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Promoter Reporter Systems for Imaging of Cells Transplanted into Post-infarcted Heart

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Abstract

Molecular imaging has been a rapidly developing field of molecular biology. Specific sophisticated approaches have been pursued to effectively monitor the fate of transplanted cells in the body, i.e. retention in target organ, migration routes and cell survival up to their final destination. One of the strategies involves direct or indirect labeling of target cells. Direct labelling is based on the incorporation of chemical agents adhering to the cell surface like fluorescence, luminescence or radioactive tracing probes. Indirect labelling requires introduction of the promoter reporter systems to the cells with variety of molecular probes. Here, we would like to comment on promoter reporter systems currently used for monitoring of different stem/progenitor cells that could be used for a therapy of post-infarction heart.

Keywords: Promoter reporter system, Fluorescence, Bioluminescence, Radiotracers, Stem/progenitor cells, Multimodal imaging, Post-infarcted heart, Cell therapy

Foreword

Recently, stem/progenitor cell therapies have been intensively pursued. An increasing body of evidence has shown the promising results with respect to transient recovery of cardiac function in a variety of animal models with the use of stem cells interventions. Unfortunately, it is still not possible to fully and functionally replace the irreversibly damaged heart tissue. Moreover, the optimal cell population for organ regeneration has not been yet identified. Prior to possible optimization strategy to find ideal cell candidates, we shall keep in mind that successful organ regeneration is a very complex process. Within such development, the administered cells require proper homing and a graft retention that would be next followed by the cell electromechanical coupling with recipient organ cardiomyocytes [1]. Recent advances in molecular imaging techniques opened many platforms that would allow tracking of transplanted cells and optimization of delivery protocols with their subsequent multimodal imaging [2].

To be able to visualize target cells by different imaging modalities, stem/progenitor cells need to be directly or indirectly labelled. Direct labelling is based on the incorporation of chemical agent into the cells or attach to the cell surface shortly before transplantation. The currently exercised labelling can be achieved using fluorescent probes, luminescence superparamagnetic iron oxide (SPIO) for MRI detection or radiotracers for radionuclide imaging [3]. As it has been highlighted, the direct labeling of cells is well suited for the short-term tracking of cells [4].

Indirect labeling most often includes introduction of the promoter reporter systems, that are suitable for long-term monitoring [5]. Stably introduced reporter system, will be expressed as long as the cells are alive and will also be passed to daughter cells [4]. It is important to note, that currently designed promoter reporter systems often combine fluorescence that can be used for easy and quick visual inspection of cell transduction efficiency *in vitro*, with luminescence or radionuclide reporters often driven by the second promoter that is used for sensitive tracking in *in vivo* models. Importantly, such multimodal

molecular imaging allows to combine high sensitivity of radionuclides with fast real-time scanning and imaging by bioluminescence and fluorescence [6], what at the end allows to identify primary localization, distribution, survival and fate of transplanted cells.

Here, we would like to focus on some examples of contemporary reporter systems that are in use to monitor different stem/progenitor cell types that could be applied for pro-regenerative therapy of post-infarction heart.

Promoter Reporter Systems Principle

The ideal reporter system should be small enough to be easily delivered using conventional delivery vehicles. It has been highlighted that reporter gene product should not trigger an immune response and should not generate toxic metabolites. Moreover, the reporter should easily reach area of interest, be stable *in vivo* and produce specific probe accumulation only at the site of its expression. The probe used to be visualized should be easily and quickly removed from the system not making a background noise. The ideal reporter assay should be sensitive, rapid, reproducible, simple and cost effective. Typical promoter reporter system should be based on phenomenon that it can be always "on" what explores so called constitutive expression of reporter gene or alternatively, inducible one, that is switched on by transcriptional factor(s) specific to a particular tissue or cell differentiation stage. First reporters described almost 40 years ago were based on the enzyme activity such as β-glucuronidase [7] or β-galactosidase [8]. Next, discovery of green fluorescent protein (GFP) [9] opened the new era and since then fluorescent dyes in all rainbow colors became available. Another natural phenomenon of spontaneous light emission by beetle (firefly), *Renilla* and modified deep- sea shrimp (Nanoluc) [10] has been readily utilized in currently used bioluminescent and biosensor reporter systems [11]. Recent advances in nuclear medicine allow for development of reporters of which visual imaging would be combined with radioisotopic probes.

