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Applications of lentiviral vectors in molecular imaging

Sushmita Chatterjee[1], Abhijit De[1]

[1]*Molecular Functional Imaging Lab, ACTREC, Tata Memorial Centre, Kharghar, Navi Mumbai, India*

TABLE OF CONTENTS

1. ABSTRACT

Molecular imaging provides the ability of simultaneous visual and quantitative estimation of long term gene expression directly from living organisms. To reveal the kinetics of gene expression by imaging method, often sustained expression of the transgene is required. Lentiviral vectors have been extensively used over last fifteen years for delivery of a transgene in a wide variety of cell types. Lentiviral vectors have the well known advantages such as sustained transgene delivery through stable integration into the host genome, the capability of infecting non-dividing and dividing cells, broad tissue tropism, a reasonably large carrying capacity for delivering therapeutic and reporter gene combinations. Additionally, they do not express viral proteins during transduction, have a potentially safe integration site profile, and a relatively easy system for vector manipulation and infective viral particle production. As a result, lentiviral vector mediated therapeutic and imaging reporter gene delivery to various target organs areas holds promise for the future treatment. In this review, we have conducted a brief survey of important lentiviral vector developments in diverse biomedical fields including reproductive biology.

2. INTRODUCTION

Lentiviral vectors (LVs) have emerged as one of the most widely used vector system for routine cell biology experiments in areas like stem cell, functional genomics and gene therapy applications. The first use of lentiviruses for gene delivery was reported by Naldini *et.al.* (1). Since then, many developments have been made in LV design to improve safety profile and gene transfer ability to various target cells. The development in LV design has been described in detail in a previous review (2). The advantages of LVs include their ability to i) transduce non-dividing cells like neuronal and glial cells of CNS (Central Nervous System), immune cells, and stem cells, ii) accommodation of large (up to 10 kb) gene sizes, and iii) stable long term expression of the transgene without detectable pathological consequences ascribed to the vector. LVs have shown a broad spectrum of applications ranging from understanding the basic biology to phenotypic corrections of diseases. For tissue targeted expression of a transgene, LVs can be pseudotyped with diverse viral envelopes by incorporating specific ligands or antibodies into the vector envelope (3, 4), else transcriptional targeting can be achieved by using tissue-specific promoter (5). Further, for finer control of the transgene expression, LVs can also be used in combination

Table 1. Differences of lentiviral vectors and their elements over different generations

Generation of viral vector	Plasmid vector	Elements
1st generation HIV[1] vector (1)	Packaging plasmid	*gag, pol, vif, vpr, vpu, rev, nef, tat*, RRE[2]
	Envelope encoding plasmid	VSV-G[3]
	Vector plasmid	5' and 3' LTR[4], RRE, transgene
2nd generation HIV vector (27)	Packaging plasmid	*gag, pol, rev, tat*, RRE
	Envelope encoding plasmid	VSV-G
	Vector plasmid	5' and 3' LTR, RRE, transgene
3rd generation HIV vector (29, 140)	Packaging plasmid	*gag*, *pol*, RRE
	Rev encoding plasmid	*Rev*
	Envelope encoding plasmid	VSV-G
	Vector plasmid	5' and 3' LTR, RRE, transgene

Abbreviations: Human Immunodeficincy Virus[1], Rev Responsive Element[2], Vesicular Somatitis Virus Glycoprotin[3], Long Terminal Repeat[4]

with drug inducible systems (6-8). In addition to the above attributes, LVs have recently been successfully employed for stable knock down of gene or microRNA (miR) by over expressing short hairpin RNA (shRNA) or miR target sequences respectively (9-11). Besides above mentioned *in vitro* studies, use of LVs in preclinical animal model is also well established for treatment of a disease, including molecular imaging applications (12, 13). At present, advantages associated with LVs have led to the progress of this technology from preclinical to clinical settings where multiple gene therapy trials are ongoing or approved with use of LV. Various gene therapy trials that are ongoing based on LV usage include treatment of β-thalassemia, ALD (Adrenoleukodystrophy), Parkinson's disease, Wiskott-Aldrich syndrome, and AIDS (Acquired Immuno Deficiency Syndrome) (14-19). Since stable, long term and efficient gene transfer is often a prerequisite for *in vivo* non-invasive monitoring of gene function, LVs have proven to be an excellent tool for molecular imaging applications (20-22). In the present review we will focus on the aspects of molecular imaging where LVs have been used as a gene delivery tool.

3. TURNING THE PATHOGEN TO A GENE DELIVERY VECTOR

The earliest LVs made were replication-competent viruses carrying transgene. Since then, a series of modifications have been made in the vector systems to ensure its safety as a gene delivery vector. The general strategy used for the production of replication defective viral particles has been by eliminating all dispensable genes from the HIV-1(Human Immunodeficiency Virus type-1) genome. Sequences needed for packaging have been separated from those encoding viral proteins. Different elements of LVs over different generations are explained in Table 1. In the first prototype, virus elements were distributed into two plasmids: (i) a plasmid that encodes HIV-1 pro-viral DNA with a deletion in the *env (envelop)* gene, and (ii) a plasmid that expresses *env* gene (23, 24). Trans-complementation of Env protein from the separate plasmid allowed production of viruses that could undergo a single round of infection, but not a second round since they do not carry the *env* gene. Later, more sophisticated HIV-1 based vectors were evolved carrying essential *cis*-acting elements for genome packaging, reverse transcription, and integration (such as LTRs or long terminal repeats and RRE or Rev Responsive Element), but no viral proteins (25). In most cases, expression of the foreign genes in these vectors is driven by a heterologous internal promoter such as cytomegalovirus (CMV) or others.

In the next phase of the LV development, HIV-1 Env protein was replaced (pseudotyped) with Vesicular Stomatitis Virus Envelope Glycoprotein (VSV-G) to expand the tropism of these recombinant infective viral particles (26). Since VSV-G binds to the ubiquitous membrane component phosphatidyl serine, a wider set of cells can be transduced by the LVs expressing VSV-G on their surface. Most LVs used today are pseudotyped with VSV-G, allowing robust transduction into many cell types.

Besides the above mentioned early replication competent prototypes of HIV vectors, researchers have introduced various elemental modifications in the plasmid systems used for lentiviral particle production. The vectors developed through these modifications are termed as generations of the viral particles. In the first-generation, replication-deficient recombinant lentiviral particles are produced from three separate elements: (i) a packaging vector plasmid expressing HIV Gag, Pol and regulatory/ accessory proteins from a strong mammalian promoter to generate viral particles; (ii) an Env plasmid encoding a viral glycoprotein; and (iii) a transfer vector plasmid. Packaging and envelop plasmids have been specifically engineered without either a packaging signal or LTRs to avoid their transmission into vector particles and reduce the production of RCL in vector preparations. The transfer vector plasmid contains the transgene(s) and all the essential *cis*-acting elements for packaging/reverse transcription/integration, but expresses no HIV proteins (2). Splitting the vector components into three plasmids means at least two recombination events are required to yield a replication-competent HIV-1-like virus during vector production. The use of VSV-G, rather than HIV-1 *env*, also reduces the chance of recombination since it eliminates homologous sequences between the *env* and transfer vector plasmids. Further, second-generation vectors have been developed by deleting accessory genes in the system. For HIV-1*vif, vpu, vpr* and *nef* are called accessory genes because they can be deleted without affecting viral replication. Second-generation LVs include only four of the nine HIV genes namely, *gag*, *pol*, *tat* and *rev* (27).

