Enhancement of Cell Adhesion and Spreading by a Cartilage-specific Noncollagenous Protein, Cartilage Matrix Protein (CMP/Matrilin-1), via Integrin α1β1*

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Cartilage matrix protein (CMP; also known as matrixin-1), one of the major noncollagenous proteins in most cartilages, binds to aggrecan and type II collagen. We examined the effect of CMP on the adhesion of chondrocytes and fibroblasts using CMP-coated dishes. The CMP coating at 10–20 μg/ml enhanced the adhesion and spreading of rabbit growth plate, resting and articular chondrocytes, and fibroblasts and human epiphyseal chondrocytes and MRC5 fibroblasts. The effect of CMP on the spreading of chondrocytes was synergistically increased by native, but not heated, type II collagen (gelatin). The monoclonal antibody to integrin abolished CMP-induced cell adhesion and spreading, whereas the antibody to integrin α2, α3, α5, β2, α5β1, or ααβ2 had little effect on cell adhesion or spreading. The antibody to integrin α1, but not to other subunits, coprecipitated 125I-CMP that was added to MRC5 cell lysates, indicating the association of CMP with the integrin α1 subunit. Unlabeled CMP competed for the binding to integrin α1 with 125I-CMP. These findings suggest that CMP is a potent adhesion factor for chondrocytes, particularly in the presence of type II collagen, and that integrin α1β1 is involved in CMP-mediated cell adhesion and spreading. Since CMP is expressed almost exclusively in cartilage, this adhesion factor, unlike fibronectin or laminin, may play a special role in the development and remodelling of cartilage.

Cartilage matrix protein (CMP) (matrilin-1) was originally isolated as a protein that binds to aggrecan and thereafter was shown to bind to type II collagen (1–3). CMP is synthesized in a cartilage-specific manner, except that eye tissues, notochord, and tendon express CMP at low levels (4–7). The role of CMP is unknown, but it may have a structural role, modulating physical properties of cartilage, or may be involved in matrix-cell interactions.

CMP exists in vivo as a homotrimer of 148 kDa, as measured by sedimentation equilibrium centrifugation, although estimates of the molecular mass of this protein by SDS-polyacrylamide gel electrophoresis (PAGE) yield a higher value. CMP migrates at positions corresponding to 215 and 60 kDa under nonreducing and reducing conditions, respectively, during SDS-PAGE (2, 8). The subunits of CMP are connected in the C-terminal region by disulﬁde bonding and the presence of the coiled-coil α-helical assembly domain (9, 10). The monomer has two type A-like (von Willebrand factor-like) or I domains connected with an epidermal growth factor-like domain in addition to the short C-terminal domain (9, 11, 12). A type A-like domain is present in several proteins such as complement factors B and C2; type VI, XII, XIV, and XVI collagens; and α subunits of several integrins (13). Some members of this protein family are involved in cell adhesion. However, whether CMP is an adhesion factor remains unknown.

Numerous adhesion proteins are recognized by integrins, which are heterodimeric proteins with two (α and β) membrane-spanning subunits. The extracellular domain of the α subunit has divalent cation-binding sites (14). Chondrocytes express integrins α1β1, α2β1, α3β1, ααβ1, ααβ3, and αββ1 (15, 16), but their ligands in cartilage have not been fully defined.

In this study, we examined the effect of coating culture dishes with CMP on cell adhesion and investigated whether CMP interacts with integrins using 125I-CMP and various anti-integrin antibodies. In addition, we examined whether collagen modulates the effect of CMP on cell adhesion. The results show that CMP markedly enhances the adhesion and spreading of chondrocytes, particularly in the presence of type II collagen, and that integrin α1β1 plays a pivotal role in the CMP-mediated cell adhesion.

