A Novel Growth-Promoting Factor Derived from Fetal Bovine Cartilage, Chondromodulin II

PURIFICATION AND AMINO ACID SEQUENCE*

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During endochondral bone formation, cartilage cells show increased matrix synthesis and rapid proliferation. We found that cartilage matrix contains at least two types of heparin binding growth-promoting components. One, with a higher affinity to heparin, was identified as chondromodulin I (Hiraki, Y., Tanaka, H., Inoue, H., Kondo, J., Kamizono, A., and Suzuki, F. (1991) Biochem. Biophys. Res. Commun. 175, 871-977). In this study, we isolated a novel growth-promoting component, chondromodulin II, which has a lower heparin affinity, from the dissipative extracts of fetal bovine epiphyseal cartilage. Chondromodulin II stimulated the proteoglycan synthesis in rabbit cultured growth plate chondrocytes, an expression of the differentiated phenotype of chondrocytes. It also stimulated DNA synthesis in chondrocytes in both the absence and the presence of fibroblast growth factor-2. The apparent molecular mass of chondromodulin II on SDS-polyacrylamide gel electrophoresis was 16 kDa. Its complete amino acid sequence was determined by overlapping sequences of the peptides released by endopeptidase digestion and CNBr cleavage. Chondromodulin II consists of 133 amino acids (calculated $M_r = 14,548$). The sequence was unique but homologous to the repeats 1 and 2 of the deduced amino acid sequence of the chicken mim-1 gene, which is specifically transactivated by the v-Myb oncogene product in promyelocytes. We also found a minor component with a higher heparin affinity, chondromodulin III, in cartilage extracts. Chondromodulin III stimulated DNA synthesis in chondrocytes in vitro, and its N-terminal sequence was identical with ribosomal protein L31 lacking the N-terminal three amino acids. These findings suggest that the growth and differentiation of chondrocytes are regulated by multiple components in the cartilage matrix.

The growth of cartilage plays a key role in endochondral bone formation during embryonic development and during the longitudinal growth of bone. Fibroblast growth factor (FGF) exhibits pleiotropic effects depending on the target tissue. Cartilage is a major source of FGF (1), although growth factors of the FGF family are also widely distributed in the body (2). Recent DNA analysis has revealed point mutations in the FGF receptor 3 gene in achondroplasia (3, 4). In individuals with this disorder, the growth cartilage of the long bones undergoes minimal proliferation. Thus, FGF signaling is considered to be important for the support of cartilage growth. FGF-2 is the most potent mitogen for chondrocytes (5) and stabilizes the phenotypic expression of these cells (6). As we have reported previously, some proteinaceous components in cartilage synergistically stimulated DNA synthesis and the growth of cultured chondrocytes in vitro as well as stimulating proteoglycan synthesis in chondrocytes (6, 7). These findings suggest that FGF, in combination with some unique growth-promoting components in cartilage, may act on chondrocytes.

In the course of the initial screening of these active components in the extracts of fetal bovine cartilage, we found at least two distinct factors in terms of their affinity to heparin (8): one was eluted from a heparin-Sepharose column with buffer containing 0.5 M NaCl, and the other was eluted with buffer containing 1.2 M NaCl. In a previous study, we purified the active component with a higher affinity to heparin from the heparin-bound fraction of cartilage extracts and named it chondromodulin I (ChM-I) (9); ChM-I is a novel 25-kDa glycoprotein that is expressed specifically in cartilage. In the present study, we purified the other component with a lower affinity to heparin to homogeneity and named it chondromodulin II (ChM-II). Chondromodulin II has an apparent molecular mass of 16 kDa on SDS-PAGE, and it stimulated proteoglycan synthesis in the cells. It also stimulated DNA synthesis in rabbit growth plate chondrocytes in vitro in both the absence and the presence of FGF-2. The complete amino acid sequence of ChM-II is reported here.

