# Pathogenesis of nephrolithiasis: recent insight from cell biology and renal pathology

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**Summary**

**Randall's plaques are very common in idiopathic calcium-oxalate nephrolithiasis. These papillary plaques have an apatite mineral structure. While these calcium deposits are generally assumed to be secondary to a purely physico-chemical phenomenon, we advance the hypothesis that they form due to a truly ectopic biomineralization in the renal tissue, and that Henle's loop epithelial cells, or pericyte-like interstitial cells, or papillary stem cells differentiating along a bone lineage might be involved.**

*KEY WORDS: CaOx renal stones, ectopic calcification, epithelial-mesenchymal transformation, papilla, Randall's plaque.*

## Calcium nephrolithiasis: a puzzling pathogenesis

In 1937 Randall (1) described calcium-phosphate plaques in stone formers, i.e. sites of interstitial crystal deposition at or near the tip of the papilla, that he conjectured might trigger calcium-oxalate (CaOx) stone formation at these sites (1). His findings were confirmed by others (2-5), but until the recent paper by Evan et al. in Indianapolis (6) this discovery was not adequately recognized as an important step forward in our understanding of the pathogenesis of renal stones. Over the last 3 decades, the putative pathogenic framework of renal stones has included a number of other hypotheses, ideas and observations, leaving the role of Randall's plaque in the background. Briefly, major breakthroughs have been:

1. the hypothesis of a physico-chemical imbalance in pre-urine somewhere in the nephron to explain crystallization;
2. the fixed particle theory, i.e. the attachment of CaOx crystals to the tubular epithelium to explain how crystals can become large enough to become trapped in the tubular lumen, thus enabling their evolution into stones;
3. the role of defective renal tubular cells in determining the pre-urine physico-chemical imbalance and/or the deposition of crystals on them;
4. the discovery of a number of crystal growth and aggregation inhibitors, including macromolecules such as bikunin, nephrocalcin and osteopontin.

Like the Phoenix, however, Randall's plaque has very recently been reborn. Evan et al. (6) have shown that it probably originates in the basement membranes of the thin loops of Henle and spreads from there through the interstitium to underneath the papillary urothelium; the same group has presented data supporting Randall's idea that CaOx stones form on the plaque in the renal pelvis (2, 3, 7).

But we still have to discover how these pieces of the puzzle fit together. We do not know whether (or where) crystals indeed form in the nephron lumen; nor do we know the fate of these putative intraluminal fixed crystals. As a matter of fact, the Indianapolis group found no crystals in Henle's loop, close to the supposedly germinating plaques (6), at the only site in the nephron where spontaneous calcium phosphate crystallization could occur (8). Moreover, the thin Henle's loop epithelia lying over the basement membrane concretions looks normal, with no signs of damage or crystals inside. So how do these basement membrane concretions form in the Henle's forceps?

Calcium phosphate crystals have oddly only been observed in Bellini's ducts in intestinal hyperoxaluric CaOx stone formers, attached to a distorted epithelium (6), close to the papillary tip, suggesting that once heterogeneous crystallization has led to the formation of a CaOx stone, this stone can easily extrude into the renal pelvis. This is a very different picture, however, from the situation seen in idiopathic CaOx stone formers, where there are no concretions on the overlying "mineralized" basement membrane (6).

In short, therefore, we are unable to combine the physico-chemical and fixed-particle theories of stone formation with the discovery of basement membrane and interstitial calcifications in the papilla (5, 6), with the hypothesis that Randall's plaque forms from these concretions, and finally with the very strong evidence that CaOx stones form and grow on Randall's plaque in the renal pelvis (1, 3, 7, 9).

Perhaps we should admit that the influence of intraluminal events in the nephron on the pathogenesis of idiopathic calcium renal stones has been overestimated. We guess that it is time to piece together many recent findings reported by different "renal stone investigators" following up very different hypotheses, and to interpret them also in the light of intriguing findings emerging in quite different areas of research concerning bone and vascular biology (10).

**Does biomineralization have a role in nephrolithiasis?**

In the last decade, some very distinguished investigators have attempted to identify the effects of high oxalate and crystal concentrations on the biology of renal tubular cells. In *in vitro* models, oxalate has been shown to trigger inflammatory, oxidative, chemotactic, and fibrogenic loops (11-13). Generally speaking, very high oxalate concentrations were used in these studies, though this makes them more relevant to primary hyperoxaluria than to idiopathic CaOx renal stones. In addition, the possibility that these conditions may trigger the transdifferentiation of tubular cells was not investigated. It would be very interesting and relevant to the present hypothesis to explore whether cultivated renal tubular cells – the origin of which is mesodermal, despite their epithelial appearance – may be induced to undergo epithelial-mesenchymal transdifferentiation under the influence of the paraphysiological oxalate concentrations observed in idiopathic CaOx stone formers. This may be the case, since these epithelial cells have the genetic program of cells of mesenchymal origin.

As a matter of fact, Myazawa et al. (14, and personal communication) have demonstrated that CaOx crystals upregulate the gene transcription for vimentin (an embryonic marker of the multipotent kidney mesenchyme) in normal rat kidney proximal cells.

