Iranian Biomedical Journal 11 (2): 119-124 (April 2007)

Short Report

The Expression of Signal Regulatory Protein-α in Normal and Osteoarthritic Human Articular Cartilage and Its Involvement in Chondrocyte Mechano-transduction Response

Mahmoud Orazizadeh[*1] and Donald M. Salter[2]

[1]*Dept. of Anatomical Sciences, Medical School, Ahwaz Jondishapour University of Medical Sciences, Ahwaz, Iran;*
[2]*MD FRCPath FRCPE, University of Edinburgh The Queen's Medical Research Institute, 47 Little France Crescent Edinburgh, Scotland, UK*

Received 30 March 2006; revised 21 June 2006; accepted 17 July 2006

[Downloaded from ibj.pasteur.ac.ir on 2024-03-28]

[DOR: 20.1001.1.1028852.2007.11.2.6.4]
[DOI: -]

ABSTRACT

Background: Signal regulatory proteins (SIRP) belong to immunoglobulin super family (IgSF) and relate to integrin signaling cascades. It has been shown that SIRPα is expressed in a variety of cells including myeloid cells and neurons. In the present study the expression of this IgSF member in articular chondrocytes was investigated. **Methods:** Using a panel of anti-SIRPα antibodies, immunohistochemistry, Western-blotting and electrophysiology methods, expression of SIRPα and its role in chondrocyte mechano-transduction were assessed. **Results:** No identifiable positive signal was obtained by using immunohistochemistry methods on frozen and paraffin sections. SIRPα is expressed by both normal and osteoarthritis cultured chondrocytes. The electrophysiological response of chondrocytes in the presence of SE7C2 mAb was significantly inhibited whereas; SE5A5 did not show any modification in this response. **Conclusions:** It seems likely that SIRPα could be associated with other proteins such as integrins, CD47 and ion channels, which contribute to the electrophysiological response of human articular chondrocytes. In any case, this study has provided a specific functional role for SIRPα in chondrocyte mechano-transduction. *Iran. Biomed. J. 11 (2): 119-124, 2007*

Keywords: Cartilage, Chondrocyte, Osteoarthritis (OA), Signal regulatory proteins (SIRP)

INTRODUCTION

Articular cartilage is subjected, *in vivo*, to frequent and repetitive mechanical loads which regulate chondrocyte function [1]. Previous studies have demonstrated that human articular chondrocytes 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 associated with an interleukin 4 dependent. In contrast, Osteoarthritis (OA) chondrocytes show a membrane depolarization to the same stimulus [2]. It has also demonstrated that the expression of cell surface molecules, such as integrins, is preferentially related to their ligand molecule(s) that is/are appropriately present in the extracellular matrix (ECM) of human articular cartilage and cell surface molecules. A complex series of steps leads from initial integrin interactions with an extracellular ligand to transmembrane effects on the localization of cytoskeletal molecules or signaling molecules, to the activation of signaling pathways, and to eventual regulation of gene expression. It is becoming increasingly apparent that full understanding of integrin functional activities may require understanding of integrin associations with other cell-surface molecules [3]. It has been also shown that several kinds of proteins, including immunoglobulin superfamily (IgSF) proteins (e.g. CD47, CD147, Signal regulatory proteins [SIRP]

*Corresponding Author; Tel/Fax: (+98-611) 333 7370; E-mail: m_orazizadeh@yahoo.com

family) that bind to integrins, may generally function in concert to mediate both cell-cell and cell-ECM interactions, which in turn stimulate cytoskeletal reorganization, migration and a cascade of intracellular signalling pathways.

SIRP belong to IgSF and relate to integrin signalling cascades. One of the SIRP [4], Src homology 2 (SH2) domain-containing protein tyrosine phosphatase substrate 1 (SHPS-1), is also known as SIRPα1 and CD172a [5]. SIRP appears ubiquitously expressed as Ig-like transmembrane glycoproteins composed of two subgroups: SIRPα and SIRPβ that overall constitute a subfamily within the Ig SF. Two subfamilies termed SIRPα and SIRPβ are distinguished by the length of the cytoplasmic region. Among SIRP, SHPS-1 was first identified as a novel tyrosine phosphorylated protein that binds to SH2-containing protein tyrosine phosphatases, SHP-1 and SHP-2, in v-Src-transformed fibroblasts (SR-3Y1 cells) [4].

