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CD147 (Extracellular Matrix Metalloproteinase Inducer-EMMPRIN) Expression by Human Articular Chondrocytes

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ABSTRACT

Background: Integrins are a family of transmembrane proteins that allow communication between the extracellular matrix and the interior of cells. Chondrocytes, cells of articular cartilage, express integrins and these molecules appear to have a variety of roles including mechanotransduction. Integrins are known to associate with a number of accessory molecules such as CD147 that may act to regulate their activity. The purpose of this study was to investigate the expression of CD147 in normal and osteoarthritis human articular cartilage and identify potential roles in mechanical signalling. **Methods:** Expression of CD147 in normal and osteoarthritis human articular cartilage was examined by the immunostaining and Western-blotting techniques. Potential roles in mechanotransduction were studied by assessing effects of function blocking antibodies on the electrophysiological response to mechanical stimulation. **Results:** CD147 was extensively expressed by chondrocytes in normal and osteoarthritic cartilage and shown by Western-blotting to have a molecular weight in the region of 35-50 kDa. Function blocking antibodies had no effect on the membrane depolarisation response of chondrocytes from osteoarthritic cartilage to mechanical stimulation. **Conclusion:** Human articular chondrocytes show extensive expression of CD147 in normal and osteoarthritic cartilage. Roles for this molecule in regulation of chondrocyte function remain to be defined. *Iran. Biomed. J. 12 (3): 153-158, 2008*

Keywords: Chondrocyte, Articular cartilage, Osteoarthritis, Extracellular matrix metalloproteinase inducer (EMMPRIN)/CD147, Integrin

INTRODUCTION

Previous studies have demonstrated that human articular chondrocytes (HAC) show changes in membrane potential following cyclical mechanical stimulation. In normal chondrocytes, a membrane hyperpolarisation response to mechanical stimulation at 0.33 Hz is mediated by α5β1 integrin and has been shown to be IL-4 dependent. In contrast, osteoarthritis chondrocytes show a membrane depolarization to the same stimulus [1]. Expression and function of integrins are related to their interaction with ligand molecule(s) appropriately present in the extracellular matrix (ECM). A complex series of steps leads from initial integrin interactions with an extracellular ligand to transmembrane effects on the localization of cytoskeletal and intracellular signaling molecules, resulting in eventual regulation of gene expression [2].

CD147, also known as ECM metalloproteinase inducer (EMMPRIN), is a highly glycosylated transmembrane glycoprotein of 50-60 kDa having typical features of an integral membrane protein of the immunoglobulin super family. It contains two extracellular Ig domains: a transmembrane domain, and a 39-amino acid cytoplasmic domain [3]. The presence of a glutamic acid residue in the transmembrane region suggests that the protein might functionally interact with other membrane proteins [4]. CD147, like CD98 associates physically with β1 integrins in the cell membrane [5] and isolated cytoplasmic β1 domains [6]. Moreover, CD147 was recently found to co-immunoprecipitate with β1 integrins (α3β1 and α6β1), and co-localize with these

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integrins in areas of cell-cell contact, within the plasma membrane of HT1080 fibrosarcoma cells [5].

CD147 is broadly expressed on human peripheral blood cells, endothelial cells, and cultured cells of hemopoietic and non-hemopoietic origin. In T cells, its expression level is dependent on the differentiation state. Thymocytes strongly express CD147 [7]. Significant expression of CD147 has also been reported in neoplasms of the bladder, liver, and lung [8]. We have recently reported the expression CD147 in cultured chondrocytes isolated from knee cartilage with elevated levels in ankle chondrocytes but these studies did not assess *in vivo* analysis or investigate potential functions [9].

Initially identified on the surface of human cancer cells, CD147 has been shown to stimulate adjacent stromal cells to produce and activate several matrix metalloproteinases (MMP) [10-12] including MMP-1, MMP-2, MMP-3, membrane type 1MMP (MT1-MMP), and MT2-MMP [11, 13]. MMP synthesis in fibroblasts in response to stimulation by CD147 is a relatively slow process, taking 24-48 h to reach maximum [14]. Different fibroblast populations appear to respond differently to the CD147 [11] perhaps as a result of varying degrees of expression. CD147 also appears to be involved in a number of other cellular activities such as chaperone functions [15], calcium transport [16], neutrophil chemotaxis [17], and blood brain barrier development [18].

