Calcium oxalate crystals trigger epithelial-mesenchymal transition and carcinogenic features in renal cells: a crossroad between kidney stone disease and renal cancer

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Abstract
Increasing evidence of association between kidney stone disease (KSD) and renal cell carcinoma (RCC) has been reported. Nevertheless, mechanism underlying such association remained unknown. Herein, we investigated the effects of calcium oxalate monohydrate (COM), a major crystalline component causing KSD, on induction of carcinogenic features in non-cancerous renal cells. COM crystals induced morphological changes from epithelial to fibroblast-like spindle shape. Additionally, COM increased spindle index and mesenchymal markers (fibronectin and vimentin) but declined epithelial markers (E-cadherin and zonula occludens-1). Moreover, COM down-regulated ARID1A, a tumor suppressor gene recently reported to be reversely associated with RCC, at both mRNA and protein levels. COM also down-regulated other RCC-related tumor suppressor genes, PTEN and VHL, but up-regulated oncogene TPX2. Finally, COM enhanced invading capability, cell-aggregate formation, chemoresistance to cisplatin, and secretion of an angiogenic factor (VEGF). These data indicate that COM crystals trigger epithelial-mesenchymal transition (EMT) and several carcinogenic features in the non-cancerous renal cells. These mechanisms may explain and strengthen the association between KSD and RCC.

Keywords: ARID1A, Carcinogenesis, EMT, PTEN, RCC, TPX2, VEGF, VHL

To the Editor,

Kidney cancers are common around the globe accounting for 2% of diagnosed cancers with increasing incidence [1]. Among all types of kidney cancers, approximately 90% are renal cell carcinoma (RCC) [1]. Its incidence is twofold higher in men than women [1]. The precise etiology of RCC remains unclear; however, several genetic backgrounds, behaviors and environments are considered as the RCC risk factors. Increasing evidence of RCC has been reported in patients with kidney stone disease (KSD) [2–4]. On the other hand, several reports have shown intratumoral deposition of calcium oxalate (CaOx) crystals in RCC [5–7]. Therefore, KSD is now considered as another risk for RCC development. Nevertheless, the precise cellular and molecular mechanisms underlying this association have not been reported previously.

KSD is a common disease worldwide caused by intrarenal deposition of solid materials, comprising mainly CaOx monohydrate (COM) crystals [8]. COM crystals cause renal cell injury, induce reactive oxygen species...
Fig. 1  COM crystals trigger epithelial-mesenchymal transition (EMT) in renal cells. A Cell morphology. B Spindle index. C, D Flow cytometry with annexin V/propidium iodide stainings. E–H Epithelial markers (E-cadherin and ZO-1). I–L Mesenchymal markers (fibronectin and vimentin). All quantitative data are presented as mean ± SD derived from three independent experiments using different biological samples. A.U. arbitrary unit.
Fig. 2 COM crystals trigger carcinogenic features of renal cells. A–C ARID1A mRNA and protein levels (GAPDH served as a loading control). D–F mRNA levels of PTEN, VHL, and TPX2, respectively. G, H Cell invasion assay. I, J Cell-aggregate formation (hanging-drop) assay. K, L Chemoresistance assay. M Level of VEGF secretion. All quantitative data are presented as mean ± SD derived from three independent experiments using different biological samples. A.U. arbitrary unit.
(ROS) overproduction, and promote oxidative modifications of cellular proteins [8]. A previous study has revealed positive staining of oxidative DNA damage marker (8-OHdG) in renal tissue around the stone [9]. Interestingly, the oxidative DNA damage is also recognized to play crucial roles in initiation and progression of several cancers, including RCC [10, 11]. Similar to RCC, KSD is more common in males than females [12]. As KSD shares some disease backgrounds with RCC, we hypothesized that its crystalline component, COM, might be responsible for triggering carcinogenic features in non-cancerous renal cells.

Herein, we investigated the effects of COM crystals on induction of carcinogenic features in non-cancerous renal cells. Several assays were performed to investigate these carcinogenic features, including morphological changes, spindle index, epithelial-mesenchymal transition (EMT), expression of RCC-related tumor suppressor genes (ARID1A, PTEN, and VHL) and oncogene (TPX2) (http://portal.gdc.cancer.gov/), cell invasion ability, cell-aggregate formation, chemoresistance, secretion of an angiogenic factor (vascular endothelial growth factor or VEGF) (see details in Additional file 1).

COM crystals induced morphological changes from epithelial to fibroblast-like spindle shape and increased spindle index (Fig. 1A, B). Without significant toxic effects (Fig. 1C, D), COM suppressed epithelial markers (E-cadherin and zonula occludens-1, ZO-1) (Fig. 1E–H) but enhanced mesenchymal markers (fibronectin and vimentin) (Fig. 1I–L). Moreover, COM down-regulated ARID1A, a tumor suppressor gene recently reported to be reversely associated with RCC, at both mRNA and protein levels (Fig. 2A–C). COM also down-regulated other RCC-related tumor suppressor genes, PTEN and VHL, but up-regulated the oncogene TPX2 (Fig. 2D–F). Finally, COM enhanced invading capability (Fig. 2G, H), cell-aggregate formation (Fig. 2I, J), chemoresistance to cisplatin (Fig. 2K, L), and secretion of the angiogenic factor VEGF (Fig. 2M).

EMT is the process in which epithelial cells structurally and functionally change to mesenchymal phenotype. This process is involved in several biological phenomena under both physiologic and pathogenic conditions, including embryogenesis, wound healing, tissue fibrogenesis, and carcinogenesis. It’s not unexpected that COM caused EMT. As EMT is one of the carcinogenic features, we hypothesized that COM crystals might induce other carcinogenic effects on the non-cancerous renal cells and may serve as a crossroad between KSD and RCC. Our hypothesis was supported by several assays to confirm such phenomenon. Together with down-regulations of the RCC-related tumor suppressor genes (ARID1A, PTEN, and VHL) and up-regulation of the oncogene (TPX2), other functional assays confirmed that COM crystals trigger several of the carcinogenic features, including invading capability, cell-aggregate formation, and chemoresistance to cisplatin. Finally, the COM-treated cells secrete greater level of an angiogenic factor, VEGF.

Our previous study has demonstrated that COM crystals trigger oxidative stress and induces oxidatively modified proteins in renal cells via ROS overproduction [8]. Therefore, COM may induce the oxidative DNA damage via this mechanism, thereby enhancing the carcinogenic features in non-cancerous renal cells. However, the precise mechanisms leading to COM-induced alterations in RCC-related tumor suppressor genes and oncogenes and increase of angiogenic factor remain unknown and deserve further investigations that may lead to better understanding of carcinogenesis induced by COM crystals.

Taken together, these data indicate that COM crystals trigger EMT and several of carcinogenic features in the non-cancerous renal cells. These mechanisms may explain and strengthen the association between KSD and RCC.

**Supplementary Information**

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Additional file 1. Supplementary methods.

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PP, WB, PP and VT designed research; PP, WB and PP performed experiments; PP, WB, PP and VT analyzed data; PP and VT wrote the manuscript; All authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article and are also available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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