The Von Hippel-Lindau Protein Interacts with Heteronuclear Ribonucleoprotein A2 and Regulates Its Expression*

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The product of the von Hippel-Lindau (VHL) tumor suppressor gene, pVHL, functions as a ubiquitin-protein isopeptide ligase in regulating HIF-1 protein turnover, thus accounting for the increased transcription of hypoxia-inducible genes that accompanies VHL mutations. The increased vascular endothelial growth factor mRNA stability in cells lacking pVHL has been hypothesized to be due to a similar regulation of an RNA-binding protein. We report the expression of the GLUT-1 3′-untranslated region RNA-binding protein, heteronuclear ribonucleoprotein (hnRNP) A2, is specifically increased in pVHL-deficient cell lines. Enhanced hnRNP A2 expression was apparent in all cell fractions, including polysomes, where a similar modest effect on hnRNP L (a GLUT-1 and VEGF 3′-untranslated region-binding protein), was seen. Steady state levels of hnRNP A2 mRNA were unaffected. Regulation of hnRNP A2 levels correlated with the ability of pVHL to bind elongin C. Proteasome inhibition of cells expressing wild type pVHL selectively increased cytoplasmic hnRNP A2 levels to that seen in pVHL-deficient cells. Finally, an in vivo interaction between pVHL and hnRNP A2 was demonstrated in both the nucleus and the cytoplasm. Collectively, these data indicate that hnRNP A2 expression is regulated by pVHL in a manner that is dependent on elongin C interactions as well as functioning proteasomes.

Von Hippel-Lindau (VHL) disease is an autosomal dominant cancer syndrome characterized by the predisposition to develop highly vascular tumors, including renal clear cell (RCC) carcinomas, cerebellar hemangioblastomas, retinal angiomas, and pheochromocytomas (1). Both germline as well as sporadic mutations of the VHL gene have been identified in patients afflicted with this disease (2–7). Sporadic renal clear cell carcinomas are highly associated with mutation or transcriptional silencing of the VHL gene and subsequent loss or inactivation of the remaining VHL allele (6, 7). Thus, VHL conforms to Knudson’s two-hit model of a tumor suppressor gene, in which gene inactivation occurs as the result of loss of function of both alleles (8).

The human VHL gene encodes a full-length protein of 213 amino acids which migrates with an apparent molecular mass of 30 kDa (9, 10). Internal translational initiation from an internal ATG at codon 54 produces a second 18-kDa gene product (11–13). Both isoforms behave identically in all reports to date. VHL protein is predominantly expressed in the cytoplasmic compartments of most tissue and cell types, although it can shuttle between the nucleus and cytoplasm (10, 14–18). The localization of pVHL appears to be regulated according to cell density: pVHL is cytoplasmic in confluent cultures, but shuttles to the nucleus under sparse culture conditions (15). Nuclear export of pVHL is reduced by inhibitors of RNA polymerase II and polyadenylation, whereas nuclear import is unaffected, resulting in the localization of pVHL to the nucleus (16, 19).

VHL has been demonstrated to form a multimeric complex with two components of the transcriptional elongation factor elongin (elongin B and C) (11, 12, 14, 20), as well as cullin-2 (21, 22) and Rbx1 (23). Significantly, the majority of VHL mutants are defective in their ability to bind elongin C, implying functional significance for this interaction in vivo (14, 20, 24, 25). Additional clues to VHL function were provided by the discovery that elongin C and cullin-2 bear homology to yeast proteins (Skp1 and Cdc53), which function as a ubiquitin E3 ligase when complexed with an F-box protein (26–29). Furthermore, anti-pVHL immunoprecipitates can support E3 ubiquitin ligase activity in vitro if supplemented with exogenous ubiquitin-conjugating enzymes (30, 31). As a consequence, a model has evolved in which the pVHL-elongin B/C-Cul-2 complex functions as a ubiquitin E3 ligase, which targets specific substrates for ubiquitin-mediated proteasomal degradation (32).

