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Establishment and characterization of McA-RH7777 cells using virus-mediated stable overexpression of enhanced green fluorescent protein

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Abstract. Hepatocellular carcinoma (HCC), the most common primary tumor of the liver, has a poor prognosis, rapid progression. The aim of the current study was to establish a stable lentiviral expression vector for enhanced green fluorescent protein (EGFP) and to evaluate biological characteristics on HCC growth and migration following transfection of HCC cells with EGFP. McA-RH7777 cells were transfected with EGFP overexpression lentiviral vector. Cell activity and mobility were monitored with a Cell-IQ Analyzer. Transwell assays were performed to detect invasiveness and flow cytometry was performed for cell cycle analysis. A subcutaneous tumor rat model was established to analyze the stability of fluorescent protein expression. The result suggested no significant differences between wild-type and EGFP-overexpressing McA-RH7777 cells with regards to cell proliferation, activity, mobility, invasiveness and cell cycle. Green fluorescence was detected over 108 days of culturing. The subcutaneous tumor rat model demonstrated that EGFP expression had no influence on tumor growth and long-term expression was stable. The stable EGFP expression of the HCC transplanted tumor rat model may share biological characteristics with human liver cancer. The model established in the current study may be suitable for various applications, including research focusing on liver cancer metastasis and recurrence, interventional therapy, imaging diagnosis and drug screenings.

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Introduction

Primary liver cancer (PLC), particularly hepatocellular carcinoma (HCC), is one of the common malignant tumors worldwide; ~748,000 new PLC cases were diagnosed worldwide in 2008 (1). China has the highest incidence of liver cancer worldwide, 55% of all liver cancers incidences are diagnosed in China, and 50% of all cancer associated mortalities are patients with liver cancer (2).

Treatment methods for HCC include surgical excision, liver transplantation, interventional therapy, molecular targeted therapy and radiotherapy. Surgical resection of early HCC can result in improved clinical results compared with advanced HCC (3). However, as early symptoms of HCC are not readily apparent, the majority of liver cancer (88%) is identified in late stages (4). Additionally, the presence of early intrahepatic spread or widespread cirrhosis of the liver allows for surgical removal in <25% of patients following a liver cancer diagnosis (5). Current clinical practice for patients that do not qualify for surgery or whose conditions do not allow for radical resections, prioritizes interventional therapy, including hepatic artery chemoembolization (6).

High metastasis and recurrence rates of HCC are major factors affecting prognosis (5,6). Following surgical screening, patients with vascular infiltration or metastasis are treated with embolization and effects of embolization on liver cancer metastasis are further addressed (7). The effects of embolization on inhibition or promotion of tumor metastasis and on different stages of liver cancer remain unknown. In addition, effects of metastasis on embolization efficacy and methods of improving curative effects of embolization while inhibiting tumor metastasis remain unclear.

The majority of clinical staging is determined by clinical examination, test results and imaging examination (8). Tumor progression is a complex process involving cells, growth factors and their receptors, adhesion and extracellular matrix molecules, tumor blood vessels and the immune system (9). It is important to determine the staging of liver cancer accurately, as different stages require varying treatments and improper staging can lead to delayed or overtreatment (10).

Green fluorescent protein (GFP) is a low molecular weight protein that emits green fluorescence at 597 nm when excited at

488 nm. It can be fused to target proteins without affecting the spatial conformation or function of the gene products (11-14). For the study of tumors, GFP marker genes can be used to determine gene expression levels and to estimate changes in gene quantity, in order to explore the roles and underlying molecular mechanisms of specific genes in tumor occurrence and development (15). Expression of GFP in tumor cells can be used to determine the initiation and progression of tumor metastasis (16).

The current study was designed to establish an enhanced (E) GFP vector and for lentivirus-mediated transfection of McARH7777 cells to produce stable gene expression. Stable expression of GFP in a rat liver cancer model using EGFP-overexpressing McA-RH7777 cells was established to evaluate biological characteristics. This study aims to provide novel ideas for clinical diagnosis and treatment of tumors.

Materials and methods

Ethics statement. All animals were treated in accordance with the Guide for the Care and Use of Laboratory Animals of the Zhongshan Hospital of the Fudan University (Shanghai, China) and all experiments were approved and performed according to the guidelines of the Ethics Committee of the Affiliated Zhongshan Hospital of Fudan University (Shanghai, China). All surgical procedures were performed under anesthesia and every effort was made to minimize suffering.