EXPERIMENTAL PROCEDURES

Antibodies—Mouse neutralizing monoclonal antibodies (mAbs) to human integrins α1 (FB12), α2 (P1E6) (17), α4 (ASC-6) (18), α5 (P1D6) (19), β2 (P4H9-A11) (20), αβ2 (JBS5), and αβ2 (P1F6) (21); rabbit anti-sera to human integrins α1, α2, α5, β1, and β3; and rabbit antisera to collagen type I were purchased from Chemicon International, Inc. (Temecula, CA). Mouse neutralizing mAbs to human integrin α1 (5ED9) (22) and β1 (DE9) (19) were purchased from Upstate Biotechnology, Inc. (Lake Placid, NY). Mouse neutralizing mAbs to human integrin α1 (P1E6) (23) and α2 (P1B5) (23) were purchased from Becton Dickinson (Lincoln Park, NJ). Fluorescein-conjugated goat IgG fraction to mouse IgG F(ab′)2 was purchased from Organon Teknika (Durham, NC).

**CMP and Anti-CMP Antiserum—**CMP was purified from a collagen fiber-rich fraction of bovine cartilage as described previously (8). SDS-PAGE analysis showed that purified CMP migrated as a single band at a position corresponding to 215 and 60 kDa under nonreducing and reducing conditions, respectively (Fig. 1), as expected from previous studies (2).

Phosphate-buffered saline (PBS; calcium- and magnesium-free) con-
Effects of Cartilage Matrix Protein on Cell Adhesion

Coating Dishes with CMP—Coating dishes with CMP was achieved by incubating each dish with a 0.1 mg/ml solution of CMP in PBS for 1 h at 37 °C. The dishes were then washed three times with PBS and air-dried.

Surface Labeling with Biotin and Immunoprecipitation—Surface labeling and immunoprecipitation were carried out using the protocol described previously (25). Briefly, cells were incubated with 125I-CMP (2000 cpm) in 1 ml of PBS for 1 h at 4 °C. The supernatant was then removed, and the cells were washed twice with PBS and air-dried.

Results

Localization of CMP—Localization of CMP was assessed using immunohistochemistry and confocal laser microscopy. Immunohistochemistry was performed using anti-CMP antibodies, followed by incubation with biotinylated anti-rabbit IgG and streptavidin-horseradish peroxidase. Immunofluorescence was observed using a confocal laser microscope.

Membrane Adhesion—Membrane adhesion was assessed using a membrane adhesion assay. Cells were seeded on a coverslip in a 24-well plate and incubated for 2 h at 37 °C. The coverslip was then removed, and the cells were stained with a 1% solution of trypan blue in PBS. The percentage of adherent cells was determined using a hemocytometer.

Cell Adhesion—Cell adhesion was assessed using an in vitro cell adhesion assay. Cells were seeded on a plastic substrate coated with CMP and incubated for 2 h at 37 °C. The substrate was then washed three times with PBS and air-dried. Adherent cells were then stained with a 1% solution of trypan blue in PBS and counted under a phase-contrast microscope.

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Effects of Cartilage Matrix Protein on Cell Adhesion

examined CMP in the resting cartilage (Fig. 2A) and the matrix-forming (prehypertrophic) zone of the growth plate (Fig. 2B) of newborn pigs, using the anti-CMP antiserum and confocal microscopy. In these cartilages, CMP was concentrated near the cell surface (Fig. 2, A and B). No stain was observed with the control serum (Fig. 2C).

**Stimulation of Cell Adhesion and Spreading by CMP**—CMP is present in the pericellular matrix (Fig. 2). In addition, CMP has type A-like domains that may be involved in cell adhesion (13). We therefore hypothesized that CMP may be an adhesion factor. To test this hypothesis, we seeded rabbit articular chondrocytes on plastic tissue culture dishes coated with various concentrations of CMP and incubated them at 37°C for 2 h in the presence of cycloheximide, an inhibitor of protein synthesis. Round and spread cells were separately counted using a phase-contrast microscope. The chondrocytes spread rapidly on the CMP-coated dishes, whereas only a few cells spread on the dishes not coated with CMP (Fig. 3A, inserts a and b). The percentage of spread cells to total cells on the CMP (20 μg/ml)-coated dishes was 42% compared with 3% on the control dishes (Fig. 3A). This stimulation of spreading was induced when dishes were preincubated with CMP at 2 μg/ml and became almost maximal at 20 μg/ml (Fig. 3A). In another experiment, adherent cells were quantified using an aqueous soluble tetrazolium/formazan assay. CMP enhanced the adhesion of chondrocytes in a similar dose-dependent fashion (Fig. 3B).