The absence of pVHL in renal carcinoma cell lines is associated with a hypoxic phenotype under normoxic culture conditions; these cells express increased levels of vascular endothelial growth factor (VEGF), platelet-derived growth factor, and glucose transporter 1 (GLUT-1) (33, 34). The overproduction of these genes likely contributes to the hypervascular phenotype characteristic of VHL disease-associated neoplasms (35). The ubiquitin E3 ligase model of pVHL action accounts for the increased transcription of hypoxia-inducible genes (VEGF, platelet-derived growth factor), which occurs under normoxic conditions with pVHL mutations (33, 34, 36–39). The transcription of these genes is regulated by the levels of the transcription factor hypoxia-inducible factor (HIF)-1 (Refs. 40 and 41, and references therein). Under conditions of normoxia, rapid ubiquitination and proteasomal-dependent degradation

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† The abbreviations used are: VHL, von Hippel-Lindau; VEGF, vascular endothelial growth factor; GLUT-1, glucose transporter 1; AURE, A+U-rich element; hnRNP, heteronuclear ribonucleoprotein; RCC, renal clear cell; E3, ubiquitin-protein isopeptide ligase; HIF, hypoxia-inducible factor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; UTR, untranslated region; PIPES, 1,4-piperazinediethanesulfonic acid; CAPS, 3-(cyclohexylamino)propanesulfonic acid; HRP, horseradish peroxidase; PAGE, polyacrylamide gel electrophoresis.
of HIF-1α is mediated by an oxygen-dependent degradation domain (42). Under hypoxic conditions, the stability of HIF-1α protein increases, resulting in enhanced transcription of hypoxia-inducible genes (43, 44). Subsequent studies have shown that pVHL and HIF-1α directly interact under normoxic conditions, resulting in the ubiquitination and proteasomal targeting of HIF-1α by the pVHL-elongin B/C-Cul-2 complex (45, 46). Thus, in the absence of pVHL or with mutations that alter its ability to function as part of a ubiquitin E3 ligase complex, HIF-1α levels rise, leading to increased transcription of hypoxia-inducible genes such as VEGF (44).

Increased GLUT-1 and VEGF mRNA stability has also been observed in cells under conditions of hypoxia (47). In cells lacking pVHL, VEGF mRNA stability has been demonstrated (33, 34). It has been hypothesized that a similar mechanism accounts for the stabilization of GLUT-1 and VEGF mRNA observed in pVHL-deficient cells (45, 47). In this model, an RNA-binding protein, instead of a transcription factor such as HIF-1α, constitutes the target by which pVHL regulates the stability of these mRNAs. The 3'UTR of both GLUT-1 and VEGF have been shown to contain AU-rich elements (AURE), which regulate mRNA turnover (48).

Previous work in our laboratory indicated that hnRNP A2 binds a cis-acting instability element in the GLUT-1 3'-UTR that plays a role in the post-transcriptional regulation of GLUT-1 expression (49). These data suggested that overexpression of hnRNPA2 might account for the change in GLUT-1 mRNA turnover associated with the absence of pVHL. In RCC cell lines that differ only in their expression of functional pVHL, we observed that hnRNPA2 expression is increased in pVHL-deficient cell lines. This pVHL-dependent reduction in hnRNPA2 expression occurs independently of cell confluence. Northern blotting demonstrated that hnRNPA2 mRNA levels were unaffected, suggesting that pVHL deficiency results in decreased hnRNPA2 protein expression through changes in protein turnover or translation. Proteasome inhibition of cells expressing wild type pVHL resulted in cytoplasmic accumulation of hnRNPA2, but not the closely related and homologous protein hnRNPA1 (50). Finally, an in vivo interaction between pVHL and hnRNPA2 is demonstrated in both proteasomally inhibited cytisolic and untreated nuclear extracts. Collectively, these data indicate that hnRNPA2 expression is regulated by pVHL, and suggest the possibility that hnRNPA2 is a target of the pVHL-elongin B/C-Ubxis ubiquitin degradation machinery.