MATERIALS AND METHODS

Cell Culture and Bioassay—Chondrocytes were isolated from the growth plate cartilage of the ribs of young male New Zealand rabbits as described previously (10). The isolated cells were suspended in a mixture (1/1, v/v) of Ham’s F-12 medium and Dulbecco’s modified Eagle’s medium (FAD medium) containing 10% fetal bovine serum (FBS). Incubation of 0.1 ml of cell suspension (1 x 10⁵ cells/ml) was plated in 96-well microplates. The cells were grown to confluence in the same medium at 37°C under 5% CO₂ in air, and the medium was renewed 1 The abbreviations used are: FGF-2, fibroblast growth factor-2; ChM-I, chondromodulin I; ChM-II, chondromodulin II; FBS, fetal bovine serum; rh, recombinant human fibroblast growth factor-2; TGF-β, transforming growth factor-β; PAGE, polyacrylamide gel electrophoresis; HPLC, high performance liquid chromatography.
Effect of cartilage extract on DNA synthesis and proteoglycan synthesis in cultured growth plate chondrocytes

The following fractionated cartilage extract materials were added to confluent culture of rabbit growth plate chondrocytes in the presence or absence of rhFGF-2 (0.4 ng/ml): 1 mg guanidine extract containing materials of 10–50 kDa (10–50 kDa extract, 200 μg/ml) and the fractions obtained by heparin affinity chromatography of 10–50 kDa extract: unbound-fraction (heparin pass, 100 μg/ml), the first protein peak eluted with 0.5 M NaCl (heparin 0.5 M I, 0.2 μg/ml), the second protein peak eluted with 0.5 M NaCl (heparin 0.5 M II, 2.4 μg/ml), and the protein peak eluted with 1.2 M NaCl (heparin 1.2 M, 6.2 μg/ml). Values are means ± S.D. for triplicate samples.

| Addition | [3H]Thymidine incorporation, dpm/well (% of control) |
|----------|-----------------------------------------------------|
| Without FGF-2 | With FGF-2 |
| None | 2437 ± 746 | (100 ± 31) | 36657 ± 2807 | (1504 ± 115) |
| 10–50 kDa extract | 30115 ± 5279 | (1236 ± 217) | 80150 ± 6192 | (3289 ± 254) |
| Heparin pass | 2105 ± 573 | (86 ± 24) | 28674 ± 1628 | (1177 ± 67) |
| Heparin 0.5 M | 1573 ± 44 | (65 ± 2) | 42384 ± 2201 | (1758 ± 90) |
| Heparin 0.5 M II | 3544 ± 773 | (145 ± 5) | 42698 ± 573 | (2352 ± 54) |
| Heparin 1.2 M | 6684 ± 355 | (274 ± 15) | 77776 ± 4025 | (3191 ± 165) |

Notes: (1) The purified ChM-II was incubated with 1% bovine serum albumin in TTBS to reduce the nonspecific binding of proteins. The extracts were added at 300 μg/ml, and the first protein peak eluted with 0.5 M NaCl (heparin 0.5 M fraction) and the second protein peak eluted with 0.5 M NaCl (heparin 0.5 M II, 2.4 μg/ml), and the protein peak eluted with 1.2 M NaCl (heparin 1.2 M, 6.2 μg/ml). Values are means ± S.D. for triplicate samples.

**TABLE I**

| Amino Acid Sequence of Bovine Chondromodulin II

| Residue | Sequence |
|---------|---------|
| 1 | M |
| 2 | F |
| 3 | D |
| 4 | N |
| 5 | P |
| 6 | Y |
| 7 | E |
| 8 | T |
| 9 | S |
| 10 | T |
| 11 | S |
| 12 | Y |
| 13 | E |
| 14 | N |
| 15 | D |
| 16 | R |
| 17 | H |
| 18 | T |
| 19 | V |
| 20 | L |
| 21 | K |
| 22 | E |
| 23 | N |
| 24 | K |
| 25 | E |
| 26 | N |
| 27 | K |
| 28 | E |
| 29 | N |
| 30 | K |
| 31 | E |
| 32 | N |
| 33 | K |
| 34 | E |
| 35 | N |
| 36 | K |
| 37 | E |
| 38 | N |
| 39 | K |
| 40 | E |
| 41 | N |
| 42 | K |
| 43 | E |
| 44 | N |
| 45 | K |
| 46 | E |
| 47 | N |
| 48 | K |
| 49 | E |
| 50 | N |
| 51 | K |
| 52 | E |
| 53 | N |
| 54 | K |
| 55 | E |
| 56 | N |
| 57 | K |
| 58 | E |
| 59 | N |
| 60 | K |
| 61 | E |
| 62 | N |
| 63 | K |
| 64 | E |
| 65 | N |
| 66 | K |
| 67 | E |
| 68 | N |
| 69 | K |
| 70 | E |
| 71 | N |
| 72 | K |
| 73 | E |
| 74 | N |
| 75 | K |
| 76 | E |
| 77 | N |
| 78 | K |
| 79 | E |
| 80 | N |
| 81 | K |
| 82 | E |
| 83 | N |
| 84 | K |
| 85 | E |
| 86 | N |
| 87 | K |
| 88 | E |
| 89 | N |
| 90 | K |

