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Expression of mRNAs for collagens and other matrix components in dedifferentiating and redifferentiating human chondrocytes in culture

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Cell cultures were initiated from epiphyseal cartilages, diaphyseal periosteum, and muscle of 16-week human fetuses Total RNAs isolated from these cultures were analyzed for the levels of mRNAs for major fibrillar collagens, two proteoglycan core proteins and osteonectin In standard monolayer cultures the differentiated chondrocyte phenotype was replaced by a dedifferentiated one the mRNA levels of cartilage-specific type II collagen decreased upon subculturing, while those of types I and III collagen, and the core proteins increased When the cells were transferred to grow in agarose, redifferentiation (reappearance of type II collagen mRNA) occurred Fibroblasts grown from periosteum and muscle were found to contain mRNAs for types I and III collagen and proteoglycan cores When these cells were transferred to agarose they acquired a shape indistinguishable from chondrocytes, but no type II collagen mRNA was observed

Cartilage, Chondrocyte, Collagen, Proteoglycan

1. INTRODUCTION

Differentiation of mesenchymal cells into chondrocytes during chondrogenesis and fracture healing involves major changes in their gene expression: a new set of genetically distinct collagens and proteoglycans is produced into the extracellular matrix (ECM) [1]. Studies on matrix production by cultured chondrocytes have demonstrated a remarkable lability of their phenotype: in monolayer culture, chondrocytes dedifferentiate and reduce the production of cartilage-specific macromolecules, type II collagen and chondroitin sulfate (ChS) proteoglycan, and start producing type I and type III collagens [2]. Interestingly the dedifferentiated cells can be reverted to reexpress the differentiated phenotype by modulating culture conditions [3,4].

Little is known of the regulation of collagen gene expression during these phenotypic changes. Studies with cultured chick chondrocytes have revealed discrepancies between transcription rates, mRNA levels and production of type I collagen chains, and thus suggest an important role for posttranscriptional control [5,6]. The biological significance of these observations remains unknown since chondrocytes do not contain type I collagen mRNA in vivo except during the very early stages of chondrogenesis [7–9]. In the present study human epiphyseal chondrocytes cultured under dedifferentiating and redifferentiating conditions were analyzed at mRNA level for phenotype-related regulation of various ECM macromolecules. Furthermore cells with potential chondrogenic capacity were analyzed similarly for possible cell shape-related changes in their gene expression.

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2. MATERIALS AND METHODS

2.1 *Cell cultures*

Standard monolayer cultures and cultures in agarose were established from epiphyseal cartilages, diaphyseal periosteum, and muscle of 16-week human fetuses. The growth medium was Dulbecco's modified Eagle medium (DMEM) containing 10% fetal calf serum. Cultures in agarose were set up as described earlier [3]

2.2. *RNA extraction and Northern analysis*

Total RNAs were extracted from cultured cells using guanidine isothiocyanate [10]. For Northern analysis total cellular RNAs (12 5 μg) were denatured with glyoxal, fractionated on 0 75% agarose gels, transferred to nylon membranes (PALL Biodyne) and hybridized using standard techniques [11]. The following cDNA clones for human procollagen mRNAs were used as hybridization probes· pHCAL1U (670 bp) [12] and pHCAL2 (1180 bp) [13] for the carboxy-propeptide domains of human proα1(I) and proα2(I) mRNAs, respectively, pHCAR3 (1470 bp) [14] for the 3′-end of proα1(II) mRNA, and pHFS3 (720 bp) [15] for the carboxy-propeptide domain of proα1(III) mRNA The other cDNA clones used as probes were clone 2B-1 (1825 bp) for human fibroblast-specific ChS proteoglycans core protein [16], 5E (2000 bp) for the human dermatan sulphate (DS) proteoglycan (PG-II, PG40) core protein [17], and pHon 164 (550 bp) for human osteonectin [18] The DNAs were labelled by the random priming technique to a specific activity of approx 1×10^8 cpm/μg. The filters were washed four times with 2 × SSC/0 1% SDS for 5 min at room temperature and twice with 0 1 × SSC/0 1% SDS for 30 min at 55–58°C (for procollagen probes) or at 45–48°C (for the other probes) Depending on the hybridization signal the filters were exposed with Kodak X-Omat films for 2–10 days.

