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Original Research

A Determination of Valosin-Containing Protein (p97/VCP) and Small VCP-Interacting Protein (SVIP) Expressions in Rat Kidney and Vero Cells

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ABSTRACT

Objective: The 97-kDa valosin-containing protein (p97/VCP), which is involved in the ubiquitin-proteasome-mediated degradation pathway, regulates a diversity of cellular activities. p97/VCP, which also physically interacts with the 26 proteasome and uses multiubiquitin chains to immediately attach the ubiquitinated substrates. Small p97/VCP-interacting protein (SVIP), was first detected to be one of numerous cofactors controlling the VCP. In rat kidney tissues and Vero cells, SVIP and p97/VCP proteins haven't been investigated. The aim of present study was to examine the expression of p97/VCP and SVIP in kidney tissues and Vero cells. Also, this study was purposed to conduce to further research on the use of two proteins as kidney cell biomarkers in cases of renal pathologies.

Methods: Hematoxylin-eosin (HE) staining was used to examine the histomorphology of rat kidney tissues. The cellular distribution and expression of SVIP and p97/VCP in rat renal tissue were investigated using the immunohistochemistry method. Also, Vero kidney cell line was used to determine cellular localization and expression of ERAD proteins by immunofluorescence and Western blotting.

Results: Immunohistochemistry analysis revealed that SVIP and p97/VCP were primarily located in the nucleus and cytoplasm of the proximal and distal tubule cells in the rat kidney. Furthermore, the immunoexpression of these proteins was discovered in the epithelial cells of the Henle loop and collecting tubules in the medulla. In the nucleus and cytoplasm of Vero kidney cells in vitro, p97/VCP immunoexpression was detected. SVIP was determined in the cytoplasm of the cells. Also, Vero cells showed intense p97/VCP expression compared to SVIP expression by Western blotting.

Conclusions: In conclusion, the current study showed that p97/VCP and SVIP had cellular localization and protein expression in the rat kidney tissue and kidney cell line (Vero). We might advise that additional research is required to ascertain the role of SVIP and p97/VCP in renal tissue.

Keywords: endoplasmic reticulum-associated protein degradation (ERAD), kidney, p97/valosin containing protein (VCP), rat, small VCP-interacting protein (SVIP), vero cells

INTRODUCTION

p97, an evolutionarily protected ATPase found in all eukaryotes, is also called valosin-containing protein (VCP) in mammals [1]. The ATPase family of enzymes assists in the folding or unfolding of proteins in all organisms, including bacteria and humans. Due to the tandem arrangement of two AAA ATPase domains (D1 and D2), p97/Cdc48p is a type II AAA+ ATPase [2].

In eukaryotic cells, proteins are degraded via two main routes. The ubiquitin-proteasome degradation pathway is one of them, while lysosomal proteolysis occurs in the lysosome's acidic environment. A protein is designated for destruction when ubiquitin attaches to its amino group on the lysine side chains. The recognition protein, ubiquitin-binding enzyme, and ubiquitin-activating enzyme work together to attach ubiquitin to the protein that will be destroyed (ubiquitination) [3-5]. The p97/VCP chaperone protein facilitates the carrying of polyubiquitinated proteins to proteosomes. A study demonstrated that p97/VCP physically connects to both the polyubiquitin and proteasome substrates as well as the polyubiquitinated chains [6].

Protein metabolism and intracellular hemostasis depend on p97/VCP. It encourages the displacement of target proteins as well as their refolding, recycling, and destruction [7,8]. By binding several cofactors and adapter proteins, p97/VCP is essential for a wide range of cellular functions. These include the Golgi assembly, chromatin-associated degradation, endosomal trafficking, cell death (autophagy and apoptosis), cell cycle, and lipid droplet biogenesis. Protein quality control processes also include ER-associated degradation (ERAD), mitochondrial, ribosome-associated, and proteasomal degradation [9-11].

26S proteasome-mediated destruction of misfolded proteins, polyubiquitination, and retrotranslocation into the cytosol are all steps in the multi-component and intricate ERAD mechanism [12,13]. The primary protein involved in the retrotranslocation of ERAD substrates through the ER membrane and into the cytosol is p97/VCP. It engages in mitophagy and autophagy in addition to ERAD [14,15]. The functional partners of p97/VCP have been found, and three of them, the VCP-interacting membrane protein (VIMP), E3 ubiquitin ligase gp78, and small VCP/p97-interacting protein (SVIP), have the VCP-interacting motif (VIM) in their structure [16,17].

In the studies, p97/VCP has been associated with several kidney diseases.In a study indicated new insights into the regulatory mechanisms of hepatocyte nuclear factor 1-beta (HNF1-β) in lupus nephritis (LN) emphasizing its effect on the Derlin-1/VCP/VIMP complex, podocyte apoptosis, and ER stress [18]. Endoplasmic reticulum stress (ERS) plays a significant function in podocyte injury of membranous nephropathy (MN), and it is intervened by the HSPa5-VCP signaling pathway. Also, VCP was identified as a requisite regulator of podocyte ER stress [19]. The 9-kDa protein of 76 amino acids, called SVIP, participates in the ERAD pathway [20]. SVIP functions as an endogenous inhibitor of the ERAD pathway and is an interacting cofactor which controls p97/VCP [17]. Furthermore, the autophagic route has been linked to SVIP, which promotes the localization of p97/VCP to the lysosomal membranes and plasma [21,22].

