Co-localization of integrins and matrix metalloproteinases in the extracellular matrix of chondrocyte cultures

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Summary. ß1-integrins were found in the cartilage matrix, suggesting their implication in the assembly of its architectural scaffold, but the mechanism for this event is not yet clear.

Matrix metalloproteinases (MMPs) may be involved in an integrin-shedding mechanism and matrix ß1-integrins may act to alter MMP activity. To begin to address this question, this study was designed to determine whether ß1-integrins and MMPs are co-localized in the chondrocytes or in the extracellular matrix of cartilage.

We investigated high-density cultures of limb buds of 12-day-old mouse embryos by double immuno-fluorescence, immunoelectron microscopy and by coimmunoprecipitation assays in order to examine the localization of ß1-integrins and matrix metallo-proteinases (MMP-1, MMP-3 and MMP-9) in cartilage.

It was found, that all investigated MMPs and ß1-integrins were specifically co-localized in high-density cartilage cultures. Immunogold and immunofluorescence labelling of both ß1-integrins and MMPs were observed not only at the surface of chondrocytes but mainly also in the pericellular space and distributed between collagen fibrils in the extracellular matrix (ECM) as well. Results of immunoprecipitation experiments suggest a functional association of MMPs and ß1-integrins in chondrocytes as already described for other cell types.

Further investigations are needed to elucidate the functional association between ß1-integrins and MMPs in chondrocytes.

Key words: High-density culture, Matrix metalloproteinase, Integrins, Immunohistology, Immunoprecipitation, Chondrocyte

Introduction

Multicellular organisms are able to function only if cell and extracellular matrix (ECM) work together. In this cooperation, integrins play an important role as receptors, whereas matrix components function as ligands. Matrix degrading enzymes like MMPs are involved in adaption, modulation and turn over processes. Without their participation this system would be inflexible, not versatile and thus not viable.

Integrins are transmembrane proteins that exhibit as adhesion receptors and play an important role as signalling receptors (Hynes, 1992). Signals generated from ligand-integrin interactions are propagated via the integrin cytoplasmic tails to signal transduction pathways within the cell (outside-in signalling). Information from within the cell can also be transmitted to the outside via integrin affinity modulation (inside-out signalling) (Longhurst and Jennings, 1998). Protein tyrosine phosphorylation has a central role in integrin-initiated cell signalling, leading to cytoskeletal organization and focal adhesion formation (Longhurst and Jennings, 1998). Many cellular events are affected by integrin-mediated signalling including cell motility, cell division, cell differentiation and programmed cell death (Howe et al., 1998).

ß1-integrins are important for cell/cell and cell/extracellular matrix interactions in various tissues including cartilage (Albeda and Buck, 1990; Loeser et al., 1995; Shakibaei et al., 1995, 1997; Shakibaei, 1998). Chondrocyte adhesion on native collagens type I and type II, fibronectin and laminin was blocked by anti-ß1-antibodies (Dürr et al., 1993; Shakibaei et al., 1997). We have previously demonstrated that ß1-integrins are not restricted to the cell surface, as they could be demonstrated on collagen fibrils in the cartilage matrix (Shakibaei et al., 1995; Shakibaei and Merker, 1999). The function and origin of integrins in the cartilage matrix are still not elucidated. A secretion mechanism or a shedding mechanism of extracellular domains or of the

whole molecules have been discussed as possible explanations. An ectodomain shedding mechanism has been described for growth factors and growth factor receptors, which changes the biological activities of membrane proteins. This ectodomain shedding of growth factors was mediated by ADAMs proteinases (adamalysin-related membrane proteinases) that contain a metalloprotease domain (Black et al., 1997; Izumi et al., 1998). Integrins can physically associate with growth factor receptors and caveolins evidenced by coimmunoprecipitation and immunofluorescent co-localization (Howe et al., 1998; Shakibaei et al., 1999; Schwab et al., 2000).

Matrix metalloproteinases (MMPs) mediate extracellular matrix remodelling/turn over by degrading extracellular matrix macromolecules (Schmitz et al., 1996). They are expressed as inactive zymogens and require activation. Activation of MMPs occurs by the removal of an aminoterminal domain by autoactivation, by other proteinases or by other MMPs (Schmitz et al., 1996; Horton et al., 1998; Yong et al., 1998). Often several proteinases, MMPs, membrane type (MT)-MMPs or tissue inhibitor of MMPs (TIMPs) are involved in activation ("activation cascade"). The activation apparatus is localized on the surface of the cell and an important consequence of this is that proteolysis is greatest in the immediate pericellular environment, where it can influence cell-cell and cell-ECM interactions (Yong et al., 1998). MMP-1, MMP-3 and MMP-9 are reported to be increased in osteoarthritic cartilage (Freemont et al., 1997; Horton et al., 1998; Saito et al., 1998).

