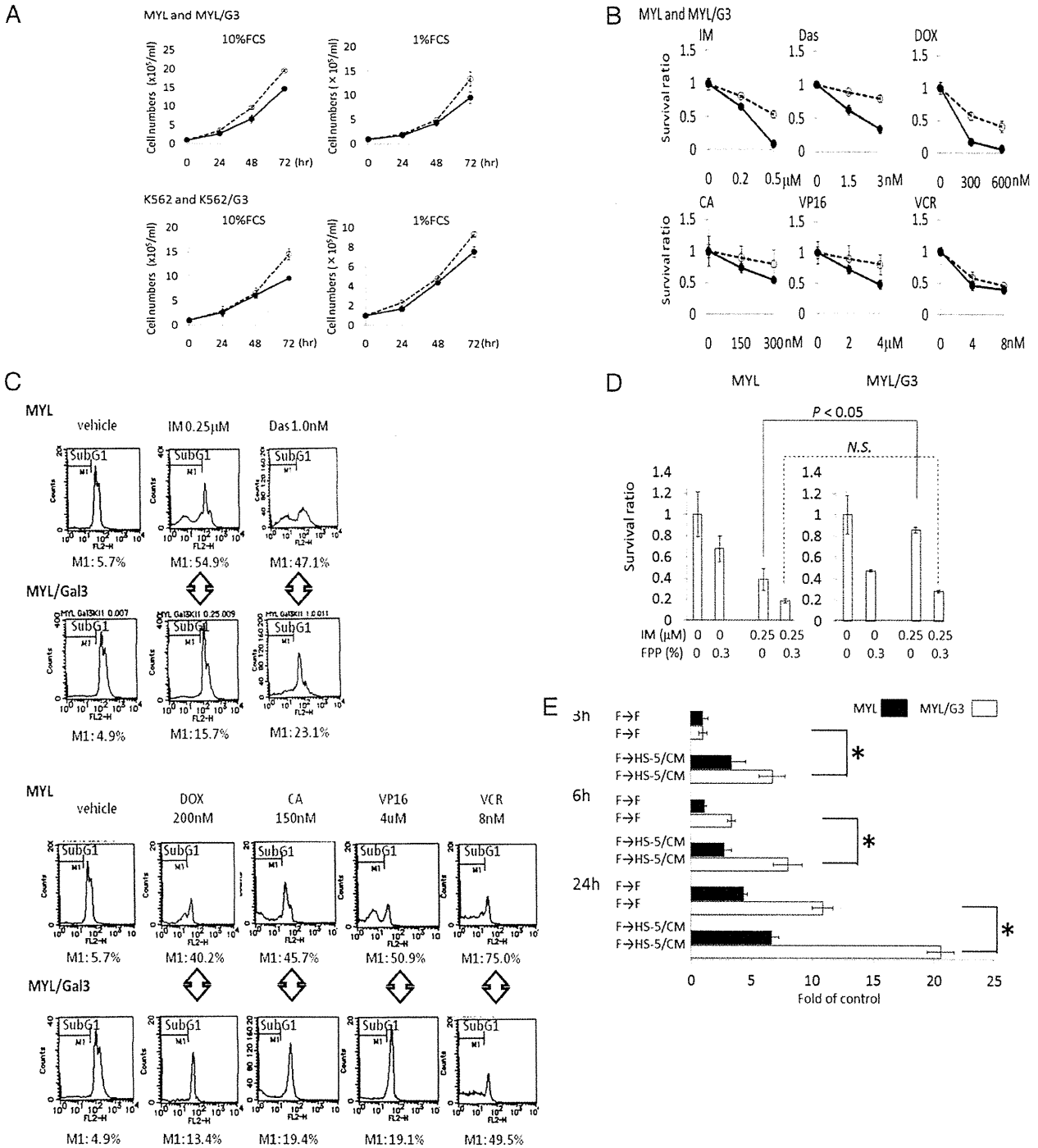

Fig. 2. Effects of Gal-3 overexpression on leukemic cell lines. (*A*) Cell proliferation potency. Solid lines represent parental cells and dotted lines represent Gal-3–overexpressing cells. (*B*) Cell-killing effects of TKIs and genotoxic agents. The *x* axis shows the drug concentration and the *y* axis shows survival cell ratio relative to untreated cells after 48 h treatment. Solid lines represent parental cells and dotted lines represent Gal-3–overexpressing cells. (*C*) Apoptosis induction determined by DNA content analyses. MYL and MYL/G3 cells were treated for 48 h with the agents indicated. The proportions of subG1 fractions (M1) were assumed to be cells undergoing apoptosis. (*D*) Gal-3 inhibitor overcomes Gal-3–induced resistance to cell death by IM in MYL cells. Cells were treated with IM and/or FPP for 48 h. *N.S.*, not statistically significant. (*E*) The effect of Gal-3 on chemotactic cell migration. The number of migrated cells in MYL after 3 h incubation was assumed to be 1.0. Asterisks indicate statistically significant differences ($P < 0.05$). F, serum-free medium; HS-5/CM, CM of HS-5 cells; F→HS-5/CM, upper chamber supplemented with serum-free medium and lower chamber filled with HS-5/CM. Bars indicate SD.

(group B). Although transplanted leukemic cells increased in a similar manner in the peripheral blood (PB) of both groups during the first 3 wk, the number of PB leukemic cells of group A mice then gradually decreased, whereas those of group B mice were preserved until death (Fig. 5*A*). The survival period of group A was significantly shorter than that of group B ($P = 0.025$; Fig.

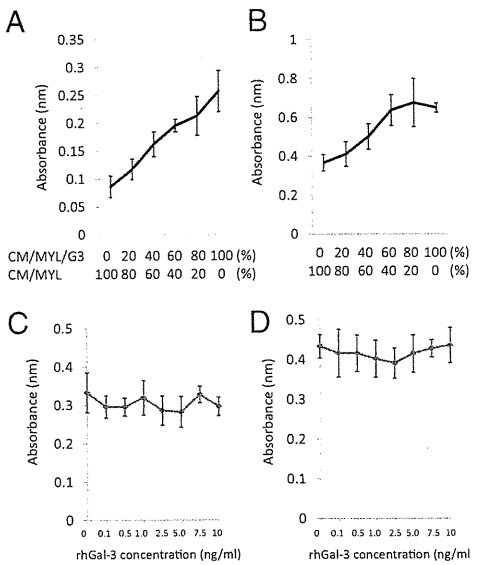

Fig. 3. CM of Gal-3–overexpressing cells contains more growth-promoting soluble factors. MYL cells (*A*) or HS-5 cells (*B*) were grown with mixtures of various concentrations of CM/MYL and CM/MYL/G3. MYL cells (*C*) or HS-5 cells (*D*) were grown in complete medium containing various concentrations of rhGal-3 for 96 h. Cell proliferation was determined by means of methylthiazol-diphenyl-tetrazolium (MTT) assay. An increasing in concentration of CM/MYL/G3 promoted the cell proliferation of MYL cells and HS-5 cells, whereas the addition of rhGal-3 up to 10 ng/mL did not.

