The Role of SETD2 and VHL in Promoting Renal Fibrosis

Yangtian Yan
Dulwich Zhuhai International High School, China

Keywords: Renal Fibrosis, SETD2, VHL, Inflammation-Related Genes, Tumour Suppressor Genes, Knockout.

Abstract: An excess accumulation of extracellular matrix is involved in renal fibrosis, which usually leads to a loss of function when scar tissues replace normal tissues. This process is stimulated by multiple different pathogenic factors that trigger the cascades of reparation converging in molecular signals responsible for initiating and driving fibrosis. SETD2 and VHL are both tumour suppressor genes, the former acts as an epigenetic modifier that is responsible for trimethylation of H3K36, and the latter works by controlling extracellular matrix formation, apoptosis response, and the epithelial-to-mesenchymal transition. Whether SETD2 and VHL play roles in promoting the occurrence of renal fibrosis is still unknown. Therefore, by creating the mouse model with the specific knockout of VHL and SETD2 genes, our experiment analyses their effects on the kidney tissue. The result indicates that there is an up-regulation for the expression level of inflammation-related genes in the mouse kidney model with both VHL and SETD2 knocked out other than with VHL-KO only, in combination with the HE, Masson, and Sirius Red staining images we performed, we can get a conclusion that these two genes, SETD2 and VHL, can trigger the occurrence of renal fibrosis. These results can provide a solid theoretical basis for the molecular mechanism of action and prospects of relevant drug screening and clinical targeted therapy.

1 INTRODUCTION

Renal fibrosis is the final manifestation of chronic kidney disease (CKD), which can be characterized by tubulointerstitial fibrosis and glomerulosclerosis (Cho, 2010). Even though a range of diseases related to kidney such as glomerulonephritis, diabetes mellitus, atherosclerosis and even polycystic kidney disease, can be the main factors causing CKD, renal fibrosis is always the common final result of CKD (Cho, 2010). It appears to be a harmful process leading to renal function deterioration inevitably, independently of the previous renal diseases which cause the original symptoms. Chronic Kidney Disease and Renal Fibrosis affect 10% of the world's population, and a significant proportion of people progress to end-stage renal failure, requiring lifelong dialysis and kidney transplants, placing a huge financial burden on patients, families, and societies. An excessive accumulation and deposition of extracellular matrix (ECM) components are the main characteristics of renal fibrosis that occur in almost every type of CKD (Liu, 2006). It is worth knowing that among many different fibrogenic factors that regulate the process of renal fibrosis, transforming growth factor-β (TGF-β) is the one that plays a central role. The epithelial to mesenchymal transition (EMT) of tubular epithelial cells means they are transformed into mesenchymal fibroblasts migrating to adjacent interstitial parenchyma, along with local and circulating cells constitute the principal mechanism of renal fibrosis (Humphreys, 2018). For the occurrence of renal fibrosis, although many in vitro studies emphasize the significance of one specific cellular event such as the activation of fibroblast, it should be kept in mind that no single type of isolated cell has the ability of initiating and sustaining an entire scale of renal fibrosis (Liu, 2006). Many experimental studies have been carried out to explain the specific pathway of renal fibrosis and some significant progress has already been made in the understanding of the cellular and molecular mechanisms of renal fibrosis.

VHL is an important tumour suppressor gene, and its mutation can promote the occurrence of renal cancer. A person with VHL has nearly a 100% chance of developing one or more VHL tumours in their lifetime (Von Hippel-Lindau, 2022). Early inactivation of VHL is commonly seen in clear-cell renal cell carcinoma (ccRCC), and insights gained

Yan, Y.
The Role of SETD2 and VHL in Promoting Renal Fibrosis.
DOI: 10.5220/0012021400003633
In *Proceedings of the 4th International Conference on Biotechnology and Biomedicine (ICBB 2022)*, pages 386-391
ISBN: 978-989-758-637-8

from the functional analysis of pVHL have provided the foundation for the routine treatment of advanced-stage ccRCC with novel targeted therapies (Gossage, 2015). It is shown that VHL can influence the content of the extracellular matrix, a proper extracellular matrix cannot be organized in cells lacking VHL. This in conjunction with enhanced production of VEGF-A favours angiogenesis and tumourigenesis (Patard, 2009). pVHL can also bind with transcription factors, which will lead to a decrease in the stability of certain mRNAs and inhibit the transcription of genes such as VEGF-A (Pal, 1997). The role of VHL in renal fibrosis is undiscovered, but the variation of VHL and the mechanism of renal fibrosis have some similarities, such as the effect on extracellular matrix.