The present study has focused on the expression pattern of CD147 by normal and osteoarthritis HAC in both *in vivo* and *in vitro* conditions. The results show very strong expression pattern of CD147 in both normal and osteoarthritis human articular cartilage. All zones from normal and different grades of osteoarthritis and chondrocyte clusters in diseased cartilage showed the same strong expression pattern. Antibodies to CD147 did not influence the electrophysiological response of chondrocytes to mechanical stimulation.

MATERIALS AND METHODS

Source of Tissue, chondrocyte culture. Human articular cartilage was obtained, with ethical approval and patients' consent, at operation from knee joint arthroplasty specimens and amputations for peripheral vascular disease. Cartilage was assessed macroscopically for the presence or absence of osteoarthritic changes and graded macroscopically for the presence or absence of osteoarthritis using the Collins/McElligott [19]. Chondrocytes were isolated by sequential enzyme digestion as described previously [20]. Cells were seeded in Iscove's modified Dulbecco's medium (Gibco, UK) supplemented with 10% fetal calf serum (Sigma, UK), 100 I.U./ml penicillin (Gibco, UK) and 100 μg/ml streptomycin (Gibco, UK) to a final density of 5×10^5/ml (for protein extraction) and 1×10^4 cells/ml (for electrophysiology) in 55-mm plastic Petri dishes (Nunc, USA). Primary, non-confluent, 1-2-week cultures of chondrocytes were used in all experiments. The day before mechanical stimulation was to be carried out, culture media containing serum was replaced by serum-free media.

Immunohistochemistry (IHC). Cryostat sections were cut from fresh frozen cartilage, obtained from either normal cartilage (n = 5) or osteoarthritic cartilage (n = 7), with a Brights cryostat, mounted on poly-L-lysine coated glass slides, allowed to come to room temperature and fixed with acetone for 10 min. Sections were stained by an avidin-biotin-immunoperoxidase technique. CD147 expression was assessed using mouse mAb anti-CD147, 8G6, at 1:5000 (a kind gift from Dr. Martin E. Hemler, Harvard Medical School, USA).

Protein extraction and Western-blotting. The methods for protein extraction and Western-blotting used have been described previously [20]. In brief, chondrocytes at rest or the following mechanical stimulation were washed with ice-cold PBS containing 100 μM Na_3VO_4 (Sigma, UK) and lysed *in situ* with ice-cold lysis buffer containing 1% Igepal (Sigma, UK), 100 μM Na_3VO_4, and protease inhibitor cocktail tablet (Boehringer Mannheim, Germany) at 4°C for 15 min. Supernatants were collected after centrifugation at 16060 ×g for 15 min. Concentration of protein within lysates was determined using Folin-Lowry assay method with Dynatech MR5000. Whole cell extract proteins were separated on a 10% SDS-PAGE under reducing and non-reducing conditions. Following electrophoresis, whole cell lysates were transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore Immobilon-P, Sigma, UK). Membranes were blocked overnight at 4°C with 2% BSA in TBST (12.5 mM Tris/HCl, pH 7.6, 137 mM NaCl, 0.1% Tween 20). After washing three times with TBST, blots were incubated at room temperature for 1 h with and then horse radish peroxidase labelled secondary antibody. Membranes were rewashed extensively and the binding was detected using Enhanced Chemiluminescence Plus (ECL+) Western-blotting detection system (Amersham, USA), according to the manufacturer's instructions.

