FULL LENGTH ARTICLE

Endothelial DLC1 is dispensable for liver and kidney function in mice

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Abstract DLC1 is a focal adhesion molecule that regulates cell polarity, proliferation, migration, and survival. DLC1 functions as a tumor suppressor and its expression is often down-regulated in various malignant neoplasms of epithelial origin. Recent studies have suggested that lack of DLC1 in endothelial cells may contribute to the development of angiosarcoma, and that DLC1 mutations have been identified in patients with nephrotic syndrome, a disease mainly due to leaky glomerular filtration barriers. To demonstrate whether lack of endothelial DLC1 induces angiosarcoma and/or damages glomerular capillaries leading to nephrotic syndrome, we have extended our analyses on endothelial cell-specific DLC1 knockout mice with focuses on their liver and kidney function. Mice were monitored up to 24 months of age. However, no histological or clinical difference was found between DLC1 knockout and wild type mice, indicating that lack of endothelial DLC1 alone does not compromise kidney and liver function in mice.

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Introduction

DLC1 (deleted in liver cancer 1) is a tumor suppressor gene that is originally identified in hepatocellular carcinoma and then in a variety of cancer types, such as lung, colon, prostate, breast, kidney and brain. In cancer cells, its expression is often lost or down-regulated due to genetic or epigenetic modifications, and re-expression of DLC1 suppresses cancer cell proliferation, migration, and colony formation. DLC1 is expressed in most tissues and DLC1 total knockout mice die by E10.5, demonstrating its essential role during embryogenesis. However, the function of DLC1 in adult tissues are not well understood.

Recent studies have shown that DLC1 deficiency may play a critical role in the development of angiosarcoma, which is a rare yet highly malignant soft tissue sarcoma of endothelial cell origin. Angiosarcoma may derive from the endothelial layer of blood or lymph vessels and can occur at anywhere in the body but most of them arise in skin, breast and liver. Hepatic angiosarcoma is also uncommon among
all primary hepatic malignancies yet it is the most frequent
malignant mesenchymal cancer of the liver.11 The patho-
genesis and risk factor of angiosarcoma are currently unclear.

Nephrotic syndrome (NS) is one of the most common
causes of glomerular disease12 and is characterized by
albuminuria, hypoalbuminemia, hyperlipidemia, and
edema.12 It may lead to chronic kidney disease that results
in end-stage renal failure requiring dialysis or renal
replacement for survival. Nephrotic syndrome is the result
of a leaky glomerular filtration barrier, which is consisting
of a three-layer structure of endothelial cells, glomerular
basement membrane (GBM), and podocytes. Dysfunction of
any of these layers will compromise the glomerular filtra-
tion barrier and lead to nepthrotic syndrome. DLC1 muta-
tions were recently identified as the potential cause of
nepthrotic syndrome in several patient families.13 Howev-
er, their direct involvement in the NS development remains
to be demonstrated.

Here, we use endothelial cell-specific DLC1 knockout
mice to examine the roles of DLC1 in the kidney and liver
structure/function with focuses on nephrotic syndrome and
hepatic angiosarcoma development.

Materials and methods

Mouse analysis

Endothelial cell-specific DLC1 knockout mice were generated
by crossing $DLC1^{lox/lox}$ from our laboratory with B6.Cg-Tg
(Tek-cre$^{Ywa/J}$) from Jackson laboratory as described.14
These mice were maintained under a specific pathogen-
free facility at the University of California–Davis. All mouse
related handlings were performed with protocols approved
by the animal ethics committee at our University. Blood and
urine were collected from mice at various ages. Liver and
kidney function chemical panel measurements were con-
ducted at the Comparative Pathology Laboratory at UC Davis.
Kidneys and livers were collected, fixed, sectioned, and
stained with hematoxylin and eosin for histological evalua-
tion. Urine samples were analyzed by SDS-PAGE and Bio-Safe
Coomassie G-250 stain to detect albuminuria.

Immunohistochemistry

Tissue sections were treated with 3% hydrogen peroxide for
30 min to block endogenous peroxidase activity. Slides were
then incubated at 4°C overnight with rabbit anti-mouse
CD31 (#77699, Cell Signaling Technology, Danvers, MA USA)
at 1:100 dilution. Signal was detected with Vectastain ABC
Elite Kit (Vector Laboratories, Burlingame, CA, USA) and
developed with diaminobenzidine substrate. Slides were
counterstained with hematoxylin.

Isolation of mouse endothelial cells

Primary endothelial cells were isolated from $DLC1^{lox/lox}$-Tek
and $DLC1^{lox/lox}$ mice as previously described.14

Figure 1  Histological evaluations of kidneys from $DLC1^{lox/lox}$-Tek and control mice. Representative H&E staining images of kidney
sections from 24 months old $DLC1^{lox/lox}$-Tek or $DLC1^{lox/lox}$ mice. Glomeruli, tubules and blood vessels are well organized (upper
panel) and their structures appear to be normal (lower panel) in mutant and control kidneys.
Statistics

Data were presented as the mean \( \pm SD \). Student’s t test was used for statistical analysis of the difference between two groups. All statistical tests were two-tailed and \( P \) values < 0.05 were considered statistically significant.

