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Tamoxifen-Inducible Cre-Recombination in Articular Chondrocytes of Adult *Col2a1-CreER^{T2}* Transgenic Mice

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Summary

Objective—To determine the specificity and efficiency of the tamoxifen (TM)-induced Cre recombination in articular chondrocytes of adult *Col2a1-CreER^{T2}* transgenic mice.

Methods—*Col2a1-CreER^{T2}* transgenic mice were bred with Rosa26R reporter mice. 2-week-old *Col2a1-CreER^{T2};R26R* mice were administered TM for 5 days and were sacrificed 1 and 6 months after TM induction. X-Gal staining was performed.

Results—Efficient Cre recombination is achieved in adult articular chondrocytes 1 and 6 months after TM induction.

Conclusion—Our findings demonstrate that the *Col2a1-CreER^{T2}* transgenic mouse model is a valuable tool to target genes specifically expressed in articular chondrocytes in a temporally-controlled manner in adult mice.

Keywords

Articular chondrocyte; Tamoxifen; Cre-recombination

Introduction

Osteoarthritis (OA) is a degenerative joint disease that is common in aging and mainly occurs in both humans and animals. Alternations of gene expression in articular chondrocytes likely play an important role in the development of OA[1-3]. Articular chondrocytes are the only cell type in articular cartilage and they produce and maintain the extracellular matrix, which is responsible for providing the appropriate function to articular tissue[4]. Articular cartilage has minimal reparative potential and degradation of articular cartilage can have severe consequences. For this reason, envisioning strategies to maintain articular cartilage is an important objective in the arthritis field. Unfortunately, very little is known regarding the mechanisms involved in establishing and maintaining the articular chondrocyte phenotype.

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In order to target chondrocyte-specific genes in a tissue-specific and inducible manner, we have recently generated a transgenic mouse model, *Col2a1-CreER^{T2}*, in which expression of the Cre recombinase is driven by the chondrocyte-specific col2a1 promoter in a TM-inducible fashion. We have previously reported that chondrocyte-specific Cre-recombination was observed in growth plate chondrocytes of these mice postnatally[5]. In the present studies, we show that Cre-recombination is maintained in articular chondrocytes of adult transgenic mice.

Methods, results and discussion

Col2a1-CreER^{T2} transgenic mice were crossed with Rosa26 reporter mice in which expression of *Escherichia coli* β-galactosidase can be induced by Cre-mediated recombination[6]. Offspring were genotyped by PCR using Cre-specific primers and primers for detecting *R26R* alleles. The sequences of PCR primers for genotyping *Col2a1-CreER^{T2}* mice are: 5′-CCTGGAAAATGCTTCTGTCCGTTTGCC-3′ (forward primer) and 5′-GAGTTGATAGCTGGCTGGTGGCAGAG-3′ (reverse primer) and the size of the PCR product is 600-bp. The sequences of PCR primers for genotyping Rosa26R reporter mice are: R1295, 5′-GCGAAGAGTTTGTCCTCAACC-3′; R523, 5′-GGAGCGGGAGAAATGGATATG-3′ and R26F2, 5′-AAAGTCGCTCTGAGTTGTTAT-3′. The 600-bp PCR product was detected in wild-type mice and the 325-bp PCR product was detected in homozygous Rosa26R mice. In heterozygous Rosa26R mice, both 600 and 325-bp PCR products were detected.

To determine if the *Col2a1-CreER^{T2}* transgenic mice can be used to target genes expressed in articular chondrocytes in adult mice, they were bred with Rosa26 reporter mice to follow the time course of reporter expression. TM induction was performed in 2-week-old *Col2a1-CreER^{T2};R26R* transgenics (1 mg TM/mouse/day for 5 days) and mice were sacrificed 1 and 6 months after the last injection. The long bones were harvested and fixed in 0.2% glutaraldehyde, decalcified, and processed for frozen sectioning followed by X-Gal staining. Nuclear Fast Red staining was performed as a counter stain. The recombination efficiency was determined by counting the X-Gal-positive cells divided by the total number of cells on the articular surface (n=4). The results showed that TM induced efficient Cre-recombination in articular chondrocytes 1 and 6 months after TM induction (Fig. 1a,b). Eighty five percent and 82% Cre-recombination efficiency was achieved in articular cartilage area of these mice respectively (Fig. 1a,b) (n=4). It should be noted that some cells in the deep zone of the articular cartilage were negative for X-Gal staining; this may be due to the poor penetration of tamoxifen into these areas.

Overall, these results suggest that *Col2a1-CreER^{T2}* transgenic mice provide a strategy for causing tissue specific gene deletion in a temporal manner. Since the percentage of X-Gal-positive articular chondrocytes is similar at 1 and 6 months after TM induction, there is likely minimal turnover or cell renewal in articular cartilage, consistent with the limited reparative potential of this tissue.