5*B*); namely, all mice of group A died by day 48, whereas only one of seven mice in group B died during the observation period. Surprisingly, the sites of disease involvement at the mice's death showed major differences between the two groups. Most mice from group A showed extensive extramedullary involvement, such as intraabdominal, mediastinal, and/or s.c. tumors isolated from

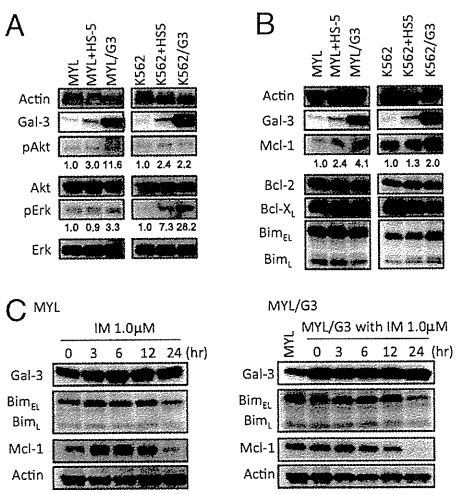

Fig. 4. Western blot analyses. Coculture with HS-5 and enforced Gal-3 overexpression induce phosphorylation of Akt and Erk (*A*), and causes Mcl-1 (*B*) to accumulate in MYL and K562. The expression levels of pAkt, Akt, pErk, Erk, Mcl-1, and Actin were calculated by using ImageJ software. The relative ratios of expression levels of pAkt/Akt, pErk/Erk, and Mcl-1/Actin of parental cells in normal cell culture were considered to be 1.0. (*C*) IM treatment (1.0 μM) for the indicated periods did not reduce Gal-3 in MYL and MYL/G3, whereas it caused accumulation of dephosphorylated Bim$_{EL}$ (faster migrated bands) and Bim$_L$. Mcl-1 accumulation was observed only when parental MYL cells were treated with IM.

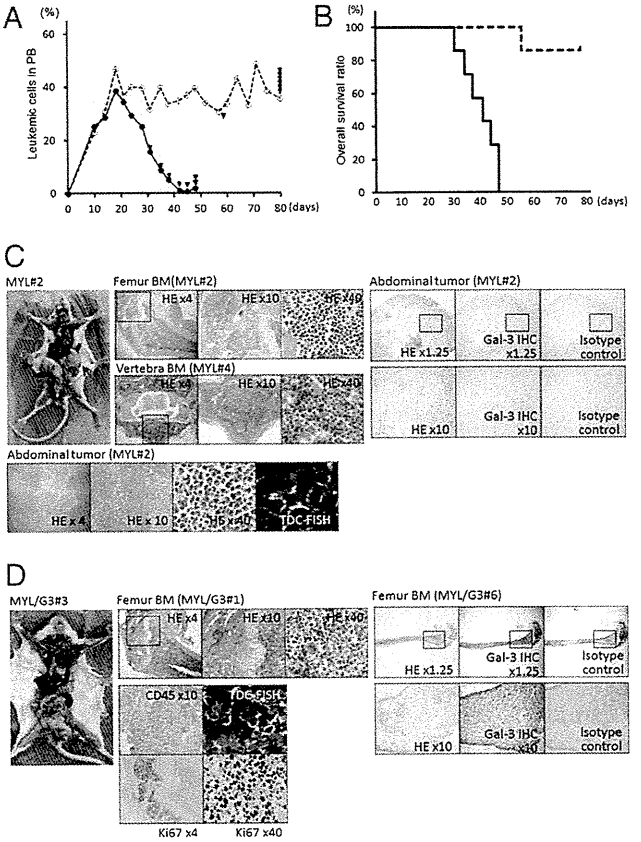

Fig. 5. In vivo role of Gal-3. (*A*) Percentages of transplanted leukemic cells in peripheral leukocytes of mice transplanted with MYL/mock cells (group A; solid line) and mice transplanted with MYL/G3 cells (group B; dotted line). The *x* axis shows days after transplantation and the *y* axis shows means ± SD of percentages of peripheral leukemic cells. Triangles indicate points of mouse deaths. (*B*) Overall survival periods of group A (solid line) and group B (dotted line). (*C* and *D*) Macroscopic and microscopic findings of mice transplanted with MYL/mock cells (*C*) or MYL/G3 cells (*D*). Data shown are representative of all mice examined (Table 1). In mice transplanted with MYL/mock cells, extensive extramedullary tumors (arrows) with Ph$^+$ Gal-3–negative leukemic cells [detected by tissue double color (TDC)-FISH and immunohistochemistry (IHC)] were identified in all mice (*C*). In contrast, none of the mice transplanted with MYL/G3 showed extramedullary involvement, whereas BM was at least partly replaced by Ph$^+$ leukemic cells expressing high level of Gal-3, which were also positive for human CD45 and Ki67 antigens (*D*). Direct invasion of leukemic cells outside BM was sometimes observed. HE, H&E staining.

BM, whereas only one of seven mice showed BM involvement at their time of death. In contrast, all mice in group B showed BM involvement, and sometimes outgrew BM, but none exhibited tumors isolated from BM (Fig. 5 *C* and *D*, Table 1, and Fig. S6). These findings suggest that Gal-3 overexpression facilitates BM homing and lodgment of CML cells. We also speculate that the reason for the shorter survival of group A mice is that the tumors expanded much faster when leukemic cells had advanced outside the BM, and that this may have had a significantly more deleterious effect on mice in group A than on mice in group B.

Discussion

The present study demonstrates that Gal-3 was specifically induced when leukemic cells were cultured with BMSCs in vitro, and that Gal-3 is predominantly expressed in CML cells, but not in acute leukemias. These findings prompted us to further investigate BMME-specific roles of Gal-3 in CML. As the results, enforced Gal-3 overexpression caused at least partial resistance to apoptotic induction by TKIs and genotoxic agents. As the

Table 1. Date of mice used in the present study

Mouse no.	Survival, d	Max. leukemic cells in PB		Organ involvement at death				Cause of death
				BM invasion		Extramedullary tumor		
		%	Day	Femoral	Vertebral	Expansion from BM	Isolated from BM	
MYL 1	31	36.8	14	—	—	—	Mediastinum	D
MYL 2	35	40.0	18	—	—	—	Abdominal cavity	D
MYL 3	38	53.6	18	—	—	Lower jaw	s.c. Tissue abdominal cavity	D
MYL 4	42	52.9	21	—	—	—	Abdominal cavity axillary LN	D
MYL 5	45	38.0	28	—	—	—	Abdominal cavity s.c. tissue	D
MYL 6	48	42.6	18	+	+	—	s.c. Tissue abdominal cavity	D
MYL 7	48	42.0	21	—	—	—	s.c. Tissue abdominal cavity, soft tissue around the testis	D
MYL/G3 1	57	73.3	14	+	+	Sacrum	—	B
MYL/G3 2	80	57.7	71	+	+	Sacrum	—	E
MYL/G3 3	80	50.0	48	+	+	—	—	E
MYL/G3 4	80	54.0	71	+	+	Sacrum	—	E
MYL/G3 5	80	48.0	18	+	+	Sacrum, right femur	—	E
MYL/G3 6	80	48.0	68	+	+	—	—	E
MYL/G3 7	80	57.14	24	+	+	—	—	E

B, Loss of >10% body wight; D, deterioration caused by tumor; E, euthanasia; LN, lymph node; Max.: maximum.