Proteolysis of ECM proteins alters integrin-mediated anchorage, focal adhesions, cytoskeletal architecture and signalling molecules like focal adhesion kinase (FAK), paxillin, talin, etc. Binding of cleaved ECM fragments by integrins also activates different pathways (Werb, 1997). The proteolytic events are exquisitely regulated and confined to localization of enzymes to receptors, adhesion sites or invasive protrusions of cells (Werb, 1997). Several examples of co-localization and functional association of MMPs and integrins in cell types other than cartilage exist, resulting in a modulation of cell surface integrin-ECM interactions by MMP activity (Brooks et al., 1996, 1998; Bafetti et al., 1998). Besides these further functions of the integrin-MMP interaction were suggested for example in cell migration (Preissner et al., 1997; Murphy and Gavrilovic, 1999; Werb et al., 1999). Therefore it is important to further investigate the functional association of MMPs and integrins.

We have found a specific co-localization of ß1-integrins and MMPs in chondrocytes by immunogold and immunofluorescence labelling and by coimmuno-precipitation assays. MMPs and ß1-integrin clusters were found in the pericellular space and on the cell surface but also between collagen fibrils in the extracellular matrix in cartilage.

Materials and methods

Materials

Primary antibodies

The polyclonal antibodies against the cytoplasmic domain of ß1-integrin were purchased from Chemicon (Chemicon International, Inc., USA) or kindly provided by Dr. Giancotti (Cellular Biochemistry and Biophysics Program, Memorial Sloan-Kettering Cancer Center, New York), as previously described (Giancotti and Ruoslahti, 1990). The monoclonal anti-MMP-1, -MMP-3 and -MMP-9 antibodies (IgG) recognizing both proenzyme and activated enzyme were obtained from R&D Systems (Abingdon, UK). Based on western blot results, these antibodies show no cross-reactivity with other MMPs.

Secondary antibodies

The secondary gold-labelled antibodies were purchased from Amersham (Brunswick, FRG) and the secondary FITC- and rhodamine-labelled antibodies from Dianova (Hamburg, FRG). The secondary antibody conjugated to alkaline phosphatase was obtained from Boehringer (Mannheim, FRG).

Methods

Cell culture

Cells were obtained as previously described (Shakibaei et al., 1993, 1995; Shakibaei and Merker, 1999). Briefly, limb buds of day 12 mouse embryos were rinsed in Hank's salt solution followed by incubation in Ca^{++}- and Mg^{++}-free solution with 0.2% dispase (Rocher, FRG). Ham's F-12 growth medium was added and the suspension was homogenized by pipetting and subsequently filtrated through a nylon mesh with a pore diameter of 20 μm. The cells were centrifuged (600 rpm) for 10 min, and the cell sediment was resuspended in medium. Ten μl of the cell sediment (~2x10^6 cells) was pipetted onto a membrane filter. The high-density cultures of limb bud mesenchymal cells were incubated under 5% CO_2 at 37 °C at the medium air interface. After a seven-day culture period of limb bud blastemal cells from 12-day-old mouse embryos at high-density, cartilage nodules had developed. Chondrocyte nodules of various sizes were surrounded by a perichondrium consisting of a few layers of fibroblast-like cells. The cartilage cells were round or polygonal with small cuspidal processes.

Immunoprecipitation

Immunoprecipitation was carried out as previously described (Shakibaei et al., 1999). Briefly, samples were rinsed in PBS before extraction via lysis buffer (50 mM

Tris-HCl pH 7.2, 150 mM NaCl, 1% Triton X-100, 1 mM sodium orthovanadate, 50 mM sodium pyrophosphate, 100 mM sodium fluoride, 0.01% aprotinin, 4 µg/ml pepstatin A, 1 µg/ml leupeptin, 1 mM phenylmethyl-sulphonylfluoride "PMSF") on ice for 30 min. The cell extract was centrifuged at 13000 rpm for 30 min and cell debris was removed. The bicinchonic acid system (Pierce, Rockford, IL, USA) was used for protein determination.