Using the 3 kb Col2a1 promoter, Ovchinnikov et al.[8] generated *Col2a1-Cre* transgenic mice which show efficient Cre-recombination in chondrocytes during mouse development. A similar approach was also reported by Nakamura et al.[9] who generated *Col2a1-CreER^{T}* transgenic mice using the same Col2a1 promoter construct as we used in our transgenic mice. Although efficient Cre-recombination was demonstrated in the embryonic and postnatal skeleton, it becomes undetectable in 12-week-old *Col2a1-CreER^{T}* transgenic mice[9]. Several factors such as differences in transgene constructs, integration sites and copy numbers may contribute to these phenotypic distinctions.

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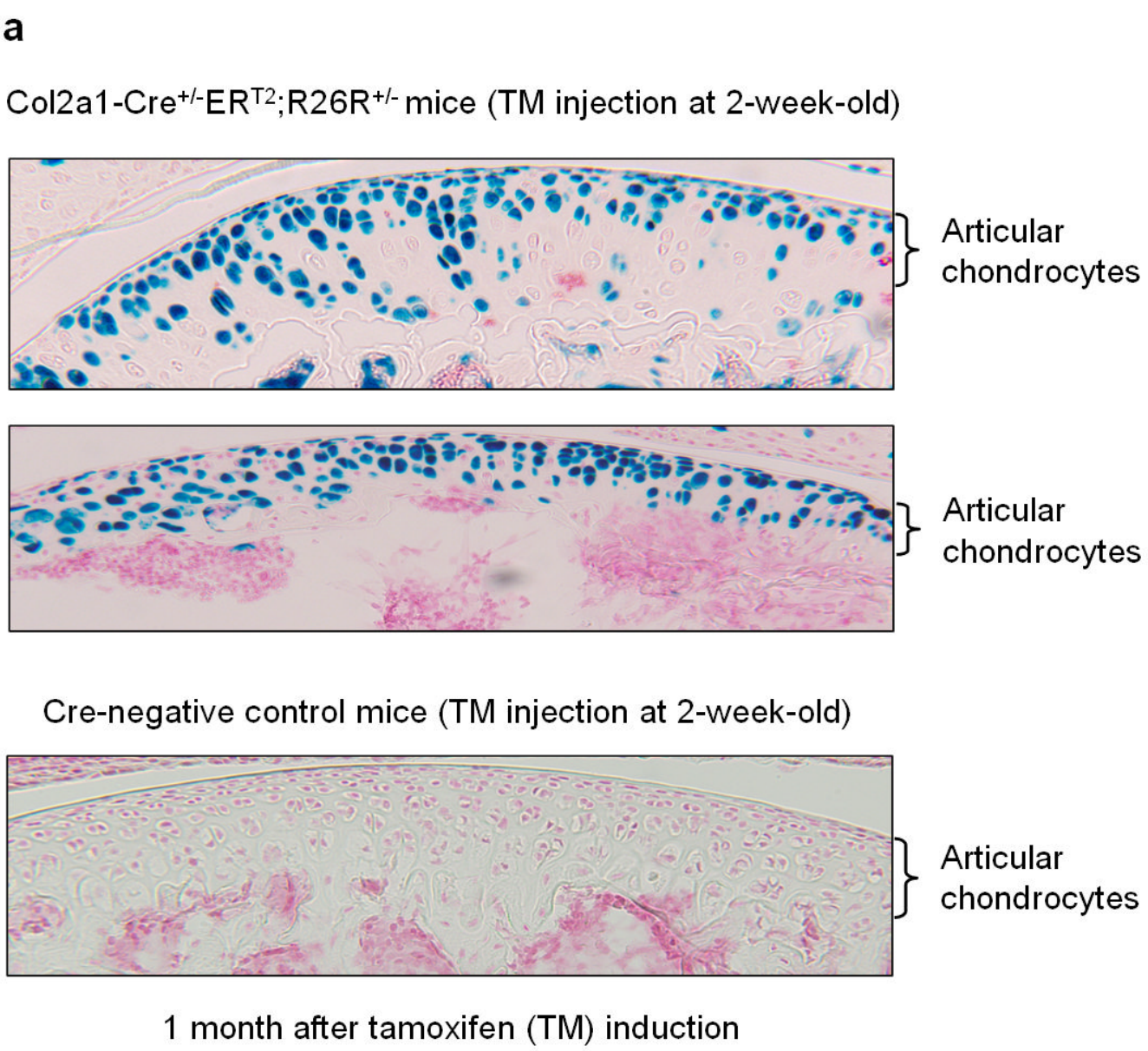