Cell lines and cell culture. Liver cancer cells (McA-RH7777) were purchased from the American Type Culture Collection (Manassas, VA, USA; CRL-1601) and cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.), 100 U/ml penicillin and 100 μg/ml streptomycin at 37˚C and 5% CO_2. Medium was replaced every 3 days.

Lentivirus-mediated expression of EGFP in McA-RH7777. McA-RH7777 cells were seeded into 96-well plates at $3x10^3$ cells/well. For EGFP overexpression, McA-RH7777 cells were transfected with lentivirus (Nanjing SenBeiJia Biological Technology Co., Ltd., Nanjing, China) harboring Lv-pGC-FU-EGFP-IRES-puromycin (Shanghai GenePharma Co., Ltd., Shanghai, China) containing an EGFP overexpression sequence. All lentivirus transfections were performed in the presence of 5 μg/ml polybrene (Nanjing SenBeiJia Biological Technology Co., Ltd.). Following culturing in DMEM supplemented with 10% FBS at 37˚C in a 5% CO_2 incubator for 96 h, transfected McA-RH7777 cells were examined for EGFP expression under an inverted fluorescence microscope (magnification, x200). The number of EGFP-positive cells was used to calculate the transfection efficiency. Successfully transfected McA-RH7777 cells (McA-RH7777-EGFP) were selected using puromycin (1 μg/ml) over 2 weeks at 37˚C in a 5% CO_2 incubator. EGFP expression was confirmed by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and western blot analysis. Untransfected McA-RH7777 cells served as negative control.

Transwell assays. Invasiveness of McA-RH7777 and McA-RH7777-EGFP cells was studied with Transwell assays (Axygen; Corning, Inc., Corning, NY, USA). Briefly, prior to addition of cells into Transwell chambers, membranes of each chamber were coated with membrane Matrigel (50 mg/l; dilution, 1:8; BD Biosciences Franklin Lakes, NJ, USA) for 30 min at room temperature prior to following experiments. Cell suspensions ($1x10^5$ cells) prepared in serum-free medium were added to the upper chambers and the lower chambers were filled with complete medium supplemented with 10% FBS. Following incubation for 24 h at 37˚C, residual cells in the upper chambers were wiped off with a cotton swab and cells that migrated to the lower surface of the membrane were fixed with 4% formaldehyde for 20 min at room temperature and stained with crystal violet for 20 min at room temperature. Cells were counted in five random fields using an inverted microscope (magnification, x200). Experiments were performed in triplicate. The relative invasion rate was determined as follows: Number of McA-RH7777-EGFP/number of migrated McA-RH7777 x100%.

Comparison of cell viability and mobility. McA-RH7777 and McA-RH7777-EGFP cells (1 ml) were seeded into 24-well plates at $1x10^4$/ml and were cultured at 37˚C in 5% CO_2. Medium was replaced every 2 days. A Cell-IQ Analyzer (Chip-Man Technologies, Ltd., Tampere, Finland) was used to monitor cell activity and mobility every 12 h for 108 days using Cell-IQ-200 Analyzer software (version IQ200; Chip-Man Technologies, Ltd.).

Flow cytometry. Flow cytometry was used to analyze the cell cycle of McA-RH7777-EGFP and McA-RH7777 cells. Cells ($1x10^6$) were collected (2,000 x g, 5 min, room temperature) following a 0.25% trypsin digest, washed with PBS and fixed with 70% ethanol for 12 h at 4˚C. The stationary liquid was removed prior to staining with RNase A (100 μl) and peridinin chlorophyll protein complex-cytochrome 5-5A (400 μl; BD Biosciences, Franklin Lakes, NJ) for 15 min at room temperature in the dark. Binding buffer (400 μl; BD Biosciences) was added to each sample and samples were analyzed on a flow cytometer and evaluated using FlowJo software (version 7.6.5; FlowJo LLC, Ashland, OR, USA). Each experiment was performed in triplicate.