Set domain containing 2 (SETD2) Set domain containing 2 (SETD2) is a histone modifier that is generally known as the single human gene responsible for trimethylation of lysine 36 of histone H3 (H3K36). H3K36me3 readers recruit protein complexes to carry out specific processes, such as transcription elongation, RNA processing, and DNA repair, to determine the impact of this histone modification. Histone H3K36 trimethylation is a highly conserved chromatin mark related to transcriptional elongation, and it accumulates mainly across the body of genes that are actively transcribed. Cells tend to become significantly vulnerable and sensitive to DNA-damaging agents after loss of the H3K36me3 mark through SETD2 mutation or loss (Li, 2016). SETD2 mutation can promote the occurrence of ccRCC, its mutation rate in clinical patients is as high as 12%, ranking third, which can lead to structural abnormalities in renal tubules. A series of researches have revealed that SETD2 is mutated or its function is lost in a range of solid cancers (Hu, 2020), lung cancer, gastrointestinal cancer, renal cancer, pancreatic cancer, osteosarcoma, and so on. Mutation, or functional loss, of the SETD2 gene produces dysfunction in corresponding tissue proteins so that a series of adverse functions will be leaded (Molenaar, 2022).

Now, little is known about the roles of SETD2 or VHL in renal fibrosis, so by analyzing the mouse renal tubular tissue with the specific knockout of VHL and SETD2 genes, the specific possible molecular mechanism of action can be elucidated, which provides a solid theoretical basis and development prospect for corresponding drug screening and clinical targeted therapy.

2 METHODS

2.1 Mice Preparation

Setd2fl/fl mice were generated as described [ref]. The Ksp-Cre mice (B6.CgTg (Cdh16-cre) 91Igr/J) and VHLfl/fl mice were purchased from The Jackson Laboratory.

Setd2fl/fl mice were mated with Ksp-Cre mice to generate Ksp-Cre; Setd2flox/flox (Setd2–KO) mice in C57BL/6 background. SETD2–KO mice were mated with VHLfl/fl mice to generate Ksp-Cre; VHLfl/fl&Setd2fl/fl mice (Setd2-VHL-KO) housing under the same condition.

2.2 DNA Extraction from Mouse Tail for Genotyping

Cutting the tip of the mouse tail and place it into an Eppendorf tube and then add 100ul to 150ul reagent A depending on the size of your tail sample. Boil the sample at 95 to 98°C for 1 hour or so till the tail is “melted”. Cool down to room temperature. Adding equal volume of reagent B and then mixing well. Centrifuging the Eppendorf tube at full speed for 5 minutes. Finally, take 1ul to 2ul supernatant for PCR.

2.3 Buffer Recipe

Reagent A: 25Mm NaOH/0.2Mm EDTA

Note: 5x stock solution which can be kept at room temp

Reagen B: 40Mm Tris HCl (pH 5.5)

2.4 RNA Isolation and Quantitative Qrt-PCR

Total RNA was isolated from fresh kidney samples. cDNA (complementary DNA) was made using the Prime Script RT reagent kit and subjected to quantitative RT-PCR. Calculating the relative abundance of mRNA by normalization to actin-beta or GAPDH mRNA.

The primer sequences are as follows: Il1rap-Forward: 5’-TGCCTGGGGGAATTGTCAC-3’, Il1rap-Reverse: 5’-CTTAGCCCGCTTCAGCTCTTT-3’; Il18r1-Forward: 5’-TCACCGATCACAAATTCATGTGG-3’, Il18r1-Reverse: 5’-TGGTGGCTGTTTCATTCCTGT-3’; Il7r-Forward: 5’-GCGGACGATCACTCCTTCTG-3’, Il7r-Reverse: 5’-AGCCCCACATATTTGAAATTCCA-3’; Il1r1-Forward: 5’-GTGCTACTGGGGCTCATTTGT-3’, Il1r1-

Reverse: 5'-GTGCTACTGGGGCTCATTTGT-3' (Fig.2).

2.5 Histology and IHC Staining

Fixing mouse kidneys in 10% formaldehyde, then embedding them in paraffin, and staining for Masson's trichrome (Sigma-Aldrich) and Picrosirius red (Abcam) separately after cutting them in 5μm thickness. Measurement of the tissue fibrotic area could be identified with contrastive images.

Fixing tissues in 10% buffered formalin and then sectioning them for hematoxylin and eosin staining.

