Cre-loxPによるAlport症候群モデルマウスの糸球体特異的なCOL4A5発現回復

Glomerular-specific restoration of COL4A5 expression in Alport syndrome model mice by Cre-loxP system

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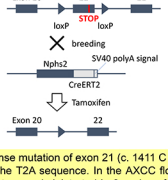
Abstract

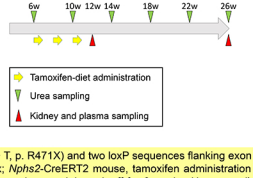
【背景】我々はこれまでに、患者と同様の変異(COL4A5　R471X)を有するAlport症候群モデルマウス(AXCCマウス)が6週齢からすでに糸球体基底膜異常を呈し、26週齢以降顕著に末期腎不全様の病態を示すことを報告した[1]。前回は本マウスの尿細管間質における慢性病変と三次リンパ組織形成に関する解析について報告したが、今回、R471X変異を有するエクソン21をloxPで挟んだAXCC floxマウスと、足細胞特異的にCreERT2を発現するマウスを交雑させ、エクソンスキッピングにより腎機能が改善するかを検討した。
【方法】タモキシフェンの反復投与(6週齢から1週毎に投薬、休薬を3サイクル)群と非投与群について経時的な尿検査を行い、12、26週齢時に腎組織、および血液を採材し、免疫染色、病理組織検査、血液生化学的検査を実施した。
【結果】投与群において、投与終了直後の10週齢から継続的に尿中Albの改善が示された。12、26週齢時の免疫染色では糸球体特異的なCOL4A5の発現回復が認められ、26週齢時には非投与群と比較して糸球体硬化、炎症細胞浸潤および線維化が軽減していた。
【結語】Cre−loxPシステムを用いたエクソンスキッピングにより、糸球体特異的にCOL4A5の発現を回復させ、腎機能を改善させることができた。

Methods

A. Exon skipping strategy



B. Protocol of Tamoxifen administration



	Genotype (flox/CreERT2)	Tamoxifen	Kidney and plasma sampling	N
1	wild/wild	-	26w	3
2	flox/CreERT2	-	12w	2
3	flox/CreERT2	+	12w	2
4	flox/CreERT2	-	26w	4
5	flox/CreERT2	+	26w	4

flox: AXCC flox, CreERT2: *Nphs2*-CreERT2
The tamoxifen-diet was administered at a concentration of 0.025%(g/g).

A) The AXCC flox mouse carries a nonsense mutation of exon 21 (c. 1411 C > T, p. R471X) and two loxP sequences flanking exon 21 in *Col4a5* allele. The *Nphs2*-CreERT2 mouse carries the CreERT2 sequence linked to the *Nphs2* gene via the T2A sequence. In the AXCC flox; *Nphs2*-CreERT2 mouse, tamoxifen administration results in glomerular-specific deletion of mutated *Col4a5* exon21. B) Starting at 6 weeks of age, tamoxifen-diet was administrated in 3 cycles of 1 week on and 1 week off for 6 weeks. Urea sampling was performed every 4 weeks. Kidney and plasma sampling were performed at 12 and 26 weeks of age. The phenotypes of AXCC flox; *Nphs2*-CreERT2 mice were investigated by the measurement of urinary and blood biochemical parameters, immunohistochemistry and light microscopy to confirm the effects of tamoxifen administration. Tamoxifen administered flox/CreERT2; flox/CreERT2, Tam(+), non-administered flox/CreERT2; flox/CreERT2, Tam(-).

Results

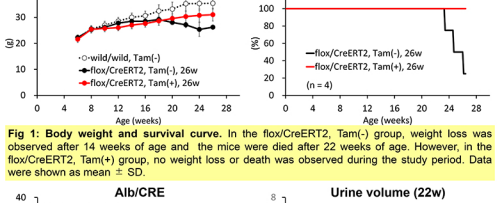


Fig 1: Body weight and survival curve. In the flox/CreERT2, Tam(-) group, weight loss was observed after 14 weeks of age and the mice were died after 22 weeks of age. However, in the flox/CreERT2, Tam(+) group, no weight loss or death was observed during the study period. Data were shown as mean ± SD.

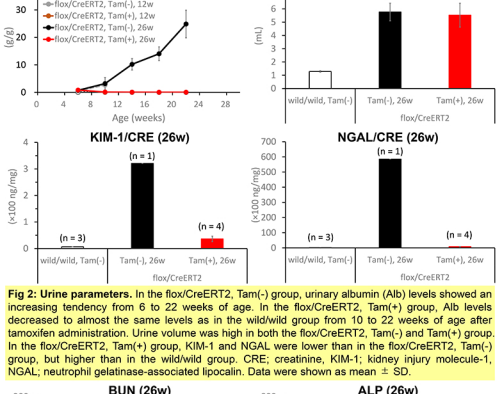


Fig 2: Urine parameters. In the flox/CreERT2, Tam(-) group, urinary albumin (Alb) levels showed an increasing tendency from 6 to 22 weeks of age. In the flox/CreERT2, Tam(+) group, Alb levels decreased to almost the same levels as in the wild/wild group from 10 to 22 weeks of age after tamoxifen administration. Urine volume was high in both the flox/CreERT2, Tam(-) and Tam(+) group. In the flox/CreERT2, Tam(+) group, KIM-1 and NGAL were lower than in the flox/CreERT2, Tam(-) group, but higher than in the wild/wild group. CRE; creatinine, KIM-1; kidney injury molecule-1, NGAL; neutrophil gelatinase-associated lipocalin. Data were shown as mean ± SD.

