pression in human OA cartilage: some investigators observed the expression in the upper part of OA cartilage (12,32), whereas others reported it in the deep zone (13,14,33–35). Our result is consistent with the latter finding, in that we identified its expression primarily in the deep zone. However, because the expression of *COL10A1* was relatively weak and fairly inconsistent among OA cartilage samples, we assume that the expression of type X collagen in OA cartilage might be of limited significance in the pathology of OA.

Previously, the appearance of type IIA procollagen mRNA or exon 2 of *COL2A1* in OA cartilage was considered to be the result of a phenotypic reversal of chondrocytes (11,12). However, this speculation is not supported by the present result. Since a result consistent with our own was reported in another recent study (23), a phenotypic reversal of chondrocytes may not be a dominant event in OA cartilage.

In light of these findings, the metabolic change of the chondrocytes in OA may be understood as follows. In the degenerated areas, a major change in the metabolism occurs in the upper region of degenerated cartilage. Such a change resembles that of the dedifferentiation process in the decline of type II collagen and aggrecan expression and the induction of type III collagen expression (Figures 2 and 3) (an illustration of the sequential changes of gene expression in articular chondrocytes during dedifferentiation is available at http://www.hosp.go.jp/~sagami/rinken/crc/index.html). However, the change is different from that process in the expression of link protein, fibronectin, and type I collagen genes. Thus, the metabolic change in the degenerated areas of OA cartilage was considered to be unique and not closely related to the one during the dedifferentiation process. Meanwhile, in the preserved areas, the expression of cartilage matrix genes is highly up-regulated. Although the phenotypic deviation is less obvious in those areas, the expression of type I collagen and type X collagen genes may be induced there in the superficial and deep zones, respectively.

Although the mechanism(s) for these metabolic changes remains entirely unknown, the change in *SOX9* expression may be related to the altered chondrocyte metabolism in OA. As shown in the correlation study, the regional difference in matrix gene expression within OA cartilage could be ascribed, at least partly, to the change in SOX9 expression. However, the present result also indicates that the general up-regulation of matrix gene expression in OA chondrocytes was not associated with the increase in *SOX9* expression. In this study, the amounts of SOX proteins were not assessed. Furthermore, the transcriptional activity of SOX9 is known to be modulated by the level of phosphorylation (36) and by the presence of coregulators (37,38). Thus, taking these factors into account may provide a better explanation of the significance of SOX proteins in the altered chondrocyte metabolism in OA.

Although the present study has clarified the metabolic change of chondrocytes in OA cartilage, it also has several limitations. First, the metabolic change was evaluated primarily by mRNA expression, and protein synthesis was not determined. The major difference in mRNA expression levels among the samples posed another problem. A large variation among human cartilage samples has been reported repeatedly in previous studies (7,8,23). For OA samples, this might reflect the diversity of the pathology, while the variation among the controls might have stemmed from differences in joint physiology that could be related to the donor's condition before death. These points should be clarified by future studies. Despite these limitations, we believe that our study has revealed several novel aspects of OA pathology. We hope that the current results may offer another clue to eventually establishing a novel strategy to treat this tenacious disease.

AUTHOR CONTRIBUTIONS

Dr. Fukui had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study design. Fukui.

Acquisition of data. Ikeda, Ohnuki, Tanaka, Hikita, Mitomi, Juji, Katsuragawa, Yamamoto, Sawabe, Yamane, Suzuki.

Analysis and interpretation of data. Fukui, Mori, Sandell, Ochi.

Manuscript preparation. Fukui.

Statistical analysis. Fukui.

REFERENCES

1. Collins DH, McElligott TF. Sulphate ($^{35}SO_4$) uptake by chondrocytes in relation to histological changes in osteoarthritic human articular cartilage. Ann Rheum Dis 1960;19:318–30.
2. Sandell LJ, Heinegard D, Hering TM. Cell biology, biochemistry, and molecular biology of articular cartilage in osteoarthritis. In: Moskowitz RW, Altman RD, Hochberg MC, Buckwalter JA, Goldberg VM, editors. Osteoarthritis: diagnosis and medical/surgical management. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2007. p. 73–106.
3. Aigner T, Gluckert K, von der Mark K. Activation of fibrillar collagen synthesis and phenotypic modulation of chondrocytes in early human osteoarthritic cartilage lesions. Osteoarthritis Cartilage 1997;5:183–9.
4. Aigner T, Vornehm SI, Zeiler G, Dudhia J, von der Mark K, Bayliss MT. Suppression of cartilage matrix gene expression in upper zone chondrocytes of osteoarthritic cartilage. Arthritis Rheum 1997;40:562–9.
5. Adam M, Deyl Z. Altered expression of collagen phenotype in osteoarthrosis. Clin Chim Acta 1983;133:25–32.

6. Miosge N, Hartmann M, Maelicke C, Herken R. Expression of collagen type I and type II in consecutive stages of human osteoarthritis. Histochem Cell Biol 2004;122:229–36.
7. Aigner T, Fundel K, Saas J, Gebhard PM, Haag J, Weiss T, et al. Large-scale gene expression profiling reveals major pathogenetic pathways of cartilage degeneration in osteoarthritis. Arthritis Rheum 2006;54:3533–44.
8. Aigner T, Zien A, Gehrsitz A, Gebhard PM, McKenna L. Anabolic and catabolic gene expression pattern analysis in normal versus osteoarthritic cartilage using complementary DNA–array technology. Arthritis Rheum 2001;44:2777–89.
9. Aigner T, Bertling W, Stoss H, Weseloh G, von der Mark K. Independent expression of fibril-forming collagens I, II, and III in chondrocytes of human osteoarthritic cartilage. J Clin Invest 1993;91:829–37.
10. Nerlich AG, Wiest I, von der Mark K. Immunohistochemical analysis of interstitial collagens in cartilage of different stages of osteoarthrosis. Virchows Arch B Cell Pathol Incl Mol Pathol 1993;63:249–55.
11. Aigner T, Zhu Y, Chansky HH, Matsen FA III, Maloney WJ, Sandell LJ. Reexpression of type IIA procollagen by adult articular chondrocytes in osteoarthritic cartilage. Arthritis Rheum 1999; 42:1443–50.
12. Nah HD, Swoboda B, Birk DE, Kirsch T. Type IIA procollagen: expression in developing chicken limb cartilage and human osteoarthritic articular cartilage. Dev Dyn 2001;220:307–22.
13. Girkontaite I, Frischholz S, Lammi P, Wagner K, Swoboda B, Aigner T, et al. Immunolocalization of type X collagen in normal fetal and adult osteoarthritic cartilage with monoclonal antibodies. Matrix Biol 1996;15:231–8.
14. Von der Mark K, Kirsch T, Nerlich A, Kuss A, Weseloh G, Gluckert K, et al. Type X collagen synthesis in human osteoarthritic cartilage: indication of chondrocyte hypertrophy. Arthritis Rheum 1992;35:806–11.
15. Poole RA, Guilak F, Abramson SB. Etiopathogenesis of osteoarthritis. In: Moskowitz RW, Altman RD, Hochberg MC, Buckwalter JA, Goldberg VM, editors. Osteoarthritis: diagnosis and medical/surgical management. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2007. p. 27–49.
16. Emmert-Buck MR, Bonner RF, Smith PD, Chuaqui RF, Zhuang Z, Goldstein SR, et al. Laser capture microdissection. Science 1996;274:998–1001.
17. Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, et al. Development of criteria for the classification and reporting of osteoarthritis: classification of osteoarthritis of the knee. Arthritis Rheum 1986;29:1039–49.
18. Buckwalter JA, Mankin HJ, Grodzinsky AJ. Articular cartilage and osteoarthritis. In: Pellegrini VD Jr, editor. AAOS instructional course lectures. Vol. 54. Rosemont (IL): American Academy of Orthopedic Surgeons; 2005. p. 465–80.
19. Poole RA. Cartilage in health and disease. In: Koopman WJ, Moreland LW, editors. Arthritis and allied conditions: a textbook of rheumatology. 15th ed. Philadelphia: Lippincott Williams & Wilkins; 2004. p. 223–69.
20. Miller DR, Mankin HJ, Shoji H, D'Ambrosia RD. Identification of fibronectin in preparations of osteoarthritic human cartilage. Connect Tissue Res 1984;12:267–75.
21. Goldwasser M, Astley T, van der Rest M, Glorieux FH. Analysis of the type of collagen present in osteoarthritic human cartilage. Clin Orthop Relat Res 1982;(167):296–302.
22. Burton-Wurster N, Horn VJ, Lust G. Immunohistochemical localization of fibronectin and chondronectin in canine articular cartilage. J Histochem Cytochem 1988;36:581–8.
23. Gebhard PM, Gehrsitz A, Bau B, Soder S, Eger W, Aigner T. Quantification of expression levels of cellular differentiation markers does not support a general shift in the cellular phenotype of osteoarthritic chondrocytes. J Orthop Res 2003;21:96–101.
24. De Crombrugghe B, Lefebvre V, Behringer RR, Bi W, Murakami S, Huang W. Transcriptional mechanisms of chondrocyte differentiation. Matrix Biol 2000;19:389–94.
25. Hollander AP, Pidoux I, Reiner A, Rorabeck C, Bourne R, Poole AR. Damage to type II collagen in aging and osteoarthritis starts at the articular surface, originates around chondrocytes, and extends into the cartilage with progressive degeneration. J Clin Invest 1995;96:2859–69.
26. Young RD, Lawrence PA, Duance VC, Aigner T, Monaghan P. Immunolocalization of type III collagen in human articular cartilage prepared by high-pressure cryofixation, freeze-substitution, and low-temperature embedding. J Histochem Cytochem 1995;43: 421–7.
27. Wotton SF, Duance VC. Type III collagen in normal human articular cartilage. Histochem J 1994;26:412–6.
28. Eyre DR, Weis MA, Wu JJ. Articular cartilage collagen: an irreplaceable framework? Eur Cell Mater 2006;12:57–63.
29. Young RD, Lawrence PA, Duance VC, Aigner T, Monaghan P. Immunolocalization of collagen types II and III in single fibrils of human articular cartilage. J Histochem Cytochem 2000;48:423–32.
30. Homandberg GA. Cartilage damage by matrix degradation products: fibronectin fragments. Clin Orthop Relat Res 2001;(391 Suppl):S100–7.
31. Wagner S, Hofstetter W, Chiquet M, Mainil-Varlet P, Stauffer E, Ganz R, et al. Early osteoarthritic changes of human femoral head cartilage subsequent to femoro-acetabular impingement. Osteoarthritis Cartilage 2003;11:508–18.
32. Kirsch T, Swoboda B, Nah H. Activation of annexin II and V expression, terminal differentiation, mineralization and apoptosis in human osteoarthritic cartilage. Osteoarthritis Cartilage 2000;8: 294–302.
33. Gibson GJ, Verner JJ, Nelson FR, Lin DL. Degradation of the cartilage collagen matrix associated with changes in chondrocytes in osteoarthrosis: assessment by loss of background fluorescence and immunodetection of matrix components. J Orthop Res 2001; 19:33–42.
34. Boos N, Nerlich AG, Wiest I, von der Mark K, Ganz R, Aebi M. Immunohistochemical analysis of type-X-collagen expression in osteoarthritis of the hip joint. J Orthop Res 1999;17:495–502.
35. Aigner T, Reichenberger E, Bertling W, Kirsch T, Stoss H, von der Mark K. Type X collagen expression in osteoarthritic and rheumatoid articular cartilage. Virchows Arch B Cell Pathol Incl Mol Pathol 1993;63:205–11.
36. Huang W, Zhou X, Lefebvre V, de Crombrugghe B. Phosphorylation of SOX9 by cyclic AMP-dependent protein kinase A enhances SOX9's ability to transactivate a Col2a1 chondrocyte-specific enhancer [published erratum appears in Mol Cell Biol 2000;20:7838]. Mol Cell Biol 2000;20:4149–58.
37. Tsuda M, Takahashi S, Takahashi Y, Asahara H. Transcriptional co-activators CREB-binding protein and p300 regulate chondrocyte-specific gene expression via association with Sox9. J Biol Chem 2003;278:27224–9.
38. Kawakami Y, Tsuda M, Takahashi S, Taniguchi N, Esteban CR, Zemmyo M, et al. Transcriptional coactivator PGC-1α regulates chondrogenesis via association with Sox9. Proc Natl Acad Sci U S A 2005;102:2414–9.