For immunoprecipitation the lysates were precleared by incubation with 25 µl of normal rabbit or mouse IgG-serum and *Staphylococcus aureus (S. aureus)* cells (Sigma, Munich, FRG). Primary antibodies against MMP-1, MMP-3, MMP-9 and the cytoplasmic domain of ß1-integrin were diluted in washing buffer (0.1% Tween 20, 150 mM NaCl, 50 mM Tris-HCl, pH 7.2, 1 mM CaCl$_2$, 1 mM MgCl$_2$, 1 mM PMSF) and lysates were incubated with primary antibodies (2 h at 4 °C) and with *S. aureus* cells (1 h at 4 °C). Some samples were also incubated with rabbit anti-mouse IgG alone as controls. *S. aureus* cells were washed five times with washing buffer and once with 50 mM Tris-HCl, pH 7.2. Finally the cells were boiled in SDS-PAGE (sodium dodecyl sulfate polyacrylamide gelelectrophoresis) sample buffer. To examine whether integrins can interact with MMP-1, MMP-3 and MMP-9 in chondrocytes, coimmunoprecipitation assays were performed. We used here anti-integrin antibodies recognizing the intracellular domain to answer the question whether the integrins in the matrix represent ectodomains formed after shedding, or complete integrins. After immunoprecipitation with anti-MMP-1, -3 and -9 antibodies, the samples were probed by immunoblotting with anti-cytoplasmic domain of ß1-integrin antibodies and vice versa, and after immunoprecipitation with anti-cytoplasmic domain of ß1-integrin antibodies, samples were probed with anti-MMP-1, -3 and -9 antibodies.

Immunoblotting

Samples were separated by SDS-PAGE (7.5% gels) under reducing conditions. Proteins were transferred onto nitrocellulose and blocked with 5% (w/v) skimmed milk powder in PBS/0.1% Tween 20 overnight at 4 °C. Incubation with primary antibodies (anti-cytoplasmic domain of ß1-integrin and anti-MMP-1, -MMP-3, -MMP-9) diluted in blocking buffer for 1 h at room temperature (RT) followed. Membranes were washed in blocking buffer and then incubated with alkaline phosphatase-conjugated secondary antibody diluted in blocking buffer for 30 min at RT. After that membranes were washed in blocking buffer and in 0.1M Tris pH 9.5 containing 0.05M MgCl$_2$ and 0.1 MNaCl. Specific immuncomplexes were detected using nitro blue tetrazolium and 5-bromo-4-chloro-3-indoylphosphate (p-toluidine salt; Pierce, Rockford, IL, USA) as substrates.

Immunofluorescence

The seven-day-old high-density cultures were embedded in O.C.T. solution (Bayer, Munich, FRG) and immediately frozen in liquid nitrogen. For double immunolabelling 10 µm-thick sections were fixed in methanol and rinsed in PBS for 3x5 min. Incubation with the first primary antibodies (anti-cytoplasmic domain of ß1-integrin antibody, diluted 1:50 in PBS) was for 1 h at RT and with the first secondary antibody goat-anti-rabbit immunoglobulin conjugated with FITC, diluted 1:50 (GAR-FITC) for 1 h at RT. The sections were incubated with the second primary antibodies (anti-MMP antibodies, diluted 1:50 in PBS) for 1 h at RT and with the second secondary antibody goat-anti-mouse immunoglobulin conjugated with rhodamine, diluted 1:50 (GAM-rhodamine) for 1 h at RT. Each step was followed by washing. The sections were examined under a light microscope (Axiophot 100, Zeiss, FRG).

Immunoelectron microscopy: Double immunolabelling against MMPs and cytoplasmic domain of ß1-integrin (postembedding technique)

The seven- and fourteen-day-old cultures were fixed in 3% paraformaldehyde or 0.25% glutaraldehyde in PBS for 1 h, washed overnight in PBS/BSA, and gradually dehydrated in ethanol followed by embedding in LR-White (London Resin, Plano, Marburg, FRG). Ultrathin sections were placed on nickel grids. For double immunolabelling the sections were pretreated with hyaluronidase (1 mg/ml) for 10 min at RT. Subsequently, the sections were washed and incubated with 1% bovine serum albumin (BSA) in 0.01M PBS, pH 7.0 and 0.5% Tween, at RT for 10 min. The sections were incubated with the first primary antibodies (normal rabbit IgG or anti-cytoplasmic domain of ß1-integrin antibody) at a dilution of 1:40 in the PBS/BSA/Tween solution overnight at 4 °C and with first secondary antibody conjugated with goat anti-rabbit immuno-globulin with 10 nm gold particles (1:30) for 1 h at RT. Every incubation step was followed by washing in PBS/BSA/Tween. After incubation with the second primary antibodies (against MMP-1, MMP-3 and MMP-9) at a dilution of 1:40 in PBS/BSA/Tween overnight at 4 °C and with the second secondary antibody conjugated with goat anti-mouse immunoglobulin with 5 nm gold particles (1:30) for 60 min at RT the sections were washed and fixed (1% glutaraldehyde) for 10 min at RT. Then they were contrasted with an aqueous saturated solution of 5% uranyl acetate for 20 min, osmium tetroxide for 5 min and 1% tannic acid for 30 min at RT. For inspection a Zeiss EM 10 electron microscope was used.

