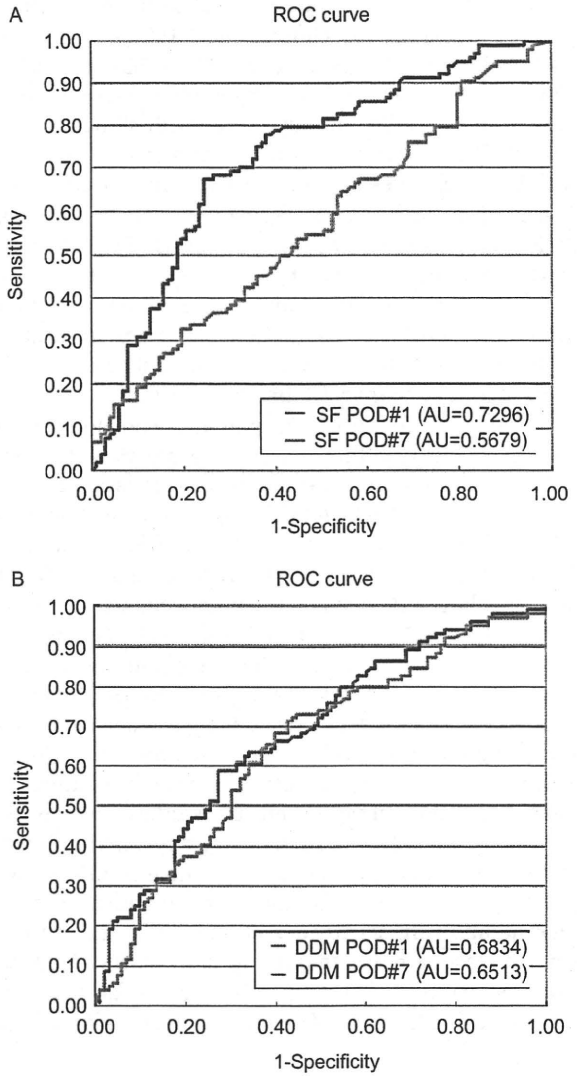

Figure 3. Receiver operating characteristics (ROC) curve for plasma soluble fibrin (SF) (A) and D-dimer (B) in the diagnosis of deep vein thrombosis. The area under the curve (AUC) of plasma SF on postoperative day 1 is 0.7296. The AUCs of plasma D-dimer on postoperative days 1 and 7 are 0.6834 and 0.6513, respectively. POD, postoperative day.

plasma levels of D-dimer are maintained for a longer period than those of SF.

This study has three main limitations. First, the number of enrolled patients was small. Second, both the sensitivity and the specificity for DVT were lower than expected. In Japan, SF and D-dimer concentrations are usually measured using LIA, while in Europe and North America, D-dimer is measured using ELISA. LIA has not been recommended instead of the ELISA method. Unfortunately, at our hospital, ELISA was not introduced, so we had to evaluate the usefulness of LIA measurement. Third, we started treatment with unfractioned heparin (UFH) or low-dose warfarin for DVT patients, which may affect SF and D-dimer levels 7 days after surgery.

In conclusion, SF evaluation on postoperative day 1 is the most useful for initial screening, with a sensitivity of 90.4% and a specificity of 33.0%. We recommend the use of SF evaluation when the time of the thrombotic event, such as an operation, is clear. This sequential approach might be useful in studies on the efficacy of antithrombotic regimens for prophylaxis of DVT in postoperative patients. SF and D-dimer assays cannot be used as a stand-alone test, and further study is needed.

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Deficiency of tenascin-C delays articular cartilage repair in mice

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SUMMARY

Objective: In human articular cartilage, tenascin-C (TN-C) expression decreases during maturation of chondrocytes, and almost disappears in adults; however, it reappears in damaged cartilage. To examine the effects of TN-C on cartilage degeneration and repair, we compared articular cartilage degeneration between wild-type (WT) and tenascin-C knockout mouse (TNKO) mice using a spontaneous osteoarthritis (OA) in aged joints and surgical OA model. In addition, we made full-thickness cartilage defects and compared the cartilage repair process between the two groups.

Methods: The surgical procedure to create degenerative OA model was performed by transecting the anterior cruciate ligament and medial collateral ligament. Full-thickness defects were created in the center of the femoral trochlea to evaluate cartilage repair. Sections of cartilage were stained with hematoxylin and eosin or safranin-O, and immunostaining for TN-C. The degrees of degeneration and repair were graded.

Results: In the WT surgical OA model, the articular cartilage was almost normal at 2 weeks, but safranin-O decreased staining at 4 weeks. In TNKO mice, safranin-O decreased staining at 2 weeks, and cartilage was injured intensely at 4 weeks.

In the cartilage repair model, TN-C was expressed after 1 week, was strongly expressed in the upper layer of regenerated tissue after 3 weeks, and disappeared after 6 weeks. The defects were restored until 6 weeks in WT mice; however, defects in TNKO mice were filled with fibrous tissue with no cartilage-like tissue.

Conclusions: This study revealed that cartilage repair in TNKO mice was significantly slower than that in WT mice and that the deficiency of TN-C progressed during cartilage degeneration.

Introduction

Tenascin-C (TN-C) is a member of the extracellular matrix glycoprotein family and is expressed during embryogenesis. Although its expression is repressed in normal adult tissues, it reappears under pathological conditions such as inflammation, infection, and tumorigenesis[1,2,3,4]. In lesions, TN-C promotes migration and proliferation of parenchymal and/or stromal cells[5,6].

Chiquet *et al.* first indicated that TN-C may participate in chondrogenesis and cartilage development[7,8]. In subsequent studies, it was found that TN-C was expressed in early mesenchymal condensations of cartilage, progressively disappeared with the accumulation of mature cartilage, and disappeared almost completely in adult articular cartilage; however, it was retained in the perichondrium, which provides a source of cells to differentiate into chondroblasts[9,10]. In addition, TN-C reappears at high levels in diseased joints, including those with osteoarthritis (OA) and rheumatoid arthritis (RA)[11,12].

Two groups have independently produced tenascin-C knockout mouse (TNKO) mice[13,14]. These mice were born alive and, originally, were described as showing no abnormalities. However, recent studies have shown that TNKO mice have several problems, such as abnormal behavior, abnormal brain chemistry[15,16], defects in the structure and repair of neuromuscular junctions[17], and defective recovery from snake venom-induced glomerulonephritis[18] and chemically induced dermatitis[19]. In addition, we demonstrated reduced neointimal hyperplasia after vascular surgery[20], decreased myofibroblast recruitment in granulation tissue after myocardial injury[21], and reduced fibrotic change in immuno-mediated hepatitis[22].

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We compared articular cartilage degeneration between wild-type (WT) and TNKO mice using a spontaneous OA in aged joints and surgical OA model to examine the effects of TN-C expression on cartilage degeneration and repair. In addition, we made full-thickness cylindrical defects in the articular cartilage in the center of the femoral trochlea and compared the cartilage repair process between the two groups.

Materials and methods

Animals

Male TNKO and WT littermates of the BALB/c strain were used and maintained according to guidelines approved by the Mie University Animal Experiment and Care Committee.

Histological and pathological assessment of young and aged mice

Complete necropsies were performed on male 8-week-old TNKO ($n=4$) and WT ($n=4$) mice and on male 24-week-old TNKO ($n=4$) and WT ($n=4$) mice. Mice were sacrificed by cervical dislocation. Examinations at necropsy included macroscopic observations, determination of body weight and tail length, and radiographs. At sacrifice, we obtained 12 tissue samples per mouse (brain, knee joint, vertebrae, spinal cord, kidney, liver, heart, lung, skin, bone, muscle, and growth plate) for histological analysis.

Spontaneous OA in aged joints model

During breeding and experiments, 24 WT and 24 TNKO mice were housed in sterilized microbarrier units under germ-free conditions. Mice received autoclaved chow and acidified water *ad libitum*. These mice were sacrificed by cervical dislocation at the age of 2 months ($n=6$ WT and 6 TNKO mice), 4 months ($n=6$ WT and 6 TNKO mice), 6 months ($n=6$ WT and 6 TNKO mice), and 12 months ($n=6$ WT and 6 TNKO mice). In all cases, the distal femoral chondyle was removed.

OA model

The surgical procedure to create an experimental OA model was performed on 8-week-old mice. 8 weeks is a standard time point for knee joint surgery in mice because the trochlear groove is of adequate size at this age[23,24]. Eight TNKO mice ($n=16$ knees) and nine WT mice ($n=18$ knees) were anesthetized with an intramuscular injection of sodium pentobarbital (0.05 mg/g body weight). Both knee joints were exposed following a medial capsular incision and gentle lateral displacement of the extensor muscle, without transection of the patellar ligament. Then, the anterior cruciate ligament and medial collateral ligament were transected using a surgical microscope and microsurgical technique. After replacement of the extensor muscle, the articular capsule and skin were independently closed with 6–0 nylon sutures. All mice were allowed to walk freely without any splintage. WT and TNKO mice were sacrificed by cervical dislocation at 1 week ($n=6$ WT and 4 TNKO mice), 2 weeks ($n=6$ WT and 6 TNKO mice), or 4 weeks ($n=6$ WT and 6 TNKO mice) after surgery.

Surgical procedures for full-thickness articular cartilage defects

Thirty-two TNKO mice ($n=64$ knees) and 50 WT mice ($n=100$ knees) were anesthetized with an intramuscular injection of sodium pentobarbital (0.05 mg/g body weight). Both knee joints were approached by means of a medial parapatellar incision (10 mm) under sterile conditions. The patella was laterally dislocated to expose the articular surface on the femoral trochlea. Full-thickness cylindrical defects were created in the center of the femoral trochlea with a hand micro-drill equipped with a 0.3-mm-diameter drill-bit. After creating the defects, the articular capsule and skin were independently closed with 6–0 nylon sutures. All mice were allowed to walk freely without any splintage. WT and TNKO mice were sacrificed by cervical dislocation at 1 day ($n=2$ WT and two TNKO mice), 1 week ($n=5$ WT and four TNKO mice), 2 weeks ($n=6$ WT and two TNKO mice), 3 weeks ($n=18$ WT and nine TNKO mice), 6 weeks ($n=12$ WT and 10 TNKO mice), or 12 weeks ($n=7$ WT and five TNKO mice) after creation of defects.