Animal experiments. For tumor growth assays, McA-RH7777 and McA-RH7777-EGFP cells ($2.0x10^6$) were injected subcutaneously into the right scapula of male Buffalo rats (age, 5 weeks; n=6 per group, Shanghai SIPPR-Bk Lab Animals Co. Ltd., Shanghai, China). All rats had free access to food and water and were housed under controlled conditions (12-h light/dark cycles; humidity, 60±5%; temperature, 22±3˚C). Rats were observed over 35 days for tumor formation, the tumor volume (V) was recorded every 7 days and calculated using the following formula: $V=0.5 \times \text{length} \times \text{width}^2$. On day 35, rats were anesthetized by intraperitoneal injection of sodium pentobarbital (30 mg/kg body weight) prior to sacrificing. Tumors were used for EGFP detection. Tumor tissues were cut into 5-μm slices and analyzed using a fluorescence microscope (magnification, x400).

Statistical analysis. Continuous variables are expressed as the mean ± standard deviation. Statistical significance of multiple groups was evaluated by one-way analysis of variance followed

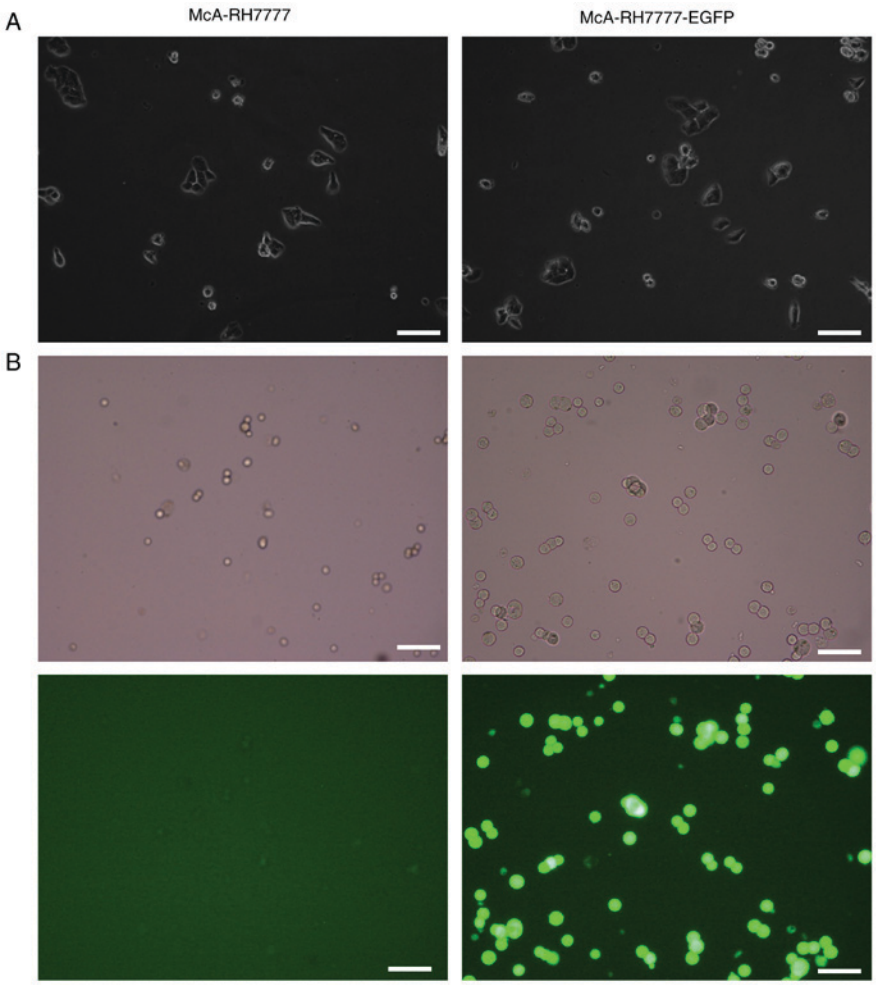

Figure 1. EGFP gene expression. (A) Morphology of McA-RH7777 cells and McA-RH7777 cells transfected with EGFP-overexpressing lentivirus; images were recorded using Cell-IQ real time monitors on day 108 following transfection. (B) Phase-contrast and fluorescence microscopy images of McA-RH7777 and McA-RH7777-EGFP cells on day 108 following transfection. Scale bar, 30 μm. EGFP, enhanced green fluorescent protein.