levels of drug resistance in Gal-3 gene transferred leukemic cells were similar to those in parental leukemic cell lines cocultured with HS-5, the inducibility of Gal-3 may at least partly explain the underlying molecular mechanisms of BMME-mediated drug resistance. As the molecular sequelae of Gal-3 overexpression, Erk and Akt, which are the essential downstream signaling molecules of Bcr-Abl (40), are activated in CML cells in a Bcr-Abl–independent manner. Simultaneously, Mcl-1 increased as the result of Gal-3 overexpression in CML cells. These results were consistent with those of previous studies showing that BMSC support activates Erk and Akt and increases Mcl-1 (41, 42), and the present study suggested Gal-3 as one of the positive mediators for these processes. Moreover, it has been reported that Gal-3 has an NWGR motif seen in the BH1 domain of Bcl-2 and may promote cell survival by interacting with Bcl-2 (27, 43). Bcl-2 family proteins have been shown to directly regulate cellular fate in the context of Bcr-Abl TK signaling, and Bim is essential for apoptosis by means of the blockade of Bcr-Abl TK signaling (36, 44–46). Because Mcl-1 protects mitochondrial integrity by binding to and keeping Bim_{EL} in check, and also inactivates other BH3-only proteins essential for genotoxic damage-induced apoptosis (47), Mcl-1 overexpression induced by Gal-3 may constitute one of the mechanisms for drug resistance of CML cells in BMME. Because Gal-3 expression induced by the BM milieu was not influenced by Bcr-Abl TK activity, Gal-3 induced by BM milieu stimuli may further augment the signaling for leukemia progression in combination with Bcr-Abl TK signaling, and also may maintain downstream pathways active even during treatment with TKIs.

In addition, the present study suggested the model that Gal-3 overexpression in CML cells exerts cell-extrinsic growth-promoting effects on CML cells as well as BMSCs, thereby accelerating the positive feedback mechanisms for leukemia proliferation and maintenance in the BM milieu in CML (Fig. S7), and promotes BM lodgment of CML cells in vivo. Although a number of studies have aimed to establish CML animal models by using xenograft models with human leukemic cells or transgene of *bcr-abl* into murine hematopoietic cells, most models have failed to recapitulate human CML-CP, which is clinically silent with persistent leukemic cell proliferation in BM and PB. Like the mice in group A in the present experiments, the survival periods of most previous CML models are frequently short as a result of progressive extramedullary involvements with or without BM leukemic lesion (48–51). The underlying molecular mechanism for this difference has remained unverified so far, but BMME-specific induction of Gal-3 expression in leukemic cells may be a clue to help solve this uncovered question. Also, soluble factors excreted by Gal-3–overexpressing CML cells, which promote this positive feedback machinery, are currently under investigation.

With respect to therapeutic applications, Gal-3 overexpression is expected to contribute to the generation of minimal residual disease as a result of the simultaneous promotion of BM lodgment and drug resistance, which makes the association between the expression levels of Gal-3 and the degree of response to TKIs a matter of considerable interest. However, because most patients with CML-CP with high levels of Gal-3 showed optimal response to TKIs (52), we underwrite the hypothesis that Gal-3 is a possible universal target in most patients with CML-CP, but is not a specific target in poor responders to TKIs. On the contrary, Gal-3 expression in leukemic cells in the advanced phase of CML and Ph^+ acute lymphoblastic leukemia is less than that of CML-CP; in addition, its expression does not differ significantly at onset and at relapse (Table S3). It is therefore important to verify that the loss of Gal-3 expression is mechanistically involved in disease stage progression and systemic organ dissemination in CML.

In conclusion, the present study disclosed that BMME-induced Gal-3 in CML cells may play an important role in drug resistance and leukemia lodgment in the BM milieu. Molecular-targeted agents against Gal-3, such as GCS-100, actually cause a decrease in Mcl-1 (53). The combined use of such compounds and TKIs is expected to be valuable for overcoming BMME-mediated protection of CML cells.

Materials and Methods

Cell Lines and Generation of Gal-3–Overexpressing Leukemic Cell Sublines. K562, BV173, KBM5 (American Type Culture Collection), MYL (54), and KCL22 (55) cell liens were established from Ph^+ patients with CML. Jurkat T is a human T-cell lymphoblast-like cell line, and HL60 (American Type Culture Collection) was established from cases of acute myelogenous leukemia; both are Ph^-. Gal-3–overexpressing subcell lines of MYL, K562, and Jurkat cells were generated by means of transfection of pEF1Galec3.neo plasmid (gift from Fu-Tong Liu, University of California, Davis, CA) (56). MYL and K562 cells were also transfected with a mock pEF1 plasmid empty vector as control, and were designated as MYL/mock and K562/mock, respectively. Following coculture assays, leukemic cells were positively isolated from HS-5 cells by using CD45 Microbeads and MiniMacs Separator (Miltenyi Biotec).

Microarray Analysis and Signal Pathway Analysis. MYL cells were cultured in normal medium on a noncoated plate as control, on a FN-coated plate, or on a plate preseeded with HS-5 for 48 h. Total RNA was isolated, and gene expression was analyzed with Affymetrix Gene Chip arrays and GeneChip Scanner 3000 (Affymetrix). Array data analysis was carried out with Affymetrix GeneChip operating software, version 1.0., and genes showing at least

a 2.0-fold difference in expression levels from control were considered to be positive. For signal pathway analysis, data were also analyzed with the Ingenuity pathway analysis software (Ingenuity Systems).

Mouse Xenografte Model for CML. Approval was obtained from the institutional review board at Kyoto University Hospital for a study using mice. Fourteen male NOD/SCID mice at 6 wk of age were sublethally irradiated (2 Gy), and 1.0×10^6 MYL/mock cells (group A) or 1.0×10^6 MYL/G3 cells (group B) were transplanted i.v. via their tail veins into seven mice each. Body weight and the percentage of leukemic cells in PB were monitored at least twice per week until day 80. For survival analysis, death was determined by spontaneous death or elective killing as a result of pain, the loss of more than 10% of maximum body weight of the individual mouse, or suffering or dying according to established criteria. All survived mice were subjected to euthanasia on day 80. We performed a macroscopic as well as microscopic analysis of BM of femoral bone and vertebra, and also of the tumors in each mouse at death. Tissue dual-color FISH was performed as previously described (57). The data shown are representative of three independent experiments.

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The current status of umbilical cord blood collection in Japanese medical centers: Survey of obstetricians

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ABSTRACT

As the first step of UCB banking, UCB collection has an important role in banking procedures. The aim of this study was to reveal the current status of UCB collection and discuss the management of the UCB bank. We conducted a questionnaire survey at medical centers collecting UCB, followed by semi-structured interviews with some respondents. Out of 38 institutes, 11 respondents (28.9%) thought that collection of UCB in addition to their routine medical services puts a burden on physicians. The obstetricians involved in the UCB collection are generally willing to participate in the procedure under current circumstances at medical institutes.

1. Introduction

Since the first report of successful umbilical cord blood (UCB) transplantation in 1988 there has been great interest in the use of cord blood as an alternative source of stem cells with which to treat cancer and genetic diseases [1,2]. With the increased recognition that the umbilical cord constitutes a viable source of stem cells, cord blood banks have been established worldwide to provide a large number of high-quality cord blood units to transplant centers [1,3]. In Japan there are 11 public cord blood banks, which form a network by communicating with each other and exchanging information. Comprehensive clinical summaries of UCB transplantation demonstrate that it is as effective as bone marrow transplantation, and without serious adverse events.

Organs including heart, lung, liver, kidney, and eye are defined by Organ Transplant Law as "internal organs" and are regulated by the Ministry of Health, Labour and Welfare (MHLW) in Japan. Accordingly, transplantation of these organs is strictly regulated by this law. A blood product for transfusion generally has associated medical charges that are similar to those of drugs. Such products are regulated by the Pharmaceutical Affairs Law (PAL), which includes the mandatory imposition of Good Manufacturing Practice (GMP) rules, and permission for manufacturing and market approval is issued by the MHLW. In contrast, bone marrow transplantation is defined as a medical practice, and is not regulated by the PAL, which means that GMP is not mandated for the preparation of products arising from this procedure. Although UCB shares characteristics with other blood products, the PAL does not apply to UCB.