Delivery of Reporter Systems

The reporter systems can be delivered *via* different routes. One of the most popular, cheap and efficient but difficult to control method of transfection is electroporation. This method causes the death of about 50% of cells and leads only to a transient overexpression of introduced sequences. Method which allows to achieve stable integration with host genome and insertion of a longer genetics construct (with high efficiency) is based on the retro- or lentiviral transduction. Lentiviral origin vectors have the advantage over retroviral ones because lentiviruses are able to stably transduce also non-dividing cells. Another system that is gaining its popularity over the recent years is based on the adenoviral transduction. These vectors have been able to transduce variety of cells, also including non-dividing ones. They lead to a transient expression, because they do not incorporate their DNA into the host genome. Unfortunately, they have been shown to trigger the immune response. Over the recent years, they have been often used to introduce reporters based on CRISPR/Cas technology.

Revealing Side Effects of Viral Promoter Reporter Systems

Viral promoter reporter systems have become very popular tools currently used, but one needs to keep in mind that introducing genetic material to the cells or individual organisms can be associated with broad spectra of side effects. The topic of side effects is extremely important, however, is definitely beyond the scope of this short communication. The toxic effects on cells during lentiviral promoter reporter system delivery can be caused by helper agents, e.g. polybrene, liposomes or cationic polymers used to increase transduction efficiency by promoting virus- cell membrane contact [12,13]. Another cytotoxic effects towards host cells can be caused by antibiotics used as selection markers. Stepanenko and Heng gave the examples of G418/geneticin which interferes with the protein synthesis and function of 80S ribosomes. Antibiotics used for cell selection may also cause translocation (at dose -600 μg/ml) of HSP27-EGFP from cytoplasm to the nucleus or co-aggregation of HSP70 (heat shock protein 70 kDa) or SUMO-1 (small ubiquitin-like modifier 1). Zeomecin, in turn, is able to oxidize lipids or at certain concentration and time initiates DNA cleavage. It has been also shown that cells may acquire resistance to the antibiotics, even if they primarily lacked the resistance to the antibiotic gene expression effects [14].

With the current state of knowledge, we are not able to foresee all the negative effects that may exert antibiotics used for positive selection of given insert introduced to our manipulated cells. At this point we should adhere to Good Laboratory Practice that require precise titration experiments to determine the lowest possible concentration of antibiotic allowing for efficient selection for each selected cell type.

Promoter Reporter Systems Used to Monitor Cells Transplanted into Myocardium

One of the best studied and sensitive promoter reporter system used to monitor cells delivery is based on the *Herpes simplex* thymidine kinase (HSV-TK). Thymidine kinase catalyzes a transfer of y-phosphate from ATP to the 5' terminus of deoxythymidine resulting in formation of deoxythymidine monophosphate (dTMP). In 1993,

De Clercq [15] observed thymidine kinase ability to phosphorylate pyrimidine and purine nucleoside derivatives. In a phosphorylated state these compounds can be trapped intracellularly. Later on it was shown that a variety of radionuclide probes (e.g., FIAU, PGCV, FHPG, FHBG) can be successfully used for double imaging *HSV1-TK* reporter probe gene expression. So far many investigations based on the *HSV-TK* promoter reporter gene system have been performed.

Liu and colleagues isolated human cardiac progenitor cell (CPC), transducing them with four variants of thymidine kinase reporter (wt-tk, sr39-tk, A168H, Ahtk2) under the control of constitutively expressed promoter – Elongation factor 1 (EF1). They have shown that none of these variants affected phenotype of transduced cells, but only two of them- A168H and sr39-tk showed much better uptake of FHBG radiotracer *in vitro* as well as *in vivo*. Additionally, cells were transduced with Firefly Luciferase and Green Fluorescent Protein Double Fusion Reporter. Next, the best variants were transplanted into the mouse heart and monitored over 28 days period. Although, PET and BLI imaging showed substantial decline of cells retention within 4 weeks, the early PET imaging revealed successful engraftment of hCPC at day 1 and this was found to be positively correlated with subsequent LVEF functional improvement at day 14. Authors concluded that transplanted cells augmented cardiac hemodynamic function due to paracrine secretion of growth factors [16].

Another study using multimodal imaging based on the reporter system combining HSV-TK with bioluminescence was performed by Wang. Here, transduced human $CD34^{+}$ cells were transplanted into post-infarction heart in mice model. The authors using combination of imaging techniques such as: BLI, MRI, micro-PET and micro-CT showed the long-term cell tracking as well as accurate localization of transplanted cells. What is important, implantation of CD34+ cells improved cardiac function with respect to the LVEF [17]. This is an important observation since many other studies have shown that e.g. mesenchymal stem cells quickly escape from the site of delivery nonetheless, may exert a positive pro-regenerative effect [18, 19].