Conventional LVs integrate transgene cassettes flanked by two LTRs into the host genome. Under normal circumstances this should be a dead-end integration event. However, there are chances for production of replication competent recombinant lentiviruses. Since LTRs have an enhancer and a promoter region, integration of the LTRs into vector genome can activate adjacent cellular gene. In a

Table 2. Essential components of lentiviral vector and their function

Components	Specific function of the element
5'R	For transactivation
5'U5	For reverse transcription
Ψ (Psi)	Packaging signal, that allows the transcibed viral RNA to be incorporated into the assembly of new virus
Partial GAG	For packaging
RRE[1]	For nuclear export
cPPT[2]	For nuclear export
WPRE[3]	For efficient transgene expression
PPT[4]	For reverse transcription
3' U3	Partly deleted U3 for self inactivating vector
3'R	Acts as poly A for termination
3'U5	For integration

Abbreviations: Rev Responsive Element[1], Central Poly Purine Tract[2], Woodchuck hepatitis virus post-transcriptional regulatory element[3], Poly Purine Tract[4]

standard viral vector genome each LTR consist of U3, R and U5. U3 acts as a viral enhancer/promoter and R in the 3'LTR acts as a poly adenylation sequence. Therefore, mRNA from provirus does not contain 5'U3 and 3'U5 regions. The LTR elements are duplicated during reverse transcription prior to integration. In order to prevent subsequent viral replication or mobilization in the transduced cells, promoter-enhancer region in the 3'LTR has been deleted. During reverse transcription the proviral 5' LTR is copied from the 3'LTR. This event transfers the deletion to the 5'LTR and thus the deleted 5'LTR of the integrated provirus is transcriptionally inactive resulting in self-inactivating (SIN) lentiviral particle (28). Later a third-generation vector with increased safety measures evolved where *rev* is provided from a separate plasmid (29). This measure turns it into a four plasmids system for generating the viral particles and they are: (i) a packaging construct containing only *gag* and *pol* genes; (ii) a plasmid expressing *rev*; (iii) a VSV-G envelope plasmid and, (iv) a transgene plasmid driven by a heterologous strong promoter such as CMV or ubiquitin. This vector system has only three of the nine genes of HIV, thereby increasing its predicted bio-safety. Functions of different elements of LVs are explained in Table 2.

Further, finer modifications were made by addition of CPPT (central polypurine tract) and WPRE (woodchuck hepatitis virus post-transcriptional regulatory element) elements in transgene vector for enhanced transduction and translational efficiency respectively (30-33). Usage of chromatin insulator sequences has also seen to shield enhancers and promoters from activation or silencing by adjacent chromatin to increase the efficiency of transgene expression (34, 35). Thus since the first use of LV, plethora of changes have been made in the vector system to increase biosafety as well as gene delivery efficiency.

4. APPLICATION OF LENTIVIRAL VECTORS IN MOLECULAR IMAGING

Molecular imaging (MI) provides the ability for simultaneous visual representation and quantification of biological processes at cellular and molecular levels *in vivo*. In contrast to cell and tissue culture, *in vivo* animal models allow the assessment of biological phenomena within intact physiological conditions (36). The emergence of MI strategies is largely due to the recent unprecedented advances in molecular and cell biology techniques, the use of transgenic animal models, availability of newer imaging probes and contrast agents that are highly specific, and successful development of small-animal imaging instrumentations. Non-invasive MI permits both the temporal and the spatial bio-distribution of a molecular probe and related biological processes to be determined in a more meaningful manner keeping the subject alive (20, 21, 37-40). As such, MI of living mice offers the following additional distinct advantages when investigating phenotypic abnormalities: (1) it reduces the number of animal cohort requirements; (2) by repetitive imaging it is possible to investigate mutants that are otherwise difficult to interpret with data taken at a single time point; (3) it allows qualitative as well as quantitative assessments of biological phenomena and, (4) it allows the researcher to explore options of multiple imaging strategies in cases where simple genetic manipulations could result in a very complex phenotype involving a large number of pathways and organs. Such advantages suggest that MI plays an important role in preclinical trials that are conducted to test drugs for their biodistribution and pharmacokinetics and unfavorable drugs can be ruled out prior to human studies.

The merger of molecular biology and medical imaging is facilitating rapid growth of MI by providing methods to monitor cellular / molecular events adapted from conventional molecular assays. MI is a highly interdisciplinary field and development in this field is due to cooperative efforts from various disciplines which include contribution of cellular/ molecular biologists for identification and validation of molecular imaging targets, chemists/radio chemists for synthesis of imaging probes and medical physicists for development of high sensitivity and high resolution imaging devices and better algorithms for further improvement of signal to noise ratio of a given imaging device. The root of MI is nuclear medicine and in many ways is a direct extension of this discipline. In nuclear medicine, radioactive-labeled tracers are used for imaging. The underlying principles can now be tailored to other imaging modalities such as optical imaging. For this, imaging probes can now also be developed by taking advantage of the available cellular / molecular targets. There are two major imaging strategies that are being employed in biomedical research: direct and indirect imaging, of which the latter is predominant in biological studies using pre-clinical animal models. The direct imaging strategy is based on imaging the target molecule directly, usually with a target-specific probe. Whereas indirect molecular imaging is based on reporter gene imaging, which involves single or multiple reporter genes and their specific reporter probe partners. The advantage of reporter gene imaging is the ability to develop and validate imaging strategies more rapidly and at considerably lower cost than direct imaging strategies. This is possible because a well-characterized reporter gene–reporter probe pair of choice can be linked to either a gene-specific promoter or a gene sequence to image many different biological and

molecular-genetic processes. Here, LVs are playing a promising role for stable and efficient delivery of the transgene or a combination thereof, a prerequisite for many molecular imaging applications (17). Now, we will discuss various tailored transgene lenti-vector designs followed by their usage in specific application areas.

4.1. Tailored designs of transgene lenti-vector for molecular imaging

4.1.1. Delivery of complex genetic structures

Requirement of vectors that can express more than one protein (e.g., therapeutic gene, reporter gene and/or a combination of the two) is of great interest in gene transfer experiments. For understanding molecular and cellular events *in vivo,* co-expression of multiple reporter genes is often required to overcome shortfalls of any single modality. In addition to that to monitor therapeutic efficacy of a gene *in vivo*, correlated expression of the therapeutic and the reporter gene is highly desired (41, 42). In order to perform multimodality imaging, much advancement has been made for designing multi-cistronic vectors. Some of the strategies used are IRES (internal ribosomal entry site), bi-directional promoter based, self cleaving peptide 2A based or dual promoter based or even fusion gene based multi-cistronic vectors (20, 38, 39, 43-47) (Figure 1A-B). In order to facilitate multimodality imaging, various groups have shown construction of fusion protein for expression of multiple reporter genes. Fusion proteins are useful, provided that all the proteins in the fusion construct retain at least moderate level of activity of each individual protein and they are not cleaved into its specific constituents by cellular protease enzymes. Bi-fusion and tri-fusion lentiviral reporter vectors have been made by various groups for multi modality imaging. Some of the bi-fusion reporter constructs made include RLuc.GFP (*Renilla* luciferase and humanized GFP) (48), tk.GFP (thymidine kinase and GFP) (49), sr39tk.RLuc (a truncated version of HSV-tk and RLuc) (39), etc. However, a major limitation of these bi-fusion vectors was the inability to image a single cell. To overcome this drawback, the tri-fusion reporter gene strategy was developed by Ray *et.al.*, which reports an array of triple fusion reporters combining humanized RLuc (hRLuc), Firefly luciferase (FLuc), a truncated sr39tk (ttk), and GFP or monomeric RFP (mRFP) (38). Of the reported fusion vectors, the most successful combination was found to be the hRLuc.mRFP.sr39tk, where the reporter activity of each component was maximally retained. However, a challenge for molecular imaging of reporter genes is loss of gene expression over the time. Studies show that DNA methylation is involved in the silencing of CMV promoter and the phenomena can be rescued by inhibiting DNA methyltransferase or histone deacetylase enzymes (50). At present there are ongoing efforts to circumvent such silencing issues.