Results

At day 3 high-density cultures of prechondrogenic mesenchymal cells from limb buds develop cartilaginous tissue that contains typical components of the cartilaginous matrix such as collagen type II and cartilage-specific proteoglycans. The formation of cartilage-specific matrix and cell surface expression of

ß1-integrins increased in culture from day three to seven. The morphology of the matrix corresponded to that of embryonic cartilage (Shakibaei et al., 1993, 1995; Shakibaei, 1998).
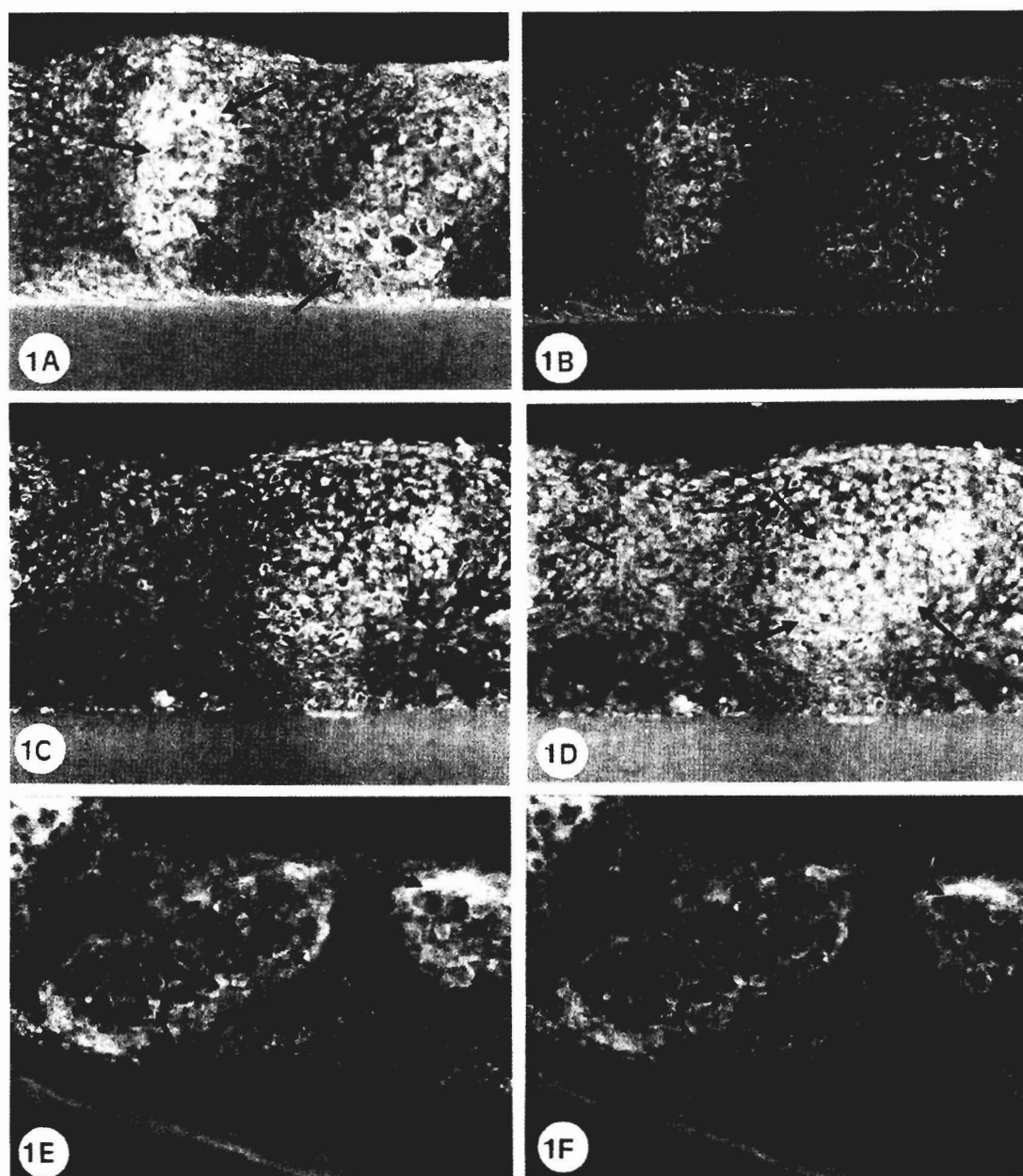
Immunofluorescence microscopy