EXPERIMENTAL PROCEDURES

Cell Lines and Cell Culture—The human 786-0 renal carcinoma cell line (obtained from ATCC) was maintained in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) supplemented with 20% fetal bovine serum (HyClone). 786-0 subclones (generously provided by Dr. William Kaelin) stably transfected with pRC/CMV (pRC-9), pRC/CMV-VHL (C162F), and pRC/CMV-VHL (WT-8) (as previously described in Ref. 10) were cultured in the presence of G418 (500 μg/ml). The UMRC6 (UMRC) human renal carcinoma cell lines (generously provided by Dr. Bert Zbar), stably transfected with either a vector control (parental) or a plasmid expressing wild type VHL cDNA, were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. For proteasome inhibition experiments, cells were treated with either 100 μM LLL-17 (Sigma) or 25 μM MG132 (Sigma) overnight.

Preparation of Subcellular Fractions—Cytosolic lysates were prepared using a method characterized by its lack of contamination by nuclear proteins (51). Briefly, cells were washed twice in 1× phosphate-buffered saline (PBS), resuspended in 1% Triton X-100 lysis buffer (50 μL 2× 107 cells) consisting of 10 mM PIPES, pH 6.8, 100 mM KCl, 2.5 mM MgCl₂, 300 mM sucrose, 1 mM Pefabloc, and 2 μg/ml leupeptin and pepstatin A. Samples were incubated for 3 min on ice and then centrifuged for 5 min at 10,000 × g. The supernatant was aliquoted and stored at −80 °C as the cytosolic fraction. The pellet was resuspended in lysis buffer and then spun through a 30% sucrose cushion.

The nuclear pellet was resuspended in 0.5 nuclear pellet volume in low salt buffer consisting of 10 mM Tris-HCl, pH 7.6, 20 mM KCl, 1.5 mM MgCl₂, 0.5 μM dithiorethiol, 0.2 mM EDTA, 25% glycerol, 2 mM Pefabloc, 1 μg/ml each leupeptin and pepstatin A. While vortexing, 1× nuclear pellet volume of high salt buffer (containing 0.5 M KCl) was added dropwise. Samples were incubated at 30 °C for 15 min at 4 °C, and then centrifuged for 30 min at 12,000 × g at 4 °C (52). The supernatant was aliquoted and stored at −80 °C as the nuclear fraction. For polysome preparations, pRC-9, C162F, and WT-8 cells were homogenized in buffer A (10 mM Tris-HCl, pH 7.6, 1 mM potassium acetate, 1.5 mM magnesium acetate, 2 mM dithiorethiol, 2 μg/ml leupeptin and pepstatin A), and nuclei removed by centrifugation. The supernatant was layered over a 30% sucrose cushion followed by ultracentrifugation at 36,000 × rpm for 5 h at 4 °C. Whole cell lysates were prepared by washing cells twice with 1× phosphate-buffered saline, and then solubilizing in 2× Laemmli SDS sample buffer (53).

Immunoprecipitation and Immunoblotting—Immunoprecipitation of pVHL with hnRNPA2 from WT-8 proteosomally inhibited cytoplasmic (100 μg) or untreated nuclear (400 μg) lysates was performed with a mouse monoclonal antibody directed against pVHL (1G32) (10). Immunocomplexes were captured with protein A-Sepharose beads (Amersham Pharmacia) for 2 h at 4 °C, and beads were washed six times in 300 mM NaCl. Proteins were resolved by 15% SDS-PAGE and electrophoretically transferred to nitrocellulose membrane in CAPS buffer, pH 11.0, with 10% methanol. Immunoblots were washed with Tris-buffered saline, 0.1% Tween 20, and blocked in 3% bovine serum albumin overnight at 4 °C. Membranes were then probed with a mouse monoclonal antibody directed against hnRNPA2 (EF67) and goat anti-mouse HRP-conjugated secondary antibody. CBirds beads were used in lieu of protein-A Sepharose in order to eliminate interfering signal generated by detection of the light chain of the secondary antibody. Depleted lysates represent supernatants of immunoprecipitations. Polysomal lysates were probed with a rabbit polyclonal anti-hnRNPA2 antibody (Act2), followed by goat anti-rabbit HRP-conjugated secondary antibody. Detection of hnRNPA2 expression was accomplished by probing with rabbit polyclonal anti-hnRNPA2 antibody (Act1), followed by goat anti-rabbit HRP-conjugated secondary antibody. As indicated, blots were probed with anti-GAPDH monoclonal antibody (6C5-American Research Products), followed by goat anti-mouse HRP-conjugated secondary antibody to control for loading. Reactive antigens were visualized with Supersignal chemiluminescent substrate (Pierce).