Immunohistochemistry for assessment

At the indicated time points, the mice were killed, and the distal femoral chondyle was removed. All tissues were then fixed in 10% neutral buffered formalin at room temperature for 2 days, decalcified with 10% ethylenediamine tetraacetic acid for 7 days, and embedded in paraffin. A minimum of five sagittal and transectional sections (3 μm thick) were prepared from the center of medial femoral condyle for assessment of OA and a minimum of five sagittal sections (3 μm thick) were prepared from the center of each defect so that the sampling error could be minimized for assessment of cartilage repair. Sections were stained with hematoxylin and eosin or safranin-O. The production, characterization, and immunostaining technique of the anti-TN-C polyclonal rabbit antibody have been described previously[25]. Briefly, sections were incubated in methanol containing 0.3% H_2O_2 for 30 min to block intrinsic peroxidase activity and then treated with 0.04% proteinase K (Sigma-Aldrich, St Louis, MO, USA)/0.1 M PBS solution for 10 min at 37°C to retrieve the antigens, followed by an overnight incubation with the primary antibody (1 μg/ml) at 4°C. After washing, sections were incubated with peroxidase-conjugated anti-rabbit IgG Fab' (1:100 dilution; DAKO, Glostrup, Denmark) for 1 h at 37°C. Finally, the immune reaction was developed with diaminobenzidine/H_2O_2 solution. The immunostaining procedure of type II collagen was performed using a standard technique (Histofine mouse stain kit; Nichirei Co., Tokyo, Japan) to block intrinsic mouse immunoglobulin activity. The sections were incubated in methanol containing 0.3% H_2O_2 for 30 min to block intrinsic peroxidase activity and then treated with 0.04% proteinase K (Sigma-Aldrich)/0.1 M PBS solution for 10 min at 37°C. After washing, sections were treated with Histofine blocking reagent A for 60 min at 37°C, followed by an overnight incubation with the primary antibody (1 μg/ml) at 37°C. After washing, sections were treated with Histofine blocking reagent B for 10 min at 37°C. After washing, the sections were incubated with Histofine simple stain mouse MAX-PO (Nichirei Co., Tokyo, Japan) for 60 min at 37°C. Finally, color was developed with diaminobenzidine/H_2O_2 solution.

Histological grading score for assessment of OA

Sections were evaluated blindly by three independent investigators using the histological grading score of Mankin[26], modified as described previously[27]. The total score ranges from 0 to 14 and includes scores from four categories: cartilage anatomy, cellular abnormality, matrix staining, and tidemark integrity. Cartilage anatomy was graded from 0 (normal tissue) to 6 (cartilaginous tissue with complete loss of cellular organization, clusters of cells, and osteoclastic activity). Cellular abnormality was graded from 0 (normal tissue) to 3 (hypercellularity). Matrix staining (with safranin-O) was graded from 0 (normal tissue or slightly decreased staining) to 4 (unstained). Tidemark integrity was graded from 0 (intact) to 1 (destruction). Based on the sum of the scores, each

section was ranked as one of four histological grades: normal, 0–2; mild, 3–6; moderate, 7–10; or severe, 11–14.

Histological grading score for assessment of cartilage repair

Sections were evaluated blindly by three independent investigators using the modified WAKITANI score[28]. In the modified WAKITANI score, safranin-O staining is used instead of toluidine blue. The WAKITANI score ranges from 0 to 14 and includes scores from five categories[29]: cell morphology, matrix staining, surface regularity, cartilage thickness, and integration of donor cartilage. Cell morphology was graded from 0 (for tissue that was normal, compared with the adjacent, uninjured cartilage) to 4 points (absence of cartilaginous tissue). Matrix staining, or the degree of metachromatic staining with safranin-O, was graded from 0 (for tissue that was normal, compared with the adjacent, uninjured cartilage) to 3 points (no metachromatic staining). Surface regularity, or the proportion of the surface of the defects that appears smooth compared with the entire surface, was graded from 0 (when more than three quarters of the surface was smooth) to 3 points (when less than one quarter was smooth). Cartilage thickness, that is, the average thickness of the cartilage in the defects compared with that of the surrounding cartilage, was graded from 0 (when the average thickness of the cartilage in the defects was more than two thirds that of the surrounding cartilage) to 2 points (when the average thickness was less than one third that of the surrounding cartilage). Integration of donor cartilage with the host adjacent cartilage was graded from 0 (no gap between donor and host cartilage) to 2 points (a complete lack of integration, referred to as dissociation).

Statistical analysis

The nonparametric Mann–Whitney *U*-test (Statview 5.0, Cary, NC) was used to compare histological grading scores of TNKO and WT mice at each period. A *P*-value <0.05 was considered significant.

Results

Gross and histological assessment of TNKO mice

Analysis of gross appearance and radiographs revealed no significant differences between WT and TNKO mice at either 8 or 24 weeks of age. Body weight at 8 and 24 weeks did not differ between WT and TNKO mice (26.7 ± 3.4 g vs 26.2 ± 2.7 g, respectively, at 8 weeks; $P = 0.773$, and 33.4 ± 2.3 g vs 33.8 ± 1.5 g, respectively, at 24 weeks; $P = 0.564$). The tail length at 8 and 24 weeks did not differ between groups (9.6 ± 0.5 cm vs 9.7 ± 0.6 cm, respectively, at 8 weeks; $P = 0.884$, and 10.6 ± 0.2 cm vs 10.4 ± 0.5 cm, respectively, at 24 weeks; $P = 0.885$) in WT and TNKO mice. In addition, all tissues examined were histologically normal (Fig. 1), and histological analysis of a variety of tissues revealed no differences between WT and TNKO mice. All sections of knee in both WT and TNKO mice scored 0 points in terms of histological grading using the modified WAKITANI and Mankin score.

Aging-dependent development of OA

In WT and TNKO mice, the articular cartilage of all mice was almost normal at 2, 4, and 6 months [Fig. 2(a) A–I, M–R]. However, at the age of 12 months, TNKO mice showed strong degeneration that sometimes included large cartilage defects and fibrillation [Fig. 2(a) S, T]. In contrast, WT mice showed slight degeneration, irregular cartilage surface, and slightly decreased safranin-O staining [Fig. 2(a) J, K]. TN-C immunostaining was found in the thin layer at the surface of cartilage [Fig. 2(a), L] only at the age of 12 months.

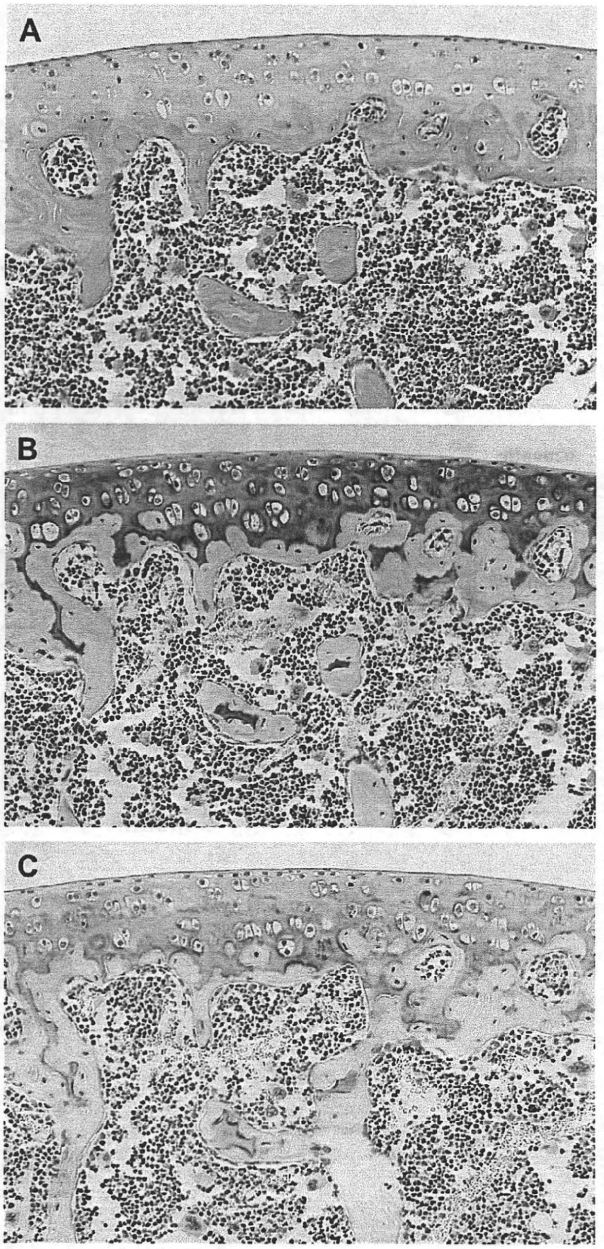

Fig. 1. Histological appearance of cartilage tissue in TNKO mice at 24 weeks (original magnification ×100). Cartilage tissue in TNKO mice was not histologically different from that in WT mice. A: hematoxylin and eosin stain; B: safranin-O stain; C: type II collagen immunostaining.