The high-density cultures were simultaneously labelled with anti-cytoplasmic domain of ß1-integrin, anti-MMP-1, -MMP-3 and -MMP-9 antibodies: ß1-integrin labelling was found in both the perichondrium and the cartilage (Fig. 1A,C,E) in agreement with other investigations (Shakibaei et al., 1995; Shakibaei and Merker, 1999). In the perichondrium (mesenchymal cells) and between the nodules, integrin-labelling was homogenous. In the cartilage nodules the labelling was restricted to the cell surface or the immediate pericellular space. MMP labelling exhibited the same distribution as anti-cytoplasmic domain of ß1-integrin-labelling. There was no difference in the distribution of immunoreactive MMP-1 (Fig. 1B), MMP-3 (Fig. 1D), or MMP-9 (Fig. 1F). Cytoplasmic domain of ß1-integrin was detected with FITC, and MMP labelling with rhodamine-labelled secondary antibody.

Immunoelectron microscopy

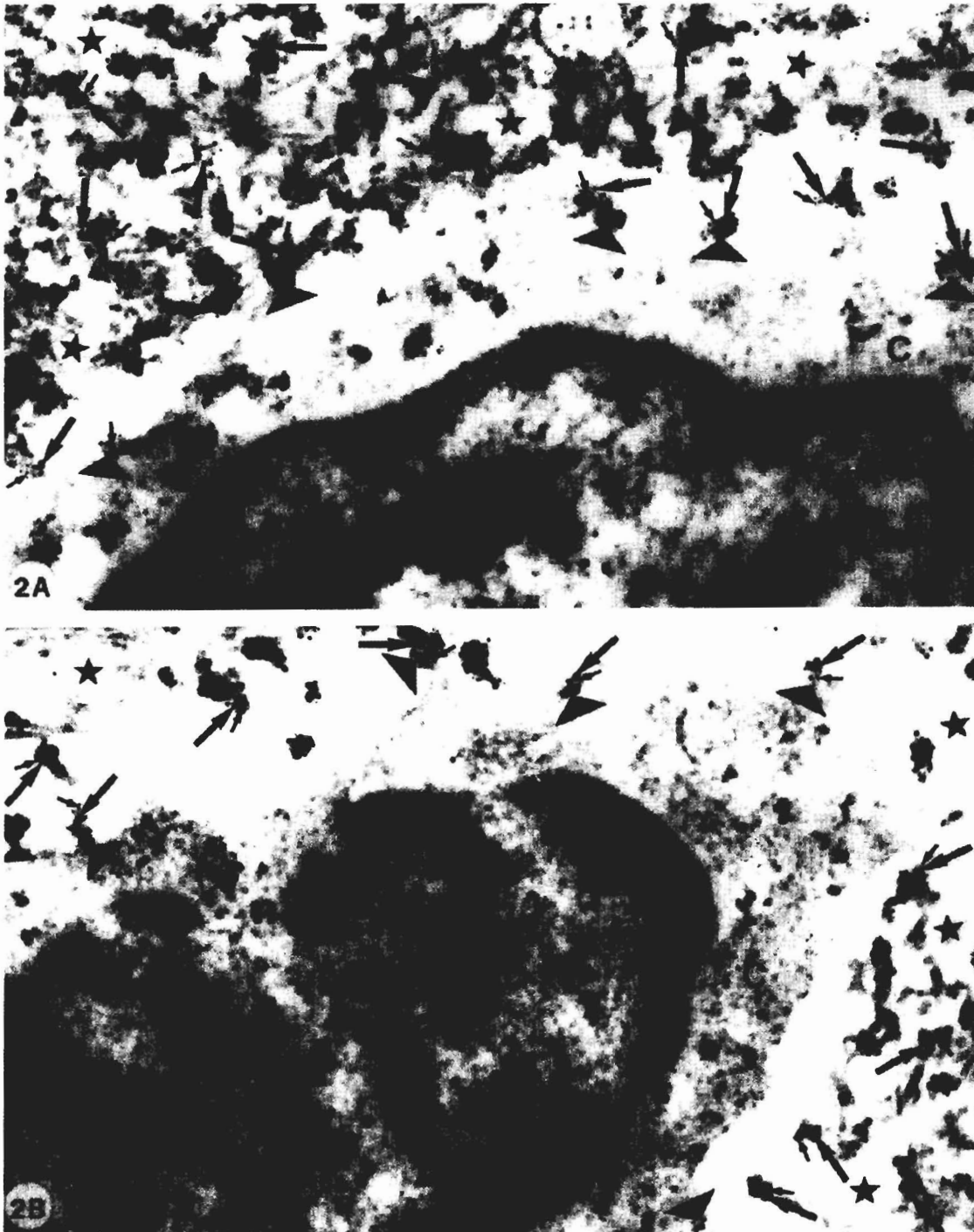Immunogold labelling with anti-MMP-1 and anti-

