INTRODUCTION:
The development of articular joint has not been well understood. There are currently two views on joint development. The widely accepted view is that the limb buds contain uninterrupted cartilaginous skeletal structures corresponding to multiple skeletal elements and bearing no obvious sign of joint development; the chondrocytes at the presumptive joint sites of the cartilaginous anlagen undergo a de-differentiation process to form joint interzone; and the joint interzone gives rise to articular joint including articular chondrocytes. The alternative view believes that the long bone anlagen develop as discrete, discontinuous, cartilaginous elements; the cartilaginous elements and presumptive joint regions originate from the same cell population that diverges into two distinct populations before, or during, overt chondrogenesis. Understanding joint development is important not only for developmental biology but also for applying the knowledge to tissue engineering/regeneration of articular cartilage. The objective of the present study was to determine expression pattern of doublecortin (Dcx) gene in the early stages of joint development. Using Dcx as a marker, the lineage differentiation of articular and endochondral chondrocytes was revealed.

METHODS:
This study was approved by the Institutional Animal Care and Use Committee. A Dcx-heterozygous LacZ knock-in mouse strain was used, in which LacZ gene replaced the second and third coding exons of Dcx and was fused to the first exon, so that LacZ expression represented the endogenous expression of Dcx. The embryos were stained with X-gal solution at a final concentration of 1 mg/ml. Another Dcx-EGFP transgenic mouse strain used contained an Enhanced Green Fluorescent Protein (EGFP) reporter inserted immediately upstream to Dcx coding sequence. Thus, EGFP expression faithfully represented endogenous Dcx expression and was captured under a fluorescent microscope. Dcx mRNA expression was detected by qRT-PCR and in situ hybridization (ISH) using Dcx cRNA probes.

RESULTS:
X-gal staining revealed that Dcx promoter-driven LacZ expression was observed in the mesenchymal progenitor cells in the mouse embryonic limb buds from E9.5 to E12.5 (Figure, left column, A to D). At these stages, Dcx promoter-driven EGFP expression was very weak, particularly at E9.5 to E10.5 (Figure, middle column, A to D). The difference between X-gal staining and EGFP signal was caused by the enzyme activity of LacZ that amplified the LacZ signal. At E13.5 when the joint interzone was formed, the blue X-gal staining and EGFP signal were concentrated in the joint interzone whereas the non-joint part of the anlagen (where the endochondral chondrocytes resided) lost LacZ and EGFP expression (Figure, left and middle columns, E). This pattern became more obvious at E14.5 (Figure, left and middle columns, F). Dcx mRNA was detected in the mouse embryonic limb buds at E9.5 and E10.5 by real-time PCR (Figure, right column, A & B). Dcx mRNA was also detected in the limb buds by in situ hybridization (Figure, right column, C & D). Of note, Dcx expression was originally found in the neurons. Thus, the neural tissues served as positive controls while the viscera were negative controls (Figure, right column, B-D). Dcx mRNA was concentrated in the digit joints at E14.5 (Figure, right column, E).

DISCUSSION:
The present study demonstrates that Dcx is expressed in the mesenchymal progenitor cells in the mouse embryonic limb buds. A population of the mesenchymal progenitor cells continues Dcx expression after they have differentiated into joint interzone cells and then articular chondrocytes. In contrast, the endochondral chondrocytes lose Dcx expression when the mesenchymal progenitor cells have differentiated into endochondral chondrocytes. These data support a concept that the articular and endochondral chondrocytes share the same mesenchymal progenitor cells that express Dcx. The results suggest that articular chondrocytes never go through an “endochondral” stage. This is because the articular chondrocyte lineage (from the progenitor cells to joint interzone cells, and then to articular chondrocytes) continuously expresses Dcx. If articular chondrocytes were derived from the endochondral chondrocytes that do not express Dcx, there must be a time (before, during, or after joint interzone formation) when the cells at the presumptive joint site do not express Dcx. In fact, we did not observe such a gap period in terms of Dcx expression at the joint site even under continuous observation of the ex vivo cultured Dcx-EGFP limbs (data not shown). Therefore, these new findings do not support the notion that articular chondrocytes are derived from de-differentiated endochondral chondrocytes. Instead, the current data support the view that the lineages of articular and endochondral chondrocytes bifurcate at the stage of endochondral chondrogenesis.

Dcx expression in articular chondrocytes is a recent discovery. There are many aspects of Dcx that remain unknown, such as how Dcx expression is regulated and whether or not Dcx plays any role in directing stem/progenitor cells to differentiate into articular chondrocyte lineage.

Figure. Left column: X-gal staining. Dcx expression was represented by the blue X-gal staining of LacZ expression in the Dcx-heterozygous LacZ knock-in mouse embryos. Arrows in (B) indicate the neural tube and nerve fibers; arrow in (C) indicates the wedge under the apical ectodermal ridge that does not express Dcx; arrow in (D) indicates the digit rays; arrows in (E) indicate the shoulder, elbow, metacarpal, and digit joints, respectively; and arrows in (F) indicate the metacarpal and digit joints, respectively. Middle column: Dcx-EGFP. Dcx expression was represented by the green fluorescent signals in the Dcx-EGFP mouse embryos. The dotted areas in (A) and (B) indicate the forelimb buds. Arrow in (C) indicates the limb; arrows in (D) indicate the digit rays; and arrows in (E) and (F) indicate the elbow, metacarpal, and digit joints, respectively. Right column: Dcx mRNA. Dcx mRNA expression in the wild-type mouse embryos. A: E10.5 embryo. N, neural tissue; FL, forelimb; HL, hindlimb; V, viscera. B: Dcx mRNA levels analyzed by qRT-PCR. C: E10.5 embryo shows Dcx mRNA expression in the forelimb bud (arrow) and hindlimb bud (arrowhead). D: Dcx mRNA in the forelimb bud (arrow). E: Dcx mRNA in the digit joints (arrows).