by Tukey's multiple comparison test and pairwise comparison by two-sided Student's t-test using GraphPad Prism software, version 5.0 (GraphPad Software, Inc., La Jolla, CA, USA). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Comparison of biological characteristics of McA-RH7777 and McA-RH7777-EGFP cells. Experimental observations indicate that the lentiviral vector carrying EGFP was successfully transfected into McA-RH7777 cells. The established McA-RH7777-EGFP cells exhibited stable expression of EGFP and puromycin resistance. Cell-IQ live cell monitoring indicated that McA-RH7777-EGFP and wild type McA-RH7777 cells exhibited stable adherent growth, fusiform, polygonal character and no growth inhibition. Lentiviral transfection had no influence on morphology and growth of McA-RH7777 cells (Fig. 1A). Following 108 days *in vitro* culturing, fluorescence intensity and expression were stable (Fig. 1B). McA-RH7777-EGFP exhibited stable expression of green fluorescence *in vitro* and fluorescence intensity was not markedly reduced in long-term culturing.

Flow cytometry analysis further indicated no significant difference between McA-RH7777 and McA-RH7777-EGFP cells with respect to the cell cycle (Fig. 2). Cell-IQ-200 Analyzer software analysis indicated no significant differences in cell growth (Fig. 3A) and mobility (Fig. 3B) between McA-RH7777 and McA-RH7777-EGFP cells. Transwell assays indicated that EGFP overexpression did not affect cell invasiveness (Fig. 3C and D).

Effects of EGFP expression on tumor growth and maintenance of green fluorescence in vivo. McA-RH7777 and McA-RH7777-EGFP cells were subcutaneously injected into rats. All animals grew subcutaneous tumors and the tumor formation rate was 100%. On day 35 of follow-up, the tumors were collected. Tumor volumes for McA-RH7777 and McA-RH7777-EGFP injected animals were 20,909.5±4,707.46 and 20,392.4±3,506.3 mm^3, respectively. Statistical analysis suggested no significant difference among the groups (Fig. 4A). The growth rate of the tumors was consistent between the two groups (Fig. 4B), suggesting that McA-RH7777-EGFP had no effect on tumor growth. Fig. 4C presents an excised tumor from an animal of the McA-RH7777-EGFP group. Following 35 days follow-up, the tumors were isolated from the sacrificed animals and tumor slices were analyzed for EGFP expression under a fluorescence microscope and expression was observed (Fig. 4D). GFP exhibited stable expression and no influence on tumor growth was suggested.

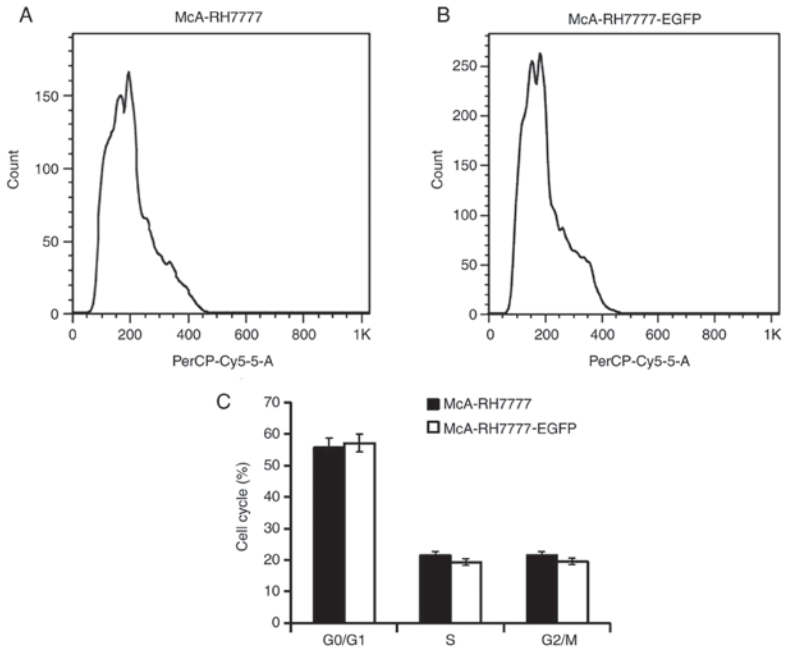

Figure 2. Cell cycle analysis using flow cytometry. Flow cytometry detection of the cell cycle in (A) McA-RH7777 and (B) McA-RH7777-EGFP cells. (C) Comparison of the cell cycles of McA-RH7777 and McA-RH7777-EGFP cells. EGFP, enhanced green fluorescent protein; PerCP, peridinin-chlorophyll.