Each of the strategy that is used for the construction of multi-cistronic LVs has several advantage as well as disadvantage. For example, the most widely used strategy for transferring two genes together is to insert an encephalomyocarditis virus (ECMV) IRES element between the two transgene of interest. IRES based LV system has been developed using a PET reporter gene HSV1-sr39tk (Herpes Simplex Virus type 1- truncated thymidine kinase) and a bioluminescence reporter gene (FLuc) showing good correlation of the two genes inserted (20). HSV1-sr39tk is useful for suicide gene therapy using GCV as well as PET-based diagnosis using ^{18}F-FHBG (^{18}F-fluoro-3-hydroxymethylbutylguanine) probe. Lentiviruses made using this vector were used for stable transduction of the growing tumor in living mice and the response of HSV1-sr39tk/ GCV (gancyclovir) suicide gene therapy efficacy was measured by luciferase imaging. The IRES based system delivers a well correlated expression of two proteins, however in many cases the expression of the second gene (downstream to IRES) exhibited 6 - 50% lower expression than the first gene (upstream to IRES) (51). In another report, various gene coupling strategies to design multi-cistronic LVs were applied and their utility were compared by multimodality non-invasive imaging (47). Vectors were designed using eGFP and FLuc reporters driven by the human CMV promoter or by a bi-directional promoter or separated by IRES or by a 10 fold repeat of a nine nucleotide cellular IRES sequence or two genes separated by a self cleaving peptide 2A derived from FMDV (foot and mouth disease virus). Results suggested that high expression of both eGFP and FLuc reporter genes for LV-IRES and LV-T2A (peptide 2A) *in vitro* was comparable to the single gene constructs. Non-invasive bioluminescence imaging was employed to compare average photon output and it was observed that gene expression by peptide 2A mediated strategy was comparable to that from LV-CMV-FLuc where as animals injected with LV-IRES and LV-fusion displayed 10 and 20 fold lower photon output respectively. Dual promoter based multi-cistronic LVs have also been developed. A bimodal LV has been constructed using myc tagged human ferritin heavy chain (myc-hFTH) and GFP (52). Ferritin is an iron accumulating reporter gene suitable for use with Magnetic Resonance Imaging (MRI). Use of such reporter genes overcomes the challenge of delivering the contrast agent to deep tissues and negative side effects of exogenous MR contrast agents are avoided. Bimodal LV system allowed simultaneous MRI and fluorescence imaging of transplanted cells. In various studies plasmid vector encoding ferritin has been used for visualization of cancer cells and tumor tissues by high magnetic field MR scanners but ferritin was demonstrated as weak T2 contrast enhancing agent until Liu *et.al.* recently showed that by encoding it in a LV particle efficient delivery and integration is possible. In addition to that *ferritin* expression was used for monitoring embryonic stem cells *in vivo* by MRI (53). Detection limit by a clinical MR scanner for ferritin was found to be as low as 2000 transduced LV-ferritin.

4.1.2. Delivery of gene silencing vectors

Often over-expression of a gene is known to be associated with disease conditions and thus lowering the gene expression can help eradicating or minimizing the disease condition. Gene silencing by RNA interference (RNAi) was first demonstrated in *C. elegans* (54) and later found to be conserved in a wide variety of species. Further, it was reported that a synthetic 21 nucleotide small interfering RNA (siRNA) can specifically inhibit the