Fig. 4 shows that CMP was effective in stimulating the spreading of rabbit growth plate chondrocytes (rGC), rabbit articular chondrocytes (rAC), human embryonic chondrocytes (hEC), rabbit fibroblasts (rFB), and human MRC5 fibroblasts (hFB).

**Synergism between CMP and Type II Collagen**—Since CMP binds to type II collagen (2), we examined whether type II collagen modulates the effect of CMP on the spreading of chondrocytes. A low concentration of CMP (0.5 μg/ml) enhanced the spreading of chondrocytes in the presence, but not absence, of type II collagen (3 μg/ml) (Fig. 5A). This synergism between CMP and collagen was observed 15 min after cell seeding and was sustained for at least 60 min (Fig. 5A). The concentration of CMP required for cell spreading was 20–200-fold higher in the absence of type II collagen than in its presence (Fig. 5B). At high concentrations (3–10 μg/ml), type II collagen alone stimulated cell spreading (Fig. 5C). CMP at 0.5 μg/ml increased this effect of type II collagen by 3–4-fold. The synergism between CMP and collagen was not observed with denatured type II collagen (gelatin) that was boiled for 5 min at pH 3.0, although gelatin alone had a greater effect on cell spreading than type II collagen alone (Fig. 5D).

**Effects of EDTA and Divalent Cations on CMP-mediated Cell Spreading**—Although CMP plus collagen produced a synergistic stimulation of cell spreading, our subsequent studies of CMP-recognizing integrins were carried out without collagen because collagen alone modulates integrin activity. The presence of collagen makes the interpretation of the data difficult. Since integrins require divalent cations to bind ligands, we examined whether EDTA inhibits cell adhesion to CMP. The addition of EDTA at 2 mM (but not 1 mM) to α-modified Eagle’s medium suppressed the spreading of chondrocytes on CMP-coated dishes (Fig. 6A). The concentrations of Mg2+ and Ca2+ in α-modified Eagle’s medium are 0.8 and 1.8 mM, respectively. When chondrocytes were suspended in 10 mM Hepes (pH 7.4) containing 0.9% NaCl, few cells were attached on CMP-coated dishes. However, the addition of Mg2+ to the buffer markedly enhanced the cell spreading on CMP-coated dishes (Fig. 6B).
This was induced at a Mg $^{2+}$ concentration of 1–2 mM and increased dose-dependently at least until 5 mM. Mn $^{2+}$ had a greater effect on cell spreading on CMP-coated dishes than Mg $^{2+}$ at 1–2 mM (Fig. 6C). Ca $^{2+}$ had less effect on cell spreading than Mg $^{2+}$ and Mn $^{2+}$ (Fig. 6D).

Inhibition of Cell Adhesion and Spreading on CMP-coated Dishes by Anti-integrin Antibodies—Since antibodies to rabbit integrins are rather difficult to obtain, we used antibodies to human integrins. Unless otherwise specified, a human fibroblast cell line (MRC5) was used because it was difficult to obtain human chondrocytes in primary cultures.

The spreading of MRC5 fibroblasts on CMP-coated dishes was suppressed by the mAb against integrin $\alpha_1$ or $\beta_1$ (Fig. 7). The mAb against $\alpha_2$, $\alpha_3$, $\alpha_5$, $\beta_2$, $\alpha_\beta_1$, or $\alpha_\beta_5$, as well as control IgG, had little effect on cell spreading on CMP-coated dishes. The inhibition of the CMP-induced cell adhesion by the mAb to integrin $\alpha_1$ or $\beta_1$, but not other mAbs, was observed with human chondrocytes using an aqueous soluble tetrazolium/formazan assay (Fig. 8). These findings suggest that the integrin $\alpha_1$ and $\beta_1$ subunits were involved in the cell adhesion and spreading on CMP-coated dishes.

