HSV1-TK/GFP/Fluc

TGL

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Chemical name:	HSV1-TK/GFP/Fluc	
Abbreviated name:	TGL	
Synonym:	TGL triple reporter	
Agent category:	Protein	
Target:	Other	
Target category:	Other – gene expression	
Method of detection:	Optical imaging, gamma imaging, SPECT	
Source of signal/contrast:	Green fluorescence protein (GFP), firefly luciferase, and ^{131}I-FIAU	
Activation:	Yes	
Studies:	• *In vitro* • Rodents	No structure is currently available in PubChem.

Background

[PubMed]

Reporter genes, also known as marker genes, possess a measurable phenotype distinguishable from the background of endogenous proteins (1). Several reporter genes express proteins that can generate signals for *in vivo* imaging, such as the herpes simplex virus type 1 thymidine kinase (HSV1-TK) gene, the green fluorescence protein (GFP) gene, and the firefly luciferase (Fluc) gene (2). The reporter gene is constructed with a "constitutive" promoter for continuous transcriptions or with an "inducible" promoter for controlled transcriptions (3). Both types of gene constructs have been used in the expression of exogenous genes and/or endogenous genes to monitor the levels of gene delivery and the efficiency in cell/tissue transduction in gene therapy.

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HSV1-TK/GFP/Fluc (TGL, Mw = 130 kDa) is a triple reporter protein used for multimodal *in vivo* imaging of gene expression (4) with single photon emission computed tomography (SPECT) or fluorescence and bioluminescence imaging. TGL is produced from the expression of a triple-fusion reporter gene (TGL gene) in which transcription and translation are processed through a single open reading frame. TGL consists of three fused protein subunits: HSV1-TK, GFP, and Fluc, where polylysine is used as a linker to connect the protein subunits and to maintain the molecular stability in the fused protein. Each subunit corresponds to a specific imaging modality. HSV1-TK is a homodimer composed of two 376-residue subunits (5), and it functions as a key enzyme in the pyrimidine-salvage pathway to catalyze the phosphorylation of thymidine (dT) to thymidine monophosphate (dTMP) in the presence of ATP and Mg^{2+}. This feature has been used to design antiviral nucleoside analogs (i.e., ganciclovir) for antiviral therapy (5) and radiolabeled substrates such as radiolabeled 2'-fluoro-2'-deoxy-1-β-D-arabino-furanosyl-5-iodo-uracil (FIAU) for SPECT imaging (6). HSV can easily infect a variety of cells in that the expressed HSV1-TK can induce significant cytotoxicity in the presence of nucleoside analogs such as ganciclovir (7). For this reason, HSV1-TK gene is also widely used as a suicide gene in cancer treatment. ^{131}I-Labeled FIAU is an active radiolabeled substrate of HSV1-TK that is detectable with SPECT (i.e., ^{131}I emits gamma-ray at 364 keV (81% abundance) with a half-life time of 8.02 days, which can be detected with a gamma camera or SPECT. GFP is a fluorescent protein of 238 amino acids that emits a bright green fluorescence (λ_{max} = 509 nm) when illuminated with a blue light (λ_{max} = 395 nm) (8). The presence of the GFP subunit allows *in vitro*/*in vivo* fluorescence imaging and cell sorting with the fluorescence-activated cell sorting (FACS) technique. Fluc is an oxygenase (Mw = 62 kDa) extracted from *Photinus pyralis* (9). In the presence of adenosine triphosphate (ATP) and O_2, Fluc oxidizes the heterocyclic substrate D-luciferin to oxyluciferin and emits light in the wavelength range of 400–620 nm (10). The Fluc subunit allows for planar whole-body bioluminescence imaging of gene-transduced cells.

Synthesis

[PubMed]

Ponomarev et al. described the preparation of the HSV1-TK/GFP/Fluc expression vector in multiple steps (4). First, a construct SFG-HSV1-TK/GFP was obtained (11). Plasmid pLTKRNL was digested with restriction enzyme *Bam*HI to generate a 2.2-kb fragment that encoded the open reading frame of HSV1-TK. This fragment was ligated into plasmid pcDNA.1/Zeo to result in the 7.8-kb plasmid pTKZeo3.2; this ligation was followed by sequential digestions with *Bam*HI and *Xma*I to produce a 1.2-kb TK fragment. At the same time, plasmid pEGFP-N1 was digested sequentially with *Bgl*II and *Xma*I to generate a 4.7-kb DNA fragment containing the open reading frame of GFP. The 1.2-kb fragment was then ligated with the 4.7-kb fragment to form the 5.9-kb expression plasmid pTKGFP, in which the TK-GFP cDNA was under control of the cytomegalovirus immediately early (IE) promoter. After the ligation, the plasmid was cloned into a retroviral vector, the Moloney murine leukemia virus-based SFG vector, to yield SFG-TK-