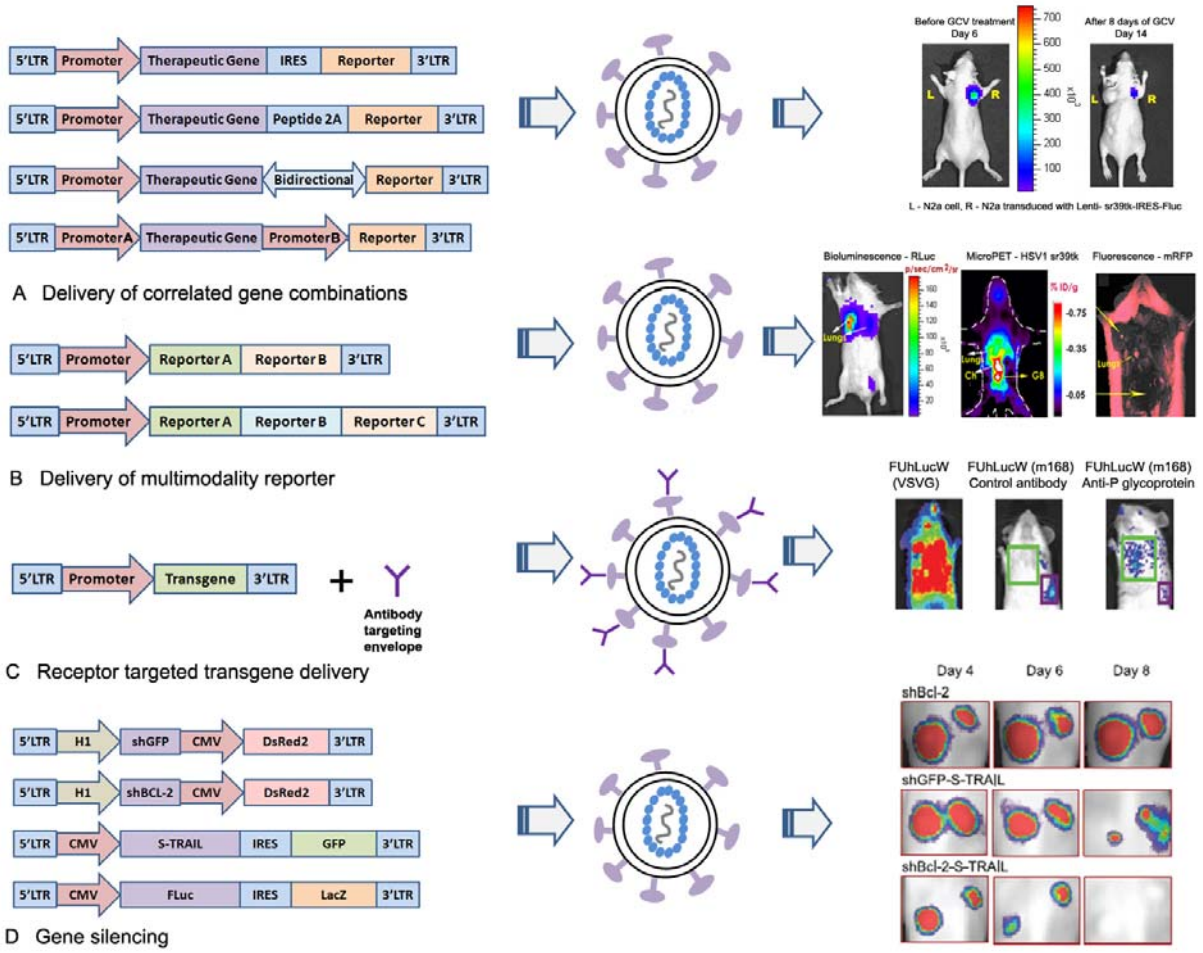

Figure 1. Schematic diagram of various applications of lentiviral vectors. (A). Components of transgene lentiviral plasmids following different strategies to deliver correlated expression of therapeutic gene and reporter gene for therapy monitoring using molecular imaging. Such customized transgene plasmids can be co-transfected with packaging and envelope plasmid to produce infectious viral particle (represented in the middle) and transduce target cell/tissues. The fig on the right exemplifies a case where using IRES based strategy, HSV1-sr39tk mediated ganciclovir (GCV) toxicity was measured using firefly luciferase imaging. (B). To circumvent the limitation of single modality imaging, often multiple reporter genes has been used. To achieve this, one can develop lentiviral transgene plasmid incorporating bi- or tri-fusion reporter compatible for PET, bioluminescence, fluorescence or MRI imaging. The images on right represent result of one such strategy which has successfully combined bioluminescence, fluorescence and micro-PET imaging performed on SCID mouse bearing A375M tumor xenograft where the cancer cells were transduced with hrl-mrfp-ttk triple fusion LV. (C). An example of tissue specific targeting of LV achieved by antibody tagged viral particles is shown. In this case, the viral envelope proteins were pseudotyped with modified Sindbis envelop antibody (m168) for specific tropism to P-glycoprotein enriched cells. The image panel on the right shows bioluminescence signal from (NOD) SCID mice implanted with B16F10 mouse melanoma cells, where an intravenous injection of m168 pseudotypes virus conjugated with the P-gp specific antibody shows enhanced tumor specificity as compared to VSVG pseudotyped vector. (D). Example of LV usage for inducing gene silencing as a therapeutic option. Dual promoter transgene plasmids were created using the H1 RNA polymerase III promoter driving either Bcl-2 targeted shRNA sequence or control GFP shRNA, linked to the CMV promoter driving DsRed2 reporter for transduced cell selection. This application also used another bi-cistronic LV construct for delivering S-TRAIL and GFP or FLuc and LacZ co-expression. Representative mouse images showing significant decrease in FLuc signal after combination therapy of LV mediated Bcl-2 downregulation and S-TRAIL expression (bottom most panel) in a human glioma tumor model. Reprinted with modifications from ref. 38, 61, 68 with permission from Cancer Research, Neoplasia and Nature Medicine respectively.

expression of a target gene (55). However, introduction of synthetic oligonucleotide siRNA in cells exerts only a transient effect, where as viral vectors such as LV have several advantages such as stable down-regulation of target gene and broad host tropism. Use of short hairpin RNA (shRNA) over siRNA is particularly useful in addressing gene specific contributions to the maintenance of the transformed phenotype in long term cell based assays, such

as soft agar or colony formation, or in tumor xenografts *in vivo*. In shRNA expression vectors, sense and antisense RNA are linked together by a small hairpin linker sequence. RNA transcribed from this cassette forms a stem-loop structure with the sense and antisense RNAs. Linker sequence is required for the transport of shRNA from nucleus to the cytoplasm with the help of the protein called exportin-5. Once shRNA is transported to the cytoplasm, it gets cleaved by dicer resulting in the formation of siRNA, which then execute the degradation of a target mRNA. Thus, shRNA LVs can serve the purpose in two ways: i.) by stable integration of the knock down cassette in the host genome in order to inhibit expression of a gene over a long period of time and ii.) simultaneously one can introduce a reporter transgene that helps in monitoring the effect of knock down *in vivo*.

Non-invasive molecular imaging technique has been applied to investigate the function of CXCR4 in an immuno-competent mouse model of primary and metastatic breast cancer (56). In this study 4T1 cells, which is an established model of stage IV breast cancer, was transduced with a lentivirus that co-expresses GFP and FLuc in order to enable non-invasive bioluminescence imaging. RNAi was directed against CXCR4 mRNA to knockdown the expression of CXCR4 in murine 4T1 cells transduced with lentiviral GFP and FLuc. Reduced expression of CXCR4 inhibited orthotopic growth of breast cancer cells *in vivo* and development of macroscopically detectable metastases from primary tumors was prevented. Thus application of LVs for *in vivo* imaging techniques play important role in monitoring the therapeutic efficacy of candidate inhibitors and analysis of genes that regulate the development and progression of primary and metastatic breast cancer. Since constitutive, long-term down-regulation of gene function by shRNA is likely to induce secondary adaptive responses which may be particularly unsuitable for studies involving gene elements essential for cancer cell growth, further development of an inducible shRNA system was made. To this line a number of inducible shRNA platforms have shown promising spatial and temporal modulation in target gene expression (57, 58). In a study a novel LV for inducible RNAi was constructed incorporating components for Tet-inducible shRNA expression (59). To study the effect of knockdown *in vivo*, HCT116 colon cancer cells constitutively expressing FLuc were stably transduced with an inducible shRNA specifically targeting the luciferase transcript. Luciferase levels and activity *in vivo* were assessed by whole body bioluminescence imaging. The inducible LV was capable of mediating robust target gene knock down in tumor xenografts *in vivo*.

Stewart *et.al.* demonstrated lentiviral delivery and intracellular processing of shRNA in a variety of cell types, including primary mammalian cells (60). For successful application of siRNA in clinical use, it is important to determine the dosing schedule required for efficacy, making insights into the kinetics of si-RNA mediated gene silencing. Therefore, mathematical modeling using simple kinetic equations for each step in the RNAi processing can shed light on many kinetic aspects of RNAi. In a report LV mediated FLuc gene was introduced in a variety of cell lines and bioluminescence imaging was used for mathematical modeling to investigate at each step, i.e. from siRNA delivery to intracellular function of RNAi with the aim of defining practical application and design of siRNA based treatment strategies both *in vitro* and *in vivo* (61).

LV has also been applied for understanding combined therapeutic effect of S-TRAIL (secretory-TNF related apoptosis inducing ligand) induced apoptosis and *bcl-2* gene down regulation in human gliomas. To evaluate the efficacy of combined therapy LV was engineered to express S-TRAIL, FLuc and shRNA against bcl-2 (62). Non-invasive real time imaging showed that combined therapy demonstrates enhanced eradication of gliomas (Figure 1D). In addition to that LVs have also been applied for understanding the effect of EGFR knock down in glioma model. EGFR and one of its variant EGFRvIII was fused to GFP and RLuc resulting in LV-EGFR-GFP-RLuc or LV-EGFRvIII-GFP-RLuc construct and effect of shRNA delivered by LV targeting different variants of EGFR in real time was investigated by non-invasive bioluminescence imaging (63). Results showed decrease in primary glioma cell proliferation in the cells bearing shRNAs targeting EGFR. Recently, recombinant LVs have also shown to be suitable to express siRNA for treatment of Alzeimer's disease and amyotrophic lateral sclerosis in animal models (64-66). Overall, these studies together show that LVs can lead to efficient gene knockdown in various cell lines and non-invasive MI modalities aids in better understanding of gene knockdown experiments.

4.1.3.. Tissue-specific gene delivery

For *in vivo* gene therapy applications the viral vector system should be easy to produce, easy to administer, and non-toxic to normal cells; it should deliver the genetic information efficiently and specifically via the bloodstream to the targeted tissues and integrate the genetic material into the target host cell so that the transgene is stably expressed. Intravenous delivery of LV has shown its localization mainly in spleen and liver (67). For most of the non-invasive imaging study of lentivirus-mediated reporter gene expression, the expression is driven by a strong constitutive promoter. The major limitation of using a constitutive promoter includes the inability to control gene expression and potential toxicity. The expression of therapeutic transgenes in cells other than the targeted ones may also give rise to significant deleterious effects and should therefore be minimized. Therefore, in many therapeutic applications, targeted approaches of viral transduction to enhance specificity are highly desirable. To achieve this, transcriptional targeting by using tissue-specific promoters to limit expression of potential transgenes to the tissue of interest has been frequently used. For specific targeting of hepato-cellular carcinoma (HCC) in a mouse tumor model novel LV construct has been used by linking sodium iodide symporter (NIS) gene to a tumor specific chimeric promoter (5). NIS is a plasma membrane protein that facilitates radio-iodine uptake and has therapeutic as well as diagnostic potential (68). For construction of chimeric promoter (EIIAPA) alpha

fetoprotein (AFP) promoter was linked to Hepatitis B virus (HBV) enhancer to achieve more stringent transcriptional targeting of HCC. To investigate the specific targeting of such LV, thyroid cell line (ARO) and HCC cell line (HepG2) were transduced with LV-CMV-NIS or LV-EIIAPA-NIS and results of *in vitro* I^{125} uptake and *in vivo* I^{124} preclinical PET imaging showed that LV-EIIAPA-NIS induced significant iodine accumulation only in HepG2 cells but not in ARO cells where as LV-CMV-NIS induced iodine uptake in both the cell lines. With such novel LV system using EIIAPA chimeric promoter transcriptional targeting of hepatoma cell line can be carried out and tumor specific expression of the transgene can be achieved.

