GENE EXPRESSION IN CHONDROGENESIS DURING FRACTURE HEALING AND THE EFFECT OF BASIC FIBROBLAST GROWTH FACTOR

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Introduction: Chondrogenesis occurring in soft callus is one of the major histological processes in the healing of fractures, though the molecular and cellular events, which regulate this process, have not been fully elucidated. In the present study, using a standardized rat closed fracture model, we examined the spatial and temporal gene expression for cartilage-related proteins and extensively characterized the chondrogenesis during fracture healing. In addition, we percutaneously injected bFGF into the fracture site and analyzed the effect of exogenous bFGF on the chondrogenesis.

Materials and Methods: Experimental animals and fracture model: Closed middiaphyseal fractures were created in the right femurs of SD rats (1). Immediately after fracture, 100µg of rhbFGF in 200µl fibrin gel was percutaneously injected into the fracture sites (FGF-injected callus; n=51). In control animals, only the carrier was injected (control callus; n=53). The rats were euthanized at 4, 7, 10, 14, 21 and 28 days postoperatively, and the fractured femora were fixed, decalcified and embedded in paraffin. In situ hybridization (ISH): Sections were hybridized with DIG labeled cRNA probes for pro-α1 (II) collagen (COL2A1), pro-α1 (X) collagen (COL10A1) and osteopontin (OPN) (2, 3). Two-color ISH: To detect chondrocytes expressing two different genes simultaneously, two-color ISH was carried out (4).

Northern blot analysis: Total RNAs fractionated on 1-% agarose gel were hybridized with cDNA probes for COL2A1, COL10A1 and OPN. Immunohistochemistry: To evaluate cell growth activity, sections were reacted with antibody for PCNA. Clearance of injected bFGF: 125I-labeled rhbFGF was injected into the fracture site, and the radioactivity remaining in the callus was counted.

Results: Control callus: In the soft callus, COL2A1 was detectable in proliferative chondrocytes from day 4 after fracture, and COL10A1 was expressed in hypertrophic chondrocytes from day 7. Pre-hypertrophic chondrocytes co-expressed COL2A1 and COL10A1. In the soft callus from day 10, OPN gene expression was found in the layers of hypertrophic chondrocytes adjacent to hard callus. Distributions of COL10A1 and OPN were mutually exclusive, indicating that OPN is expressed in terminally differentiated chondrocytes and contributes to the calcification process at the front of endochondral ossification. Exogenous bFGF markedly increased the size of the soft callus, in which enlargement of the cartilage area was evident on days 14 (Fig.1A, B) and 21. On day 14, both COL2A1 and COL10A1-expressing cells were more widely distributed (Fig.1C, D), and the amounts of COL2A1 and COL10A1 genes in the callus were both approximately 2-fold increased compared with controls (Fig.2A, B, D, E). Temporal patterns of expression for COL2A1, COL10A1, and OPN were, however, principally identical to those found in control calluses. The number of PCNA-positive cells was apparently increased in the non-cartilaginous area in the soft callus on day 4. At days 1, 4 and 7 after the injection, the radioactivities remaining in the calluses were 81%, 24% and 6%, respectively.

Discussion: In this model of fracture healing, gene expressions for COL2A1, COL10A1 and OPN were site- and stage-specific and highly reproducible. Detection of the molecular alteration of these genes enabled us to evaluate the precise in vivo action of bFGF on the chondrogenesis in fracture callus. The results demonstrate that exogenous bFGF markedly enhances the proliferation of chondroprogenitor cells in the soft callus without suppressing their differentiation into chondrocytes, contributing to the formation of large cartilage tissues. The administration of bFGF does not affect the synthesis of cartilage matrix proteins nor the maturation or calcification of the cartilage matrices. From these findings we conclude that, during fracture healing, bFGF has a capacity to enlarge the cartilage tissues having normal architecture without affecting their maturation.

Fig. 1: Sections on day 14 were stained with toluidine blue (A, B), and hybridized with COL2A1 antisense probe (C, D). Areas showing metachromasia in FGF-injected callus (B) were larger than those in control (A). In FGF-injected callus (D), COL2A1 signals were widely distributed compared with control (C).

Fig. 2: Northern analysis of total RNA from fracture callus in control group and FGF-injected group (n.f.: normal femur, D4: day4, D7: day7, D14: day14, D21: day21, D28: day28 post-fracture). Panels (D), (E) indicate relative gene expressions of COL2A1 and COL10A1, respectively.


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