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Research review paper

Secreted blood reporters: Insights and applications

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ABSTRACT

Secreted reporters detected in body fluids (blood, serum or urine) have shown to be simple and useful tools for *ex vivo* real-time monitoring of *in vivo* biological processes. Here we explore the most commonly used secreted blood reporters in experimental animals: secreted alkaline phosphatase, soluble marker peptides derived from human carcinoembryonic antigen and human chorionic gonadotropin, as well as *Gaussia* luciferase. We also comment on other recently discovered secreted luciferases and their potential use as blood reporters for multiplexing applications.

Contents

Introduction

Secreted blood reporter proteins are valuable tools for rapid and sensitive detection, quantification, and non-invasive monitoring of biological phenomena in pre-clinical animal experiments (Hughes et al., 2009; Pham et al., 2009; Wurdinger et al., 2008). Traditional enzyme-based reporter systems using cytosolic markers are sensitive, but require either a tissue lysis step which prevents their use for long-term monitoring, or frequent systemic anesthesia and substrate injection for detection of photon absorption. By contrast, the levels of secreted reporters can be evaluated repeatedly to generate multiple sets of data over time using conditioned medium from the same viable cell population or small amount of blood, serum, or urine without sacrificing the animal. This property allowed time-course study of embryo development, viral dissemination, gene transfer, tumor growth and response to therapy, as well as the fate of genetically engineered cells in animal models (Maelandsmo et al., 2005; Msaouel et al., 2009; Tannous, 2009).

Initial attempts were made using growth hormone as a secreted reporter in rats and humans. The restricted expression pattern of this hormone permits its use in mammalians (Kingston et al., 1986). However, the rate of its secretion varies significantly among tissue types and under different experimental conditions. Also, growth hormone is not "neutral," i.e. it may interfere with metabolism, development, and other cellular signaling pathways (Larsen et al., 1986). These weaknesses make this protein an unreliable reporter. The first engineered secreted reporter was from human placental alkaline

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phosphatase, initially derived by Berger et al. (1988) and used for quantification experiments in cell culture. Bettan et al. (1999) then applied this reporter for *ex vivo* analysis in a mouse model. To avoid immune response, at least two forms of mouse specific alkaline phosphatase were later produced (Maelandsmo et al., 2005; Wang et al., 2001). More recently, virus-encoded soluble marker peptides have been exploited to monitor viral therapy. Edmonston vaccine strain of measles virus (MV-Edm) was engineered to express a soluble peptide derived from the human carcinoembryonic antigen (hCEA) or human chorionic gonadotropin (hCG) (Liu et al., 2007; Peng et al., 2002b; Pham et al., 2009). Infection of this oncolytic virus was easily detected by measuring the concentration of these polypeptides in tissue culture supernatant or in body fluids (Msaouel et al., 2009; Peng et al., 2002a).

Light emission from bioluminescence provides a rapid and sensitive method to detect gene expression in a quantitative manner. The *Photinus pyralis* firefly luciferase (Fluc) (de Wet et al., 1987) and the *Renilla reniformis* luciferase (Rluc) (Lorenz et al., 1991) have been mostly studied. The typical luciferase reaction occurs intracellularly and therefore a lysis step is required for detection in cultured cells. Several attempts have been made to engineer secreted luciferases in mammalian cells (Kim et al., 2005; Liu et al., 1997). More recently, naturally secreted luciferases from marine copepods such as *Gaussia princeps* and marine ostracod *Vargula hilgendorfii* have been cloned and optimized for mammalian gene expression (Tannous et al., 2005; Thompson et al., 1989). Here we address the three most popular and promising secreted blood reporters, the secreted alkaline phosphatase, soluble peptide markers, as well as the *Gaussia* luciferase, and their use for non-invasive *ex vivo* monitoring of *in vivo* biological processes. Further, we comment on other recently discovered secreted proteins and their potential use as blood reporters for multiplexing applications in experimental animal models and potential applications in humans.