A Novel Cell Delivery System Using Magnetically Labeled Mesenchymal Stem Cells and an External Magnetic Device for Clinical Cartilage Repair

Takaaki Kobayashi, M.D., Mitsuo Ochi, M.D., Ph.D., Shinobu Yanada, M.Sc., Masakazu Ishikawa, M.D., Ph.D., Nobuo Adachi, M.D., Ph.D., Masataka Deie, M.D., Ph.D., and Koji Arihiro, M.D., Ph.D.

Purpose: The purpose of this study was to investigate whether it is possible to successfully accumulate magnetically labeled mesenchymal stem cells (MSCs), under the direction of an external magnetic force, to the desired portion of osteochondral defects of the patellae after intra-articular injection of the MSCs. **Methods:** MSCs were cultured from bone marrow and were labeled magnetically. Osteochondral defects were made in the center of rabbit and swine patellae, and magnetically labeled MSCs were injected into the knee joints either under the direction of an external magnetic force or with no magnetic force applied. In the rabbit model we evaluated the patellae macroscopically and histologically, and in the swine model we observed the patellae arthroscopically. **Results:** Accumulation of magnetically labeled MSCs to the osteochondral defect was shown macroscopically and histologically in the rabbit model and was shown by arthroscopic observation to be attached to the chondral defect in the swine model. **Conclusions:** We showed the ability to deliver magnetically labeled MSCs to a desired place in the knee joint. **Clinical Relevance:** Our novel approach is applicable for human cartilage defects and may open a new era of repairing cartilage defects caused by osteoarthritis or trauma by use of a less invasive technique. **Key Words:** Cell delivery system—Mesenchymal stem cell—Magnetic force—Cartilage repair—Arthroscopic observation.

Articular cartilage has very limited healing potential. Although there are several treatment options for cartilage defects, no treatment has been established as a gold standard procedure. One recent strategy for cartilage repair is by transplantation of mesenchymal stem cells (MSCs).[1-4] MSCs are the cell population of undifferentiated cells isolated from adult tissue that have the capacity to differentiate into mesodermal lineages, such as bone, cartilage, fat, muscle, or other tissues.[5,6] The MSCs from bone marrow can be cultured and differentiated into the desired lineage in vitro with the application of specific growth factors or bioactive molecules. We previously examined the effectiveness of a cell delivery system using an internal magnet and magnetic

From the Department of Orthopaedic Surgery, Graduate School of Biomedical Sciences, Hiroshima University (T.K., M.O., S.Y., M.I., N.A., M.D.), and Department of Anatomical Pathology, Hiroshima University Hospital (K.A.), Hiroshima, Japan.

Supported by a grant from the Ministry of Education, Culture, Sports, Science and Technology of Japan (No. 16209045). The authors report no conflict of interest.

Address correspondence and reprints request to Mitsuo Ochi, M.D., Department of Orthopaedic Surgery, Hiroshima University, 1-2-3 Kasumi, Minami-ku Hiroshima, Hiroshima 734-8551 Japan. E-mail: ochim@hiroshima-u.ac.jp

0749-8063/08/2401-7164$34.00/0
doi:10.1016/j.arthro.2007.08.017

Note: To access the supplementary videos accompanying this report, visit the January issue of Arthroscopy at www.arthroscopyjournal.org.

liposomes, or magnetic beads, in vitro.[7-10] However, for clinical applications, it is indispensable to develop an external magnetic device, as well as to use materials that have been safely used in humans. Recently, there have been many reports of labeling MSCs with ferumoxides.[11-13] Ferumoxides are dextran-coated superparamagnetic iron oxide nanoparticles approved by the US Food and Drug Administration as a magnetic resonance contrast agent for hepatic imaging of humans. By use of this technique, it has become easy to make magnetically labeled MSCs. We originally made an external magnetic device that generated a high magnetic force of 0.6 T (Tamagawa, Miyagi, Japan) for the purpose of human tissue repair. In this study, using this device, we hypothesized that we could successfully accumulate magnetically labeled MSCs, which have been shown to have a capacity to differentiate into chondrocytes,[12] to the desired portion of the osteochondral defect of the rabbit's patella after intraarticular injection of MSCs. We also hypothesized that this novel, less invasive approach could be done under arthroscopic control in swine knees.

METHODS

Our research methods were reviewed and approved by the Ethical Committee of Hiroshima University, Hiroshima, Japan.

Cell Culture

The method for isolation and in vitro expansion of bone marrow–derived MSCs is well known and has been previously described. A modification of the culture method of Kotobuki et al.[14] was used. In brief, 5 mL of bone marrow from the tibia of adult human donors was aspirated with 1 mL of heparin sodium when they underwent anterior cruciate ligament reconstruction, and this was centrifuged for 5 minutes at 1,500 rpm; the subsequent supernatant, including heparin sodium, was discarded. The extract was resuspended in 6 mL of culture medium, composed of Dulbecco's modified Eagle medium (Invitrogen [Gibco], Paisley, Scotland) with 10% fetal bovine serum (Sigma-Aldrich, St Louis, MO) and 1% antibiotics (penicillin, streptomycin, and Fungizone; BioWhittaker, Walkersville, MD). We seeded 2 mL of the suspension onto 100-mm culture dishes (Falcon; BD Biosciences, Franklin Lakes, NJ), and 8 mL of culture medium was added to each dish. The dishes were incubated for 3 weeks under a humidified atmosphere and 5% carbon dioxide at 37°C. The medium was not changed for the first 7 days. When the medium was changed, the suspended cells and the supernatant were discarded, and fresh culture medium was added to the dish, where the adherent cells were left. After the first change of medium, the medium was then changed every 3 days. About 2 weeks after seeding, the cells had proliferated and reached confluence. The cells were then harvested by treatment with 0.25% trypsin and 0.02% ethylenediaminetetraacetic acid (EDTA) (2.5-g/L trypsin/1-mmol/L EDTA solution; Nacalai Tesque, Kyoto, Japan). To expand the MSCs, 2 to 3 • 10^5 of the harvested cells were seeded on 100-mm culture dishes. On reaching confluence again, the cells were reseeded under the same conditions. We referred to these adherent cells as MSCs. We confirmed that these cells had the capacity to differentiate into osteocytes, adipocytes, and chondrocytes (data not shown) as shown in previous experimental studies.[8,14]

Magnetic Labeling of MSCs

MSCs were labeled overnight with 25 • g Fe/mL ferumoxides and 375 ng/mL poly-L-lysine (PLL) (poly-L-lysine hydrobromide; molecular weight, 388 kd [P-1524; Sigma-Aldrich]) as a transfection agent.[11] In brief, 2.2 • L of ferumoxide stock solution (11.2 mg Fe/mL; Tanabe Seiyaku, Osaka, Japan) was added per milliliter of culture medium, without cells, and mixed well. PLL was then added at 3.75 • L/mL from a 0.1-mg/mL stock solution. The medium was mixed and incubated for 60 minutes at room temperature with occasional gentle mixing. Labeling was initiated by removal of the medium from the adherent MSCs and then adding to the medium containing the ferumoxides-PLL mixture. After incubation overnight in the medium, the MSCs were collected after trypsinization, and some cells were stained with Prussian blue to evaluate magnetic labeling of MSCs.

External Magnetic Device

A variable DC electromagnet (model TM-SP12010SC-014; Tamagawa) was manufactured for the purpose of generating an external magnetic force (Fig 1A). The disk-shaped electromagnet consists of a solenoid iron coil and is designed to be able to generate a magnetic field efficiently. The generating magnetic field is symmetric about the arbor of the disk (Fig 1B). In brief, the magnetic field is directed to the center of the disk surface, and its magnitude decreases away from the surface (Fig 1C). The magnitude of the magnetic field

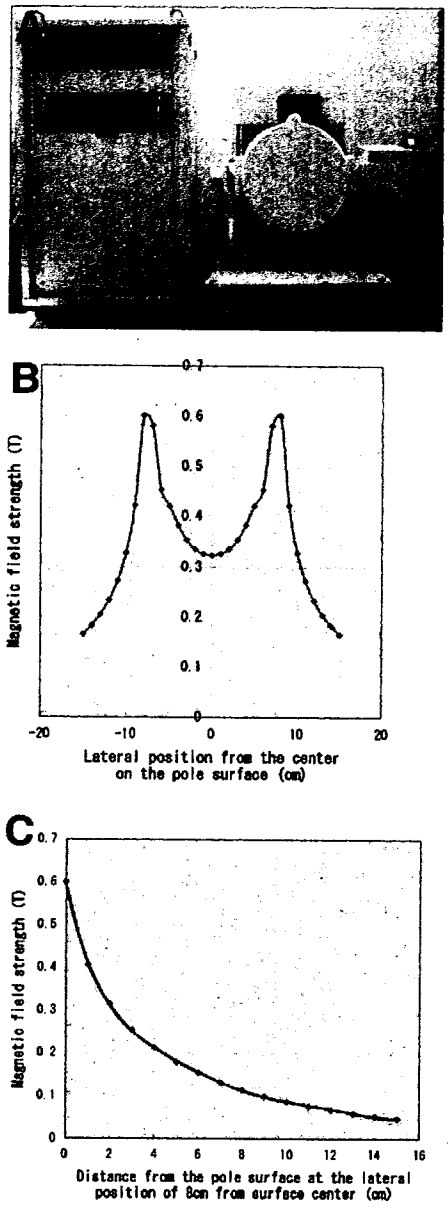


FIGURE 1. (A) An external magnetic device generated a magnetic force (maximum, 0.6 T) to the round wall (40 cm in diameter). (B) The maximum magnetic field is 0.6 T, and (C) the magnitude of the magnetic field decreases away from the surface.

is increased by intensifying the electric current through the electromagnet, and is limited by the temperature of the coil. When a sample lies 8 cm from the center of the pole, the maximum magnetic field is 0.6 T (Fig 1B), and the maximum gradient of the amplitude of the magnetic field is 25 T/m. The disk is able to change height and direction.