Progression of OA in WT and TNKO surgical OA model mice

In WT and TNKO mice, articular cartilage was almost normal, although some cartilage showed irregular surface areas and cell clusters at 1 week [Fig. 3(A, B, H, I)]. There were no differences between 1 week and 2 weeks in WT mice [Fig. 3(C, D)], but safranin-O decreased staining in TNKO mice at 2 weeks [Fig. 3(J, K)]. At 4 weeks, safranin-O decreased staining in WT mice [Fig. 3(E–G)],

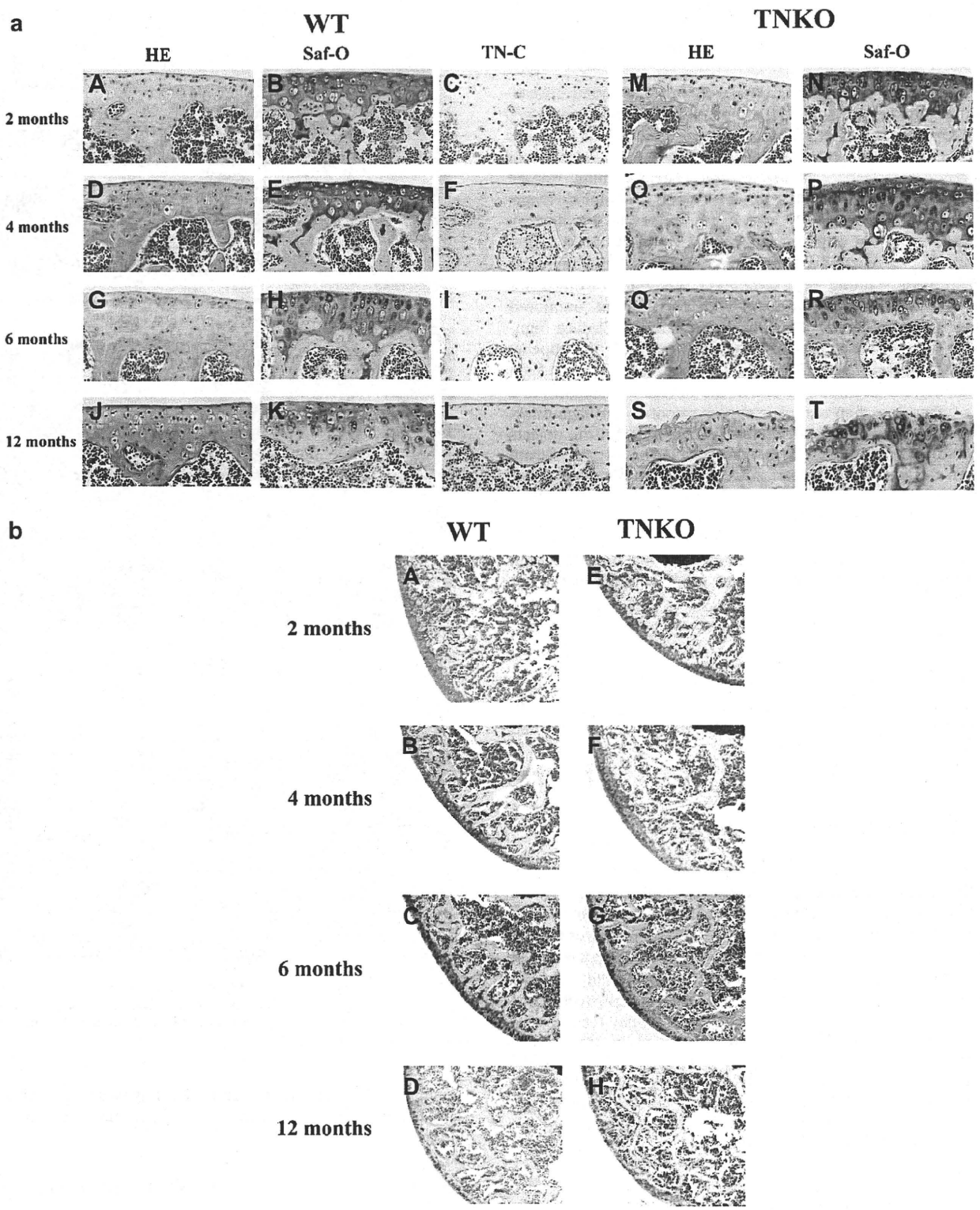

Fig. 2 (a). Histological appearance of cartilage tissues in WT mice at the age of 2 months (A–C), 4 months (D–F), 6 months (G–I), and 12 months (J–L), and in TNKO mice at the age of 2 months (M, N), 4 months (O, P), 6 months (Q, R), and 12 months (S, T) (A, D, G, J, M, O, Q, S: hematoxylin and eosin stain; B, E, H, K, N, P, R, T: safranin-O stain; C, F, I, L: TN-C immunostaining). Original magnification ×100 for all stains.The cartilage of all mice was almost completely degenerated until the age of 4 months, and both groups showed degenerated cartilage at the age of 6 months. However, the degeneration of cartilage in TNKO mice was more conspicuous than that in WT mice at the age of 12 months. (**b**) Histological appearance of cartilage tissues of the medial chondyle in WT mice at the age of 2 months (A), 4 months (B), 6 months (C), and 12 months (D), and in TNKO mice at the age of 2 months (E), 4 months (F), 6 months (G), and 12 months (H) (A–H; safranin-O stain). Original magnification ×40.

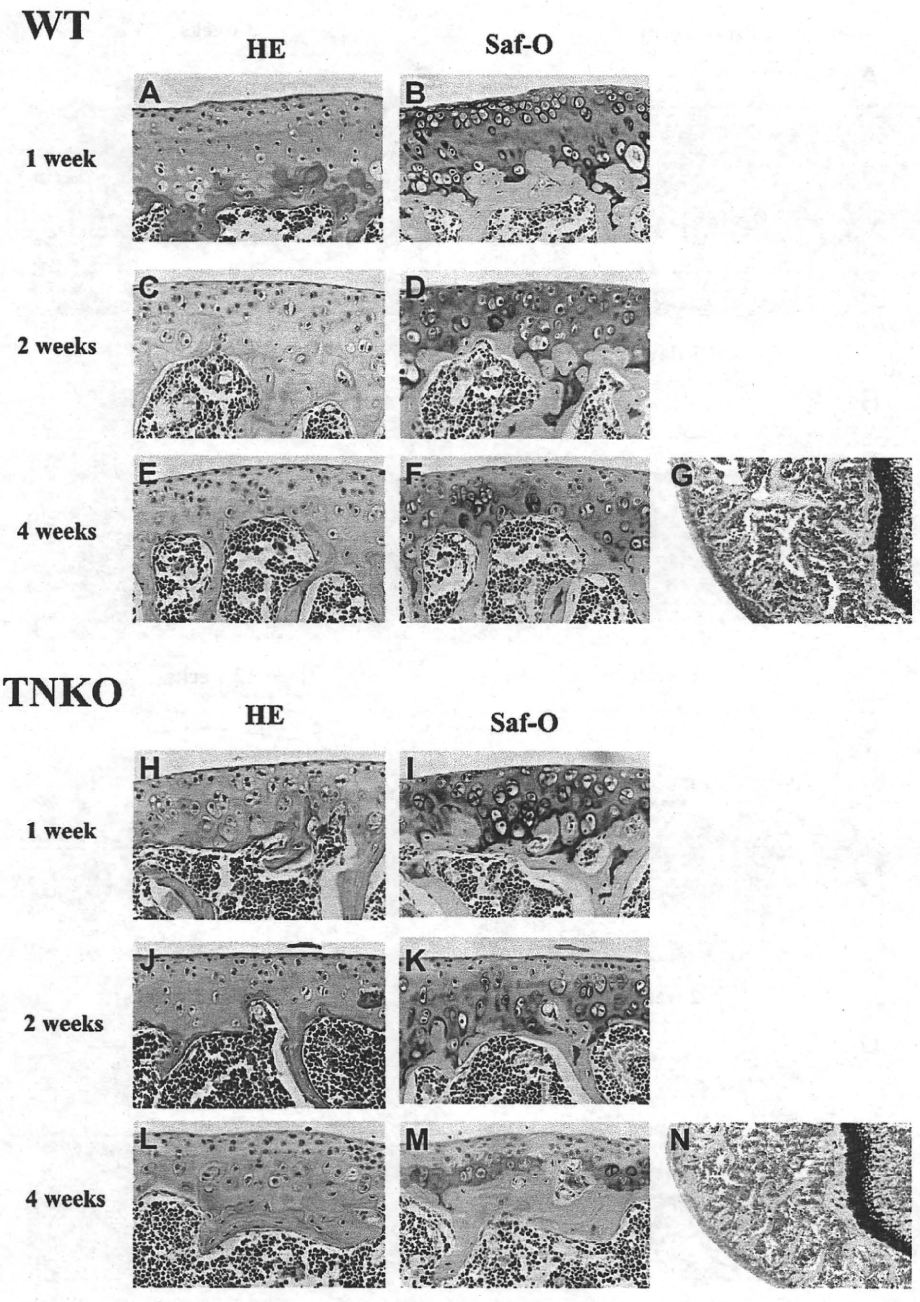

Fig. 3. Histological appearance of cartilage tissues in WT mice at 1 week (A, B), 2 weeks (C, D), and 4 weeks (E–G) after surgery, and in TNKO mice at 1 week (H, I), 2 weeks (J, K), and 4 weeks (L–N) after surgery (A, C, E, H, J, L: hematoxylin and eosin stain; B, D, F, G, I, K, M, N: safranin-O stain). A–F, H–M: hematoxylin and eosin and safranin-O stain; original magnification ×100; G, N: original magnification ×40. The cartilage of all mice was slightly degenerated 1 week after surgery. The degeneration in TNKO mice was more conspicuous than that in WT mice at 2 and 4 weeks after surgery.

but cartilage was injured intensely and cartilage degeneration progressed in TNKO mice [Fig. 3(L–N)].