Secreted alkaline phosphatase

The secreted alkaline phosphatase (SEAP) is the most commonly used secreted reporter for monitoring *in vivo* processes. Alkaline phosphatases are normally membrane-bound, thus not secreted. By introducing a termination codon at sequence encoding the membrane anchoring domain, the 513-amino acid cell-surface form was converted into a truncated (489-amino acid), fully active protein (Berger et al., 1988). This recombinant reporter gene can be constitutively expressed and efficiently released from transfected cells. Changes in SEAP levels detected in conditioned medium of Chinese hamster ovary cells are directly proportional to changes in intracellular SEAP mRNA and cell number (Bettan et al., 1999; Cullen and Malim, 1992). Since then, SEAP has been widely used as a secreted serum reporter for accurate long-term monitoring of gene expression in a variety of gene transfer studies, from direct injection of plasmid DNA into muscles of adult mice (Abruzzese et al., 1999) to electroporating into large animals (e.g. pigs) (Brown et al., 2008), and delivering the plasmid DNA in the form of bioreducible multilayered polyelectrolyte films in subcutaneous model in rats (Blacklock et al., 2009). A single intrathecal injection of naked plasmid DNA in rats yields a sensitive detection of SEAP in serum for up to 4 months (Hughes et al., 2009). More recently, SEAP has been applied in screening mini-peptide-coding sequences to increase gene transfer activity (Cutrera et al., 2011). Further, SEAP expression in serum is a useful indicator in early and long-term measurement of tumor growth and response to therapeutics. In different human cancer xenograft models (Bao et al., 2000; Chaudhuri et al., 2003; Nilsson et al., 2002), SEAP was detectable in mouse plasma as soon as 1 day after injection, long before evident tumor was present. In all these studies, changes in SEAP levels reflected changes in tumor volume and cell numbers.

SEAP has also been used in detection of endoplasmic reticulum (ER) stress which is implicated in a number of diseases such as diabetes and neurodegeneration (Zhao and Ackerman, 2006). In transgenic mice constitutively producing SEAP, *in vivo* induction of systemic ER stress using intraperitoneally administration of thapsigargin caused rapid down-regulation of serum SEAP levels (Hiramatsu et al., 2006). SEAP serum assay has been used in measuring promoter and transcription factors activation and inhibition. Its activity detected in serum and protein extract of pancreas in transgenic mice could represent the activity of pancreatic and duodenal homeobox gene-1 promoter (Shiraiwa et al., 2007). Real-time monitoring of the nuclear factor kappa B (NFκB) activation, a transcription factor that plays a major role in many human disorders, including immune diseases and cancer, was achieved first using SEAP (Meng et al., 2005). In addition, SEAP has been extended to clinic use and was recently used to evaluate systemic and cervical antibody levels after vaccination. In 50 women vaccinated with HPV16/18 AS04-adjuvanted vaccine, serum SEAP levels correlated with antibody levels measured using ELISA (Kemp et al., 2008). In addition to the measurement of SEAP in blood, it is a useful reporter for *in situ* histo-enzymatic revelation to correlate the level of its secretion and the number of transduced cells (Shimajiri et al., 2011).

Human SEAP is immunogenic in rodents, which are the most commonly used laboratory animals. Circulating human SEAP levels are transient and decrease rapidly after reaching a maximum level in animal models, since it elicit cytotoxic T lymphocyte or neutralizing antibody responses that can suppress its own expression. To improve SEAP stability in transgenic mice, the mouse SEAP (mSEAP) has been developed by modification of the murine embryonic alkaline phosphatase (Wang et al., 2001) or isolation, the cDNA using mouse placental RNA as template (Maelandsmo et al., 2005). After intravenous administration in mice, both forms of mSEAP continued to express at high levels for >1 month, and no antibodies to mSEAP were detected. This species-specific form of SEAP has high potential in preclinical transplantation studies. When mSEAP-expressing muscle precursor cells were injected into the muscle of dystrophin-deficient *mdx* mice, there was a good correlation between the level of circulating mSEAP, the number of mSEAP-positive fibers, and the expression of dystrophin in regenerating fibers (Gerard et al., 2009).