However, as widely used LVs are pseudotyped with VSV-G envelope, by introducing specific promoters or enhancers into transgene LV, systemic delivery of viral particles in organism can still home in and potentially integrate in broad host cell types. To limit the expression of transgenes only in appropriate target cells a combination of targeted transduction and tissue specific transcription is utilized. Along this line, a LV targeted at the transductional level through a modified chimeric Sindbis virus envelope protein (termed m168) pseudotyping has been described. Human P-glycoprotein (P-gp) expressed on the surface of melanoma cells is targeted by the m168 pseudotyped LV bearing antibody to P-gp and the vector demonstrates preferential *in vivo* targeting to melanoma tumors after intravenous injection (69) (Figure 1C). However, transcription of the transgene was ubiquitous and resulted in residual expression in non-target tissues, primarily liver and spleen. Further, a LV has also been engineered to synergistically enhance specificity of transgene expression (70). As a proof of concept, this dual targeted vector was used for targeting bone metastases of prostate cancer. Transductional targeting was achieved by Sindbis envelop protein pseudotyping, that directs infection to prostate cells via monoclonal antibodies. These monoclonal antibodies recognize the PSCA (Prostate Stem Cell Antigen) which is over expressed in prostate cancer and some bladder and pancreatic cancer. In addition to that transcriptional targeting is achieved with a prostate-specific antigen promoter called PSE-BC, which further drives the expression preferentially in prostate cells. It was shown that this vector system could effectively target bone metastases *in vivo* even after systemic injection and poses an advantage over transcriptionally or transductionally targeted vectors. Transgene expression of the dual targeted vector was 1000 times lower in the liver and 34 times lower in the spleen than that of a VSV-G pseudotyped vector carrying UbiC promoter. By changing the tissue specific promoter and targeting antibody such vector systems can be applied for targeting other tissues as well. Targeted LVs that circulate in the system, but home only to specific tissues allow early therapeutic intervention in diseases such as cancer.

Further, to reciprocate the weak tissue-specific promoter activity it may be important to enhance the transcription of tissue-specific promoter. One of the amplification approaches referred as two-step transcriptional activation (TSTA) can potentially be used to augment the transcriptional activity of cellular promoter uses the GAL4-VP16 fusion protein (71). In many studies the strong trans-activating properties of the GAL4-VP16 fusion protein has been used to achieve amplification where the promoter is weak to drive measurable expression of the transgene in a target cell line. It has been shown that at high levels of doxycycline concentration (110ng/ml) tetracycline responsive element promoter show very high gene expression that is comparable to, or only slightly less than, a strong constitutive promoter such as EF1A or CAGG in same set of cell line (72). TSTA system has also been used in a LV construct and *in vivo* bioluminescence imaging using FLuc reporter gene has been used to demonstrate the performance of the approach (73).To demonstrate efficacy of the approach, a prostate specific promoter was linked to the GAL4-VP16 fusion protein. 5 GAL4 binding sites were positioned upstream of the FLuc reporter gene. This vector was able to transduce LNCap prostate cancer cells efficiently, while maintaining cell type specificity, and mediate tissue-specific sustained, long term gene expression in living animals. The intensity of the bioluminescence signal in the tumor was 125 fold higher than the corresponding signal intensity using control vector. Thus use of targeted lentivirus vectors combined with bioluminescence imaging offers the feasibility of monitoring efficient, long-term, tissue-specific, and sustained gene expression in living subjects.

4.2. Applications of lentiviral vector for understanding various disease biology

4.2.1. Application in stem cells and regenerative medicine

Stem cells hold immense potential for its use in cell therapy applications as they have the ability for both self-renewal and multi-lineage differentiation. For example, it has been shown that clinical symptoms of Parkinson's disease in the mouse model can be improved by using dopaminergic neurons derived from embryonic stem cells and induced pluripotent stem cells (74). In addition, stem cells are also used for differentiation into chrondrocytes to repair osteoarthritis (75), cardiomyocytes to reduce ischemic heart disease and peripheral arterial disease (76-79), and insulin producing cells to potentially treat diabetes (80). For successful translation of the stem cell therapy in human, it is important to study their engraftment, differentiation and migration in research subjects and thus lentiviral mediated molecular imaging proved pivotal.

Stem cells are difficult to transfect but with the introduction of LVs there is significant improvement towards reporter gene imaging of stem cells. Lentiviral based gene transfer efficiency in stem cells has been compared with plasmid based systems and found to be much more efficient (81, 82). Expression of reporter genes and their interaction with reporter probes did not adversely affect embryonic stem (ES) cell viability, proliferation, and differentiation. To verify, Wu *et.al.* (83) conducted global gene profile analysis of murine embryonic stem cells transduced with LV triple fusion construct and differentially regulated pathways were identified. Transcriptional profiling of control *vs.* triple fusion reporter (FLuc-mRFP-ttk) transduced ES cells identified 207 unique

genes that were up-regulated and 333 unique genes that were down-regulated in reporter expressing cells as compared to the control. Despite the transcriptional changes, no significant functional differences between native and reporter expressing ES cells was observed in terms of their viability, proliferation, chronotropicity or differentiation as revealed by imaging studies. It is therefore postulated that the use of ES cells may be the most effective strategy to develop lines that may be valuable in regenerative medicine. Human embryonic stem cells (hMSCs) have also been used to develop hepatocyte like cells and ability of these cells for engraftment, survival and proliferation in immunodeficient mice was evaluated (84). Further purity of this differentiated cell population was enhanced by liver specific LVs in conjunction with laser micro-dissection and pressure catapulting (LMPC). These cells were further transduced with lentiviral triple fusion reporter vector and were injected under the liver capsule or parenchyma in NOD-SCID mice. Results obtained by bioluminescence signal from implanted cells suggest that transduction with liver specific LV is effective and stable in differentiated cells over time. Development of such hepatocyte like cell line from hESCs may provide a valuable tool for pharmacology and toxicology studies, as well as for use in cell-based therapeutics.

Stem cell therapy has also been thoroughly evaluated for cardiovascular disease (85). Human mesenchymal stem cells (hMSCs) have been reported to play an important role in cardiac injury as they facilitate both myocardial repair and neo-vascularization in animal models (86, 87). Differentiation of hMSCs into cardiac endothelial cells and smooth muscle cells has been shown in many *in vitro* studies (88-90). In order to understand the fate of transplanted hMSCs *in vivo* by imaging methods LV expressing 2 distinct double-fusion reporter genes (*viz*.GFP.FLuc under the control of endothelial-specific promoter Tie-2 and mCherry.RLuc under constitutively active U3 region from the murine stem cell virus-long terminal repeat) was constructed. Multimodality imaging with bioluminescence imaging, fluorescence imaging and cardiac magnetic resonance imaging (MRI) demonstrated that transplanted hMSCs differentiate into endothelial cells in the injured hearts. The signal intensity showed dynamic correlation with the original number of injected hMSCs and thus enabled to track engraftment and proliferation of transplanted cells. In another study the potential of bone marrow-derived, hMSCs as a source of mural cells was explored (91).

Recruitment of mural cells facilitates stabilization of endothelial cells during neo-angiogenesis. Human umbilical vein endothelial cells (HUVEC) were transduced with a LV harboring a luciferase-IRES-EGFP cassette. Co-implantation of hMSCs and HUVEC resulted in formation of a network of human mature blood vessels in immunodeficient mice which could be assessed quantitatively by *in vivo* bioluminescence imaging for more than 120 days. In addition to application of MSCs in cardiac disease, tropism capacity of MSCs has also been investigated in breast cancer model by bioluminescence imaging (92). MSCs were transduced with LV carrying dual reporter gene and the pattern of the distribution of dual reporter labeled MSCs were examined in animals with subcutaneous tumor versus lung metastasis by bioluminescence imaging. Results from this study provided evidence of targeting and engraftment of MSCs to both lung metastasis lesion and subcutaneous tumor lesion when administered systemically. It was also shown that MSCs that migrate to lung-metastasis lesion tend to differentiate into osteoblasts, whereas MSCs that migrate to subcutaneous tumor lesion tend to differentiate into adipocytes.