Binding of CMP to Integrins—We next examined whether $^{125}$I-CMP added to the cell lysates is coprecipitated with integrin subunits using antibodies to the $\alpha_1$, $\alpha_2$, $\alpha_\beta$, $\beta_2$, and $\beta_5$ subunits. After iodination, CMP did not form trimers even under nonreducing conditions during electrophoresis in the presence of SDS, as described under “Experimental Proce-

FIG. 6. Effects of EDTA and divalent cations on spreading of chondrocytes on CMP-coated dishes. A, rabbit articular chondrocytes were incubated for 2 h with EDTA (0–5 mM) in medium A on 20 $\mu$g/ml CMP-coated dishes (closed triangles) or on uncoated dishes (open triangles). B–D, rabbit articular chondrocytes were incubated for 1 h with Mg $^{2+}$, Mn $^{2+}$, or Ca $^{2+}$, respectively, at various concentrations in 10 mM Hepes, pH 7.4, containing 0.9% NaCl, 1 mg/ml BSA, and 10 $\mu$g/ml cycloheximide on 20 $\mu$g/ml CMP-coated dishes (hatched bars) or on uncoated dishes (open bars). The values are the means ± S.D. of triplicate determinations.

FIG. 7. Inhibition of spreading of human MRC5 fibroblasts on CMP-coated dishes by mAbs to human integrins. MRC5 fibroblasts were incubated for 2 h on dishes coated with CMP in medium A containing mouse IgG or various mAbs to human integrins at 10 $\mu$g/ml. The values are the means ± S.D. of three to nine determinations.

$^{2}$ The percentage of spread articular chondrocytes to total cells on dishes coated with $^{125}$I-CMP (10 $\mu$g/ml) at 2 h was 13 ± 2% compared
FIG. 8. Inhibition of adhesion of human embryonic chondrocytes to CMP by mAbs to human integrins. Human embryonic chondrocytes were incubated for 2 h on dishes coated with CMP in medium A containing 3 or 10 μg/ml mouse IgG (open circles) or mAb to human integrin α1 (3E5D9) (closed circles), α2 (P1E6) (open triangles), α3 (P1B5) (closed triangles), or β1 (DE9) (open squares). After gentle washing, adherent cells were quantified using an aqueous soluble tetrazolium/formazan assay. The values are the means ± S.D. of triplicate determinations.

Fig. 9. Precipitation of 125I-CMP and the integrin α1 subunit using the antiserum to integrin α1. A. 125I-CMP was incubated with rabbit antisera (1%) to various human integrins or human type I collagen, or nonimmune rabbit serum, and then the material precipitated with protein G-Sepharose was analyzed by SDS-PAGE. The gel with the fractionated samples was dried and exposed to x-ray film. B. MRC5 cells were labeled with biotin. The lysates of biotin-labeled cells were incubated with the antiserum (1%) to human type I collagen or to nonimmune serum (1%) in the presence (panel a) or absence (panel b) of the lysates of MRC5 fibroblasts pretreated with pure collagenase. The proteins bound to protein G-Sepharose were resolved by Laemmli buffer. Laemmli buffer-resolved proteins were fractionated by SDS-PAGE. The gel with the fractionated samples was dried and exposed to nitrocellulose membranes. The membranes were blocked with BSA and incubated for 1 h in streptavidin-conjugated horseradish peroxidase. Biotinylated proteins were visualized using enhanced chemiluminescence.

DISCUSSION

The biological role of CMP is not known, although it is one of the major noncollagenous proteins in growth plates, tracheal cartilage, and other cartilages (4, 8, 29). It constitutes up to 5% of the wet weight of tracheal cartilage (4). CMP may stabilize the cartilage matrix and alter the tensile strength and elasticity of the matrix by binding to collagen and aggregan. Although articular chondrocytes do not usually synthesize CMP, CMP synthesis is markedly enhanced in arthritic joints (8), suggesting that CMP may be involved in the destruction and/or remodeling of cartilage.

Our immunohistochemical analyses showed that unlike aggregan and type II collagen, CMP is concentrated near the chondrocyte surface in vivo. This observation, as well as the presence of the type A-like domain on CMP, suggested that CMP could be involved in the matrix-cell interaction. We tested this hypothesis and showed for the first time that CMP adheres to chondrocytes and fibroblasts.