The expression level of SEAP is relatively low; thus a relatively large amount of blood (up to 100 μl in some cases) is required for the assay. The high molecular weight of SEAP (64 kDa) significantly lessens its release into blood and almost completely blocks its excretion into urine (Hiramatsu et al., 2005). Most blood samples contain naturally occurring serum alkaline phosphatases which can interfere with the SEAP assay. Also, some treatments may cause liver damage and increase the presence of circulating liver-derived alkaline phosphatases in the experimental animals leading to non-specific SEAP activity. However, the heat stability of SEAP and resistance to phosphatase inhibitors (such as L-homoarginine) permit the sample to be pre-treated by either incubation at 65 °C or with this inhibitor to eliminate endogenous alkaline phosphatase activity (Fig. 1a). The requirement for sample processing and this inactivation step limits throughput and can be especially problematic for screening assays (Bettan et al., 1999). Since native mSEAP is sensitive to L-homoarginine and heat labile, high background activity cannot be inactivated by these strategies. Also, most mSEAP remains cell-associated due to glycan phosphatidylinositol linkage to the cell surface. A mutant (H451E) of mSEAP has been reported to enhance secretion and heat stability (Christou and Parks, 2011).

Soluble marker peptides

Many viruses have shown oncolytic activity in preclinical tumor models. A major challenge in cancer virotherapy studies is the lack of a convenient method to monitor the *in vivo* spread and elimination

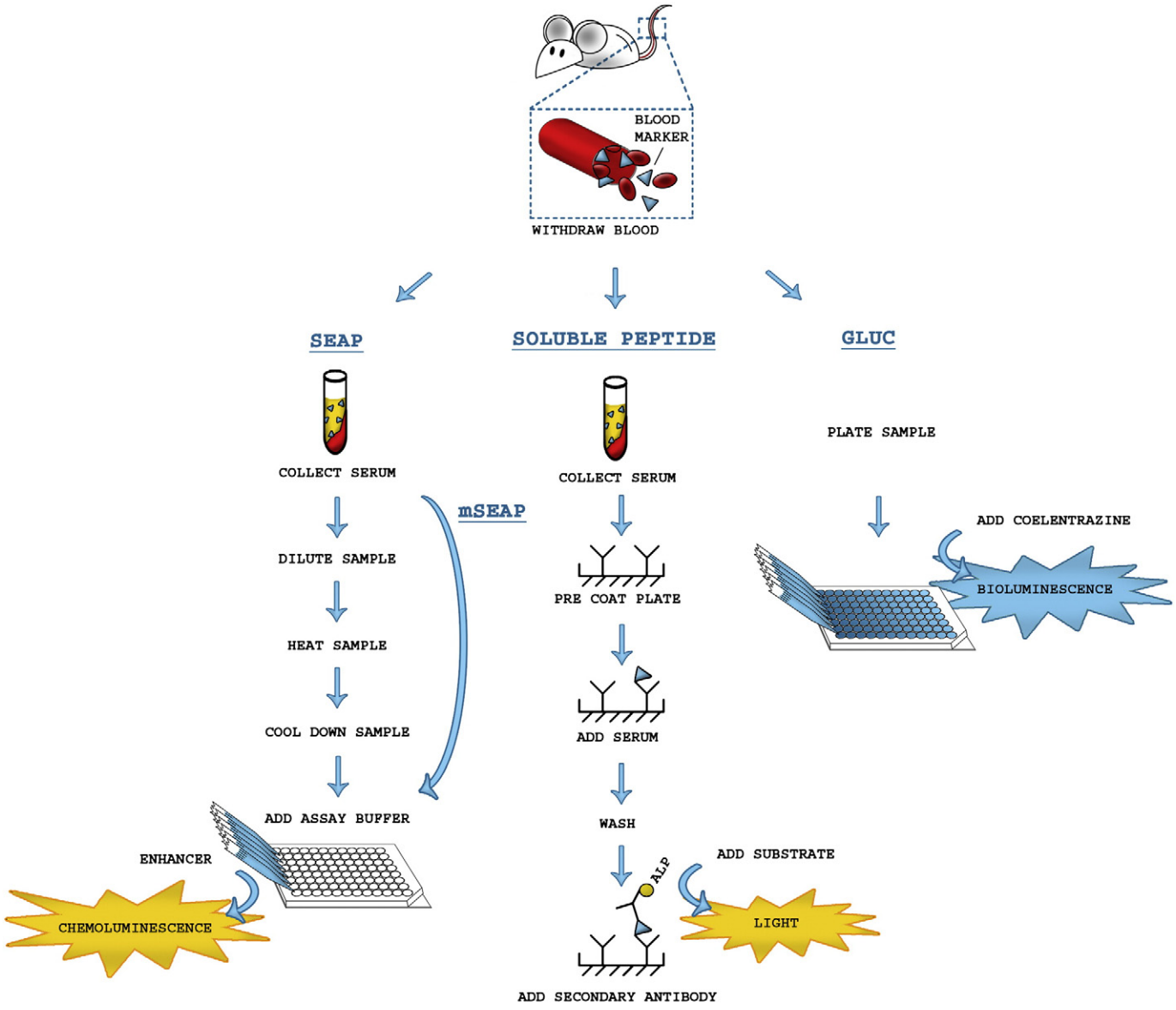

Fig. 1. Experimental set up for the secreted alkaline phosphatase, soluble peptides and *Gaussia* luciferase assays in body fluids.

