Published in final edited form as:

Cytometry A. 2012 October ; 81(10): 910–915. doi:10.1002/cyto.a.22160.

Ferritin as a Novel Reporter Gene for Photoacoustic Molecular Imaging

Seung Han Ha^{1,2}, Andrew R. Carson^{1,2}, and Kang Kim^{1,2,3,4,*}

¹Center for Ultrasound Molecular Imaging and Therapeutics, University of Pittsburgh and University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania 15213

²Heart and Vascular Institute, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania 15213

³Department of Bioengineering, University of Pittsburgh, Pittsburgh, Pennsylvania 15213

⁴McGowan Institute of Regenerative Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania 15219

Abstract

Reporter genes may serve as endogenous contrast agents in the field of photoacoustic (PA) molecular imaging (PMI), enabling greater characterization of detailed cellular processes and disease progression. To demonstrate the feasibility of using ferritin as a reporter gene, human melanoma SK-24 (SK-MEL-24) cells were co-transfected with plasmid expressing human heavy chain ferritin (H-FT) and plasmid expressing enhanced green fluorescent protein (pEGFP-C1) using lipofectamineTM 2000. Non-transfected SK-MEL-24 cells served as a negative control. Fluorescent imaging of GFP confirmed transfection and transgene expression in co-transfected cells. To detect iron accumulation due to ferritin overexpression in SK-MEL-24 cells, a focused high-frequency ultrasonic transducer (60 MHz, f/1.5), synchronized to a pulsed laser (fluence < 5 mJ/cm²) was used to scan the PA signal at a wide range NIR wavelengths (850–950 nm). PA signal intensity from H-FT transfected SK-MEL-24 cells was about 5–9 dB higher than nontransfected SK-MEL-24 cells at 850–950 nm. Immunofluorescence and RT-PCR analysis both indicate high levels of ferritin expression in H-FT transfected SK-MEL24 cells, with little ferritin expression in nontransfected SK-MEL-24 cells. In this study, the feasibility of using ferritin as a reporter gene for PMI has been demonstrated *in vitro*. The use of ferritin as a reporter gene represents a novel concept for PMI using an endogenous contrast agent and may provide various opportunities for molecular imaging and basic science research.

Keywords

photoacoustics; molecular imaging; photoacoustic molecular imaging; endogenous contrast agent; reporter gene; ferritin

Molecular imaging, a combination of molecular cell biology and medical imaging, is a rapidly expanding field combining the disciplines of cell biology, molecular biology, chemistry, pharmacology, genetics, biomedical physics, engineering, and medicine (1). Recently, remarkable advances in molecular cell biology have accelerated the development of molecular imaging techniques to allow the visualization and characterization, and

quantify functional biologic processes at the cellular and subcellular level in living subjects, greatly advancing the field beyond the simple anatomic imaging offered by previous imaging techniques (2–4).

Photoacoustic (PA) imaging techniques (5,6) for biomedical applications, especially in molecular imaging, have been rapidly developing (7–9). PA imaging probes using exogenous contrast agents for photoacoustic molecular imaging (PMI) have been developed, but the field is still in its infancy (8,10–13). PMI using gold nanorods (GNRs) of mutually exclusive wavelength or specific fluorescent dyes have also been used to detect multiple molecular targets simultaneously *in vivo* (11,14). Exogenous contrast agents, in the form of magnetic nanoparticles that bind a receptor found on cancer cells, have been used to detect circulating tumor cells (CTC) using PA imaging in mice (15). Despite these exciting findings, exogenous contrast agents still have limitations. Exogenous contrast agents such as nanoparticles (NPs) are free to interact with plasma proteins, which can alter surface properties and effect binding (16). Moreover, NPs can be engulfed by phagocytic cells and accumulate in the liver, spleen, or lymph (10,17). This limitation has led to precise and delicate procedures of surface modification for NPs such as dextran coating, to limit nonspecific binding while preserving specific targeting (18). Despite these advances in targeting and masking, exogenous contrast agents are still challenged by potentially low sensitivity and low binding efficacy to target ligands (19). In addition, there are challenges posed by the need for substrates with desirable pharmacokinetics, potential toxicity, and delayed signal changes (20–22).