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Generally, UCB banking involves the following procedures: (i) donor recruitment, informed consent, and testing of maternal donors; (ii) collection of the cord blood units; (iii) processing, freezing, and testing of the cord blood units; and (iv) release of cord blood units to a transplant center [1]. At many Japanese hospitals patients have the option of donating their cord blood free of charge to the public cord blood banking system. Currently there are 11 public cord blood banks in Japan, providing access to genetically unrelated cord blood. Alternatively, for a certain fee patients are able to have their cord blood stored with a commercial or private company for future use within their own family (private cord blood banking) [4,5].

Although the medical cost of UCB transplantation is covered by national health insurance, the banking procedure is not covered by such official systems in Japan. To maintain the sterility of cellular products and to manage the tracking record to deal with possible infectious diseases, Good Tissue Practice (GTP) has been introduced recently in the USA [6] and Japan [7]. Compliance to FDA 21 CFR part 1271 is obligated because UCB is regarded as a "tissue" and all cord blood banks have to follow the legislation. UCB is also defined as a tissue in the UK. Cord blood banks have been given licenses by the UK Government: the Human Tissue Authority operates under the European Union's Tissues and Cells Directive to regulate collection and use of UCB. Under GTP and also GMP regulations it is expected that cellular products remain clean and that the consistency of product characteristics are well controlled throughout preparation [8]. However, UCB are generally not processed in accordance with GTP and GMP because they are not regulated by the PAL in Japan. Thus, there are concerns about the quality of UCB stem cells and the management of infectious disease. Infection control is critical for securing the safety of cord blood transplantation [8]. UCB cells infection control is in fact secured by autonomous standards [9] of the UCB banks and by the efforts of the collecting centers. The regional cord blood banks provide both SOPs [10] and training. In Japan, pregnant women are screened for infectious diseases at their prenatal visit. Safety is secured by history taking and blood test prior to delivery, covering infections including rubella, toxoplasma, HTLV-1, chlamydiosis, HIV, hepatitis B and C, and syphilis. The results of these tests are available when screening UCB donors, and infected donors are excluded from UCB collection, even though they havenot been specially screened for UCB donation. Furthermore, SOPs require a blood test within 24 h prior to delivery or within a week after delivery. Moreover, the patient's anamnesis, including family medical history with up to three degrees of kinship, and the risks of hereditary disorder and infectious disease, are evaluated at all institutes.

To our knowledge, so far no critical issues relating to infection have been reported, and additionally the clinical outcomes of UCB stem cell transplantation seem reasonable [11]. These facts made us interested in how obstetricians involved in the collection of cord blood cells think and how they perform the procedure, since the collection of cord blood is the first step in the process of UCB transplantation, in terms of the "manufacture" of drug products. Therefore, quality is important. To investigate these issues, in this study we conducted a questionnaire survey with representative obstetricians involved in collecting UCB at medical centers that have contracts with a public cord blood bank. Subsequently, a semi-structured interview survey was performed with some respondents to further understand how they manage UCB.

2. Methods

2.1. Questionnaire survey

We first conducted an initial survey of obstetricians at medical centers collecting UCB in connection with the Japanese public cord blood bank network, by sending a confidential self-complete-type questionnaire by mail. The self-administered questionnaire was developed with reference to items used in previous studies [8], and was finalized after expert review by bank personnel. The questionnaire items were as shown in Table 1. Currently, there are 106 medical centers collecting UCB in Japan, which communicate with the 11 public cord blood banks (Fig. 1). We chose four public cord blood banks in two metropolitan areas in Japan that are supplied by a large number of medical centers (shown in bold in Fig. 1) and sent the questionnaire to 53 such centers, covering 50% of the total number of medical centers collecting cord blood for the public cord blood bank network. The medical centers contacted comprised 13 supplying UCB to Keihan cord blood bank, 17 supplying Hyogo cord blood bank, 12 supplying The Metro Tokyo Red Cross Cord Blood Bank, and 11 supplying Tokai University Cord Blood Bank. The survey was conducted in July to September 2008. We asked the medical centers for the questionnaire to be filled out by the obstetricians who mainly collected UCB in the institute.

2.2. Semi-structured interview survey

Subsequently we conducted semi-structured interviews with representatives of eight medical centers collecting cord blood, comprising five institutes supplying Hyogo cord blood bank, one institute supplying Tokyo Red Cross Cord Blood Bank, and two institutes supplying Tokai University Cord Blood Bank. We selected these medical centers because they agreed to be interviewed when the questionnaire survey was performed. A semi-structured individual interview was conducted including the following question items: (i) Standard Operating Procedures (SOPs) and training courses prior to collecting cord blood; (ii) burden and risks associated with UCB collection; (iii) infection testing for donor eligibility; and (iv) coverage of UCB banking by national health insurance.

3. Results

3.1. Questionnaire survey

A total of 38 institutes out of 53 responded to the questionnaire, a response rate of 72%. The results are as shown in Table 1.

The number of physicians collecting UCB was 10 or fewer in 90% of the institutes sampled. There were five people

Table 1
Questionnaire survey results.

Number (%) of respondents ($n = 38$)	Yes	No
1. How many physicians participate in UCB collection at your institute?		
5 or fewer	17(44.7)	
6–10	17(44.7)	
11–15	3(7.9)	
15 or more	1(2.6)	
2. Is UCB collected under sterile conditions?*	30(78.9)	6(15.8)
3. Are there SOPs which relate to cord blood collection?	32(84.2)	6(15.8)
In cases where SOPs exist, is there any documents relating to the method of transportation to the cord blood bank? ($n = 32$)*	24(75.0)	7(21.9)
4. Do you perform a training course prior to collecting cord blood?	15(39.5)	23(60.5)
In cases where no training course exists, do you think it would be appropriate to initiate such a program? ($n = 23$)*	11(28.9)	9(39.1)
5. How many cases do you collect in a month at your institute?		
10 or fewer	22(57.9)	
11–20	5(13.2)	
21–30	5(13.2)	
31–40	3(7.9)	
41–50	3(7.9)	
6. Do you have enough physicians involved in UCB collection at your institute?	36(94.7)	2(5.3)
7. Does the collection of cord blood put any burden on your regular clinical work as an obstetrician?*	11(28.9)	26(68.4)
8. Do you think there are any risks for the pregnant woman relating to UCB collection?	6(15.8)	32(84.2)
9. Do you perform routine screening for possible infectious disease in the pregnant woman?	34(89.5)	4(10.5)

* In cases where the number of Yes and No answers does not add up to the indicated sample size, some respondents failed to provide an answer.

