Placenta (2006), **27**, 411–421
doi:10.1016/j.placenta.2005.04.002

Expression of von Hippel Lindau (pVHL) Protein in Placentae from Normal Pregnant Women and Women with Preeclampsia

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Paper accepted 6 April 2005

The hypoxia inducible transcription factors, HIF-1α and -2α proteins, are overexpressed in placentae from women with preeclampsia (Biol Reprod 2001;64:499–506; Biol Reprod 2001;64:1019–1020). Normally, these proteins are regulated in an oxygen-dependent manner being rapidly degraded by the ubiquitin-mediated proteasomal pathway. Recent studies have shown that the tumor suppressor protein, von Hippel Lindau (VHL), targets HIF for ubiquitinylation under nonhypoxic conditions. The objectives of the present work were: (1) to investigate VHL protein expression in normal pregnant (NP), preeclamptic (PE), and preterm (without PE) placentae, (2) to test whether VHL protein is hypoxia inducible in term and first trimester placental villous explants, and (3) to analyze the ontogeny of VHL protein expression in the human placenta. To begin evaluating the potential contribution of VHL to HIF overexpression in preeclamptic placentae, we analyzed the levels of the VHL protein in both normal and preeclamptic placentae ($n = 7$ each). We hypothesized a deficiency of VHL protein in preeclamptic placentae. Eight biopsy sites were tested in each placenta and protein extracts were made. Western analysis was performed using VHL specific antibodies. Human renal adenocarcinoma (ACHN) cell extracts and extracts from COS-7 cells transfected with a VHL expression vector were used as positive controls. In a total of 112 biopsy sites that were analyzed (56 each for normal and preeclamptic placentae), the composite densitometry ratios (PE/NP) for the long (28 kDa) and short (19 kDa) forms of VHL were 1.09 ± 0.2 and 1.16 ± 0.11, respectively (both p = NS vs 1.0). A ratio of 1.0 indicates equal expression by preeclamptic and normal placentae. The same placentae exhibited composite densitometry (PE/NP) ratios of 1.97 ± 0.23 and 1.68 ± 0.20 for HIF-1α and -2α proteins, respectively (both $p < 0.05$ vs 1.0). In a separate analysis, the protein expression of the short form of VHL was also comparable among NP, PE and preterm ($n = 6$) placentae. VHL immunoreactivity was localized to cells within the basal plate and the syncytiotrophoblast. Despite induction of HIF proteins by hypoxia in first and term villous explants, there was no significant upregulation of VHL proteins. Finally, the expression of both the short and long forms of VHL protein decreased with gestational age (both $p < 0.05$ by ANOVA), and in villous tissue from first trimester placentae VHL immunoreactivity was predominantly localized to the cytotrophoblast. These results suggest that (1) deficiency of VHL protein does not account for HIF-α overexpression in preeclamptic placentae, (2) VHL protein is not regulated by hypoxia in either first trimester or term placental villous explants, and (3) VHL protein expression in the placenta decreases as a function of gestational age.

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Keywords: Preeclampsia; Placenta; Villous explants; Hypoxia inducible transcription factors; von Hippel Lindau protein; Hypoxia

INTRODUCTION

Placental hypoxia contributes to normal placental development and pathology [1]. The hypoxia inducible transcription factors, HIF-1α and -2α, are major transducers of hypoxia signaling in most tissues including the human placenta leading to the regulation of numerous genes [2–4]. Throughout most of the first trimester of human pregnancy, the intervillous space is relatively hypoxic [5–7]. Accordingly, both HIF-1α and -2α proteins (but not mRNA) are increased in the syncytiotrophoblast, villous cytotrophoblast and fetoplacental vasculature at this gestational stage [2]. The HIF-α proteins are then downregulated at the end of the first trimester when intervillous blood flow and placental oxygenation begin to rise [2,5,7].

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Table 1. Clinical characteristics of the patients who provided placentas for studies presented in Figures 2 and 3

Variable	Normal pregnancy ($n = 7$)	Preeclampsia ($n = 7$)[a]
Maternal age (years)	28 ± 3.4	29 ± 2.5
Race	Six Caucasians, one African American	Five Caucasians, one African American, one Native American
Gestational age at delivery (weeks)	39.7 ± 0.2	35.8 ± 1.2*
Birth weight (g)	3538 ± 284	1998 ± 323*
Parity	7/7 Nulliparous	7/7 Nulliparous
Mode of delivery	7/7 Cesarean section	7/7 Cesarean section
Systolic blood pressure (mmHg)	118 ± 4	160 ± 7*
Diastolic blood pressure (mmHg)	74 ± 4	95 ± 3*
Proteinuria	None	7/7[b]
Uric acid (mg/dl)	NA[c]	6.6 ± 0.7

Means ± SEM. *$p < 0.01$ vs normal pregnancy by unpaired t-test.
[a] One patient had HELLP syndrome.
[b] ≥2+ on dipstick in six patients, protein/creatinine ratio of 3.0 in one patient.
[c] NA, not available.