RNA sequencing and analyses

For IHC staining, treating paraffin-embedded tissues with 0.01 mol/L sodium citrate (pH 6.0) to deparaffinized, rehydrate, and subject them to a heat-induced epitope retrieval step. 0.3% (v/v) hydrogen peroxide in distilled water was used to block the activity of endogenous peroxidase. Then incubating the sections with 0.3% Triton X-100 in PBS (137 mmol/L NaCl, 2.7 mmol/L KCl, 10 mmol/L Na2HPO4, 2 mmol/L KH2PO4, pH 7.4) for 15 minutes, followed by 1 hour's 10% goat serum in PBS.

3 RESULTS

To ensure that the mouse gene model is needed for the experiment, we carried out PCR tests to identify the genotype. It can be seen that the fourth column is the model of SETD2 and VHL double knockout. At this time, the band size of SETD2 is 130bp and that of VHL is 500bp. The third column is the model of only knocking out the VHL gene; the band sizes of SETD2 and VHL are 75bp and 500bp respectively. The second column represents a model with only SETD2 knockout. (Fig.1).

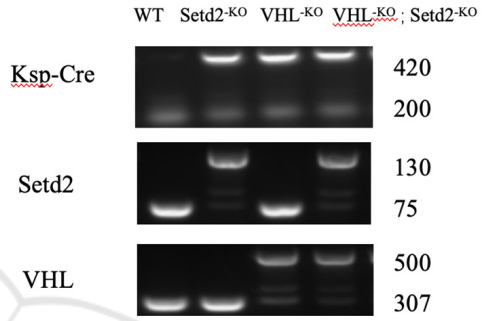

Figure 1: Results of mouse DNA PCR test.

QueryID	forward	reverse
Il1rap	TGCCTGGGGGAATTGTCAC	CTTAGCCCGCTTCAGCTCTTT
Il18r1	TCACCGATCACAAATTCATGTGG	TGGTGGCTGTTTCATTCCTGT
Il7r	GCGGACGATCACTCCTTCTG	AGCCCCACATATTTGAAATTCCA
Il1r1	GTGCTACTGGGGCTCATTTGT	GTGCTACTGGGGCTCATTTGT

Figure 2: Inflammation related genes are up-regulated in SETD2 knockout mice.

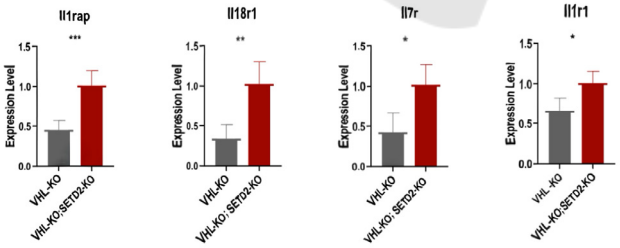

Figure 3: Results of qPCR test of inflammation related genes.

Many studies have shown that inflammation shares some of the same biological mechanisms as a range of other conditions, such as fibrosis (Inflammation, 2022). Increased expression levels of inflammation-related genes suggest the presence of diseases with the same mechanism. The gray column of each graph in Fig. 3 represents the expression level of inflammation-related genes in the VHL-KO model, and the red column represents the expression level in the VHL-KO and SETD2-KO models. It can be seen that Il1r1 and Il7r have significant rises from the gray column to the red column, and the before and after comparisons of Il18r1 and Il1rap are more obvious. The results of the qPCR test for four inflammation-related genes indicate that the Knockout of SETD2 in VHL knockout mouse

kidneys results in renal fibrosis in mice because we can see that the expression level of these inflammation-related genes all experience an up-regulation in the mouse kidney model with both VHL and SETD2 knocked out other than VHL-KO only (Fig.3).

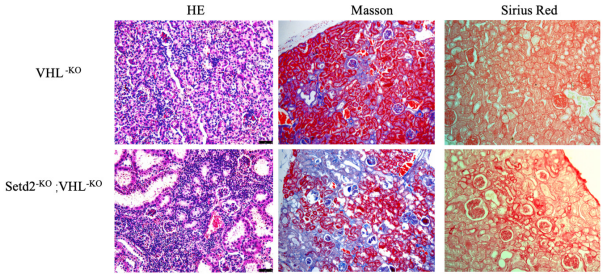

Figure 4: Knockout of SETD2 in VHL knockout mouse kidneys results in renal fibrosis in mouse(scale bars, 80um).

Knockout of SETD2 in VHL knockout mouse kidneys results in renal fibrosis in mice (scale bars, 80um)

By staining the renal tubular tissue of the mouse kidney model with VHL knockout only and the mouse kidney model with both SETD2 and VHL knockout at the same time, we can see that there is more cellular fibrosis in the double knockout mouse model (Fig.4). In HE staining, the blue stained part represents the nucleus. In the double knockout model experiment, we can find a significant increase in the number of nuclei in the tissue. In Masson's staining, blue represents fibers, and Sirius Red staining is on a yellow background, in which the red part is the fibrotic part. Both of these two staining images show a vivid upward trend in the occurrence of fibrosis from VHL-KO only to SETD2-KO and VHL-KO. Therefore, we can conclude that simultaneous mutations of SETD2 and VHL can induce the occurrence of renal fibrosis.