The phenomenon of tubular epithelial cell differentiation into cells with the mesenchymal phenotype is well known. Studies suggest that renal interstitial myofibroblasts originate from renal tubular cells undergoing epithelial-mesenchymal transformation (15). The phenomenon of differentiation is restricted neither to the kidney, nor to the epithelial cell, since it also occurs in liver Ito cells (16) and in a subpopulation of smooth muscle cells in the intima of arteries. Both these cell populations are thought to be pericyte-like cells. Notably, vascular pericytes have the ability to undergo osteoblastic differentiation and mineralization (17, 18) and seem to play a crucial part in ectopic vascular calcification. Long thought due to passive degeneration, vascular calcification instead derives from a complex and strictly-regulated process of biomineralization resembling osteogenesis (19). There is evidence to indicate that proteins controlling bone mineralization are also involved in regulating vascular calcification. Cultured artery smooth muscle cells are also induced to become osteogenic by inflammatory stimuli, reactive oxygen species and hypoxia (20).

A similar phenomenon may occur in the renal papilla, where CaOx crystals and/or oxalate at paraphysiologically high concentrations or, more likely, a high pre-urine CaOx supersaturation in conjunction with an unfavorably low oxygen tension may trigger inflammation in cells at the bend of the long loop of Henle. This would make these cells transdifferentiate towards the osteogenic lineage, causing the synthesis of typical bone osteoid proteins (osteopontin, osteocalcin, BMP-2, etc.) and a true biological hydroxyapatite mineralization of the Henle's loop basement membrane (beneath the differentiating cells). While both hydroxyapatite and brushite have been identified in stones, depending on the clinical phenotype (21), any existence of brushite in calcified tissue has been ruled out (22). Reports that Randall's plaque and the preceding crystalline structures in the basement membrane and papillary interstitium are composed of bone-like hydroxyapatite crystals (6,23) thus support the hypothesis that they are the consequence of an active process of biomineralization, and therefore that the Henle's loop cells are capable of differentiation.

Concerning the active process of biomineralization, it is worth noting that osteopontin has been found localized in the Golgi apparatus precisely of thin loop of Henle cells in the normal rat kidney (24). Osteopontin was detected in the lamellae and striations of the organic matrix in human calcium oxalate monohydrate stones (25); this observation and in vitro findings have led to the suggestion that osteopontin is a powerful inhibitor of CaOx crystallization (26), which may actually be the case, though we speculate that it might also well reflect the biomineralization process occurring at the tip of Henle's loop and in the papillae.

Although almost all *in vitro* studies on epithelial mesenchymal differentiation and oxalate toxicity used proximal tubular cells as a model, it would be hardly surprising to find that Henle's cells also undergo differentiation after injury. For the purposes of our hypothesis, however, more interest lies in the findings recently reported by Kumar et al. (27) that inner medullary collecting duct cells grown in a calcifying media tend to form calcific nodules that are positive for typical bone proteins, osteopontin and bone sialoprotein. This is specifically realistic considering that proximal and collecting tubule epithelia and Henle's tubules have the same embryonic, mesenchymal origin.

**Multipotent interstitial cells may also have a role**

An alternative possibility is that the interstitial renal cells (pericyte-like cells) thought to be involved in the process of fibrogenesis because of their differentiation into myofibroblasts (15, 28, 29) may also differentiate into the bone lineage when exposed to the inflammatory environment created by oxalate on the neighboring Henle cells: a cross-talk between these two cells has been postulated (30). In the same way as for calcifying vascular cells of the arterial wall (31), and for mesenchymal procursor cells to a site of osteoinduction (32), a subsequent putative step in which these cells become condensed – a stage preceding biomineralization, which is always associated with an epithelium and an epithelial basement membrane *in vivo* – may explain why hydroxyapatite mineralization occurs in the Henle's loop basement membrane.

Finally, there is a third cell type that might hypothetically develop Randall's plaque. Given its particular conditions of low oxygen tension, the papilla is a niche for stem cells (33), which have been shown to differentiate into myofibroblasts and cells expressing neuronal markers, and to spontaneously form cellular spheres. These renal stem cells can migrate to other parts of the kidney, and to the medullary tubular epithelia in particular (33, 34). Since stem cells recovered from other tissues can differentiate along the bone lineage, the third cell population potentially capable of mineralizing in the kidney is that of the papillary stem cells.

Why the phenomenon occurs at papillary level might be because of the particular physiological conditions of the renal papilla. We have already seen that oxygen tension is very low at papillary level; indeed, ischemic lesions of the papilla in diabetic and analgesic-induced nephropathies are well known and calcium deposits are so typical of the latter condition (with a recorded 47% incidence of nephrocalcinosis [35]) that they are used as a means for its CT diagnosis. Thus, in a subischemic environment, the Henle's loop cells or the pericytes, or the stem cells might be very sensitive even to mild toxic insult, or to high calcium, oxalate or phosphate concentrations. Cell differentiation could occur as a consequence, leading to Randall's plaque formation.

If this hypothesis is confirmed, it should open a new window on renal lithiasis, possibly pointing towards new therapeutic approaches.

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