IMMATURE OSTEOBLASTS EXPRESS THE PRO-ALPHA2(XI) COLLAGEN GENE DURING BONE FORMATION IN VITRO AND IN VIVO.

+*Urabe, K; *Jingushi, S; *Okazaki, K; *Ikenoue, T; *Sakai, H; *Iwamoto, Y  
+*Department of Orthopaedic Surgery, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan. Maidashi 3-1-1, Higashi-ku, Fukuoka, Japan 812-8582, +81-92-642-5487, Fax: +81-92-642-5507, urabek@ortho.med.kyushu-u.ac.jp

INTRODUCTION

The type XI collagen molecule is a heterotrimer composed of three distinct subunits: α1(XI), α2(XI) and α3(XI). Type XI collagen has been primarily identified as a minor extracellular matrix component in cartilage. However, expression of the pro-α2(XI) collagen gene (COL11A2) has recently been detected in various non-cartilaginous tissues, such as muscle, brain and calvaria. However, it is still unclear which osteoblasts predominantly express COL11A2 during the process of osteoblast differentiation during bone formation. In this study, we analyzed the temporal and spatial expression of COL11A2 in cultured osteoblast-like cells and in the healing of rat femoral shaft fractures by Northern blot analysis and in situ hybridization in order to identify when osteoblasts express COL11A2 during bone formation in vitro and in vivo. We also demonstrated the splicing pattern of exons 6-8 of α2(XI) mRNA during bone formation both in vitro and in vivo.

MATERIALS AND METHODS

Fetal rat calvarial cell culture

Primary cultures of fetal rat calvarial (FRC) cells were prepared as described by Owen et al (1). Cells were harvested on days 0, 5, 9, 14 and 19 after confluence. Total cellular RNA was extracted and the expression of COL11A2 was detected by Northern blot analysis. A full-length (774-bp) fragment of mouse pro-α2(XI) collagen cDNA was used as a probe (2).

Fracture callus

In Sprague-Dawley rats (300 - 350 g), bilateral closed femoral fractures were performed by using total RNA from cultured osteoblasts and fracture calluses. A set of primers was prepared from exon 5 and exons 9-10. RT-PCR was performed by using total RNA from cultured osteoblasts and fracture calluses.

RESULTS

COL11A2 expression in the FRC cells

During the primary culture, mineralized nodules were observed from day 9 after confluence. COL11A2 was highly expressed on days 0 and 5, but the level of expression was found to have rapidly decreased on days 9, 14 and 19 (Fig. 1).

COL11A2 expression during new bone formation in rat femoral fracture callus

On day 7 after fracture, intramembranous ossification proceeded, and newly formed trabecular bone was observed on the cortex (Fig. 2A). COL11A2 signals were detected in osteoblastic cells in the newly formed trabecular bone (Fig. 2B). In the fibrous periosteum and subperiosteal cells, COL11A2 signals were not expressed. On day 9 after fracture, the distribution of COL11A2 signals was restricted in the osteoblastic cells located superficially in the newly-formed trabecular bone. On day 14, the number of positive cells was decreased.

DISCUSSION

COL11A2 was expressed during the early differentiation phase of this culture. Our previous study showed that when COL11A2 was highly expressed, pro-α1(I) collagen gene expression was at high levels and the AP gene had begun to be expressed, but the OC gene had not yet been detected. These results indicate that immature cultured-osteoblasts express COL11A2.

As expected, high levels of COL11A2 mRNA were seen in the proliferating chondrocytes during chondrogenesis and endochondral ossification in the rat femoral fracture callus. On the other hand, COL11A2 was detected in the osteoblasts lined on newly-formed trabecular bone. According to the maturation and remodeling of the trabecular bone, the distribution of the signals for COL11A2 was limited to the superficial osteoblasts of the newly-formed trabecular bone. The character of osteoblasts in the subperiosteal region of the trabecular bone is more immature than that in the deeper layer. Therefore, our results suggest that COL11A2 mRNA is expressed in the immature osteoblasts in the rat fracture callus, as well as in the cultured FRC cells.

The pro-α 2(XI) collagen transcript in cartilage lacks the 6, 7 and 8 exons. On the other hand, FRC cells and hard callus contained several mRNAs corresponding to longer splice variants including exons 6-8. The expression of COL11A2 including at least one of exons 6-8 may be important for bone formation.

REFERENCES