A protein of the SIRP family, like other IgSF members, consists of three domains: the extracellular domain, the transmembrane domain and the intracellular domain. The extracellular domain is further divided into three Ig- like regions: an amino-terminal Ig variable region and two Ig constant regions. The second and third IgC can be removed by alternative splicing. At least 15 human members [5], varying in the form of subtle amino acid differences in the IgV region, have been identified in the SIRP family. SHPS-1 has relative molecular weight of 90-120 kDa and is differentially glycosylated in a species and tissue specific manner [5]. Because the differential pattern of SHPS-1 glycosylation, the species-specific difference of molecular weight may be due to the number of potential N-glycosylation sites [4]. Various mitogens such as epidermal growth factor and platelet-derived growth factor induce the tyrosine phosphorylation of SHPS-1 to recruit the tyrosine phosphatases and several SH2-containing adapters to the plasma membrane [4]. These receptors can transducer extracellular signals regulating a diverse array of responses including proliferation, differentiation and cell survival [6]. SHPS-1 is ubiquitously expressed in all tissues examined (heart, brain, spleen, lung, liver, muscles, kidney, and testis), being most abundant in brain and spleen [5]. Using immunohistochemistry with a specific anti-SHPS-1 antibody, it has been shown that SHPS-1 is strongly expressed in myeloid cells (macrophages, monocytes, granulocytes and dendritic cells) and neurons [7].

Previous studies have shown that the interaction between integrin and ECM proteins causes tyrosine phosphorylation of SIRPα. This suggested that SIRPα might be a member of the group of proteins that are at focal adhesion contacts and undergo tyrosine phosphorylation in response to the interaction of integrins with the ECM [8].

The discovery that a major ligand for mouse SIRPα is the integrin-associated protein (CD47/IAP) was reported [8]. Studies by Seiffert *et al*. [9] revealed that the extracellular regions of human SIRPα adhere to a number of primary haematopoietic cells and cell lines and also identified CD47/IAP as prominent extracellular ligands.

In the present study, using a panel of anti- SIRPα Ab, immunohistochemistry and Western-blotting methods, the expression of this IgSF member in articular chondrocytes was investigated and then its role in mechano-transduction was assessed.

MATERIALS AND METHODS

Source of tissue, chondrocyte culture. Human articular cartilage was obtained from Royal Infirmary Hospital, Edinburgh (Scotland, UK), with ethical approval and patients' consent, at operation from knee joint arthroplasty specimens and amputations for peripheral vascular. Cartilage was assessed macroscopically for the presence or absence of osteoarthritic changes and graded macroscopically for the presence or absence of OA using the Collins/McElligott system [10]. Chondrocytes were isolated by sequential enzyme digestion as described previously. 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 and culture media containing serum was replaced by serum-free media.

Antibodies. A panel of mouse mAb anti-SIRPα antibodies SE12B6, SE7C2 and SE5A5 (kindly donated by Dr. H.J Büring, University of Tübingen,

[Downloaded from ibj.pasteur.ac.ir on 2024-03-28]
[DOR: 20.1001.1.1028852.2007.11.2.6.4]
[DOI: -]

Germany) and a rabbit polyclonal anti-SIRPα (Affinity Bioreagents [ABR, Inc.]) were applied.

Immunohistochemistry. The technique for paraffin sections has been previously described [10]. For frozen sections, samples of normal and osteoarthritic human articular cartilage (Collins grading system) were snap frozen in liquid nitrogen. The sections were cut 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. Then, the sections were stained by an avidin-biotin-immunoperoxidase technique.

Protein extraction and Western-blotting. The methods for protein extraction, immunoprecipitation and Western-blotting used have been described previously [10]. In brief, cells at rest or 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 at 4°C for 15 min. Lysis buffer contained 1% Igepal (Sigma, UK), 100 μM Na_3VO_4, and protease inhibitor cocktail tablet (Boehringer Mannheim, Germany). Supernatants were collected after centrifugation at 16060 ×g for 15 min. Concentration of protein within lysate was determined using Folin-Lowry assay method with Dynatech MR5000.