Cartilage repair process in WT and TNKO mice

In WT mice, the deeper halves of the defects were filled with blood clots without TN-C expression after 1 day [Fig. 4(B)]. After 1 week, cartilage regeneration started [Fig. 5(A, B)]. TN-C immunostaining was observed at the base of the defects and the trabecular surfaces [Fig. 4(C)]. After 2 weeks, the defects were completely filled with fibrous tissue. Safranin-O staining was found in a limited part of the regenerated tissue [Fig. 5(C, D)]. In addition, TN-C expression was seen at the edge of the defects, and was positive in immature chondrocytes but not in mature chondrocytes stained by safranin-O [Fig. 4(D) and Fig. 5(D)]. 3 weeks later, the defects were filled with regenerated cartilage-like tissue resembling hyaline cartilage. The cells seemed to be well-differentiated chondrocytes and were surrounded by metachromatic matrix. However, the margins of the regenerated cartilage-like tissue were not completely integrated with adjacent host cartilage, and the repaired cartilage-like tissue was thicker than normal cartilage [Fig. 5(E, F)]. TN-C expression was observed in the upper layer of the

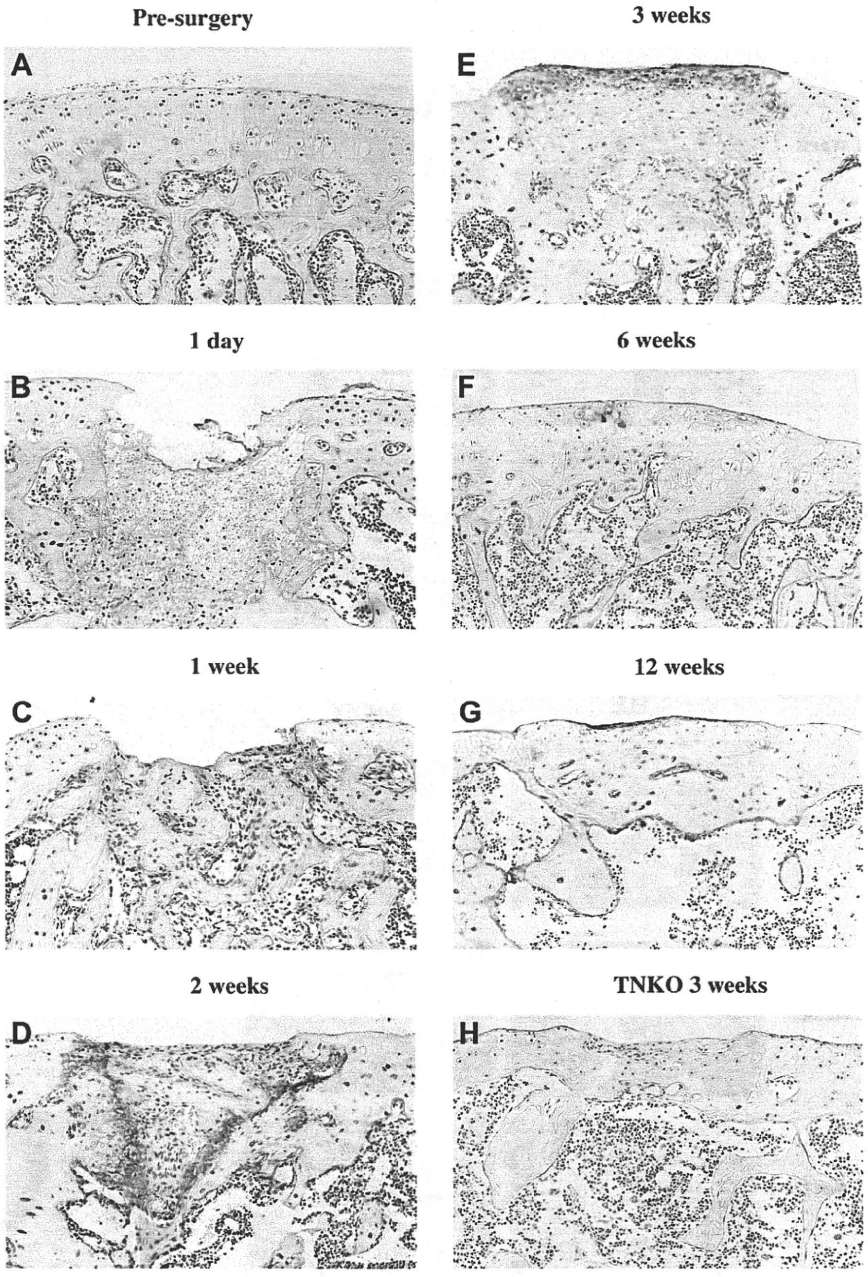

Fig. 4. TN-C immunostaining of cartilage tissues in WT mice at pre-surgery (A), 1 day (B), 1 week (C), 2 weeks (D), 3 weeks (E), 6 weeks (F), and 12 weeks (G) and in TNKO mice at 3 weeks (H) (original magnification ×100). In WT mice, TN-C expression was found from 1 week after surgery to 3 weeks after surgery, and disappeared after 6 weeks. However, TN-C reappeared after 12 weeks. In TNKO mice, TN-C expression was not found.

cartilage-like tissue, but not in the mature cartilage after 2 weeks [Fig. 4(E)]. After 6 weeks, the thickness of the regenerated cartilage tissue was reduced to the same size as the adjacent normal cartilage [Fig. 5(G, H)] and TN-C expression disappeared [Fig. 4(F)]. After 12 weeks, the appearance of the cartilage was comparable with that of hyaline cartilage, although the regenerated cartilage-like tissue was eroded and metachromatic staining was abnormally faint or absent in some areas [Fig. 5(I, J)]. TN-C reappeared in the thin layer at the surface of the regenerated cartilage [Fig. 4(G)]

The cartilage repair process in TNKO mice was different from that in WT mice. After 1 day and 1 week, no difference was found in cartilage repair between WT and TNKO mice [Fig. 5(K, L)]. After 3 and 6 weeks, the defects were completely organized by fibrous tissue; however, safranin-O staining and regenerated tissue that resembled hyaline cartilage were not observed. The surface was also irregular [Fig. 5(O–R)]. After 12 weeks, the defects were not filled with the regenerated cartilage-like tissue [Fig. 5(S, T)].

Comparison of histological grading scores in WT and TNKO mice

Figure 6 shows the histological grading scores between groups. In the spontaneous OA in aged joints model, significant differences were only seen at the age of 12 months. At the age of 2 months, the score was 0.333 ± 0.52 points in both WT and TNKO mice

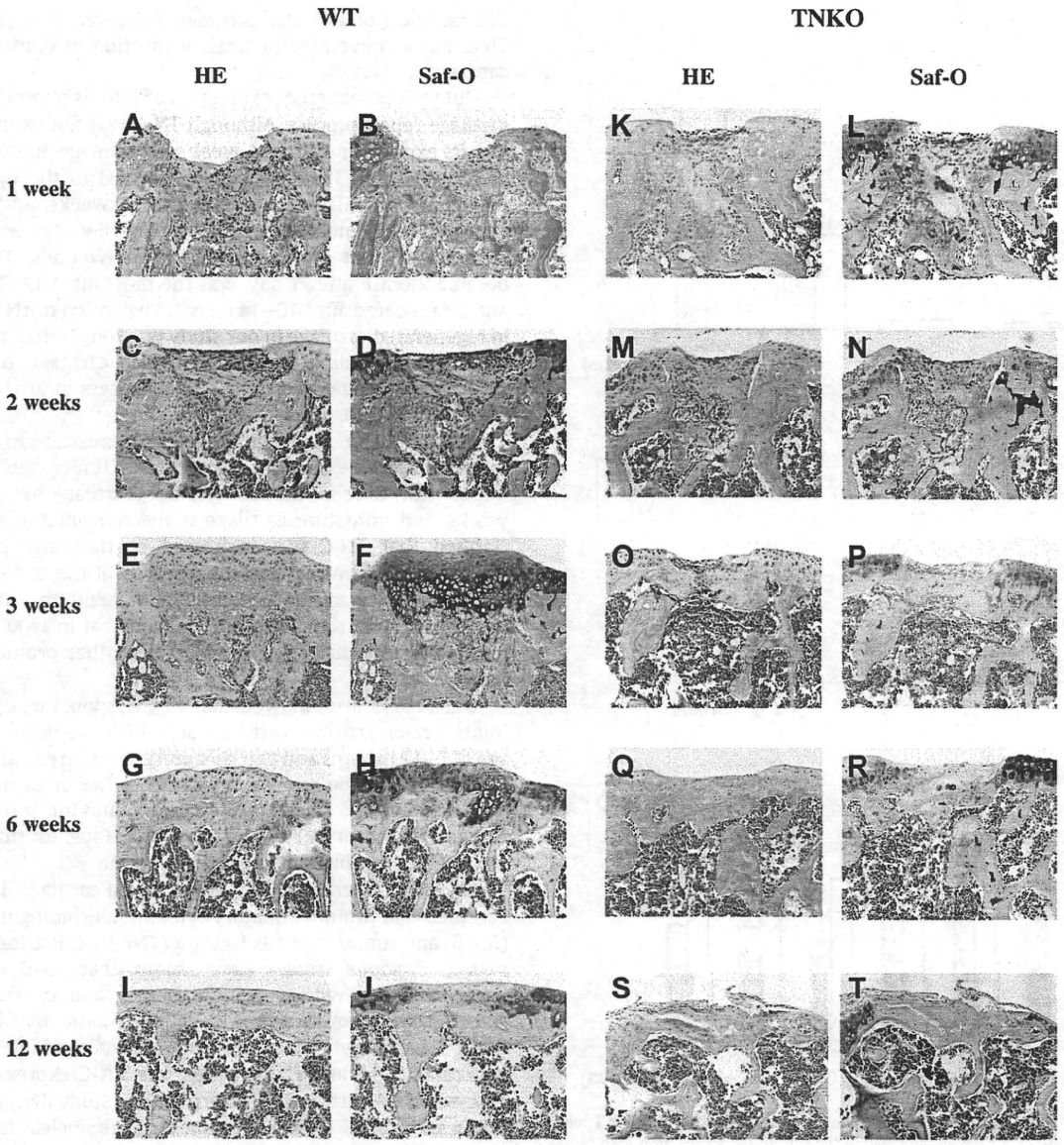

Fig. 5. Histological appearance of articular cartilage tissues in WT mice at 1 week (A, B), 2 weeks (C, D), 3 weeks (E, F), 6 weeks (G, H), and 12 weeks (I, J) after surgery, and in TNKO mice at 1 week (K, L), 2 weeks (M, N), 3 weeks (O, P), 6 weeks (Q, R), and 12 weeks (S, T) after surgery (A, C, E, G, I, K, M, O, Q, S: hematoxylin and eosin stain; B, D, F, H, J, L, N, P, R, T: safranin-O stain). Original magnification ×100 for all stains. Hematoxylin and eosin or safranin-O stain in TNKO mice was not different from that in WT mice. In WT mice, the defects were restored by cartilage-like tissue until 6 weeks after surgery. In TNKO mice, the defects were filled with fibrous tissues without cartilage-like tissue. The deficiency of TN-C appeared to delay articular cartilage repair in mice.