Accumulation Capacity of Magnetically Labeled MSCs Under Influence of Magnetic Force

Magnetically labeled MSCs (9 • 10^6/300 • L Dulbecco's phosphate-buffered saline solution [PBS]) were injected into a tissue culture flask two thirds full with PBS. In the magnetic force group, injection of magnetically labeled MSCs was performed under the influence of an external magnetic force (0.6 T). In the control group, cell injection was performed without the influence of a magnetic force.

Rabbit Model

Surgery and Treatment: Six male Japanese white rabbits weighing 2.5 to 3.0 kg were used in this study. All rabbits were killed by the intravenous administration of pentobarbital sodium, and knee joints were opened through a medial parapatellar approach. Patellae were turned over, and an osteochondral defect (3 mm in diameter and 2 mm in depth) was made in the center of the patellae with a cylindrical drill. The joint capsules were closed in a routine manner, and the knees were removed. After surgery, 100 • L of PBS was injected into the knee joint. The knees were put on the external magnetic device (8 cm from the pole center, with patellae in the direction of the generated magnetic force). In the magnetic force group, magnetically labeled MSCs (3 • 10^6 cells/50 • L PBS) were injected into the knee joints under the influence of a magnetic force (0.6 T) and were kept in position by use of the magnetic force for 4 hours. In the control group, magnetically labeled MSCs were injected into the knee joints without the influence of a magnetic force and were kept in the same position for 4 hours.

Evaluation: After treatment, the knees were fixed with 4% formalin for 2 days, and the patellae were then removed with the joint capsule. The knee joints were opened through a lateral parapatellar approach and were observed macroscopically. The patellae were removed and immersed in 4% formalin for 24 hours, decalcified with 10% EDTA for 4 weeks, and then embedded in paraffin. Histologic sections were stained with H&E, and Prussian blue staining was used for microscopic analysis.

Swine Model

In this study, 4 fresh-frozen porcine knees were used (age range, 3 to 6 months). Before testing, the knees were stored at • 20°C and thawed for 48 hours at 4°C. The knee joints were opened through the medial parapatellar approach. Patellae were turned over, and full-thickness, critical-sized chondral de-

fects (10 mm in diameter) were made in the center of the patellae. The joint capsules were closed in a routine manner, and an arthroscope (Smith & Nephew Endoscopy, Andover, MA) was inserted into the knee joints. After surgery, the knees were put on the external magnetic device (with the patellae being in the direction of the generated magnetic force). In the magnetic force group, magnetically labeled MSCs ($8 \cdot 10^5$ cells/100 • L PBS) were injected into the knee joint under the influence of a magnetic force (0.6 T) by use of a 1-mL injector (whose needle was bent to the chondral defects). When magnetically labeled MSCs were injected into the knee joints, arthroscopic findings were recorded, and then the arthroscope was removed. At 90 minutes after injection under the influence of the magnetic force, the chondral defect sites were again observed arthroscopically and recorded. During arthroscopy, the inflow of fluid was turned on intermittently. In the control group, magnetically labeled MSCs were injected into the knee joints under arthroscopic observation without the influence of a magnetic force, and the arthroscopic findings were recorded.

RESULTS

Magnetic Labeling of MSCs

We confirmed that almost 100% of MSCs were stained blue with Prussian blue stain.

Accumulation Capacity of Magnetically Labeled MSCs Under Influence of Magnetic Force

In the control group (Fig 2B and Video 1 [online only, available at www.arthroscopyjournal.org]), magnetically labeled MSCs fell down vertically with gravity. In the magnetic force group (Fig 2C and Video 2 [online only, available at www.arthroscopyjournal.org]), magnetically labeled MSCs fell down not vertically but diagonally, moved in the direction of the generated magnetic force, and accumulated to the side wall of the flask under the influence of gravity and the magnetic force.

Rabbit Model

Macroscopic Examination: In the control group, magnetically labeled MSCs, visible as brown particles, were scattered in the joints and were attached to the bottom side of the joint capsules (6/6 knees) (Fig 3C). In the magnetic force group, magnetically labeled MSCs had accumulated to the osteochondral defect of the patellae and the capsule around the patellae (6/6 knees) (Fig 3D).

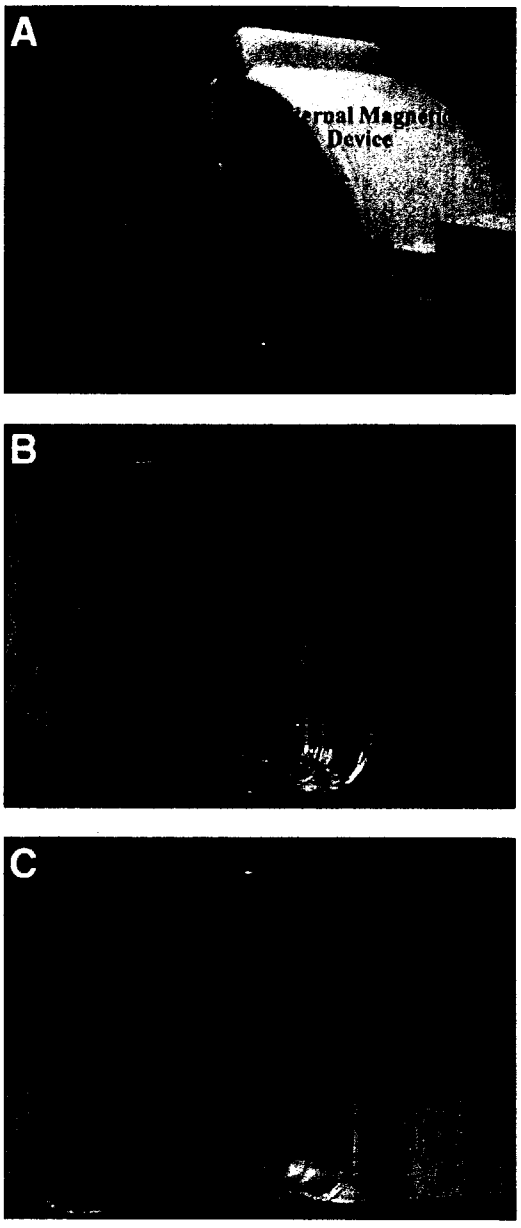


FIGURE 2. (A) External magnetic device. (B) Magnetically labeled MSCs ($9 \cdot 10^6$ cells/300 • L PBS) were injected into a flask without a magnetic force, and the MSCs fell down vertically (Video 1 [online only, available at www.arthroscopyjournal.org]). (C) Under the influence of a magnetic force (Video 2 [online only, available at www.arthroscopyjournal.org]), magnetically labeled MSCs were induced to the side wall of the flask, where the magnetic force was directed.

Histologic Findings: In the control group, no cells, excluding red blood cells, were shown to be on the osteochondral defect of the patellae as de-

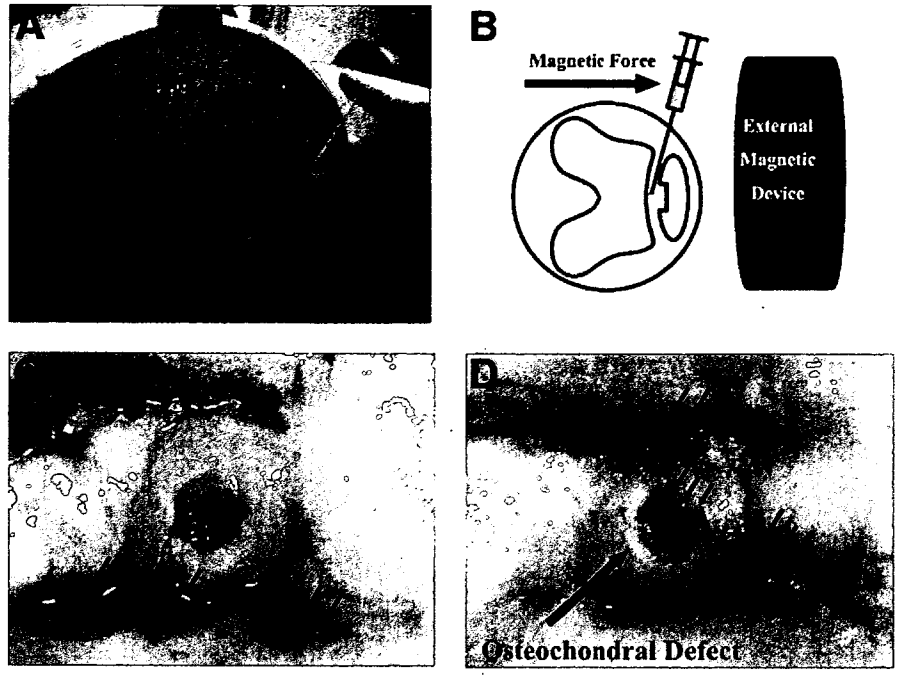


FIGURE 3. Rabbit model. (A, photograph of the experimental procedure; B, schema of the procedure.) Osteochondral defects were made in the center of the patellae, and magnetically labeled MSCs ($3 \cdot 10^6$ cells/50 • L PBS) were injected into the knee joint with or without the influence of a magnetic force. Macroscopic findings are shown 4 hours after injection, and magnetically labeled MSCs are visible as brown particles (arrows). In the control group, the MSCs did not accumulate to the defect and were attached to the bottom side of the capsules as a result of gravitation (C). In the magnet force group, magnetically labeled MSCs accumulated to the osteochondral defect of the patellae (D).

termined by H&E staining (Fig 4A and 4B) and Prussian blue staining (Fig 4C and 4D). In the magnetic force group, many cells were visible on the osteochondral defect of the patellae with H&E staining (Fig 5A and 5B) and were stained blue with Prussian blue, indicating that the cells contained iron and were magnetically labeled MSCs (Fig 5C and 5D).