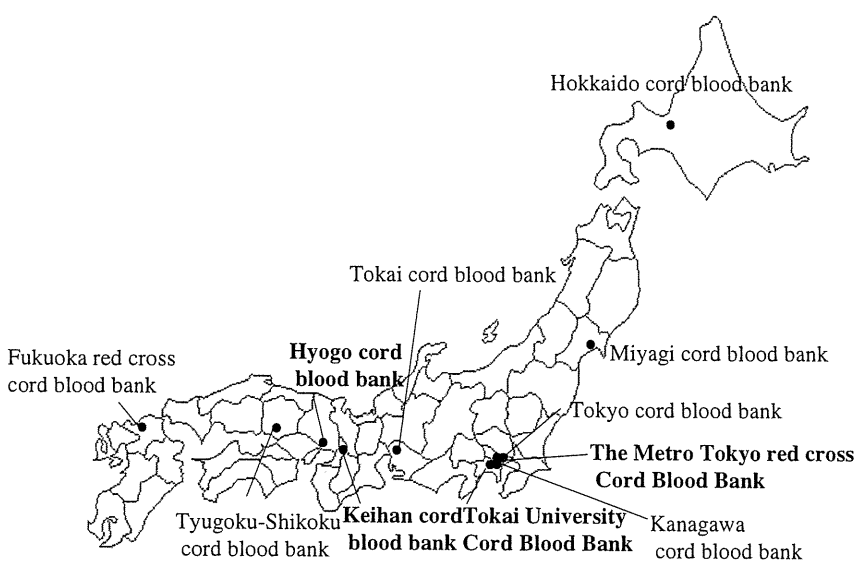

Fig. 1. Location of 11 public cord blood banks of Japan cord blood bank network.

or fewer at 17 institutes (44.7%) and six to 10 at another 17 institutes (44.7%). With regard to the sterile collection of UCB, 30 respondents (78.9%) thought that the procedure is performed in a sterile fashion. On the other hand, six respondents (15.8%) gave a negative answer and two respondents (5.3%) did not answer this question. Although 32 respondents (84.2%) indicated that there are SOPs for cord blood collection at their institute, six respondents (15.8%) did not. Fifteen respondents (39.5%) indicated that there is a training course for collecting UCB at their institute. In contrast, 23 respondents (60.5%) indicated that there is no training course at their institute; however, 11 of these 23 respondents felt that training is necessary prior to the collection of UCB.

With regard to the collection opportunities per month, 22 institutes (57.9%) indicated that there were 10 or fewer opportunities, five institutes (13.2%) were in the 11–20 range, and another five institutes (13.2%) indicated 21–30 opportunities. In addition, 36 respondents (94.7%) felt that the number of physicians participating in collection of UCB at their institute was appropriate. Twenty-six respondents (68.4%) thought that collection of UCB in addition to their routine medical services does not put a burden on physicians.

Thirty-two respondents (84.2%) indicated that UCB collection does not pose a risk to pregnant women prior to or after delivery. Screening tests for infection are performed prior to delivery at 34 institutes (89.5%).

3.2. Semi-structured interviews

The interviewees for the semi-structured interviews comprised representatives from eight medical centers, including one private university hospital, one public hospital, three private hospitals and three private clinics. Eight respondents answered the interview.

3.3. SOPs and training courses

All eight respondents answered that all SOPs documents are supplied by the related public bank and that they use the latest versions of the procedures. One institute uses documents after some modification from the original version. With regard to the sterile collection of UCB, the collection kits are sent to the institutes by the cord blood banks. Six respondents answered that if cord blood is simply collected, a training course is not mandatory. However, a training course prior to collecting UCB would be of great help for yielding large amounts of UCB cells and preventing bacterial contamination.

3.4. Burden and risks associated with UCB collection

With regard to the burden and risks associated with UCB collection, it seems that the task does not pose any burden to the respondents in this study, since the bank prepares the SOPs and supplies equipment required for collection. One respondent pointed out that, since UCB transplantation is performed on volunteer donors, it is worthwhile even though it creates extra work. In addition, all respondents suggested that neither risks nor burdens are posed to volunteer donors because collection is stopped immediately if something goes wrong with the procedure. It suffices to say that two respondents answered that the preparation of documents is a burden. Another respondent answered that it takes time to explain the procedure to the donor.

3.5. Infection testing for donor eligibility

With regard to infection testing, all the eight respondents indicated that no additional examination was required since pregnant women are screened for infectious diseases at their prenatal visit during the first trimester. Furthermore, at six of the eight institutes blood samples are tested for infection upon delivery of the baby.

3.6. Coverage of UCB banking by national health insurance

Because the UCB banking process is currently not covered by national health insurance in Japan and therefore the preparation of UCB may not be performed sustainably, we included this issue in the interview. One respondent pointed out that volunteer donation required no insurance coverage, as regular blood donation for transfusion is not covered by insurance. This respondent indicated that there is a need for the organized management of the banks because voluntary participants in cord blood banks may not always be able to manage them in a stable and reliable manner. Another respondent pointed out that since preserved UCB cells are not always used for transplantation and are sometimes destroyed, insurance coverage is inapplicable.

3.7. Other comments

Five respondents indicated that the standard of preservation is strict. Because neither UCB of insufficient volume nor UCB with any bacterial contamination is preserved, the number of collected UCB samples that are cryopreserved is much less than the total number of UCB samples collected. Five respondents answered that the UCB is not transported to the bank at weekends. Two respondents answered that transportation relies on volunteers. Two respondents indicated that the operation of the banks needs more financial support to maintain good medical performance.

4. Discussion

In this study we revealed the current status of UCB collection in Japanese medical centers and elicited opinions from the obstetricians involved in cord blood collection by conducting a questionnaire survey and semi-structured interviews. To eliminate selection bias, we choose four public cord blood banks in two metropolitan areas covering 50% of all centers. Out of a Japanese population of 130 million, the number of newborns in 2008 was 1.09 million and the number of cord blood transplants performed in that year was 928 [12]. Since the first UCB transplant was performed more than 20,000 such transplants have been reported worldwide [13] and in Japan the cumulative number of UCB transplants by 2009 was 6019. The number of cord blood provided each year is increasing every year (Fig. 2) (data from the Japan cord blood bank network). The frequency of UCB collection varied depending on the scale of the medical centers and the motivation of the obstetricians.

Several questions were suboptimal worded and answers reflect confusion. It seems that six respondents (15.8%) might have a misunderstood the SOPs or did not know what the abbreviation SOPs stands for. It might have affected that SOPs is not technical term in medicine and this abbreviation is not widely used in Japan. We found that six institutes have no SOPs, including four institutes which have only five or fewer physicians participate in the UCB collection, or ten or fewer cases are collected in a month. Responders at these institutes stated that they have no SOPs. Then six respondents (15.8%) who gave a negative answer seem to have evaluated themselves strictly about sterile conditions. In the free comment column of the questionnaire survey two respondents pointed out that the level of sterility was not clear. Recently, cell manufacturing at the GMP level has been advocated in Japan, so it seems that six respondents were considering GMP when responding to the questionnaire.

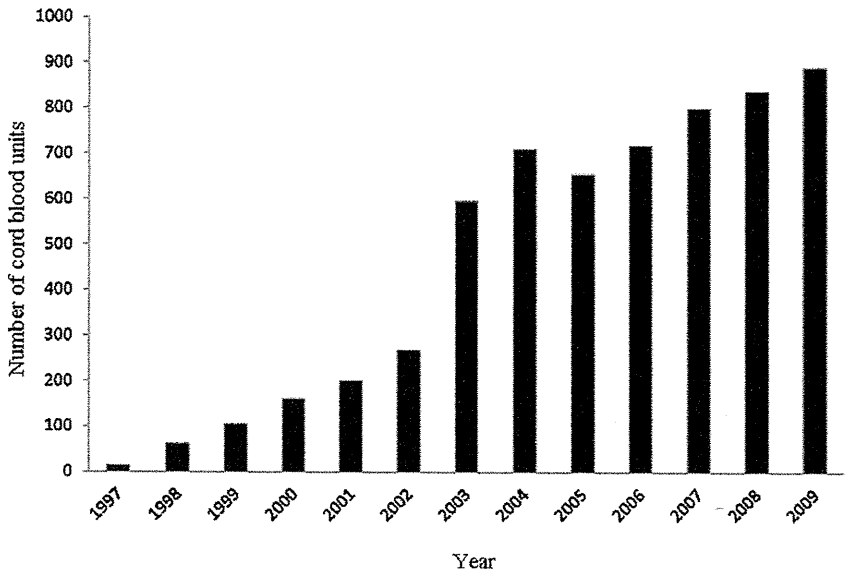

Fig. 2. The number of cord blood provided each year in Japan. Source: Japan cord blood bank network, 2010.

