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Virus-based reporter systems for monitoring transcriptional activity of hypoxia-inducible factor 1

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Abstract

Being key regulator of oxygen homeostasis hypoxia-inducible factor 1 (HIF-1) plays significant roles in cancer progression as well as in cardiovascular diseases. The modulation of HIF-1α activity in vivo may represent a valuable therapeutic approach to these disorders [Hofer, T., Desbaillets, I., Hopfl, G., Wenger, R.H., Gassmann, M., 2002. Characterization of HIF-1 alpha overexpressing HeLa cells and implications for gene therapy. Comp. Biochem. Physiol., Toxicol. Pharmacol. 133, 475–481]. In order to monitor HIF-1 transcriptional activity, we have developed HIF-1α-responsive reporter constructs, in which *lacZ* gene expression is driven by minimal *Hsp70* gene promoter or minimal immediate early promoter of cytomegalovirus (CMV) and a combination of hypoxia response elements from regulatory regions of *PGK1*, *ENO1* and *LDHA* genes. For the efficient delivery to a wide variety of cell types we chose retroviral and lentiviral vectors as carriers of the reporter cassette. We demonstrate that the obtained reporter system i) has a high inducibility in response to treatments leading to HIF-1α activation, ii) shows upregulation in response to HIF-1 activation and downregulation following inhibition of HIF-1α expression by small interfering RNA, iii) follows the dynamics of endogenous HIF-1 target gene expression. The retrovirus- and lentivirus-based reporters can be used for high-throughput screening of HIF-1α modulators and for the study of crosstalk between HIF-1 and different related signal transduction pathways. Potential applications for the reporters are discussed.

Keywords: Hypoxia response element; β-galactosidase; HIF-1α siRNA

1. Introduction

HIF-1 is the major transcription factor responsible for the induction of specific genes under conditions of low oxygen level, or hypoxia. In addition to hypoxic conditions HIF-1 can be activated through numerous signaling pathways by cytokines, hormones, reactive oxygen and nitrogen species, and in particular, under various conditions associated with tumor growth (Maxwell et al., 2001). HIF-1 is composed of alpha and beta subunits. The constitutive HIF-1β is present in the nucleus, while the expression, localization and functional activity of HIF-1α subunit are subject of complex regulation. To activate the transcription of target genes, HIF-1α forms complex with HIF-1β and binds to a specific sequence (hypoxia response

Abbreviations: HIF-1, hypoxia-inducible factor 1; HRE, hypoxia response element; VEGF, vascular endothelial growth factor; ENO1, enolase 1; LDHA, lactate dehydrogenase A; PGK1, phosphoglycerate kinase 1; pVHL, Von Hippel Lindau protein; LTR, long terminal repeat; SIN, self-inactivating vector; dsDNA, double stranded DNA; HIV-1, human immunodeficiency virus type 1; mHsp70, minimal *Hsp70* gene promoter; mCMV, minimal immediate early promoter of cytomegalovirus; DFO, desferrioxamine mesylate; *lacZ*, β-galactosidase gene; βGal, β-galactosidase protein; X-Gal, 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside; ONPG, *o*-nitrophenyl β-D-galactopyranoside; siRNA, small interfering RNA; u, unit(s); bp, base pair(s); nt, nucleotide(s); h, hour(s).

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element, HRE) in regulatory regions of these genes. The genes that are upregulated through HRE include vascular endothelial growth factor (*VEGF*), erythropoietin, enzymes of glucose metabolism (glucose tranporter-1, aldolase A, lactate dehydrogenase A (*LDHA*), phosphoglycerate kinase 1 (*PGK1*), enolase 1 (*ENO1*)) and many others (Semenza et al., 1996; Semenza, 2003). In normoxia, the activity of HIF-1α is controlled by interactions with the Von Hippel Lindau protein (pVHL), which blocks its transcriptional activity and promotes rapid proteosomal degradation (reviewed in Semenza, 2001).

Deregulation of HIF-1 transcriptional activity is detected in variety of pathological conditions. Dramatic activation of HIF-1 is typical to majority of human cancers (Zhong et al., 1999; Maxwell et al., 2001). It is frequently associated with increased tumor vascularization (Zagzag et al., 2000; Giatromanolaki et al., 2001) leading to failures in the cancer treatment (Birner et al., 2000; Aebersold et al., 2001). Hence, inhibition of HIF-1 activity represents a reasonable approach to the treatment of cancers with high levels of the HIF-1α protein (reviewed in Semenza, 2002, 2003).