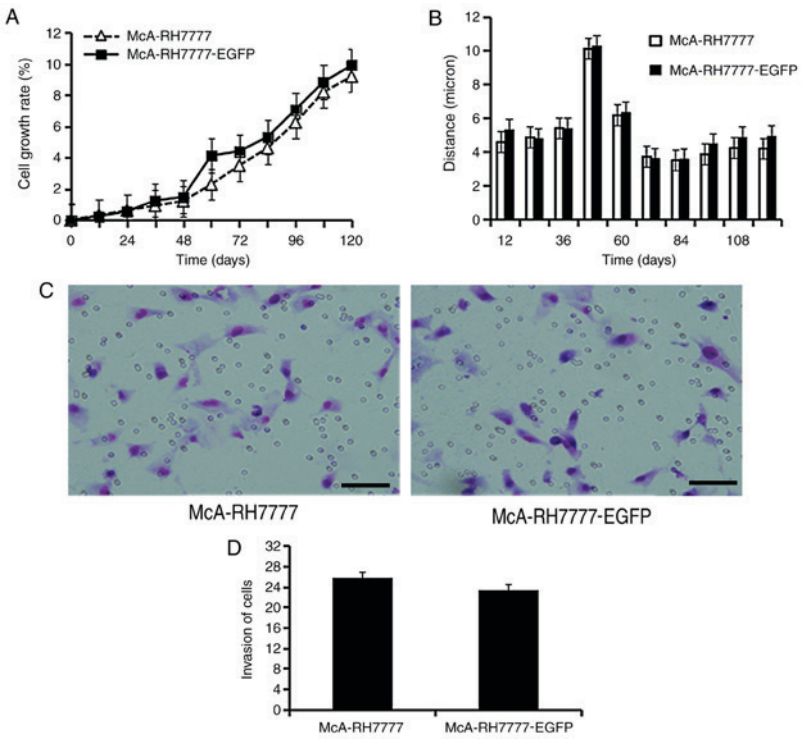

Figure 3. Cell activity and mobility. (A) Cell growth rate and (B) comparison of cell mobility ability in McA-RH7777 and McA-RH7777-EGFP cells. (C) Light microscope images and (D) Transwell invasion assay of McA-RH7777 and McA-RH7777-EGFP cells. Scale bar, 40 μm. EGFP, enhanced green fluorescent protein.

Discussion

Metastasis and recurrence of HCC begins with the shedding of single cells from a primary lesion, making it difficult to accurately trace the path that tumor cells follow (17). Fluorescence detection of tumor cells may aid understanding of the effects of various interventions on a tumor in a timely and accurate manner (18). In the field of malignant tumor metastasis, research progress has been limited due to a lack of technology to detect the transfer of cells. However, with the development of suitable optical imaging technology, it is now possible to detect tumor cells and gene expression accurately (19-21).

GFP is a low molecular weight protein; its chromophore is formed by internal amino acid dehydrogenation cyclization

