Published in final edited form as:

Mol Imaging Biol. 2009 ; 11(6): 408–414. doi:10.1007/s11307-009-0222-3.

Death and Proliferation Time Course of Stem Cells Transplanted in the Myocardium

Hui Qiao^{1,3}, Suleman Surti¹, Seok Rye Choi¹, Karthik Raju^{1,3}, Hualei Zhang^{2,3}, Datta E. Ponde¹, Hank F. Kung¹, Joel Karp¹, and Rong Zhou^{1,3}

¹Department of Radiology, University of Pennsylvania, Philadelphia, PA 19104, USA

²Department of Bioengineering, University of Pennsylvania, Philadelphia, PA 19104, USA

³Laboratories of Molecular Imaging, Department of Radiology, University of Pennsylvania, Philadelphia, PA 19104, USA

Abstract

Purpose—Noninvasive positron emission tomography (PET) imaging of reporter gene is combined with quantitative real-time polymerase reverse transcription (RT-PCR) method to study the time course of death and proliferation of stem cells transplanted in the myocardium.

Methods—Male murine embryonic stem cells (ESCs) were stably transfected with a mutant version of herpes simplex virus type 1 thymidine kinase (HSV1-sr39tk) reporter gene; 5×10^6 such cells were injected into the myocardium of female athymic rats. While the transplanted cells was monitored by *in vivo* 9-(4-[F-18]fluoro-3-hydroxymethylbutyl)guanine ([F-18]FHBG) PET imaging of the heart, their absolute number was estimated by RT-PCR from hearts harvested at 3–5 h, 24 h, days 4, 7, and 14 after transplantation.

Results—(1) Forty percent of injected cells were retained in the heart while majority of injected cells were lost within a few hours after injection. Cell death was peaked at 24 h when 18% of donor cells retained in the heart were dead. (2) The substantial cell loss was reversed by significant proliferation of ESCs. This led to the recovery of cell number to 3.4 million (70% of injected dose) at day 4 and first visual observation of *in vivo* [F-18] signal in the heart. (3) A robust correlation ($R^2=0.9$) between percent of injected dose per gram of tissue derived from *in vivo* PET signal and the number of donor cells estimated by RT-PCR was revealed.

Conclusions—The time course of transplanted stem cells surviving in the heart reveals a process of substantial cell loss within 24 h of injection and subsequent recovery of cell number through proliferation. Such proliferation can be noninvasively monitored by reporter gene imaging.

Keywords

Embryonic stem cells; PET; Reporter gene; RT-PCR; *Sry* gene; HSV1-tk

Introduction

The time course of death and proliferation of stem cells after they are grafted in the myocardium would reveal their ability to survive and to regenerate in the harsh environment of a beating

heart. A variety of imaging methods have been utilized to examine this time course noninvasively. Methods available to visualize cells fall into two categories [1]: direct labeling and reporter gene approach. The former loads into stem cells an imaging-detectable probe that would remain inside the cell during tracking. The latter inserts into stem cells a gene that expresses an enzyme or receptor detectable by an imaging modality. The direct labeling method would be less sensitive to monitor the proliferation because cell division leads to dilution or loss of the imaging probe. In contrast, reporter gene approach can potentially overcome this limitation by stable integration into each stem cell [2,3]. Nuclear imaging modalities due to their high sensitivity have been leading the effort of cell tracking in the heart: by transfecting stem cells with herpes simplex virus type 1 thymidine kinase (HSV1-tk), Wu *et al.* first demonstrated the feasibility of reporter gene approach to visualize the stem cells in the heart by positron emission tomography (PET) imaging [4] and to monitor their proliferation in the heart upon stable transfection [3]. Other reporter genes such as human sodium/iodide symporter have also been examined for cardiac applications [5,6]. However, the percentage of injected cells that retained and survived in the heart over time was not estimated in previous studies [3,7] in which the imaging was typically initiated a few days after injection. Furthermore, the correlation between *in vivo* imaging signal and the absolute number of grafted cells surviving in the heart was not examined. Finally, the detection limit of the reporter gene-based method has not been reported and will be an important consideration when determining the feasibility of using this approach to detect a specific number of cells.