Northern Blotting—Total cellular RNA was extracted by acid guanidium-phenol-chloroform extraction (54). RNA was size-fractionated by formaldehyde-agarose gel electrophoresis, blotted onto Hybond-N nylon membrane (Amersham Pharmacia) in 20× SSC, and baked under vacuum for 2 h at 80 °C. Filters were prehybridized overnight at 42 °C in 50% formamide, 0.8 M NaCl, 0.1 M PIPES, 0.1% Sarkosyl, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% polyacrylamide, 10 μg/ml yeast poly(A) RNA (20 μg/ml), and salmon sperm DNA (200 μg/ml). Hybridizations were performed at 42 °C for 24 h in prehybridization mix containing 10% dextran sulfate and 1× 106 cpm/ml cDNA probes for hnRNPA2 and β2-microglobulin, which had been labeled with [32P]dCTP (3000 Ci/mmol) using a random primer method (55). Membranes were washed with 0.1× SSC containing 0.02% sodium pyrophosphate and 0.5% Sarkosyl four times for 30 min each at 56 °C. Blots were dried and exposed at −70 °C to Fuji XAR film using one intensifying screen.

Densitometry—Protein and mRNA bands were quantified by densitometric scanning of autoradiographs and immunoblots using NIH Image software.

RESULTS

Increased hnRNPA2 Expression in pVHL-deficient RCC Cell Lines—The mechanism by which pVHL deficiency increases GLUT-1 mRNA stability is unknown. It was hypothesized that the effect on GLUT-1 mRNA stability is mediated through a specific GLUT-1 mRNA-binding protein (24). Previous work identified hnRNPA2 as a GLUT-1 mRNA 3'-UTR-binding protein (49). For these reasons, we examined hnRNPA2 protein levels in three RCC cell lines, which differ only in their expression of VHL.

Cytosolic lysates derived from 786-0 RCC cells, which lack functional VHL protein, pRC-9 cells (786-0 cells trans-
whole cell lysates (20,000 cells) of 786-0, pRc-9, and WT-8 cells were immunoblotted in parallel as indicated above for hnRNP A1 and hnRNP A2. Cytosolic (25 μg) fractions of UMRC parental (PAR) and UMRC wild type (WT) cells were immunoblotted in parallel as indicated above for hnRNP A1 and hnRNP A2.

**Fig. 1.** Overexpression of hnRNP A2 in cells lacking pVHL. A, cytosolic (25 μg) fractions of 786-0, pRc-9, and WT-8 cells were separated by 15% SDS-PAGE and analyzed by immunoblotting in parallel with the polyclonal antibodies specific for hnRNP A1 (Act-1) and hnRNP A2 (Act-2) as described under “Experimental Procedures.” B, whole cell lysates (20,000 cells) of 786-0, pRc-9, and WT-8 cells were immunoblotted in parallel as indicated above for hnRNP A1 and hnRNP A2. C, cytosolic (25 μg) fractions of UMRC parental (PAR) and UMRC wild type (WT) cells were immunoblotted in parallel as indicated above for hnRNP A1 and hnRNP A2.

**Fig. 2.** pVHL-dependent differences in hnRNP A2 expression are independent of cell confluence. Figure shows anti-hnRNP A2 immunoblot of pRC-9 and WT-8 cytosolic lysates. pRC-9 and WT-8 cells were grown to the indicated cell density, cytosols extracted, and protein (25 μg) separated by 15% SDS-PAGE. Cytosols were immunoblotted for expression of hnRNP A2 with Act-2. Lysates were also blotted in parallel with anti-GAPDH monoclonal antibody to control for loading.
PvHL Interacts with and Regulates HnRNP A2