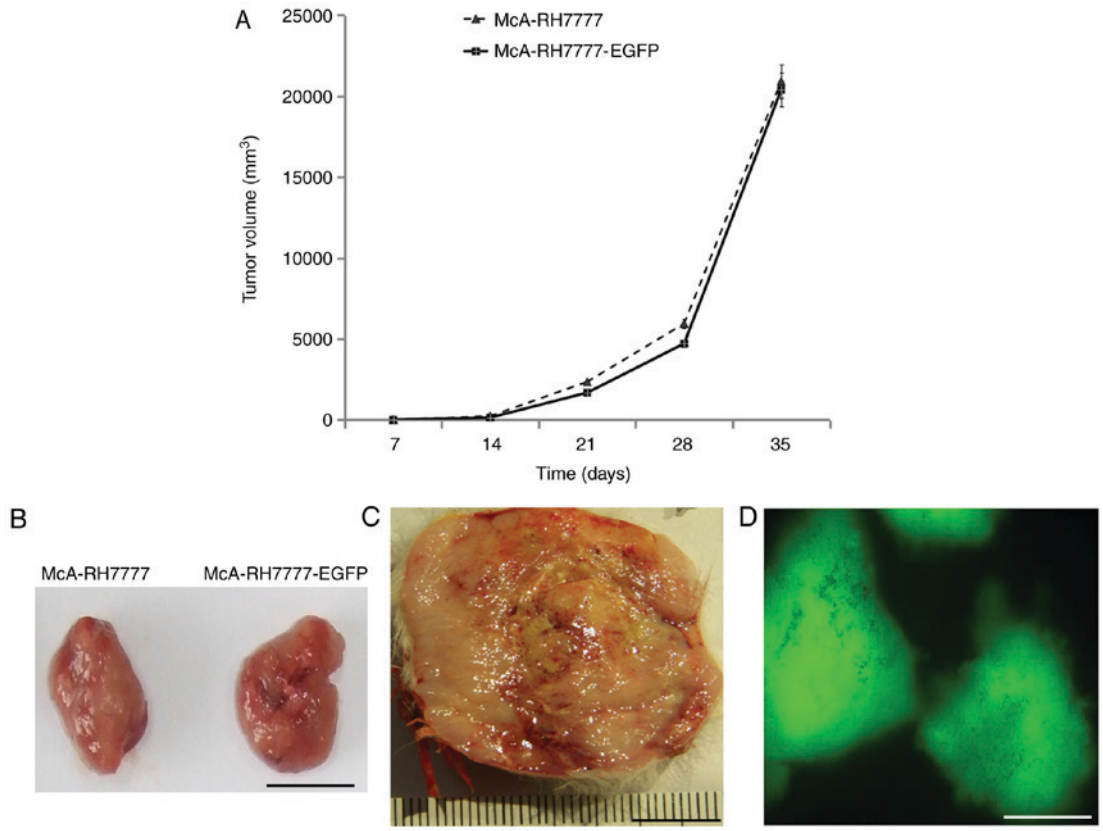

Figure 4. Effects of EGFP expression on tumor growth and fluorescence intensity *in vivo*. (A) Tumor volume measured following subcutaneous injection of McA-RH7777 and McA-RH7777-EGFP cells into rats. (B) Tumor tissues from animals injected with McA-RH7777 and McA-RH7777-EGFP following 35 days of growth. Scale bar, 1 cm. (C) Image of an excised tumor following 35 days of growth. Scale bar, 0.25 cm. (D) Fluorescence microscopy highlighting green fluorescence detection 35 days following subcutaneous injection. Scale bar, 50 μm. EGFP, enhanced green fluorescent protein.

and oxidation, including tyrosine, glycine and serine (22-24). Under blue light excitation, GFP emits green fluorescence and the detection is intuitive and accurate. Using a gene carrier to import the GFP gene into cells allows the direct observation of these cells under a fluorescence microscope. GFP reporter genes can be transfected into tumor cells, which then divide, grow and pass fluorescence on to next generations (25). GFP may be fused to other target proteins and rarely affects the spatial conformation and function of the gene products (26). GFP expression allows for quantitative analysis of gene expression, accurately reporting the location and quantity of target gene expression in tumor cells (27). Researchers have used GFP in applications, including drug evaluation and studies of tumor mechanisms (16).

In fluorescence-labeling, lentiviral transfection has a broad spectrum of applications and high transfection efficiencies. Lentiviral transfection allows for stable fluorescent protein expression (28,29). Lee *et al* (30) have observed that retrovirus-mediated transfection of GFP is able to produce stable expression in target cells in the mouse bodies for >3 months. Long-term external observations have revealed that this model retains the biological characteristics of the original system while stably expressing fluorescent protein.

In the current study, lentiviral-mediated transfection was performed to establish McA-RH7777-EGFP cells expressing EGFP *in vitro* over 108 days of culturing, indicating that cells stably expressed EGFP. *In vitro* experiments further suggested that tumor characteristics, including cell invasion and proliferation, were retained in McA-RH7777-EGFP cells. A rat tumor model established using subcutaneous injection suggested that the tumor formation rate was 100% and tumor growth indicated no significant differences between McA-RH7777-EGFP and McA-RH7777. There was also no significant difference between cells with respect to proliferation and activity. In addition, on day 35 of growth, the tumor tissue exhibited stable expression of green fluorescence. *In vivo* and *in vitro* experiments confirmed that biological characteristics of the transfected cells were not significantly different compared with wild-type cells. Within the chosen cancer cell line, EGFP expression provided a simple, intuitive and effective method to evaluate the invasion and metastasis of tumor cells.