Main Points

- Studies have shown that ERAD plays a crucial role to avoid cellular damage upon ER stress in a variety of renal involvements.
- The immunostaining of p97/VCP was detected in the cortex and the medulla of the kidney. In detail, cellular localization of p97/VCP protein was seen in the nucleus and cytoplasm of the proximal and distal tubule cells, particularly in the cortex. Furthermore, the positive staining of p97/VCP protein was detected in the loop of Henle. Our results showed that the cortex and the medulla of the kidney were stained with SVIP. Particularly renal tubules that include proximal and distal tubule cells have shown positive staining for SVIP. In the medulla, the epithelial lining cells of the Henle loop were stained with SVIP.
- In the present study, we showed the presence of SVIP and p97/VCP proteins in the Vero kidney cell line by immunofluorescence and western blotting. Furthermore, Vero cells showed intense p97/VCP protein expression compared to SVIP protein expression.
- Results suggest that our study can contribute to further studies on using of these proteins as renal cell biomarkers in renal pathologies. We can suggest that further studies are needed for the determination of the function of the p97/VCP and SVIP in the kidney and molecular mechanisms of renal tissue.

Studies have shown that ERAD plays a crucial role to avoid cellular damage upon ER stress in a variety of renal involvements [23-26].

There are few researches on the functions and expression of SVIP and p97/VCP in kidney tissue. As far as our knowledge, we have not encountered researches in the data in which the expressions of SVIP and p97/VCP proteins were defined by immunohistochemical analysis in rat renal tissues. Therefore, we aimed to examine the cellular localization and immunoexpression of SVIP and p97/VCP in rat kidneys. In addition, expression differences in the cortex and the medulla of kidney were evaluated. Furthermore, we were examined the expression of p97/VCP and SVIP two ERAD pathway proteins on Vero kidney cell line (a normal, adult African green monkey kidney cell line). Since the proteins showed immunostaining in kidney tissue, their in vitro expression was further evaluated using Vero cells. Vero cells were selected as a well-established epithelial cell model suitable for studying protein expression and cellular mechanisms. Moreover, the target proteins are highly conserved across species, supporting the suitability of this model for in vitro analysis.

MATERIALS AND METHODS

Animals

The permission for this experimental study was received from the "Animal Ethics Committee" of Nesa Experimental Animals Reproduction Laboratory (Decision No: 130, Date: 2025-09-22). 30 male Wistar albino rats weighed 200–220 grams were provided by the Nesa Experimental Animals Reproduction Laboratory. The number of animals was determined based on the need to ensure reproducible immunohistochemical localization across samples, considering potential biological and tissue variability. Wistar albino rats were housed under an ambient temperature of 22–25 °C and with dark and light cycles of 12 and 12 h. The Wistar albino rats were anesthetized with xylazine hydrochloride (10 mg/kg) and ketamine hydrochloride (50 mg/kg). Then, the kidney tissues were removed and fixed.

Paraffin Embedding

The kidney tissue samples were fixed in 10% formalin (Merck) solution for 24 hours, then rinsed in running tap water for 3 hours. After, samples were dehydrated in escalating series of ethanol (Merck), cleared in xylene (Merck), and embedded in paraffin (Patolab, Germany) for histological and immunohistochemical analysis. The paraffin blocks were cut at 4-5 μm thickness by a microtome (Thermo Fisher Scientific, USA).

Histological Analysis

Hematoxylin and Eosin (H&E) (Gill's hematoxylin, Merck-Alcoholic eosin, Bio-Optica) staining of the sections was applied to interpret the histological structure of the kidney tissue. In brief, the slides were incubated at 58 °C for 2 hour for H&E staining. Then, the slides were incubated with xylene for 40 min and after rehydrated in the decreasing series of ethanol for 3 min each time. Following rinsing the slides, we stained them with hematoxylin for 5 min followed by eosin for 1 min. After washing slides with water, sections were dehydrated in increasing ethanol series. Then, sections were cleared in xylene for 30 min and mounted with entellan (Millipore Sigma, USA). The H&E stained samples were analysed with a light microscope (Olympus BX43, Japan) and captured on camera (Olympus DP26, Japan).

