ALKBH1 reduces DNA N^6-methyladenine to allow for vascular calcification in chronic kidney disease

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Vascular calcification is a common complication of chronic kidney disease (CKD), and one of the main risk factors for increased cardiovascular morbidity and mortality in patients with CKD. In this issue of the *JCI*, Ouyang and Su et al. report that Alkb homolog 1 (ALKBH1), a DNA demethylase, reduced DNA N^6-methyladenine (6mA) in vascular smooth muscle cells (VSMCs) and leukocytes, thus leading to aortic arch calcification in the patients with CKD. During the progression of vascular calcification, increased ALKBH1 expression was linked to decreased 6mA levels, findings that the authors noted in both patients with CKD and CKD mouse models. The kidney and vascular disease risk factor soluble urokinase receptor (suPAR) was also elevated in the plasma. Notably, lower 6mA levels induced BMP2-mediated osteogenic reprogramming in the VSMCs. These findings present a function of ALKBH1 in vascular calcification and provide a framework for therapeutic strategies.

Vascular calcification and CKD

Cardiovascular disease is the leading cause of death in patients with chronic kidney disease (CKD). Vascular calcification, the pathological deposition of minerals in the vasculature, is one of the strongest predictors of cardiovascular risk. Two major types of vascular calcifications are distinguished according to the locations in the blood vessels and association with atherosclerosis. Atherosclerotic calcification occurs in the arterial intima that comprises a layer of endothelia cells, and medial artery calcification is mediated by smooth muscle cells and more prevalent in patients with diabetes and CKD (1, 2). Vascular calcification is considered a pathological process driven by osteogenesis in blood vessels, since osteoblast differentiation factors such as alkaline phosphatase (ALP), bone morphogenetic protein-2 (BMP2), and osteocalcin (OCN) have been identified in calcified human tissues and in animal models of vascular calcification (3–5). Traditionally, in patients with advanced CKD, vascular calcification coincides with hyperphosphatemia (6). In a cell-based model for calcification, higher phosphorus concentration induces the expression of Runx2 and osteocalcin, two key factors associated with osteoblast differentiation (7).

The findings of Ouyang and Su et al. in this issue of the *JCI* center around another area of vascular calcification research with a focus on DNA N^6-methyladenine (6mA) (8), a type of epigenetic modification that includes DNA methylation, histone modification, noncoding RNAs (especially microRNAs), and chromatin changes (9). 6mA was thought to only exist in prokaryotes, where it was used as a genetic mark to distinguish the host DNA from foreign pathogenic DNA and thus to protect the host genome through the restriction-modification system. Recent studies have manifested that 6mA is also present in eukaryotes, such as green algae, *Caenorhabditis elegans,* and *Drosophila melanogaster*. 6mA levels can be regulated by methyltransferases, such as the DNA methyltransferase (DNMT) family, and demethylases, such as the AlkB family (10). While it is controversial whether 6mA exists in mammals (11–13), emerging evidence has demonstrated that 6mA plays an important role in human diseases. For example, Xie et al. reported that ALKBH1-mediated 6mA demethylation was prevalent in human glioblastoma and regulated cancer cell growth (14). Wu et al. found that ALKBH1-6mA was highly associated with patients with atherosclerosis and hypertension (15), while another study described overexpression of ALKBH1 in human mesenchymal stem cells to regulate 6mA and subsequent osteogenic differentiation (16). However, the role of ALKBH1 in patients with CKD with vascular calcification is largely unknown and the link between induced ALKBH1, decreased 6mA, and high soluble urokinase receptor (suPAR) represents a segue connecting epigenetic modification to an effector pathway for CKD and vascular injury.