To address these issues, we have combined with the reporter gene approach a genomic real-time polymerase reverse transcription (RT-PCR) method to estimate the number of grafted cells in a sex-mismatched transplantation in which stem cells of male origin are grafted into a female recipient heart [8,9]. This approach allows us to examine the time course of stem cell death and proliferation and is a necessary step to validate the reporter gene approach.

Materials and Methods

ESCs Stably Expressing HSV1-sr39tk Reporter Gene

Murine embryonic stem cells (ESCs; R1 line) stably transfected with HSV1-sr39tk are designated as R1-sr39tk. Details of transfection, uptake of 9-(4-[F-18]fluoro-3-hydroxymethylbutyl)guanine ([F-18]FHBG) tracer by R1-sr39tk cells are provided in the Supplemental materials.

Intramyocardial Injection of Stem Cells

To improve the survival of grafted cells [10], R1-sr39tk cells were heat shocked by incubation at 43°C for 30 min 1 day prior to injection.

All animal procedures were approved by the local Institutional Animal Care and Use Committee. Adult athymic rats (female, 150–200 g) were purchased from Frederick Cancer Center (Frederick, MD, USA). Five million R1-sr39tk cells suspended in 100 μ L serum-free culture media were injected in one spot into the mid-anterior wall of the left ventricle. An insulin syringe with a 29.5-gauge needle that is bent 45° at 3 mm from the tip was used for injection; the needle tip penetrated about 2 mm into the epicardium to deliver the cells.

Experimental Groups and Time Points for Imaging and TUNEL Staining

Three animals were used for the autoradiographic study described in the Supplemental materials.

In a pilot study, PET imaging was performed at days 0 (3–5 h), 1, 2, 3, 4, and 7 ($n=2$ for each time point) after injection of 5×10^6 R1-sr39tk cells. This study identified that day 4 is the earliest time to detect *in vivo* [F-18] PET signal in the heart.

In the study of combined PET imaging with RT-PCR, rats were randomly assigned into five groups ($n=6$ in each group) for follow-ups at 3–5 h, 24 h, 4 days, 7 days, and 14 days after the injection of five million R1-sr39tk cells. Four rats from each group were euthanized for RT-PCR analysis after imaging. Based on the pilot study, PET imaging was performed at days 4, 7, and 14 only. The remaining two rats in each group were used to estimate the percentage of dead donor cells by Terminal dUTP Nick-End Labeling (TUNEL) staining. Fifteen additional animals (not receiving cell injection) were used to generate the standard curve for the RT-PCR assay. A total of 60 rats were used.

Analysis of PET Images Acquired from Animals and Phantoms

In Vivo Studies—PET imaging was performed on a small animal PET (A-PET) scanner based on the Philips Mosaic System (Philips Medical Systems, Cleveland, OH, USA) [11, 12]. A discrete $2 \times 2 \times 10 \text{ mm}^3$ L-YSO Anger-logic detector housed in a 21-cm diameter bore provides a transverse field of view (FOV) of 12.8 cm. A-PET operates exclusively in 3D volume imaging mode and images were reconstructed using the row action maximum likelihood algorithm [13]. A spatial resolution of 2 mm in the central region of the FOV and system sensitivity of 5.45 cps/kBq was achieved. Animals were sedated by isoflurane (1% in oxygen at flow rate of 1 L/min) during imaging. Vital signs including electrocardiography and core temperature were monitored (SA Instruments, Stony Brook, NY, USA), and core temperature was maintained at 35–37°C using a heating pad.

[N-13]ammonia and [F-18]FHBG [14] were synthesized at the Penn Cyclotron Facility. [N-13]ammonia at a dose of $1.2 \pm 0.2 \text{ mCi}$ in 0.5 mL saline was injected into the tail vein and the acquisition started immediately and lasted for 15 min. Thirty minutes after the injection of ammonia, [F-18]FHBG was injected and data acquisition started 1 h later and lasted for 30 min. To examine whether a larger tracer dose leads to earlier detection of injected cells, a range of FHBG dose from 1.5 to 2.75 mCi (representing an 83% increase) in 1 mL saline was used in the pilot study, while a fixed dose of $1.6 \pm 0.2 \text{ mCi}$ [F-18] FHBG was used for imaging before the RT-PCR study.

