Von Hippel-Lindau gene expression on the human fallopian tube epithelium during the menstrual cycle

YAN-YAN LU, WEI-JIE ZHU and BAO-GUO XIE

Department of Developmental and Regenerative Biology, College of Life Science and Technology, Jinan University, Guangzhou, Guangdong 510632, P.R. China

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Abstract. The Von Hippel-Lindau gene (VHL) is a tumor suppressor gene, which is widely expressed in kidney, lung, breast, ovary, and cervix. VHL gene mutations can induce VHL disease and tumorigenesis. However, whether this gene is expressed in the human fallopian tube has not been evaluated. The objectives of this study were to investigate whether the VHL gene is expressed in human fallopian tube, and to investigate its expression changes during the menstrual cycle. Twenty-seven patients undergoing abdominal hysterectomy with adnexectomy for benign uterine disease were enrolled in the study. Human fallopian tubes were divided into proliferative stage (n=14) and secretory stage (n=13) according to the stage of the menstrual cycle they were isolated from. The expression of the VHL gene and protein was studied by reverse transcription-polymerase chain reaction (RT-PCR), western blotting and immunohistochemistry, respectively. The results revealed positive expression of the VHL protein in the cytoplasm of ciliated cells of the human fallopian tube. The mRNA and protein expression of VHL in the fallopian tubes was higher in the proliferative compared to the secretory phase of the menstrual cycle, but this difference was not significant (P>0.05). Overall, this study presents data on the VHL mRNA and protein expression in the human fallopian tube, which may be relevant to the process of differentiation of ciliated and secretory cells.

Introduction

The fallopian tube is the tubular organ connecting the periovarian space to the uterus, and performs several interrelated functions. It captures the cumulus-oocyte complex, maintains viability, and constrain the spermatozoa to the fertilization site.

Correspondence to: Professor Wei-Jie Zhu, Department of Developmental and Regenerative Biology, College of Life Science and Technology, Jinan University, 601 Huang Pu Da Dao Xi, Guangzhou, Guangdong 510632, P.R. China E-mail: tzhuwj@jnu.edu.cn

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Moreover, it provides the biological environment for fertilization, contributes to removal or addition of oocyte coatings, and nourishes the egg (1).

The Von Hippel-Lindau gene (VHL) was isolated by positional cloning and identified as a tumor suppressor gene since 1993 (2). VHL, which is a classical tumor suppressor gene, can regulate the mRNA stability of a number of target genes through selective degradation of RNA-bound proteins, including the endothelial growth factor (EGF), transforming growth factor- α (TGF- α), hypoxia-inducible factor 1α (HIF- 1α), and carbonic anhydrase 9 (3,4). VHL disease is an inherited neoplastic disease characterized by a predisposition to develop vascularized tumors or cysts in numerous tissues (5). Moreover, mutations in the VHL gene are associated with cancer development of a number of organs, including kidney, lung, breast, ovary, and cervix (6). The VHL protein (pVHL) has been ascribed several distinct biochemical activities, and was shown to be involved in the regulation of the cell cycle (7). pVHL is a multifunctional protein, related to the inhibition of cell growth, angiogenesis, fibronectin matrix assembly, activation of p53, and proteolysis; nearly all these processes contribute to its tumor suppressor function (8). In addition, pVHL is also a component of the mechanism that transduces local differences in oxygen tension at the fetalmaternal interface to the cytotrophoblasts, affecting their biologic behavior (9). However, whether the VHL gene is expressed in the human fallopian tube is presently unclear.

This study was carried out to investigate the expression of VHL at the mRNA and protein level in human healthy fallopian tube samples, isolated from the proliferative and the secretory stages of the menstrual cycle, and to elucidate whether VHL expression levels change during the different stages of the menstrual cycle.

Materials and methods

Tissue samples. Samples of human healthy fallopian tube tissues were obtained by abdominal hysterectomy with salpingectomy for benign uterine disease. Twenty-seven fresh tissue samples of the proliferative (n=14) and the secretory phase (n=13) of the menstrual cycle were collected. One part of each sample was immediately frozen in liquid nitrogen or stored at -80° C until further use for reverse transcription-polymerase chain reaction (RT-PCR) and western blotting. The other part of the

samples was fixed in 10% formalin and embedded in paraffin for immunohistochemistry (IHC) analysis.