Table 2. Clinical characteristics of the patients who provided placentas for studies presented in Figure 6

Variable	Normal pregnancy ($n = 10$)	Preeclampsia ($n = 10$)
Maternal age (years)	24.9 ± 1.6	29 ± 2.1
Race	Eight Caucasians, two African Americans	Seven Caucasians, three African Americans
Gestational age at delivery (weeks)	39.2 ± 0.4	34 ± 1.1*
Parity	10/10 Nulliparous	8/10 Nulliparous
Birth weight (g)	3210 ± 119.5	2433 ± 312*
Systolic blood pressure (mmHg)	116 ± 4	152 ± 4*
Diastolic blood pressure (mmHg)	72 ± 3	96 ± 2*
Proteinuria	0-trace (in 5/10)	10/10[a]
Uric acid (mg/dl)	NA[b]	7.09 ± 0.5

Means ± SEM. *$p < 0.05$ vs normal pregnancy by unpaired t-test.
[a] ≥2+ on dipstick in nine patients and one had 344 mg/24 h.
[b] NA, not available.

Both HIF-1α and -2α proteins are significantly increased in preeclamptic placentae where they are primarily expressed in the nuclei of syncytiotrophoblast and fetoplacental blood vessels suggesting transactivational activity [8,9]. HIF-1α protein overexpressed in preeclamptic placentae is capable of binding to the DNA hypoxic response element in vitro, and is associated with aberrant placental expression of the VEGF receptors Flt-1 and Flk-1, as well as tyrosine hydroxylase in vivo [10]. Thus, in women destined to develop preeclampsia, overexpression of HIF-α proteins in the placenta likely contributes to the dysregulation of numerous genes that perturb placental function leading to impairment of trophoblast invasion during early gestation and elaboration of various proteins deleterious to the endothelium during late gestation.

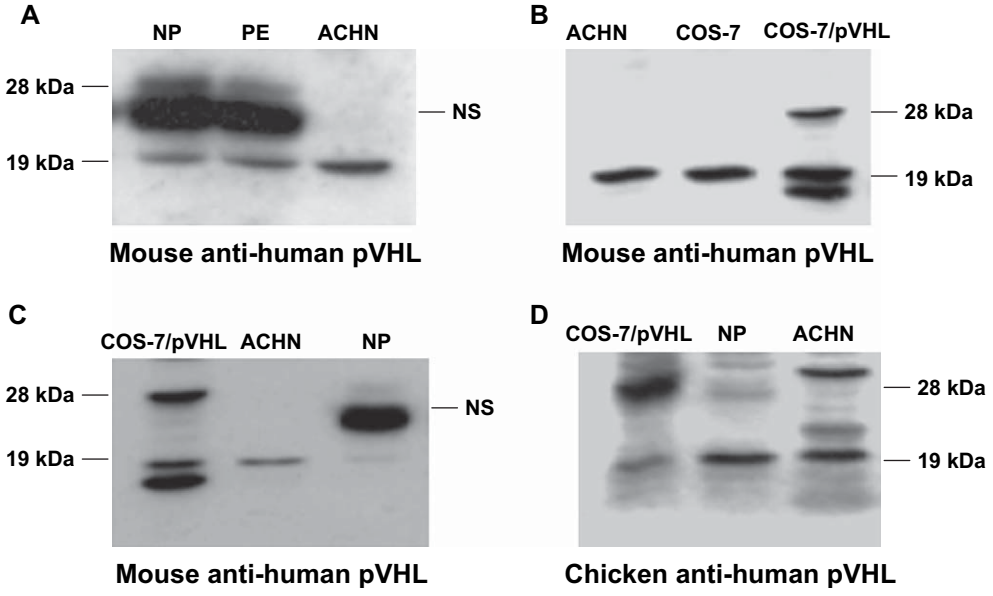

Figure 1. Characterization of the VHL antibodies. (A) Total proteins from a normal pregnant (NP) and preeclamptic (PE) placental biopsy were analyzed by Western blotting using a mouse anti-human VHL monoclonal antibody. Human renal adenocarcinoma cell extracts (ACHN) were used as positive control for the short form of VHL (19 kDa). (B) To detect the long form of VHL (28 kDa), COS-7 cells were transfected with a full-length VHL-containing expression vector pCR3.1 and the extract was analyzed by Western blot using the same VHL antibody as in (A), above. Note that untransfected COS-7 cells expressed the short form of VHL. (C) Total proteins from a NP placental biopsy were processed along with extracts of ACHN and COS-7/VHL transfected cells and analyzed by Western blotting. Only faint signals were detected in this particular placental biopsy. (D) A similar blot was probed with an antibody raised in chicken against human VHL. NS = nonspecific binding.