of the virus, as well as to measure the profile of viral gene expression and kinetics over time (Msaouel et al., 2009). One approach to this problem is to introduce into the virus a second secreted marker which can be accurately and non-invasively detected over time. Measurement of the amount of these inert peptide markers in serum *ex vivo* could therefore provide important feedback on *in vivo* kinetics of viral infection and spread (Fig. 1b) (Peng et al., 2002a; Ricci et al., 2008). Important characteristics for choosing a soluble peptide includes lack of biological activity, constant circulation half-life, minimal immunogenicity, and the existence of a validated assay that allows its detection in blood/serum (Msaouel et al., 2009).

Edmonston vaccine strain of measles virus (MV-Edm) has been effectively engineered to express different soluble marker peptides, including the ones derived from the human carcinoembryonic antigen (hCEA) and β subunit of human chorionic gonadotropin (βhCG) (Peng et al., 2002b; Pham et al., 2009). The replication-competent virus was engineered to express the soluble extracellular N-terminal domain of hCEA or βhCG as an additional transcription unit before the viral N gene. Viral replication and gene expression following infection of the target cell result in encoded marker peptide production and secretion (Peng et al., 2006; Ricci et al., 2008). When expression cassettes for both hCEA and βhCG as well as the reporter gene human sodium iodide symporter (hNIS; a membrane ion channel detected non-invasively by radioisotopic single photon emission computed tomography imaging) were inserted into the same recombinant adenoviral vector backbone, strong correlation between hNIS image intensity and serum level of both soluble markers was noted (Liu et al., 2007; Liu et al., 2008). In athymic rats, blood and urine levels of βhCG were highly concordant with serum levels of hCEA at all time points. More recently, these soluble peptides have been successfully used in Lewis inbred rats to monitor transplanted heart (Liu et al., 2007; Pham et al., 2009).

Iankov et al. (2009) used the same concept and engineered an oncolytic measles virus to express a human light immunoglobulin chain reporter gene for the treatment of multiple myeloma. The vector-encoding λ protein would recombine with myeloma IgG-κ immunoglobulin, which is only expressed in tumor cells. In human myeloma xenograft mouse models, the level of specific chimeric IgG-κ/λ correlated

with response to the therapy. This strategy allows discrimination between tumor and normal cells using secreted blood markers (Iankov et al., 2009).

Real-time monitoring of the profile and kinetics of viral gene expression could facilitate tailoring of individualized treatment protocol to generate a specific dose size intervals between repeat treatment cycles both at the pre-clinical and clinical levels (Msaouel et al., 2009). In a phase-1 clinical trial of an intraperitoneal administration of an oncolytic measles virus strain in recurrent ovarian cancer patients, a dose-dependent CEA elevation was observed in peritoneal fluid and serum without any development of anti-CEA antibodies (Galanis et al., 2010). Soluble marker peptides were used in two additional trials in patients with recurrent glioblastoma multiforme and multiple myeloma (Galanis et al., 2010; Msaouel et al., 2009).

Gaussia luciferase

Our laboratory has established a novel naturally secreted reporter from the marine copepod *G. princeps* (Gluc) (Tannous et al., 2005). This 185 amino acid monomeric luciferase is the smallest luciferase cloned (19.9 kDa). The Gluc gene possesses a secretory signal consisting of 16 amino acids and therefore it is naturally secreted in an active form upon expression in mammalian cells. Gluc does not require any cofactors for activity (e.g., ATP) and catalyzes the oxidation of the substrate coelenterazine in a reaction that leads to emission of blue light (480 nm). The levels of Gluc in the conditioned medium are linear with respect to cell number, growth and proliferation (Badr et al., 2007; Wurdinger et al., 2008). Compared to Fluc or Rluc, human codon-optimized Gluc generates over 1000-fold higher bioluminescent signal intensity when expressed in mammalian cells. Gluc also has a much shorter assay time and increased sensitivity and linear range over other secreted blood reporters (Fig. 1c, Table 1) (Tannous, 2009) (Table 2).

