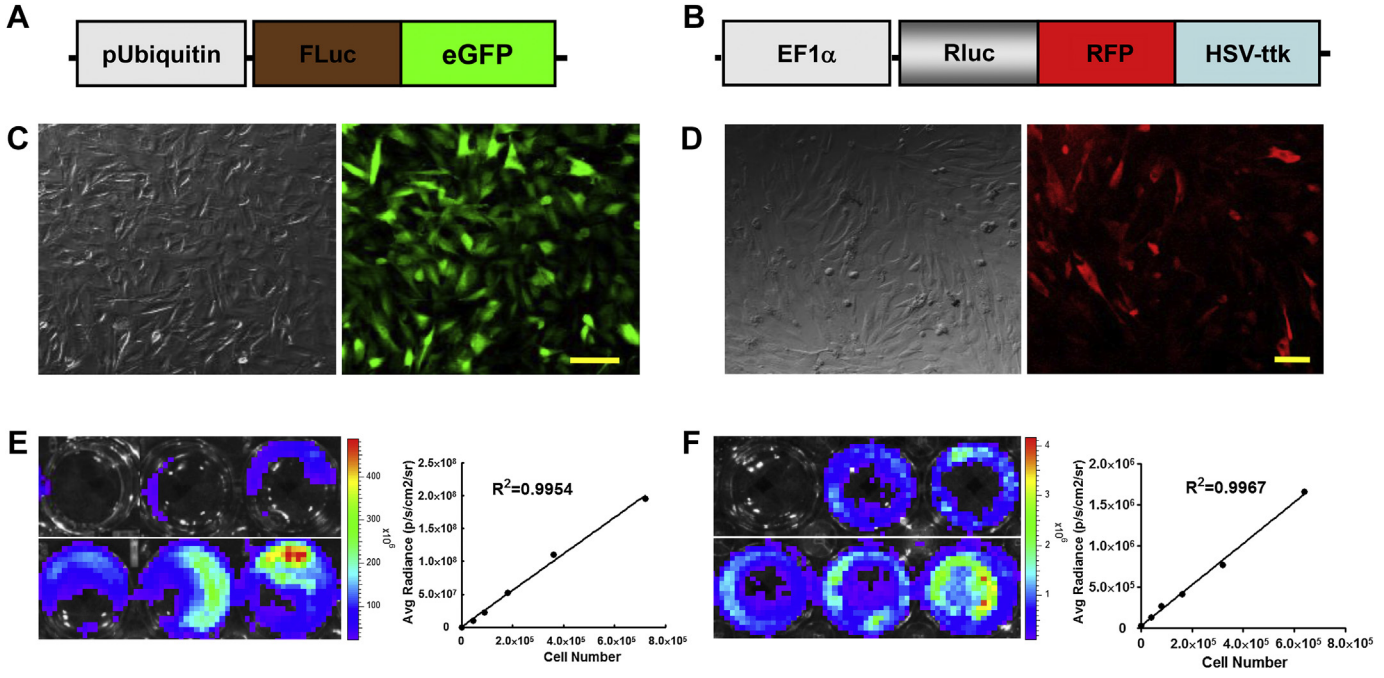

Fig. 1. Transduction of MAD-MB-231 cells, hUC-MSCs with double fusion (DF) and triple fusion (TF) reporter genes, respectively. (A) Schema of the DF reporter gene containing Fluc and eGFP driven by an ubiquitin promoter. (B) Schema of the TF reporter gene contains Rluc-RFP-HSV-ttk driven by an EF-1α promoter. (C) Transduced MDA-MB-231 cells are strongly positive for eGFP on fluorescence microscopy. (D) RFP was expressed robustly in hUC-MSCs. (E & F) *Ex vivo* imaging analysis of stably transduced MDA-MB-231 cells and hUC-MSCs shows a robust correlation between cell numbers and Fluc/Rluc reporter gene activity. Scale bar = 100 μm.