($P > 0.999$). At the age of 4 months, the score was 0.500 ± 0.84 points in WT mice and 0.500 ± 0.55 points in TNKO mice ($P = 0.784$). At the age of 6 months, the score was 4.167 ± 1.33 points in WT mice and 5.333 ± 2.07 points in TNKO mice ($P = 0.276$). At the age of 12 months, the score was 5.833 ± 1.17 points in WT mice and 9.833 ± 2.56 points in TNKO mice ($P = 0.007$) [Fig. 6(A)].

In the surgical OA model, 1 week after surgery, the score was 1.750 ± 0.44 points in WT mice and 2.250 ± 0.50 points in TNKO mice ($P = 0.312$). 2 weeks later, the score was 2.500 ± 1.23 points in WT mice and 4.500 ± 1.52 points in TNKO mice ($P = 0.037$). 4 weeks later, the score was 2.750 ± 1.04 points in WT mice and 6.117 ± 1.94 points in TNKO mice ($P = 0.038$) [Fig. 6(B)].

In the full-thickness cartilage defects model, 1 day after surgery, the score was 14 points in both WT and TNKO mice. 1 week later, the score was 12.50 ± 1.55 points in WT mice and 12.79 ± 1.02 points in TNKO mice ($P = 0.838$). Two weeks after, the score was 9.47 ± 2.01 points in WT mice and 10.73 ± 1.14 points in TNKO mice ($P = 0.042$). Three weeks after surgery, the score was 6.70 ± 3.42 points in WT mice and 10.87 ± 2.39 points in TNKO mice ($P < 0.001$). Six weeks after surgery, the score was 7.71 ± 3.72 points in WT mice and 9.27 ± 3.01 points in TNKO mice ($P = 0.018$). Twelve weeks after surgery, the score was 7.86 ± 1.77 points in WT mice and 10.27 ± 2.20 points in TNKO mice ($P < 0.001$) [Fig. 6(C)].

Discussion

TN-C is known to regulate multiple cellular functions during tissue remodeling. Despite this function, previous studies have shown that initial examination of TNKO mice revealed no

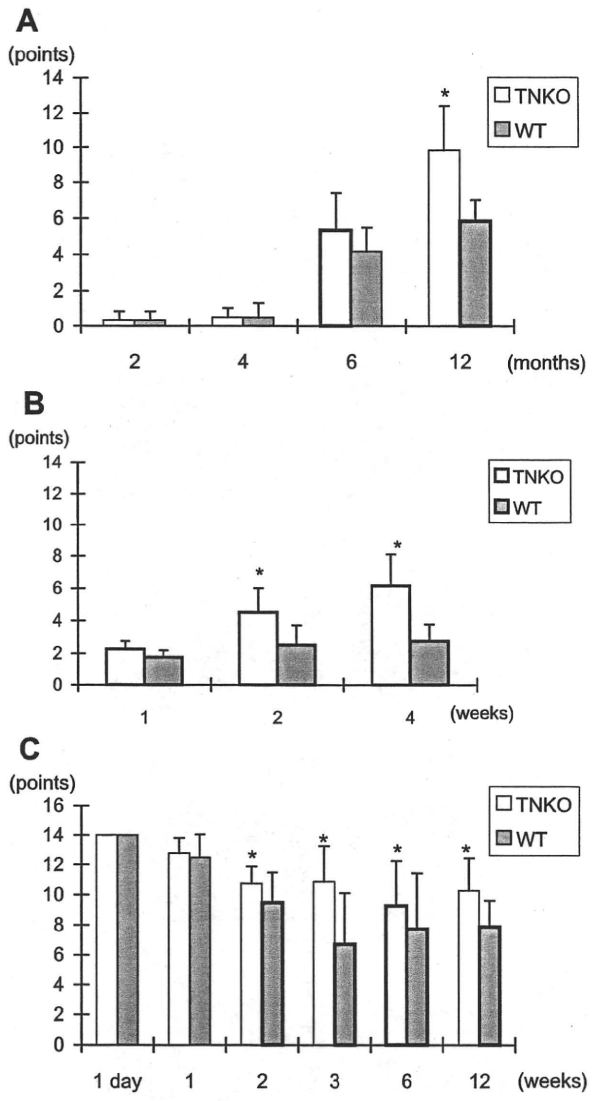

Fig. 6. Histological grading scores for cartilage degeneration and repair of WT mice and TNKO mice are expressed as the mean of the sum of the scores from each histological section through the joints (A: Mankin score[26] in aging model, B: Mankin score[26] in OA model, C: modified Wakitani score[28] for cartilage repair). Error bars indicate standard deviation. $^{*}P < 0.05$.

phenotypic difference in the gross organization or histology of tissues with the highest expression of TN-C[13,14]. In this study, extensive histological evaluation of samples from 12 different tissues, including articular cartilage obtained from our originally produced TNKO mice, revealed no indication of developmental or growth deficiencies in TNKO mice.

Other studies have examined the cartilage of TNKO mice[30,31]. Immunohistological studies have confirmed that in healthy articular cartilage, TN-C staining is barely observed in the superficial and upper-middle zones and is primarily seen in the territorial matrix of the articular chondrocytes[11]. In moderate and severe OA, lesions revealed increased territorial and interterritorial immunostaining, mainly in cartilage areas showing a great reduction of safranin-O staining[9,11,32]. In addition, we previously demonstrated a correlation between levels of TN-C in joint fluids and OA severity as shown on radiographs[33]. Thus, results of previous studies suggest that TN-C plays an important role in the degeneration of articular cartilage. However, it is still unclear if TN-C has a preventive or healing function in damaged articular cartilage.

Our results show that expression of TN-C is observed during the cartilage repair process. Although TN-C was not expressed after 1 day, its expression started 1 week after damage and was increased in 2 weeks. TN-C was strongly expressed in the upper layer of regenerated cartilage-like tissue after 3 weeks, and almost disappeared in regenerated mature cartilage 6 weeks later. Mackie *et. al.* reported that in full-thickness skin wounds, TN-C staining became intense after 1 day, was the most intense after 5–7 days, and disappeared after 10–14 days[34]. The period of TN-C expression in regenerated cartilage in our study was longer than that shown in skin wound healing. Because articular cartilage has a limited capacity for regeneration, the repair process in articular cartilage may be slower than that in the skin.

Athough the cartilage defects were almost completely restored at 6 weeks in WT mice, in TNKO mice, the defects were filled with fibrous tissues without cartilage-like tissue at 3 weeks, and immature cartilage tissue remained at 6 weeks. We clarified that TN-C was expressed at the early phase of the cartilage repair process and disappeared at the end phase in full-thickness articular cartilage defects. In addition, cartilage repair in WT mice was completed earlier than that in TNKO mice. These results suggest that TN-C is the protein that promotes cartilage repair.

Our results also showed that in the spontaneous OA in aged joints model, articular cartilage of TNKO mice degenerated at the age of 6–12 months and that this process occurred earlier than that in WT mice. In the surgical OA model, articular cartilage of TNKO mice degenerated 2 weeks after surgery, and this process occurred earlier than that in WT mice. These results suggest that TN-C is the protein that inhibits cartilage degeneration.

OA is characterized by degeneration of cartilage. It is generally accepted that proinflammatory cytokines, including interleukin-1β (IL-1β) and tumor necrosis factor α (TNF-α), cause loss of cartilage matrix. Previous studies have shown that TN-C expression is upregulated by various growth factors and cytokines such as Transforming growth factor (TGF)β1[35], basic fibroblast growth factor[36], hepatocyte growth factor[37], TNF-α[23], and interleukins[38]. In particular, IL-1β and TNF-α upregulate TN-C expression in chondrocytes of OA cartilage[12,39]. Our previous study demonstrated that TNF-α stimulated TN-C expression through nuclear factor-kappa B (NF-κB) signaling with RelA activation in cultured chondrocytes of OA[32]. Previous studies have suggested that the signaling involved in RelA activation affects cell proliferation[40–42]. TN-C is also known to promote proliferation in various cells[21,31]. These findings suggest that deposited TN-C can promote chondrocyte proliferation through NF-κB signaling in OA cartilage.

TN-C has previously been shown to be associated with the mesenchymal cell condensation that precedes either chondrogenesis or intramembranous osteogenesis, and to stimulate chondrogenic cell differentiation in chick limb mesenchymal cultures[10,30]. TN-C seems to promote the differentiation of chondrocytes from mesenchymal cells.

In conclusion, the present study revealed that TN-C expressed at the early phase of cartilage repair completely disappeared in regenerated mature cartilage, and reappeared in the thin layer at the surface of regenerated cartilage with degeneration. Moreover, the present study revealed that the deficiency of TN-C progresses during cartilage degeneration in the spontaneous OA in aged joints and surgical OA model. We showed that cartilage repair in full-thickness cartilage defects in TNKO mice was delayed compared with WT mice. These results strongly suggest that TN-C promotes chondrogenesis and cartilage repair in damaged and degenerated

cartilage. In terms of the reason that TN-C is expressed strongly in knee OA in human[9,11,31,32], we consider that TN-C is expressed at high levels in conjunction with cartilage remodeling such as occurs with degeneration and repair. Further studies are needed to evaluate the effect of TN-C in cartilage.

Conflict of interest
No benefits or funds were received in support of this study.