As a secreted blood reporter, Gluc has been extensively used to monitor different biological processes including tumor growth and response to therapy, viral infection and replication, as well as viability of circulating cells (Wurdinger et al., 2008). In one application, Gluc blood assay was used to monitor MDA-MB-231 BR human breast cancer metastasis and treatment response in experimental animal models (Chung et al., 2009). Good correlation between the primary tumor volume and Gluc concentrations in blood and urine was observed. More importantly, Gluc blood assay revealed early detection of tumor growth and metastasis in mice and in large animal models (e.g. sheep) for breast, airway and lung cancer which were not attainable by typical *in vivo* imaging techniques (Griesenbach et al., 2011). When Gluc was constructed into a piggyBac transposon, gene expression in mice could be detected up to 80 days after transfection, suggesting that this transposon is useful for organ-selective somatic integration and sustained gene expression in mammals, and could potentially contribute to basic genetic studies and gene therapies (Nakanishi et al., 2010).

Gluc offers a tool for evaluating transcriptional regulation associated with signaling pathways which are dysregulated in many human disorders including inflammation and cancer. A reporter system was generated by cloning Gluc under the control of tandem repeats of NFκB responsive elements. This reporter demonstrated to be a highly sensitive for non-invasive continuous monitoring of the kinetics of NFκB activation and inhibition over time using blood or urine samples in mice (Badr et al., 2009; Badr et al., 2011a; Yang and Richmond, 2009). Recently, an apoptosis blood assay was generated by fusing GFP to the N-terminus of Gluc (including its signal sequence) separated by a short peptide consisting of aspartic acid, glutamic acid, valine, and aspartic acid (DEVD). This peptide is recognized and cleaved by caspase-3, whose activation reflects both the intrinsic and extrinsic apoptosis pathways. Under normal conditions, this fusion protein resides in the cytoplasm in an inactive form. During apoptosis, DEVD is cleaved, freeing Gluc which can now enter the secretory pathway, release from cells, and be detected in the conditioned medium in culture or blood of animals *ex vivo*. This reporter has been proved to be useful in real-time monitoring of apoptosis both in subcutaneous and metastatic tumor models (Niers et al., 2011).

Table 1
Application of secreted blood reporters.

Secreted reporters	Strategy	Applications	References
Secreted alkaline phosphatase	Constitutive expression driven by promoters	Monitor gene expression in gene transfer studies	Brown et al., 2008 Hughes et al., 2009 Cutrera et al., 2011 Nilsson et al., 2002
		Monitor tumor growth and response to therapy	Chaudhuri et al., 2003
	Expression under the control of transcription factors/ gene promoters	Monitor transcription factors, signaling pathways, such as NFκB, ER stress	Meng et al., 2005 Hiramatsu et al. 2006
	Expression using modified murine embryonic alkaline phosphatase	Long-term expression of SEAP in mice	Wang et al. 2001 Gerard et al. 2009
	Expression using alkaline phosphatase isolated from mouse plancental RNA		Maelandsmo et al., 2005
Soluble peptide markers	Measles virus to express human carcinoembryonic antigen	Non-invasively monitor virus expression and kinetics	Peng et al. 2002a Pham et al. 2009
	Measles virus to express human chorionic gonadotropin β subunit		Ricci et al. 2008
	Measles virus to express human sodium iodide symporter	Use the image intensity of a membrane protein as control	Liu et al. 2008
	Measles virus to express human light immunoglobulin chain	Discrimination between tumor and normal cells	Iankov et al. 2009
Gaussia luciferase	Constitutive expression driven by promoters	Monitor cell growth and response to therapy, viral infection/replication, viability of circulation cells	Wurdinger et al. 2008 Tannous 2009 Chung et al. 2009 Marquardt et al. 2011
	Expression under the control of transcription factors/gene promoters	Monitor transcription factors, signaling pathways, ER stress etc.	Badr et al. 2007 Yang and Richmond 2009 Badr et al. 2011a
	Fusing to microRNA target site at the 3′ UTR	Monitor expression and activity of miRNAs	Kim et al. 2009
	Fusing a short peptide recognized and cleaved by caspase-3	Real-time *in vivo* monitoring of apoptosis	Niers et al. 2011
	Isolation Gluc variant that catalyzes stable light emission	High-throughput screening	Maguire et al. 2009

Table 2
Comparison of secreted blood reporter.