Mechanical stimulation of chondrocytes and electrophysiological measurement. Membrane potentials of cells were recorded using a single electrode bridge circuit and calibrator as previously described [21, 22]. Microelectrodes with tip resistances of 40 to 60 meg ohms and tip potentials of approximately 3 mV were used to impale the cells. Membrane potentials of the isolated cells were measured, and results were accepted if, on cell impalement, there was a rapid change in voltage to the membrane potential level that remained constant for at least 60 s. The membrane potentials of 5-10 cells were measured prior to and following the additional of the reagent to be tested and/or mechanical stimulation. Each experiment was undertaken at least three times on cells from different donors.

The technique and apparatus used for mechanical stimulation of primary HAC have been previously described in detail [22]. Plastic tissue culture dishes (58 mm diameter, NUNC, USA) containing sparse primary monolayer cultures of HAC were placed in a sealed pressure chamber with inlet and outlet ports. The chamber was pressurized using helium gas from a cylinder, at a frequency determined by an electronic timer that controlled the inlet and outlet valves. The standard stimulation regime used was a frequency of 0.33 Hz (2 s on and 1 s off) at 37°C, for 20 min at a pressure of 16 kPa above atmospheric pressure. This system produces 3700 microstrain on the base of the culture dish.

Statistics. The mean, standard and standard error of the mean of cell membrane potentials were determined in each experiment. For statistical comparisons, when the F-ratio of the two variances reached significant, the non-parametric Mann-Whitney test was used. When the ratio did not reach significant, the student's *t*-test was used.

RESULTS

In vivo expression of CD147/ EMMPRIN in normal and* osteoarthritis *human articular cartilage. Both normal and osteoarthritic cartilage showed a similar strong expression pattern of CD147 (Fig. 1). All chondrocytes in sections from different donors showed a similar strong staining pattern. No identifiable difference was found between the expression pattern of normal and osteoarthritis articular cartilage. Chondrocytes in different zones (superficial, surface, middle and deep) showed a similar expression pattern of CD147. Strong immunoreactivity of CD147 at a high dilution (1:5000) of mAb 8G6 was observed, suggesting strong expression of CD147 in normal and osteoarthritis articular cartilage. Mild and severe grades of osteoarthritis did not show differences in expression pattern of CD147.

In vitro expression of CD147 (EMMPRIN) in normal and osteoarthritis HAC. To assess the biochemical characteristics of CD147 in articular cartilage, a series of Western-blotting experiments was performed. Normal chondrocytes were isolated from ankle joint (5), knee joint (3) or hip joint (3) cartilage. Osteoarthritis chondrocytes were isolated from knee joint (4) or hip joint (4). In preliminary studies, cell lysate extracted from cultured HAC were run under both reducing (not shown) and non-reducing (Fig. 2) conditions and probed with mouse anti-CD147, 8G6, mAb at a range of dilutions. Under reducing conditions, some samples showed a band of ~50 kDa, but other samples did not express the same band but expressed multiple faint bands. Under non-reducing conditions, however, all samples showed a broad band of between 35 and 50 kDa and this was consistent in all samples (Fig. 2). Expression of CD147 appeared similar in both normal and osteoarthritis chondrocytes.

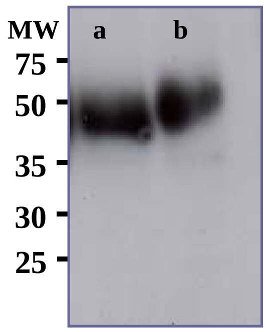

Fig. 2. Detection and comparison of CD147 (EMMPRIN) in normal and osteoarthritis human articular cartilage. Equal amounts (40 μg/lane) of total extracted proteins prepared from cultured human articular chondrocytes derived from normal (lane a, femoral head) and osteoarthritis (lane b, tibia plateau, osteoarthritis grade III) samples were run in a 10% SDS-PAGE under non-reducing conditions. Detection of CD147 was assessed by probing the blots with mouse mAb anti-CD147 8G6 at 1:5000. The blots shown are representative of three different donors (normal and osteoarthritis). Molecular weights (MW) in kDa are indicated on the left.