Phosphorus and ALKBH1-regulated 6mA in vascular calcification

High serum phosphorus can increase DNA methyltransferase activity and methylation of SM22α promoter, which results in

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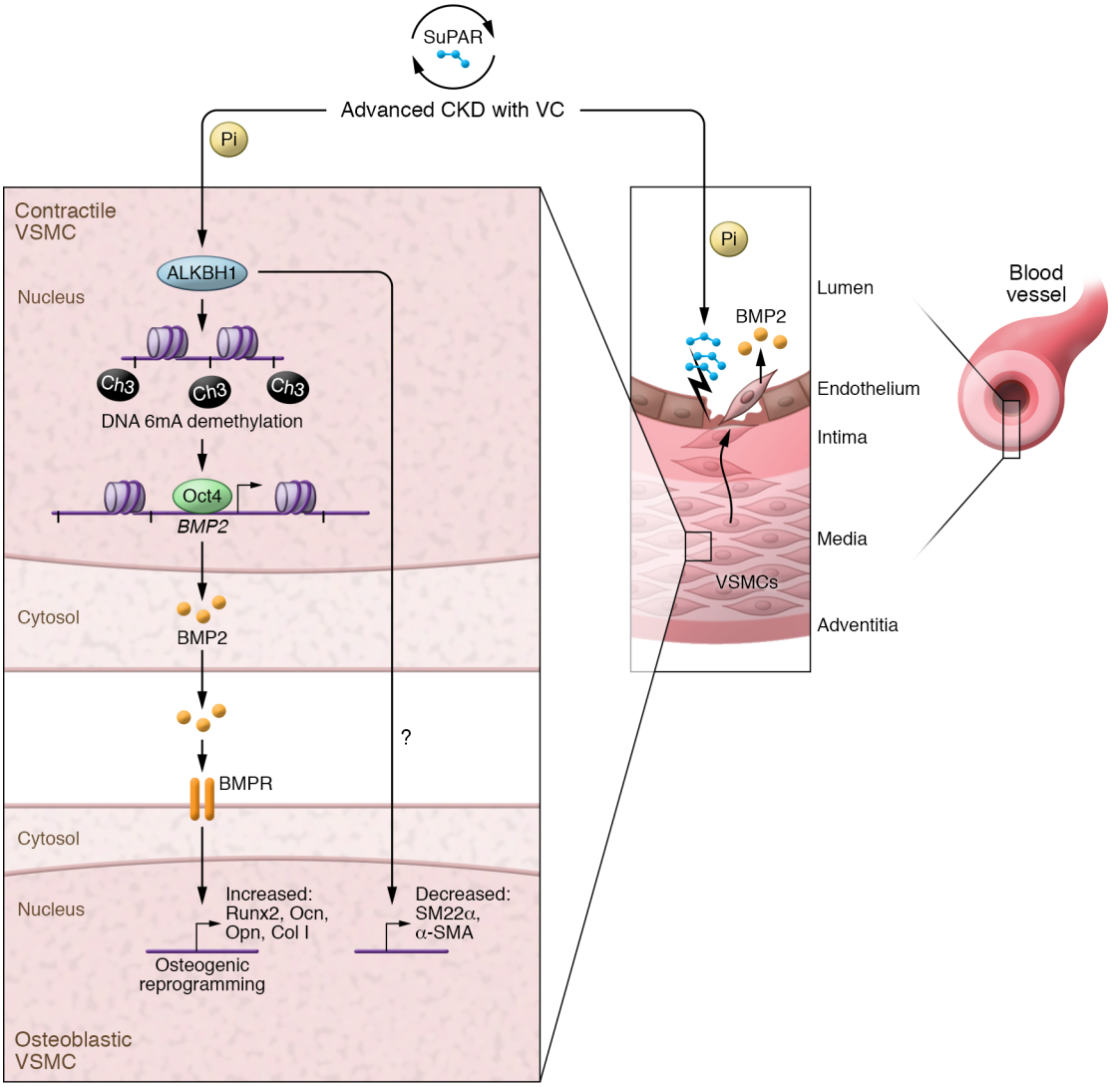

Figure 1. Mechanism of ALKBH1 in CKD with vascular calcification. Hyperphosphatemia in advanced CKD with vascular calcification (VC) induces ALKBH1 expression, leading to a reduced 6mA level that promotes Oct4 binding to the *BMP2* promoter and activates *BMP2* transcription in VSMC. High serum suPAR levels in patients with CKD may also aggravate the ALKBH1/Oct4/BMP2 cascade. Meanwhile, suPAR can induce the migration of VSMCs from the media to the intima, where VSMCs are exposed to the calcification stimuli, triggering the ALKBH1/Oct4/BMP2 cascade. As a result, BMP2 is secreted from VSMCs, and the BMP2/BMPR signaling pathway stimulates the expression of osteoblast markers such as Runx2, OCN, OPN, and Collagen I, resulting in osteogenic reprogramming and vascular calcification.