Phantom Studies—In order to estimate the activity concentration present in the grafted cells at the time of their first visual observation on the PET images, we performed a simple phantom study using two groups of three thin wall capillaries with ID of 0.75 and 1.75 mm, respectively, placed in a cylinder. The activity concentration ratio between the capillary and background is 4:1, 6:1, and 8:1 for each capillary size (Fig. 2f). Dynamic scans for 1, 2, 3, 4, 5, and 30 min were collected.

Estimation of Image Contrast—PET images were analyzed using the AMIDE software (<http://amide.sourceforge.net/>). For the pilot study, each data set was examined to identify the [F-18] hot spot within the myocardial wall that is delineated by [N-13]ammonia in three orthogonal views. When such a hot spot was detected, an ellipsoidal volume of interest was placed to encompass the greatest dimension of the hot spot in three orthogonal views of the [F-18] FHBG images (VOI_h) and was also placed on septal region of the myocardial wall (VOI_{ref}). Both VOIs have the same size. The image contrast was estimated by:

$$\text{Contrast} = \frac{\text{meanVOI}_h - \text{meanVOI}_{ref}}{\text{meanVOI}_{ref}}.$$

When no hot spot was detected, VOI_h was placed on the mid-anterior region of the left ventricle (where cells were injected) while VOI_{ref} remained at the septum.

Phantom images from the 30-min scan were analyzed by placing a VOI on the hot spot (as VOI_h) and also on the background (as VOI_{ref}) to calculate the contrast as defined above. The size of the VOI used for phantoms is similar to the VOI used for *in vivo* images.

Estimation of %ID/g—Average counts per minute per voxel from the VOI_h was converted to tracer activity (millicuries per milliliter) using a calibration constant obtained by scanning of a 50-mm sphere filled with known [F-18] activity. The tracer activity was divided by the injected dose to obtain percent injected dose per gram of tissue (%ID/g), an index of [F-18] FHBG uptake in grafted cells.

Quantitative RT-PCR Detection of Y Chromosome

Design of Taqman Probes—The design of the Taqman probes for the internal standard (Xbp1) and male *Sry* gene are described in Supplemental materials. The internal standard is necessary to correct variations in DNA loading in samples.

Sample Preparation—Triplicate samples of the following three categories were prepared: (1) To test the sensitivity and specificity, DNA extracted from donor cells was mixed with DNA extracted from female hearts at 0:1, 1:0, 1:3, 2:3, 1:30, 1:300, and 1:3,000 (*w/w*). (2) To obtain a standard curve between the numbers of donor cells *versus* the amount of cellular DNA determined by RT-PCR, 1, 5, 10, 20, and 40 million donor cells (in a pellet) were mixed with the heart tissue and homogenized together to extract DNA (Qiagen, Valencia, CA, USA). (3) To estimate the number of donor cells retained in the heart, the left ventricle was dissected and snap-frozen in liquid nitrogen. The frozen tissue was homogenized to extract DNA. DNA concentration was determined on a Lambda-20 spectrophotometer (PerkinElmer, Waltham, MA, USA).

Each sample contains 30 ng of DNA from homogenates, 1× *Sry* (or Xbp1) primer, 1× Taqman universal master mix, and water in a total volume of 20 μL. All samples were run together on 364-well plates placed in an ABI 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Wells without any DNA were run as negative controls. Denaturing was initiated at 95°C for 10 min, and the following steps were repeated for 40 cycles: denaturing at 95°C for 15 s and annealing/extension at 60°C for 45 s.

Estimation of Number of Donor Cells—The amount of DNA from donor cells in a given homogenate sample was estimated by multiplying total DNA extracted from the sample by the ratio of donor DNA/total DNA obtained from RT-PCR. The number of donor cells was then obtained from the calibration curve.

TUNEL Staining and Quantification of R1-sr39tk Cell Death

Ten-micrometer sections from three levels (with a gap of 30 sections) were cut from a slab tissue containing the injection site. TUNEL staining was performed on nine sections (three sections per level) per heart using an *in situ* cell death detection kit (Roche, Indianapolis, IN, USA) to label the 3'-end of fragmented DNA with fluorescein-conjugated dUTPs. Positive control slides with DNase treatment and negative control slides without Tdt were included. Nuclei were identified by 4',6-diamidino-2-phenylindole (DAPI) counterstaining.