Many reports suggest that administration of embryonic stem cell (ESC) derived endothelial cells (ESC-ECs) may enhance perfusion in the murine ischemic hind limb (93, 94). To monitor the localization of ESCs and ESC-ECs in the ischemic hind limb molecular imaging was applied for the first time by Huang *et.al.* (77) . Purified ESC-ECs were transduced with a LV carrying an ubiquitin promoter driving FLuc and enhanced green fluorescent protein and bioluminescence imaging demonstrated that in comparison to the parental ESCs, ESC-ECs preferentially localize in the ischemic hind limb. In the similar line, another study investigated the potential of human induced pluripotent stem cells (hiPSCs) derived endothelial cells to promote the perfusion of ischemic tissue in a murine model of peripheral arterial disease (79). LV was used for labeling these cells with a fusion reporter (FLuc-GFP) for bioluminescence imaging based long term tracking. Non-invasive monitoring demonstrates that hiPSC-ECs survived in the ischemic hind limb for at least 2 weeks (Figure 2).

Re-programming of fibroblast to embryonic stem cell like state was pioneered by Takahashi and Yamanaka (95). These so-called induced pluripotent stem cells (iPS) provide a powerful *in vitro* model system for the study of the molecular mechanisms of reprogramming (96-98) and have been successfully used in proof-of-principle cell-based therapies in mouse models of disease (74, 99). However the derivation of iPS typically requires multiple individual viral vectors to deliver the constellation of transcription factors needed to induce reprogramming. This presence of multiple viral integrations across the genome inhibited the production of induced pluripotent stem cells (95). Moreover, many cells receive only one, two, or three factors, making it difficult to study the biochemistry of reprogramming on a homogenous population of cells. Many studies have shown generation of iPS without integrations; however the efficiency of iPS derivation was significantly reduced, and the reprogrammed cell types were limited (100, 101). In a study, a single LV was generated expressing four transcription factors, Oct4, Klf4, Sox2, and c-Myc, from a single multi-cistronic transcript (102). Constitutive or inducible expression of the cassette generated iPS able to differentiate into all three primary germ layers. The major advantage of a single vector based approach is the possibility of inducing reprogramming with limited numbers of viral integrations. In addition to that, LV construct have been made in which the reprogramming cassette is flanked by loxP sites that enables subsequent

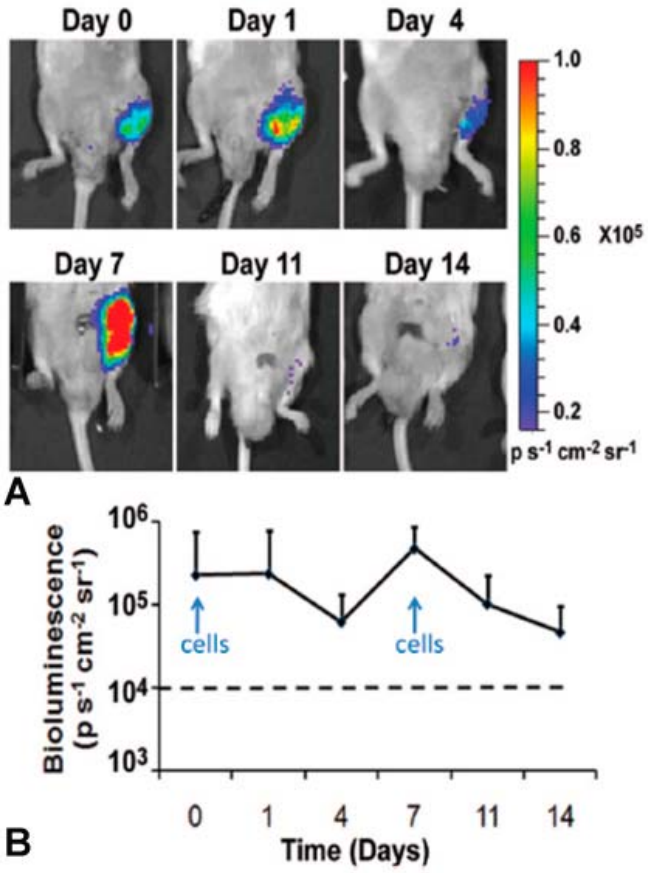

Figure 2. Localization and survival of LV transduced human induced pluripotent stem cells-endothelial cells in the ischemic hind limb. (A). Tracking of hiPSC-ECs in ischemic limb by non-invasive bioluminescence imaging (B). Quantification of bioluminescence signal in ischemic limb of mice after cell injection. Reprinted from ref. 77 with permission from Arteriosclerosis, Thrombosis and Vascular Biology.

excision of the transgene upon de novo expression of the CRE recombinase in the iPS-derived cells (103). Use of such inducible LV system represents a powerful tool and provides opportunity for application of iPS technology in clinical settings.

4.2.2. Application in gene therapy

Gene therapy applications involve transfer of a therapeutic gene into specific target cells with the motive to repair a faulty gene copy or introduce a new function (104). One of the most important requirements for successful gene therapy applications is optimum expression level of the therapeutic gene in the target tissue and tissue targeted LV play important role in fulfilling these requirements. To understand the location, magnitude, and persistence of the transgene expression, optimization of gene therapy applications are mandatory. It is possible to monitor the location, magnitude, and time variation of gene expression in living subjects by development of tools that allow *in vivo* evaluation of therapeutic gene delivery (105, 106). In general viral vectors are most commonly used for *in vivo* delivery of the transgene (therapeutic or reporter gene) for cancer therapy studies (107, 108). One of the treatment approaches is suicide gene therapy which involves selective killing of the cells by incorporating a non-human suicide gene into the chromosome of the infused cells. HSV1-tk (Herpes simplex virus type 1 thymidine kinase) is one of the most commonly studied suicide gene for the treatment of cancer (104, 109). Cells containing HSV1-tk may be selectively killed by infusion of ganciclovir (GCV) which is metabolized to monophosphate GCV by HSV1-tk. GCV monophosphate is further converted to GCV triphosphate by host kinases. GCV-triphosphate causes premature DNA chain termination and apoptosis. The HSV1-tk gene can function either as therapeutic (using GCV as substrate) or as a reporter gene (using ^{18}F-FHBG as substrate). Thus HSV1-tk transgene expression can be monitored non-invasively and is highly desirable for both clinical and preclinical investigations as it allows image guided therapy response approach. The mutant variant of the wild-type HSV1-tk gene is sr39tk and it has enhanced binding affinity for the pro-drug GCV (110). sr39tk also shows increased PET sensitivity with the reporter probe ^{18}F-FHBG (111-113). Johnson *et.al.* used LV construct for stable expression of sr39tk or enhanced green fluorescent protein (eGFP) under the control of the cytomegalovirus immediate early promoter (CMV) in prostate cancer cell lines and tumors were established with variable fractions of

transduced cells for PET (114). This study showed that a minimum of 100,000 tumor cells expressing sr39tk was needed to detect sr39tk gene expression by PET using ^{18}F-FHBG as a reporter probe. However, the point to be noted on this context is that the minimal cell number requirement may vary depending on the cell type to use or the organ location where the tumor is implanted.