Acknowledgment

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Clinical Outcomes of the KYOCERA Physio Hinge Total Knee System Type III After the Resection of a Bone and Soft Tissue Tumor of the Distal Part of the Femur

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Background and Objectives: The KYOCERA Physio Hinge Total Knee System Type III (PHKIII) was developed to reconstruct bony defects of the distal femur. The PHKIII is originative in that the metallic parts are fully made of titanium alloy, and this prosthesis has a unique semi-rotating hinge joint and was designed especially for people with the Asian physical body-type. The clinical outcomes of the PHKIII after the resection of musculoskeletal tumors of the distal femur were evaluated.
Methods: There were 41 males and 28 females with a median age of 48-years. The median duration of follow-up was 57 months.
Results: Eleven early complications and 37 late complications were observed, including 10 recurrences, 7 deep infections, 7 aseptic loosenings, 4 stem breakages, 4 displacements of shaft cap, and one wear of rotation sleeve. Twenty four prosthesis (35%) required a secondary operation because of complications. The five-year overall prosthetic survival rates, -prosthetic survival rate without aseptic loosening, and -limbs preservation rate were 85%, 90%, and 86%, respectively. The mean functional score according to the classification system of the Musculoskeletal Tumor Society was 20.5 points (68%).
Conclusions: Although continuous follow-up is required, reconstructions using PHKIII are considered to achieve more acceptable functional results.
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KEY WORDS: **limb salvage surgery; clinical outcomes; musculoskeletal tumors; distal femur**

INTRODUCTION

Because of advances in imaging, surgical techniques, radiation therapy and adjuvant chemotherapy protocols, there has been a considerable improvement in the prognosis for patients with musculoskeletal sarcoma over the past 25 years [1]. Limb salvage is considered to be the standard procedure for the majority of the patients with a sarcoma involving the extremities [1,2]. The distal femur is a common site for primary and metastatic bone tumors, and therefore, it is a frequent site in which limb salvage surgery is performed.

Various types of prostheses have been developed and applied for the reconstruction of bone defects after tumor resection [3]. The current model of the prostheses include a modular segment, a wrought stem, a kinetic rotating hinge, a circumferential porous coating around the prosthesis at the bone-prosthesis junction, and a loophole for soft tissue attachment. The advantages of the current modular prosthesis include their durability, intraoperative flexibility to fill the bony defects, and the immediate structural stability they provide to permit immediate weight bearing.

However, these prostheses are generally designed for the Caucasian physical body-type, and are frequently too large in size and too heavy in weight for Asian-pacific patients. Mensch and Amstutz measured the linear dimension of predominantly Caucasian cadaver knees and described that the width of the femur, depth of the lateral femoral condyle, width of the tibia, and depth of the medial tibial plateau were 75, 64.6, 74.9, and 48.9 mm, respectively [4]. In contrast, Miyake et al. examined these bones in Japanese cadaver knees and showed that they were 71.5, 60.9, 71.4, and 45.4 mm, respectively [5]. The average size of Japanese knees was found to be 5–10% smaller than that of Caucasians. Therefore, in 1997, the Japanese Muscloskeletal Oncology Group developed a new modular prosthesis, the KYOCERA Physio Hinge Total Knee System Type III (PHK III).

The PHKIII is originative in that the metallic parts of the prosthesis are fully made of titanium alloy, and this prosthesis has a unique semi-rotating hinge joint and was designed especially for people with the Asian physical body-type. The purpose of this study was to evaluate

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the clinical outcome of the treatment with the PHK III after the wide resection of a musculoskeletal tumor of the distal femur in 69 Japanese patients.

PATIENTS AND METHODS

Patients

Between April 1997 and May 2002, 108 patients with bone and soft tissue tumors of the distal femur were treated by surgeons of the JMOG, using the PHK III. The records of 80 of the 108 patients were collected using a questionnaire administered to the members of the JMOG. The collected data included the demographic details, histological diagnosis, tumor location, stage, grade, adjuvant therapy, surgical methods, size of the prosthesis components, complications, post-operative limb function, range of motion of knee joint, and oncological outcomes at the final follow-up. Since the PHK III was used for revision arthroplasty in 11 patients, these revision cases were excluded from the present study. Therefore, the performance of PHK III used for 69 patients was retrospectively reviewed and evaluated. There were 41 males and 28 females who ranged in age from 10 to 79 years (median, 48 years-old). The duration of the follow-up ranged from 6 to 134 months (mean, 57 months). There were 58 primary malignant bone tumors, 4 giant cell tumors, 5 metastatic bone tumors and 2 direct bone invasion of soft tissue sarcomas. The primary malignant bone tumors included 40 osteosarcomas, 8 chondrosarcomas, 8 malignant fibrous histiocytomas, one Ewing's sarcoma and one leiomyosarcoma of the bone. Staging according to Enneking's surgical staging system [6] was as follows: stage IA, 3 patients; stage IB, 2 patients; stage IIA, 5 patients; stage IIB 44 patients; stage IIIA 1 patient; stage IIIB, 10 patients; unknown, 4 patients. Chemotherapy was performed in 47 patients, and irradiation was administered in combination with the chemotherapy in 5 patients. Informed consent was obtained from all patients according to the guidelines of the each institutional ethics review board.

Prosthesis

PHK III is a full modular prosthetic system with a rotating-hinge joint, which was created in order to reconstruct distal femoral bone defects after a tumor resection, and designed for Asian patients, including the Japanese with the smaller anatomical architecture of the knee joint (Fig. 1a). The PHK III has a unique semi-rotating hinge joint which allows a maximal flexion of 142° and an internal/external-rotation of 5° (Fig. 1b and c). The metallic parts of the PHK III are made of light-weight and high-strength titanium alloy (Ti-6Al-4V) with good bio-compatibility and bio-stability and allow scanning by magnetic resonance imaging (MRI). As a result, the PHK III is extremely light in weight. When the PHK III is used for an 11 cm bony defect of the distal femur, the total weight is about 660 g, whereas reconstruction of a 12 cm bony defect using the HMRS reaches a weight of about 1200 g. The metallic surface of the hinge shaft and the rotator, which creates friction between high density polyethylene, is fabricated using a surface-hardening treatment by azote-ionic inpouring to increase the durability of the hinge joint. The rotation sleeve, plate and shaft sleeve are made of ultra high molecular weight polyethylene. The PHKIII requires the use of polymethylmethacrylate cement for the fixation of the femoral stem and tibia component (Fig. 1c).

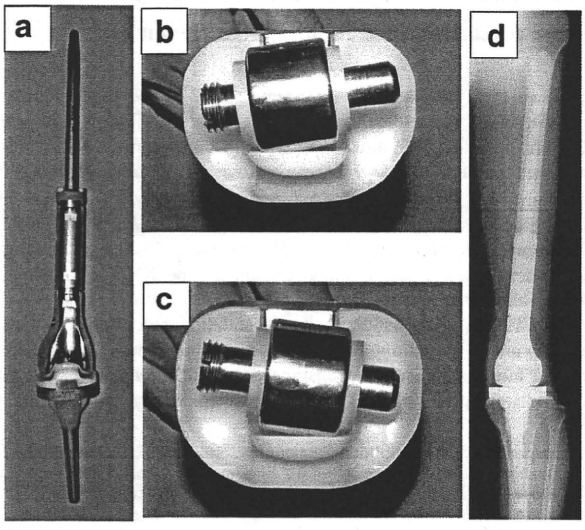

Fig. 1. The photographs showing (a) an overview of the PHK III and a unique semi-rotating hinge joint (b and c) which allows internal/external-rotation of 5°. (d): Radiography showing anterior–posterior view of PHK III used for the osteosarcoma patient.

Surgical Procedure and Postoperative Rehabilitation

All operations were performed under general anesthesia. All surgical interventions were performed by trained surgeons specialized for orthopedic oncology. All surgical resections followed the guidelines of the Japanese Orthopedic Association outlined by Enneking [6,7]. The length of the resected distal femoral bone were 9 cm in 4, 11 cm in 11, 13 cm in 16, 15 cm in 7, 17 cm in 11, 19 cm in 12, 21 cm in 7, and 23 cm in 1. The following surgical margins were provided: a wide margin in 60 patients, a marginal margin in 4, an intralesional margin in one and the margin was unknown in 4. Extra-capsular resections were performed in 16 patients, whereas intra-capsular resections were performed in 50. More than 2 segments of musculus quadriceps femoris were resected in 67 patients. The musculus quadriceps femoris was not totally resected in any of the patients. The prostheses were implanted using modern cementation techniques. A local musculocutaneous flap was required in 3 patients, and a free vascularized musculocutaneous flap was required in one patient to cover a defect of the skin after the resection of the tumor. All patients received intravenous antibiotics preoperatively and postoperatively. Deep drains were used routinely and antibiotics were given while the drains were in place. All patients were kept at bed rest, and immobilized with the extremity in 30° of flexion in a bulky dressing for the first 24 hr. Thereafter, the patients were started on a regimen of gentle passive range of motion (ROM) and isometric exercises such as straight leg raisings. The use of a machine which administered continuous passive motion and a knee brace were used, depending on the institution. Full weight bearing was permitted one week after the surgery.

Assessment and Statistical Analysis

The complications, overall prosthetic survival rate, prosthetic survival rate without aseptic loosening, limbs preservation rate and functional outcome were evaluated in the 69 patients.

The overall prosthetic survival was defined as the time from the surgical reconstruction using the PHK III to the date of revision or amputation due to prosthetic failure. Prosthetic failure was defined as replacement of the any of the prosthetic components including minor parts of the prosthesis or complete removal of the implant. Patients in whom the implant was removed for a local recurrence or deep infection were not considered prosthetic failures in the prosthetic survival analysis. The prosthetic survival rate without aseptic loosening was defined as the time from the surgical reconstruction to the date of aseptic loosening assessed by radiographic examination. The limb preservation rate was defined as the time from the surgical reconstruction to the date of amputation due to complications. When analyzing the survival rate, the death of the patients without any prosthesis-related event was

conducted as censoring at the date of patient's death. The relationships between various characteristics and prosthetic survival were assessed by a univariate analysis (Log-rank test). A P-value of <0.05 was considered to be significant. Analyses were performed using the StatView statistical software program (version 5.0; SAS Institute Inc. Cary, North Carolina).