In the field of molecular imaging, reporter genes are powerful tools for monitoring gene expression that enable researchers to monitor cancer biology, screen drugs, monitor gene therapy, or track the fate of cells (1,23). Changes in gene expression often forerun anatomic changes during disease progression, and monitoring these changes using endogenous contrast agents might allow detection of events occurring in the initial stages of disease (24). Changes in gene expression and activity can be monitored by assaying for messenger ribonucleic acid (mRNA), protein, or by directly assaying protein activity (25). The best strategy for detection will depend on the gene being monitored and the disease state.

Reporter genes such as green fluorescent protein (GFP), luciferase, chloramphenicol acetyl transferase (CAT), and β -galactosidase have been widely used to monitor gene expression *in vitro* and *in vivo* (26–28). GFP is a fluorescent protein, which is directly detected by fluorescent microscopy and can be used to visualize gene expression as well localize gene expression within living cells (29–31). Luciferase can be used for bioluminescence imaging both *in vitro* and *in vivo*, and it is very sensitive and quantifiable (32). Despite their wide use and theoretical potential of *in-vivo* imaging, both of these reporter genes are challenging to apply *in vivo* because of strong light-scattering, low signal-to-background ratios, autofluorescence, and poor tissue penetration (33).

Much research has focused on overcoming these disadvantages via the development of new optical imaging methods to detect reporter genes or other endogenous contrast agents (34). It has been recently demonstrated that PMI can be used to detect the expression of specific proteins that may be useful candidates for reporter genes with which to image gene expression and biochemical events *in vivo* (35, 36).

Ferritin, one of the major proteins of iron metabolism, has been reported as an endogenous reporter gene for molecular imaging (3). Ferritin is a highly conserved iron storage protein that plays a prominent role in maintaining intracellular iron homeostasis (37). Fascinatingly, it has been reported that iron containing materials can be detected by PA techniques (38). Here, we demonstrate *in vitro* the feasibility of using ferritin as a novel endogenous reporter

gene for PMI, which would be functional in the absence of externally administered contrast agents.

MATERIALS AND METHODS

Cells and Transfection

Human melanoma SK-24 (SK-MEL-24) cells were obtained from ATCC (American Type Culture Collection, Manassas, VA). SK-MEL-24 cells were cultured in Eagle's minimum essential medium (EMEM) (ATCC, Manassas, VA) supplemented with 15% fetal bovine serum (FBS). SK-MEL-24 cells were then split into 60 mm tissue culture plate so as to produce cells at 50% confluence on the day of transfection. SK-MEL-24 cells were transfected with plasmid DNA using lipofectamine™ 2000 (Invitrogen Corporation, Carlsbad, CA) as per manufacturer's instructions. Briefly, 9 l of lipofectamine™ 2000 reagent and 8 µg of plasmid DNA were mixed in 3 ml EMEM media, which was added to each 60 mm tissue culture plate containing SK-MEL-24 cells and incubated for 4–6 h, followed by replacement of the media with EMEM containing 15% FBS. SK-MEL-24 cells were incubated for 48 h prior to any assays for transgene expression or PA imaging. SK-MEL-24 cells were transfected with either heavy-chain ferritin (H-FT) plasmid, in which the human H-FT gene was cloned behind the strong viral CMV promoter of pCDNA3.1 (Invitrogen, Carlsbad, CA), or p-EGPF-C1 (Clontech, Mountain View, CA), or not transfected.

Immunofluorescence

Transfected or nontransfected SK-MEL-24 cells were blocked for 30 min at 4°C in blocking media (Phosphate buffered saline (PBS) with 1% bovine serum albumin (BSA) and 1% horse serum (HS)). Blocking solution was then removed and SK-MEL-24 cells were exposed to labeled anti-ferritin antibody (Iockland, Gilbertsville, PA), at a 1:5,000 dilution in blocking media, for 8 h at 4°C. SK-MEL-24 cells were then washed with PBS and fixed in 4% paraformaldehyde for 15 min. To confirm H-FT gene expression from SK-MEL-24 cells, each sample was observed by fluorescence microscopy using an inverted microscope (X81, Olympus, USA) and images were acquired using a CCD camera (DP71, Olympus, USA). All images were acquired and processed using identical settings. In addition, we were able to observe a 60–80% transfection efficiency by using cells co-transfected with *p*-EGPFC1, which expresses GFP as a reporter (30).