Expression in a PvHL-Dependent Manner

In this report, we demonstrate that hnRNP A2 levels in RCC cell lines vary inversely as a function of PvHL expression. Neither altered subcellular distribution nor different rates of cell growth account for the elevated expression of hnRNP A2 observed in PvHL-deficient cells. The effects of PvHL on hnRNP A2 levels were not due to altered sequestration, as equivalent effects were seen with cells directly lysed in SDS-PAGE loading buffer. The levels of hnRNP L, which has been shown to form complexes in vivo with hnRNP A2 (49), were also reduced by PvHL, but this effect was more modest than the alteration in hnRNP A2 protein expression and was restricted to polysomal fractions. Moreover, increased hnRNP A2 protein expression in these cells does not correlate with changes in mRNA levels, implying that PvHL affects the rate of hnRNP A2 protein turnover or translation. Pharmacologic inhibition of proteasome function increased cytoplasmic hvhl complexed almost entirely with hnRNP A2.

Discussion

Interaction of hvhl and HnRNP A2 in Vivo—To discuss the possibility that hvhl regulates hnRNP A2 protein turnover, we examined whether VHL physically interacts with hnRNP A2 in vivo, as it does with HIF-1α (45, 47, 60). Due to low levels of hnRNP A2 in the cytosol of WT-8 cells, we examined their association following treatment with MG132. Immunoprecipitates of hvhl from proteasomally inhibited WT-8 cytosol were shown to contain hnRNP A2 (Fig. 7A, left panel). Indeed, hnRNP A2 immunoprecipitation quantitatively depleted hvhl from the cytosol (Fig. 7A, right panel). In contrast, no hvhl was found in hvhl A1 immunoprecipitates (data not shown). This interaction between hvhl and HnRNP A2 can also be shown in the nuclear fraction of WT-8 cells (Fig. 7B). These data not only imply that VHL and hnRNP A2 interact in vivo in the nucleus and cytosol, but also indicate that, in the context of proteasome inhibition, cytoplasmic VHL is complexed almost entirely with hnRNP A2.

In the absence of elongin C interaction, hnRNP A2 levels were modestly modulated by hvhl in the absence of elongin C

Proteasomal Inhibition Increases Cytoplasmic HnRNP A2 Expression in a Hvhl-Dependent Manner—The data with the C162F RCC cell line suggested that the interaction of hvhl with elongin C was necessary to regulate hnRNP A2 protein levels. To test the hypothesis that hvhl regulated hnRNP A2 levels through modulating protein turnover, the effect of proteasomal inhibition on hnRNP A2 gene expression was evaluated (Fig. 6). Overnight treatment with the proteasome inhibitor MG132 increased cytoplasmic hvhl A2 in WT-8 RCC cells, while pRc-9 RCC cells were unaffected (Fig. 6A). Following proteasomal inhibition, cytoplasmic hvhl A2 levels in WT-8 cells approached that seen in pRc-9 cells. Similar results were obtained with a proteasome inhibitor with a different mechanism of action, LLL (Fig. 6B). Immunoblots were then reprobed with polyclonal antibody directed against hvhl A1. The specificity of this effect on hvhl A2 expression is further demonstrated by lack of change in nuclear or cytosolic levels of hnRNP A1 in either cell type (Fig. 6, A and B). Thus, proteasomal inhibition selectively increased cytoplasmic hvhl A2 in a Hvhl-dependent manner, suggesting that VHL mediates proteasomal degradation of hvhl A2.

FIG. 5. elongin C binding is involved in hvhl regulation of HnRNP A2 expression. A, whole cell lysates (20,000) of pRc-9, C162F, and WT-8 cells were separated by 15% SDS-PAGE and analyzed by immunoblotting with the polyclonal antibodies specific for hnRNP A1 (Act-1), hvnl A2 (Act-2), and GAPDH (6C5) as described in “Experimental Procedures.” B, nuclear (10 µg) fractions of pRc-9, C162F, and WT-8 cells were immunoblotted as indicated above for hvnl A1, hvnl A2, and GAPDH.

FIG. 4. HnRNP A2 mRNA levels are Hvhl-independent. Figure shows Northern blot for hnRNP A2 RNA. Total RNA (30 µg) was size-separated, blotted, and probed with 32p-labeled hnRNP A2 cDNA. β2-Microglobulin loading control is depicted below. Data are representative of results obtained from three RNA isolations each probed twice for hnRNP A2 mRNA levels.