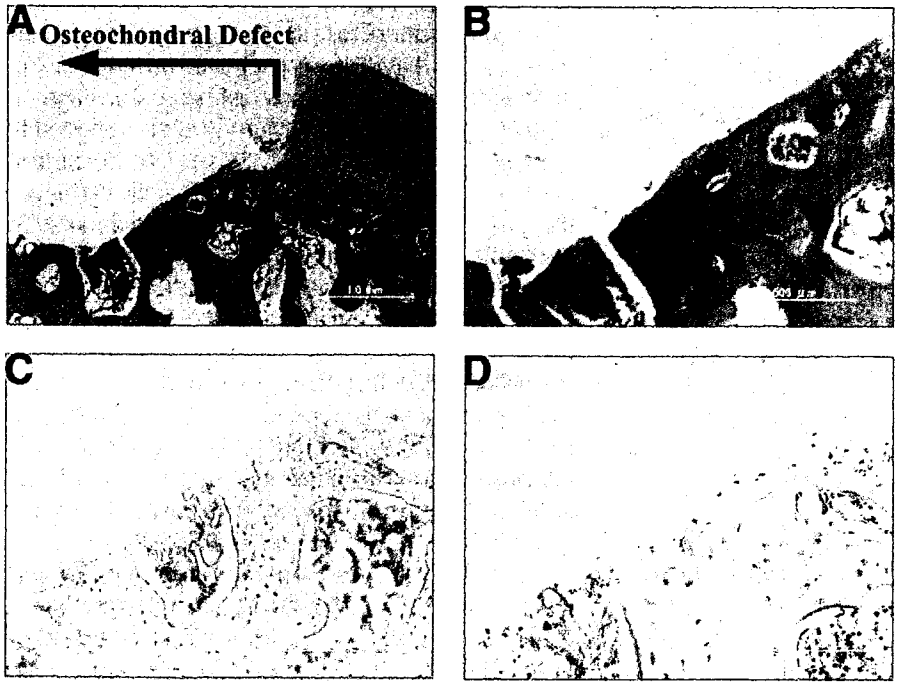


FIGURE 4. Microscopic findings of control group (rabbit model). There were no cells, excluding red blood cells, on the osteochondral defect of the patellae stained with (A, original magnification • 40; B, original magnification • 100) H&E and (C, original magnification • 100; D, original magnification • 200) Prussian blue stain.

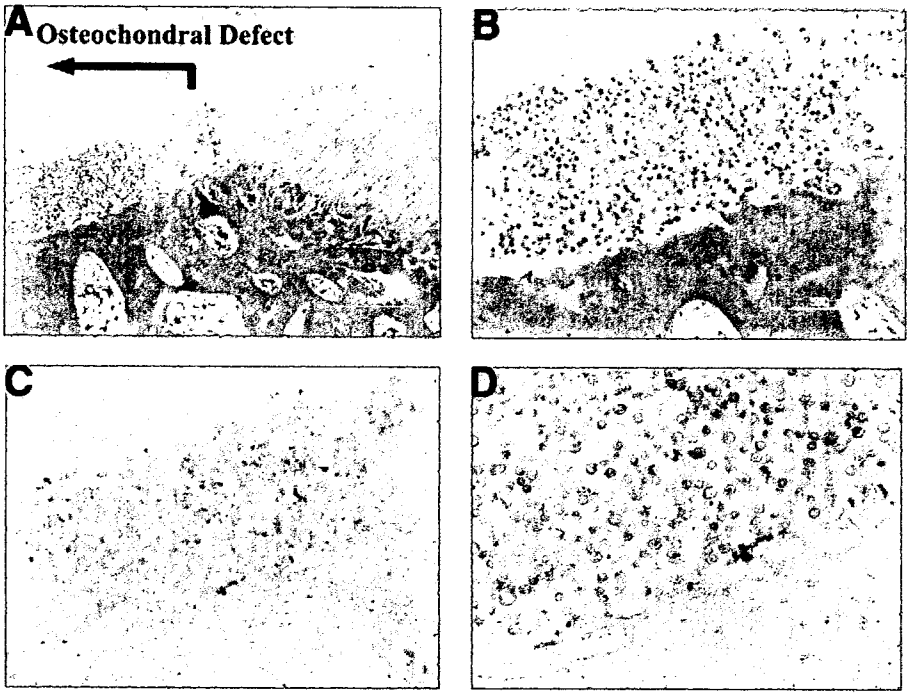


FIGURE 5. Microscopic findings of magnetic force group (rabbit model). Many cells were seen on the osteochondral defect with H&E stain (A, original magnification • 40; B, original magnification • 100), and the cells were stained blue with the Prussian blue stain (C, original magnification • 100; D, original magnification • 200), indicating that the cells contained iron and magnetically labeled MSCs.

Swine Model

In the control group, MSCs were immediately scattered in the joints after injection (Fig 6B and Video 3 [online only, available at www.arthroscopyjournal.org]). In the magnetic force group, arthroscopic observation showed that magnetically labeled MSCs had accumulated to the chondral defect of the patellae (Fig 6C and Video 4 [online only, available at www.arthroscopyjournal.org]). Under the influence of the magnetic force, metallic equipment (arthroscopic system and injector's needle) was not affected by the magnetic force, because we used nonmagnetic metals as arthroscopic tools. Only the video camera for recording arthroscopic observation was affected, if the camera was within 30 cm of the external magnetic device. At 90 minutes after MSC injection, MSCs were attached to the chondral defect site against gravity and inflow of the fluid (Fig 6D and Video 5 [online only, available at www.arthroscopyjournal.org]).

DISCUSSION

This study clearly showed that our system using arthroscopic control and an external magnetic device could deliver magnetically labeled MSCs to an osteochondral defect of the patellae. This is the first report to visualize targeting of magnetically labeled MSCs under the influence of a magnetic force to accumulate to the desired place in the knee joint.

Recently, many researchers have reported methods of transplanting MSCs to repair cartilage defects.[1-4] MSCs can be easily gathered from the marrow of an ilium or tibia, and large amounts of cells can be obtained by culture outside the human body. Furthermore, MSCs have the capacity to differentiate into mesodermal lineages, such as bone, cartilage, fat, muscle, or other tissue.[5,6] Murphy et al.[1] reported that intra-articular injection of MSCs to an osteoarthritic knee stimulated regeneration of articular cartilage in the goat model, and Tatebe et al.[4] reported that MSCs were able to survive on the osteochondral defects and differentiated to cartilage. Nishimori et al.[2] reported that intra-articular injection of MSCs along with a bone marrow stimulation procedure repaired chronic osteochondral defects in the rat model. In a clinical trial, Wakitani et al.[3] reported that the implantation of MSCs together with high tibial osteotomy for the human osteoarthritic knee was better than only high tibial osteotomy on the arthroscopic and histologic grading scale. On the other hand, Agung et al.[15] reported that injured cartilage was repaired after intra-articular injection of MSCs in the rat model but that the injection of too many MSCs generated free bodies of scar tissue in the joint. We therefore conceived a

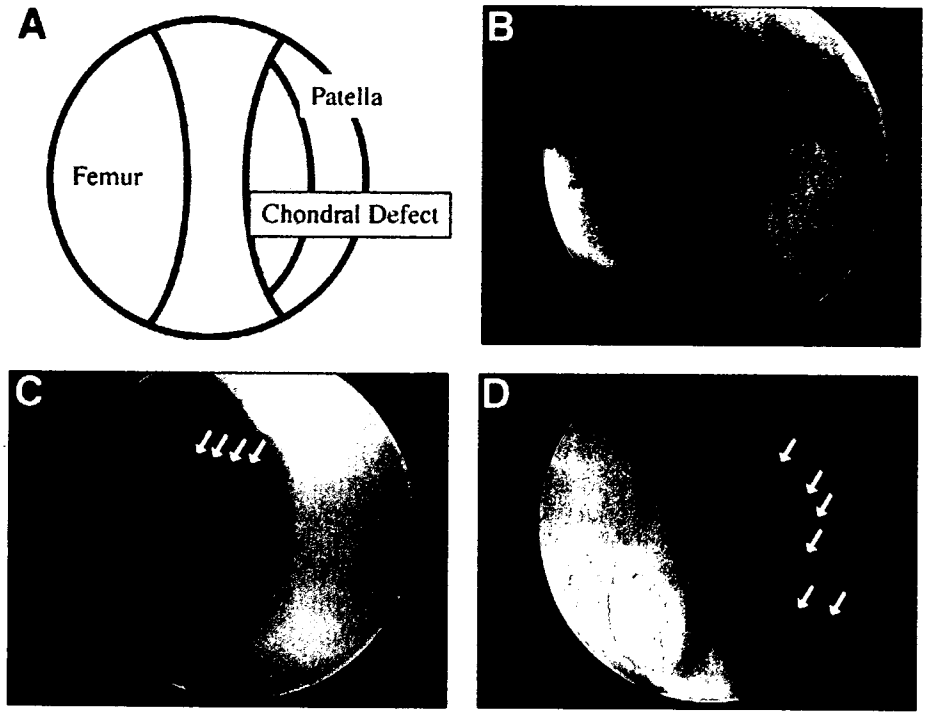


FIGURE 6. Arthroscopic findings of swine model. Magnetically labeled MSCs are visible as brown particles (arrows). (A) Chondral defects of the patellae are shown on the right side of the photographs and videos. (B) In the control group, magnetically labeled MSCs were scattered in the joint (Video 3, online only, available at www.arthroscopyjournal.org). (C) In the magnetic force group, magnetically labeled MSCs accumulated in the chondral defect of the patellae (Video 4, online only, available at www.arthroscopyjournal.org). (D) At 90 minutes after the injection of MSCs, MSCs were attached biologically to the chondral defect site (Video 5 [online only, available at www.arthroscopyjournal.org]).

new method of accumulating a relatively small number of MSCs to a desired area using a magnetic force with the aim of avoiding side effects such as the production of free bodies. We have previously reported a drug delivery system and cell delivery system using a magnetic force.[7-10,16] Tanaka et al.[16] reported that a magnetic liposomal delivery system using transforming growth factor • 1 is useful for cartilage repair of the rabbit knee, and Yanada et al.[8] concluded that MSC-RGDS (arginine [R]–glycine [G]–asparatic acid [D]–serine [S]) peptide–bead complexes are useful for cartilage repair in vitro. These studies used a permanent neodymium magnet. For our study, we developed a new external magnetic device for the treatment of humans.