**RESULTS AND DISCUSSION**

First, we attempted to confirm the bioactivity in the guanidinium extracts of cartilage from which contaminating FGF had been separated by heparin affinity chromatography. Fetal bovine epiphyseal cartilage was homogenized and extracted with 1 M guanidinium chloride. The resultant extract was fractionated with 45–65% acetone. Precipitates were solubilized in 4 M guanidinium chloride and fractionated by ultrafiltration. Materials with a molecular mass of 10–50 kDa were concentrated (9). A portion of this 10–50 kDa extract was fractionated into four on a heparin affinity column by batch elution. As shown in Table I, the 10–50 kDa extract strongly stimulated [3H]thymidine incorporation in the growth plate chondrocytes, largely due to contaminating FGF-like activity that was tightly bound to heparin (1). FGF-2 is the most potent mitogen for chondrocytes (7, 16). FGF-2 stimulated DNA synthesis in quiescent primary chondrocytes in a dose-dependent manner, and treatment with an optimal dose of FGF-2 (0.4 ng/ml) resulted in a 5–15-fold increase of [3H]thymidine incorporation in the cells, depending on the batch of isolated cells (Table I and Fig. 1, inset). Interestingly, the extract enhanced the effect of an optimal dose of FGF-2 that had produced maximal stimulation of [3H]thymidine incorporation. This synergistic activity was recovered in the 0.5–1.2 M NaCl eluate (heparin 1.2 M) from the heparin affinity column, and this fraction alone stimulated [3H]thymidine incorporation in the cells to 2 times the basal level. The 10–50 kDa extract strongly stimulated proteoglycan synthesis in chondrocytes, an expression of the differentiated phenotype (Table I and Fig. 1A). The half-maximal dose was about 40 μg/ml. This activity was also recovered in the heparin
tripticate samples.

6 show the dose-response curve of rhFGF-2. Values are means 20 h after addition of the samples.

with [3H]thymidine from 22 to 26 h after addition of the samples. (In the absence (Mf cultured chondrocytes. The cells were incubated with purified ChM-II (9).) or presence (Mf). Proteoglycan synthesis in cultured chondrocytes. The cells were incubated with medium containing 0.3% FBS for 24 h. The medium was then replaced to confluence in 96-well microplates. They were preincubated in FAD medium supplemented with 0.3% FBS and test samples. medium containing 0.3% FBS for 24 h. The medium was then replaced to confluence in 96-well microplates. They were preincubated in FAD medium supplemented with 0.3% FBS and test samples.

FIG. 1. Dose-response curves of fractionated cartilage extracts and purified ChM-II. Rabbit growth plate chondrocytes were grown to confluence in 96-well microplates. They were preincubated in FAD medium containing 0.3% FBS for 24 h. The medium was then replaced with FAD medium supplemented with 0.3% FBS and test samples. A, proteoglycan synthesis in cultured chondrocytes. The cells were incubated with the 10–50-kDa extract (●), heparin 0.5 M (□), heparin 1.2 M (○), and purified ChM-II (□) and labeled with [35S]sulfate from 3 to 20 h after addition of the samples. B, [3H]thymidine incorporation in cultured chondrocytes. The cells were incubated with purified ChM-II in the absence (□) or presence (□) of rhFGF-2 (0.4 ng/ml) and labeled with [3H]thymidine from 22 to 26 h after addition of the samples. Inset shows the dose-response curve of rhFGF-2. Values are means ± S.D. for triplicate samples.

1.2 M fraction (Table I and Fig. 1A). In contrast, FGF-2 had no effect on proteoglycan synthesis in the cells. As we have reported previously, ChM-I accounts for these bioactivities of the heparin 1.2 M fraction (9).