Quantitative Real-time Reverse-transcription PCR

Real-time reverse-transcription PCR (RT PCR) was performed to quantify expression of H-FT. Briefly, SK-MEL-24 cells were homogenized and RNA was extracted using TRIzol® reagent (Invitrogen, Carlsbad, CA) as per manufacturer's instructions and quantified on a SmartSpec™ Plus spectrophotometer (Bio-Rad, Hercules, CA). Ferritin specific primers were used to amplify the human H-FT cDNA, using the following sequences: 5'-CATCAACCGGATCAAC-3' (forward), 5'-GATGGCTTTCACCTGCTCAT-3' (reverse). Real-time PCR was performed on a ABI Prism 7000 sequence detection system (Applied Biosystems, Foster City, CA) using the Absolute Blue SYBR Green kit (Thermo Fischer Scientific, Epsom, Surrey, UK). H-FT expression in all samples was calculated by the ΔC_t method as fold over glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression as an internal control, and compared to nontransfected SK-MEL-24 cells.

In Vitro Photoacoustic Imaging

As depicted in Figure 1, a Nd:Yag pulsed laser (Brilliant, Quantel, France) pumps an optical parametric oscillator (Vibrant HE532I, OpoTek, Carlsbad, CA) to generate 5 ns pulses at 10

Hz. The range of wavelength was tuned from 850 to 950 nm. The laser beam was directed to illuminate on the surface of SK-MEL-24 cells in the tissue culture plate (fluence < 5 mJ/cm²). The laser light beam was circular Gaussian with a diameter of approximately 10 mm, which was large enough to uniformly illuminate the sample of the scanning area in the tissue culture plate. The tissue culture plate was held by a sample holder connected to *x-y-z* manual translational stage for positioning. A focused high-frequency ultrasonic transducer (60 MHz, f/1.5, NIH Resource Center for Medical Ultrasonic Technology, USC, PI:K.K. Shung) was used to scan the PA signal on the surface of the tissue culture plate. A motorized *x-y* translational stage was used to scan horizontally the samples. The ultrasonic transducer output was amplified by an amplifier (5900PR, Olympus NDT, Waltham, MA), digitized and recorded by a digital oscilloscope (WaveSurfer 452, LeCroy, Chestnut Ridge, NY) synchronized to the laser. Between microscopic and PA images, manual co-registration was achieved using landmark of tissue culture plate covers as guides. PA signal intensity was obtained scanning over an area of 250 μ m by 250 μ m in 25 μ m step increments within the microscopic fluorescent imaging area, which is about half of the horizontal beam diameter of the ultrasonic transducer. The PA signal was averaged 10 times at each scanning grid point. The PA signal was normalized to the measured average pulse energy (J-50MB-YAG Energy MaxTM Sensor, Coherent, Portland, OR). All PA signal intensity was obtained from the same batch of SK-MEL-24 cells and the cell confluence on each plate was similar ($\sim 7 \times 10^5$ cells/plate). Three independent sets of the experiments were performed in each case. For PA spectral imaging, the laser light was illuminated at different wavelengths, from 850 nm to 950 nm with increment of 10 nm, on the same samples (13).

RESULTS

Ferritin immunofluorescence was performed on H-FT transfected and nontransfected SK-MEL-24 cells to confirm expression of H-FT and is presented in Figure 2. In Figure 2, the upper panels (A–C) present bright field images and the lower panels (D–F) present immunofluorescence images following immunofluorescence staining using FITC labeled antiferritin antibody. The left column indicates nontransfected SK-MEL-24 cells (A and D) and right two columns represent SK-MEL-24 cells transfected with ferritin plasmid (C and F) or co-transfected with ferritin and eGFP plasmids (B and E). Increased fluorescence indicates increased H-FT levels in transfected SK-MEL-24 cells (E and F) compared to control nontransfected cells (D). Subsequently, the sample plate was positioned in the sample holder for PA imaging (Fig. 1). The small boxes (solid yellow) in Figure 2 represent the area where PA images were believed to be taken and presented in Figure 3, and the large boxes (dotted white) present the maximum possible area of mis-registration.