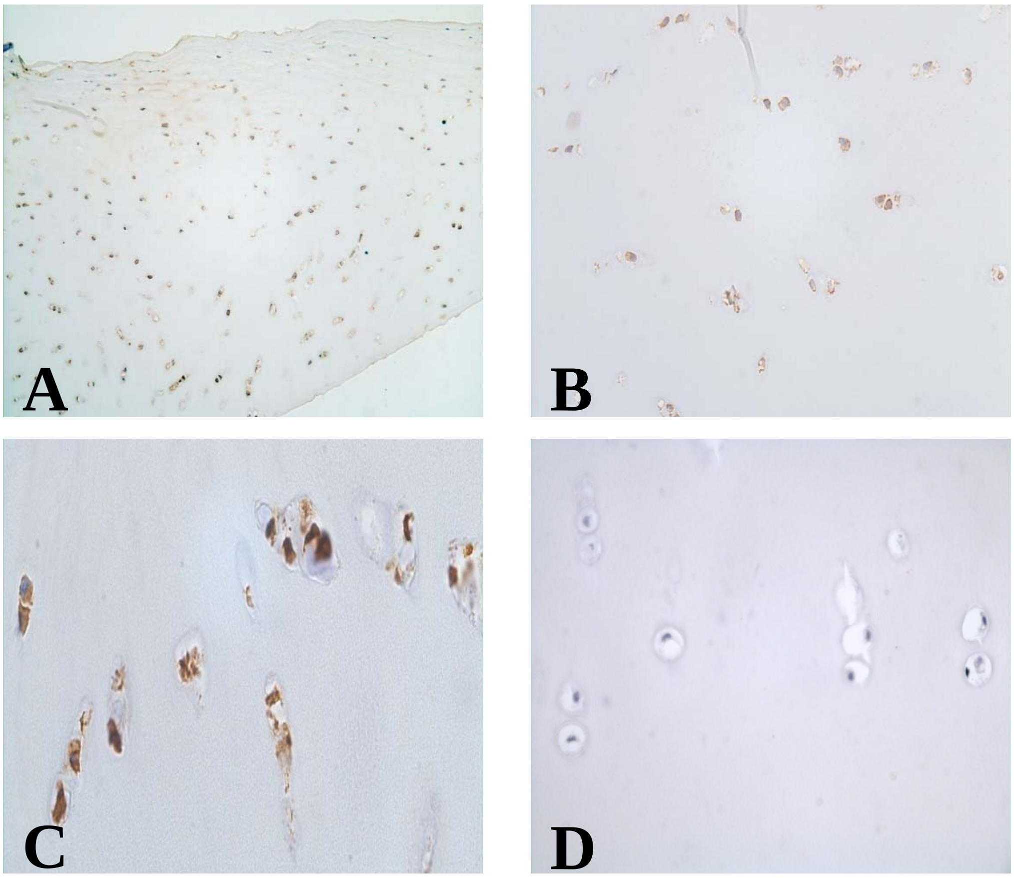

Fig. 1. Immunoreactivity of mAb anti-CD147, 8G6, in normal and osteoarthritis human articular cartilage sections. Immunoreactivity was assessed by using mouse anti-CD147, 8G6, at 1:5000. All sections showed strong expression of C1D47 (EMMPRIN) in different zones. **(A)** Tibial plateau, normal, superficial-deep, (age, sex not recorded), ×100. Chondrocytes in all zones showed strong expression pattern of CD147. **(B)** Femoral head, normal, mid-zone (male, 76 years), ×200. Mid-zone chondrocytes showed strong expression pattern for CD147. **(C)** Femoral condyle, osteoarthritis II, mid zone (female, 78 years), ×400. Osteoarthritis chondrocytes in mid-zone of FAII showed strong expression of CD147. **(D)** Femoral condyle, normal, mid zone (female, 63 years), ×200. In comparison to negative control (D), chondrocytes in all zones of normal and osteoarthritis sections showed a similar strong expression pattern of CD147 immunostaining.