The physiological significance of the CMP-induced spreading of fibroblasts is not known. However, CMP may function as an adhesion factor for fibroblast cells in eye tissues, notochord, and tendon that contain CMP at low levels (5–7).

Biochemical studies have shown that CMP binds to type II collagen in vitro (2), and immunostained transmission electron microscopy has shown that CMP binds to the exterior of the collagen fibril in vivo (2). CMP distributes along type II colla-
gen fibers with a periodicity of 59 nm (2). However, the biological significance of the CMP-type II collagen complex is not known. We showed here that native, but not denatured, type II collagen increases the effect of CMP on the spreading of chondrocytes. This finding suggests that CMP attached on type II collagen fibers more efficiently concentrates CMP receptors/integrins at adhesion plaques than CMP alone attached uniformly on a plastic or gelatin surface. We also observed that fibronectin and laminin did not produce a synergistic stimulation of cell spreading in the presence of type II collagen (data not shown), although fibronectin and laminin bind to collagen; this distinguishes the role of CMP in cartilage from those of fibronectin and laminin.

CMP forms two types of filamentous networks in the pericellular matrix: one that contains type II collagen and another that does not contain type II collagen (30). The collagen-independent CMP filaments may also serve as a scaffold for cell adhesion in vitro because CMP at high concentrations (10–20 μg/ml) enhanced cell spreading in the absence of type II collagen in vitro.

In tracheal cartilage, CMP binds to aggrecan noncovalently and covalently, and the covalent cross-linking to the aggrecan core protein increases with age (3). The CMP-aggrecan complex is unlikely to enhance the adhesion or spreading of chondrocytes because aggrecan suppresses cell adhesion in vitro (31).

In this study, we examined whether integrins are involved in the CMP-induced cell adhesion. In our assays, the CMP-induced cell spreading required Mg^{2+} or Mn^{2+}. Ca^{2+}had less effect on the cell spreading than Mg^{2+} and Mn^{2+}. This is consistent with results from extensive studies of the effect of divalent cations on integrin activity (32, 33). The mAb to human integrin α_{1} or β_{1} suppressed the adhesion and spreading of human chondrocytes and human MRC5 fibroblasts on dishes coated with CMP. The other tested mAbs to various integrins had a marginal effect on cell adhesion or spreading on CMP-coated dishes. These findings suggest that integrin α_{1}β_{1} plays a pivotal role in the CMP-mediated cell adhesion and spreading.

Of the various antibodies to integrin subunits examined here, only the antibody to integrin α_{1} coprecipitated [125I]-CMP in cell lysates. Unlabeled native CMP competed for the binding to integrin α_{1} with [125I]-CMP. Although the anti-integrin β_{1} mAb inhibited the effect of CMP on cell adhesion, this mAb (data not shown), as well as the antisera to integrin β_{1}, did not consistently precipitate [125I]-CMP in cell lysates. Under these conditions, the antibody to integrin β_{1} may coprecipitate the α_{1} subunit only at low levels. In addition, the affinity of integrin β_{1} for CMP may decrease in the absence of the intact plasma membrane. In any case, our findings suggest that CMP selectively binds to the integrin α_{1} subunit even in the presence of detergents (Nonidet P-40 and SDS).

Integrin α_{1}β_{1}, as well as α_{2}β_{1}, binds to collagen under some experimental conditions. However, the precipitation of CMP with anti-integrin α_{1} antibodies is not due to the binding of CMP to collagen because [125I]-CMP used in this study did not bind to type I, II, III, IV or V collagen. Furthermore, the lysates were prepared after cells were dispersed from the cell layer with bacterial pure collagenase at a high concentration (0.8 mg/ml) and trypsin. No type I collagen was detected in the cell lysates by immunoblotting.\(^3\)

Chondroadherin is also an adhesion protein prominently expressed in cartilage and binds to integrin α_{1}β_{1} (34). Chondroadherin can promote cell adhesion, but not spreading (34), whereas CMP enhances both cell adhesion and spreading. These findings suggest that CMP and chondroadherin have different roles in cartilage.