Immunohistochemistry and Hscore Analysis

We performed immunohistochemical staining to determine the expression of the SVIP, and p97/VCP proteins in the kidney tissue. The serial sections cut from the paraffin-embedded blocks were incubated at 58 °C for 1 hour and, deparaffinized in xylene. Then the slides were rehydrated in a graded series of ethanol before being microwaved (LG wave MS20421) twice for 5 min each to dissolve them in 10 mM citrate buffer (Merck). Endogenous peroxidase activity was blocked with 3% hydrogen peroxide after three 5-min washings with phosphate-buffered saline (PBS). To prevent an unintended reaction, the sections were blocked with a blocking serum (Thermo Scientific TP-125-HL, UK, Ultra V Block) for 20 min after being rinsed with PBS. After incubation, the block solution was withdrawn and two primary antibodies p97/VCP (1:600, ab11433, Abcam), and SVIP (1:60, HPA039807, Sigma) were incubated overnight at 4 °C. After that, the slides were rinsed for 20 min in PBS. After being treated with Biotinylated goat anti-rabbit antibody (Thermo Scientific TP-125-HL), and were applied in slides for 25 min. Streptavidin peroxidase (Thermo Scientific TP-125-HL) was incubated for 25 min. Then slides were rinsed with PBS. After being treated with DAB chromogen and counterstained with Mayer's hematoxylin (Merck), covered with entellan (Merck, Germany). The sections were analyzed histologically with a light microscope (Olympus BX43, Japan). The slides were photographed using a digital imaging system (Olympus DP26, Japan) and evaluated with a computerized system (Olympus DP21, Japan). The intensities of p97/VCP and SVIP were

evaluated using H-SCORE analyses, according to an already established method (27). The intensities of p97/VCP and SVIP immunoreactivities were semiquantitatively analyzed using the intensity categories. In each slide, 5 randomly selected areas were assessed under a light microscope (40x objective), and the percentage of the cells for each intensity within these areas was detected at different times by two investigators who were not informed about the source and type of the tissues. The average score of both observers was used.

Cell Culture

Vero cell line (ATCC, CCL-81) were grown in Dulbecco Minimal Essential Medium (DMEM; Capricorn Scientific) supplemented with 10% fetal bovine serum (FBS; Gibco), 1% antibiotics (streptomycin/penicillin; Gibco). Vero cells were incubated at 37°C with 5% CO_2. For immunofluorescence, the Vero cells were fixed in 4% formaldehyde. Additionally, for the Western blot method, the Vero cells were lysed. Each transfection was carried out at least three duplicates.

Immunofluorescence

The Vero cell line were seeded in 6-well plates on glass coverslips (NEST Scientific). Then, the cells were fixed in 4% formaldehyde for 30 min. Following rinsing with PBS, the coverslips were treated in a blocking solution (Thermo Scientific TP-125-HL, UK, Ultra V Block). Then the cells were treated with the indicated primary antibodies SVIP (1:60, HPA039807, Sigma) and p97/VCP (1:600, ab11433, Abcam) overnight at 4 °C. Following rinsing with PBS, the samples were then treated with a FITC-conjugated antirabbit secondary antibody (1:700, ab6717, Abcam) and Texas red-conjugated goat antimouse secondary antibody (1:700 dilution, ab6787, Abcam) for one hour. DAPI containing mounting medium (Abcam) was used to mount the samples. The intracellular localization of the p97/VCP and SVIP proteins was analyzed using a fluorescent microscope (Olympus BX53).

Western Blotting

The Vero cell line was prepared for Western blot technique. The cell pellets were resuspended with protease inhibitor containing RIPA buffer. Protein concentration was detected performing the standard Bradford assay. After boiling in the sample buffer, 30 **µg** of total cellular protein was loaded on a 4–12 % Bis-Tris gel (Invitrogen). The semi-dry transfer was performed onto a nitrocellulose membrane (Scie-Plas, UK). 3% nonfat dry milk in PBS was performed. Detection of SVIP and p97/VCP was applied employing primary antibodies: SVIP (1:750, HPA039807, Sigma), p97/VCP (1:1500, ab11433, Abcam, UK), and β-actin (1:2000, ab6276, Abcam) applied overnight. After incubation, PBS-Tween was performed to wash the membrane. After, the secondary antibodies were performed. Immunodetection was determined applying a chemiluminescence kit (ab133406, Abcam, UK). Using the gel documentation system (UVP), the Western blot bands were displayed.

Statistical Analysis

The western blot bands and the band densities were analysed using IMAGEJ Version 1.46. All data are indicated as the mean ± standard error (SEM) of three independent experiments.

RESULTS

Histomorphological Structure

The renal tissue samples of rats stained with H&E are displayed in Figure 1. The renal sections showed a healthy histological structure with tubular cells and a glomerular structure. There was not any congestion, tubular cell damage, or necrosis in any samples. The cortex, medulla, and calyces of the renal tissues were in normal histological architecture. The glomerulus, a cluster of capillaries encircled by a Bowman's capsule made of two layers of epithelial tissue, makes up the renal corpuscle. The proximal and distal tubules with simple cuboidal epithelium were shown in Figure 1. The proximal convoluted tubule cells' cuboidal cells feature a brush border. a brush border made up of microvilli that are straight, compact, and somewhat lengthy. The Henle loop in normal histological structure was seen in the medulla of the kidney.

Immunohistochemical Localization of P97/Vcp and Svip in Rat Kidney

There are currently no details available regarding the renal site of p97/VCP. Because of this, we examined the slide of rat kidneys with immunohistochemistry. The cortex and medulla of rat renal tissue were immunostained with p97/VCP. Particularly in the cortex, p97/VCP was mostly found in the nucleus and cytoplasm of the proximal and distal tubule cells in the rat kidney, according to an immunohistochemical investigation. In the medulla, it was seen in the epithelial cells of the collecting tubules and the Henle loop (Figure 2).