However, residual proteoglycan synthesis-stimulating activ-

ity was also recovered in the fraction corresponding to the second protein peak eluted by 0.5 M NaCl (heparin 0.5 M II) as shown in Table I. The presence of TGF-β in cartilage has been documented (17, 18), although there is no definitive evidence that it is activated in cartilage under physiological conditions. Proteoglycan synthesis in chondrocytes was markedly stimulated by TGF-β (19). Neutralizing antisera against bovine bone-derived TGF-β1 and -β2 failed to inhibit the proteoglycan synthesis-stimulating activity of heparin 0.5 M II, while these sera completely blocked the actions of the corresponding TGF-β subtypes (Table II). These findings suggested that a novel growth factor was recovered in this fraction. Therefore, we decided to purify the novel factor on the basis of its proteoglycan synthesis-stimulating activity in the 10–50-kDa extract. To facilitate purification of the activity, the 10–50-kDa extract (141 mg) was subfractionated on Sephacryl S-200 in the presence of 4 M guanidinium chloride and 1 M NaCl. The first protein peak was collected (17.3 mg), since all bioactivity of the extract was recovered in this fraction (9). The heparin-bound materials eluted by 0.5 M NaCl (heparin 0.5 M; 11.4 mg) and then the heparin-bound materials eluted by 1.2 M NaCl (heparin 1.2 M; 2.66 mg) were collected as described under “Materials and Methods.” The heparin 0.5 M fraction showed a dose-response curve with a slope shallower than that of the 10–50-kDa extract, while the heparin 1.2 M fraction stimulated proteoglycan synthesis with a dose-response profile parallel to that of the 10–50-kDa extract (Fig. 1A). The half-maximal dose of the heparin 0.5 M fraction was about 4 μg/ml. Finally, the heparin 0.5 M fraction was subjected to reverse-phase HPLC. The elution profile is shown in Fig. 2A. Bioassay defined the presence of a novel component (ChM-II; 60 μg), which had a longer retention time than ChM-I.

Chondromodulin II purified from the heparin 0.5 M fraction had an apparent molecular mass of 16 kDa (Fig. 3A). The purified ChM-II stimulated proteoglycan synthesis in the growth plate chondrocytes in culture in a dose-dependent manner (Fig. 1A). The half-maximal dose of ChM-II was about 75 ng/ml. Insulin-like growth factor-I or -II also stimulates proteoglycan synthesis in rabbit growth plate chondrocytes, the half-maximal dose being about 40 ng/ml.2 However, SDS-PAGE analysis (Fig. 3A) indicated that the purified ChM-II preparation did not contain insulin-like growth factor-I or -II to the extent that could account for its proteoglycan synthesis-stimulating activity. ChM-II also stimulated [3H]thymidine incorporation in chondrocytes (Fig. 1B). As shown in the inset of Fig. 1B, FGF-2 markedly stimulated [3H]thymidine incorporation in chondrocytes. Although it was assumed that FGF-2 in the 10–50-kDa extract was eliminated by heparin affinity chromatography, we examined the ChM-II preparation for possible 2 Y. Hiraki, unpublished data.

**Table II**

Effects of TGF-β1 and TGF-β2 antisera on proteoglycan synthesis in chondrocytes stimulated by cartilage extract

| Addition                  | [35S]Sulfate incorporation (% of control) |
|---------------------------|-------------------------------------------|
| None                      | 100                                       |
| TGF-β1                    | 176                                       |
| TGF-β2                    | 183                                       |
| Heparin 0.5 M II          | 206                                       |

1 or TGF-β2 antisera on proteoglycan synthesis in chondrocytes stimulated by cartilage extract

| Addition                  | [35S]Sulfate incorporation (% of control) |
|---------------------------|-------------------------------------------|
| None                      | 100                                       |
| TGF-β1                    | 176                                       |
| TGF-β2                    | 183                                       |
| Heparin 0.5 M II          | 206                                       |

Addition | [35S]Sulfate incorporation (% of control) |
|---------|-------------------------------------------|
| None    | 100                                       |
| TGF-β1  | 176                                       |
| TGF-β2  | 183                                       |
| Heparin 0.5 M II | 206   |

FIG. 1. Dose-response curves of fractionated cartilage extracts and purified ChM-II. Rabbit growth plate chondrocytes were grown to confluence in 96-well microplates. They were preincubated in FAD medium containing 0.3% FBS for 24 h. The medium was then replaced with FAD medium supplemented with 0.3% FBS and test samples. A, proteoglycan synthesis in cultured chondrocytes. The cells were incubated with the 10–50-kDa extract (●), heparin 0.5 M (□), heparin 1.2 M (○), and purified ChM-II (□) and labeled with [35S]sulfate from 3 to 20 h after addition of the samples. B, [3H]thymidine incorporation in cultured chondrocytes. The cells were incubated with purified ChM-II in the absence (□) or presence (□) of rhFGF-2 (0.4 ng/ml) and labeled with [3H]thymidine from 22 to 26 h after addition of the samples. Inset shows the dose-response curve of rhFGF-2. Values are means ± S.D. for triplicate samples.
contamination of FGF-2 by immunoblotting with a monoclonal antibody (bFM-2) against bovine brain-derived FGF-2 (12, 13). As shown in Fig. 3B, immunoblotting clearly visualized the 10–50-ng rhFGF-2 used as the reference substance. In contrast, no immunoreactive material was detected in the ChM-II preparation (2.5 mg), suggesting that there was not more than a nanogram order of contamination of FGF-2 in the preparation. Further, the purified ChM-II enhanced [3H]thymidine incorporation in the presence of an optimal dose (0.4 ng/ml) of FGF-2 (Fig. 1B). This enhancing effect cannot be accounted for by contamination with FGF-2, since additional FGF-2 did not stimulate [3H]thymidine incorporation any further. The purified ChM-II preparation had no effect on [3H]thymidine incorporation in cultured fibroblasts (data not shown). These results suggest that weak growth-promoting activity is also associated with ChM-II, although this agent plays a role primarily in the phenotypic expression of chondrocytes.