Under a Nikon fluorescence microscope, three FOVs per section were captured into the image analysis software (ImageJ, NIH, Bethesda, MD, USA). The number of donor cells in the FOV was estimated by applying an appropriate threshold to generate a binary image in which nuclei

of individual donor cells were outlined and counted automatically by the software. The number of TUNEL-positive cells in the same FOV was counted manually. The percent of TUNEL staining positive cells was averaged over three to nine sections for each heart (the number of sections containing donor cells is fewer at earlier time points); two hearts were used for each time point.

Statistics

Data was presented as the mean ($\pm$ SD). A two-tailed Student's *t* test was performed when comparing two groups and a *P* value of less than 0.05 was considered to be statistically significant.

Results

Tracer Uptake and Autoradiography Studies

The retention of FHBG in R1 cells stably expressing sr39tk was 40-fold of that in nontransfected R1 cells (Fig. s 1 of the Supplemental materials). Autoradiography and histological staining confirmed the presence of R1-sr39tk ESCs in the myocardium (Fig. s 2 of the Supplemental materials).

Sensitivity and Specificity of RT-PCR

High specificity and sensitivity in the detection of male DNA was obtained by RT-PCR (Fig. 1): as few as 10 pg of male DNA (equivalent of two donor cells) was detected by Sry primer/probe which is unresponsive to DNA from the female heart tissues (Fig. 1a). In contrast, Xbp1 detects both male and female DNA regardless of their ratio (Fig. 1b). Consequently, threshold cycles (Ct) are inversely proportional to the ratio of male/female DNA in samples for Sry detection, but independent of this ratio for Xbp1 detection (Fig. 1c). A standard curve that relates the number of donor cells to the amount of DNA reveals a linear relationship with a strong correlation ($R^2=0.94$; Fig. 1d).

In Vivo Imaging Quantification of R1-sr39tk ESCs Survival in the Heart

The time course of the image contrast (Fig. 2) reveals that the contrast was well below 1 at days 0, 1, 2, and 3 while it increased sharply to 1.3 at day 4 when a hot spot in the heart was first visualized on [F-18] PET images. The hot spot indeed localized in the myocardium as revealed by overlaying the [F-18]FHBG and [N-13]ammonia images. The [F-18] signals became stronger and expanded at days 7 and 14.

To estimate the sensitivity of our instrument in the detection of a hot spot of 1–2 mm in diameter, a simple phantom measurement was performed (Fig. 2f). For a 1.75-mm diameter (the larger capillary) region, the detection sensitivity of our PET scanner is at a 6:1 uptake level, while for a smaller, 0.75-mm diameter region, it can detect about an 8:1 uptake level. It is also noted that the contrast value of 1.3 was obtained from the larger capillary with 8:1 concentration activity; the same contrast value was achieved for the hot spot on day 4 [F-18] images acquired at the same scanning time, suggesting that the uptake ratio between the hot spot and surrounding myocardium could reach or exceed 8:1 at day 4. Thus, the phantom study allows a simple scaling of our measured contrast and estimation of the *in vivo* uptake level.

RT-PCR Quantification of Cell Survival and its Correlation with %ID/g

The %ID/g was quantified from *in vivo* images acquired from days 4 to 14 and was presented in Fig. 3a. The time course of donor cells surviving in the heart suggests that two million cells (40% of the injected dose) were retained in the heart within 3–5 h after injection. The cell number recovered to 3.5 million (71% of injected dose) at day 4 when *in vivo* PET signal from

donor cells was first detected. A robust correlation ($R^2=0.9$) was observed between the number of donor cells quantified by RT-PCR and the %ID/g quantified from [F-18] PET images (Fig. 3b).

Time Course of Graft R1-sr39tk Cells Death

By labeling the free 3'-OH termini of cleaved genomic DNA, TUNEL assay detects both apoptotic and necrotic cells [15]. As shown in Fig. 4, at 3–5 h after injection, the fraction of dead cells is small (1.3%). Cell death reached a peak of 17.8% at 24 h and subsequently dropped to 3.5% at day 4, 2% at day 7, and less than 1% at day 14. After correction for the percentage of dead cells, the number of surviving cells at each time point is summarized in Table 1.