The functional assessments were performed according to the scoring system of musculoskeletal tumor society (MSTS) [8].

RESULTS

Complications

Out of the 69 prosthetic surgical treatments, 11 early complications (16%) were found (Table I). Of the 7 patients with skin necrosis, 5 patients underwent minor surgery under local anesthesia and 2 patients with a relatively wide skin defect underwent a free vascularized flap. The peroneal nerve palsy improved within 3 months in all patients.

Thirty-seven late complications were found. Of the 10 patients with local recurrence, 8 patients required a wide resection of the recurrent tumor, and finally 4 patients underwent amputation of the affected limbs. Out of the 7 patients who had a deep infection, 4 patients required surgical debridement and partial exchange of the components, and 3 patients underwent an amputation of the affected limbs. Of the 7 patients with aseptic loosening of the femoral stem, 4 patients required revision surgery. Because the 4 of 15 femoral stems with a 10 mm diameter had broken, the stems were exchanged to those with an 11 m diameter during the revision surgery. Four patients with displacement of the shaft caps underwent an exchange to a new shaft cap. Three patients with a patellar tracking abnormality were treated conservatively using a brace. A patient with an undisplaced avulsion fracture of the tibia tuberculum was treated conservatively using a cast. A patient with wear of rotation sleeve was treated by the exchange of rotation sleeve.

TABLE I. Details of Complications after Reconstruction of Defect in the Distal Femur Using the PHKIII

Complication	No. of complication (%)	No. of re-operation (%)	No. of amputation (%)
Early complication			
Skin necrosis	7 (10%)	7 (10%)	0
Peroneal nerve palsy	4 (6%)	0	0
Total	11(16%)	7 (10%)	0
Late complication			
Recurrence	10 (14%)	8 (12%)	4 (6%)
Deep infection	7 (10%)	4 (6%)	3 (4%)
Aseptic loosening	7 (10%)	4 (6%)	0
Stem breakage	4 (6%)	4 (6%)	0
Displacement of shaft cap	4 (6%)	3 (4%)	0
Patellar tracking abnormality	3 (4%)	0	0
Fracture	1 (1%)	0	0
Wear of rotation sleeve	1 (1%)	1 (1%)	0
Total	37(54%)	24 (35%)	7(10%)

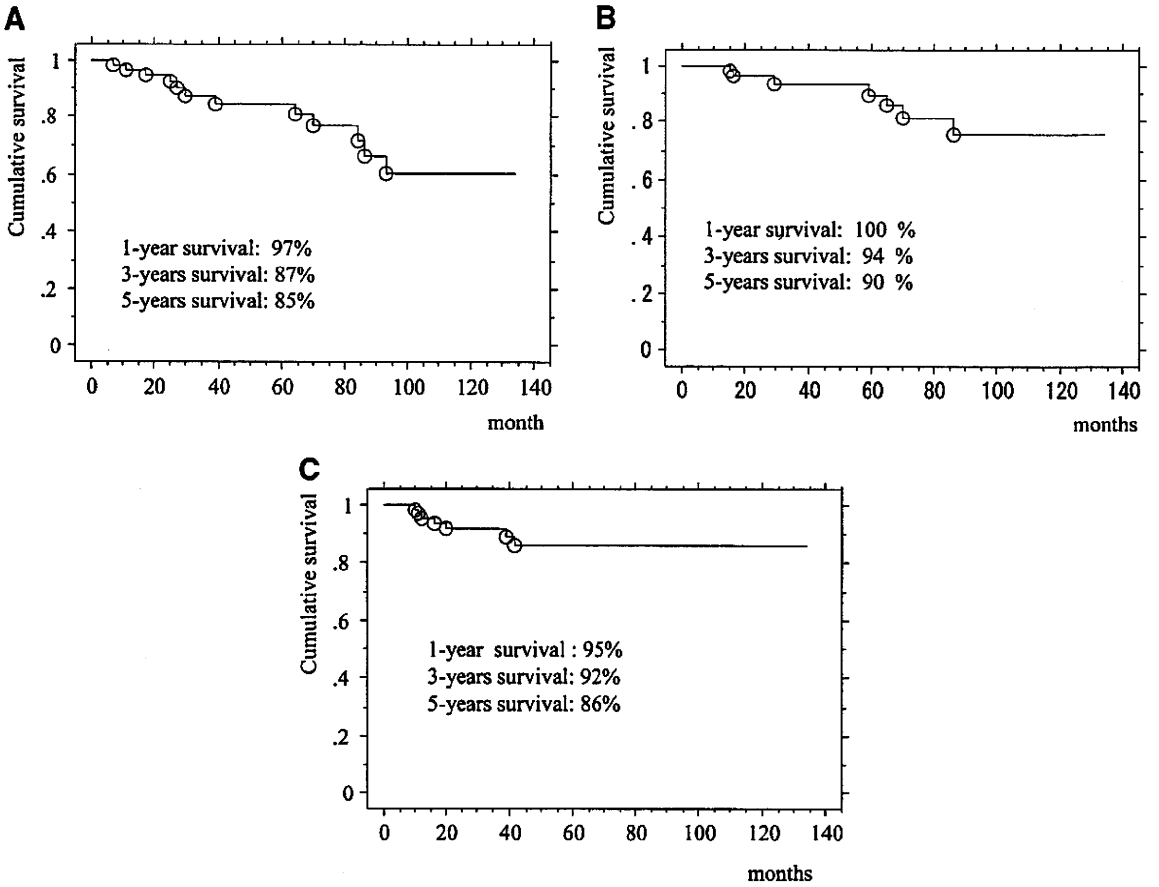

Fig. 2. Kaplan–Meier curve showing (a) the overall prosthetic survival of 69 prostheses, (b) the prosthetic survival without aseptic loosening after reconstruction using the PHK III, and (c) overall limb preservation rate.

Overall Prosthetic Survival Rate and the Factors which Affect the Prosthetic Survival

There were 22 prosthetic surgeries which required additional surgery. Excluding failure because of local tumor recurrence or deep infection, there were 12 prosthetic failures which required revision surgery. The reasons for prosthetic failures included 4 stem breakages, 4 aseptic loosening, 3 displacements of the shaft cap, and one wear of rotation sleeve (Table I). Based on Kaplan–Meier estimates, the 1-, 3-, and 5-years overall prosthetic survival rates were 97%, 87%, and 85%, respectively (Fig. 2a). When the factors which affect the overall prosthetic survivals were investigated, a univariate analysis showed better prosthetic survival in female gender ($P = 0.012$) (Table II).

Prosthetic Survival Rate Without Aseptic Loosening and Overall Limbs Preservation Rate

The prosthetic survival without aseptic loosening and the overall limb preservation rate were examined. 7 cases of aseptic loosening were observed between 15 months and 46 months postoperatively after prosthetic reconstruction. Thus, the 1-, 3-, 5-years prosthetic survival rates without aseptic loosening were 100%, 94%, and 90%, respectively (Fig. 2b). A total of 7 patients underwent a subsequent amputation (Table I). The time to amputation varied from 10 months to 42 months, with a mean of 21.4 months. Therefore, the 1-, 3-, 5-year overall limb preservation rate were 95%, 92%, and 86% (Fig. 2c).

TABLE II. The Relationships Between Various Factors and Prosthetic Survival

	No.of patients	3-yrs survival	5-yrs survival	*P*-value*
Gender				
Male	28	78	78	0.012†
Female	41	95	89	
Age				
<50	39	90	86	0.77
≧50	30	82	82	
BMI				
<25	56	88	84	0.99
≧25	9	83	83	
Femur resection				
<40	32	89	89	0.81
≧40	23	86	86	
Capusular resection				
Intracapsular	50	86	82	0.57
Extracapsular	16	92	92	
Irradiation				
Yes	5	100	—	Incomputable
No	64	87	84	
Chemotherapy				
Yes	47	89	85	0.94
No	22	81	81	
Diameter of stem				
10 mm	15	93	84	0.98
Except for 10 mm	53	85	85	
Length of stem				
130 mm	55	86	86	0.26
170 mm	13	92	77	
Replacement of patella				
Yes	28	90	90	0.74
No	41	86	81	

*Log-rank test.
†$P < 0.05$ significant.

TABLE III. Results of the Limb Function Evaluated with the MSTS Scoring System

	MSTS score						
Factor	5	4	3	2	1	0	Mean
Pain	44	10	9	1	1	1	4.4
Function	10	9	26	5	12	4	2.8
Acceptance	16	14	18	10	7	1	3.3
Supports	22	6	16	1	17	4	3.0
Walking ability	24	14	21	1	3	3	3.7
Gait	18	13	18	4	10	3	3.2
						Total	20.5 (68%)

Functional Outcome and the Factors Which Affect the Limb Function

At the most recent follow-up, the mean range of passive motion of the knee joint was: extension −0.6° (±2.6), flexion 90° (±26). In 6 patients, an extension lag was observed with a mean lag of 15° (±26°). At the latest follow-up examination, the functional score according to the classification system of the MSTS ranged from 3 to 30 points. The mean was 20.5 points, which represents function that is 68% of normal. When this was evaluated in detail, the score of pain seemed to show a relatively high score and function showed a relatively low score (Table III).