4 CONCLUSION

By creating the mouse model with the specific knockout of VHL and SETD2 genes, we analyze its effects on the kidney tissue. From the up-regulation for the expression level of inflammation-related genes (including Il1rap, Il18r1, Il7r, and Il1r1) in the mouse kidney model with both VHL and SETD2 knocked out other than with VHL-KO only, combined with the HE, Masson and Sirius Red staining images we performed, we can get a conclusion that these two genes of SETD2 and VHL can also trigger the occurrence of renal fibrosis.

These inflammation-related genes also play a role in the study of fibrosis. Pro-inflammatory proteins can be synthesized by the induction of Interleukin 1 (IL-1) during tissue damage or infection, IL-1 does this by forming a complex with an interleukin 1 receptor and an accessory protein at the cell membrane (Rouillard, 2016). This gene is responsible for encoding the interleukin-1 receptor accessory protein (Il1rap). Il1rap can recognize IL-1 and it is the co-receptor for signaling pathways. Interleukin 18 receptor 1 (Il18r1) is a protein-coding gene that encodes a cytokine receptor that belongs to the interleukin-1 receptor group. Interleukin 18 (Il18) is specifically bound to this receptor, which is also a pro-inflammatory cytokine (IL18R1, 2022). Instructions for making the interleukin-7 (Il-7) receptor alpha chain are provided by the Interleukin-7 receptor subunit alpha (Il7r) gene. These Il-7 receptors can be embedded in the cell membrane of cells in the immune system (New11., 2008). They are usually found in B cells, T cells, and also the early blood-forming cells that give rise to them. Interleukin-7 (Il-7) is a protein that can interact with the Il-7 receptor at the cell surface to regulate the activity of immune system cells (Plumb, 2017). Signaling across the Il-7 receptor helps mature B cells and T cells to develop properly and it also stimulates the later proliferation of these cells (Corfe, 2012). Similarly, the interleukin-1 receptor type 1 (Il1r1) gene encodes a cytokine receptor which also belongs to the IL-1 receptor family. It is a significant mediator involved in many cytokine-induced immune and inflammatory responses (IL1R1, 2022). Some studies suggested that pro-inflammatory stimuli can induce this receptor and it may be involved in the function of helper T cells (IL1R1 protein overview, 2022). These series of responses caused by the cytokine interleukin released by inflammatory-related molecules are proof of the persistence of inflammation, and also directly or indirectly reflect the development of fibrosis.

The results indicate that there is an up-regulation for the expression level of inflammation-related

genes in the mouse kidney model with both VHL and SETD2 knocked out other than with VHL-KO only. It is known that renal fibrosis is a kind of pathophysiological change, which is a gradual process of renal function from health to injury, then to loss of function. Due to the stimulation of trauma, infection, inflammation, blood circulation disorders, immune response, and other pathogenic factors, cells of the kidney are damaged, and a large amount of collagen deposition and accumulation appear in the later stage of development [2], causing the renal parenchyma to gradually harden and form scars until the kidney completely loses organ function [3]. The process of hardening in the kidney is also the process of renal fibrosis. In the three staining methods we used, HE, Masson, and Sirius Red staining, the image results indicate an increasing tendency of tissue fibrosis from the mouse model with VHL-KO only to that with both SETD2-KO and VHL-KO. Therefore, we can get the conclusion that the knockout of SETD2 in VHL knockout mouse kidneys results in renal fibrosis in mice.

However, SETD2 and VHL are well known as tumour suppressor genes and their effects of mutation are mainly found and studied in clinical patients with renal clear cell carcinoma (ccRCC). In this experiment, we did not study carcinogenic effects, so some potential limitations about the specific knockout of these two genes may also be present throughout the process. More experiments need to be done and these results have to be repeatedly deliberated to give out a more reliable conclusion.

Taken together, these results obtained can provide a solid theoretical basis for the mechanism of molecular action and prospects for corresponding drug screening and clinical target therapy.

REFERENCES

Cho MH. Renal fibrosis. Korean J Pediatr. 2010 Jul; 53(7):735-40. doi: 10.3345/kjp.2010.53.7.735. Epub 2010 Jul 31. PMID: 21189948; PMCID: PMC3004484.