	SEAP	Soluble peptide	Gluc
Time to complete the assay	Hours	Hours	Minutes
Sensitivity	ng/ml	ng/ml	1000-Fold more than SEAP
Dynamic range to cell number	<3 Orders of magnitudes	>4 Orders of magnitudes	>5 Orders of magnitudes
Assay in the whole blood	No, requires sample processing	No, requires sample processing	Yes
Half-life	3 h leading to accumulation	Hours	20 min
Detected in urine	No	Yes, also in peritoneal fluid	Yes, cleared by kidneys
Localized with *in vivo* imaging	*In situ* histology	No	Yes, non-invasive imaging
Immunogenicity	Need species-specific	No	Yes
Tested in clinic	No	Yes	No
Cost	$$	$$$	$

Gluc was also shown to be a sensitive marker for viral infection, replication, and reactivation. In one study, a mouse cytomegalovirus reporter that expresses Gluc under control of a strong major immediate early promoter was constructed. Gluc blood assay revealed virus reactivation 3 days after latent infection, preceding the detection of infectious virus by approximately 4 days (Marquardt et al., 2011). In another study, the efficiency of lentivirus infection and replication in tumors was monitored using the Gluc blood assay (Badr et al., 2011a; Wurdinger et al., 2008).

A major challenge in the cell therapy field is the ability to monitor the fate of implanted cells *in vivo*. By engineering neural precursor cells to express Gluc, viability, growth and proliferation of these cells could be monitored in real-time. This assay has the unique advantage in that the Gluc activity in blood reports from all viable circulating cells and not only from subpopulation of cells localized at a single site, typically imaged with other techniques.

MicroRNAs (miRNA) comprise a large group of endogenous noncoding short sequence RNA that can block mRNA translation or negatively regulate mRNA stability and thereby play a central role in the regulation of gene expression (Cortez and Calin, 2009). miRNAs are known to be extremely stable. Recently, endogenous naturally expressed miRNA circulating in blood has been tested as diagnostic markers in both fresh and archived serum or plasma (Reid et al., 2010). At the pre-clinical level, several recent studies utilized Gluc to monitor *in vivo* miRNA biogenesis by cloning seed sequences of a particular miRNA to the 3′-untranslated region of Gluc under the control of a constitutively active promoter. The Gluc blood assay allowed continuous detection of *in vivo* miR-122, miR142, or miR-34a activities (Kim et al., 2009).

One limitation for the Gluc assay in general is the rapid decay of its bioluminescence reaction, thereby requiring a luminometer with a built-in injector for assaying its activity. Our laboratory has isolated a Gluc variant (GlucM43I) that catalyzes a stable light emission output (half-life of bioluminescence reaction is ≥10 min versus approximately 3 min for wild type) in the presence of a detergent Triton X-100 using a screen of a mutant library created by DNA shuffling and error-prone PCR (Maguire et al., 2009). This Gluc variant proved to be a useful reporter for high-throughput screening applications where sensitivity and stable light emission are desired, and could potentially replace the wild-type Gluc for blood assay allowing semi-throughput screening/validation of novel therapeutics *in vivo* (Badr et al., 2011b; Marquardt et al., 2011).

Trends in development of novel secreted reporters

An ideal reporter for *ex vivo* monitoring of *in vivo* biological processes should encode a protein that is secreted from various tissues into the bloodstream where its endogenous level is minimal and distinguishable, be expressed in a stable manner over time in immunocompetent animals, and be easy to detect with rapid, specific and sensitive assays. The secreted protein should not be retained in different tissues through heparin-binding or matrix attachment region or through its interaction with its naturally occurring receptor (Baumgartner et al., 1998). Another important factor in the development of a blood reporter is the potential inactivation of these non-endogenous proteins through interaction with serum proteins such as albumin, limiting their detection *ex vivo* (Hiramatsu et al., 2005).