There are some limitations to our study, however. First, we used ferumoxides to make magnetically labeled MSCs. Ferumoxides are approved by the US Food and Drug Administration as a magnetic resonance contrast agent, but it is not confirmed whether MSCs labeled with ferumoxides have the same chondrogenic capacity as nonlabeled MSCs. Arbab et al.[12] reported that labeling with ferumoxides does not inhibit the chondrogenic differentiation capacity of MSCs, but Kostura et al.[13] reported contradictory results. We confirmed that magnetically labeled MSCs have the capacity to differentiate into chondrocytes in the chondrogenic differentiation medium that contained transforming growth factor • 1 and bone morphogenetic protein 2 (data not shown). Second, this study was conducted over a short time. We have only shown that magnetically labeled MSCs accumulated in the osteochondral defect of the patella and did not confirm whether articular cartilage was formed in the osteochondral defect. A further long-term study is needed. Third, we used human MSCs, because we aimed at clinical applications in humans. We investigated whether magnetically labeled human MSCs accumulated in the desired place. For another long-term study in animals, it would be necessary to use immunodeficient animals or the host's MSCs. Fourth, we used a magnetic field of 0.6 T, but we do not know the most effective strength of the magnetic field. However, we confirmed that our external magnetic device was able to control the direction of magnetically labeled MSCs. If we use a greater magnetic force, there may be effects on operation tools, such as probes, arthroscopic equipment, and television monitors. In this study, however, the operation tools were not affected by the magnetic field. (It should be noted that we used nonmagnetic tools for arthroscopy and an arthroscopic system that did not use a magnet in the arthroscope.) We definitely need to perform another study to investigate the optimal strength of the mag-

netic field for clinical applications. The last aspect is the efficiency of magnetically labeling in magnetic resonance imaging (MRI). Because ferumoxides act as a magnetic resonance contrast agent for hepatic imaging in humans, there may be some artifacts on MRI. Given that this labeling procedure was originally developed for monitoring MSCs,[17] magnetic labeling may have the merit of monitoring implanted MSCs on MRI.

Another important factor is the location of the osteochondral defect. Although it is relatively easy to apply this new method to lesions in the patella or patella groove, it is not easy to apply it to femoral condyle lesions or tibial plateau lesions. However, we think that this new method may be applicable for these lesions by managing the direction of the external magnetic force and by effectively using gravity by changing the knee position of the patients.

Although there were some limitations to our study, this system has the potential to become a novel stem cell delivery method in humans. Our novel, less invasive approach is applicable to human cartilage defects and may open a new era of repairing cartilage defects caused by osteoarthritis or trauma. Furthermore, we think that this new system may be applicable not only for the treatment of osteochondral defects but also for the treatment of brain or spinal cord injuries, fractures, and myocardial infarctions.

CONCLUSIONS

We have shown the ability to deliver magnetically labeled MSCs to a desired place in the knee joint. This supports the hypothesis of our study.

Acknowledgment: The authors thank Paul Reay for valuable comments.

REFERENCES

1. Murphy JM, Fink DJ, Hunziker EB, Barry FP. Stem cell therapy in a caprine model of osteoarthritis. Arthritis Rheum 2003;48:3464-3474.
2. Nishimori M, Deie M, Kanaya A, Exham H, Adachi N, Ochi M. Repair of chronic osteochondral defects in the rat. A bone marrow-stimulating procedure enhanced by cultured allogenic bone marrow mesenchymal stromal cells. J Bone Joint Surg Br 2006;88:1236-1244.
3. Wakitani S, Imoto K, Yamamoto T, Saito M, Murata N, Yoneda M. Human autologous culture expanded bone marrow mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees. Osteoarthritis Cartilage 2002;10:199-206.
4. Tatebe M, Nakamura R, Kagami H, Okada K, Ueda M. Differentiation of transplanted mesenchymal stem cells in a large osteochondral defect in rabbit. Cytotherapy 2005;7:520-530.
5. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143-147.
6. Jiang Y, Jahagirdar BN, Reinhardt RL, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002;418:41-49.
7. Ochi M, Adachi N, Nobuto H, Yanada S, Ito Y, Agung M. Articular cartilage repair using tissue engineering technique—Novel approach with minimally invasive procedure. Artif Organs 2004;28:28-32.
8. Yanada S, Ochi M, Adachi N, Nobuto H, Agung M, Kawamata S. Effects of CD44 antibody- or RGDS peptide-immobilized magnetic beads on cell proliferation and chondrogenesis of mesenchymal stem cells. J Biomed Mater Res A 2006;77:773-784.
9. Nakashima Y, Deie M, Yanada S, Sharman P, Ochi M. Magnetically labeled human natural killer cells, accumulated in vitro by an external magnetic force, are effective against HOS osteosarcoma cells. Int J Oncol 2005;27:965-971.
10. Hamasaki T, Tanaka N, Ishida O, et al. Characterization of labeled neural progenitor cells for magnetic targeting. Neuroreport 2005;16:1641-1645.
11. Bulte JW, Arbab AS, Douglas T, Frank JA. Preparation of magnetically labeled cells for cell tracking by magnetic resonance imaging. Methods Enzymol 2004;386:275-299.
12. Arbab AS, Yocum GT, Rad AM, et al. Labeling of cells with ferumoxides-protamine sulfate complexes does not inhibit function or differentiation capacity of hematopoietic or mesenchymal stem cells. NMR Biomed 2005;18:553-559.
13. Kostura L, Kraitchman DL, Mackay AM, Pittenger MF, Bulte JW. Feridex labeling of mesenchymal stem cells inhibits chondrogenesis but not adipogenesis or osteogenesis. NMR Biomed 2004;17:513-517.
14. Kotobuki N, Hirose M, Takakura Y, Ohgushi H. Cultured autologous human cells for hard tissue regeneration: Preparation and characterization of mesenchymal stem cells from bone marrow. Artif Organs 2004;28:33-39.
15. Agung M, Ochi M, Yanada S, et al. Mobilization of bone marrow-derived mesenchymal stem cells into the injured tissues after intraarticular injection and their contribution to tissue regeneration. Knee Surg Sports Traumatol Arthrosc 2006;14:1307-1314.
16. Tanaka H, Sugita T, Yasunaga Y, et al. Efficiency of magnetic liposomal transforming growth factor-beta 1 in the repair of articular cartilage defects in a rabbit model. J Biomed Mater Res A 2005;73:255-263.
17. Daldrup-Link HE, Rudelius M, Piontek G, et al. Migration of iron oxide-labeled human hematopoietic progenitor cells in a mouse model: In vivo monitoring with 1.5-T MR imaging equipment. Radiology 2005;234:197-205.

卒後
研修講座

変形性膝関節症のリスクファクター*

大森　豪**

[整形外科 59 巻 3 号：297～304，2008]

はじめに

変形性膝関節症（膝 OA）は年齢に伴う膝関節の退行性疾患であり，日常診療の場において変形性関節症の中では腰椎に次いで頻度が高い．本症の病態は，関節軟骨の変性と摩耗を主体として骨や軟骨下骨，滑膜，半月板，靱帯といった関節構成体に炎症反応や増殖性変化，変形性変化が生じ，結果的に関節破壊の進行にいたる一連の過程として理解される．膝 OA は“common disease”であり，その発症と進行には多数の因子が関与している．これらの因子は，膝関節に限局する局所因子と全身性因子あるいは遺伝要因と環境要因などに区分され，疫学や生体力学，生化学，画像解析などさまざまなアプローチで研究が行われている．

膝 OA の治療や予防を考えるうえでリスクファクターを理解することはきわめて重要であり，本稿では膝 OA の発症・進行因子について，これまでに明らかになっているものおよび残された課題を含めて概説する．なお，ここで述べる膝 OA とは X 線像にて診断された radiographic OA であり，膝痛などの症状を有する symptomatic OA ではない．

1 年齢と性別

男女とも 40 歳代以降年齢とともに膝 OA の頻度は増加し，70 歳代では男性で 30～40%，女性で 50～60% に達する[1]（図 1）．40 歳以降の各年代では女性が 1.5～2.5 倍発症率が高くなっており，これらの点から加齢および女性は膝 OA の危険因子といえる．興味深いのは 40 歳以下の年代では，Lawrence ら[2]は男性で 5.5%，女性で 3.9%，NHANES-I[3]でも 35～44 歳の群で男性 1.75%，女性 1.44% と逆に男性の発症率がわずかながら高く，比較的若年者の膝 OA 発症に靱帯，半月，軟骨損傷といった膝外傷が潜在的に影響している可能性を示唆する所見と考えられる．

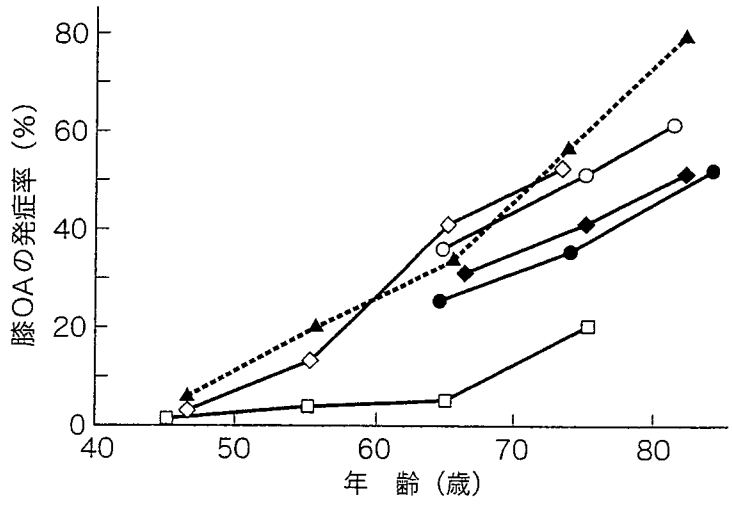
2 人　種

これまでの報告では，欧米の白人，日本人，中国人における年代別の膝 OA 発生率はいずれも男女とも加齢とともに増加する（図 1）．また，NHANES-I[3]では米国内の黒人は白人に比べて男性で 1.4 倍，女性で 2.8 倍膝 OA に対する危険度が大きいことも示されている．近年，異なる二つの人種を同一の解析手法で比較した研究が行われ，Zhang ら[4]は Framingham study のプロトコルを用いて調査を行い，中国人女性が白人女性に比べて有意に膝 OA が多いことを示した．また，Yoshida ら[5]も同様の手法で日本人女性は白人女性に比べて 1.9 倍膝 OA の危険度が高いと述べており，今後，同様の研究がすすむに従い人種間の相違や特徴が明らかになることが期待される．

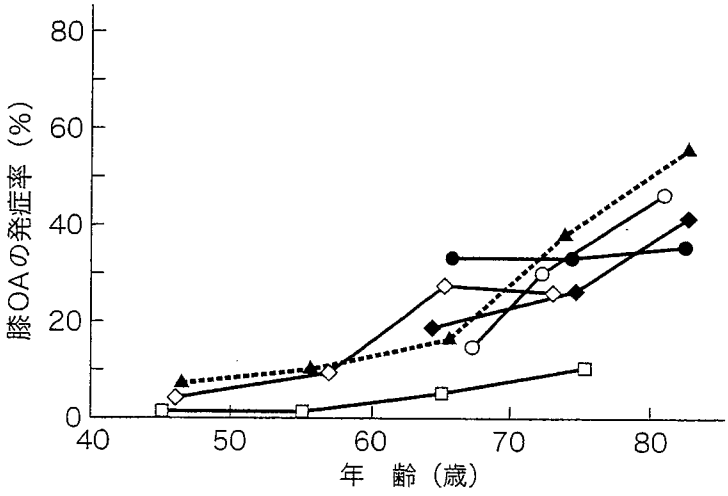
Key words：medial knee osteoarthritis, risk factor, review