Patients with healthy fallopian tube tissues were operated for reasons unrelated to tubal dysfunction, and their mean \pm standard deviation (SD) age was 36 (4.38) years (range, 32-43 years). Their menstrual day was coincident with the histological diagnosis of the stage of the endometrium according to the criteria of Noyes *et al* (10). Patients were excluded if they had received exogenous steroid treatment or chemotherapy within the last 6 months before surgery. All patients signed informed consent letters, and the study protocol was approved by the Local Ethics Committee of Jinan University (Guangzhou, China).

RNA isolation and RT-PCR. Total RNA was extracted from 27 fresh fallopian tube tissues using an RNA isolation kit by Omega Bio-Tek Inc. (Norcross, GA, USA) according to the manufacturer's protocol. The mRNA concentration and purity were determined by 1.0% agarose gel electrophoresis, and the optical density $(OD)_{260/280}$ ratio was >1.8. Aliquots of mRNA (20 μ g) from each sample were reverse transcribed using oligo (dT)18 primer and the M-MLV reverse transcriptase (Promega Corp., Madison, WI, USA). The housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control to normalize the results for variations in the input cDNA amount or the RT efficiency. The forward primer for the amplification of the VHL gene (PCR product, 300 bp) was 5'-GTCGAAGAGTACCGCCCTGAAG-3' and the reverse, 5'-GTGTCCCTGCATCTCTGAAGAG-3'. PCR amplification was carried out in the following conditions: initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 94°C for 15 sec, annealing at 60°C for 15 sec, and extension at 72°C for 1 min. The PCR products were verified by electrophoresis on a 1.5% agarose gel, and densitometry analysis was carried using the Bio-Rad Gel Doc 2000 Imaging system (Bio-Rad Laboratories, Hercules, CA, USA). Densitometrical values were used to calculate the relative expression ratio VHL/GAPDH.

Western blotting. The fresh fallopian tube tissues were homogenized and lysed on ice using cell lysis buffer (Beyotime Institute of Biotechnology, Shanghai, China) and a protease inhibitor cocktail. Following centrifugation at 10,000 x g for 15 min at 4°C, protein concentrations were determined using the Bicinchoninic Acid Protein Assay kit (Pierce Biotechnology, Inc., Rockford, IL, USA). Total proteins were denatured in Laemmli buffer (Bio-Rad Laboratories), fractionated using 10% one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes. Blots were blocked for 2 h in TBST solution (20 mmol/l Tris pH 7.6, 137 mmol/l sodium chloride, 0.1% Tween-20) containing 10% non-fat dry milk, and incubated with primary antibodies targeting human pVHL (1:1,000; Cell Signaling Technology, Inc., Danvers, MA, USA) overnight at 4°C, or β-actin (1:3,000; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) for 1 h at room temperature with rocking. Subsequently, the blots were washed three times for 10 min each in TBST, followed by incubation for 1 h at room temperature with anti-rabbit and anti-mouse horseradish peroxidase-linked species-specific immunoglobulin (IgG). The bound antibodies were detected with the enhanced chemiluminescence (ECL) system BeyoECL Plus (Beyotime Institute of Biotechnology). Band intensities were quantified by scanning densitometry, using the Bio-Rad Quantity One software (Bio-Rad Laboratories).

IHC. Paraffin-embedded sections of fallopian tissues at the proliferative (n=14) and secretory (n=13) phase were examined by IHC. Positive staining was evaluated with the standard streptavidin-biotin system (Mai-xin Bio, Fuzhou, China). The $4-\mu$ m thick sections were deparaffinized in xylene, hydrated through graded alcohol, and incubated in antigen retrieval solution (0.01-mol/l sodium citrate buffer, pH 6.0) at 60°C for 16 min. Endogenous peroxidase activity was blocked by incubating the samples in 3% hydrogen peroxide for 10 min. Non-specific antibody binding was blocked by incubation with normal goat serum for 10 min. Mouse anti-human VHL monoclonal antibody (1:100; Mai-xin Bio) was used as the primary antibody, and the sections were incubated with the antibody overnight at 4°C. The secondary antibodies were biotinylated anti-mouse IgGs, and the reaction was developed with the streptavidin-peroxidase system. The diaminobenzidine Substrate-Chromogen system (Mai-xin Bio) was used as the color-developing substrate. The samples were observed under a fluorescence microscope (E400; Nikon, Tokyo, Japan).