DISCUSSION

One of the unique properties of the PHKIII is that the metallic parts of the PHKIII are fully made of light-weight and high-strength titanium alloy (Ti-6Al-4V). Here has been, and is still, concern about the high elastic modules of the metallic alloys as compared to bone, and the variable fatigue resistance of the prosthesis, because both properties may eventually lead to prosthesis failure through loosening or breakage. Long and Rack indicated that the titanium alloy, Ti-6Al-4V, has acceptable strength levels as defined by either the percent elongation or the percent reduction of area in a standard tensile test [9]. The smooth fatigue strength of the titanium-alloy is as high as that of CoCrMO. The problems related to implant stiffness-related stress shielding of bone have resulted from the high elastic modulus of alloys. However, titanium alloy has a lower elastic modulus value closer to bone, approximately half that of CoCrMo alloy [9]. In addition, the titanium alloy has superior biocompatibility and corrosion resistance [9]. Furthermore, the titanium alloy allows scanning by MR imaging, which is an important diagnostic modality that can be used to detect the local recurrences of the tumor. Therefore, we considered that the titanium alloy is generally preferable to a cobalt-based alloy for the tumor prosthesis, and developed the PHK III.

Failure analysis allows the investigator to determine the causes of poor outcome and potentially to improve future outcomes. Out of the 37 late complications, local tumor recurrences were observed in 10 of the 69 patients (14%). This local recurrence rate seems to be slightly high compared to that of other previous reports [10–27]. The histological diagnoses of the 10 recurrent cases were osteosarcoma in 6, MFH of bone in one, high-grade soft tissue sarcoma in 2 and chondrosarcoma in one. Four of the 10 recurrent cases were in non-osteosarcoma patients. MFH of bone and soft tissue sarcomas generally exhibit poor prognosis with a high local recurrence rate, in comparison to osteosarcoma [1]. Therefore, these types of histological diagnoses seem to exert an influence on the high recurrence rate in the present study. In all of the 10 patients who had local recurrences, MRI was a useful diagnostic modality although minor distortions and halation of images were visible. Thus, the PHK III which is made of titanium alloy is considered to have a great advantage for the detection of local tumor recurrence.

TABLE IV. Summary of the Clinical Results of Prosthetic Reconstruction of the Distal Femur after Resection of a Muskuloslkeletal Tumor

			Prosthetic survival rate‡				Limb preservation rate‡			Complications							
Author	Prosthesis* (no.)	Hinge type†	1-yr	3- yrs	5- yrs	10- yrs	1- yr	3- yrs	5- yrs	Loosen-ing	Breakage of femoral component	Infect-ion	Recur-rence	Hinge trouble	% of amputation	Functional outcome (MSTS)	Follow-up (months)
Robert[21]	Stanmore(133)	F	93	87	72	—	99	90	89	6.0%	2%	7%	8%	—	10%	—	34
Capanna[20]	KMFTR/HMRS (95)	F	—	—	—	—	—	—	—	0%	6%	12%	5%	30%	6%	—	51
Unwin[23]	Stanmore(218)	F	—	91‡	82‡	68	—	—	—	5.0%	3%	2%	4%	—	—	—	58
Torbert [19]	Not clearly shown(57)	—	97‡	90	84	66	—	—	—	—	—	—	—	—	5%	—	56
Muschler[16]	Custom(37)	Semi-c	89	73‡	57	—	—	—	—	16.0%	3%	3%	14%	—	5%	—	49
Unwin[22]	Stanmore(493)	F	—	—	—	—	—	—	—	46.0%	10%	8%	30%	—	7%	—	46
Biau[25]	GUEPAR (56)	F	—	—	85	55	—	—	—	17.9%		7%	11%	—	—	—	62
Kawai[14]	HSS(40)	Semi-c	91‡	85	67	48	—	93	90	40.0%	3%	10%	—	5%	8%	80%	96
Ham[17]	Spherocentric(3) Endo (12)	Semi-c(3) R(12)	93‡	87‡	87‡	80‡	—	—	—	—	—	7%	13%	47%	13%	—	61
Kawai[13]	Finn(25)	R	91‡	88‡	88	—	—	—	—	—	—	—	—	—	—	—	—
Kawai[12]	HSS(51), Finn(31)	HSS: Semi-c, Finn: R	—	82	71	50	—	94	92	21.9%	1%	6%	4%	5%	9%	—	HSS:84 Finn: 38
Bickels[15]	Modular Prosthesis(73) Custom(27) Expandable(10)	R	—	—	93	88	—	—	96	5.4%	—	5%	5%	—	4%	—	—
Heisel [11]	MUTAS(50)	Semi-c	—	—	—	—	—	—	—	22.0%	0%	12%	6%	10%	—	72	46
Griffin [24]	KMFTR/HMRS(74)	F	96‡	84‡	77‡	—	97‡	92‡	92‡	2.0%	6%	10%	5%	—	—	—	73
Sharma [18]	KMFTR/HMRS(77)	R		—	84	79				0%	0%	8%	7%	4%	7%	56	52
Present series	PHKIII(69)	Semi-R	97	87	85	—	95	92	86	10%	6%	10%	14%	6%	10%	68%	57

*Stanmore, Stanmore prosthesis; KMFTR/HMRS, Kotz Modular Femur-Tibia Reconstruction System/Howmedica Modular Reconstruction System; GUEPAR, GUEPAR prosthesis; HSS, Hospital for Special Surgery-modular-linked system; Finn, Finn prosthesis; Spherocentric, spherocentric type of semiconstrained knee endoprosthesis(Howmedica); Endo, axial rotating Endo knee (Waldemar Link); PHKIII, KYOCERA Physio Hinge Total Knee System Type III.

†F, fixed hinge; Semi-c; semi-constrained hinge; R, rotating hinge; Semi-R, semi-rotating hinge.

‡Numerical character determined based on the Kaplan–Meier curve indicated in the figure.

Prosthetic replacement following excision of a bone tumor can be complicated by infection because patients often are subjected to extensive soft-tissue dissection and long surgical times and are immune-suppressed [24]. Prosthesis-associated infection rates are reported to be 2–12% [11,12,14–18,20–26] (Table IV). In the present series, a deep infection was observed in 7 patients (10%). Although the majority of patients were immunosuppressed at the time of surgery and for prolonged periods afterwards, this relative high rate of infection is still hardly acceptable. This appeared to be due to, in large part, the thinness of the soft tissue covering the prosthesis. The use of muscle rotation flaps or a vascularized musculocutaneous flap should be considered when only the rectus femoris muscle have been preserved [12].

Radiographic aseptic loosening of the prosthesis is the most common reason for prosthesis revision [11,12,14,22]. The rotating hinge mechanism theoretically provides a more congruent articulation, leading to a decrease in wear and transmitted interfacial stresses to the femoral bone. Thus, special attention was paid to determine the prosthetic survival rate without aseptic loosening. In the present study, seven prostheses (10%) were identified as radiographically loose, and four of the seven loose prostheses required revision surgery. Thus, the 1-, 3-, 5-years prosthetic survival rates without aseptic loosening were 100%, 94%, and 90%, respectively. Previous reports indicated that the incidence of aseptic loosening varied from 0 to 46% [4,12,14–16,18,20–23,25]. A recent large retrospective study demonstrated that the overall risk of revision for any reason fell by 52% when the rotating hinge implant was used [27]. At the present time, definitive advantage on aseptic loosening in PHKIII is not observed. However, a further long-term follow-up study is needed before any definitive conclusions can be drawn.

There were 4 incidents of breakage of the femoral stem. Breakages of the femoral stem could be attributed to the fact that all of the 4 broken stems were small sized stems (10 mm diameter). Four of 15 stems with a 10 mm diameter had broken at the base. Some reports also described an association between the increased risk of stem breakage and the smaller stem size [20,24]. After excluding the 10 mm stem component from the PHKIII system in May 2001, no breakage of the femoral stem was observed. But, further careful follow-up is needed for the remaining 11 prostheses with 10 mm stem.

There were 4 displacements of the shaft caps. Because they were apparently due to material fragility of the shaft cap, a more robust shaft cap was developed in September 1998. After reinforcement of the shaft cap, there have been no displacements of the shaft caps. Wear of rotation sleeve was observed in a patient leading to revision surgery. Further long-term follow-up is needed to deliver the judgment whether the semi-rotator hinge joint of PHK III itself is a durable design, or not.

There were 12 prosthetic failures which required revision surgery. Based on the Kaplan–Meier estimates, the 1-year, 3-years, and 5-years prosthetic survival rates were 97%, 87%, and 85%, respectively for all 69 prosthesis. Prosthetic 5-year survivals for distal femoral replacement in previous studies ranged 57%–93% [12–18,19,21,23–25] (Table IV). Direct comparison of survival results in the current series to other published reports is difficult due to the heterogeneity with respect to the patient population. However, the prosthetic survival rate in the present study is comparable to that reported in the previous literature.

A univariate analysis showed better prosthetic survival in female gender. The similar result is indicated in the other previous report [14]. I supposed that the loading to the prosthesis in female is less than that in male due to the lower activity in daily life. Some previous reports demonstrated that resection of more than 40% of the femur was found to be a significant negative prognostic factor for prosthetic survival [10,12–14,17]. However based on the Kaplan–Meier survival analysis, there was no statistically significant difference in failure rate based on the length of the resection. These results suggest that the PHKIII has a stable clinical performance which was rarely affected by the patients' characteristics and the surgical procedure.

Because prosthetic replacements are usually associated with a great risk of complications, there is significant risk of amputation following prosthetic replacement [26]. In the current study, the 1-, 3-, 5-year overall preservation of the limbs were 95%, 92%, and 86%. Although the overall risk of amputation in the current study is comparable to the other series [12,13,15–18,19–22] (Table IV), further long-term follow-up is required.

The final goal of prosthetic reconstruction after a resection of a distal femoral tumor is to obtain better limb function. In the current study, the mean functional score according to the classification system of the MSTS was 68% of normal, and which is comparable to that seen in the previous series [11,14,18]. This result suggest that the reconstruction using PHKIII promise the acceptable functional outcome for the patients with musculoskeletal tumor at the distal femur.

In summary, we developed new type of tumor prosthesis, PHK III for the people with the typical Asian physical body-type to reconstruct bony defects of the distal femur. Although more continuous follow-up is required to determine the clinical performance of the PHKIII, reconstructions using PHKIII are considered to achieve acceptable clinical outcome.

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