Cord blood banks have a minimum volume limit as the first selection criterion for UCB donations in order to store clinically useful UCB units. In our interview survey we found out that because neither UCB of insufficient volume nor UCB with any bacterial contamination was preserved, the number of collected UCB units that went forward to cryopreservation was much less than the total number collected. This indicates that quality-control standards at medical centers are strict. Actually only high-quality UCB is used in transplantation because of the strict selection criteria. Consequently, the clinical outcome of UCB transplantation in Japan seems to be above average comparing other countries. Routinely updated SOPs for UCB collection are supplied adequately to the medical centers by the cord blood banks, and this contributes to a high level of quality control for UCB collection. Judging from clinical results [14–18] it is thought that Japan cord blood banks provide UCB for transplantation within the appropriate system.

The Japanese cord blood bank service is different from the European and North American ones. The Japanese system is not a government initiative but is voluntarily established by parent organizations around the country. UCB banking is not covered by national health insurance in Japan. Only cord blood transplantation is covered by insurance and 174,000 yen is paid to the bank of parent organization by the hospital. They add up to only a tiny fraction of the price that entails a lot of costs about total banking processing, and obstetrician's extra work is not paid for. However, limited appreciation rewards are paid and the equipment is provided free of charge by the bank to the collecting center. Management of each public bank is supported by limited funds from the government, and it is very difficult to acquire extra financial support from elsewhere to improve the quality of the UCB banking procedure. We wanted to investigate physicians' views and opinions of the lack of cover by national health insurance.

Blood can be collected from the cord while the placenta is still *in utero* after vaginal delivery (*in vivo* collection) or after the placenta has been delivered (*in vitro* collection). The technique of collection varies between different cord blood banks and medical centers. In the questionnaire survey only 11 respondents (28.9%) thought that collection of UCB in addition to their routine medical services puts a burden on physicians. Thirty-two respondents (84.2%) answered that UCB collection does not pose a risk to pregnant women prior to or after delivery. In the interview survey all respondents suggested that UCB collection procedures seem to impose little burden on the obstetrician and are of almost no risk to donors because collection is stopped immediately if something goes wrong with the procedure. Two respondents in the interview survey answered that preparation of documents or providing explanation to the donor is a burden. However, many obstetricians have strong motivation, and they feel no additional burden and risks. In addition, they were satisfied with the current circumstances. They deemed UCB collection to be worthwhile despite the procedure being insufficiently paid, and they were satisfied with donors being volunteers. These results suggested that the obstetricians involved in the UCB collection are generally willing to participate in the procedure under current circumstances at medical institutes. It seems that obstetricians also feel discomfort at the proposal of collecting UCB for a fee. The banks are additionally funded by subsidies, funds from the parent organization such as Hyogo College of Medicine for Hyogo cord blood bank, and donations. In addition, some operation relies on volunteers. In the interview survey it was expressed that the reliance of some banking procedures, such as transportation of collected UCB to the banks, being partly managed by volunteers leads to the fragile operation of UCB banking.

Recently, discussions have started over whether UCB should be regulated as a medical product in Japan in line

with the regulations in foreign countries. In Japan, if UCB were to be re-defined as a blood product for transfusion, as are biopharmaceuticals derived from specific living organs, then the banking process would be covered by national health insurance and other frameworks would be in place to ensure quality control, such as GMP. UCB collection requires certain quality control; however, if UCB preparation was regulated like a drug, then UCB would be regulated by the PAL. Clinical trials are needed under Good Clinical Practice (GCP) to obtain regulatory approval under PAL. Such clinical trials would take time and have huge costs. Taken together, as the clinical outcomes of cord blood transplantation in Japan are comparable with those from other countries, it may be difficult to define UCB as a drug and for it to be regulated by the PAL. Nevertheless, steady financial support from the government is critical for operating cord blood banks and UCB collecting sites to ensure the future sustainable performance of cord blood transplantation.

The questionnaire was not designed to demonstrate potential flaws in the collection process and no data were obtained about on-site storage or shipping irregularities that would impact product quality. The result of this survey simply represent only about obstetricians' consciousness. However, if not all, this survey should help us learn about comprehension of the banking quality control. The interview survey presented here had some limitations including that it consisted of a small, select sample of the quite large number of participating obstetricians in the hospitals. Although there might have been some bias, the answers from the interview survey were very informative that could not have been obtained by questionnaire survey only. With limited time and budget these eight medical centers covered the two Japanese metropolitan areas of Kanto and Kansai evenly. The data obtained in this study could also provide a basis for future studies.

Conflict of interest

The authors declare no conflict of interest

Acknowledgments

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Using a Smartphone while walking: a measure of dual-tasking ability as a falls risk assessment tool

Sir—Falls are relatively common among older people; 30% of 65-year-old community-dwelling adults experience at least one fall per year. Of these falls, 6% result in fractures [1, 2]. Falls typically occur during locomotion; therefore, previous studies have focused on identifying the changes in locomotor performance which occur with increasing age [3, 4].

In every-day life, locomotion typically occurs under complicated circumstances with cognitive attention focused on other tasks. Lundin-Olsson *et al.* [5] reported a novel method for predicting falls based on the dual-task (DT) performance of subjects. In recent years, numerous studies have evaluated DT walking in elderly people. However, Beauchet *et al.* [6] reported that reliable conclusions cannot be drawn from the prediction of falls based on DT results due to the lack of standardisation in DT paradigms. We considered that the two main limitations of the previous research using DT protocols [7–12] were: (i) insufficient evaluation of the performance of the secondary task and (ii) the lack of standardisation of the DT protocols.

The aim of the present study was to evaluate the use of a Smartphone-based application for assessing dual-tasking ability as a tool for predicting the risk of falls in a community-dwelling elderly population.

Methods

Participants

Participants for this study were recruited through advertisements placed in local newspapers. A total of 318 community-dwelling older individuals (mean age, 78.9 [7.3] years) participated in this study. The exclusion criteria ensured that none of the participants had any indications of the following clinical conditions: (i) serious visual impairment, (ii) inability to ambulate independently (those individuals requiring the assistance of a walking frame were excluded), (iii) a score of <7 on the Rapid Dementia Screening Test [13], (iv) symptomatic cardiovascular disease, (v) Parkinson's disease or stroke, (vi) peripheral neuropathy of the lower extremities or (vii) severe arthritis. Written informed consent was obtained from each subject in accordance with the guidelines approved by the Kyoto University Graduate School of Medicine and the Declaration of Human Rights, Helsinki, 1975. Each participant was categorised as either a high-risk (HR) or low-risk (LR) elderly individual on the basis of whether they had experienced at least one fall within the past year (self-reported). A fall was defined as any event that led to unplanned, unexpected contact with a supporting surface during walking. On the basis of this classification, the participants were divided into HR ($n = 90$) and LR ($n = 228$) groups (Table 1).