Statistical analysis. Statistical analysis was performed with the SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA). Data are expressed as mean \pm SD and were analyzed with one-way analysis of variance (ANOVA). P<0.05 was considered to indicate a statistically significant difference.

Results

RT-PCR analysis. VHL expression was analyzed in the human fallopian tube by RT-PCR. The *GAPDH* mRNA was first detected in twenty-seven fresh fallopian tube samples, and those that were positive were then used for detection of the VHL mRNA level (Fig. 1). The VHL mRNA levels in the human fallopian tubes are presented in Fig. 2. The VHL mRNA level in the fallopian tube was higher at the proliferative phase compared to the secretory phase of the menstrual cycle. There was no difference in the expression of VHL between the proliferative and the secretory phase (P>0.05).

Western blotting. The pVHL and β -actin protein expression levels were revealed by western blotting analysis in the fresh fallopian tube tissues. A single band for each protein was observed in all fresh fallopian tube tissues (Fig. 3A). The pVHL levels in the 27 fresh fallopian tube samples are shown in Fig. 3B. Although pVHL expression in fallopian tubes was increased at the proliferative phase compared to the secretory one, this difference was not statistically significant (P>0.05).

IHC. Positive staining corresponding to pVHL was detected by IHC in the cytoplasm of ciliated cells of the fallopian tube epithelium (Fig. 4). Positive staining for pVHL was detected in the 27 ciliated cells of the fallopian tube tissues.



Figure 1. Gel image of polymerase chain reaction (PCR) products. Reverse transcription (RT)-PCR analysis of the 3-phosphate dehydrogenase (*GAPDH*) and von Hippel-Lindau (*VHL*) genes was performed in human fallopian tubes. Both genes are expressed in the human fallopian tube samples.



Figure 2. Von Hippel-Lindau (*VHL*) mRNA expression in human fallopian tubes at the proliferative and the secretory phase of the menstrual cycle were measured by reverse transcription polymerase chain reaction (RT-PCR). The 3-phosphate dehydrogenase (*GAPDH*) gene was used as an internal control. The *VHL* mRNA level in human fallopian tubes is higher at the proliferative compared to the secretory phase, but the difference is not significant (P>0.05).

Discussion

The VHL gene locates at the short arm of the human chromosome 3. It is ubiquitously expressed in a variety of organs, with particularly high levels of expression in the urogenital system, brain, kidney, sensory ganglia, and bronchial epithelium (11). pVHL, encoded by the VHL gene, is a multifunctional protein essential for endothelial extracellular matrix deposition, and inhibits cell motility (12). Moreover, pVHL can promote the assembly of actin and vinculin, and induce cell differentiation and growth arrest through integration of cell-cell and cellextracellular matrix (ECM) signaling (13).

It was reported that pVHL is associated with embryogenesis and placenta development. The fallopian tube is the organ that provides the microenvironment for gamete transport, fertilization and preimplantation of the embryo. However, whether the *VHL* gene is expressed in human fallopian tubes has not been reported to date. pVHL can control ciliogenesis by stabilizing microtubule growth of the ciliated cells (14). In this study, pVHL-positive staining was detected in the cytoplasm of ciliated epithelial cells of human fallopian tubes. These results indicate that pVHL may be involved in the development of the ciliated epithelial cells and the cell differentiation of cilia in the human fallopian tube.

The epithelium of human fallopian tube undergoes morphological and functional changes during the menstrual cycle. The number of tubal epithelial cells is low during the secretory phase of the menstrual cycle, and increase from the proliferative phase until the periovulatory period. At the periovulatory period, both secretory and ciliated cells are of equal size, with the secretory cells forming domes between the tufts of cilia (15). At approximately the time of ovulation, the secretory cells reach their peak activity, consequently reducing in height relative to the ciliated cells in tubal epithelium (16). The secretory cells can differentiate into ciliated cells, although this may be also considered as prima facie evidence for the existence of a multipotent precursor that can differentiate into other cell types via an intermediate cell type (17). A previous study indicated that pVHL can regulate the formation of primary cilia in the renal epithelium (18). Restoration of pVHL expression in VHL-negative human cancer cell lines was shown to enhance the frequency of ciliated cells, and knockdown of VHL in immortalized mouse kidney epithelial cells resulted in loss of primary cilia; these results suggest that VHL is necessary for the formation of the primary cilium (14,19). VHL-induced cell differentiation in renal epithelial cells was also reported (13). In this study, we demonstrated that the VHL gene is expressed in the human fallopian tube during the menstrual cycle, and that its expression at the proliferative phase is higher than the secretory phase, although the difference was not significant in our data. These results suggest that the VHL gene may regulate the differentiation of ciliated and secretory cells.