As previously reported (9), ChM-I was the major protein in the heparin 0.5 M fraction associated with growth-promoting activity for chondrocytes (Fig. 2B). As shown in Fig. 3B, immunoblotting clearly visualized the 10–50-ng rhFGF-2 used as the reference substance. In contrast, no immunoreactive material was detected in the ChM-II preparation (2.5 µg), suggesting that there was not more than a nanogram order of contamination of FGF-2 in the preparation. Further, the purified ChM-II enhanced [3H]thymidine incorporation in the presence of an optimal dose (0.4 ng/ml) of FGF-2 (Fig. 1B). This enhancing effect cannot be accounted for by contamination with FGF-2, since additional FGF-2 did not stimulate [3H]thymidine incorporation any further. The purified ChM-II preparation had no effect on [3H]thymidine incorporation in cultured fibroblasts (data not shown). Thus, ChM-II seems irrelevant as a growth factor. It is possible that the extraordinarily high pI of the ribosomal protein may lead to erroneous stimulation of chondrocytes in culture. No ChM-III was found in the heparin 0.5 M fraction (Fig. 2A). Interestingly, Chester and co-workers (21) reported overexpression of human ribosomal protein L31 mRNA in familial adenomatous polyposis and colorectal tumors. The gene is also expressed at abnormally high levels in various hematopoietic malignant tumor cells, but its expression was markedly down-regulated upon terminal differentiation of immature leukemic cell lines such as HL-60 promyelocytic leukemia cells and K562 erythroleukemia cells in vitro (22). These findings indicate that the ribosomal protein L31 is associated with growth regulation and differentiation. Recently, Fujita and co-workers (23) reported that the heparin-binding ribosomal protein L22 was copurified with FGF-1 from...
Asn-Glu-Ile-Arg-Thr. The seventh residue was assumed to be (8). The N-terminal amino acid sequence determined at that glycansynthesis in rabbit growth plate chondrocytes in culture, which has a molecular mass of 16 kDa, stimulated proteophannear the N terminus, had six cysteine residues, and was primary amino acid sequence of ChM-II, which had one tryp-lapping sequences of these fragments defined the complete resultant acetylated ChM-II was digested with trypsin. Over-nally, ChM-II was acetylated to protect lysine residues. The age yielded two internal amino acid sequences of ChM-II. Fi-thiohydantoinformation method (15). Cyanogen bromide cleav-quence was determined from one of the peptides isolated by the peptides were determined, and the C-terminal amino acid se-
a Bakerbond C8 column. The amino acid sequences of the tide fragmentsthat were separated by reverse-phase HPLC on
bovine brain. Thus, it is possible that these heparin-binding ribosomal proteins may have functional relevance in growth regulation through a potential interaction with FGF signaling.

To determine the amino acid sequence, ChM-II was first reduced and protected by S-carboxymethylation. First, the N-terminal amino acid sequence of 37 residues was determined (Fig. 4). Then lyslendopeptidase digestion yielded several peptide fragments that were separated by reverse-phase HPLC on a Bakerbond C8 column. The amino acid sequences of the peptides were determined, and the C-terminal amino acid se-
quence was determined from one of the peptides isolated by the thiohydantoin formation method (15). Cyanogen bromide cleavage yielded two internal amino acid sequences of ChM-II. Fi-
ally, ChM-II was acetylated to protect lysine residues. The resultant acetylated ChM-II was digested with trypsin. Overlapping sequences of these fragments defined the complete primary amino acid sequence of ChM-II, which had one try-
tophan near the N terminus, had six cysteine residues, and was rich in lysine residues (Fig. 4).