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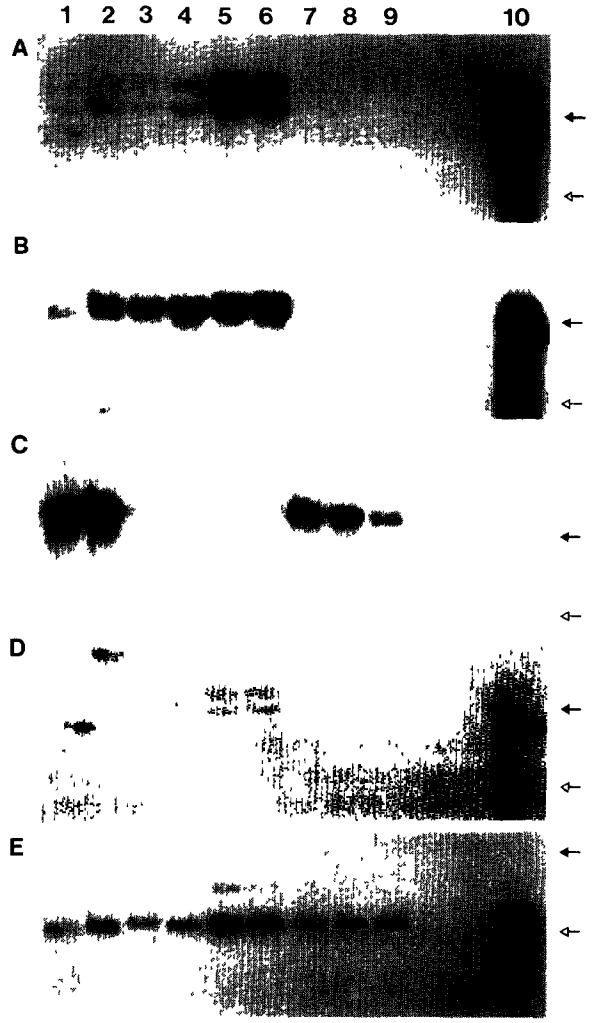

Fig 1. Changes in procollagen and osteonectin mRNA levels during dedifferentiation and redifferentiation of chondrocytes in culture For Northern analysis total RNA was isolated from epiphyseal cartilages (lane 1), from chondrocytes in monolayer cultures in passage one (2), three (3), and five (4), from fibroblasts cultured from fetal muscle (5) and periosteum (6), from chondrocytes in passage one cultured in agarose (7 and 8), from chondrocytes transferred to agarose after five passages in monolayer (9), and from calvarial bone (10). RNA samples were hybridized with cDNA probes for proα1(I) collagen (panel A), proα2(I) collagen (B), proα1(II) collagen (C), proα1(III) collagen (D), and osteonectin (E) The exposure times were two days (panels A and B), one day (panel C), or five days (panels D and E) The positions of the 28 S rRNA (arrows) and 18 S rRNA (open arrows) are shown on the right

3. RESULTS

Chondrocyte dedifferentiation was readily accomplished by culturing freshly isolated epiphyseal chondrocytes in monolayer cultures: a gradual change in morphology from round and cuboidal cells into fibroblast-like cells by the fifth subculture was observed. Northern analysis of total RNAs isolated from freshly isolated epiphyseal chondrocytes and from cells in the first, third, and fifth subculture revealed gradual disappearance of the mRNA for type II procollagen, and a concomitant increase in mRNAs for types I and III procollagen (fig.1, lanes 1–4). The dedifferentiation could be inhibited by culturing the cells in agarose (lanes 7 and 8). When dedifferentiated chondrocytes (grown in monolayer for 5 passages) were transferred to agarose cultures they reexpressed the original phenotype as shown by the reappearance of type II collagen mRNA and marked reduction of mRNAs for types I and III collagens (lane 9).