Further therapeutic efficacy of HSV1-tk gene therapy has also been investigated in brain tumors using various viral vector systems (115, 116). In case of such therapeutic interventions measuring tumor mass is not reliable and accurate as tumor mass also contains necrotic mass and inflammatory cells together with viable tumor cells where as imaging modalities can objectively measure viable tumor cells which is easy to quantify. In a study rat glioma cell line (C6) was infected with LV containing the HSV1-tk and FLuc gene and therapeutic efficacy of HSV1-tk/GCV activating system was monitored *in vivo* as well as *in vitro* by bioluminescence (12). The study showed strong correlation between bioluminescence signal and therapeutic efficacy of HSV1-tk/GCV and with the application of bioluminescence imaging high therapeutic efficacy of HSV1-tk/GCV system was demonstrated.

In another study, glioma cells (U87 and A172) were transduced with LV-FLuc-DsRed2 and neuronal progenitor cells (NPCs) were transduced with LV-S-TRAIL. Combined therapy of miR-21 antagonism and NPC mediated S-TRAIL delivery showed synergistic increase in caspase activity and significantly decreased cell viability in human glioma cells *in vitro* as well as *in vivo* by optical imaging method (13). Most of the present gene therapy applications are based on *ex vivo* transduction of the cells followed by reintroduction into patients but in certain cases gene therapy with such *ex vivo* cell manipulation is not possible. The major hold back for *in vivo* gene therapy is inefficient gene delivery systems which often lack the specificity for the target tissue. In order to limit expression of the transgene in target tissue various LV have been designed by introducing tissue specific promoters. However since most of these vectors are pseudotyped with envelopes that have broad host tropism (e.g. VSV-G), they enter all cells of an organism and do not maintain tissue specific expression. In a study dual-targeted LV was designed for targeting bone metastases of prostate cancer (70). Dual targeting was achieved by using Sindbis envelop protein pseudotyping specifically transducing prostate cells via monoclonal antibodies and a prostate-specific antigen promoter called PSE-BC was used for preferential expression in prostate cells. The dual targeted vector showed 1,000 times lower expression of the transgene in liver and 34 times lower in the spleen than that of a VSV-G pseudotype LV carrying Ubi-C promoter. Dual targeted LV technology represents a significant improvement in specificity of gene delivery and allows *in vivo* targeting of tumor metastases after intravenous administration.

Kaikkonen *et.al.* reported construction of a recombinant LV displaying avidin or streptavidin fused to the transmembrane anchor of VSV-G protein 18 pseudotyped with the baculovirus major envelope glycoprotein gp64. These avidin labeled LVs were conjugated to biotinylated radionuclides and engineered to express a ferritin transgene. Such novel LV system generated allowed for the first time dual imaging of virus biodistribution and transduction pattern by single-photon emission computed tomography (SPECT) and magnetic resonance imaging (MRI) after stereotactic injection into rat brain (117). Linking a therapeutic gene with ferritin gene by various methods e.g. fusion protein, IRES or Peptide 2A to monitor the treatment efficacy is also possible.

4.2.3. Application in reproductive biology

Human pregnancy remains a very challenging area for the conduction of clinical research. The problems associated with pregnancy often include preeclampsia, fetal growth restriction, prematurity or postmaturity contributing significantly to fetal as well as maternal morbidity and mortality. Generally it is considered that placenta plays an important role in many reproductive disorders but disrupting placental functions to conduct invasive studies is a challenge. In various reports the efficiency of transgene expression in placenta has been evaluated by various methods including plasmid based EBV (Epstein Barr Virus) vectors (118), adeno-associated viral vectors (119), and LVs (120). Among these studies LV mediated transgene expression in placenta was found to be most efficient. Placental transgene expression is well tolerated by the maternal immune system (120). Thus LVs have played a very important role by enabling efficient placenta specific transgene expression (121-124). Since many of the genes are expressed only at specific stages of placental development, application of non-invasive methods for monitoring gene expression kinetics at various stages is highly desirable.

There are a very few reports showing non-invasive imaging of placenta specific gene transfer. The first proof-of-principle study for the quantitative image guided analysis of gene expression in the placenta throughout pregnancy in mice model was demonstrated by Fan *et.al.* (125). In this study, a reporter LV construct was used expressing FLuc-tdTomato fusion protein for trophectoderm-specific transduction and transplanted into day 3 of pseudo-pregnant recipients. Animals were monitored for the transgene expression by non-invasive bioluminescence imaging at different time points during pregnancy (Figure 3). Blastocyst viability or continuation of the pregnancy was not affected by expression of FLuc in trophoblasts and repeated exposure of its substrate (luciferin). The feasibility of uniform gene expression in all placentas of the same litter was also confirmed by selecting optimally lentiviral transduced blastocysts. Use of such advanced bioluminescence imaging techniques are useful for a broad range of applications including trophoblast-specific gene manipulations in utero, study of discrete biological functions and the detection of protein functions and other posttranslational modification events in the placentas of living animals.

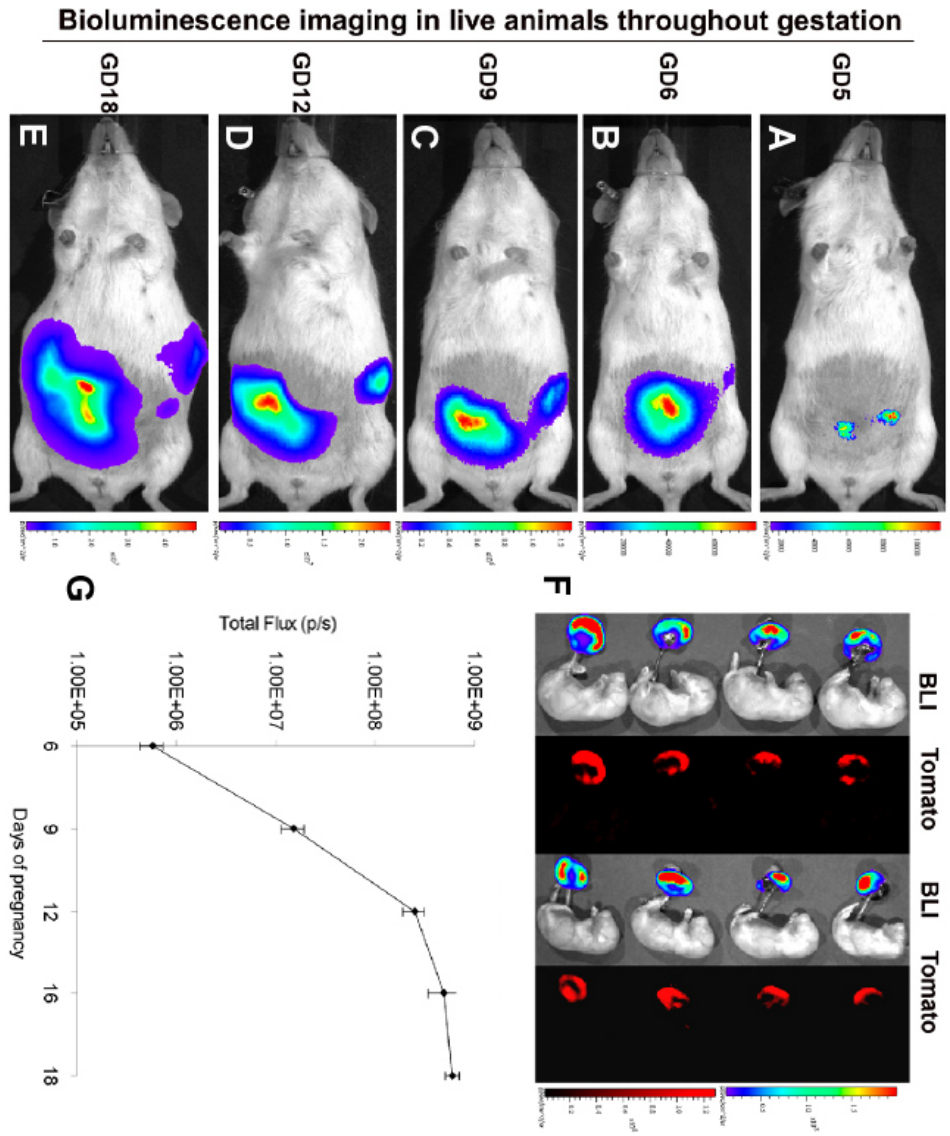

Figure 3. An example of LV mediated molecular imaging application for monitoring plancental development (A-E). Image showing serial bioluminescence imaging at different stages of pseudo-pregnant mice in which implanted blastocysts were optimally transduced with LV-FLuc.tdTomato reporter. (F) Placenta specific reporter gene expression in pseudo-pregnant mice. Expression of the reporter gene only in placenta and no corresponding fetus shows viral transduction of trophoblast-specific lineage. (G) Quantitation of the bioluminescence signal showing increase in signal intensity between day 6 (GD6) and 12 (GD12). Reprinted from ref. 123 with permission from Plos one.