pVHL, as a tumor suppressor protein, regulates the proper extracellular fibronectin matrix assembly and the cell cycle. The fallopian tube epithelium can secrete fibronectin, and localizes at the luminal surface of the cells, especially on the tips of the cilia (20). Fibronectin is a regulator of various cellular activities, by promoting cell migration and spread, and extracellular matrix assembly. A previous study indicated that pVHL binds to fibronectin, and that VHL expression is associated with the deposition of assembled fibronectin (21). Overexpression of pVHL may increase fibronectin expression post-transcriptionally, as well as the secretion of extracellular fibronectin (22). Additionally, VHL gene mutations associated with disease result in fibronectin assembly defects, and pVHL-deficient cells fail to perform proper extracellular fibronectin matrix assembly (23). Therefore, pVHL physically interacts with fibronectin under physiological conditions, and this interaction can influence the ability of cells to assemble the extracellular fibronectin matrix. In the present study, the pVHL expression in the human fallopian tubes was not different between the proliferative and the secretory phase of the menstrual cycle. These results indicate that pVHL may not affect the fallopian tube during menstrual cycle through the interaction with fibronectin.

Integrins are $\alpha\beta$ heterodimeric transmembrane proteins that provide a link between cells and the surrounding matrix through tightly regulated interactions with ligands. Integrins have also been used as molecular markers of endometrial receptivity. Sülz *et al* reported that a similar 'window of implantation' may exist in the fallopian tube, based on their observation of regulated expression of $\beta3$ integrin in



Figure 3. Western blotting was used to analyze the von Hippel-Lindau protein (pVHL) and β -actin expression in the human fallopian tubes. (A) The expression of pVHL is shown at the proliferative phase (lanes 1-4) and the secretory phase (lanes 5-8). (B) pVHL expression levels are elevated in human fallopian tubes at the proliferative phase compared to the secretory phase. There is no statistically significant difference between the proliferative and secretory phase expression levels (P>0.05). RU, relative units.



Figure 4. Immunochemistry was used to detect the Von Hippel-Lindau protein (pVHL) in the human fallopian tube epithelium. Positive staining corresponding to pVHL appears in the cytoplasm of the ciliated cells. The ciliated cells at the secretory phase are indicated by black arrows. Magnification, x200.

the human fallopian tube epithelium (24). A previous study demonstrated that VHL induces cell differentiation through cell-cell and ECM signaling (13). Cell-ECM signaling is mediated by integrins, and integrin signaling influences cell survival, proliferation and differentiation, which indicates that integrins may contribute to VHL-mediated cell differentiation (25). VHL-mediated tight junction and adherens junction assembly was associated to the downregulation of integrins (26). In addition, the presence of β 1-integrin fibrillar adhesions in VHL(+) cells at late confluence allowed the cells to firmly anchor to the substrate, and thus may be an important mechanism in controlling cell migration (27). In our study, the expression levels of VHL mRNA and protein in the fallopian tubes were not significantly different during the two stages of the menstrual cycle studied. This result indicates that VHL may regulate the differentiation of ciliated cells in the fallopian tube through integrin signaling.

The environment in the fallopian tube can affect the fertilization potential of the sperm and the embryo development. The transport of the spermatozoa and the pre-embryo is deemed to be aided by muscular contractions in the wall of the fallopian tube and the cilia in the tubal mucosa. Regulation of muscular activity and formation of cilia in the fallopian tube were shown to be affected by sex steroids, nitric nerves, and prostaglandins (PG) (28). PGE2 can increase HIF-1 α levels (29). Moreover, the HIF1- α subunit was identified and targeted for rapid proteasome-dependent degradation by the VHL E3 ubiquitin ligase complex at normal oxygen concentrations (30). Under normal conditions, the HIF1- α protein is rapidly degraded via the VHL-ubiquitin-proteasome pathway (31). Therefore, *VHL* gene expression in the fallopian tube.