The cortex and medulla of the rat kidney included immunostaining for SVIP. The proximal and distal tubule cells exhibit strong SVIP staining in the cortex. In the medulla, it was

clearly seen that the cellular localization of SVIP was in the loop of Henle (Figure 3).

Accordingly, the H-Score results demonstrated that the expression of p97/VCP protein is higher compared to the SVIP protein in rat kidney tissues. (Figure 4A).

Expressions of p97/VCP and SVIP in Vero Cells

The likely location of SVIP and p97/VCP in the Vero kidney cell line is unclear now. The existence of p97/VCP and SVIP proteins in the Vero kidney cell line was determined by immunofluorescence and western blotting.

The protein expressions of p97/VCP and SVIP have been determined in Vero cells by the Western blot technique (Figure 4B). Vero cells showed intense p97/VCP protein expression compared to SVIP protein expression (Figure 4C).

Furthermore, to verify the presence of p97/VCP and SVIP in kidney cells, immunofluorescence was applied in addition to the Western blotting method. We identified the immunoexpression and cellular localization of SVIP and p97/VCP in Vero cells. p97/VCP was detected in the cytoplasm and nucleus of Vero kidney cells. SVIP was also determined in the cytoplasm of Vero cells (Figure 5).

DISCUSSION

In the present study, we examined the p97/VCP and SVIP protein expressions in rat kidney and Vero cells. We demonstrated for the first time the cellular localization and immunoexpression of SVIP protein in rat kidney and Vero cells. The findings are limited to normal kidney tissue conditions, and no disease models or physiological stress conditions were examined. Future studies including functional tests and pathological models may help to better define the biological significance of these proteins in kidney physiology.

p97/VCP, also known as CDC48, was first identified by Moir et al. in 1982 [28]. It has a crucial function in arranging the concentrations of cellular proteins. Cell transformation, signal transduction, and cell cycle progression are just a few examples of how it is engaged in crucial cellular activities [29]. By means of a network of protein quality control mechanisms, p97/Cdc48p maintains protein homeostasis [7]. ERAD, a route that breaks