Fig. 1. Double immunofluorescence labelling of MMPs and cytoplasmic domain of ß1-integrin. Cytoplasmic domain of ß1-integrin **(A, C, E)** is detected with FITC and MMP-1 **(B)**, MMP-3 **(D)**, MMP-9 **(F)** labellings with rhodamine-labelled secondary antibody exhibit almost the same distribution. Immunolabelling was found in both perichondrium and cartilage nodules. In the nodules (arrows) the labelling was restricted to the cell surface and immediate pericellular space. x 160

cytoplasmic domain of ß1-integrin on day seven of the culture period was observed mainly on the cell membrane and outside the chondrocytes (Fig. 2A). The distribution of immunoreactive MMP-3 and MMP-9 with immunoreactive ß1-integrin was similar to that of MMP-1 and ß1-integrin (data not shown). The distribution was not affected by culture age since fourteen-day-old cultures were also investigated. Labelling with anti-MMP-3 and anti-cytoplasmic domain of ß1-integrin on day fourteen of the culture period was observed mainly on the cell membrane and outside the chondrocytes (Fig. 2B). The distribution of different MMP-1 and MMP-9 and ß1-integrin labellings did not differ from those found on day seven of the

Fig. 2.
Immunoelectron microscopic demonstration of MMP-1, after seven days **(A)** and of MMP-3 after fourteen days **(B)** in cartilage organoid culture. Double immunolabelling with anti-cytoplasmic domain of ß1-integrin antibody (10 nm gold particles: large arrows) and anti-MMP antibody (5 nm gold particles: small arrows). Both gold-labelled integrin and MMP antibodies are co-localized and concentrated to the cell membrane (arrowheads) as well as distributed in the ECM (*) between collagen fibrils. Only few gold particles are observed in the cytoplasm of the chondrocytes (C). n: nucleus. x 80,000.

culture period (data not shown). Almost everywhere, the large (anti-ß1-integrin-coupled) and small (anti-MMP-coupled) gold particles formed clusters (Fig. 2A,B). Many clusters were localized close to the cell surface but also between the irregular collagen fibrils in the pericellular space. The pretreatment with hyaluronidase increased the staining in the matrix and on the cell membrane, indicating that some sites of integrins and MMPs were masked, as reported earlier (Shakibaei and Merker, 1999). No immunogold labellings were observed in negative controls with normal rabbit IgG. Taken together, these findings suggest that MMPs and ß1-integrins are co-localized and are distributed in the extracellular matrix and on the chondrocyte membrane in cartilage.

