Lethal (3) malignant brain tumor like 2 (L3MBTL2) protein protects against kidney injury by inhibiting the DNA damage–p53–apoptosis pathway in renal tubular cells

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DNA damage contributes to renal tubular cell death during kidney injury, but how DNA damage in tubular cells is regulated is not fully understood. Lethal (3) malignant brain tumor like 2 (L3MBTL2), a novel polycomb group protein, has been implicated in regulating chromatin architecture. However, the biological functions of L3MBTL2 are largely undefined. Here we found that L3MBTL2 was expressed in the nuclei of renal tubular epithelial cells in mice. Ablation of L3mbtl2 in renal tubular cells resulted in increases in nuclear DNA damage, p53 activation, apoptosis, tubular injury and kidney dysfunction after cisplatin treatment or unilateral ureteral obstruction. *In vitro*, inhibition of L3MBTL2 sequentially promoted histone γH2AX expression, p53 activation and apoptosis in cisplatin-treated mouse proximal tubular TKPTS cells. Inhibition of p53 activity attenuated the apoptosis induced by L3mbtl2 deficiency after cisplatin treatment both *in vivo* and *in vitro*. Intriguingly, unlike other polycomb proteins, L3MBTL2 was not recruited to DNA damage sites, but instead increased nuclear chromatin density and reduced initial DNA damage load. Thus, L3MBTL2 plays a protective role in kidney injury, in part by inhibiting the DNA damage-p53-apoptosis pathway.

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Kidney injury is a major health problem. In response to various stimuli, including chemical, hemodynamic, hypoxic, and mechanical stress, tubular epithelial cells are subject to morphologic changes, loss of polarity, DNA damage, and cell death.[1–4]

In rodent models of kidney injury induced by cisplatin or unilateral ureteric obstruction (UUO), apoptosis plays an important role in the development of tubular injury.[5,6] Mechanisms underlying cisplatin-induced acute kidney injury (AKI) are very complex, but nuclear DNA injury and activation of apoptotic cascades are involved.[7–10] The pathogenesis of obstructive kidney injury also involves nuclear DNA damage as a response to various insults, including oxidative stress.[11–14] However, how the DNA damage is controlled at the molecular level in renal tubular cells is largely unknown.

Lethal (3) Malignant Brain Tumor Like 2 (L3MBTL2) is a member of the MBT protein family composed of 9 members in humans and mice.[15,16] The MBT domains were first identified in the *Drosophila* tumor-suppressor protein L(3) MBT, and mutations of the *L(3)mbt* gene lead to development of lethal malignant brain tumor in the larva.[16]

Research has revealed that L3MBTL2 is an integral component of the atypical polycomb repressive complex 1 (PRC1)–family complexes, containing E2F6, RING2, HP1γ, and MBLR in cancer cells[17,18] and in mouse embryonic stem cells.[15] A number of PcG proteins, including BMI1 and RING2, were recruited to DNA damage sites and participated in DNA damage repair.[19–24] However, these observations were called into question by a recent study that found no evidence that BMI1, MEL18, RING1, and RING2 are recruited to double-strand breaks.[25]

Although L3MBTL2 is required for early embryonic development,[15]its biological role in adults remains unknown. L3MBTL2 is widely expressed in many organs, including the kidney.[15] In the present study, we found that L3MBTL2 is expressed in renal tubular epithelial cells in mice. Deletion of

L3mbtl2 in renal tubular epithelial cells did not alter normal kidney development and function, but it rendered renal tubular cells more susceptible to DNA damage and apoptosis, leading to increased tubular cell death and kidney injury after cisplatin treatment or UUO.

RESULTS

Generation of *L3mbtl2* renal tubule conditional knockout (cKO) mice

In adult mouse kidneys, L3MBTL2 was detected predominantly within the nuclei of the tubular epithelial cells (Supplementary Figure S1). We then generated tubular cell L3mbtl2 knockout mice by intercrossing floxed *L3mbtl2* mice with *Ksp-Cre* transgenic mice (Supplementary Figure S2). *Ksp-Cre* has been shown to efficiently ablate floxed Smad2, β-catenin, or Dicer in renal tubules, including proximal tubules.[26–28]

Protein levels and L3mbtl2 mRNA were dramatically reduced in L3mbtl2 cKO kidneys, compared with wild-type (WT) kidneys (Figure 1a and b). Immunofluorescence confirmed a drastic decrease in L3MBTL2 expression in all the tubules, including the proximal tubules, as determined by costaining with megalin, in *L3mbtl2* cKO kidneys (Figure 1c and Supplementary Figure S2B).