Since placenta specific genes have highly stage specific functions, a controllable, transient expression system for functional analysis can help in understanding the placental gene functions in development or its role in case of any reproduction related problems. Lentivirus mediated trophoblast specific transduction methods are adapted to transiently express CRE recombinase and demonstrated feasibility of placenta-specific gene knock ins and knock outs (121). An inducible, placenta specific gene expression system was described by Fan *et.al.* that enables high-level, transient transgene expression. Such model allows non-invasive bioluminescence monitoring in mouse placenta at different stages of pregnancy (126). In this study an inducible placenta-specific gene expression system was used which is based on the third generation tetracycline-responsive transcriptional activator protein Tet-on 3G and an improved cognate promoter, Tet-on 3G-response element promoter (TRE3G) (127). Transgenic mice constitutively expressing Tet-on3G were created using a novel integrase-based site-specific approach (128). Blastocysts from these mice were then transduced with the LV-TRE3G-FLuc-EF1-copGFP virus transferred into wild type pseudo-pregnant females for placenta specific Dox-inducible gene expression. Systemic administration of Dox at various time points during pregnancy led to transient, placenta-specific firefly luciferase expression even on day

5 of pregnancy in a Dox dose dependent manner. The quick induction and decay of Dox-induced signal in the blastocysts observed by bioluminescence imaging suggests the potential of this approach to study the role of gene functions in pre-implantation stage (trophectoderm) in embryo development and placentation.

Development of such novel LV construct for studying placental development using non-invasive optical imaging methods opens up new avenues for understanding molecular function and regulations during placental development. The approach developed can be suitably extended for studying related disorders including pre-implantation functions and placental effects on fetal development. LV mediated gene knockdown studies in placenta will also be valuable to understand and confirm the roles played by various placental development related gene signatures in future (129).

5. LIMITATIONS AND BIOSAFETY

Although there are various advantages of LVs, few problems related to their limited capacity as a transgene carrier, low titer of these viral vectors and low expression level of the transgene (vs. Baculovirus or adenovirus) still persist. For clinical translation of LV system there are also concerns remained regarding possible generation of RCLs during vector production, mobilization of the vector by endogenous retroviruses in the genomes of patients, insertional mutagenesis leading to cancer, germ line alteration resulting in trans-generational effects and dissemination of new viruses from trial patients. Like other transgene vectors, LVs also have the risk of causing moderate to severe side effects with respect to the immune system. As HIV patients have circulating antibodies against the viral proteins, gene therapy based on LVs must exclude HIV patients. In a clinical trial, patients with X-SCID received CD34+ haematopoietic stem cells that had been genetically modified with a Murine Leukemia Virus (MLV) vector expressing the common v-chain from IL2RG (interleukin2 receptor v) (130). Although gene therapy was successful, a subset of patients subsequently developed leukemia like disease that appeared to be due to insertional mutagenesis by the vector. Since LVs also integrate randomly into the genome thus they can cause such side effects. Lentiviral vectors apparently integrate into transcriptionally active genes (131, 132) and thus are less likely to disturb the regulation and expression of host genes. At present, available experimental data using mouse models suggest that LVs are less oncogenic than MLV vectors which are also prone to insertional mutagenesis.

6. FUTURE PROSPECTS

Many indigenous and commercial LVs available are made to add flexibility in designing the expression cassette. Their utility for cell engineering including difficult to transfect cell lines and primary cells like immune cells, single-cell embryos, early blastocysts, or embryonic stem cells have expanded their scope in many areas including transgenic animal generation. LV transgenesis is far more efficient, less technically demanding, and less time consuming than the standard method of pronuclear injection of naked DNA. At present none of the reports show monitoring of LV mediated transgenesis by imaging methods, which is expected to rise in future.

Another facet that will see potential growth in future is LV usage in clinic. At present there are several ongoing trials based on use of LV for treatment of β-thalassaemia, adrenoleukodystrophy, Parkinson's disease, Wiskott-Aldrich syndrome, and AIDS (14-19, 133-135). The first Phase I clinical trial attempted in 2003 using HIV based LV was for gene therapy in AIDS syndrome (17). In this study a VSV-G pseudotyped vector named VRX496 was used, without any HIV-1 accessory proteins. Five patients who were non responders to two antiviral regimens were enrolled in the study. A single intravenous infusion of gene-modified autologous CD4+ T cells was well tolerated in all patients and immune function improved in four subjects. Clinical benefits of LV mediated gene therapy have also been demonstrated in X-linked adrenoleukodystrophy (136). Another example for clinical application of LV includes phase I/II clinical trial of β-globin gene therapy for β-thalassaemia that began in 2007 (133). In this study a self-inactivating LV containing large elements of the β-globin locus control region, and chromatin insulators (cHS4) was used to transduce CD34+ cells (137). After approximately 3 years of treatment, the patients had corrected β-globin gene and stable blood haemoglobin levels (137). No insertional mutagenesis has been reported from these trials so far, leaving patients with definite therapeutic benefit. Although LVs show promise for future use in clinical studies, biological complexities and HIV vector backbone complicate their choice for clinical applications. It will be essential for basic scientists to work in close association with clinical scientists in developing novel vectors design, executing, and evaluating the results via thorough experimental analysis to declare LV for safe clinical practices.

7. PERSPECTIVE

To the biomedical research community, LVs have been proven as an excellent research tool for stable gene transfer and expression in both non-dividing cells and terminally differentiated cells (138, 139). The admixture of LV development with reporter-based MI methodologies has suitably enhanced there applicability in multiple disease areas including some of which are described here. Over the years, adding good safety profile and reasonable gene carrying capacity of LVs has made it suitable to deliver multiple reporter genes, thus opened up the scope of applying multi-modality imaging as well. Scattered reports also indicated that LVs have the propensity to become excellent tool for generating transgenic animals. Such widespread applicability has created a special place for LVs within the research community.

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Abbreviations: LVs: lentiviral vectors, CNS: central nervous system, env: envelop, CMV: cytomegalovirus, RCLs: replication competent lentivirus, LTRs: long terminal repeats, SIN: self inactivating, WPRE: woodchuck hepatitis virus post-transcriptional regulatory element, RLuc: Renilla luciferase, mrfp: monomeric RFP, FMDV: foot and mouth disease virus, ECMV: encephalomyocarditis virus, TSTA: two-step transcriptional amplification (activation), NIS: sodium iodide symporter, AFP: alpha feto protein, HCC: hepatocellular carcinoma, iPS: induced pluripotent stem cells, LMPC: laser micro-dissection and pressure catapulting, hMSCs: Human mesenchymal stem cells, HUVEC: Human umbilical vein endothelial cells, HSV1-tk: Herpes simplex virus type 1 thymidine kinase, NPCs: neuronal progenitor cells, SPECT: single-photon emission computed tomography, MRI: magnetic resonance imaging

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Send correspondence to: Abhijit De, KS325, Molecular Functional Imaging Lab ACTREC, Tata Memorial Centre Sector 22, Kharghar Navi Mumbai , 410210, India, Tel: 91-22-2740 5038, Fax: 91-22-2740 5085, E-mail: ade@actrec.gov.in