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이중 형질주입을 이용한 세포-특이적 녹다운 마우스의 제작

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Double Transduction of a Cre/LoxP Lentiviral Vector: A Simple Way to Generate Cell-Specific Knockdown Mice

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Background: Genetically engineered mice with gene overexpression or knockout have been used to elucidate the precise roles of specific genes in the pathogenesis of a disease. For downregulated genes, transgenic mice generated by conventional knockout techniques using oocytes or embryonic stem cells are most commonly used, but this method has certain limitations. We have devised a simple way to knock down a specific gene in a cell-specific pattern in adult mice by lentivirus (LV)-assisted transfer of short hairpin RNA.

Methods: In vitro, for LV transfection, the LV suspension containing LV-Hoxb7 Cre and/or LV-Aquaporin3 shRNA (shAQP3) was added to cultured mouse renal collecting duct cells (CDs) and mouse mesangial cells (MMCs). In vivo, there were three models to check efficiency of each virus. First, LV-Hoxb7 Cre is injected into the loxP-EGFP mice to check efficiency of the Hoxb7 promotor. Second, LV-loxP shAQP3 is injected into the Hoxb7 Cre transgenic mice to check efficiency shAQP3. Third, C57BL6/J mice were assigned to one of the four groups, and were injected with PBS (Con), LV-Hoxb7 Cre, LV-loxP shAQP3, or LV-Hoxb7 Cre+LV-loxP shAQP3. Western blot, immunofluorescence staining, real-time PCR were performed to confirm expressions of AQP3, EGFP, mCherry.

Results: In vitro, LV-Hoxb7 Cre, worked only in CDs due to the presence of Hoxb7 in CDs but not in MMCs. Moreover, combined infection of CDs with LV-Hoxb7 Cre and LV-loxP shAQP3 significantly inhibited the protein expression of AQP3 along with the disappearance of EGFP protein expression, suggesting that LV-Hoxb7 Cre and LV-loxP shAQP3 used in the present study worked together effectively. In vivo, Kidney CD-specific AQP3-knockdown mice were generated by consecutive injection of LV-Hoxb7 Cre and LV-loxP shAQP3 in adult C57BL6/J mice. In mice treated with LV-Hoxb7 Cre alone, mCherry protein expression occurred only in CDs, while LV-loxP shAQP3 injection alone resulted in an increase in EGFP expression in all cells. In kidney, AQP3 expression in mice injected with LV-Hoxb7 Cre or LV-loxP shAQP3 alone did not differ, but consecutive injection of LV-Hoxb7 Cre and LV-loxP shAQP3 significantly reduced AQP3 expression. However, the expressions of AQP3 in other organs did not differ between the groups.

Conclusion: These findings suggest that double transduction of Cre- and loxP-based LV can be a simple way to generate cell-specific knockdown mice, and this method may also be applicable to other species.

Key Words: Lentiviral 벡터, Cre/LoxP, Hoxb7 프로모터
Lentiviral vector, Cre/LoxP, Hoxb 7 promotor