Corfe SA, Paige CJ. The many roles of IL-7 in B cell development; mediator of survival, proliferation and differentiation. Semin Immunol. 2012 Jun;24(3):198-208. doi: 10.1016/j.smim.2012.02.001. Epub 2012 Mar 14. PMID: 22421572.

Gossage L, Eisen T, Maher ER. VHL, the story of a tumour suppressor gene. Nat Rev Cancer. 2015 Jan;15(1):55-64. doi: 10.1038/nrc3844. PMID: 25533676.

Humphreys BD. Mechanisms of Renal Fibrosis. Annu Rev Physiol. 2018 Feb 10;80:309-326. doi: 10.1146/annurev-physiol-022516-034227. Epub 2017 Oct 25. PMID: 29068765.

Hu M, Zhang Q, Lai J, Liu X. SETD2, an epigenetic tumor suppressor: a focused review on GI tumor. Front Biosci (Landmark Ed). 2020 Jan 1;25(4):781-797. doi: 10.2741/4834. PMID: 31585917.

IL1R1 protein overview: Sequence, structure, function and protein ... (n.d.). Retrieved August 11, 2022, from https://www.sinobiological.com/resource/il1r1/proteins

Inflammation: What is it, causes, symptoms & treatment. Cleveland Clinic. (n.d.). Retrieved August 2022, from https://my.clevelandclinic.org/health/symptoms/21660-inflammation.

Liu Y. (2006). Renal fibrosis: new insights into the pathogenesis and therapeutics. Kidney international, 69(2), 213–217. https://doi.org/10.1038/sj.ki.5000054

Li J, Duns G, Westers H, Sijmons R, van den Berg A, Kok K. SETD2: an epigenetic modifier with tumor suppressor functionality. Oncotarget. 2016 Aug 2;7(31):50719-50734. doi: 10.18632/oncotarget.9368. PMID: 27191891; PMCID: PMC5226616.

Molenaar, T. M., & van Leeuwen, F. (2022). SETD2: from chromatin modifier to multipronged regulator of the genome and beyond. Cellular and molecular life sciences: CMLS, 79(6), 346. https://doi.org/10.1007/s00018-022-04352-9

New11. Palmer, M. J., Mahajan, V. S., Trajman, L. C., Irvine, D. J., Lauffenburger, D. A., & Chen, J. (2008). Interleukin-7 receptor signaling network: an integrated systems perspective. Cellular & molecular immunology, 5(2), 79–89. https://doi.org/10.1038/cmi.2008.10

Patard JJ, Rioux-Leclercq N, Masson D, Zerrouki S, Jouan F, Collet N, Dubourg C, Lobel B, Denis M, Fergelot P. Absence of VHL gene alteration and high VEGF expression are associated with tumour aggressiveness and poor survival of renal-cell carcinoma. Br J Cancer. 2009 Oct 20;101(8):1417-24. doi: 10.1038/sj.bjc.6605298. Epub 2009 Sep 15. PMID: 19755989; PMCID: PMC2768461.

Pal, S., Claffey, K. P., Dvorak, H. F., & Mukhopadhyay, D. (1997). The von Hippel-Lindau gene product inhibits vascular permeability factor/vascular endothelial growth factor expression in renal cell carcinoma by blocking protein kinase C pathways. The Journal of biological chemistry, 272(44), 27509–27512. https://doi.org/10.1074/jbc.272.44.27509

Plumb AW, Sheikh A, Carlow DA, Patton DT, Ziltener HJ, Abraham N. Interleukin-7 in the transition of bone marrow progenitors to the thymus. Immunol Cell Biol. (2017) 95:916–24. doi: 10.1038/icb.2017.68

Rouillard AD, Gundersen GW, Fernandez NF, Wang Z, Monteiro CD, McDermott MG, Ma'ayan A. The harmonizome: a collection of processed datasets gathered to serve and mine knowledge about genes and proteins. Database (Oxford). 2016 Jul 3;2016. pii: baw100.

U.S. National Library of Medicine. (n.d.). IL18R1 interleukin 18 receptor 1 [homo sapiens (human)] -

gene - NCBI. National Center for Biotechnology Information. Retrieved August 11, 2022, from https://www.ncbi.nlm.nih.gov/gene/8809.

U.S. National Library of Medicine. (n.d.). IL1R1 interleukin 1 receptor type 1 [homo sapiens (human)] - gene - NCBI. National Center for Biotechnology Information. Retrieved August 5, 2022, from https://www.ncbi.nlm.nih.gov/gene/3554/

Von Hippel-Lindau (VHL). Johns Hopkins Medicine. (n.d.). Retrieved September 19, 2022, from https://www.hopkinsmedicine.org/health/conditions-and-diseases/von-hippellindau-vhl