FIG. 3. HvnL expression regulates polysomal hnRNP A2 and L levels. pRc-9 and WT-8 polysomes (A, 0.2) were separated on 15% SDS-PAGE and analyzed by immunoblotting in parallel for expression of hnRNP A2 with Act-2, hnRNP L (1D11), hvnl (1G32), and GAPDH (6C5).

FIG. 2. Interaction of hvnl and HnRNP A2 in Vivo—To discuss the possibility that hvnl regulates hnRNP A2 protein turnover, we examined whether VHL physically interacts with hnRNP A2 in vivo, as it does with HIF-1α (45, 47, 60). Due to low levels of hnRNP A2 in the cytosol of WT-8 cells, we examined their association following treatment with MG132. Immunoprecipitates of hvnl from proteasomally inhibited WT-8 cytosol were shown to contain hnRNP A2 (Fig. 7A, left panel). Indeed, hnRNP A2 immunoprecipitation quantitatively depleted hvnl from the cytosol (Fig. 7A, right panel). In contrast, no hvnl was found in hvnl A1 immunoprecipitates (data not shown). This interaction between hvnl and HnRNP A2 can also be shown in the nuclear fraction of WT-8 cells (Fig. 7B). These data not only imply that VHL and hnRNP A2 interact in vivo in the nucleus and cytosol, but also indicate that, in the context of proteasome inhibition, cytoplasmic VHL is complexed almost entirely with hnRNP A2.

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of hnRNP A2 mRNA levels were unaffected by VHL gene expression in three experiments. Therefore, pVHL most likely regulates hnRNP A2 expression at the level of mRNA translation or protein turnover. However, several lines of data strongly support a mechanism of increased hnRNP A2 protein turnover, including the demonstration that hnRNP A2 and pVHL directly interact in vivo and that hnRNP A2 levels increase with proteasomal inhibition, but only in pVHL-expressing cell lines.

Consistent with this possibility, we demonstrate that C162F cells, in which the ability of pVHL to bind the elongin B/C complex is abrogated, exhibit elevated expression of hnRNP A2 relative to cells expressing wild type pVHL. The interaction of pVHL with elongin C has been shown to be necessary for conferring pVHL function as an E3 ubiquitin ligase (32, 59). In this model, the failure of pVHL to interact with components of ubiquitin degradation machinery (elongins B and C, Cul-2, and Rbx1) results in cytosolic hnRNP A2 accumulation, as hnRNP A2 cannot be targeted by pVHL to the proteasome for proteolytic degradation. Such a model would predict (and our data support) that hnRNP A2 and pVHL interact in vivo, and that this association is heightened by proteasome inhibition.

Further supporting a role for altered hnRNP A2 protein turnover is the demonstration that polysomal hnRNP L levels are affected by pVHL expression. Since hnRNP A2 and L have been shown to exist as a complex on the polysomes, these data raise the possibility that pVHL mediates proteasomal targeting of this hnRNP A2/L mRNA complex and degradation. This would explain why the effects of pVHL expression on hnRNP L levels were only consistently seen in polysomal fractions, presumably through its association with hnRNP A2 (49). In this model, only hnRNP L that is associated with hnRNP A2 is degraded by pVHL targeting to the proteasome.

The model that pVHL plays a role in targeting specific mRNA-protein complexes to the proteasome is consistent with both increased expression of hnRNP A2/L and the stability of VEGF and GLUT-1 mRNA that occurs in its absence (33, 62). For example, hnRNP L has been shown to bind the 3'-UTR of VEGF and play a role in stabilizing this mRNA (61). A similar function may exist for hnRNP L binding to GLUT-1 mRNA (49). In contrast, hnRNP A2 binding to GLUT-1 mRNA has been correlated with increased mRNA turnover in brain tumor extracts (49, 63). The demonstrated relationship between increased hnRNP A2 levels and GLUT-1 mRNA stability in pVHL-deficient RCC cells is not consistent with these data and may reflect tissue-specific differences. Such a tissue-restricted effect would be consistent with the selective tissue pathology associated with VHL disease (17, 18).