Results and discussion

Since recent studies have reported that DLC1 mutations were identified as the potential cause of NS\(^{13}\) and dysfunctional blood vessels in the glomeruli might lead to albuminuria,\(^{15}\) a major feature of NS, and that DLC1 deficiency is implicated in the development of angiosarcoma\(^{8,9}\) and the liver is one of the frequent sites of angiosarcoma,\(^{10}\) we decided to investigate the effects of endothelial DLC1 deficiency on the kidney and liver structure/function using DLC1\(^{lox/lox}\):Tek-Cre (DLC1\(^{lox/lox}\)-Tek) mice. We had previously examined the DLC1\(^{lox/lox}\)-Tek mice and reported that their angiogenesis processes are compromised as demonstrated by gel plug and aortic ring sprouting assays.\(^{14}\) In the current study, mice were further monitored up to 24 months of age. However, we did not observe any noticeable difference between DLC1\(^{lox/lox}\)-Tek and DLC1\(^{lox/lox}\) mice on their overall gross appearance, behavior, and health under the specific pathogen-free (SPF) living condition. Histologically, well organized renal structures were presented in kidneys from both DLC1\(^{lox/lox}\)-Tek and control mice at 24 months of age (Fig. 1). Glomerular, tubular and vascular structures appear to be normal (Fig. 1). Mild interstitial infiltrates were occasionally detected in DLC1\(^{lox/lox}\)-Tek and DLC1\(^{lox/lox}\) kidneys (not shown). In livers, mild lipidosis and inflammatory cell infiltrates were observed in both DLC1\(^{lox/lox}\)-Tek and DLC1\(^{lox/lox}\) mice likely due to aging (Fig. 2). Blood vessels were indistinguishable between DLC1\(^{lox/lox}\)-Tek and DLC1\(^{lox/lox}\) samples (Fig. 2). The distribution patterns of CD31 positive endothelial layers in livers and kidneys were very similar between DLC1\(^{lox/lox}\)-Tek and DLC1\(^{lox/lox}\) mice by immunohistochemistry staining (Fig. 3A). Because anti-DLC1 antibodies were not able to detect mouse endogenous DLC1 by immunohistochemistry staining, we had isolated primary endothelial cells from mice and confirmed the lack of DLC1 expression in endothelial cells from DLC1\(^{lox/lox}\)-Tek mice by immunoblotting analysis (Fig. 3B). Clinically, blood tests for liver and kidney function were shown in Figure 4. No statistically significant difference was found in all 8 markers between DLC1\(^{lox/lox}\)-Tek and DLC1\(^{lox/lox}\) mice. However, if one-tailed, instead of two-tailed, distribution was applied, the \( p \) values of serum alanine transaminase, aspartate transaminase, albumin, BUN, and creatinine levels were lower than 0.05, showing statistical significance. Nonetheless, the levels of these markers in DLC1\(^{lox/lox}\)-Tek mice were still within the ranges of normal liver/kidney functions. Albuminuria was not detected even in 24 months old DLC1\(^{lox/lox}\)-Tek mice (Fig. 3C), whereas albuminuria was
prominent in a 10 weeks old TNS2 knockout mouse, a nephrotic syndrome mouse model. Altogether, lack of endothelial DLC1 does not compromise kidney and liver function, and shows no sign of nephrotic syndrome or hepatic angiosarcoma.

Although the functions of DLC1 in tumorigenesis (especially in carcinoma) have been well recognized, the critical roles of DLC1 in endothelial cells are only revealed recently. These findings include (1) knockout or depletion of DLC1 promotes endothelial cell migration and reduces tube formation activities; (2) down-regulation of DLC1 enhances cell survival, cell cycle and reduces contact inhibition of growth in confluent endothelial cells; (3) silencing of endothelial DLC1 reduces cell stiffness and mimicked leukocyte transmigration kinetics; (4) depletion of endothelial DLC1 disrupts cell polarization in directed collective migration and inhibits the formation of angiogenic sprouts. These findings are mainly concluded from analyses of DLC1-silenced human umbilical vein endothelial cells (HUVECs). However, our previous and current studies using endothelial cell-specific DLC1 knockout mice have demonstrated that endothelial DLC1 is not absolutely required for embryogenesis, liver and kidney function, mouse survival as well as preventing angiosarcoma formation. It is likely that additional “hit(s)” are needed to induce angiosarcoma and our DLC1lox/lox-Tek mouse line will be a useful animal model for identifying and validating the candidates.

While our study demonstrates that endothelial DLC1 is not required for normal renal function, this study does not rule out the potential involvement of DLC1 in nephrotic syndrome through DLC1’s role in the podocyte, which is
another important cell type in the glomerulus. Podocytes adhere to the GBM through their foot processes assembling cell-matrix junction and establishing intercellular junctions that form slit diaphragm filtration barrier, which is essential for normal renal function. Because podocytes have very limited, if any, regenerative capacities, damaged cells are replaced by healthy podocytes migrating to the injury site. Dysregulated focal adhesions and/or cell-cell junctions will impair podocyte function and repair. For example, lack of or mutated α-actinin-4 focal adhesion protein in podocytes leads to progressive proteinuria and glomerular disease. Therefore, we are in the process of generating and analyzing podocyte-specific DLC1 knockout mice to understand the role of DLC1 in podocytes relating to nephrotic syndrome.

Conflict of interests

The authors disclose no potential conflicts of interest.

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