Coimmunoprecipitation assay

Immunoprecipitates using the anti-MMP-1, -3 and -9 antibodies contained ß1-integrins that could be detected subsequently by immunoblotting as a specific doublet with apparent molecular weight of 110 kDa (Fig. 3A). Furthermore, immunoprecipitation using the ß1-integrin antibody contained MMP-1, -3, and -9 that could be
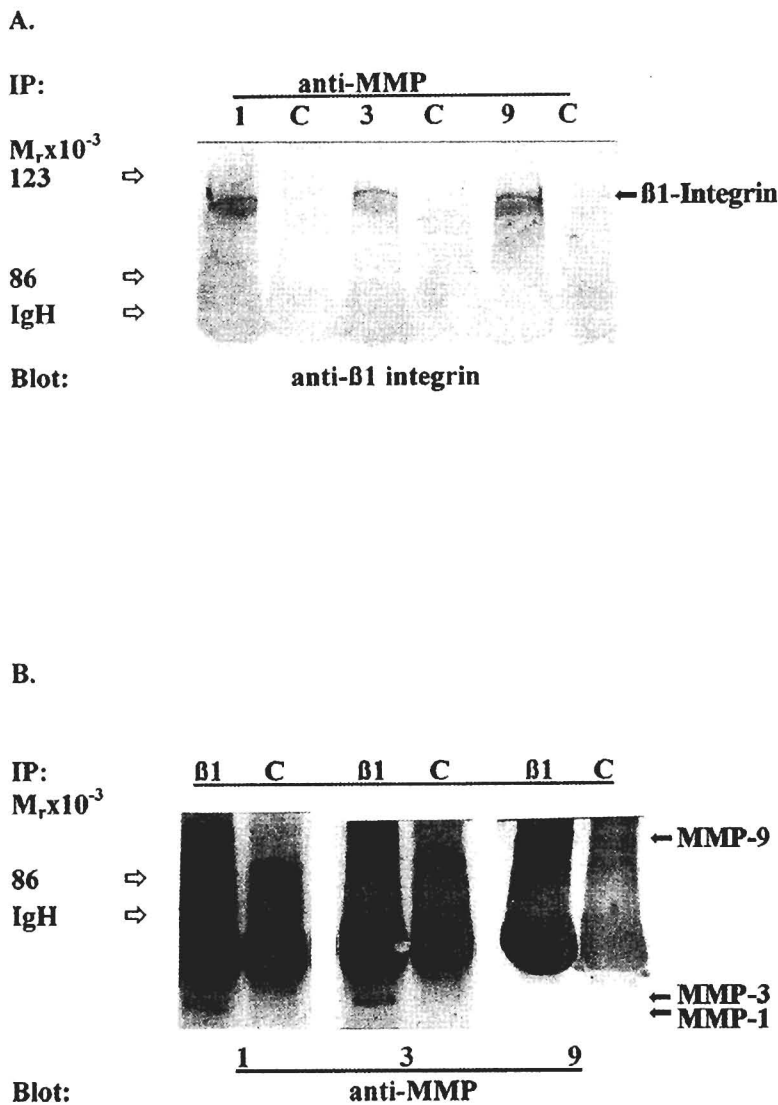
Fig. 3. Coimmunoprecipitation assays and immunoblotting of cytoplasmic domain of ß1-integrin and MMP-1, -3, and -9. Immunoprecipitates were separated and analysed by immunoblotting with anti-cytoplasmic domain of ß1-integrin or anti-MMP antibodies. A. Immunoprecipitation with anti-MMP antibodies or normal IgG serum followed by immunoblotting with anti-cytoplasmic domain of ß1-integrin antibody. 1: anti-MMP-1 immunoprecipitate, 3: anti-MMP-3 immuno-precipitate, 9: anti-MMP-9 immunoprecipitate, C samples precipitated with control antibody. B. Immunoprecipitation with anti-cytoplasmic domain of ß1-integrin antibody (ß1) or control antibody (C) followed by immunoblotting with anti-MMP antibodies. 1: anti-MMP-1 antibody; 3: anti-MMP-3 antibody; 9: anti-MMP-9 antibody.

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detected subsequently by immunoblotting as specific bands of apparent molecular weights of 51, 57, and 92 kDa, respectively (Fig. 3B). The results indicate that the ß1-integrins interact with MMP-1, -3 and -9 and the ß1-integrins in the matrix represent complete integrins. Samples precipitated with control antibodies did not reveal any specific bands (Fig. 3A,B).

Discussion

In the present study we used chondrocyte high-density cultures because they reflect *in vivo* cartilage conditions more closely than monolayer cultures. Previous studies indicate that the morphology of the matrix corresponds closely to that of embryonic cartilage (Shakibaei et al., 1993, 1995; Shakibaei, 1998), and that integrins are not restricted to the cell surface but rather can also be demonstrated on collagen fibrils in the cartilage matrix by immunoelectron microscopy and immunoprecipitation assays of cell-free cartilage preparations (Shakibaei et al., 1993, 1995; Shakibaei and Merker, 1999). The findings of the present study confirm this phenomenon and show that immunoreactive ß1-integrins are also in extracellular matrix. Others have determined the presence or expression of MMP-1, MMP-3 and MMP-9 in cartilage (Freemont et al., 1997; Saito et al., 1998). In this study we showed by electron microscopy that immunoreactive MMP-1, MMP-3 and MMP-9 are in cartilage, mainly in the pericellular space close to the cell surface. Because the antibodies used recognized both zymogen and activated MMP forms, in the present study we could not distinguish between zymogen and activated form. Our results suggest that one function of the MMPs involves an interaction with extracellular ß1-integrins and MMPs may also be responsible for the shedding of ß1-integrin molecules. Indeed, it has been reported that a variety of cell membrane molecules can be secreted, such as heparan sulfate proteoglycans (syndecane) (Rapraeger et al. 1986), cell adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) (Pigott et al., 1992) and vascular cell adhesion molecule-1 (VCAM-1) (Pigott et al., 1992). Furthermore, it is known that also other cell membrane receptors can be shed, such as cytokines and growth factor receptors (Rose-John and Heinrich, 1994) and the transferrin receptor (Chitambar et al., 1991).