* Risk factor of knee osteoarthritis

** G. Omori（教授）：新潟大学超域研究機構（Center for Transdisciplinary Research, Niigata University, Niigata）．



a．女



b．男

図 1．疫学調査による膝 OA の年齢別発症率

3 肥　満

膝関節には立位で体重の 1～1.5 倍，階段昇降で 2～3 倍，ジャンプ動作では 4～6 倍の荷重がかかるとされ，機械的要因の観点から体重と膝 OA の関連性は明らかである．疫学調査においても膝 OA と肥満の有意な関連を示す報告は多く，肥満の指標として BMI［body mass index：体重（kg）/身長（m）^{2}］を用いた欧米の調査[3,6]では BMI が 25 以上で 1.8～3.8 倍，BMI が 30 以上で 3.8～4.8 倍，35 以上の超肥満では 4～7.8 倍の相対危険度が報告されている．日本では，われわれが行った調査（松代膝検診）[7]において BMI が 24 以上で危険度が 2.1 倍に高くなり，また Yoshimura ら[8]は和歌山県のコホートに対し case-control-study を行い，過去の肥満の既往が膝 OA 発症に関連することを述べている．男女差については，男性のほうが肥満の影響が大きいとする報告と女性のほうが大きいとする報告があり一定の見解は得られていない．また，肥満が膝 OA に影響するメカニズムとして膝関節への荷重負荷増大による機械的作用と高脂血症や糖尿病などの代謝性疾患による作用が考えられているが，次項に述べるように代謝性疾患による影響は少なく機械的作用が主体と考えられる．

4 代謝性疾患

偽痛風の原因であるピロリン酸カルシウム結晶（CPPD）やほかのカルシウムリン酸結晶（BCP）と膝 OA との関連性は古くから指摘されており，膝 OA の 50～60％に関節液中に

表 1. 職業および日常活動性の膝 OA への影響

職業および日常活動性	膝 OA への影響
炭鉱労働者	男で影響あり
港湾労働者	男で影響あり
膝屈曲を要する職業（大工，トラック運転手など）	男で 2.5，女で 3.5（OR*）
力を要する職業（農夫，大工など）	男で 1.8，女で 3.1（OR）
膝屈曲＋力仕事	男で 2.2，女で 0.3（OR）
しゃがみ込み動作（1 日 30 分以上）	6.9（OR）
膝つき動作（1 日 30 分以上）	3.9（OR）
階段昇降（1 日 10 段以上）	2.7（OR）
しゃがみ込み動作（1 日 1 時間以上）	女で 1.2（OR）
階段昇降（1 日 30 段以上）	女で 1.19（OR）
椅子の腰掛け（1 日 2 時間以上）	女で 0.77（OR）
しゃがみ込み動作（1 日 2 時間以上）	女で 2.4（OR），男で 2.0（OR）

*OR：オッズ比

CPPD が存在するといわれている[9]．しかし，全身的な高尿酸血症の影響については明らかになっていない．また高脂血症，糖尿病，高血圧についても関連性があるとする報告とないとする報告があり一定の見解は得られておらず，現時点で代謝性疾患の膝 OA への直接的な関与は少ないと考えられる[10,11]．

5 喫　煙

これまでの疫学調査では，タバコおよび葉巻の喫煙習慣と膝 OA の発症は逆相関が認められ，喫煙は膝 OA に無関係とするものから予防的効果の可能性すら指摘する報告もある[12,13]．しかしニコチンやタール，アンモニアといったタバコに含まれる成分が膝関節に及ぼす生物学的な影響についてはまったく解明されていない．

6 職業，生活様式，日常活動性と運動

職業や日常動作と膝 OA との関連性については多数の研究があり，膝の屈伸を伴う重労働の影響が大きいとする報告が多い（表 1）．地域での生活習慣については，グリーンランドの狩猟民族やジャマイカの裸足生活者には膝 OA が多いという報告もある[14,15]．

運動と膝 OA との関連では，ジョギングのように軽度～中等度の負荷にとどまる場合は影響が少ないとされている[16]．これに対し膝関節への負荷が増大する運動強度の高い種目では，次項に述べる半月板損傷や軟骨損傷，靱帯損傷といった膝外傷の合併との関連で検討され，膝 OA に大きく影響するといった報告が多い．Sandmark ら[17]の行った調査によると，クロスカントリースキーやアイスホッケーでは，男性で 2.9 倍相対危険度が増すと述べている．

7 膝 外 傷

膝 OA に影響する外傷としては靱帯損傷，半月板損傷，軟骨損傷，骨折があるが，未治療の膝外傷については診断が明確とならないため特定することは困難である．半月板損傷の影響は，生体力学研究により半月板切除による膝関節への著明な応力集中が証明されているが[18]，臨床的には治療として行った切除術後の OA 変化を検討するものが多く，変性半月板断裂，半月板切除量が多いことが成績不良因子としてあげられている[19]．また，前十字靱帯損傷に関しては，保存的治療例または放置例において受傷後 10～20 年の経過で高率に膝 OA が発症することが報告されている[20,21]．しかし，これらの臨床研究では対象者の年齢が 40～50 歳と比較的若いこと，X 線像上の OA 所見のわりに臨床症状が少ないことが指摘されている．われわれも膝半月板切除後 30 年以上の長期経過例を評価し，膝 OA 発症例に臨床症状が少なく可動域が良好な例が多いことを経験している[22]．さらに，既述した膝 OA の発症率が 40 歳代までは男性が女性より多くその後逆転することを考えると，膝外傷後にみられる膝関節の OA 変化は外傷に対する関節の生体変化である可能性が示唆される．そして，これが最終的に真の膝 OA となりさらに進行するかどうかは，

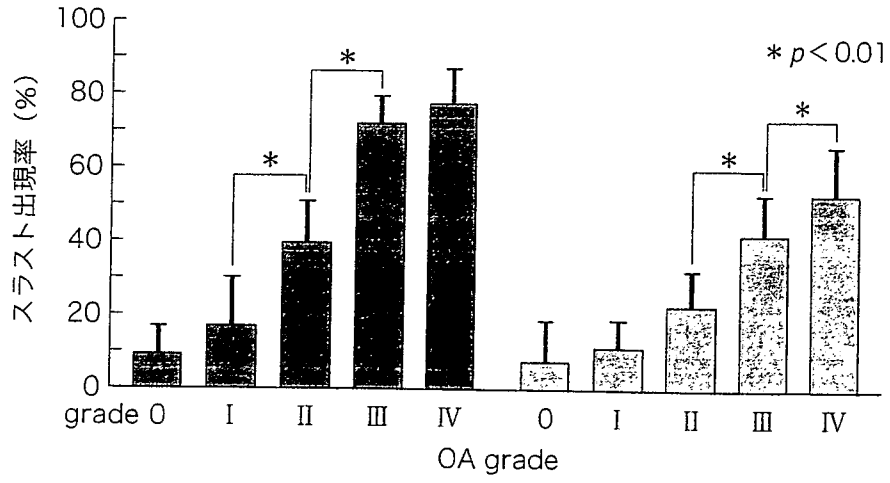


図 2. 膝 OA grade とスラスト運動出現率

その個人のもつ膝 OA のリスクファクターによって左右されると考えられる．しかし，この点に関する科学的なエビデンスはなく今後明らかにすべき課題である．

8 下肢筋力

大腿四頭筋力が膝 OA の進行に伴い低下することは，疫学的な横断調査や患者を対象とした臨床研究において多数の報告がみられ，両者に関連性があることは明らかであるが，因果関係については不明な点が多い．最近の研究では単なる大腿四頭筋力低下ではなく，日常生活動作（ADL）における大腿四頭筋反応時間[23]や，膝屈筋とのバランス[24]，関節位置覚[25]，スラスト運動を含めた関節安定性[26]などほかの要素を含めて膝 OA との関連性を述べたものが多い．われわれは松代膝検診における縦断解析より，大腿四頭筋力低下が後述するスラスト運動を介して膝 OA 発症に影響することを明らかにしている[27]．また大腿四頭筋力と膝 OA の進行との関係については，これまでのところ有意な関連性を示した基礎研究は見当たらず，大腿四頭筋力強化により疼痛や ADL が改善したという臨床研究がみられるのみである．

このように，現在臨床の場において大腿四頭筋力強化が膝 OA の予防や治療として有効であると推奨されているが，そのエビデンスは意外に乏しく，今後明らかにすべき多くの課題が残されている．

9 下肢アライメント，スラスト運動

生体力学的研究から膝内反により膝関節内側の荷重負荷が増大することが証明されているが，近年，膝内反アライメントと内側型膝 OA の関連性がわれわれ[27]や Sharma ら[28]により疫学調査や臨床研究から示されている．また，スラスト運動は立脚歩行初期における急激な内反運動で，われわれは歩行解析を行った膝 OA 患者にスラスト運動が多くみられたことから膝 OA の有力なリスクファクターと考えている．松代膝検診でも膝 OA の進行とともにスラスト運動の出現が増加し，さらに縦断解析によりスラスト運動が膝 OA 発症に関与していることが明らかとなった（図 2）．欧米ではスラスト運動は膝内反モーメントとして評価され，歩行解析を用いた臨床研究から膝 OA との関連性が指摘されているが，近年，疫学研究においてスラスト運動と膝 OA との関連性を述べた報告もみられる[29,30]．

10 骨粗鬆症

従来，変形性関節症と骨粗鬆症は逆の病態と考えられ，膝 OA についても高骨密度との関連性を示した報告が多い[31~33]．しかしその一方で，高骨密度は膝 OA 発症に関与するが，膝 OA の進行には逆に低骨密度が影響するという報告[34]や，脛骨近位の内反変形と腰椎骨密度と相関するという研究[35]もみられる．また，動物

表 2. これまでに報告された膝 OA の候補遺伝子

膝 OA の候補遺伝子として報告されたもの	膝 OA および類縁疾患との関連
COMP (cartilage oligomeric matrix protein)	偽性軟骨無形成症
COL11A1 (human type-XI procollagen gene)	Stickler 症候群
COL2A1 (human type-II procollagen gene)	軟骨形成不全，脊椎骨端異形成症など多数
VDR (vitamin D receptor gene)	骨粗鬆症，骨棘形成
aggrecan	手指 OA
COL9A1 (human type-IX procollagen gene)	股関節 OA
COL9A3 (human type-IX procollagen gene)	股関節 OA
IGF1 (insulin-like growth factor 1)	手指 OA，脊椎 OA
CRTL1 (cartilage matrix protein gene 1)	手指 OA，股関節 OA
ER (estrogen receptor)	骨粗鬆症
PAPSS2 (3'-phosphoadenosine 5'-phosphosulfate synthase)	脊椎骨端異形成症
ASPN (asporin)	膝 OA
AnK	CPPD 沈着
CALM1 (calmodulin1)	股関節 OA
FRZB (serected frizzeled-related protein-3)	股関節 OA（女性）
IL-1	股関節 OA
MATN3 (matrilin 3)	手指 OA
IL-4L	股関節 OA
ADAM12 (metalloprotease)	股関節 OA