Pei and his group monitored differentiation of stem cells into cardiac cells using promoter reporter system based on the cardiac specific alpha MHC (myosin heavy chain) driving expression of HSV-TK. This is possible when the reporter is switched on as consequence of activation of tissue specific gene [4]. They used BMSC isolated from the rat and after lentiviral transduction delivered them into the post infarction rat myocardium. The FHBG uptake measured at 4, 17, 31, and 46 days after administration showed a significant increase of signal at day 31 what might evidence that BMSC cell trans-differentiation into cardiomyocytes. Such phenomenon was further confirmed by immunostaining with GATA 4 and troponin I [20].

An interesting example of triple fusion promoter reporter system where the ubiquitin promoter allows expression of three reporter proteins as a single output including firefly luminescence, green fluorescent protein and the truncated mutant of HSV-TK- sr39tk(sr39ttk) has been shown by Parashurama et al. [21]. Their studies were performed in an acute phase of post-infarcted mice and rats which were treated with lentivirally transduced Bone Marrow Stromal Cells (BMSC). The imaging was performed after cell transplantation at 2, 4, 8 and 14 days post intervention and chosen as the endpoint indicated by other studies [22]. The analysis of imaging demonstrated that signal intensity in a control group of mice (without MI) was strong at day 2^{nd} and subsequently decreased to the assigned endpoint, suggesting cell death, while the signal obtained in MI group was significantly enhanced over the time. The same transduced MSC population administered to rats demonstrated survival at day 7 and increase of the signal at day 14.

An alternative for HSV-TK promoter reporter system has been described by Miyagawa et al. [23] who proposed to use Sodium/iodide symporter (NIS). Sodium/iodide symporter (NIS) is a transmembrane glycoprotein that mediates active transport of iodide and sodium across the cytoplasmic membranes. It occurs naturally in a thyroid gland which facilitates accumulation of iodide. As authors highlighted, NIS could be used as a promoter reporter gene because it is normally not expressed in cardiac cells but has an ability to uptake radionuclides such: I^{123} or I^{131}. The reporter gene tested in these studies was based on the constitutively expressed promoter – CMV, that drove NIS expression and was packed into adenoviral vectors. Such vectors were directly injected into rat myocardium and the whole body imaging was performed using I^{123} or ^{99}mTc radiotracers. The results confirmed accumulation of radiotracers in the heart as well as in two other organs where NIS has been naturally expressed: thyroid and stomach. Therefore, as authors claim, further advancement of this reporter system would involve application of cardiac tissue specific promoter like myosin light chain instead of constitutively expressed one. This idea was further developed and confirmed by Kang and colleagues, who generated transgenic mice expressing NIS under the control of alpha-myosin heavy chain (alpha-MHC) reporter and demonstrated the significant uptake of 99mTc-pertechnetate in myocardium [24].

Conclusions and Perspectives

The multimodal imaging based on the promoter reporter systems has been developed over the recent years in *in vivo* as well as *in vitro* models. Currently, those ones used *in*

vivo allow not only tracking of transplanted cells but also monitor gene therapy by e.g. visualization of transplanted cells fate. It is important to note, that although *in vitro* studies confirmed that a variety of cardiac specific promoters and specific reporters like, ACTC [25], TNNT2 [26] can be successfully used as promoters, they have not been tested yet in *in vivo* models.

The most commonly used engineered vector vehicles in order to deliver reporter system to the target cells are based on viruses: lenti-, retro- or adenoviruses. They all have their own advantages, and disadvantages like difficulty to control insert copy number or optimal timing of the reporter gene expression *in vivo*. Taking into account the recent development of CRISPR/Cas system, one may expect that next generation of promoter reporter systems would be based on genome editing technology. So far, with regard to pluripotent stem cells, only two of promoter reporters based on the *Sox2* [27] and *Oct4* [28] have been proposed.

The multimodal imaging allows to minimize limitations that are linked with single imaging. As indicated by previous studies the limitation of PET had been a low spatial resolution and-exposure to radiation, whereas for bioluminescence and fluorescence a time kinetics has been critical as they could early reach a quenching of the signal [29]. It needs to be emphasized, that the multimodal imaging technology has a great chance to become prominent player in future studies of stem/progenitor cell therapies in post-infarction heart not only due to minimization and better resolution of imaging devices but also with a progress of novel probes identification as well as their promoters which are vital for success of this technology.

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Conflict of Interests

The authors declare no conflict of interests.

Authors Contributions

KF- original draft preparation, MK- checking and editing.

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