Whether CMP has a special role in cartilage in the presence of other adhesion proteins is not known. It is noteworthy, however, that genetic variation at the CMP gene locus was found to be significantly associated with hip radiographically evident osteoarthritis in 55–65-year-old men, whereas a significant association between hip or knee radiographically evident osteoarthritis in men or women and the cartilage link protein gene was not observed (35). Typically, hip radiographically evident osteoarthritis is most frequently present in men in the 55–65-year age group. Radiographically evident osteoarthritis particularly of the hip is often considered to arise due to anatomic abnormalities. During endochondral bone formation, the length and shape of the bone are determined. If CMP protein plays a distinctive role in the matrix-cell interaction during endochondral bone formation, genetic variation at the CMP gene locus may alter the shape of the skeleton. In addition, CMP may modulate the matrix-cell interaction in articular cartilage of osteoarthritic joints (8).

cDNAs encoding CMP-like proteins (matrilin-2 and -3) were recently cloned (36–38). Matrilin-2 is expressed in a variety of organs, but not in cartilage (36), whereas matrilin-3 is expressed in a cartilage-specific manner (37, 38). Matrilin-3 and CMP form disulfide-linked hetero-oligomers in bovine epiphyseal cartilage (39). These proteins may also function as adhesion factors since their modular structure is similar to that of CMP.

In conclusion, CMP was found to be an adhesion factor for fibroblasts and chondrocytes. CMP is the first protein observed to synergistically increase the effect of collagen on adhesion and spreading. Integrin α_{1}β_{1} seems to be involved in the CMP-induced cell adhesion. The cartilage-specific adhesion protein CMP may play an important role in the development and repair of skeletal tissues.

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REFERENCES
1. Paulsson, M., and Heinegard, D. (1979) Biochem. J. 183, 539–545
2. Winterbottom, N., Tondravi, M. M., Harrington, T. L., Klier, F. G., Vertel, B. M., and Goetinck, P. F. (1992) Dev. Dyn. 193, 266–276
3. Hauser, N., Paulsson, M., Heinegard, D., and Morgelin, M. (1996) J. Biol. Chem. 271, 32247–32252
4. Paulsson, M., and Heinegard, D. (1982) Biochem. J. 207, 207–213
5. Tsonis, P. A., and Goetinck, P. F. (1988) Exp. Eye Res. 46, 753–764
6. Stirpe, N. S., and Goetinck, P. F. (1989) Development (Camb.) 107, 23–33
7. Hauser, N., DiCesare, P. E., and Paulsson, M. (1995) Acta Orthop. Scand. 266, (suppl.) 19–21
8. Okimura, A., Okada, Y., Makihira, S., Pan, H., Yu, L., Tanne, K., Imai, K., Yamada, H., Kawamoto, T., Noshiro, M., Yan, W., and Kato, Y. (1997) Arthritis Rheum. 40, 1029–1036
9. Kiss, I., Deak, F., Holloway, R. G. Jr., Delius, H., Mebust, K. A., Frimberger, E., Argraves, W. S., Tsonis, P. A., Winterbottom, N., and Goetinck, P. F. (1989) J. Biol. Chem. 264, 8126–8134
10. Hauser, N., and Paulsson, M. (1994) J. Biol. Chem. 269, 25747–25753
11. Argraves, W. S., Deak, F., Sparks, K. J., Kiss, I., and Goetinck, P. F. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 464–468
12. Jenkins, R. N., Osborne-Lawrence, S. N., Sinclair, A. K., Eddy, R. L., Jr., Ryers, M. G., Shows, T. B., and Duhay, A. D. (1990) J. Biol. Chem. 265, 19624–19631
13. Colombatti, A., and Bonaldo, P. (1991) Blood 77, 2305–2315
14. Ruoslahti, E., and Pierschbacher, M. D. (1987) Science 238, 491–497
15. Woods, V. L., Jr., Schreck, P. J., Gesink, D. S., Puchech, H. O., Amiel, D., Akesson, W. H., and Lotz, M. (1994) Arthritis Rheum. 37, 557–544
16. Saltzer, D. M., Godolphin, J. L., and Gourlay, M. S. (1995) J. Histochem. Cytochem. 43, 447–457
17. Wayner, E. A., Carter, W. G., Piotrowicz, R. S., and Kunicki, T. J. (1988) J. Cell Biol. 107, 1881–1891
18. Pattaramalai, S., Skubitz, K. M., and Skubitz, A. P. (1996) Exp. Cell Res. 222, 281–290
19. Bergelson, J. M., Chan, B. M., Findberg, R. W., and Hemler, M. E. (1993) J. Clin. Invest. 92, 232–239
20. Wayner, E. A., Gil, S. G., Murphy, G. F., Wilke, M. S., and Carter, W. G. (1993) J. Cell Biol. 121, 1141–1152
21. Wayner, E. A., Orlando, R. A., and Chereesh, D. A. (1991) J. Cell Biol. 113, 55–65.