実験では骨吸収抑制薬により膝 OA にみられる骨棘形成が抑制されたという研究[36]もある．さらに Baltimore aging study[37]では，変形性関節症と骨粗鬆症の関連性は罹患関節によって異なると述べている．したがって現状では膝 OA と骨粗鬆症の関連性は示唆されるが，その作用機序は明らかではない．

11 性ホルモン

女性ホルモンであるエストロゲン（estrogen：ERT）は，骨に対しては骨吸収を抑制し形成を維持する作用がある．膝 OA に対しても ERT 補充療法が試行され，発症と進行に予防的に作用可能性を示した報告が散見されるが，統計学的な有意差は認められていない[38,39]．近年，ERT レセプターに対する遺伝子学的研究からも ERT は膝 OA に対する予防的効果が報告されており[40]，前項の骨粗鬆症との関連性においても今後の研究がまたれている．

12 微量栄養素

ビタミン A，C，E およびベータカロチンは，活性酸素による軟骨破壊の抑制と修復をうながす点で膝 OA の発症に対しては有効ではないが，進行の抑制や膝痛の軽減に効果があるという報告が多い[41,42]．近年，Wang ら[43]は 10 年間の縦断研究からビタミン C 摂取が軟骨変性に予防的に作用することを明らかにしている．また，ビタミン D は骨代謝の観点から膝 OA との関連性が研究されている．McAlindon ら[44,45]は Framingham study において血中 25-ハイドロキシビタミン D 濃度の低下は膝 OA の進行を助長すると報告したが，近年，Felson ら[46]は Framingham と Boston の二つのコホートに対する縦断調査からビタミン D と膝 OA の進行との間に有意な関連性は見出せなかったとしており，一定の見解が得られていない．

13 遺 伝 子

膝 OA は common disease のため遺伝形式は多因子遺伝であり，原因遺伝子よりも感受性遺伝子（susceptibility gene）として研究される場合が多い．膝 OA の遺伝性についての報告は，Kellgren ら[47]が手指遠位指節間（DIP）関節の OA である Heberden 結節と膝 OA の合併例を調べ，「generalized OA (GOA)」という疾患概念を提唱し遺伝的素因を示唆したのが最初である．その後，軟骨形成不全症や Stickler 症候群など膝 OA の一つのモデルとも考え

られる疾患の原因遺伝子としてCOMP（cartilage oligomeric matrix protein）やCOL2A1（human type-Ⅱ collagen gene）などが同定され，これを足がかりにして，変形性関節症に関する多くの遺伝子多型（genetic polymorphism）が発見された[48~50]（表2）．さらに，これらの遺伝子多型の相関解析が行われているが，膝OAに関しては近年asporinの報告があるものの，いまだ特定されてはいない．また，ビタミンD受容体遺伝子（vitamin D receptor gene：VDR）は骨粗鬆症と関連性があることから，膝OAへの影響についても注目され多くの研究が行われており[51~53]，VDR遺伝子は初期の膝OAに関連する（Chingford study）や膝関節の骨棘形成に影響する（Rotterdam study）など多くの報告がなされているが一定の結論にいたっていない．いずれにせよ，今後膝OAに対する感受性遺伝子の研究はさらに加速すると思われるが，本疾患の複雑な病態から考えると大規模な集団に対する遺伝子解析が望まれる．

おわりに

現在，わが国においてX線像上膝OAと診断される人は1,000万人を超えると推計される．膝関節は起立歩行といった人間のもっとも基本的な動作の要であり，その機能破綻はわれわれの日常生活動作（ADL）や生活の質（QOL）に大きな影響を与える．したがって，膝OAに対する予防や治療方法の確立に向けて今後も病態解明の努力が必要と考えられる．

文　献

1) Omori G：Epidemiology of knee osteoarthritis. Acta Med Biol **53**：1-11, 2005
2) Lawrence JS, Bremner JM, Brief F：Osteoarthrosis；prevalence in the population and relationship between symptoms and X-ray changes. Ann Rheum Dis **17**：388-396, 1958
3) Anderson JJ, Felson DT：Factors associated with osteoarthritis of the knee in the first national health and nutrition examination survey. Am J Epidemiol **128**：179-189, 1988
4) Zhang Y, Xu L, Felson DT et al：Comparison of the prevalence of knee osteoarthritis between the elderly Chinese population in Beijing and whites in the United States；the Beijing osteoarthritis Study. Arthritis Rheum **44**：2065-2071, 2001
5) Yoshida S, Aoyagi K, Felson DT et al：Comparison of the prevalence of radiographic osteoarthritis of the knee and hand between Japan and Unite States. J Rheumatol **29**：1454-1458, 2002
6) Felson DT, Anderson JJ, Naimark A et al：Obesity and knee osteoarthritis；the Framingham study. Ann Intern Med **109**：18-24, 1988
7) Aoda H, Nakamura K, Omori G et al：Independent predictors of knee osteoarthritis in an elderly Japanese population；multivariate analysis. Acta Med Biol **54**：33-41, 2006
8) Yoshimura N, Nishioka S, Kinoshita H et al：Risk factors for knee osteoarthritis in Japanese women；heavy weight, previous joint injuries, and occupational activities. J Rheumatol **31**：157-162, 2004
9) Martin K, Lethbridge-Cejku M, Muller DC et al：Metabolic correlates of obesity and radiographic features of knee osteoarthritis；data from the Baltimore longitudinal Study of aging. J Rheumatol **24**：702-707, 1997
10) Davis MA, Ettinger WH, Neuhaus JM：The role of metabolic factors and blood pressure in the association of obesity with osteoarthritis of the knee. J Rheumatol **15**：1827-1832, 1988
11) Hart DJ, Doyle DV, Spector TM：Association between metabolic factors and knee osteoarthritis in women；the Chingford Study. J Rheumatol **22**：1118-1122, 1995
12) Felson DT, Anderson JJ, Naimark A et al：Does smoking protect against osteoarthritis? Arthritis Rheum **32**：166-172, 1989
13) Jarvholm B, Lewold S, Malchau H et al：Age, body weight, smoking habit and the risk of severe osteoarthritis in the hip and knee in men. Eur J Epidemiol **20**：537-542, 2005
14) Anderson S：The epidemiology of the knee in Greenland. Scand J Rheumatol **7**：109-112, 1978
15) Bremner JM, Lawrence JS, Miall WE：Degenerative joint disease in a Jamaican rural population. Ann Rheum Dis **27**：326-332, 1968
16) Panush R, Hanson C, Caldwell J et al：Is running associated with osteoarthritis?；an eight-year follow-up study. J Clin Rheumatol **1**：35-39, 1995

17) Sandmark H, Vingard E : Sports and risk factors for severe osteoarthritis of the knee. Scand J Med Sci Sports **9** : 279-284, 1999
18) Fukubayashi T, Kurosawa H : The contact area and pressure distribution pattern of the knee. Acta Orthop Scand **51** : 871-879, 1980
19) Englund M, Roos M, Lohmander LH : Impact of type of meniscal tear on radiographic and symptomatic knee osteoarthritis. Arthritis Rheum **48** : 2178-2187, 2003
20) Von Past A, Roos EM, Roos H : High prevalence of osteoarthritis 14 years after an anterior cruciate ligament tear in male soccer players ; a study of radiographic and patient ; relevant outcomes. Ann Rheum Dis **63** : 269-273, 2004
21) Segawa H, Omori G, Koga Y : Long-term results of non-operative treatment of anterior cruciate ligament injury. Knee **8** : 5-11, 2001
22) 古賀良生，佐藤　卓，祖父江展ほか：半月摘出例の関節症進行因子について．整・災外 **48** : 39-44, 2005
23) Fitzgerald GK, Piva SR, Irrgang JJ et al : Quadriceps activation failure as a moderator of the relationship between quadriceps strength and physical function in individuals with knee osteoarthritis. Arthritis Rheum **51** : 40-48, 2004
24) Hortobagyi T, Westerkamp L, Beams S et al : Altered hamstrings-quadriceps muscle balance in patients with knee osteoarthritis. Clin Biomech **20** : 97-104, 2005
25) Van Baar M, Assendelft WJ, Dekker J et al : Effectiveness of exercise therapy in patients with osteoarthritis of the hip or knee ; a systemic review of randomized clinical trials. Arthritis Rheum **42** : 1361-1369, 1999
26) Thorstensson CA, Henriksson M, Porat A et al : The effect of eight week of exercise on knee adduction moment in early knee osteoarthritis ; a pilot study. Osteoarthritis Cartilage **15** : 1163-1170, 2007
27) 大森　豪，古賀良生，遠藤和男：疫学調査から見た変形性膝関節症の発症要因．日整会誌 **80** : 927-932, 2006
28) Sharma L, Song J, Felson DT et al : The role of knee alignment in disease progression and functional decline in knee osteoarthritis. JAMA **286** : 188-195, 2001
29) Amin S, Luepongsak N, McGibbon CA et al : Knee adduction moment and development of chronic knee pain in elders. Arthritis Rheum **51** : 371-376, 2004
30) Chang A, Hayes K, Dunlop D et al : Thrust during ambulation and the progression of knee osteoarthritis. Arthritis Rheum **50** : 3897-3903, 2004
31) Hannan MT, Anderson JJ, Zhang Y et al : Bone mineral density and knee osteoarthritis in elderly men and women ; the Framingham study. Arthritis Rheum **36** : 1671-1680, 1993
32) Hart DJ, Mootoosamy I, Doyle DV et al : The relationship between osteoarthritis and osteoporosis in the general population ; the Chingford study. Ann Rheum Dis **53** : 158-162, 1994
33) 須藤啓広，宮本　憲，田島正稔ほか：変形性膝関節症の疫学的調査．整形外科 **50** : 1033-1038, 1999
34) Zhang Y, Hannan MT, Christine E et al : Bone mineral density and risk of incident and progressive radiographic knee osteoarthritis in women ; the Framingham study. J Rheumatol **27** : 1032-1037, 2000
35) Terauchi M, Shirakura K, Katayama M et al : The influence of osteoporosis on varus osteoarthritis of the knee. J Bone Joint Surg **80-B** : 432-436, 1998
36) Hayami T, Pickarski M, Wesolowski GA et al : The role of subchondral bone remodeling in osteoarthritis ; reduction of cartilage degeneration and prevention of osteophyte formation by alendronate in the rat anterior cruciate ligament transection model. Arthritis Rheum **50** : 1193-1206, 2004
37) Hochberg MC, Lethbridge-Cejku M, Tobin JD : Bone mineral density and osteoarthritis ; data from the Baltimore longitudinal study of aging. Osteoarthritis Cartilage **12** : 45-48, 2004
38) Zhang Y, McAlindon TE, Hannan MT et al : Estrogen replacement therapy and worsening of radiographic knee osteoarthritis ; the Framingham study. Arthritis Rheum **41** : 1867-1873, 1998
39) Spector TD, Nandra D, Hart DJ et al : Is hormone replacement therapy protective for hand and knee osteoarthritis in women? : the Chingford study. Ann Rheum Dis **56** : 432-434, 1997
40) Fytili P, Giannatou E, Papanikolaou V et al : Association of repeat polymorphisms in the estrogen receptors alpha, beta, and androgen receptor genes with knee osteoarthritis. Clin Genet **68** : 268-277, 2005
41) Henrotin Y, Deby-dupont G, Deby C et al : Production of active oxygen species by