Discussion

The major findings of this study are the following. (1) The majority (60%) of injected cells were not retained in the heart after intramyocardial injection. Cell death that peaked at 24 h after injection further decreased the number of surviving cells. (2) The substantial cell loss occurred within 24 h after injection was reversed by significant proliferation of ESCs. This led to the recovery of the cell number to 3.4 million (70% of injected dose) at day 4 and first detection of *in vivo* signals. (3) A robust correlation ($R^2=0.9$) between % ID/g derived from *in vivo* PET images and the number of donor cells estimated by RT-PCR was obtained.

The rapid loss of the majority of injected cells within hours after injection is not likely associated with cell death since the fraction of dead cells at 3–5 h was quite small (<2%). Instead, cells might be washed out into circulation and might efflux (back up) along the needle track. The dead space of the syringe and needle would retain a fixed number of cells each time during injection. Increasing the initial cell retention in the heart would be necessary to enhance the graft formation and could be achieved by delivering cells in a scaffold. Cell death peaked at 24 h after injection leading to a nadir point of donor cells surviving, 16% of the injected dose. The substantial cell loss within 24 h of injection is consistent with previous reports [8, 15] and led to inability to detect the surviving donor cells by PET imaging. The proliferation of ESCs, however, enabled the recovery of cell number to 70% of injected dose at day 4, when 3.4 million cells were surviving in the heart and the *in vivo* [F-18] signal was first visually detected. The robustness of this detection is supported by the phantom study, which suggests that the activity concentration ratio between the hot spot and background was ≥ 8 . The detection threshold of stem cells is determined by the level of reporter gene expression in each cell and its access to the tracer. Aspects of our experimental design that would affect the threshold are summarized below. First, while the stable transfection allows long-term tracking and the contribution from each cell is accounted for, it results in a lower level of reporter gene expression per cell compared to transient transfection, which leads to multiple copies of episomal DNA in a single cell. Second, as the result of intramyocardial transplantation of cells, the tracer is not uniformly accessible to individual cells; and increase of tracer dose did not lead to earlier detection in our hands. These factors might have led to a relatively high detection threshold (3.4 million cells) obtained in this study compared to that achieved when the cells are transiently transfected or are directly labeled with a radioactive probe such as [Cu-64]PTSM [16]. Since the dose of stem cells ranging from 0.5 to 10 million has been used in rat models of myocardial infarction [4,17], the HSV1-sr39tk combined with [F-18]FHBG PET may have limited utility in tracking a small number of cells. However, it provides a true survival marker for monitoring transplanted cells once their number reaches the detection threshold.

Our study has limitations. First, while the %ID/g obtained from static images is strongly correlated with the absolute number of donor cells, a kinetic study is required to calculate the flux of the phosphorylation reaction catalyzed by the HSV1-TK enzyme. The flux is proportional to the enzyme concentration, i.e., the level of HSV1-tk expression, whereas the

concentration of a product in the enzymatic reaction is not [18]. Recent work from Green *et al.* [19] demonstrated that dynamic imaging combined with kinetic modeling is able to estimate the phosphorylation rate of HSV1-TK, and compared to %ID, such rate seems to better correlate with TK activity measured by the standard assay. Second, cardiac gating and attenuation correction were not applied in this study. Such procedures are expected to reduce errors in estimations of %ID/g. Finally, grafted ESCs undergo differentiation, which may lead to variations in reporter gene expression level; therefore, the reporter gene approach is more suitable for tracking a single population of cells such as ESC-derived cardiomyocytes.

In conclusion, quantitative RT-PCR method permitted the estimation of the absolute number of grafted cells surviving in the heart and revealed a process of substantial cell loss within 24 h of injection followed by recovery afterwards through proliferation. Once reaching the *in vivo* detection threshold, a robust correlation between cell number and % ID/g validates that the index derived from imaging can serve as a surrogate for the number of surviving cells in the heart.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank UPenn Small Animal Imaging Facility (SAIF) and Eric Blankemeyer for the technical assistance with the A-PET scanner and UPenn Cyclotron Facility and Drs. Alexander Schmitz and Richard Freifelder for the assistance with [F-18]FHBG production. The research was supported by NIH grants, R21EB-2473 and R01-HL081185 (RZ), and was funded, in part, under a grant with the Pennsylvania Department of Health (RZ). The department specifically disclaims responsibility for any analyses, interpretations, or conclusions.