Whole cell extracts proteins were separated on a 7.5% SDS-PAGE under reducing conditions. Following electrophoresis, whole cell lysate was transferred onto polyvinylidene fluoride 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 for 1 h at room temperature with rabbit polyclonal anti-SIRPα (Affinity Bioreagents [ABR Inc.]) and then HRP was labelled as secondary antibody. Membranes were rewashed extensively and the binding was detected using Enhanced Chemiluminescence Plus Western- blotting detection system (Amersham, USA), according to the manufacturer's instructions.

Electrophysiological measurements and mechanical stimulation of chondrocytes. Membrane potentials of cells were recorded using a single electrode bridge circuit and calibrator as previously described [11, 12]. 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 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 human articular chondrocytes have been previously described in detail [12]. Plastic tissue culture dishes (55 mm diameter, NUNC**,** USA) containing sparse primary monolayer cultures of human articular chondrocytes monolayer 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) for 20 min, 37°C, 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

Immunohistochemistry. To identify whether chondrocytes express SIRPα, a panel of mouse mAb anti- SIRPα antibodies SE12B6, SE7C2 and SE5A5 and one rabbit polyclonal anti-SIRPα (ABR) were applied. In preliminary studies all antibodies were tested by immunohistochemistry on frozen and paraffin sections. The antibodies did not show positive signal at all on either paraffin or frozen sections at a range of concentrations. Different zones including superficial, surface, middle and deep zones in each section were assessed separately with three different expert persons.

[Downloaded from ibj.pasteur.ac.ir on 2024-03-28]
[DOR: 20.1001.1.1028852.2007.11.2.6.4]
[DOI: -]

Table 1. Effect of function blocking anti- SIRPα mAb, SE5A5 and SE7C2, at 1:1000, on the depolarisation response of OA cultured chondrocytes to 0.33 Hz cyclical mechanical stimulation.

Reagent	n	Membrane potential (-mV) (Mean ± SEM)			% change	*P* value
		Resting	Anti-SIRPα alone	Anti-SIRP + 0.33 Hz MS		
Nil	5	24.8 ± 0.8	-	16.4 ± 0.9	-33	0.0002
SE5A5	5	29.2 ± 1.0	29.0 ± 1.1	23.2 ± 0.8	-20	0.002
SE7C2	5	26.2 ± 0.8	27.4 ± 0.9	28.2 ± 1.3	7	NS*

*NS: not significant; Compared with antibody alone.

Expression of SIRPα in cultured human articular chondrocytes. Normal chondrocytes were isolated from 1 tibial plateau, female, age 71; 3 femoral heads, 2 males and 1 female, age range 70-83, mean 78. OA chondrocytes were isolated from 1 femoral head, grade II, male, age 83; 2 tibial plateaux, grades II and III, both female, age range 62-87, mean 75 (Table 1).

Extracts from normal and OA chondrocytes were analyzed by running onto 7.5% SDS-PAGE under reducing conditions. Polyclonal rabbit anti-SIRPα (ABR) at 1:1000 showed strong band at molecular weight of approximately 70-80 kDa, that it was consistent with the range of the molecular weight of this molecule (Fig. 1).

Both normal and OA chondrocytes showed a similar band at ~70-80 kDa, which is consistent with the known range molecular weight of this receptor. Chondrocytes isolated from different donors did not show any detectable difference in the expression pattern of SIRPα in the several experiments (Fig. 1). The results in Figure 1 shows reproducible similarity in detectable levels of SIRPα in chondrocytes derived from normal and OA chondrocytes.

Effect of anti- SIRPα Ab, SE5A5 and SE7C2, on the OA chondrocyte depolarization response to 0.33 Hz cyclical mechanical stimulation. A series of experiments was undertaken to establish whether SIRPα is involved in the chondrocyte electro-physiological response to 0.33 Hz cyclical mechanical stimulation.

Two function-blocking mAb against SIRPα SE5A5 and SE7C2, at 1:100, were applied. The monoclonal Ab were incubated with OA cultured chondrocytes in separate experiments at 37°C for 10 min prior to 0.33

Hz cyclical mechanical stimulation for 20 minutes.

