FEBS Letters 587 (2013) 826–832

journal homepage: www.FEBSLetters.org

Tumor suppressor protein VHL inhibits Hedgehog–Gli activation through suppression of Gli1 nuclear localization

Hyun Kook Cho [a], So Young Kim [a], Kook Hwan Kim [a], Hyeong Hoe Kim [b], JaeHun Cheong [a,*]

[a] Department of Molecular Biology, College of Natural Sciences, Pusan National University, Busan 609-735, Republic of Korea
[b] Department of Experimental Medicine, Pusan National University Hospital, Busan 602-739, Republic of Korea

ARTICLE INFO

Article history:
Received 18 November 2012
Revised 19 January 2013
Accepted 23 January 2013
Available online 8 February 2013

Edited by Lukas Huber

Keywords:
VHL
Gli
Hedgehog signaling
Tumor suppression

ABSTRACT

The transcription factor Gli1 acts in the last known step of the Hedgehog signaling, and deregulation of Gli1 is implicated in human cancers. VHL protein is widely expressed in both fetal and adult tissues and acts as a tumor suppressor. Here, we demonstrate the molecular mechanism through which VHL inhibits the Hedgehog–Gli pathway. VHL decreased Gli1-mediated promoter transactivation as well as the expression of Hedgehog/Gli pathway target genes. Nuclear translocation of cytosolic Gli1 protein was inhibited by VHL via protein–protein interaction. These results indicate that overexpression of VHL may antagonize Hedgehog–Gli activation at the post-translational level in Hedgehog pathway-induced cancers.

Structured summary of protein interactions:
VHL-30 physically interacts with **GLI1** by anti tag coimmunoprecipitation (View Interaction: 1, 2)
GLI1 and **VHL** colocalize by fluorescence microscopy (View interaction)
VHL-19 physically interacts with **GLI1** by anti tag coimmunoprecipitation (View Interaction: 1, 2)
VHL physically interacts with **GLI1** by pull down (View interaction)

1. Introduction

The Hedgehog signaling pathway is one of the most fundamental signal transduction pathways in embryonic development and is highly conserved from flies up through humans [1]. The pathway is initiated by the binding of Hedgehog (Hh) ligand to PTCH1 receptor on the target cell surface, resulting in activation of SMO and transmission of the Hh signal through the protein complex [2]. Finally, activated glioma-associated oncogene homologue (Gli), a zinc finger transcription factor, translocates to nucleus whereupon it increases the expression of Gli target genes such as Gli1, PTCH1, Cyclin D1, Snail, BCL2, VEGF, and so on [3]. Gli1 and Gli2 mostly function as transcriptional activators while Gli3 functions as a repressor [4]. Recent findings have shown that Hh signaling is also important in the regulation of proliferation, survival, and growth of adult tissues [5]. Aberrant activation of the Hedgehog signaling pathway has been implicated in several human cancers, including medulloblastoma, rhabdomyosarcoma, basal cell carcinoma, breast cancer, small-cell lung carcinoma, hepatocellular carcinoma, colon cancer, pancreatic cancer, prostate cancer, and digestive tract cancer [6–15]. Furthermore, various studies have shown that transcriptional activation of Gli1 and Gli2 is important for cell proliferation, cell cycle progression, and anti-apoptosis [14,16–19]. Other studies have identified several negative regulators of Gli proteins. According to recent models, Gli1 activity is mainly regulated by nuclear-cytoplasmic shuttling [20–23].

Von Hippel–Lindau (VHL) disease is the result of inheriting a mutation in the *VHL* tumor-suppressor gene [24]. *VHL* mutations, including missense, non-sense, splice site, deletion, and insertion mutations, are associated with several types of tumors, which suggests that VHL protein exerts tumor suppressor activity in various cancers [25]. The *VHL* gene located on chromosome locus 3p25 is translated into two biological active proteins, pVHL30 and pVHL19. Internal translation is initiated at the 54th codon of VHL mRNA, thus generating pVHL19 [26]. Signs of VHL disease, including retinal angiomas, endolymphatic sac tumors, central nervous system hemangioblastomas, clear-cell renal cancers, and pancreatic neuroendocrine tumors, suggest that VHL protein has multiple functions [27]. Specifically, VHL protein interacts with several proteins and acts as a tumor suppressor [28]. The best characterized target of VHL protein is the α-subunit of HIF-1α and HIF-2α, which are hypoxia-inducible factor (HIF) family members [29]. Interaction of VHL protein with the HIF-1α subunit results in ubiquitylation and degradation of HIF-1α under normoxic conditions [30].