References

1. Zhou R, Acton PD, Ferrari VA. Imaging stem cells implanted in infarcted myocardium. *J Am Coll Cardiol* 2006;48:2094–2106. [PubMed: 17112999]
2. Acton PD, Zhou R. Imaging reporter genes for cell tracking with PET and SPECT. *Q J Nucl Med Mol Imaging* 2005;49:349–360. [PubMed: 16407818]
3. Cao F, Lin S, Xie X, et al. *In vivo* visualization of embryonic stem cell survival, proliferation, and migration after cardiac delivery. *Circulation* 2006;113:1005–1014. [PubMed: 16476845]
4. Wu JC, Chen IY, Sundaresan G, et al. Molecular imaging of cardiac cell transplantation in living animals using optical bioluminescence and positron emission tomography. *Circulation* 2003;108:1302–1305. [PubMed: 12963637]
5. Miyagawa M, Anton M, Haubner R, et al. PET of cardiac transgene expression: comparison of 2 approaches based on herpesviral thymidine kinase reporter gene. *J Nucl Med* 2004;45:1917–1923. [PubMed: 15534063]
6. Miyagawa M, Beyer M, Wagner B, et al. Cardiac reporter gene imaging using the human sodium/iodide symporter gene. *Cardiovasc Res* 2005;65:195–202. [PubMed: 15621047]
7. Qiao H, Zhang H, Zheng Y, et al. Embryonic stem cell grafting in normal and infarcted myocardium: serial assessment with MR imaging and PET dual detection. *Radiology* 2009;250:821–829. [PubMed: 19244049]
8. Muller-Ehmsen J, Whittaker P, Kloner RA, et al. Survival and development of neonatal rat cardiomyocytes transplanted into adult myocardium. *J Mol Cell Cardiol* 2002;34:107–116. [PubMed: 11851351]
9. Sheikh AY, Lin SA, Cao F, et al. Molecular imaging of bone marrow mononuclear cell homing and engraftment in ischemic myocardium. *Stem Cells* 2007;25:2677–2684. [PubMed: 17628019]
10. Zhang M, Methot D, Poppa V, Fujio Y, Walsh K, Murry CE. Cardiomyocyte grafting for cardiac repair: graft cell death and anti-death strategies. *J Mol Cell Cardiol* 2001;33:907–921. [PubMed: 11343414]

11. Surti S, Karp JS, Perkins AE, Freifelder R, Muehllehner G. Design evaluation of A-PET: a high sensitivity animal PET camera. *IEEE Trans Nucl Sci* 2003;50:1357–1363.
12. Surti S, Karp JS, Perkins AE, et al. Imaging performance of A-PET: a small animal PET camera. *IEEE Trans Med Imag* 2005;24:844–852.
13. Daube-Witherspoon ME, Matej S, Karp JS, Lewitt RM. Application of the row action maximum likelihood algorithm with spherical basis functions to clinical PET imaging. *IEEE Trans Nucl Sci* 2001;48:24–30.
14. Ponde DE, Dence CS, Schuster DP, Welch MJ. Rapid and reproducible radiosynthesis of [18F] FHBG. *Nucl Med Biol* 2004;31:133–138. [PubMed: 14741578]
15. Skuk D, Caron NJ, Goulet M, Roy B, Tremblay JP. Resetting the problem of cell death following muscle-derived cell transplantation: detection, dynamics and mechanisms. *J Neuropathol Exp Neurol* 2003;62:951–967. [PubMed: 14533784]
16. Adonai N, Nguyen KN, Walsh J, et al. Ex vivo cell labeling with ⁶⁴Cu-pyruvaldehyde-bis(N4-methylthiosemicarbazone) for imaging cell trafficking in mice with positron-emission tomography. *Proc Natl Acad Sci U S A* 2002;99:3030–3035. [PubMed: 11867752]
17. Van't Hof W, Mal N, Huang Y, et al. Direct delivery of syngeneic and allogeneic large-scale expanded multipotent adult progenitor cells improves cardiac function after myocardial infarct. *Cytotherapy* 2007;9:477–487. [PubMed: 17786609]
18. Auricchio A, Zhou R, Wilson JM, Glickson JD. *In vivo* detection of gene expression in liver by ³¹P nuclear magnetic resonance spectroscopy employing creatine kinase as a marker gene. *Proc Natl Acad Sci U S A* 2001;98:5205–5210. [PubMed: 11296261]
19. Green LA, Nguyen K, Berenji B, et al. A tracer kinetic model for 18F-FHBG for quantitating herpes simplex virus type 1 thymidine kinase reporter gene expression in living animals using PET. *J Nucl Med* 2004;45:1560–1570. [PubMed: 15347725]

Figure 1.