Secreted reporters are advantageous when can be detected in urine, since this specimen can be sampled more easily, frequently, and noninvasively in large animals. However, the bottleneck for a protein to pass into urine is the glomerular basement membrane in the kidney. Filtration rates of individual macromolecules are dependent on their molecular size. Proteins with a molecular mass more than 60 kDa can be rarely passed through. In contrast, a protein with a molecular mass smaller than 20 kDa often has a good chance to be filtered into the urinary space (Hiramatsu et al., 2005; Salgado et al., 2010).

Another field that can benefit from repeated data acquisition from live cells is toxicology. A secreted reporter allows non-invasive monitoring of the toxic effect of a given compound or treatment strategy over a prolonged exposure time in animals by withdrawing a few microliters of blood or urine and assaying for their activity. It also allows monitoring of potential drug interaction, dependent on the time as well as the order in which the drug is administered (Haugwitz et al., 2008; Tannous, 2009).

Various bioluminescent marine organisms exist with naturally occurring secreted luciferases. Among them, *Vargula* luciferase (Vluc) was the first to be cloned from the small marine ostracod crustacean *Vargula* (formerly Cypridine) *hilgendorfii* (Thompson et al., 1989). In the presence of the substrate vargulin, Vluc emits a blue light (emission maxima at 462 nm). However, the unavailability of the vargulin substrate limited its use (Thompson et al., 1995). Another naturally secreted luciferase was recently cloned from the marine copepod *Metridia longa* (Mluc). The cDNA of Mluc is 897 bp and encodes a 219-amino acid polypeptide with a M.W. of 23.9 kDa (Takenaka et al., 2008). Mluc has a more "glow-like" bioluminescence characteristic (Haugwitz et al., 2008). However, Mluc was reported to be rapidly inactivated by rat serum, fetal bovine serum, or human serum. Albumin was identified as the potential factor for the inhibition of its activity (Hiramatsu et al., 2005). This problem led to the limited use of Mluc as a blood reporter. In addition, several attempts were made to create a secreted Rluc variant by tagging it with a secretion signal peptide sequence. Liu et al. (1997) fused the 5′ end of the Rluc gene in frame with a short DNA sequence encoding the signal peptide of the human interleukin-2 (IL-2) protein. When expressed in mammalian cells, this construct encodes a protein product which can be secreted as a functional Rluc enzyme (Liu et al., 1997). Unfortunately, the specific activity of the secreted form of Rluc was only 7% of the cytosolic Rluc in mammalian cells. This may be in part due to cysteine residues in Rluc sequence which are oxidized in the secretory pathway environment (Liu et al., 2000).

Simultaneous monitoring of multiple biological processes in the same experimental animal facilitates understanding of disease development and expedites findings of novel therapeutics and translation

into the clinic. The discovery of new secreted reporters which either utilize different substrates, emit at different regions of the spectrum, or detect using specific assays allows their potential use for multiplex applications. For instance, different secreted reporters/soluble peptides can be used to report for different molecular event such as activation of different promoters, viral transduction, gene expression (etc.) simultaneously. In order for secreted reporters to be suited for multiplexing, the detection of their signals must be distinguishable from each other, and their chemical reactions must be compatible or separable. Recently, a multiplex blood assay was developed using Gluc and SEAP secreted reporters to monitor the kinetics of NFκB activation during tumor development (Badr et al., 2009). Since Gluc and Vluc utilizes different substrates, they can be potentially multiplexed together along with SEAP, giving that Vluc is a suited blood reporter. Many luciferases which are expressed intracellularly and emit different colored light (green and red-emitting luciferases from click beetle (Caysa et al., 2009; Takeuchi et al., 2010) and the American or Italian firefly (Branchini et al., 2007)) have been cloned. Secreted variants of these luciferases could be engineered and multiplexed with Gluc for multicolor applications using spectral unmixing (Gammon et al., 2006; Michelini et al., 2008).

Secreted blood reporters have proven to be sensitive tools for real-time *ex vivo* monitoring of *in vivo* biological processes. Since secreted reporters are first processed in the secretory pathway (i.e. ER and Golgi) before released from the cells, conditions which interfere with this pathway such as protein folding and ER stress could affect protein secretion leading to false data interpretation (Hiramatsu et al., 2006). Careful consideration of the secretory pathway is a must when applying blood assays in different disease models.

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