As shown in Table 1, in the presence of anti-SIRPα mAb SE7C2, the electrophysiological response of cultured OA human chondrocytes to 0.33 Hz cyclical mechanical stimulation was inhibited, indicating that this protein has a role in chondrocyte mechano-transduction. Another anti-SIRPα mAb (SE5A5) did not show significant inhibitory effect on depolarisation response of osteoarthritic chondrocytes following 0.33 Hz mechanical stimulation (Table 1).

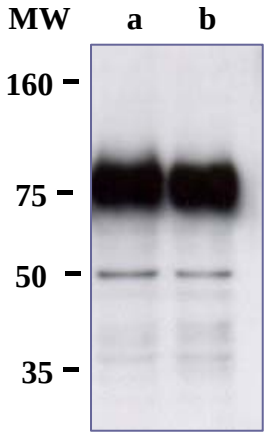


Fig. 1. Detection and comparison of SIRPα in normal and OA human articular cartilage. Equal amounts (40μg/lane) of total extracted proteins prepared from cultured human articular chondrocytes derived from normal (**lane a**), OA (**lane b**) chondrocytes were run onto 7.5% SDS-PAGE under reducing conditions. The detection of SIRPα was assessed by probing the blots with rabbit polyclonal anti-SIRPα (AB) at 1:1000. Normal (lane a, femoral head, male, age 70) and OA (lane b, tibia OA grade III, female, age 87) chondrocytes. Both normal and OA chondrocytes did not show detectable difference in the pattern of SIRPα expression.

DISCUSSION

SIRPα shows heterogeneity in molecular weight (~65-120 kDa) in various tissues due to differential glycosylation [2]. These observations and a similar structure of SIRPα and cell adhesion molecules suggest a role for SIRPα as a signal transducer

[Downloaded from ibj.pasteur.ac.ir on 2024-03-28]
[DOR: 20.1001.1.1028852.2007.11.2.6.4]
[DOI: -]

molecule in various cell types. It has been shown previously that adhesion of cultured fibroblasts to various ECM proteins induces the tyrosine phosphorylation of SIRPα and its association with SHP-2 in a manner dependent on Src family kinases and Focal adhesion kinase [8].

This is the first study that showed the expression of SIRPα by both normal and OA cultured articular chondrocytes. No detectable difference was found between the expression of SIRPα by normal and diseased chondrocytes. Chondrocytes in OA are known to show significant phenotypic differences from those of normal cartilage [13]. These differences in phenotype, including altered expression of integrins, cytokines, and growth factors, as well as production of ECM, are believed to represent a reparative or remodelling response of chondrocytes following damage to cartilage [14].

Different SIRPα epitopes may have different functional roles, and this could be due to their relative ability to cluster and induce a signaling cascade in chondrocytes. SE5A5 and SE7C2 mAb recognized different epitopes on the same antigen [9, 15] and one of them was involved in chondrocyte mechano-transduction at least in this system. Therefore, both SE7C2 and SE5A5 characterized as function-blocking mAb but have different non-competitive epitopes. SE7C2 recognizes a site on the SIRPα molecule necessary for chondrocyte response to mechanical stimulation, whereas SE5A5 does not.

It seems likely that SIRPα, under specific circumstances, undergoes a transient state, including interaction with integrins and/or phosphorylation on tyrosine residues and in part participates in mechanical-induced response of articular chondrocytes mechano-transduction. Alternatively, SIRPα could be associated with other proteins, which contribute to the electrophysiological response of chondrocytes such as ion channels. In any case, this study has provided a specific functional role for SIRPα in chondrocyte mechano-transduction.

This study demonstrated that SIRPα, a ligand for CD47, is expressed by human articular chondrocytes. The electrophysiology observations, which are obtained from the present study, give evidence that SIRPα as a component of the mechano-transduction pathway is also responsive for the regulation of intracellular signaling after mechanical stimulation.

[Downloaded from ibj.pasteur.ac.ir on 2024-03-28]

[DOR: 20.1001.1.1028852.2007.11.2.6.4]

[DOI: -]

ACKNOWLEDGEMENTS

This work has supported by a scholarship from the Ministry of Health and Medical Education, Tehran, Iran.