human chondrocytes. Br J Rheumatol **32**: 562-567, 1993
42) Sowers M, Lachance L: Vitamins and arthritis; the roles of vitamins A, C, D, and E. Rheum Dis Clin North Am **25**: 315-332, 1999
43) Wang Y, Hodge AM, Wluka AE et al: Effect of antioxidants on knee cartilage and bone in healthy, middle-aged subjects; a cross-sectional study. Arthritis Res Therapy **9**: R66, 2007
44) McAlindon TE, Felson DT, Zhang Y et al: Relation of dietary intake and serum levels on vitamin D to progression of osteoarthritis of the knee among participants in the Framingham study. Ann Int Med **125**: 353-359, 1996
45) McAlindon TE, Biggee BA: Nutritional factors and osteoarthritis; recent developments. Curr Opin Rheumatol **17**: 647-652, 2005
46) Felson DT, Niu J, Clancy M et al: Low levels of vitamin D and worsening of knee osteoarthritis. Arthritis Rheum **56**: 129-136, 2007
47) Kellgren JH, Lawrence JS, Bier F: Genetic factors in generalized osteoarthritis. Ann Rheum Dis **22**: 237-255, 1963
48) 池田敏之，馬淵昭彦，張　軍衛ほか：変形性膝関節症の遺伝的背景―感受性遺伝子の同定へ向けて．別冊整形外科 **42**：17-20，2002
49) Mabuchi A, Ikeda T, Fukuda A et al: Identification of sequence polymorphisms of the COMP (cartilage oligomeric matrix protein) gene and association study in osteoarthrosis of the knee and hip joints. J Hum Genet **46**: 456-462, 2001
50) Ikeda T, Mabuchi A, Fukuda A et al: Association analysis of single nucleotide polymorphisms in cartilage-specific collagen genes with knee and hip osteoarthritis in the Japanese population. J Bone Miner Res **17**: 1290-1296, 2002
51) Huan J, Ushiyama T, Inoue T et al: Vitamin D receptor gene polymorphisms and osteoarthritis of the hand, and knee; a case-control study in Japan. Rheumatology **39**: 79-84, 2000
52) Utterlinden AG, Burger H, Huang Q et al: Vitamin D receptor genotype is associated with radiographic osteoarthritis at the knee. J Clin Invest **1001**: 259-263, 1997
53) Keen RW, Hart DJ, Lanchbury JS et al: Association of early osteoarthritis of the knee with a Taq polymorphism of the vitamin D receptor gene. Arthritis Rheum **40**: 1444-1449, 1997

*　　*　　*

■ 原著

膝伸展筋力の加齢変化と変形性膝関節症との関連

渡辺博史[1] 古賀良生[2] 大森豪[3] 遠藤和男[4]
田中正栄[5] 縄田厚[6] 穐丸舞[6]

新潟こばり病院リハビリテーション部[1]，同整形外科[2]，新潟大学超域研究機構[3]，
新潟医療福祉大学[4]，新潟県健康づくり・スポーツ医科学センター[5]，株式会社アルケア[6]

要旨 住民検診で協力を得られた1,214名（24－91歳）に対し，膝伸展筋力の測定を行い，膝伸展筋力の加齢変化と変形性膝関節症（膝OA）発症との関連を検討した．膝伸展筋力は，男女とも60歳代以降で有意な低下を認め，膝OA gradeの進行とともに低下する傾向であった．そして女性では，肥満や加齢の影響を除外しても膝OAと膝伸展筋力との関連を認め，女性の膝OA発症における膝伸展筋力低下の影響が示唆された．

Abstract In the epidemiological study on the knee osteoarthrosis 1,214 subjects (24-91 years old) were measured their knee extensor muscle strength and OA grade by standing X-ray. The strength of knee extensor was significantly reduced by the age of 60 years, and also related to the severity of knee OA grade. Especially in females this relation of muscle weakness to the OA knee grade was significant even after the consideration of the effect of their obesity and aging.

Key words：膝伸展筋力（knee extensor muscle strength），加齢（aging），変形性膝関節症（osteoarthritis of the knee）

はじめに

人口の高齢化に伴い運動器の退行性疾患の増加が問題となっている．変形性膝関節症（以下膝OA）は，その退行性疾患として日常診療で接する機会の多い疾患である．また膝OAの発症および進行防止に膝伸展筋力強化が重要とされている．

本研究は，膝伸展筋力の測定を行い，性別・年代別および膝OA grade（以下grade）別で比較し，膝伸展筋力の加齢変化と膝OA発症との関連について検討した．

対象

新潟県十日町市松代地区の2006年住民検診にて協力を得られた1,214名で，性別の内訳は女性695名，男性519名であった．対象者の年代別構成を図1に示す．男女とも70歳代が最も多く，平均年齢は女性66.5±12.2歳，男性67.9±13.1歳であった．

Relationship between osteoarthritis of the knee and aging change of the knee extensor muscle strength
Hiroshi WATANABE, RPT, Yoshio KOGA, MD, Go OMORI, MD, Kazuo ENDO, MD, Masaei TANAKA, RPT, Atsushi NAWATA and Mai AKIMARU

連絡先：〒950－2022 新潟県新潟市西区小針3－27－11 新潟こばり病院リハビリテーション部 渡辺博史 電話 025－232－0111

方　法

対象者全員に膝伸展筋力を測定し，対象者のうち757名（女性443名，男性314名）に，立位膝関節前後X線撮影（以下X線）を行った．筋力測定は，われわれが開発した簡易筋力測定器を使用し右側のみ測定した．筋力測定の肢位は，膝屈曲約30°の長座位で膝窩部に測定器を設置し，骨盤帯と下腿遠位部を非収縮性バンドで固定した．筋力はこの肢位で対象者が膝伸展運動を行った際，膝窩部で測定器を押し付ける力を5秒間計測し，その最大値を膝伸展筋力の測定値とした（図2）．測定値の体重比を体重支持指数（以下weight bearing index: WBI）値として算出し，測定値およびWBI値を性別，年代別，grade別で比較した．膝OAの病期分類は，X線像からKellgrenの分類[2]を参考に5段階で同一の医師が判定した（表1）．またgradeが0，Ⅰを非OA群，Ⅱ以上をOA群の2群に分けて（表2），膝伸展筋力との関連について検討した．統計処理は，性別では対応のないt

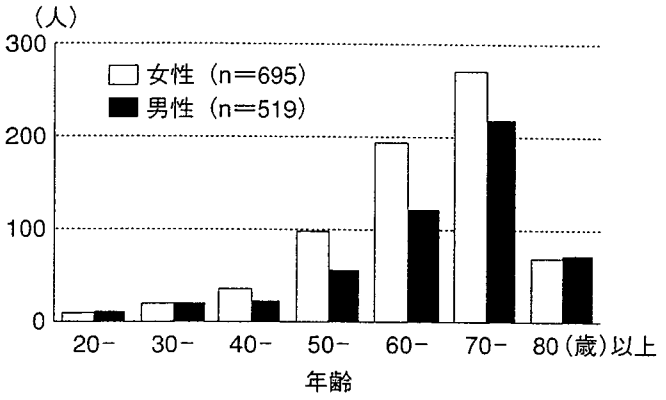


図1　対象者の性別年代別構成
男女とも70歳代が最も多く，平均年齢は女性66.5歳，男性67.9歳であった．

表1　X線像による膝OAの病期分類

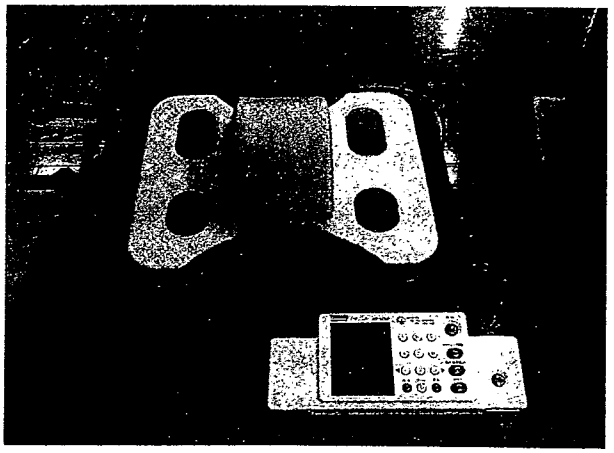
OA grade	裂隙狭小化	骨棘形成等
Grade 0	(−)	(−)
Grade Ⅰ	(−)	(+)
Grade Ⅱ	<1/2	(+)
Grade Ⅲ	>1/2	(+)
Grade Ⅳ	閉鎖	(+)

Kellgrenに準じ5段階評価で同一の医師が判定した．

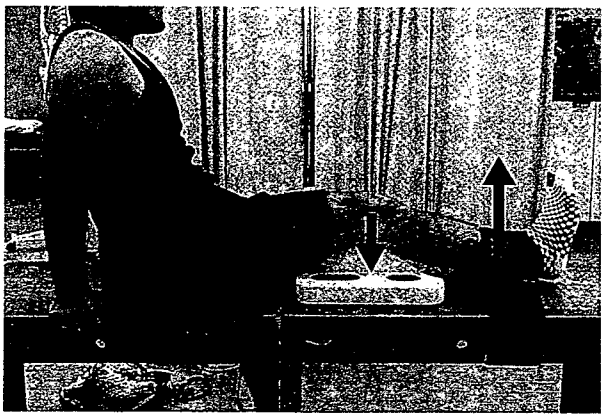
表2　OAについての2群の内訳

	非OA群（grade 0, Ⅰ）	OA群（grade Ⅱ以上）
女性	208名（63.7±11.5歳）	235名（71.2±8.9歳）
男性	161名（64.9±12.9歳）	153名（73.4±8.4歳）

男女とも2群間で年齢に有意差を認め，OA群が高かった（$p<0.01$）．



簡易筋力測定器



測定肢位：膝屈曲30°，長座位

図2　筋力測定（右膝）
骨盤帯と下腿遠部を非収縮性バンドで固定して，膝伸展運動させた際の膝窩部に発生する力を測定した．