A representative set of PA images at 900 nm is depicted in Figure 3, which can be compared to immunofluorescence images presented in Figure 2. In Figure 3, the PA image with reduced brightness (A) from nontransfected SK-MEL-24 cells is also associated with low levels of fluorescent staining using H-FT antibody (Fig. 2D). Figures 3B and 3C present PA images with increased intensity following transfection or cotransfection of H-FT and are also associated with increased staining by H-FT anti-body (Figs. 2E and 2F). Note PA signal intensity in each image is presented in the dB scale normalized to PA peak intensity of H-FT transfected SK-MEL-24 cells. Note also a standard linear interpolation was applied to PA raw data in pixels for better presentation. Overall, similar observations were made at different wavelengths between 850 and 950 nm. In Figure 4, the average PA signal intensity of the scan area (250 μ m $\times$ 250 μ m) was traced for nontransfected SK-MEL-24 cells (blue circles), H-FT and eGFP co-transfected SK-MEL-24 cells (red triangles), or H-FT transfected SKMEL-24 cells (green squares) at wavelengths from 850 to 950 nm. PA signal intensity from transfected SK-MEL-24 cells was much higher (5–9 dB; 2- to 3-fold increase

in linear scale) than nontransfected SK-MEL-24 cells throughout the entire wavelength ranges of 850–950 nm.

The RT PCR results of nontransfected SK-MEL-24 cells and ferritin transfected SK-MEL-24 cells are plotted in Figure 5 and are reported as fold over-expression relative to endogenous ferritin expression from nontransfected control cells. Transfected or co-transfected SK-MEL-24 cells express about 40- to 50-fold higher levels of ferritin than nontransfected SK-MEL-24 cells ($P < 0.005$ transfected vs. nontransfected SK-MEL-24 cells; $P < 0.005$ co-transfected vs. nontransfected SK-MEL-24 cells).

DISCUSSION

The H-FT gene was selected as a reporter gene based on the hypothesis that PA signal intensity at NIR will increase as a result of iron accumulation due to increased H-FT expression levels. We have demonstrated that PA signal intensity can be used to differentiate H-FT transfected SK-MEL-24 cells from nontransfected SK-MEL-24 cells over a wide range of wavelengths in the NIR range. Increased H-FT gene expression from the H-FT transfected SK-MEL-24 cells was confirmed by the immunofluorescence and RT PCR, with little expression seen in nontransfected control SK-MEL-24 cells. Increased H-FT levels in SK-MEL-24 cells after ferritin gene transfection were sufficient to increase PA signal intensities throughout the NIR wavelength range of 850–950 nm, possibly due to increased cellular iron uptake and sequestration by ferritin. We hypothesized that the relative levels of H-FT gene expression as reported by fluorescence imaging and RT PCR would accurately correlate with PA image intensity, and we have found this to be the case in our study.

However, several issues could have hindered precise correlation between these different techniques for quantification of H-FT expression in both nontransfected and transfected SK-MEL-24 cells. We have found strong correlation between the H-FT signal as reported by PA, fluorescence imaging, and RT PCR; however, the degree of increase reported by these three different methodologies does not precisely match. These differences in relative expression might be due to fact that RT PCR, immunofluorescence, and PA imaging are designed to measure mRNA, protein, and protein activity, respectively, and these aspects may not precisely match at any given time (13). Temporal and quantitative differences reported by these different methodologies might be explained by the time required to translate mRNA into protein, time to process and transport protein to the surface, and the kinetics of iron binding (39). These three distinct methodologies also vary in sensitivity, and signal-to-noise ratios (SNR). However, in efforts to minimize the expected variations from these, three independent experiments were performed where consistent observations to the presented results were obtained.

It also should be noted that in our current experimental set up, the region of interest (ROI) for PA imaging was smaller than the scanned area of the fluorescence images. In these experiments, fluorescence images were first scanned to find an area with uniform coverage of SK-MEL-24 cells, and that area was noted and scanned by PA. The PA scan was smaller than the fluorescence image due to relatively slow scanning time using a single element transducer. The PA images were scanned with increments of 25 μm step size, which is about half of the beam width of the transducer in the scanning direction. The current experimental setup consists of two separated imaging systems that cannot achieve precise co-registration, in micron scale, between PA and fluorescence images. With the current experimental setup, only manual visual co-registration is possible. A PA setup with confocal capabilities would facilitate co-registration in future studies and/or potentially enable individual cell imaging (40).