In a previous study, we reported that cartilage-derived fac-
tor, which has a molecular mass of 16 kDa, stimulated proteoglycan synthesis in rabbit growth plate chondrocytes in culture (8). The N-terminal amino acid sequence determined at that time was Gly-Pro-Trp-Ala-Ile-Xaa-Ala-Gly-Lys-Ser-Ser-Asn-Glu-Ile-Arg-Thr. The seventh residue was assumed to be cysteine that had been oxidized during purification. The N-terminal sequence of ChM-II completely matched the sequence above, suggesting that ChM-II was identical to cartilage-derived factor. In the study above (8), we presented the amino acid composition of cartilage-derived factor assuming its molecular mass to be 16 kDa. The amino acid composition of ChM-II was compatible with that of cartilage-derived factor.

Screening of data bases indicated that ChM-II had a novel amino acid sequence. We were unable to identify any previously characterized sequence motifs. However, ChM-II had a similarity to the repeats 1 and 2 of the mim-1 gene product (24) (Fig. 5). The similarity spread over the entire region of the repeats 1 and 2, each of which contains seven cysteine residues. The positions of the six cysteine residues in ChM-II were all indicated that MIM-1 protein may participate in the regulation of granulopoietic differentiation as a local growth factor (24, 27). However, no biological function of MIM-1 protein has yet been defined.

Although ChM-II has a sequence showing 57% homology to the mim-1 repeats, we assume that ChM-II is a product of a gene distinct from mim-1. It seems unlikely that ChM-II is derived from a larger protein with internal repeats, such as MIM-1, since we were unable to find a peptide with a similar amino acid sequence in our preparation of cartilage extract. Our preliminary sequence data for ChM-II cDNA indicated that the CUA codon corresponding to the C-terminal leucine residue was directly followed by a TAG stop codon (data not shown), while the putative cleavage products of MIM-1 have an extension of 11 amino acids at their C terminus. However, it is possible that there is some evolutionary relationship between the mim-1 gene and the gene encoding ChM-II. Although ChM-II was purified on the basis of its action on the growth and phenotypic expression of chondrocytes, its sequence similarity to MIM-1 implies that ChM-II may act on the growth and differentiation of other cell types, such as the cells involved in hematopoietic differentiation (28, 29). Further studies along these lines are in progress.

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Vol. 271 (1996) 10194–10199

Molecular cloning and characterization of SmLIM, a developmentally regulated LIM protein preferentially expressed in aortic smooth muscle cells.

Mukesh K. Jain, Kenji P. Fujita, Chung-Ming Hsieh, Wilson O. Endege, Nicholas E. S. Sibinga, Shaw-Fang Yet, Saori Kashiki, Wen-Sen Lee, Mark A. Perrella, Edgar Haber, and Mu-En Lee

Page 10197, Fig. 4: The signal indicated by an arrow in the BIOS somatic cell hybrid blot was generated by a pseudogene on chromosome 3. We have since correctly mapped the human SmLIM gene to chromosome 12 by PCR analysis against a Gene-Bridge 4 radiation hybrid panel. This localization is consistent with the localization reported by Weiskirchen et al. (Weiskirchen, R., Erdel, M., Utermann, G., and Bister, K. (1997) Genomics 44, 83–93).

Page 10197, Fig. 5, legend: The last sentence should read: “A 1.0-kb transcript is shown.”

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A novel growth-promoting factor derived from fetal bovine cartilage, chondromodulin II. Purification and amino acid sequence.

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The sequence for chondromodulin II can be obtained from the PIR data base, accession number JH0270, rather than from GenBank™ as stated in the article.

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Cardiac hypertrophy induced by mitogen-activated protein kinase kinase 7, a specific activator for c-Jun NH2-terminal kinase in ventricular muscle cells.

Yibin Wang, Bing Su, Valerie P. Sah, Joan Heller Brown, Jiahuai Han, and Kenneth R. Chien

Page 5425, Fig. 4: An incorrect image has been used as panel b. The correct figure is shown below. This does not alter our interpretations or conclusions of the published study.

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FIG. 4. Co-activation of JNK and p38 pathways led to suppression of hypertrophy and induction of cytopathic response and cell death. Cardiac myocytes were either infected with viral vectors expressing MKK6bE (a), MKK7D (b), or MKK3bE (c) alone or co-infected with vectors expressing MKK6bE and MKK7D (d) or MKK3bE and MKK7D (e). The cellular morphology was examined and photographed under light microscopy.