The McA-RH7777-EGFP cell line may be used to construct liver cancer animal models. The dynamic process of tumor cell formation and growth in the evaluated system may be stable, continuous and yielding high efficiency, allowing for accurate observation using optical imaging. The system may further be used to evaluate the mechanism and efficacy of various targeted therapy drugs, to accurately reveal tumor stages and to identify and evaluate new treatment methods. Owing to the high visibility, stable EGFP expression provides an effective tool for detecting tumor cells and performing tumor molecular research.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

WZ, SQ, GY and LZ generated and analyzed the data. BZ, JW, RL, XQ and ZY designed the experiments and drafted the manuscript. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

All animals were treated in accordance with the Guide for the Care and Use of Laboratory Animals and all experiments were approved and performed according to the guidelines of the Ethics Committee of the Affiliated Zhongshan Hospital of Fudan University (Shanghai, China). All surgical procedures were performed under anesthesia and every effort was made to minimize suffering.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Fidler MM, Bray F and Soerjomataram I: The global cancer burden and human development: A review. Scand J Public Health 46: 27-36, 2018.
2. Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D: Global cancer statistics. CA Cancer J Clin 61: 69-90, 2011.
3. Ye JZ, Wang YY, Bai T, Chen J, Xiang BD, Wu FX and Li LQ: Surgical resection for hepatocellular carcinoma with portal vein tumor thrombus in the Asia-Pacific region beyond the Barcelona clinic liver cancer treatment algorithms: A review and update. Oncotarget 8: 93258-93278, 2017.
4. Jaka H, Mshana SE, Rambau PF, Masalu N, Chalya PL and Kalluvya SE: Hepatocellular carcinoma: Clinicopathological profile and challenges of management in a resource-limited setting. World J Surg Oncol 12: 246, 2014.
5. Geuskens M and Uriel J: Subcellular immunolocalization of alphafetoprotein (AFP) in cell lines established from Morris hepatoma 7777 and 8994. Lack of effect of dexamethasone on the ultrastructural detection of AFP in the 8994 cells. Oncodev Biol Med 3: 291-300, 1982.
6. Belanger L, Commer P and Chiu JF: Isolation of rat alpha1-fetoprotein messenger RNA from Morris hepatoma 7777. Cancer Res 39: 2141-2148, 1979.
7. Yen CW, Hsu LS, Chen CW and Lin WH: Hepatocellular carcinoma with thoracic metastases presenting as hemothorax: A case report and literature review. Medicine (Baltimore) 97: e10945, 2018.
8. Vieth V, Schulz R, Heindel W, Pfeiffer H, Buerke B, Schmeling A and Ottow C: Forensic age assessment by 3.0T MRI of the knee: Proposal of a new MRI classification of ossification stages. Eur Radiol 28: 3255-3262, 2018.
9. Khialeeva E, Chou JW, Allen DE, Chiu AM, Bensinger SJ and Carpenter EM: Reelin deficiency delays mammary tumor growth and metastatic progression. J Mammary Gland Biol Neoplasia 22: 59-69, 2017.
10. Engelen SM, Beets GL and Beets-Tan RG: Role of preoperative local and distant staging in rectal cancer. Onkologie 30: 141-145, 2007.
11. Miyayama S, Matsui O, Nishida H, Yamamori S, Minami T, Shinmura R, Kozaka K, Notsumata K, Toya D, Tanaka N, *et al*: Transcatheter arterial chemoembolization for unresectable hepatocellular carcinoma fed by the cystic artery. J Vasc Interv Radiol 14: 1155-1161, 2003.