This finding is in agreement with the function of MMPs. MMPs cleave ECM molecules and are important proteinases of ECM remodelling. MMP-1 and MMP-3 are involved in cartilage collagen degradation (Saito et al., 1998). MMPs are synthesized in the cytoplasm, activated at the cell surface and exported into the ECM (Freemont et al., 1997; Yong et al., 1998). By other investigators it was shown that MMPs were present in extracellular matrix vesicles by substrate gel electrophoresis and western blot analysis. Matrix vesicles are involved in maturation of cartilage ECM (Schmitz et al., 1996). But in our study vesicles could not be found in the cartilage matrix by electron microscopy. We used here a postembedding technique (LR-white) for immunogold labelling. With this technique the classical tri-laminar cell membrane of chondrocyte is not evident and therefore no membrane surrounded vesicles like matrix vesicles can be distinguished.

In this study we have shown by immunoelectron microscopic and immunofluorescence investigations a specific co-localization of ß1-integrins and particular MMPs, implicating a functional association between MMPs and ß1-integrins, which was then demonstrated by results of coimmunoprecipitation assays. The detailed functional association remains to be elucidated, but one can assume that the MMP/integrin complex plays an important role in cell/matrix interactions of chondrocytes as described for other cell types (Brooks et al., 1996; Bafetti et al., 1998). It requires further investigations to discover if or which other proteins participate in the integrin/MMP complex. Furthermore, integrins are known to form complexes with many intracellular proteins and membrane proteins like the IGF-I receptor and caveolins (Shakibaei et al., 1999; Schwab et al., 2000, 2001).

Some indications for a functional association of MMPs and integrins in other cell types have been reported previously. It was found that MMP-2 and αvß3 (vitronectin receptor) are specifically co-localized and functionally associated on angiogenic blood vessels and melanoma cells *in vivo* (Brooks et al., 1996). MMP-2 binds in a proteolytically active form directly to integrin αvß3 on the surface of invasive cells, facilitating cell-mediated collagen degradation. The naturally occurring MMP-2 breakdown product (PEX) inhibits binding of MMP-2 to the vitronectin receptor (Brooks et al., 1998). Integrin receptors are also involved in regulation of particular MMP gene expression. For example, the vitronectin receptor, participates in regulation of MMP-2 at the transcriptional level in melanoma cells (Bafetti et al., 1998). The fibronectin receptors (α5ß1 and α4ß1) take part in regulation of MMP-1, MMP-3 and MMP-9 in fibroblasts, and integrin α2ß1 is a positive regulator of collagenase MMP-1 and collagen α1 (I) gene expression (Werb et al., 1989; Huhtala et al., 1995; Riikonen et al., 1995). It is reported that cleavage of the ß1-integrin subunit of the main collagen receptor in platelets by a metalloproteinase inhibits the interaction of platelets with collagen (Kamiguti et al., 1997).

At the moment we can only hypothesize about the functional association between MMPs and integrins in chondrocyte cultures. Perhaps MMPs affect also integrin signalling pathways passed through membrane-anchored integrins in chondrocytes as described for other proteins that co-localize with ß1-integrins in chondrocyte cultures, for example, caveolins or the IGF-I receptor (Murphy and Gavrilovic, 1999; Shakibaei et al., 1999; Schwab et al., 2000, 2001). Signalling by integrins leads to activation of the Ras-MAP kinase signalling pathway in human chondrocytes. Previously, we showed that activation of the MAP kinase pathway regulates the

activity of a number of intracellular signalling proteins through phosphorylation and chondrocyte differentiation (Shakibaei et al., 1999). More recently, it has been shown that the specific inhibition of the MAP kinase signalling pathway leads to apoptosis of human chondrocytes *in vitro* (Shakibaei et al., 2001).

The question of how integrins get into the cartilage matrix has not been answered yet. Since we used anti-ß1-integrin antibodies recognizing the intracellular c-terminal domain we can exclude an ectodomain shedding comparable to ectodomain shedding of growth factors by MMPs, because the antibody would not recognize shedded ectodomains (Black et al., 1997; Izumi et al., 1998). Evidently the whole ß1-integrin molecule or a part comprising the intracellular domain is released from the cell membrane and co-localized with MMPs in the ECM. Possibly, MMPs take part in or initiate this integrin release that enables chondrocytes to break off focal contacts to the ECM and may change integrin-mediated signalling. Is integrin shedding simply a mechanism for down-regulation of integrin receptors and how is it regulated? Integrin shedding may also be a result of apoptosis of chondrocytes. It has been reported that the loss of integrin-mediated ECM-anchorage can terminally result in apoptosis (Howe et al., 1998). Perhaps released integrins in the ECM act as receptors for MMPs to direct and organize ECM remodelling (e.g. direct the collagen digestion, because integrins are distributed between the collagen fibrils) or to further activate MMPs or otherwise to alter MMP activity.

The results of this study suggest a functional association of MMPs and ß1-integrins. Further investigations are needed to answer some of the many questions that arise about the functional significance of this phenomenon for chondrocytes.

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