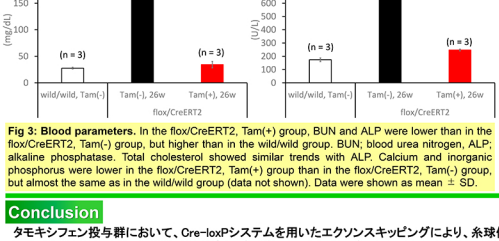


Fig 3: Blood parameters. In the flox/CreERT2, Tam(+) group, BUN and ALP were lower than in the flox/CreERT2, Tam(-) group, but higher than in the wild/wild group. BUN; blood urea nitrogen, ALP; alkaline phosphatase. Total cholesterol showed similar trends with ALP. Calcium and inorganic phosphorus were lower in the flox/CreERT2, Tam(+) group than in the flox/CreERT2, Tam(-) group, but almost the same as in the wild/wild group (data not shown). Data were shown as mean ± SD.

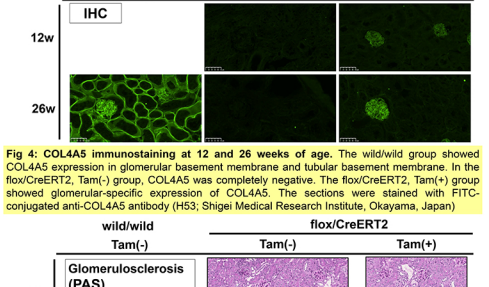


Fig 4: COL4A5 immunostaining at 12 and 26 weeks of age. The wild/wild group showed COL4A5 expression in glomerular basement membrane and tubular basement membrane. In the flox/CreERT2, Tam(-) group, COL4A5 was completely negative. The flox/CreERT2, Tam(+) group showed glomerular-specific expression of COL4A5. The sections were stained with FITC-conjugated anti-COL4A5 antibody (H53; Shigei Medical Research Institute, Okayama, Japan)

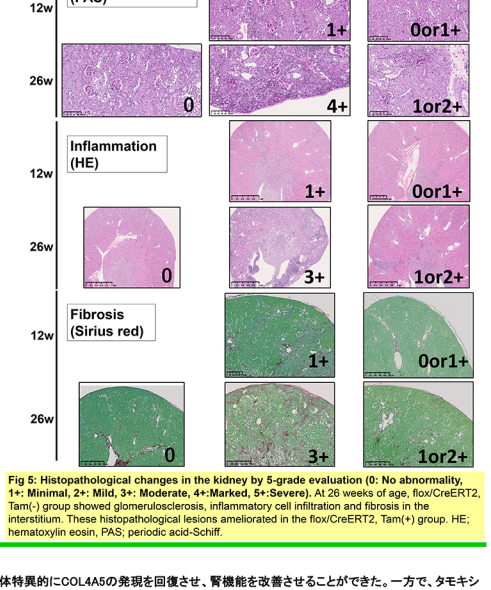


Fig 5: Histopathological changes in the kidney by 5-grade evaluation (0: No abnormality, 1+: Minimal, 2+: Mild, 3+: Moderate, 4+:Marked, 5+:Severe). At 26 weeks of age, flox/CreERT2, Tam(-) group showed glomerulosclerosis, inflammatory cell infiltration and fibrosis in the interstitium. These histopathological lesions ameliorated in the flox/CreERT2, Tam(+) group. HE; hematoxylin eosin, PAS; periodic acid-Schiff.

Conclusion

タモキシフェン投与群において、Cre−loxPシステムを用いたエクソンスキッピングにより、糸球体特異的にCOL4A5の発現を回復させ、腎機能を改善させることができた。一方で、タモキシフェン投与群で野生型と同程度までは病態が改善していないことが、血中、尿中パラメータの値や病理評価により明らかになった。以前我々はAXCCマウスの腎乳頭部に上皮の壊死脱落や管腔の拡張、繊維化などの病変が強く出ることを報告しているが、腎乳頭部でのCOL4A5発現は回復していないことから、尿量の改善が認められないことと併せ、腎乳頭部の病変がタモキシフェン投与群での病態に強く関連している可能性が考えられる。今後、尿細管特異的なCOL4A5発現マウスを作製、解析することで、尿量、さらには病態の改善が認められるかどうか、改善のメカニズム解析も含めて検討していく予定である。

【参考文献】1. Hashikami K., Asahina M., Nozu K., Iijima K., Nagata M., Takeyama M. Establishment of X-linked Alport syndrome model mice with a Col4a5 R471X mutation. Biochem Biophys Rep. 2019;17:81–86. doi: 10.1016/j.bbrep.2018.12.003.