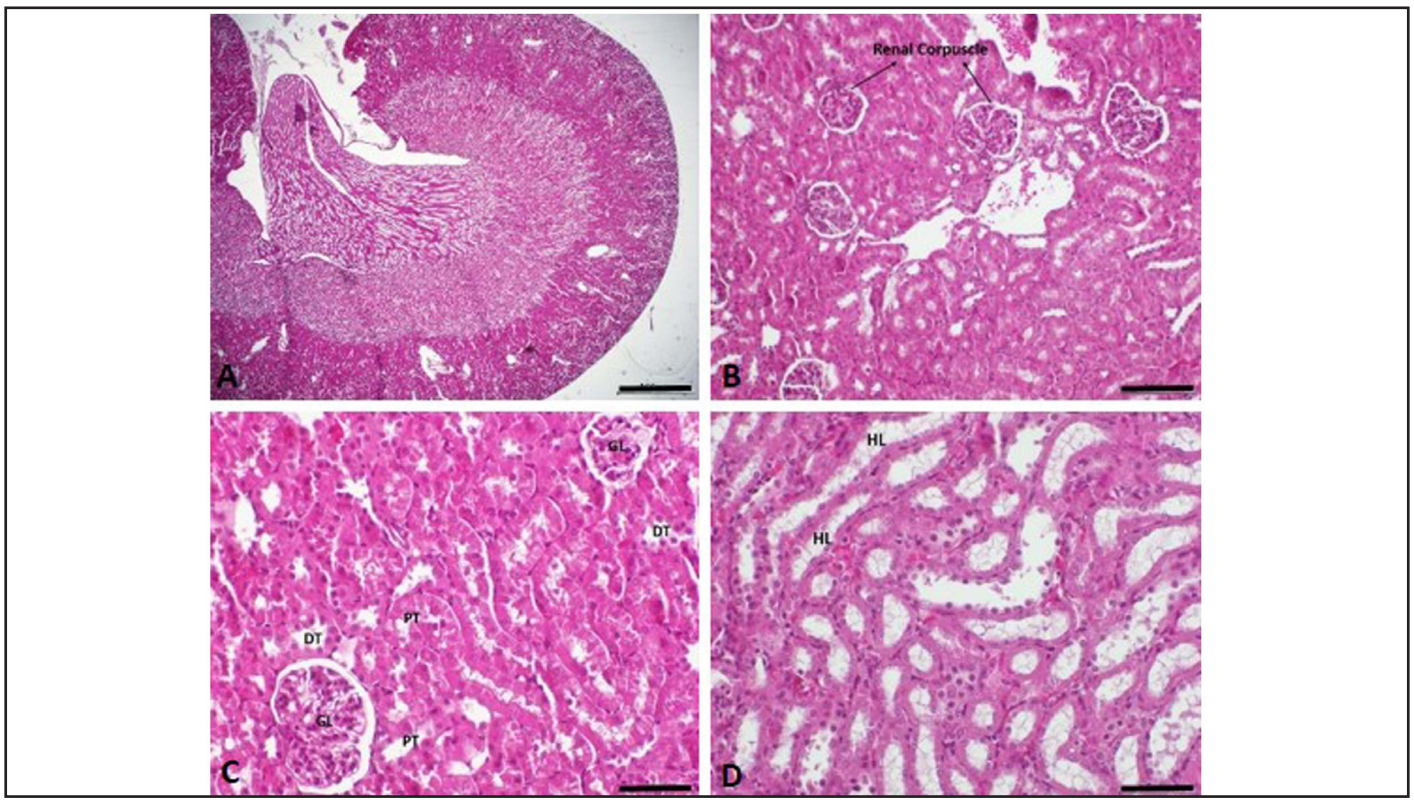

Figure 1. Histomorphological images of the rat kidney tissue sections. (Pt, Proximal tubule; Dt, Distal tubule; Gl, Glomerulus; HL, Loop of Henle). Hematoxylin and Eosin staining. Scale bars= 2, 200, 100, 100 µm (A-D).

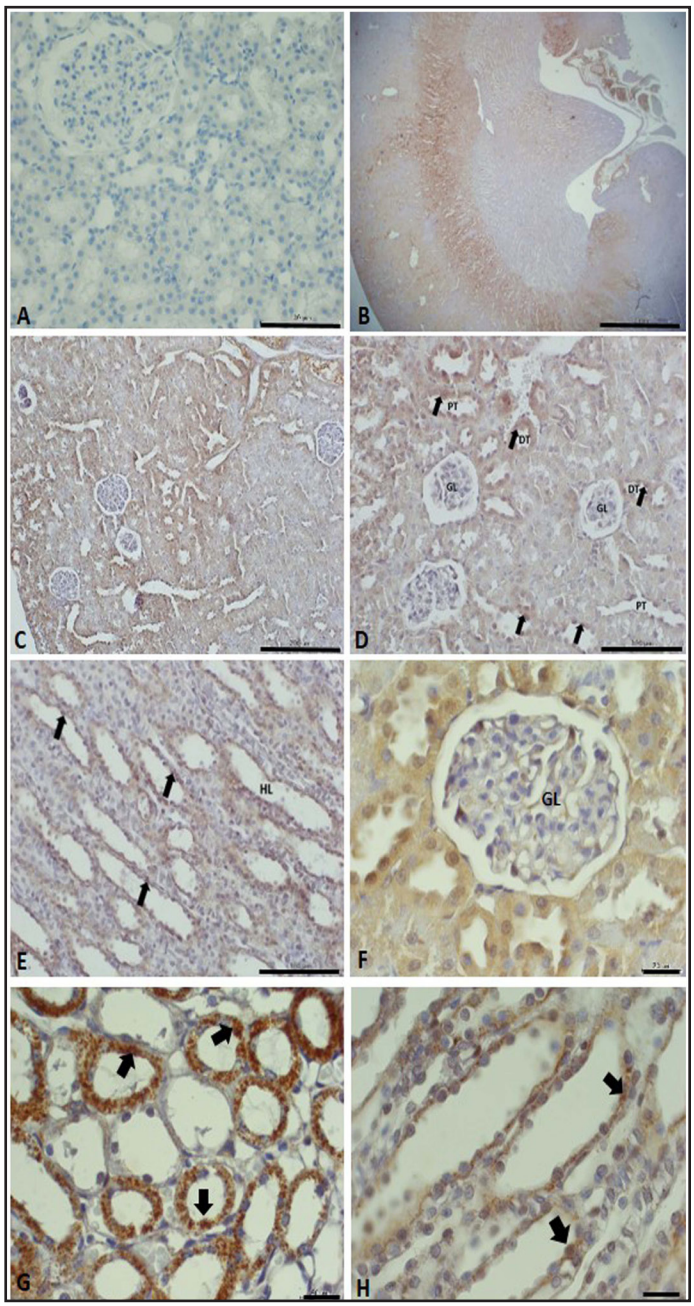

Figure 2. Immunohistochemical expressions of p97/VCP in the rat kidney. Particularly in the cortex, p97/VCP was mostly found in the nucleus and cytoplasm of the proximal and distal tubule cells in the rat kidney, according to an immunohistochemical investigation. In the medulla, it was seen in the epithelial cells of the collecting tubules and the Henle loop. (Pt, Proximal tubule; Dt, Distal tubule; Gl, Glomerulus; HL, Loop of Henle). A) Negative control. Scale bar= 100 µm. B) The histology of kidney. Scale bar= 2 µm. C-D) The histology of cortex. Scale bars= 200,100 µm. E) The histology of medulla. Scale bar= 100 µm. F) The histology of cortex. Scale bar= 20 µm. G-H) The histology of medulla. Scale bars= 20 µm.