GFP. Second, two modified constructs, SFG-NES-HSV1-TK/GFP and SFG-Δ45-HSV1-TK/GFP, were obtained (6). To generate SFG-NES-HSV1-TK/GFP, the Xenopus cDNA for encoding the 20 amino acids in the nuclear export signal (NES) sequence of the mitogen-activated protein (MAP) kinase kinase (MAPKK) was inserted into the N-terminus of HSV1-TK/GFP between His-9 and Ala-10 using *Mlu*L. To generate SFG-Δ45-HSV1-TK/GFP, the Δ45-HSV1 cDNA lacking the first 45 amino acids (135 nucleotides) of the original HSV1-TK was amplified with selective primers and subsequently cloned between the *Nco*l sites of SFG-HSV1-TK/GFP. Third, the commercially available cDNA of fused *Aequorea victoria* eGFP and Fluc (eGFP/Fluc) was obtained from the plasmid pEGF/Pluc and subcloned into the retroviral vector SFG-Ntp to yield the construct SFG-GL (4). Finally, SFG-NES-HSV1-TK/GFP or SFG-Δ45-HSV1-TK/GFP was treated with *Nco*l and the fragment containing NES-HSV1-TK or Δ45-HSV1-TK was subcloned into the *Nco*I site of the SFG-GL to produce SFG-NES-HSV1-TK/GFP/Fluc (SFG-NES-TGL) or SFG-Δ45-HSV1-TK/GFP/Fluc (SFG-Δ45-TGL) (4). [^{131}I]-FIAU with >97% radiochemical purity was prepared by the iododestannylation reaction using the tin precursor and carrier-free ^{131}I.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Ponomarev et al. assessed the integrity of the fused reporter proteins with Western blot analysis (4). The molecular weight of the NES-TGL and Δ45-TGL proteins as determined with Western blot analysis matched the predicted molecular weights. These proteins were further examined with anti-HSV1-TK, anti-GFP, and anti-Fluc antibodies to demonstrate the coexistence of the triple subunits in the full reporter proteins. Ponomarev et al. then imaged the cytoplasmic localizations of the NES-TGL and Δ45-TGL reporter proteins with fluorescence microscopy (4). Human glioma U87 cells were transduced with the retroviral vectors SFG-NES-TGL or SFG-Δ45-TGL *in vitro*, followed by a bulk culture for 48 h. The obtained U87-NES-TGL cells or U87-Δ45-TGL cells were sorted for positive GFP expression using FACS with 488-nm excitation beam and 510-nm emission filter. The subcellular localization of NES-TGL and Δ45-TGL was visualized with fluorescence microscopy with similar excitation emission parameters. The GFP/Fluc signal appeared to be lower in the nucleus than in cytoplasm, but it was still detectable. Reporter gene expression in these transduced U87 cells was quantified with FACS, a bioluminescence assay, and a radiotracer [^{14}C]-FIAU assay. The level of GFP fluorescence was found to be ~1.3×10^3 relative fluorescent units (RU) in the U87-NES-TGL cells and ~2.1×10^3 RU in the U87-Δ45-TGL cells. The influx rate constant *Ki* of ^{14}C-labeled FIAU was ~1.4 media ml/min per g in the U87-NES-TGL cells and ~1.7 media ml/min per g in the U87-Δ45-TGL cells. The bioluminescence was ~2.8×10^3 relative bioluminescence unit (RLU) in the U87-NES-TGL cells and ~4.9×10^3 RLU in the U87-Δ45-TGL. The wild-type U87 cells were used as a control. Their measured fluorescence, influx rate, and bioluminescence were negligible.

Animal Studies

Rodents

[PubMed]

Ponomarev et al. used three imaging modalities to examine the expression of the triple reporter proteins *in vivo* (4). *Nu/nu* mice (20–25 g) were implanted subcutaneously with U87-NES-TGL cells on the right shoulder, with U87-Δ45-TGL cells on the left shoulder, and with wild-type U87 cells on the left flank. The tumors grew to an average size of 5.4 ± 2.1 mm and were imaged with whole-body fluorescence, bioluminescence, and gamma camera imaging. For bioluminescence imaging, mice were injected intravenously with 100 µl of D-luciferin solution at a dose of 150 mg/kg, and the images were acquired for 0.5–10 s. For the gamma camera imaging, mice received an intraperitoneal injection of 0.9% NaI solution (1 ml) one day before imaging to block the thyroid uptake of radioactive iodide. Then the mice were injected intravenously with [^{131}I]-FIAU at a dose of 7.4 MBq per animal. The gamma camera images were collected 24 h after [^{131}I]-FIAU injection to allow sufficient clearance of body background radioactivity. The GFP fluorescence was collected with a charge-coupled device (CCD) camera. The average level of gene expression in the implanted U87-NES-TGL tumor was detected as 707 ± 36 in fluorescence, 486 ± 47 × 10^6 photons/cm^2 per sr in bioluminescence, and 0.47 ± 0.08% injected dose (ID)/g in [^{131}I]-FIAU accumulation. The average level of gene expression in the implanted U87-Δ45-TGL tumor was detected as 740 ± 47 in fluorescence, 508 ± 36 × 10^6 photons/cm^2 per sr in bioluminescence, and 0.86 ± 0.06% ID/g in [^{131}I]-FIAU accumulation. As a control, the average level of gene expression in the implanted wild-type U87 cells was found at background level in fluorescence or bioluminescence imaging and at 0.03 ± 0.01% ID/g in [^{131}I]-FIAU accumulation (the background level). After completion of the triple modality imaging, the mice were euthanized and the tissues were harvested for *in vitro* fluorescence imaging. Bright green fluorescent areas were found in the tumor tissues, demonstrating the presence of TGL labeling.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

No publication is currently available.

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