Validating this model requires demonstration that pVHL mediates hnRNP A2 protein turnover. These studies will be limited by the stability of hnRNP A2 protein, which in prelim-

FIG. 6. Proteasome inhibition results in hnRNP A2 cytoplasmic accumulation in WT-8 cells. Figure shows proteasome inhibition of pRC-9 and WT-8 cells. A, pRC-9 and WT-8 cells were treated overnight with 25 μM MG132. Cytosolic (25 μg) and nuclear (10 μg) fractions were analyzed by immunoblotting for hnRNP A1 and hnRNP A2 in parallel. B, pRC-9 and WT-8 cells were treated overnight with 100 μM LLnL. Cytosolic (25 μg) and nuclear (10 μg) fractions were analyzed by immunoblotting for hnRNP A1 and hnRNP A2 in parallel.

FIG. 7. hnRNP A2 and VHL associate in vivo. A, coimmunoprecipitation of hnRNP A2 and VHL from MG132-treated WT-8 cells. Cytosolic lysate (100 μg) derived from MG132-treated WT-8 cells was immunoprecipitated (IP, ip) with α-VHL monoclonal antibody (1G32), and then analyzed by immunoblotting with α-hnRNP A2 monoclonal antibody (EF67). Input indicates start material, while Depl refers to supernatant unbound to beads. Reciprocal experiment is indicated in right panel. B, coimmunoprecipitation of hnRNP A2 and VHL from untreated WT-8 cells. Nuclear lysate (400 μg) extracted from WT-8 cells was immunoprecipitated with 1G32, and then analyzed by immunoblotting with EF67.
inary studies has a half-life of greater than 12 h in both cell lines (data not shown). Moreover, we have been unable to
detect an increase in the Mₜ of hnRNPA2 with proteasomal
inhibition expected with ubiquitination (data not shown). The
failure to demonstrate ubiquitinated forms of hnRNPA2 does
not eliminate the possibility that proteasomal targeting of an
mRNA-hnRNPA2 complex might occur through ubiquitination
of another protein in that complex. Immunoprecipitation of the
hnRNPA2 complexes from cells that differ solely in pVHL
expression may resolve some of these issues.

Independent of the mechanism by which pVHL reduces
hnRNPA2 levels, its specificity is notable. No effect is seen on
hnRNPA1 levels, which shares identical domain organization,
nuclear import/export signals, and considerable amino acid
homology (70% overall). The N-termina RNA binding domains
of hnRNPA1 and A2 share 85% homology (50), in accordance
with their high affinity binding of polyuridine sequences (64).
Despite these similarities, hnRNPA1 and A2 exhibit disparate
association with proteasome and regulate the high ambient levels of cytoplas-
cipherly, these data suggest that the association of VHL muta-
tions with clear cell carcinomas of the kidney is due to its
critical role in regulating the high ambient levels of cytoplas-
ic hnRNPA2 in normal proximal tubular epithelium. In this
model, the absence of pVHL-elongin C interaction causes
hnRNPA2 levels to rise above a threshold level, resulting in
renal carcinogenesis. In closing, the demonstration of a specific
interaction of GLUT-1 mRNA stability.

[50x271]correlates with the subcellular distribution and function of
[50x292]renal carcinogenesis. In closing, the demonstration of a specific
[50x302]hnRNP A2 levels to rise above a threshold level, resulting in
[50x313]model, the absence of pVHL-elongin C interaction causes
[50x323]mic hnRNP A2 in normal proximal tubular epithelium. In this
[50x334]critical role in regulating the high ambient levels of cytoplas-
[50x355]tively, these data suggest that the association of VHL muta-
[50x376]of particular interest, given its association with neoplastic
[50x722]inary studies has a half-life of greater than 12 h in both cell
[50x418]arise in proximal tubular epithelial cells (6). It is intriguing
[50x460]ulation of hnRNP A2 by pVHL is associated with its RNA
[50x512]whereas the reverse was true for the granulocyte macrophage
[50x544]Despite these similarities, hnRNP A1 and A2 exhibit disparate
[50x554]with their high affinity binding of polyuridine sequences (64).
[50x565]of hnRNP A1 and A2 share 85% homology (50), in accordance
[50x670]not eliminate the possibility that proteasomal targeting of an
[50x691]inhibition expected with ubiquitination (data not shown). The

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