12. Bae SI, Yeon JE, Lee JM, Kim JH, Lee HJ, Lee SJ, Suh SJ, Yoon EL, Kim HR, Byun KS and Seo TS: A case of necrotizing pancreatitis subsequent to transcatheter arterial chemoembolization in a patient with hepatocellular carcinoma. Clin Mol Hepatol 18: 321-325, 2012.
13. Ozcinar B, Guven K, Poyanli A and Ozden I: Necrotizing pancreatitis after transcatheter arterial chemoembolization for hepatocellular carcinoma. Diagn Interv Radiol 15: 36-38, 2009.
14. Kim W, Clark TW, Baum RA and Soulen MC: Risk factors for liver abscess formation after hepatic chemoembolization. J Vasc Interv Radiol 12: 965-968, 2001.
15. Hoffman RM and Bouvet M: Imaging the microenvironment of pancreatic cancer patient-derived orthotopic xenografts (PDOX) growing in transgenic nude mice expressing GFP, RFP, or CFP. Cancer Lett 380: 349-355, 2016.
16. Paris S and Sesboue R: Metastasis models: The green fluorescent revolution? Carcinogenesis 25: 2285-2292, 2004.
17. Yang SL, Luo YY, Chen M, Zhou YP, Lu FR, Deng DF and Wu YR: A systematic review and meta-analysis comparing the prognosis of multicentric occurrence and vs. intrahepatic metastasis in patients with recurrent hepatocellular carcinoma after hepatectomy. HPB (Oxford) 19: 835-842, 2017.
18. Juratli MA, Siegel ER, Nedosekin DA, Sarimollaoglu M, Jamshidi-Parsian A, Cai C, Menyaev YA, Suen JY, Galanzha EI and Zharov VP: In Vivo Long-Term Monitoring of Circulating Tumor Cells Fluctuation during Medical Interventions. PLoS One 10: e0137613, 2015.
19. Ntziachristos V, Ripoll J, Wang LV and Weissleder R: Looking and listening to light: The evolution of whole-body photonic imaging. Nat Biotechnol 23: 313-320, 2005.
20. Iyer M, Berenji M, Templeton NS and Gambhir SS: Noninvasive imaging of cationic lipid-mediated delivery of optical and PET reporter genes in living mice. Mol Ther 6: 555-562, 2002.
21. Maggi A and Ciana P: Reporter mice and drug discovery and development. Nat Rev Drug Discov 4: 249-255, 2005.
22. Ebe T: Green fluorescent protein as a marker gene expression. Tanpakushitsu Kakusan Koso 52: 1766-1767, 2007 (In Japanese).
23. Bottin A, Larche L, Villalba F, Gaulin E, Esquerre-Tugaye MT and Rickauer M: Green fluorescent protein (GFP) as gene expression reporter and vital marker for studying development and microbe-plant interaction in the tobacco pathogen Phythphthora parasitica var. nicotianae. FEMS Microbiol Lett 176: 51-56, 1999.
24. Yeh E, Gustafson K and Boulianne GL: Green fluorescent protein as a vital marker and reporter of gene expression in Drosophila. Proc Natl Acad Sci USA 92: 7036-7040, 1995.
25. Apfel J, Reischmann P and Muller O: A new fluorescence-based reporter gene vector as a tool for analyzing and fishing cells with activated wnt signaling pathway. ISRN Oncol 2013: 603129, 2013.
26. Shorter SA, Pettit MW, Dyer PDR, Coakley Youngs E, Gorringe-Pattrick MAM, El-Daher S and Richardson S: Green fluorescent protein (GFP): Is seeing believing and is that enough? J Drug Target 25: 809-817, 2017.
27. Hoffman RM: Application of GFP imaging in cancer. Lab Invest 95: 432-452, 2015.
28. Doerner A, Rhiel L, Zielonka S and Kolmar H: Therapeutic antibody engineering by high efficiency cell screening. FEBS Lett 588: 278-287, 2014.
29. Feki A, Berardi P, Bellingan G, Major A, Krause KH, Petignat P, Zehra R, Pervaiz S and Irminger-Finger I: Dissemination of intraperitoneal ovarian cancer: Discussion of mechanisms and demonstration of lymphatic spreading in ovarian cancer model. Crit Rev Oncol Hematol 72: 1-9, 2009.
30. Lee K, Majumdar MK, Buyaner D, Hendricks JK, Pittenger MF and Mosca JD: Human mesenchymal stem cells maintain transgene expression during expansion and differentiation. Mol Ther 3: 857-866, 2001.