A recent study indicated that HIF-1α activates the Hedgehog pathway under hypoxic conditions [31]. This suggests that the tumor suppressor VHL may regulate the Hedgehog signaling

* Corresponding author. Fax: +82 51 513 9258.
E-mail address: molecule85@pusan.ac.kr (J. Cheong).

0014-5793/$36.00 © 2013 Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies.
http://dx.doi.org/10.1016/j.febslet.2013.01.050

pathway through suppression of HIF-1α. However, a direct effect of *VHL* on Hedgehog/Gli pathway-related cancer development has not yet been confirmed. Therefore, we investigated whether or not VHL can inhibit the Hedgehog pathway. We found that VHL suppressed the Hedgehog/Gli pathway, leading to repression of Hedgehog target gene expression. Furthermore, the protein–protein interaction between VHL and Gli1 inhibited nuclear localization and transcriptional activity of Gli1. These results suggest that VHL may act as a tumor suppressor in some Hedgehog-related cancers.

2. Materials and methods

2.1. Plasmid constructs and reagents

The Gli1-luc reporter, PTCH1-luc reporter, and BCL2-luc reporter constructs (containing the Gli1, PTCH1, and BCL2 promoters fused to luciferase reporter, respectively) were provided by Dr. Fritz Aberger (University of Salzburg, Salzburg, Austria). The pcDNA3/HA-hGli1, pcDNA3/GFP-hGli1, pcDNA3/GFP-Gli1/NESmut expression plasmids were constructed as previously described [23]. VHL constructs (pcDNA3/HA-VHL19, pcDNA3/HA-VHL30, pcDNA3/RFP-VHL30, and pcDNA3/GST-VHL30) were kindly provided by Dr. K. H. Kim. LMB (leptomycin B) and SAG was purchased from Sigma–Aldrich. The transfection reagents jetPEI and jetPRIME were purchased from Polyplus Transfection.

2.2. Cell culture

Huh7, HEK-293, and SKBR3 cells were maintained in DMEM containing 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) penicillin–streptomycin (PS) (GIBCO BRL) at 37 °C in a humid atmosphere of 5% CO_2, respectively. NIH3T3 cells were cultured in DMEM containing 10% fatal calf serum (FCS) and 1% (v/v) PS (GIBCO BRL) at 37 °C in a humidified atmosphere containing 5% CO_2.

2.3. SDS–PAGE and Western blotting

Whole cell lysates (50 μg) were subjected to SDS–PAGE (8–12%) and transferred onto a PVDF membrane (Millipore) by semi-dry electroblotting. Membranes were then incubated with anti-actin