Smartphone data collection

The Android-based Smartphone Android Dev Phone 2 (HTC Corp., Taiwan) was used as a measurement device. Android is a popular operating system for Smartphones. The phone is lightweight (123 g with battery) and has

Table 1. Characteristics of the participants in the GR and LR groups

Characteristic	HR ($n = 90$) Mean (SD)	LR ($n = 228$) Mean (SD)	*P*-value	Effect size	95%CI
Age	79.1 (7.4)	78.8 (7.2)	0.733	0.04	−1.41 to 2.00
Height, cm	153.3 (6.7)	153.6 (6.4)	0.703	0.05	−1.81 to 1.22
Weight, (kg)	53.7 (10.2)	54.2 (9.8)	0.695	0.05	−2.78 to 1.86
Gender, female, *n* (%)	62 (56.3%)	146 (64.0%)	0.435		
ST walking time, s	13.3 (5.3)	11.6 (4.3)	0.001*	0.34	0.70 to 2.87
DT walking time, s	27.8 (28.2)	20.9 (22.9)	0.019*	0.24	1.14 to 12.67
ST android, score	39.7 (14.5)	36.9 (12.5)	0.081	−0.23	−0.35 to 6.09
DT android, score	29.5 (12.4)	34.8 (9.5)	<0.001*	0.56	−7.78 to −2.84
DT time lag, %	52.2 (35.1)	39.4 (32.4)	0.001*	0.36	5.00 to 20.49
DT point lag, %	34.8 (46.4)	3.8 (34.1)	<0.001*	0.67	21.89 to 39.97
DT total lag, %	86.9 (52.6)	42.9 (47.7)	<0.001*	0.84	32.51 to 55.54
TUG, s	15.8 (12.6)	11.8 (5.3)	0.000*	0.32	2.01 to 6.03
One leg standing, s	7.5 (12.8)	10.4 (11.4)	0.046*	0.25	−5.74 to −0.05
FR, cm	21.5 (8.2)	24.5 (9.5)	0.006*	0.32	−5.12 to −0.89
Five-chair stand test, s	12.9 (6.9)	10.7 (4.5)	0.001*	0.34	1.03 to 3.59

*Indicates statistical significance, Student's *t*-test, $P < 0.05$.
CI, confidence interval; DT, dual-task; ST, single task; DT time lag (%) = 100 * (DT walking time − ST walking time)/ST walking time. DT point lag (%) = 100 * (DT Android score − ST Android score)/ST Android score. DT total lag (%) = DT point lag + DT time lag. TUG, time up and go.

triaxial accelerometers. The use of Android-based applications is advantageous because they are free to develop, offer flexible design options, and can be easily and rapidly distributed over the Internet. The author (K.O.) developed an Android application (RollingBall) for the assessment of fall risk (available for download at http://www.kuhp.kyoto-u.ac.jp/~kazuya/RollingBall.apk) in which a small blue ball (1.5 cm in diameter) is moved on a large white circle (4 cm in diameter) by tilting the phone. The inclination of the phone is determined by the triaxial accelerometers (Figure 1). The Android application also calculates a score based on coordinate data of the ball on the circle; higher scores indicate that the blue ball is nearer to the centre of the circle. The application was based on the 'walking while carrying a ball on a tray' task, previously demonstrated to be a good predictor of falling (Yamada M., unpublished data).

Participants used the application in single- (ST) and dual-task conditions. In the ST condition, participants used the application for 15 s while stationary (ST Android test). The instructions were as follows: 'Using your left hand (or the hand without a cane), please control the Smartphone to keep the blue ball in the centre of the white circle'. The score calculated by the application was recorded as a variable. In the DT condition, the participants walked 15 m at a comfortable speed while using the Android application. The participants were instructed as follows: (i) They should walk at a comfortable speed while positioning and maintaining the blue ball at the centre of the white circle with the left hand (or the hand without a cane). (ii) It was not necessary to constantly look at the Smartphone screen. (iii) The exercise should be performed safely to ensure that no accidents, such as falls, occurred. The score calculated by the application and the time taken to walk 15 m were recorded as variables. Before the tests were carried out, a trained evaluator gave standardised verbal instructions regarding the test procedure and a visual demonstration of the tests. The test–retest reliability, determined using the inter-class correlation coefficient (ICC [1.1]). was 0.976. The tests were performed in a random order. The score under each condition was calculated as an average of the scores obtained from the two trials. The reduction in performance due to walking, the DT lag, was calculated as follows for both the application score (DT point lag) and

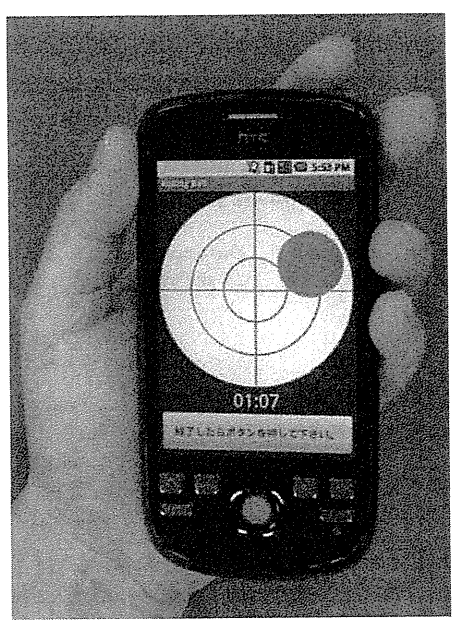

Figure 1. The developed Android application allowed users to control the position of a small blue circle (1.5 cm in diameter) on a large white circle (4 cm in diameter). The score was automatically calculated on the basis of the coordinate tracking data of the blue circle.

walking time (DT time lag) variables [14]:

$$\text{DT lag}(\%) = 100 * \frac{(\text{DT condition} - \text{ST condition})}{\text{ST condition}}$$

The DT total lag was then calculated using the following equation:

$$\text{DT total lag } (\%) = \text{DT point lag} + \text{DT time lag}$$

Data collection for other physical performance tests

In addition to DT walking, the participants were subjected to five other physical performance tests that are widely used to identify HR elderly adults: 10 m walk under an ST condition (ST walking) [15], timed up and go (TUG) test [16], functional reach (FR) [17], one-leg stand [18] and five-chair stand tests [19]. The tests were performed in random order. For each performance task, the participants performed two trials and an average score was calculated.

Statistical analysis

Differences in the physical performance variables between the HR and LR groups were analysed using a Student's *t*-test. To compare physical performance in the two groups, effect sizes were determined. The effect size was calculated as: (HR mean − LR mean)/standard deviation. The relationship between the scores from the Smartphone test and the five previously validated tests was assessed using Pearson's correlation coefficient. All data analysis was carried out in the Statistical Package for Social Science (Windows version 11.0). A *P*-value of <0.05 was considered statistically significant for all analyses.

Results

Descriptive statistics for patient characteristics in the two fall risk groups are summarised in Table 1. Participants in the HR and LR groups were comparable and well-matched in terms of their age, height, weight and gender. With the exception of the ST Android, DT walking time, one-leg standing and FR test results ($P > 0.05$), all physical performance tests demonstrated that the elderly participants in the LR group had significantly better scores than those in the HR group. The largest effect size was the DT total lag in all physical performance tests. The results for DT total lag were weakly, but significantly, correlated with those for ST walking time ($r = 0.267$, $P < 0.001$) and those for the TUG ($r = 0.194$, $P = 0.001$), one-leg standing ($r = -0.195$, $P = 0.001$), FR ($r = -0.202$, $P < 0.001$) and five-chair stand ($r = 0.161$, $P = 0.005$) tests.