In conclusion, this study aimed to investigate VHL gene and protein expression in the human fallopian tube tissues during the menstrual cycle. Expression levels of VHL mRNA and protein were not significantly different between the proliferative and the secretory phase. Our result may enhance the current understanding on the mechanism of fallopian tube associated-disease and functions. Additional studies are needed to investigate whether the VHL mRNA and protein expression levels can be the target of novel therapies against fallopian tube associated-disease.

References

- 1. Croxatto HB: Physiology of gamete and embryo transport through the fallopian tube. Reprod Biomed Online 4: 160-169, 2002.
- Latif F, Tory K, Gnarra J, Yao M, Duh FM, Orcutt ML, Stackhouse T, Kuzmin I, Modi W, Geil L, *et al*: Identification of the von Hippel-Lindau disease tumor suppressor gene. Science 260: 1317-1320, 1993.
- Kamada M, Suzuki K, Kato Y, Okuda H and Shuin T: von Hippel-Lindau protein promotes the assembly of actin and vinculin and inhibits cell motility. Cancer Res 61: 4184-4189, 2001.
- 4. Lu N, Hui H, Yang H, Zhao K, Chen Y, You QD and Guo QL: Gambogic acid inhibits angiogenesis through inhibiting PHD2-VHL-HIF-1α pathway. Eur J Pharm Sci 49: 220-226, 2013.
- Ohh M, Park CW, Ivan M, Hoffman MA, Kim TY, Huang LE, Pavletich N, Chau V and Kaelin WG: Ubiquitination of hypoxia-inducible factor requires direct binding to the beta-domain of the von Hippel-Lindau protein. Nat Cell Biol 2: 423-427, 2000.
- Zhou MI, Wang H, Foy RL, Ross JJ and Cohen HT: Tumor suppressor von Hippel-Lindau (VHL) stabilization of Jade-1 protein occurs through plant homeodomains and is VHL mutation dependent. Cancer Res 64: 1278-1286, 2004.
- Schraml P, Struckmann K, Hatz F, Sonnet S, Kully C, Gasser T, Sauter G, Mihatsch MJ and Moch H: VHL mutations and their correlation with tumour cell proliferation, microvessel density, and patient prognosis in clear cell renal cell carcinoma. J Pathol 196: 186-193, 2002.
- Shuin T, Yamasaki I, Tamura K, Okuda H, Furihata M and Ashida S: Von Hippel-Lindau disease: molecular pathological basis, clinical criteria, genetic testing, clinical features of tumors and treatment. Jpn J Clin Oncol 36: 337-343, 2006.
- Rajakumar A, Doty K, Daftary A, Markovic N and Conrad KP: Expression of von Hippel Lindau (pVHL) protein in placentae from normal pregnant women and women with preeclampsia. Placenta 27: 411-421, 2006.
- Noyes RW Hertig AT and Rock J: Dating the endometrial biopsy. Am J Obstet Gynecol 122: 262-263, 1975.
 Richards FM, Schofield PN, Fleming S and Maher ER: Expression
- Richards FM, Schofield PN, Fleming S and Maher ER: Expression of the von Hippel-Lindau disease tumour suppressor gene during human embryogenesis. Hum Mol Genet 5: 639-644, 1996.
 Tang N, Mack F, Haase VH, Simon MC and Johnson RS: pVHL
- Tang N, Mack F, Haase VH, Simon MC and Johnson RS: pVHL function is essential for endothelial extracellular matrix deposition. Mol Cell Biol 26:2519-2530, 2006.
- 13. Davidowitz EJ, Schoenfeld AR and Burk RD: VHL induces renal cell differentiation and growth arrest through integration of cell-cell and cell-extracellular matrix signaling. Mol Cell Biol 21: 865-874, 2001.