Effect of anti- CD147 mAb on the osteoarthritis chondrocyte depolarization response to 0.33 Hz cyclical mechanical stimulation. To establish whether CD147 had roles in chondrocyte mechano-transduction, a series of experiments in which the effect of anti-CD147 antibody on the electro-physiological response to mechanical stimulation were carried out. The monoclonal antibody anti-CD147, 8G6, had no any effect on resting membrane potential of articular chondrocytes and had no effect on the depolarization response that occurs following 0.33 Hz cyclical mechanical stimulation (Table 1).

DISCUSSION

CD147 expressed by HAC *in vivo* and *in vitro* was confirmed by IHC and Western-blotting. Normal and osteoarthritis chondrocytes showed comparable levels of expression of EMMPRIN by using an anti-CD147 monoclonal antibody. This antibody has previously been shown to inhibit CD147 dependent hemophilic interactions and production of secreted MMP-2 by breast cancer cells. The above antibody did also influence the electrophysiological response of osteoarthritis chondrocytes to mechanical stimulation. CD147, is a 45-60 kDa highly glycosylated transmembrane protein showing differential molecular weight in various cell types. These differences in molecular weight of CD147 are mainly due to the varying extent of glycosylation since the protein backbone corresponds to an approximate molecular weight of 27-32 kDa [3]. Accordingly, the molecular weight of CD147 in chondrocytes is in the broad range of 35-50 kDa, which is similar to that of CD147 expressed in other human cells [13].

Table 1. Effect of function blocking monoclonal anti-CD147 antibody (8G6), at 1.5 μg/ml on the depolarization response of osteoarthritis chondrocytes to 0.33 Hz cyclical mechanical stimulation.

		Membrane potential (-mV) (mean ± SEM)				
Reagent	**n**	**Resting**	**anti-CD147 (0.33 Hz M)**	**anti-CD147 +**	**% change**	***P* value**
Nil	5	30.0 ± 1.9	-	17.4 ± 1.3	-42	0.0001
8G6	5	28.2 ± 0.9	30.4 ± 1.5	17.8 ± 1.2	-36	0.0001

It has been previously shown that antibodies to CD147 can neutralize CD98-induced cell aggregation [23]. There are also some striking parallels between CD147 and CD98 another integrin-associated protein. CD147 is physically associated with β1 integrin in the cell membrane [5], as is CD98 with isolated cytoplasmic β1 domains [6]. Levels of CD98 and CD147 correlate on T cells, with high levels in the thymus, low levels in resting mature T cells, and higher levels on activated mature T cells [24]. Thus, it appears that CD147, like CD98, is acting as a chaperone for multimembrane-spanning transporter molecules. In the case of CD98, these are amino acid transporters whilst with CD147 these are the monocarboxylate transporter family of proton-linked monocarboxylic acid transporters [25] leading to the suggestion that CD98 forms one component of a ''sensory complex'', containing β1 integrins, CD98, and CD147, together with all their associated molecules [23].

In the current study we have found no evidence to support our hypothesis that CD147 may be involved in integrin-dependent mechanotransduction in chondrocytes. It is however likely that a molecule that appears to be highly expressed would have important functions. Possible roles for CD147 in chondrocytes include the production and activation of several MMP including MMP3 [11-13] that are involved in tissue remodeling and may have importance in osteoarthritis. CD147 expression in chondrocytes may be important for healthy cartilage metabolism under physiologic conditions but altered activity may be a component of degenerative and inflammatory joint disease such as osteoarthritis and rheumatoid arthritis. In this regard, Tomita *et al.* [26], by analyzing synovial tissue, have shown that CD147 may be one of the important factors in progressive joint destruction in RA. CD147 also interacts with annexin II [27] a molecule that is also expressed by osteoarthritis chondrocytes [28, 29]. Annexins form Ca^{2+} channels and influx Ca^{2+} into the chondrocytes, suggesting possible roles in controlling or altering Ca^{2+} homeostasis in cartilage [29]. Thus, studying the interactions between CD147 and annexin II during the early events of osteoarthritis may indicate novel mechanisms underlying osteoarthritis cartilage degeneration. Nevertheless further studies are required to characterize CD147/EMMPRIN in articular cartilage metabolism and signal transduction.

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