SK-MEL-24 cells were transiently transfected with H-FT plasmid to serve as a model with which to demonstrate the feasibility of using ferritin as a reporter gene and long-term studies were not preformed in this study. Future studies will focus on monitoring long term expression of H-FT signal in stably transfected SK-MEL-24 cells to enable longitudinal monitoring and better apply the reporter gene *in vitro* and *in vivo*.

In summary, the feasibility of using ferritin as a reporter gene for photoacoustic imaging has been demonstrated *in vitro*. The novel idea of using ferritin as a reporter gene for molecular imaging represents a new concept in PMI. This validation approach will also have direct applications in confirming the *in vivo* importance of biological phenomena such as altered gene expression, changes in signal transduction pathways, protein–protein interactions, and other molecular aspects previously evaluated only at the *in vitro* level. The use of ferritin as a reporter gene may provide various opportunities for preclinical *in-vivo* applications including cell tracking, monitoring transduction rates in gene therapy, and allow for longitudinal monitoring of gene expressions at defined locations, provided with anatomical images by nature of PA imaging techniques, to further characterize and further understand disease states.

Acknowledgments

Grant sponsor: Ministry of Knowledge Economy (MKE) of Korea (International Collaborative R&D Program); Grant number: 2010-TD-500409-001; Grant sponsor: NIH; Grant number: R21HL093176; Grant sponsor: National Center for Research Resources (NCRR).

Literature Cited

1. Massoud TF, Gambhir SS. Molecular imaging in living subjects: seeing fundamental biological processes in a new light. *Genes Dev.* 2003; 17:545–580. [PubMed: 12629038]
2. Gambhir SS. Molecular imaging of cancer with positron emission tomography. *Nat Rev Cancer.* 2002; 2:683–693. [PubMed: 12209157]
3. Cohen B, Dafni H, Meir G, Harmelin A, Neeman M. Ferritin as an endogenous MRI reporter for noninvasive imaging of gene expression in C6 glioma tumors. *Neoplasia.* 2005; 7:109–117. [PubMed: 15802016]
4. Blasberg RG, Gelovani-Tjuvajev J. *In vivo* molecular-genetic imaging. *J Cell Biochem Suppl.* 2002; 39:172–183. [PubMed: 12552617]
5. Xu MH, Wang LV. Photoacoustic imaging in biomedicine. *Rev Sci Instrum.* 2006; 77:041101.
6. Li C, Wang LV. Photoacoustic tomography and sensing in biomedicine. *Phys Med Biol.* 2009; 54:R59–R97. [PubMed: 19724102]
7. Emelianov SY, Li PC, O'Donnell M. Photoacoustics for molecular imaging and therapy. *Phys Today.* 2009; 62:34–90. [PubMed: 20523758]
8. La Zerda AD, Zavaleta C, Keren S, Vaithilingam S, Bodapati S, Liu Z, Levi J, Smith BR, Ma TJ, Oralkan O, et al. Carbon nanotubes as photoacoustic molecular imaging agents in living mice. *Nat Nanotechnol.* 2008; 3:557–562. [PubMed: 18772918]
9. Lu W, Huang Q, Ku G, Wen X, Zhou M, Guzatov D, Brecht P, Su R, Oraevsky A, Wang LV, et al. Photoacoustic imaging of living mouse brain vasculature using hollow gold nanospheres. *Biomaterials.* 2010; 31:2617–2626. [PubMed: 20036000]
10. Eghtedari M, Liopo AV, Copland JA, Oraevsky AA, Motamedi M. Engineering of hetero-functional gold nanorods for the *in vivo* molecular targeting of breast cancer cells. *Nano Lett.* 2009; 9:278–291.
11. Li PC, Wang CR, Shieh DB, Wei CW, Liao CK, Poe C, Jhan S, Ding AA, Wu YN. *In vivo* photoacoustic molecular imaging with simultaneous multiple selective targeting using antibody-conjugated gold nanorods. *Opt Express.* 2008; 16:18605–18615. [PubMed: 19581946]