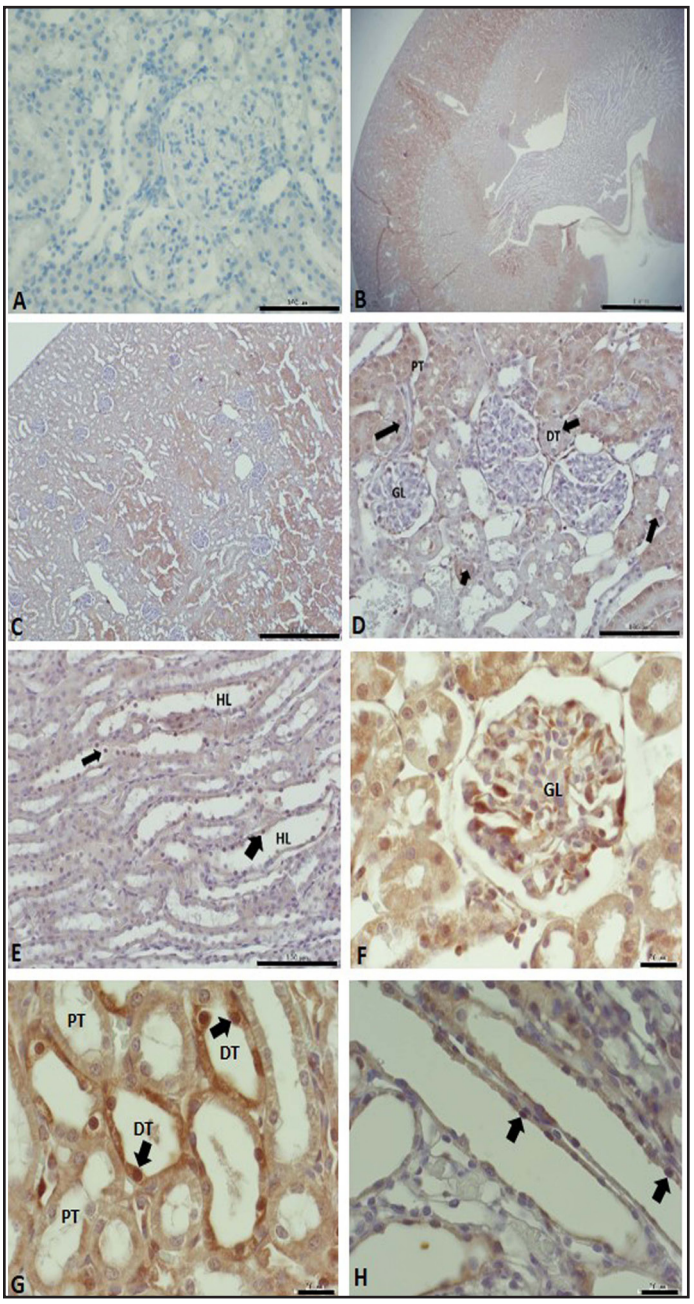

Figure 3. Immunohistochemical expressions of SVIP in the rat kidney. The proximal and distal tubule cells exhibit strong SVIP staining in the cortex. In the medulla, it was clearly seen that the cellular localization of SVIP was in the loop of Henle. (Pt, Proximal tubule; Dt, Distal tubule; Gl, Glomerulus; HL, Loop of Henle). A) Negative control. Scale bar= 100 µm. B) The histology of kidney. Scale bar= 1 µm. C-D) The histology of cortex. Scale bars= 200,100 µm. E) The histology of medulla. Scale bar= 100 µm. F) The histology of cortex. Scale bar= 20 µm. G) The histology of cortex. Scale bar= 200 µm. H) The histology of medulla. Scale bars= 20 µm.