HIF-1 plays a key role in the pathogenesis of ischemic disorders. Under hypoxic conditions, HIF-1 triggers the transcriptional induction of a number of angiogenic growth factors, including *VEGF* (Semenza, 2001). Overexpression of the *VEGF* in ischemic tissues, has been successfully applied as angiogenic gene therapy (Schratzberger et al., 2000). The above observations suggest that HIF-1α activating drugs would potentially elicit a more physiological induction of local angiogenesis, that, in turn, would increase the proportion of successful therapeutic angiogenesis applications (reviewed in Hofer et al., 2002).

In order to accurately monitor HIF-1 transcriptional activity under different conditions, we developed a series of virus-based reporter systems. We combined the existing knowledge about the HIF-1-mediated transcription of target genes with the advances in reporter gene technology to develop new powerful reporter system. We created a reporter cassette based on multiple HREs from the genes of glucose metabolism. This reporter cassette was cloned into retroviral and lentiviral vectors, allowing transduction of virtually any cell type within 24–48 h, as lentivirus can infect even non-dividing cell cultures with up to 100% efficiency. In addition, unlike plasmid transfection methods that frequently produce rearrangements and amplifications of the transgenes, the retro/lentivirus mediated gene transfer results in uniform integration into the genome of individual intact expression cassettes whose copy number can be easily controlled by variation in the multiplicity of infection. The obtained readout systems could be used to identify small molecules or genetic elements (including small interfering RNAs (siRNAs)) that affect signal transduction pathways modulating the activity of HIF-1. In addition, we expect our system to be useful in transgenic models for gene therapy, when the reporter gene is replaced by a gene of interest for targeted expression in hypoxic regions.

2. Materials and methods

2.1. Vectors

pSIP-mHsp70-lacZ and pUSTdS-mCMV-lacZ (Ivanov et al., in preparation) are self-inactivating (SIN) retroviral vectors (Yu et al., 1986) with full-length 5′ long terminal repeat (LTR) and truncated 3′LTR devoid of viral enhancer. Upon infection of target cells, the 3′LTR replaces 5′LTR, leading to integration of the proviral DNA into the host genome as an insert with no functional viral promoters. Similarly, the pLV-mCMV-lacZ represents a SIN construct, though it includes a backbone of the HIV-1-based lentiviral vector (Pfeifer et al., 2002). The reporter constructs rely on minimal promoters from human *Hsp70* gene (mHsp70) and from immediate early gene of cytomegalovirus (mCMV). The sequence of mHsp70 is: 5′-gcgggtctccgtgacgactataaaagcccaggggcaagcggtccg; the sequence of mCMV is: 5′-taggcgtgtacggtgggaggtctatataagcagagctcgtttagtgaaccgtcagatcgcctggagacgccatccacgctgttttgacctccatagaagacaccgggaccgatccagcct.

2.2. Construction of HIF-1α-responsive reporter plasmids

2.2.1. HRE1 element

The element consists of HRE-containing portions from three hypoxia-inducible genes: *PGK1* gene—5′-gacgtgacaaacgaagccgcacgtc, *ENO1* gene—5′-agggccggacgtggggccccagagcgacgctgagtgcgtgcgggactcggagtacgtgacggagcccc and *LDHA* gene—5′-acacgtgggttcccgcacgtccgc. An 88 nt long 5′HRE-*Xho*I (5′-agagactcgagacgtgacaaacgaagccgcacgtcagggccggacgtggggccccagagcgacgctgagtgcgtgcgggactcggagt) and an 87 nt long 3′HRE-*Sph*I (5′-ctctctgcatgcggacgtgcgggaacccacgtgtggggctccgtcacgtactccgagtcccgcacgcactcagcgtcgctctggggc) partially complementary oligos were annealed and the single stranded regions were filled in using Klenow DNA polymerase. The resulting 137 bp double stranded DNA (dsDNA) fragment (HRE1, see Fig. 1A) was cleaved with *Xho*I and *Sph*I restriction endonucleases and ligated into pSIP-mHsp70-lacZ plasmid according to standard ligation protocol (for map see Fig. 2A).

2.2.2. HRE12 element

Two self-complementary 79 nt oligos (5′HRE-*Xba*I: 5′-ctagaggacgtgacaaacagaagccacacgtcctagggacgtggggagtgcgtgaggagtacgtgaggacacgtgggta and 3′HRE-*Spe*I: 5′ctagtacccacgtgtcctcacgtactcctcacgcactccccacgtccctaggacgtgtggcttctgtttgtcacgtcct) were annealed resulting in dsDNA fragment with *Xba*I and *Spe*I compatible cohesive ends. The self-ligation products of these duplexes could not be recleaved by either of these restriction endonu-