12. Kim K, Huang SW, Ashkenazi S, O'Donnell M, Agarwal A, Kotov NA, Denny MF, Kaplan MJ. Photoacoustic imaging of early inflammatory response using gold nanorods. *Appl Phys Lett*. 2007; 90:223901.
13. Ha S, Carson A, Agarwal, Kotov NA, Kim K. Detection and monitoring of the multiple inflammatory responses by photoacoustic molecular imaging using selectively targeted gold nanorods. *Biomed Opt Express*. 2011; 2:645–657. [PubMed: 21412469]
14. Kruger RA, Kiser WL, Reinecke DR, Kruger GA, Miller KD. Thermoacoustic molecular imaging of small animals. *Mol Imaging*. 2003; 2:113–123. [PubMed: 12964308]
15. Galanzha EI, Shashkov EV, Kelly T, Kim JW, Yang L, Zharov VP. *In vivo* magnetic enrichment and multiplex photoacoustic detection of circulating tumor cells. *Nat Nanotechnol*. 2009; 4:855–860. [PubMed: 19915570]
16. Moghim SM, Hunter AC, Murray JC. Long-circulating and target-specific nanoparticles: Theory to practice. *Pharmacol Rev*. 2001; 53:283–318. [PubMed: 11356986]
17. Thorek DL, Chen AK, Czupryna J, Tsourkas A. Superparamagnetic iron oxide nanoparticle probes for molecular imaging. *Ann Biomed Eng*. 2006; 34:23–38. [PubMed: 16496086]
18. Minchin RF, Martin DJ. Minireview: Nanoparticles for molecular imaging—An overview. *Endocrinology*. 2010; 151:474–481. [PubMed: 20016027]
19. Jain PK, Huang X, El-Sayed IH, El-Sayed MA. Review of some interesting surface plasmon resonance-enhanced properties of noble metal nanoparticles and their applications to biosystems. *Plasmonics*. 2007; 2:107–118.
20. Hans ML, Lowman AM. Biodegradable nanoparticles for drug delivery and targeting. *Curr Opin Solid St M*. 2003; 6:319–327.
21. Boisselier E, Astruc D. Gold nanoparticles in nanomedicine: Preparations, imaging, diagnostics, therapies and toxicity. *Chem Soc Rev*. 2009; 38:1759–1782. [PubMed: 19587967]
22. Kelly KL, Coronado D, Zhao LL, Schatz GC. The optical properties of metal nanoparticles: The influence of size, shape, and dielectric. *J Phys Chem B*. 2003; 107:668–677.
23. Shah K, Jacobs A, Breakefield XO, Weissleder R. Molecular imaging of gene therapy for cancer. *Gene Ther*. 2004; 11:1175–1187. [PubMed: 15141158]
24. Kang JH, Chung JK. Molecular-genetic imaging based on reporter gene expression. *J Nucl Med*. 2008; 49:164S–179S. [PubMed: 18523072]
25. Sharma V, Luker GD, Piwnica-Worms D. Molecular imaging of gene expression and protein function *in vivo* with PET and SPECT. *J Magn Reson Imaging*. 2002; 16:336–351. [PubMed: 12353250]
26. Wood KV. Marker proteins for gene expression. *Curr Opin Biotechnol*. 1995; 6:50–58. [PubMed: 7894082]
27. Schenborn E, Groskreutz D. Reporter gene vectors and assays. *Mol Biotechnol*. 1999; 13:29–44. [PubMed: 10934520]
28. Chalfie M, Tu Y, Euskirchen G, Ward WW, Prasher DC. Green fluorescent protein as a marker for gene expression. *Science*. 1994; 263:802–805. [PubMed: 8303295]
29. Misteli T, Spector DL. Applications of the green fluorescent protein in cell biology and biotechnology. *Nat Biotechnol*. 1997; 15:961–964. [PubMed: 9335045]
30. Müller-Taubenberger, A. Chapter 14, Application of fluorescent protein tags as reporters in live-cell imaging studies. In: Eichinger, L.; Rivero, F., editors. *Methods in Molecular Biology*, Vol. 346: *Dictyostelium discoideum* Protocols. SpringerLink; 2006. p. 229–246.
31. Chudakov DM, Lukyanov S, Lukyanov KA. Fluorescent proteins as a tool kit for *in vivo* imaging. *Trends Biotechnol*. 2005; 23:605–613. [PubMed: 16269193]
32. Greer LF, Szalay AA. Imaging of light emission from the expression of luciferases in living cells and organisms: A review. *Luminescence*. 2002; 17:43–74. [PubMed: 11816060]
33. Troy T, Jekic-McMullen D, Sambucetti L, Rice B. Quantitative comparison of the sensitivity of detection of fluorescent and bioluminescent reporters in animal models. *Mol Imaging*. 2004; 3:9–23. [PubMed: 15142408]