Aggravated kidney injury in L3mbtl2 cKO mice, 72 hours after cisplatin treatment

The *L3mbtl2* cKO mice showed normal kidney sizes, structures, and function.We therefore induced AKI by a single injection of cisplatin. Cisplatin did not alter L3MBTL2 protein expression (Supplementary Figure S3A). No apparent tubular injury was observed in either *L3mbtl2* cKO or WT kidneys at 24 hours after cisplatin treatment. However, cisplatin treatment for 72 hours induced widespread tubular cell death and patchy loss of tubular cells in *L3mbtl2* cKO mice, whereas these features were much less apparent in WT kidneys (Figure 1d, e, and f). Remarkably, expression of neutrophil gelatinase–associated lipocalin (NGAL), a marker for kidney injury,[29] in the kidney and urine, was induced much more in *L3mbtl2* cKO than in WT mice (Figure 1g). Serum creatinine and blood urea nitrogen levels were significantly higher in L3mbtl2 cKO mice than in WT mice at 72 hours after cisplatin treatment (Figure 1h and i). Taken together, the results suggest that ablation of L3mbtl2 in renal tubules exacerbated cisplatin-induced kidney injury.

Increased DNA damage and p53 activation in tubular cells in L3mbtl2 cKO kidneys 24 hours after cisplatin treatment

Cisplatin induces nuclear DNA damage. If the DNA damage is very severe, apoptotic cascades will be activated.[8,30] Expression of γH2AX, a marker for DNA damage[31] in the kidney, was not yet altered 24 hours after cisplatin treatment in WT mice, but it was already induced to a large extent in *L3mbtl2* cKO mice (Figure 2a and d). Given that the proximal tubules are the principal sites of cisplatin-induced nephrotoxicity, we examined DNA damage in proximal tubular cells by co-staining of γH2AX with megalin. γH2AX and megalin double-positive cell numbers increased 3.82-fold in *L3mbtl2* cKO mice, compared with WT kidneys, 24 hours after cisplatin treatment (Figures 2b and d). Double-positive cell numbers in WT kidneys dramatically increased from 24 hours to 72 hours after cisplatin treatment; 2.35-fold more double-positive cells were seen in *L3mbtl2* cKO kidneys than in WT kidneys 72 hours after cisplatin treatment (Figures 2b, c, and d). The numbers of positive tubular cells for 53BP1, another DNA damage marker, were also increased in *L3mbtl2* cKO kidneys (Supplementary Figure S4).

The central player in activation of DNA damage checkpoints is p53. Initial responses to DNA damage include p53 phosphorylation.[32] We found that p53 phosphorylation at Ser15 was much higher in *L3mbtl2* cKO than in WT kidneys 24 hours after cisplatin treatment (Figure 2e). Phosphorylated and total p53 levels were also increased by *L3mbtl2* deletion 72 hours after cisplatin treatment (Figure 2f). These results indicate that deletion of *L3mbtl2* promoted DNA damage and p53 activation, and these effects were already evident 24 hours after cisplatin treatment.

Increased tubular cell apoptosis in L3mbtl2 cKO kidneys 72 hours after cisplatin treatment

DNA damage, and subsequent p53 activation, may lead to apoptosis governed by a number of p53 target proapoptotic genes, including *Puma, Noxa, Bak,* and *Bax*.[10,33,34] Furthermore, p53 has been found to mediate tubular cell apoptosis induced by cisplatin or ischemia/reperfusion.[6,35–39] We therefore examined proapoptotic genes and apoptosis in cisplatin-treated kidneys. Cisplatin treatment for 24 hours induced *Puma* and *Bax*, but not *Noxa* and *Bak* mRNA expression in WT kidneys. *Puma, Noxa,* and *Bak* expression was significantly higher in *L3mbtl2* cKO kidneys than in WT kidneys (Figure 3a). At 72 hours after cisplatin injection, *Noxa, Bak* and *Bax* expression was higher in *L3mbtl2* cKO than in WT kidneys (Figure 3b). Interestingly, p53 mRNA was increased in *L3mbtl2* cKO kidneys compared with WT kidneys 72 hours after cisplatin treatment (Figure 3b).

Cleaved caspase-3 expression was not detected in either WT or *L3mbtl2* cKO kidneys 24 hours after cisplatin treatment (Supplementary Figure S5). However, caspase-3 and poly(ADP-ribose) polymerase (PARP) cleavages were induced in WT kidneys 72 hours after cisplatin treatment; these were further increased in *L3mbtl2* cKO kidneys (Figure 3c). More cleaved caspase-3 and TUNEL (terminal deoxynucleotidyl transferase [TdT]-mediated dUTP nick end labeling)–positive tubular cells were observed in L3mbtl2 cKO kidneys than in WT kidneys 72 hours after cisplatin treatment (Figure 3d and e). These results suggest that apparent apoptosis occurred in the kidney 72 hours, but not 24 hours, after cisplatin treatment. Deletion of L3mbtl2 increased cisplatin-induced tubular apoptosis, and this effect was evident 72 hours after cisplatin treatment.

Prevention of apoptosis and kidney injury in *L3mbtl2* cKO mice by inhibition of p53 activity after cisplatin treatment

To further determine the role of p53 in the increased apoptosis and kidney injury in *L3mbtl2* cKO mice, we used