vascular smooth muscle cell (VSMC) calcification (17), while inhibition of DNA methyltransferase activity accelerates phosphorus-induced mineralization of VSMCs (18). A role of DNA methylation, especially that of 6mA in vascular calcification, has been elusive. Ouyang and Su et al. have now identified 6mA as an epigenetic modification in patients with CKD with vascular calcification that controls osteogenic reprogramming in VSMCs (8). At the core of this translational study was a single-center CKD cohort of 198 patients with elevated serum suPAR levels in Donghua Hospital of Sun Yat-sen University, China. During the progression of calcification in patients, leukocytes and VSMCs showed gradual decreases in 6mA. Notably, ALKBH1 was upregulated, accounting for the reduced 6mA levels (8). Increased suPAR levels have been identified as a strong clinical indicator for renal disease and are associated with artery calcification (19–22). Ouyang and Su et al. generated elegant CKD mouse models in which elevated suPAR levels established a physiological relevance between vascular and renal disease. The authors targeted ALKBH1 to study a possible intervention. Knockdown of ALKBH1 either in vivo or in vitro potently reduced vascular calcification, whereas its overexpression had the opposite effect (8). The authors also demonstrated that exposing primary mouse VSMCs to high phosphorus medium greatly increased the expression levels of osteoblast differentiation markers, including osteopontin (OPN), osteocalcin (OCN), and Collagen I, and reduced the expression of VSMC markers, including SM22α and α-SMA. Further, these changes of gene expression were alleviated by ALKBH1 knockdown or aggravated by ALKBH1 overexpression (8).

This study presents us with opportunities for targeting the pathogenic mechanisms of vascular calcifications by interfering with ALKBH1 function. Ouyang and Su et al. delineated that ALKBH1 could regulate the transcriptional level of BMP2, an essential signaling molecule for osteogenesis (8). Through approaches of bioinformatics and molecular biology, Ouyang and Su et al. revealed that 6mA demethylation, which is ALKBH1-regulated, promoted Oct4 binding to the BMP2 promoter and activated BMP2 transcription (8). Finally, overexpression of ALKBH1 in WT but not in Oct4-deficient VSMCs substantially enhanced the expression of osteoblast markers, and thus increased calcification (8).

In summary, the findings by Ouyang and Su et al. provide insight into our understanding of the molecular mechanism of vascular calcification in patients with kidney disease in which ALKBH1 is playing a major role. Whether ALKBH1 could directly inhibit SM22α and α-SMA remains unknown and warrants further study (Figure 1).

Future directions

This study provides a basis for many additional lines of investigation. It will be interesting to explore if high suPAR is directly involved in the regulation of the ALKBH1/Oct4/BMP2 cascade. suPAR is a circulating kidney disease risk factor (20, 21). It is also associated with artery calcification and cardiovascular disease, conditions seen often in CKD (19). In the present study, serum suPAR level substantially increased to 4809 pg/mL in patients with CKD with vascular calcification (8), while the level in healthy people is typically maintained between 2000 to 3000 pg/mL. The response of VSMCs to calcifying stimuli remains largely unknown. In atherosclerosis, the medial VSMCs may migrate to the intima and contribute to initial atherosclerotic plague formation (23). Given that suPAR can induce VSMC migration in a dose-dependent manner (24), it will be worth exploring whether suPAR drives VSMCs to the intima, where VSMCs are exposed to the stimuli that trigger the ALKBH1-mediated cascade to enhance BMP2 secretion and induce osteogenic reprogramming (Figure 1). Since the current study revealed a lower level of 6mA in the leukocytes of the patients, it will be valuable to determine if the leukocytic 6mA levels can be used as a biomarker for early disease diagnosis, which we anticipate may complement results from drug discovery efforts focused on the ALKBH1 enzyme.