34. Barone PW, Parker RS, Strano MS. *In vivo* fluorescence detection of glucose using a single-walled carbon nanotube optical sensor: Design, fluorophore properties, advantages, and disadvantages. *Anal Chem.* 2005; 77:7556–7562. [PubMed: 16316162]
35. Li L, Zemp RJ, Lungu G, Stoica G, Wang LV. Photoacoustic imaging of lacZ gene expression *in vivo*. *J Biomed Opt.* 2007; 12:020504. [PubMed: 17477703]
36. Paproski RJ, Forbrich AE, Wachowicz K, Hitt MM, Zemp RJ. Tyrosinase as a dual reporter gene for both photoacoustic and magnetic resonance imaging. *Biomed Opt Exp.* 2011; 2:771–780.
37. Arosio P, Levi S. Ferritin, iron homeostasis, and oxidative damage. *Free Radic Biol Med.* 2002; 33:457–463. [PubMed: 12160928]
38. Stolik S, Reguera E, Tomas SA, Sanchez-Sinencio F. Photoinduced charge transfer in molecular materials studied by optical absorption using photoacoustic spectroscopy. *Russn J Phys Ch.* 2011; 85:702–705.
39. Karmann K, Min W, Fanslow WC, Pober JS. Activation and homologous desensitization of human endothelial cells by CD40 ligand, tumor necrosis factor, and interleukin1. *J Exp Med.* 1996; 184:173–182. [PubMed: 8691131]
40. Zhang HF, Maslov K, Stoica G, Wang LV. Functional photoacoustic microscopy for high-resolution and noninvasive *in vivo* imaging. *Nat Biotechnol.* 2006; 24:848–851. [PubMed: 16823374]

Figure 2.

Fluorescence microscopy images of the ferritin gene over-expression in the SK-MEL-24 cells. Top panel shows the bright field (A—C) and corresponding fluorescent images are presented in the bottom panel (D—F). The left panel (A, D) shows nontransfected SK-MEL-24 cells as control. The middle and right panels show H-FT and eGFP co-transfected SK-MEL-24 cells (B, E) and H-FT transfected SK-MEL-24 cells (C, F), respectively. The small box (solid yellow) represents the area where PA image (see Fig. 3) was believed to be taken, and the large box (dotted white) presents the maximum possible area of mis-registration (scale bar unit: 100 μm). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 3.

The comparison of the PMI images between nontransfected SK-MEL-24 cells and transfected SK-MEL-24 cells at 900 nm. (A) indicates nontransfected SK-MEL-24 cells as control. (B) indicates H-FT and eGFP co-transfected SK-MEL-24 cells. (C) indicates H-FT transfected SK-MEL-24 cells. PMI intensity in each image is presented in dB normalized to PA peak intensity of H-FT transfected SK-MEL-24 cells. Note a standard linear interpolation was applied to PA raw data in pixels for better presentation (scale bar unit: 25 μm). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 4.

The comparison of the average PA signal intensities between nontransfected SK-MEL-24 cells and transfected SK-MEL-24 cells at NIR of 850—950 nm. Blue circles indicate non-transfected SK-MEL-24 cells as control. Red triangles indicate HFT and eGFP co-transfected SK-MEL-24 cells. Green squares indicate H-FT transfected SK-MEL-24 cells (■; H-FT, ▲; H-FT and eGFP, ●; Ctrl). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 5. RT PCR results. Fold-over of H-FT with eGFP transfected SK-MEL-24 cells (red bar) and H-FT transfected SK-MEL-24 cells (green bar) to nontransfected SK-MEL-24 cells (unit; fold over control, $*P < 0.005$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]