TEMPORAL EXPRESSION PATTERNS OF BMP RECEPTORS DURING PERIOSTEAL CHONDROGENESIS

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Introduction: Chondrogenesis is a critical event in autologous periosteal transplantation, a viable approach for cartilage repair (1,2,3). It is also important for fracture (cartilage) callus formation during fracture healing (4). Periosteal chondrogenesis is, however, a poorly understood phenomenon. We have established an organ culture model system to study periosteal chondrogenesis in vitro. In this model periosteal explants produce cartilage when grown in agarose and this process is enhanced by exogenously added TGF-β1 (5). During our search for the expression of TGF-β related mRNAs in this model we observed that regulation of BMP2 within the first 12 hrs of culture in agarose and it remains upregulated for at least one week. The stimulation of BMP2 in this system is transcriptionally regulated (6). Since BMPs exert their biological function by interactions with cell surface receptors, we have hypothesized that the expression of BMP receptors also will be regulated positively early during periosteal chondrogenesis. BMP receptors consist of heterodimers of type I (BMPRI) and type II (BMPRII) receptors. There are, however, two type I receptors, BMPRIA and BMPRIB (7). We have studied the temporal expression patterns of BMPRIA, BMPRIB and BMPRII mRNAs in periosteal explants during growth in agarose in the presence of TGF-β1 by RT-PCR, as a first step to determine if their expressions are all regulated during periosteal chondrogenesis.

Materials and Methods: Periosteal explants, harvested from the proximal tibiae of 2 month-old rabbits, were cultured in agarose in the presence of TGF-β1 as described by O’Driscoll (5). Total RNA was prepared from pooled periosteal explants, either before culture (0d) or after culture, using Trizol reagent, and 2 µg of total RNA was treated with DNase I before being converted to cDNA using random primers and Superscript II reverse transcriptase as described by Sanay et al (8). PCR, either for single amplification or for coamplification, was performed with initial denaturation at 94 °C for 7 minutes, followed by 30 cycles of annealing at 60 °C for 2 minutes, elongation at 72 °C for 3 minutes and denaturation at 94 °C for 1 minute. PCR was performed 2µH of rabbit specific primers for BMPRIA, BMPRIB and BMPRII mRNAs using cDNAs prepared from periosteal explants cultured in agarose. The radioactive bands were quantitated using phosphor imager. Data are shown as mean ± 1S.D. All work in this study was approved by the Mayo Clinic Institutional Animal Care and Use Committee.

Results: Acquisition of species-specific sequence is a prerequisite for RT-PCR. Since our working model is the rabbit, we have isolated rabbit specific partial cDNA sequences of BMPRIA and IB (in the ligand binding domain) and BMPRII (in the cytoplasmic tail) by gene digging (9) using cDNA prepared from rabbit tibiae. Using the rabbit specific primers for these mRNAs and a cDNA prepared from 0d periosteal explants (used right after the harvest and not cultured), we amplified, by PCR, each mRNA separately for different periods. This was done to determine not only the presence of these mRNAs in the periosteum but also the linear range of amplification. Whether the expected size band was really amplified from the target mRNA was determined by direct sequencing. Next we coamplified desired segments of either BMPRIA and BMPRII or BMPRIA and BMPRII using the same cDNA preparation to determine the relative basal level expression of these mRNAs in periosteum. Our results indicate that the basal level of expression of BMPRIA, while higher than that of BMPRII (Fig.1A), is lower than that of BMPRII (Fig.1B). To determine the relative level of expression of BMPRIA and BMPRII mRNAs in periosteum during chondrogenesis, we coamplified segments of both mRNAs using cDNAs prepared from periosteal explants cultured over a period of time in agarose with TGF-β1. The results, presented in Fig.2A, indicate that BMPRIA mRNA expression is maintained at a higher level than BMPRII all through the time points tested, although there is an initial down regulation (within 12 hrs of culture) followed by an upregulation. BMPRII mRNA expression, on the other hand, has a relatively short but distinct upregulatory period early during periosteal chondrogenesis. It is upregulated within 24 hrs of culture, reaching a peak around day 5 and falls below the basal level by day 14. Using the same cDNAs BMPRII mRNA was coamplified with BMPRIA mRNA. The results, shown in Fig.2B, indicate a parallel expression pattern for both mRNAs. Initially there is a down regulation within 12 hrs followed by an upregulation by 24hrs reaching a peak between day 5 and day 10. BMPRII mRNA level, however, is higher than that of BMPRIA in almost all the time points tested.

Discussion: Our RT-PCR data show that periosteum expresses BMPRIA, BMPRIB and BMPRII mRNAs at different levels. When the periosteum is subjected to conditions conducive to chondrogenesis (agarose and TGF-β1) the expression of each of these three mRNAs is upregulated, although to a different extent, during the early stage of periosteal chondrogenesis. While the expression of BMPRIA and BMPRII persists over a prolonged period of time, the expression of BMPRIB is barely detectable beyond day14. In this system collagen type II, a marker for matrix-depositing chondrocytes, is induced between day10 and day14, indicating a time point when osteochondroprogenitor cells in periosteum are differentiated into chondrocytes (10). In the developing limb BMPRIB is expressed in precartilaginous condensation zones and is needed for the initial steps of chondrogenesis and its expression precedes collagen type II expression (11). This suggests existence of similar regulatory mechanisms for both embryonic and periosteal chondrogenesis.


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