Sensitivity and specificity of RT-PCR and calibration curve. The amplification plots (**a**, **b**) of category 1 samples show changes in ΔRn (normalized reporter dye fluorescence) *versus* the number of amplification cycles. For Sry detection, cycles of amplification to reach the threshold is dependent on the ratio of male/female DNA while the threshold cycle (Ct) has a narrow spread for Xbp1 detection suggesting independence of this ratio. Samples containing only female DNA do not reach threshold as indicated by *arrows* while the *arrowhead* points to samples with the least male/female DNA ratio. Based on the average DNA content of 8.8 ± 0.4 pg per donor cell and 4.4 ± 0.9 pg per cardiomyocyte isolated from adult rat, the 10-pg detection limit is equivalent to detection of two donor cells in a mixture of 6,800 cells. Ct *versus* ratios of male/female DNA is plotted for Sry and Xbp1 detection (**c**). The calibration curve (**d**) covers a wide range of donor cell number (one to 40 million), which permits the proliferation of ESCs in the heart over a time course of 14 days to be quantified.

Figure 2.

a Contrast of [F-18] images acquired at days 0–14 (no error bars for days 0, 1, 2, and 3 because the sample size is two for these points). Coronal and sagittal views of the heart at day 4 (**b–c**) and axial view at week 1 (**d**) and week 2 (**e**) after the injection of five million R1-sr39tk cells. As a perfusion tracer, [N-13]ammonia (*gray scale*) provides the anatomical reference of the heart (myocardial wall in particular) to facilitate the localization of the F-18 signals (*color scale*). **f** Phantom study. Phantoms consist of three small capillaries (ID=0.75 mm) and three large capillaries (ID=1.75 mm) containing the activity concentration of 4:1, 6:1, and 8:1, respectively. After 30 min scan, contrast values are estimated for the three visible capillaries using the equation defined in the “Materials and methods” section: 0.3 for the large 6:1 capillary, 0.5 for the small 8:1, and 1.3 for the large 8:1 capillary.

Figure 3. Survival kinetics of grafted cells estimated by %ID/g derived from *in vivo* [F-18] PET images and by RT-PCR method (a). The correlation of the two quantifications (b).

Figure 4.

Death of grafted R1-sr39tk cells over time estimated by TUNEL assay. TUNEL-positive nuclei are *green* while other nuclei are *blue* (DAPI). At 3–5 h (a) and 24 h (b) time points, when the graft size is small, donor cells were identified through their unique nuclear morphology: large and round nuclei compared to host cardiomyocytes whose nuclei are spindle-shaped (arrows). At day 4 (c), day 7 (d), and day 14 (e), when the graft size becomes larger, the donor cells are easily identified by their small size and aggregation. Bar=100 μ m. Percent of TUNEL-positive donor cells over time (f 1.3 $\pm$ 0.7% at 3–5 h, 17.8 $\pm$ 2.9% at 24 h, 3.5 $\pm$ 1.9% at day 4, 2.0 $\pm$ 1.6% at day 7, and 0.8 $\pm$ 0.4% at day 14). * P <0.05 when compared to other time points.

Number of surviving donor cells in the heart estimated by RT-PCR with and without correction for cell death after the injection of 5M male R1-sr39tk cells into female recipient hearts

Table 1

Time after injection	3–5 h	24 h	Day 4	Day 7	Day 14
RT-PCR, mean (±SD) M	2.0 (±0.8)	1.0 (±0.4)	3.5 (±1.9)	16.7 (±7.1)	41.9 (±11.7)
Fraction of injected dose, mean (±SD)%	40 (±17)	20 (±9)	71 (±38)	334 (±142)	837 (±235)
With correction for cell death, mean (±SD) M	2.0 (±0.8)	0.8 (±0.3)	3.4 (±1.8)	16.4 (±7.0)	41.6 (±11.6)

M million