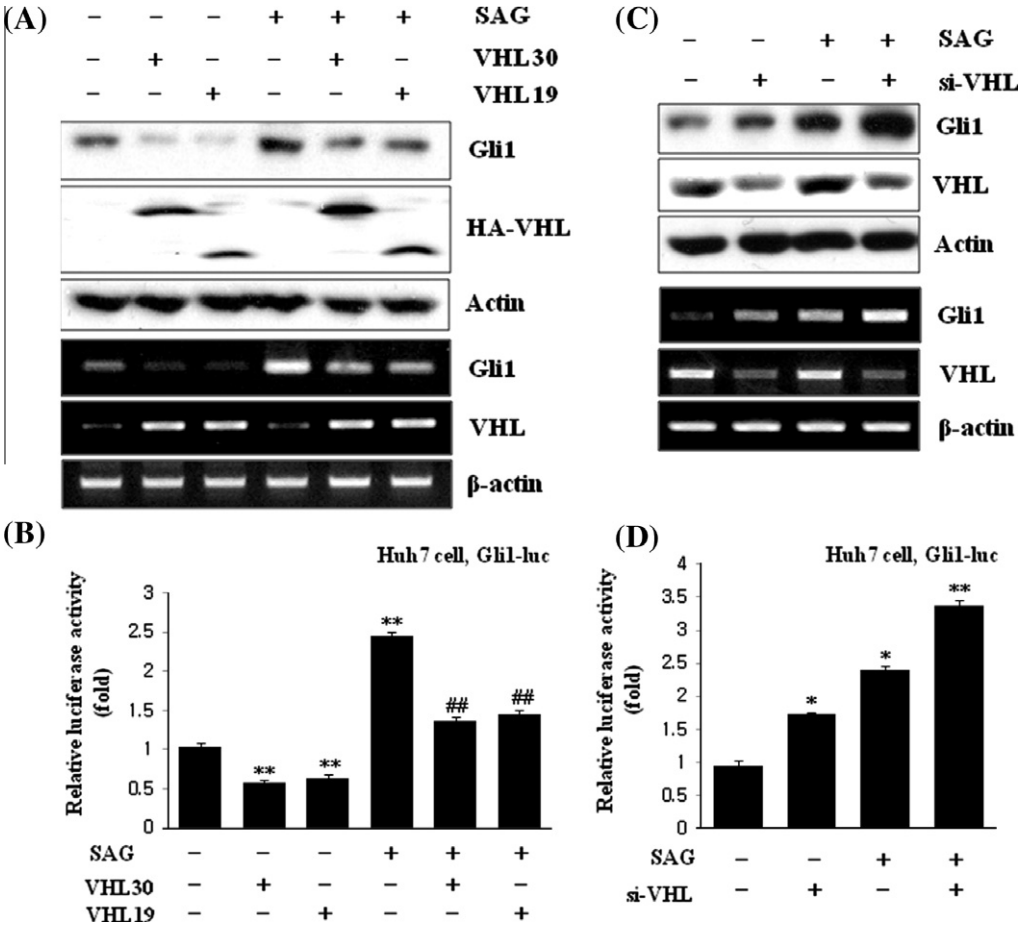

Fig. 1. VHL decreases Gli1 transcriptional activity. (A) Effect of VHL on Gli1 expression. Huh7 cells were transiently transfected with empty vector, VHL30, or VHL19 expression plasmids. After 12 h of transfection, the cells were treated with or without SAG (1 μM) dissolved in distilled water for 24 h. The cell lysates were analyzed for expression of Gli1, HA-VHL or Actin by Western blotting (top panels). Total RNAs were prepared from the cells, and then Gli1 or VHL mRNA levels were detected by RT-PCR with β-actin as a loading control (bottom panels). (B) Transcriptional repression of VHL on Gli1 expression. Huh7 cells were transfected with expression vectors encoding VHL30 or VHL19, along with Gli1 promoter luciferase reporter plasmid. After 12 h of transfection, the cells were treated with or without SAG (1 μM) for 24 h. The cell lysates were analyzed for luciferase activity. Luciferase activity was normalized for transfection efficiency based on corresponding β-galactosidase activity. Values are means ± S.D. ($n = 3$). $^{**}P < 0.01$ compared with mock transfectants, $^{\#\#}P < 0.01$ compared with SAG treated mock transfectants. (C) Effect of VHL-knockdown on Gli1 expression. Huh7 cells were transiently transfected with negative siRNA or siVHL. After 12 h of transfection, the cells were treated with or without SAG (1 μM) for 24 h. The cell lysates were analyzed for expression of Gli1, VHL or Actin by Western blotting (top panels). Total RNAs were prepared from the cells and then Gli1 or VHL mRNA levels were detected by RT-PCR with β-actin as a loading control (bottom panels). (D) Transcriptional activation of VHL-knockdown on Gli1 expression. Huh7 cells were transiently transfected with negative siRNA or siVHL, along with Gli1 promoter luciferase reporter plasmid. After 12 h of transfection, the cells were treated with or without SAG (1 μM) for 24 h. The cell lysates were analyzed for luciferase activity. Luciferase activity was normalized for transfection efficiency based on corresponding β-galactosidase activity. Values are means ± S.D. ($n = 3$). $^{**}P < 0.01$, $^{*}P < 0.05$ compared with mock transfectants.