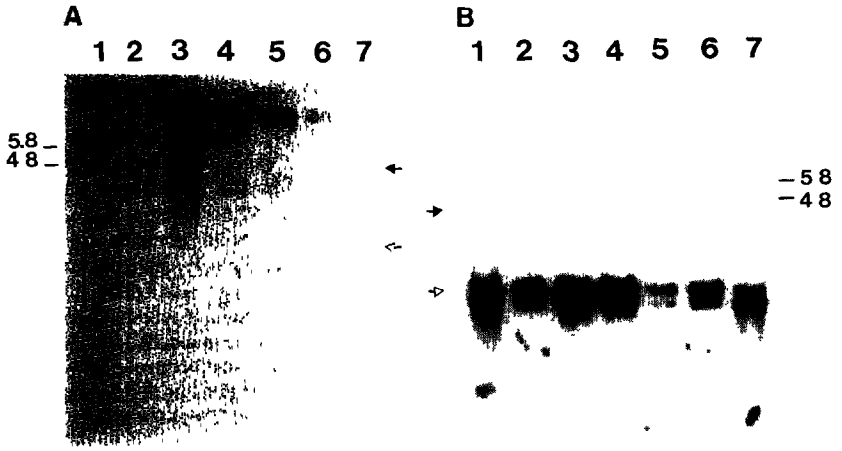

Fig 2 Changes in proteoglycan core protein mRNA levels during dedifferentiation of chondrocytes in culture Total RNAs were isolated from epiphyseal cartilages (lane 1), from chondrocytes in monolayer cultures in passage one (2), three (3), and five (4), from fibroblasts cultured from fetal muscle (5) and periosteum (6), and from calvarial bone (7). Aliquots were fractionated on agarose gels, transferred to nylon membranes and hybridized with cDNA probes for core protein of the large fibroblast ChS proteoglycan (panel A) and core protein for the small DS proteoglycan (B) The exposure times were ten days (panel A) or five days (panel B) The positions of the 28 S rRNA (arrows) and 18 S rRNA (open arrows) are shown The additional molecular size markers used are the 4 8 kb and 5 8 kb proα1(I) collagen mRNAs

Northern analysis of periosteal and muscle fibroblasts grown in monolayer revealed high levels of type I and type III collagen mRNAs in both cell types (fig.1, lanes 5 and 6). In attempts to induce a chondrocytic phenotype these cells were transferred into agarose and treated with 5–15 ng/ml of TGF-β in the medium as suggested by an earlier study [19]. This caused the cells to acquire a round shape, but no type II collagen mRNA was observed.

Dedifferentiating chondrocytes were also analyzed for changes in mRNA levels of 3 other ECM components. The three mRNA species for the fibroblast-specific ChS proteoglycan core gradually appeared during chondrocyte dedifferentiation (fig.2, panel A). These mRNAs were also present in periosteal and muscle fibroblasts, but not in epiphyseal chondrocytes. The levels of mRNAs for DS proteoglycan core were highest in dedifferentiating chondrocytes (panel B). Considerably lower levels were seen in fibroblasts and epiphyseal chondrocytes. Osteonectin mRNA was observed in all samples, with the highest levels in muscle and periosteal fibroblasts (fig.1, panel E).

4. DISCUSSION

A completely new pattern of collagens and other ECM macromolecules is expressed when mesenchymal cells differentiate into chondrocytes [1]. Several in vivo studies have shown that these changes are correlated with the appearance and disappearance of the specific mRNAs coding for these molecules [7–9]. In cell culture, chondrocytes readily dedifferentiate, but can also be induced to redifferentiate with concomitant changes in ECM production [3,4]. The present study demonstrates, using human epiphyseal chondrocytes and procollagen cDNA probes, association of the cartilage-specific type II collagen mRNA with the differentiated phenotype, and type I and type III collagen mRNAs with the dedifferentiated (fibroblastic) phenotype. This is in good correlation with the in vivo results, and with protein chemical data with cultured rabbit chondrocytes, where the change in morphology has previously been associated with reduction in type II collagen, and increase in types I and III collagen production [3,4].

Studies with chick chondrocytes have given partially contradictory results: presence of (modified or unprocessed) type I collagen mRNAs suggests post-transcriptional regulation of collagen production [5,6]. Our results suggest that most of the regulation occurs at transcriptional level. How much these differences are dependent on the origin of chondrocytes (avian vs mammalian) and on the culture conditions remains to be shown.

The expression patterns of the mRNAs for the other ECM macromolecules also exhibited some dependency on the phenotype. The mRNA for the (fibroblast-specific) ChS proteoglycan core was coexpressed with type I and type III collagen mRNAs. The diffuse band migrating at 28 S rRNA resembles that seen earlier [16] and may represent degraded RNA. Although the mRNA for the small DS proteoglycan was present in all cell types studied highest levels were associated with dedifferentiating chondrocytes. Production of osteonectin by osteoblasts and hypertrophic chondrocytes has suggested that this molecule plays a role in the mineralization process [18,20]. In the present study osteonectin mRNA was detected in all the cultures, with the highest levels in rapidly dividing fibroblasts. This is in accordance with suggestions that osteonectin (or SPARC) has also other roles in extracellular matrices [21].

Research on cartilage diseases would greatly benefit from systems enabling differentiation of chondrocytes from progenitor cells in cell culture. We have shown that human chondrocytes propagated under dedifferentiating conditions can redifferentiate and express cartilage-specific mRNAs at levels comparable to in vivo situations. Although periosteal and mesenchymal fibroblasts exhibit chondrogenic potential in vivo, attempts to induce expression of the cartilage-specific type II collagen gene in these cells in culture failed. Recently growth factors associated with cartilage induction have been characterized by molecular cloning [19,22]. In the future it should be possible to find the correct combination of growth factors and culture conditions capable of supporting and inducing the chondrocyte phenotype in culture, and thus to obtain sufficient quantities of (re)differentiated chondrocytes for analysis of cartilage-specific macromolecules and their mRNAs.

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