- 14. Schermer B, Ghenoiu C, Bartram M, Müller RU, Kotsis F, Höhne M, Kühn W, Rapka M, Nitschke R, Zentgraf H, *et al*: The von Hippel-Lindau tumor suppressor protein controls ciliogenesis by orienting microtubule growth. J Cell Biol 175: 547-554, 2006.
- Lyons R, Saridogan E and Djahanbakhch O: The reproductive significance of human Fallopian tube cilia. Hum Reprod Update 12: 363-372, 2006.
 Crow J, Amso NN, Lewin J and Shaw RW: Morphology and
- Crow J, Amso NN, Lewin J and Shaw RW: Morphology and ultrastructure of fallopian tube epithelium at different stages of the menstrual cycle and menopause. Hum Reprod 9: 2224-2233, 1994.
- Comer MT, Leese H and Southgate J: Induction of a differentiated ciliated cell phenotype in primary cultures of Fallopian tube epithelium. Hum Reprod 13: 3114-3120, 1998.
- Esteban MA, Harten SK, Tran MG and Maxwell PH: Formation of primary cilia in the renal epithelium is regulated by the von Hippel-Lindau tumor suppressor protein. J Am Soc Nephrol 17: 1801-1806, 2006.
- Lutz MS and Burk RD: Primary cilium formation requires von hippel-lindau gene function in renal-derived cells. Cancer Res 66: 6903-6907, 2006.
- Makrigiannakis A, Karamouti M, Petsas G, Makris N, Nikas G and Antsaklis A: The expression of receptivity markers in the fallopian tube epithelium. Histochem Cell Biol 132: 159-167, 2009.
- 21. Ohh M, Yauch RL, Lonergan KM, Whaley JM, Stemmer-Rachamimov AO, Louis DN, Gavin BJ, Kley N, Kaelin WG Jr and Iliopoulos O: The von Hippel-Lindau tumor suppressor protein is required for proper assembly of an extracellular fibronectin matrix. Mol Cell 1: 959-968, 1998.
- Zhou Q, Pardo A, Königshoff M, Eickelberg O, Budinger GR, Thavarajah K, Gottardi CJ, Jones J, Varga J and Selman M: Role of von Hippel-Lindau protein in fibroblast proliferation and fibrosis. FASEB J 25: 3032-3044, 2011.
 Lonser RR, Glenn GM, Walther M, Chew EY, Libutti SK,
- Lonser RR, Glenn GM, Walther M, Chew EY, Libutti SK, Linehan WM and Oldfield EH: von Hippel-Lindau disease. Lancet 361: 2059-2067, 2003.
- 24. Sülz L, Valenzuela JP, Salvatierra AM, Ortiz ME and Croxatto HB: The expression of alpha (v) and beta3 integrin subunits in the normal human Fallopian tube epithelium suggests the occurrence of a tubal implantation window. Hum Reprod 13: 2916-2920, 1998.
- 25. Guo W and Giancotti FG: Integrin signalling during tumour progression. Nat Rev Mol Cell Biol 5: 816-826, 2004.
- 26. Ji Q and Burk RD: Downregulation of integrins by von Hippel-Lindau (VHL) tumor suppressor protein is independent of VHL-directed hypoxia-inducible factor alpha degradation. Biochem Cell Biol 86: 227-234, 2008.
- 27. Esteban-Barragán MA, Ávila P, Álvarez-Tejado M, Gutiérrez MD, García-Pardo Á, Sánchez-Madrid F and Landázuri MO: Role of the von Hippel-Lindau tumor suppressor gene in the formation of β1-integrin fibrillar adhesions. Cancer Res 62: 2929-2936, 2002.
- 28. Mastroianni L Jr: The fallopian tube and reproductive health. J Pediatr Adolesc Gynecol 12: 121-126, 1999.
- 29. Wanggren K, Lalitkumar P, Stavreus-Evers A, Stabi B and Gemzell-Danielsson K: Prostaglandin E2 and F2alpha receptors in the human Fallopian tube before and after mifepristone treatment. Mol Hum Reprod 12: 577-585, 2006.
- Semenza GL: Hypoxia-inducible factor 1 (HIF-1) pathway. Sci STKE 2007: cm8, 2007.
 Maxwell PH, Wiesener MS, Chang GW, Clifford SC,
- 31. Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, Wykoff CC, Pugh CW, Maher ER and Ratcliffe PJ: The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature 399: 271-275, 1999.