REFERENCES

1. Parkkinen, J.J., Ikonen, J., Lammi, M.J., Laakkonen, J., Tammi, M. and Helminen, H.J. (1993) Effects of cyclic hydrostatic pressure on proteoglycan synthesis in cultured chondrocytes and articular cartilage explants. *Arch. Biochem. Biophys. 300: 458-465*.
2. Salter, D.M., Millward-Sadler, S.J., Nuki, G. and Wright, M.O. (2001) Integrin-interleukin-4 mechano-transduction pathways in human chondrocytes. *Clin. Orthop. (391 Suppl): S49* - S60.
3. Miyamoto, S., Akiyama, S.K. and Yamada, K.M. (1995) Synergistic roles for receptor occupancy and aggregation in integrin transmembrane function. *Science 267: 883-885*.
4. Fujioka, Y., Matozaki, T., Noguchi, T., Iwamatsu, A., Yamao, T., Takahashi, N., Tsuda, M., Takada, T. and Kasuga, K. (1996) A novel membrane glycoprotein, SHPS-1, that binds the SH2-domain-containing protein tyrosine phosphatase SHP-2 in response to mitogens and cell adhesion. *Mol. Cell Biol. 16: 6887-6899*.
5. Kharitonenkov, A., Chen, Z., Sures, I., Wang, H., Schilling, J. and Ullrich, A. (1997) A family of proteins that inhibit signaling through tyrosine kinase receptors. *Nature 386: 181-186*.
6. Ullrich, A. and Schlessinger, J. (1990) Signal transduction by receptors with tyrosine activity. *Cell 61: 203-212*.
7. Adams, J.C. (2001) Thrombospondins: multifunctional regulators of cell interactions. *Annu. Rev. Cell Dev. Biol. 17: 25 -51*.
8. Brown, E.J. and Frazier, W.A. (2001) Integrin-associated protein (CD47) and its ligands. *Trends Cell Biol. 11 (3): 130-135*.
9. Seiffert, M., Cant, C., Chen, Z., Rappold, I., Brugger, W., Kanz, L., Brown, E.J., Ulrich, A. and Buring, H.J. (1999) Human signal-regulatory protein is expressed on normal, but not on subsets of leukemic myeloid cells and mediates cellular adhesion involving its counterreceptor CD47. *Blood 94: 3633-3643*.
10. Orazizadeh, M. and Salter, D.M. (2005) Differential immunohistochemical expression pattern of Galectin-3 in normal and osteoarthritic (OA) human articular cartilage. *Iran. J. Immunol. 2: 78-86*.
11. Wright, M.O., Stockwell, R.A. and Nuki, G. (1992) Response of plasma membrane to applied

hydrostatic pressure in chondrocytes and fibroblasts. *Connect. Tissue Res. 28: 49-70*.

12. Wright, M.O., Nishida, K., Bavington, C., Godolphin, J.L., Dunne, E., Walmsley, S. and Salter, D.M. (1997) Hyperpolarization of cultured human chondrocytes following cyclical pressure-induced strain: evidence of a role for alpha 5 beta 1 integrin as a chondrocyte mechanoreceptor. *J. Orthop. Res. 15: 742-747*.
13. Aigner, T., Vornehm, S.I., Zeiler, G., Dudhia, J., Von der, M. and Bayliss, M.T. (1997) Suppression of cartilage matrix gene expression in upper zone chondrocytes of osteoarthritic cartilage. *Arthritis Rheum. 40: 562-569*.
14. Ostergaard, K. and Salter, D.M. (1998) Immunohistochemistry in the study of normal and osteoarthritic articular cartilage. *Prog. Histochem. Cytochem. 33: 93-165*.
15. Seiffert, M., Brossart, P., Cant, C., Cella, M., Colonna, M., Brugger, W., Brown, E.J., Ulrich, A. and Buring, H.J. (2001) Signal-regulatory protein alpha (SIRPalpha) but not SIRPbeta is involved in T-cell activation, binds to CD47 with high affinity, and is expressed on immature CD34(+)CD38(-) hematopoietic cells. *Blood 97 (9): 2741 -2749*.

[Downloaded from ibj.pasteur.ac.ir on 2024-03-28]

[DOR: 20.1001.1.1028852.2007.11.2.6.4]

[DOI: -]