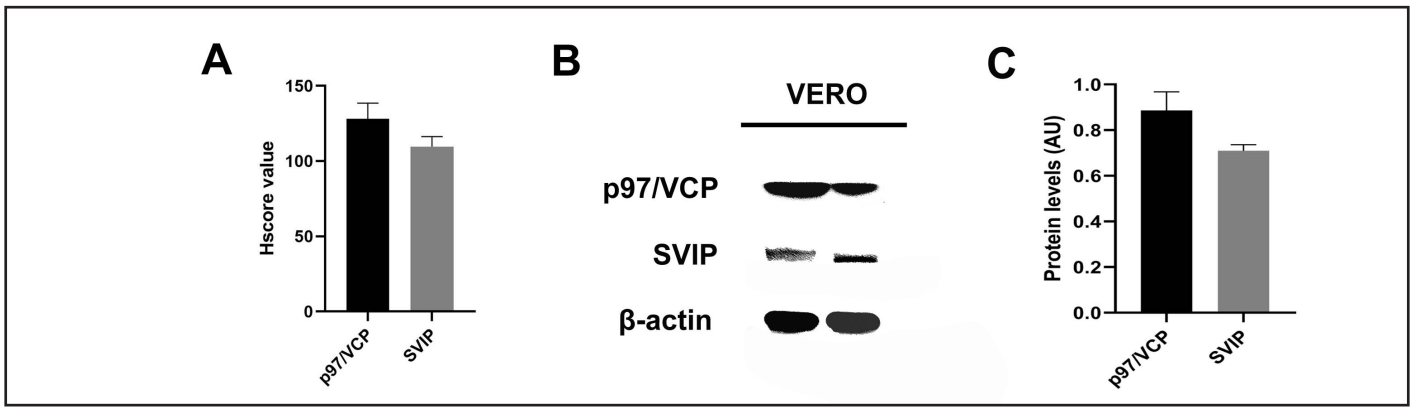

Figure 4. H-Score values of p97/VCP and SVIP immunostaining intensities in the rat kidney. **A.** Western blot analysis of the p97/VCP and SVIP proteins in Vero cells. **B.** The expression of p97/VCP and SVIP proteins, using β-actin as loading control, was assessed in Vero cells **C.** The data are represented as mean ± SEM.

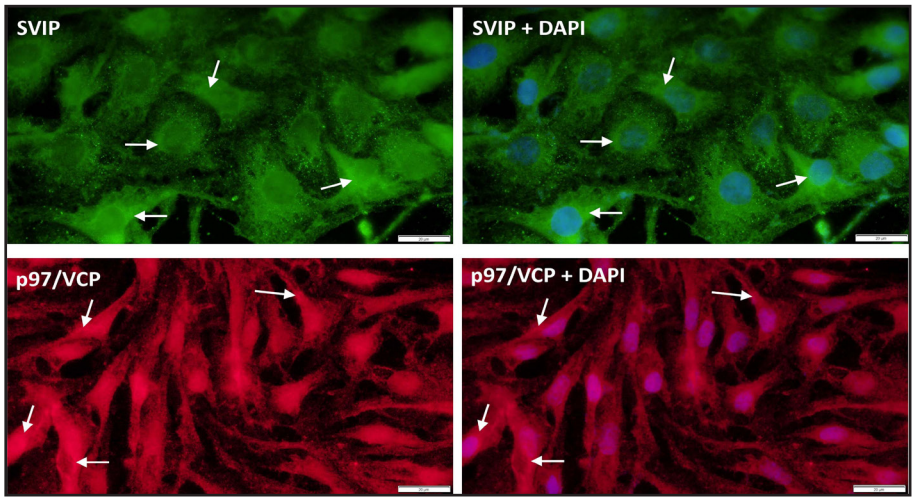

Figure 5. Immunofluorescence staining of p97/VCP and SVIP proteins. Localization of the p97/VCP (arrow) was determined in the cytoplasm and nucleus in Vero cells. Also, localization of the SVIP (arrow) was detected in the cytoplasm of Vero cells. Scale bars: 20 μm.

down misfolded ER proteins, is the process that has received the most attention in this setting [30-32]. When misfolded proteins emerge through a hypothesized retrotranslocation pathway, membrane-associated p97 catches them [33]. VCP/p97 separates ubiquitylated proteins from binding proteins or removes them from membranes or cellular structures.Importantly, the level of VCP/p97 need fluctuates and may be influenced by the location, structure, or solubility of the substrate [34,35].

VCP/p97 plays a key role in maintaining protein homeostasis by facilitating co-translational degradation at the ribosome as well as proteasomal degradation of big cohorts of misfolded or damaged proteins in various compartments, such as ERAD, the outer mitochondrial membrane, and the nucleus. In addition to performing quality control, VCP/p97 controls important signaling pathways [7,36]. The role of VCP/p97 in chromatin-associated processes that maintain genomic integrity and cell cycle progression. The number of substrates that are arranged in this way is expanding [7, 37]. VCP/p97 has been connected to several membrane trafficking procedures, such as the reassembly of the Golgi after mitosis [38]. More recently, it has been demonstrated to regulate the synthesis of lipid droplets [39]. It has been identified in human skeletal muscle, ovary, breast, liver, heart, lung, and other organs [40].

A member of the AAA family, p97/VCP, serves as a chaperone that participates in the formation, breakdown, and proper functioning of protein complexes. The p97/VCP localization in

the sperm head, midpiece, and tail has been demonstrated in the literature [27]. The location and colocalization of ubiquitin and p97/VCP in the developing rat retina have been demonstrated in another investigation in the literature [41]. Studies in the literature have shown that p97/VCP expression varies at different stages of spermatogenic development and is present in mouse gonad embryos [42].

For p97 functions, the adrenal gland and kidney have significant roles. In a study, p97 downregulation on chromosome 9 was indicated to be impacted by protein tyrosine phosphatase receptor mutation on chromosome 9. A downregulation of p97/VCP has to be involved in a broad diversity of cellular inactivity. VCP downregulation, which had resulted in a broad range of cell inactivity, was judged to be induced by hypoxic stress. Nephrectomy together with adrenalectomy strengthened the clinical manifestations of p97 downregulation [43]. An experimental study indicated that endoplasmic reticulum stress plays a significant function in podocyte injury of membranous nephropathy and is intervened by an HSPa5-VCP signaling pathway, in that the most predominant proteins are those connected to catalytic activity and cellular metabolism [19].