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1. Goodman WG, et al. Vascular calcification in chronic kidney disease. *Am J Kidney Dis*. 2004;43(3):572–579.
2. Libby P, et al. Progress and challenges in translating the biology of atherosclerosis. *Nature*. 2011;473(7347):317–325.
3. Bostrom K, et al. Bone morphogenetic protein expression in human atherosclerotic lesions. *J Clin Invest*. 1993;91(4):1800–1809.
4. Levy RJ, et al. The identification of the vitamin K-dependent bone protein osteocalcin as one of the gamma-carboxyglutamic acid containing proteins present in calcified atherosclerotic plaque and mineralized heart valves. *Atherosclerosis*. 1983;46(1):49–56.
5. Tanimura A, et al. Calcification in atherosclerosis. I. Human studies. *J Exp Pathol*. 1986;2(4):261–273.
6. Wojcicki JM. Hyperphosphatemia is associated with anemia in adults without chronic kidney disease: results from the National Health and Nutrition Examination Survey (NHANES): 2005-2010. *BMC Nephrol*. 2013;14:178.
7. Jono S, et al. Phosphate regulation of vascular smooth muscle cell calcification. *Circ Res*. 2000;87(7):E10–E17.
8. Ouyang L, et al. ALKBH1-demethylated DNA N6-methyladenine modification triggers vascular calcification via osteogenic reprogramming in chronic kidney disease. *J Clin Invest*. 2021;131(14):146985.
9. Hou YC, et al. The epigenetic landscape of vascular calcification: an integrative perspective. *Int J Mol Sci*. 2020;21(3):980.
10. Luo GZ, et al. DNA N(6)-methyladenine: a new epigenetic mark in eukaryotes? *Nat Rev Mol Cell Biol*. 2015;16(12):705–710.
11. Douvlataniotis K, et al. No evidence for DNA N^6-methyladenine in mammals. *Sci Adv*. 2020;6(12):eaay3335.
12. Wu TP, et al. DNA methylation on N(6)-adenine in mammalian embryonic stem cells. *Nature*. 2016;532(7599):329–333.
13. Xiao CL, et al. N(6)-Methyladenine DNA modification in the human genome. *Mol Cell*. 2018;71(2):306–318.
14. Xie Q, et al. N(6)-methyladenine DNA modification in glioblastoma. *Cell*. 2018;175(5):1228–1243.
15. Wu L, et al. Association of N(6)-methyladenine DNA with plaque progression in atherosclerosis via myocardial infarction-associated transcripts. *Cell Death Dis*. 2019;10(12):909.
16. Zhou C, et al. DNA N(6)-methyladenine demethylase ALKBH1 enhances osteogenic differentiation of human MSCs. *Bone Res*. 2016;4:16033.
17. Montes de Oca A, et al. High-phosphate-induced calcification is related to SM22alpha promoter methylation in vascular smooth muscle cells. *J Bone Miner Res*. 2010;25(9):1996–2005.
18. Azechi T, et al. 5-aza-2′-deoxycytidine, a DNA methyltransferase inhibitor, facilitates the inorganic phosphorus-induced mineralization of vascular smooth muscle cells. *J Atheroscler Thromb*. 2014;21(5):463–476.
19. Fuhrman B. The urokinase system in the pathogenesis of atherosclerosis. *Atherosclerosis*. 2012;222(1):8–14.
20. Hayek SS, et al. Soluble urokinase receptor and acute kidney injury. *N Engl J Med*. 2020;382(5):416–426.
21. Hayek SS, et al. Soluble urokinase receptor and chronic kidney disease. *N Engl J Med*. 2015;373(20):1916–1925.
22. Wei C, et al. Circulating urokinase receptor as a cause of focal segmental glomerulosclerosis. *Nat Med*. 2011;17(8):952–960.
23. Ross R. Atherosclerosis — an inflammatory disease. *N Engl J Med*. 1999;340(2):115–126.
24. Duru EA, et al. Role of formic receptors in soluble urokinase receptor-induced human vascular smooth muscle migration. *J Surg Res*. 2015;195(2):396–405.