\(^3\) S. Makihira, unpublished data.
22. Luque, A., Sanchez Madrid, F., and Cabanas, C. (1994) FEBS Lett. 346, 278–284.
23. Carter, W. G., Wayner, E. A., Bouchard, T. S., and Kaur, P. (1990) J. Cell Biol. 110, 1387–1404.
24. Okada, Y., Shinmei, M., Tanaka, O., Naka, K., Kimura, A., Nakanishi, I., Bayliss, M. T., Iwata, K., and Nagase, H. (1992) Lab. Invest. 66, 680–690.
25. Shen, M., Kawamoto, T., Yan, W., Nakamasu, K., Tamagami, M., Koyano, Y., Noshiro, M., and Kato, Y. (1997) Biochem. Biophys. Res. Commun. 236, 284–288.
26. Kato, Y., Nakashima, K., Iwamoto, M., Murakami, H., Hiranuma, H., Koike, T., Suzuki, F., Fuchihata, H., Ikehara, Y., Noshiro, M., and Jikko, A. (1993) J. Clin. Invest. 92, 2323–2330.
27. Cory, A. H., Owen, T. C., Barltrop, J. A., and Cory, J. G. (1991) Cancer Commun. 3, 207–212.
28. Towbin, H., Stoehein, T., and Gordon, J. (1992) Bio/Technology 24, 145–149.
29. Paulsson, M., Inerot, S., and Heinegård, D. (1984) Biochem. J. 221, 623–630.
30. Chen, Q., Johnson, D. M., Haudenschild, D. R., Tondravi, M. M., and Goetinck, P. F. (1995) Mol. Biol. Cell 6, 1743–1753.
31. Yamagata, M., Suzuki, S., Akiyama, S. K., Yamada, K. M., and Kimata, K. (1989) J. Biol. Chem. 264, 8012–8018.
32. Elices, M. J., Urry, L. A., and Hemler, M. E. (1991) J. Cell Biol. 112, 169–181.
33. Loeser, R. F., Carlson, C. S., and McGee, M. P. (1995) Exp. Cell Res. 217, 248–257.
34. Camper, L., Heinegård, D., and Lundgren Akerlund, E. (1997) J. Cell Biol. 138, 1159–1167.
35. Meulenbelt, I., Bijkerk, C., de Wildt, S. C., Miedema, H. S., Valkenburg, H. A., Breedveld, F. C., Pols, H. A., Te Koppele, J. M., Sloos, V. F., Hofman, A., Slagboom, P. E., and van Duijn, C. M. (1997) Arthritis Rheum. 40, 1760–1765.
36. Deak, F., Pichea, D., Bachratí, C., Paulsson, M., and Kiss, I. (1997) J. Biol. Chem. 272, 9268–9274.
37. Wagener, R., Kobbe, B., and Paulsson, M. (1997) FEBS Lett. 413, 129–134.
38. Bussocca, D., and Trueb, B. (1997) FEBS Lett. 415, 212–216.
39. Wu, J. J., and Eyre, D. R. (1998) J. Biol. Chem. 273, 17433–17438.

Effects of Cartilage Matrix Protein on Cell Adhesion

11423