In this investigation, we examined the expression of the proteins p97/VCP rat renal tissue. Regarding the renal location of p97/VCP, there are no details at this time. The immunostaining of p97/VCP was determined in the cortex and the medulla of the kidney. In detail, cellular localization of p97/VCP protein was seen in the nucleus and cytoplasm of the proximal and distal tubule cells, particularly in the cortex. Furthermore, the positive staining of p97/VCP protein was determined in the loop of Henle.Therefore, p97/VCP is important protein in this area, which aligns with the necessity for cellular quality control in the highly specialized and stressed environment of the medullary loop of Henle.

A protein called SVIP, which is also implicated in ERAD, regulates the actions of the ATPase p97/VCP by binding to it. The first study to demonstrate that SVIP is a protein which connects to and interacts with p97/VCP was conducted by Nagahama et al. SVIP controls the suppression of ERAD and takes a role in autophagy and vacuole formation [44]. An ERAD-associated protein is p97/VCP. The adapter ER protein SVIP, which straightly binds to p97/VCP, is found in close proximity to the ER membrane surface. p97/VCP is inhibited by SVIP via interaction [17,45]. According to reports, androgens control ERAD and p97/VCP connects to androgen receptors [46]. Additionally, it has been discovered to be an androgen-responsive gene in SVIP, and androgens have been reported as controlling its expression [45]. In prostate cancer cells, it has been demonstrated that androgen-mediated downregulation of SVIP expression occurs [46].

A multifunctional protein known as SVIP, the first endogenous ERAD inhibitor, has been connected to VLDL trafficking, and secretion, lysosomal dynamic stability, and autophagy [21,47]. Recent research indicates that SVIP is continually expressed at a high level in the adrenal gland throughout postnatal development, indicating that SVIP might work along the adrenal gland's developmental duration [48]. According to a study published in the literature, SVIP is mainly expressed by adult and fetal Leydig cells. The experimental study examined the immunoexpression of SVIP in fetal testis. Additionally, using immunofluorescence, specific peritubular and Sertoli cells were found to express SVIP [20].

In our previous study, we showed the autophagic and UPS (Ubiquitin, SVIP, p97/VCP) protein expressions in MetS rat pancreas [49]. In the current study, we examined the immunoexpression and cellular localization of SVIP in rat kidneys. Our results showed that the cortex and the medulla of the kidney were stained with SVIP. Particularly renal tubules that include proximal and distal tubule cells have shown positive staining for SVIP. In the medulla, the epithelial lining cells of the Henle loop were stained with SVIP. Since proximal tubule cells are key sensors and initiators of injury in both acute and chronic kidney disease, SVIP may act as a crucial mediator in mitigating injury by modulating autophagy (a protective mechanism), ERAD, and preventing the fibrotic progression that leads to chronic kidney disease. Also, when SVIP malfunctions in the renal cortex, the accumulation of misfolded proteins may trigger cellular stress, disrupting transport mechanisms in the distal tubule.

In an experimental study, they investigated ER motility in VERO cell line and indicated that ER tubules move in both directions along microtubules by using cytoplasmic dynein and kinesin-1 [50]. In the current study, we demonstrated the presence of SVIP and p97/VCP proteins in the Vero kidney cell line by immunofluorescence and western blotting. Furthermore, Vero cells showed intense p97/VCP protein expression compared to SVIP protein expression.

Limitations

This study assessed kidney structure and evaluated its expressions for p97/VCP and SVIP immunohistochemically, immunocytochemically and with western blotting. The findings are limited to normal kidney tissue conditions, and no disease models or physiological stress conditions were examined. Future studies including functional tests and pathological models may help to better define the biological significance of these proteins in kidney physiology. Animal experiments were performed using only male rats. Therefore, possible sex-dependent biological differences in protein expression or localization were not addressed and may be considered in future studies. Finally, cell culture experiments were performed using a kidney epithelial cell line derived from African green monkeys. While this cell line is commonly used in kidney-related research, species differences should be considered when generalizing the results to rat kidney tissue.

CONCLUSIONS

In summary, the cellular localization and the protein expression of SVIP and p97/VCP were demonstrated in the current study. In recent studies, p97/VCP has been associated with several kidney diseases. We believe that present study can contribute to further studies on using of these proteins as renal cell biomarkers in renal pathologies. We can suggest that further studies are needed for the detection of the role of the small VCP-interacting protein (SVIP) and p97/VCP in the kidney and molecular mechanisms of renal tissue.

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Conflict of Interest: The authors declare no conflict of interest.

Informed Consent: Data sets of this study are available from the corresponding author upon reasonable request.

Data Availability Statement: Data will be made available on request.

Ethics Committee Statement: All animal experimental procedures were approved by the Nesa Experimental Animals Reproduction Laboratory Animal Ethics Committee (Approval No: 130, Date: 2025-09-22).

Author Contributions: Conception: EA; Design: EA; Supervision: BK; Fundings: EA; Materials: IC, MK; Data Collection and/or Processing: EA, IC, MK; Analysis and/or Interpretation: EA, IC; Literature: US; Review: US; Writing: EA, BK; Critical Review: BK.

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