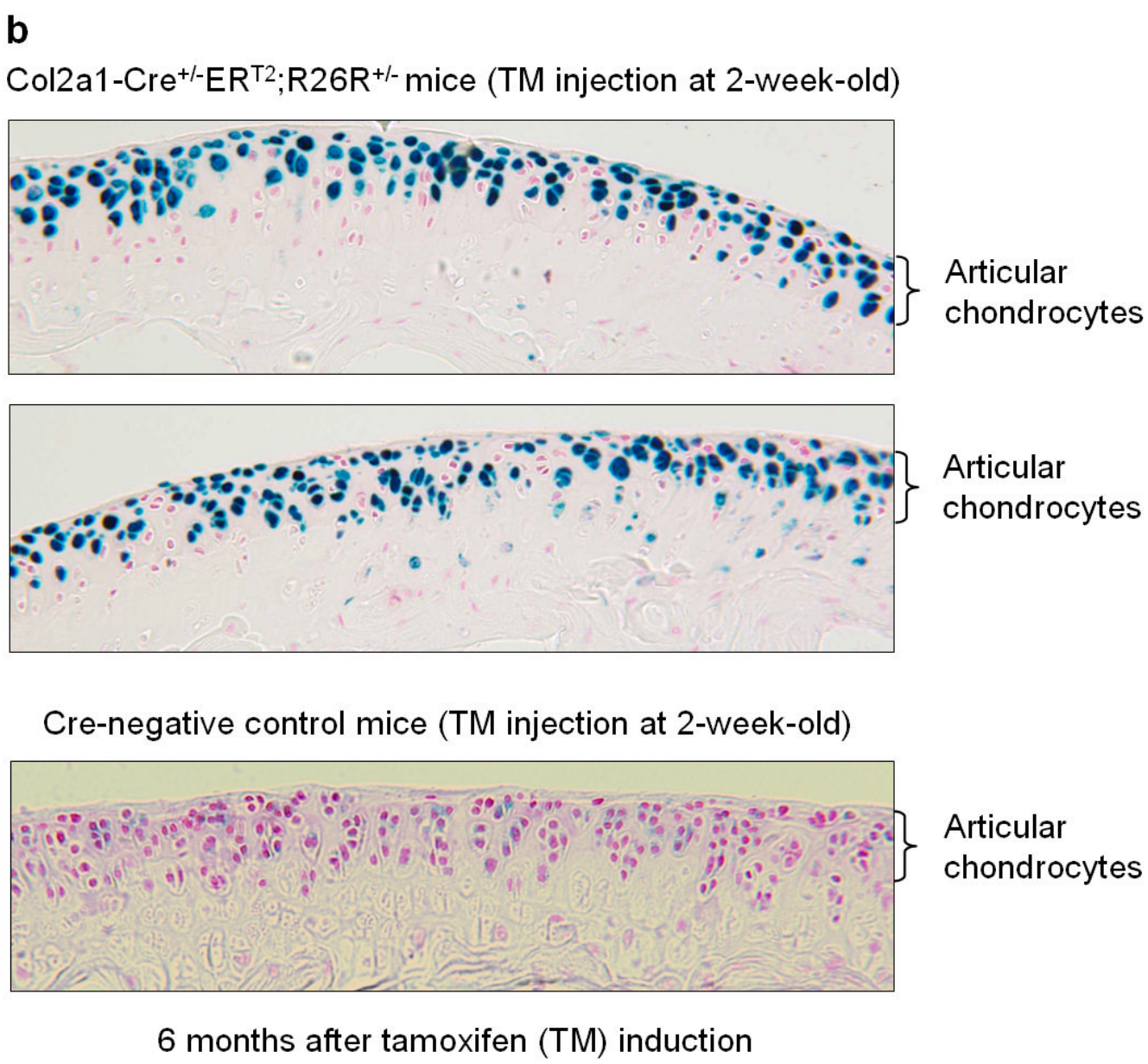

Fig. 1.

Histological analyses of the *Col2a1-Cre*$^{+/-}$*ER*^{T2}*;R26R*$^{+/-}$ mice. 2-week-old *Col2a1-Cre*$^{+/-}$*ER*^{T2}*;R26R*$^{+/-}$ double transgenic mice and Cre-negative control littermates were injected with TM (1 mg/mouse/day for 5 days). Mice were sacrificed 1 (**a**) and 6 months (**b**) after injections were completed and samples of long bones were fixed, decalcified and processed for frozen section preparation followed by X-Gal staining. Sections were then counterstained by Nuclear Fast Red. Articular chondrocytes of double transgenic mice that received TM showed X-Gal positive staining (**a,b**). Recombination efficiency was evaluated by counting the X-Gal-positive cells divided by total cell numbers in articular cartilage area from 3 non-consecutive histological sections of four transgenic mice (n=4). High Cre-recombination efficiency was achieved in articular chondrocytes of the transgenic mice 1 and 6 months after TM induction.