Discussion

This is the first study to examine the use of a Smartphone device for DT-based fall risk assessment. The present findings support the conclusion of previous experimental studies that measurement of changes in gait while dual tasking is an effective tool in the clinical assessment of fall risk [7–12]. Several characteristics of the Smartphone application developed here are considered to contribute to increasing the demands on the attention of HR elderly participants during DT walking. First, the application represents a simple manual task (i.e. maintaining a small circle in a central position on a large circle) that participants can easily understand and perform. Second, the application provides the ability to measure performance in both the principal and secondary tasks. This constitutes an improvement over previous DT-related reports, which did not sufficiently evaluate the participants' performance in secondary tasks [7–12]. Changes in physical performance during dual tasking are considered to be due to the competing demands for the participant's attention required to successfully complete both tasks [20, 21]. Therefore, performance in both the principal and secondary tasks needs to be evaluated. The results for DT total lag weakly correlated with those from previously validated physical performance tests. Our results reveal that the Smartphone test evaluates the risk of falls by using a different parameter from that used in previously validated physical performance tests.

In addition to the benefits of the developed Smartphone application as a clinical assessment tool, we assessed whether this application could be used as tool for public health promotion. The Smartphone application has a number of advantages over conventional DT-based fall risk assessment tests. First, it is able to measure performance in both principal and secondary tasks. Second, because the application is downloadable from the Internet, it can be readily accessed and distributed throughout the world. Third, the simplicity and portability of the application permits self-assessment of fall risk by concerned individuals in non-clinical settings. However, there is a serious limitation in this study. The developed Smartphone application could not predict falling in older adults as this study was based on the participants having experienced falls in the previous year.

Key points

- A Smartphone-based application was used to assess dual-tasking ability as a measure of the risk of falls.
- The results for DT total lag weakly correlated with those for previously validated physical performance tests.
- This is the first study to examine the use of a Smartphone device for the assessment of the risk of falling.

Conflicts of interest

None declared.

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Long-term effect on mortality of a multicomponent cognitive behavioural group intervention to reduce fear of falling in older adults: a randomised controlled trial

SIR—Fear of falling and avoidance of activity due to fear of falling are common in older people. Prevalence rates for fear of falling in community-living older persons range from 20 to 60% [1–8] and for avoidance of activity due to fear of falling from 15 to 55% [1, 6, 7, 9–11]. Fear of falling and related avoidance of activity may lead to adverse consequences, like functional decline [8, 12, 13], restriction of social participation [9], decreased quality of life [2, 8, 12], increased risk of falling [8, 10, 13] and institutionalisation [12]. Indeed, fear of falling is suggested to be a potential health problem of equal importance to a fall [12].

Several interventions showed to reduce fear of falling or to improve confidence regarding performing activities without falling [14]. Particularly two multicomponent cognitive behavioural group interventions explicitly aimed at reducing excessive fall-related fear and unnecessary avoidance of activity showed beneficial outcomes in randomised controlled trials in community-living older people [15, 16]. In the first study confidence in performing activities without falling and mobility range were improved directly and 12 months after a multicomponent cognitive

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Effect of resistance training on physical performance and fear of falling in elderly with different levels of physical well-being

SIR—Several factors are involved in the maintenance of activities of daily living (ADL) in older adults. Skeletal muscle mass and strength are important factors for maintaining independence and quality of life in elderly. Several recent cross-sectional studies have shown the associations of muscle strength with physical fitness and disability [1, 2]. Loss of muscle mass (sarcopenia) is prevalent in older adults [3] and represents an impaired state of health with mobility disorders, increased risk of falls and fractures, impaired ability to perform ADL, disabilities and loss of independence [4–6].

Fear of falling is common in older adults. The prevalence varies from 21 to 85%, is higher in women than in men, and increases with age [7]. The risk factors of fear of falling are shown to be physical frailty [8], perception of poor health [9], obesity, cognitive impairment, depression, poor balance [10] and history of at least one fall [7].

Resistance training is an effective intervention to improve the physical function in older adults by increasing strength and physical performance [11]. However, it is still controversial whether resistance training is effective for all levels of elderly people. For example, we reported that decreased muscle power is a reliable predictor of falls only in frail elderly [12].

We hypothesised, therefore, that there is a differential effect of resistance training on physical performance according to the level of physical well-being. The aim of this study was to compare the effects of resistance training on skeletal muscle mass, physical performance and fear of falling in robust and frail elderly.

Methods

Participants

Participants were recruited by an advertisement in a local press. We used the following criteria to screen participants in an initial interview: aged ≥65 years, community dwelling, has visited a primary care physician within the previous 3 years, score of ≥8 by Rapid Dementia Screening Test [13], able to walk independently, willing to participate in group exercise classes for at least 6 months, access to transportation and no regular exercise in the previous 12 months.

We also used the interview to exclude participants based on the following exclusion criteria: severe cardiac, pulmonary, or musculoskeletal disorders, pathologies associated with an increased risk of falls (i.e. Parkinson's disease or stroke) and use of psychotropic drugs. We obtained written informed consent from each participant in accordance with the guidelines approved by the Kyoto University Graduate School of Medicine and the Declaration of Human Rights, Helsinki, 1975.

Frailty definition

The frailty classification was based on a composite of previous work. The Timed Up and Go (TUG) is a simple test developed to screen basic mobility performance and has been shown to be significantly associated with ADL in frail older adults [14]. It has been reported that elderly with a TUG score greater than 13.5 s can have an increased risk of falling [15]. Frailty was defined as a TUG score >13.5 s. Based on key components of the screening examination (TUG score greater than 13.5 s), 159 elderly adults were classified as the frail group, whereas 178 elderly adults were classified as the robust group because they had a TUG score of ≤13.5 s.

Resistance training

All participants underwent resistance training sessions twice a week for 50 weeks. All participants performed the seated row, leg press, leg curl and leg extension exercises on resistance-training machines. Training loads were chosen using the 10-repetition maximum (10-RM, the maximal weight that can be lifted 10 times). Participants used the 10-RM for 3 sets of 10 repetitions for each machine exercise. Participants were required to adjust the training weight to ensure failure at the 10-RM. It took approximately 1 h to finish all sessions, with 15-min warm-up at the beginning and 10-min cool-down stretch at the end.

Bioelectrical impedance analysis measurement

A bioelectrical impedance data acquisition system (Physion MD; Physion Co. Ltd, Kyoto, Japan) was used to determine

the bioelectrical impedance of the right upper and lower limbs [16]. This system applies a constant current of 800 mA at 50 kHz through the body. Participants lay supine with their arms and legs extended and relaxed during bioelectrical impedance measurement. Leg lean mass (LLM) per whole-body weight was used for the analysis.

Measurement of physical performance

All participants underwent five measurements upon entry into the study (pre-test), which included 10-m walk test, TUG test, single leg standing (SLS), functional reach (FR) and 5-chair stand. The order of performing these tests was random. For each performance task, the participants performed two trials, and an average score was calculated from these two trials. All baseline and pre-test measurements were completed prior to randomisation.

Measurement of fear of falling

Falls Efficacy Scale (FES) [17] is the most frequently used surrogate measure for fear of falling in older adults. The reliability and validity of FES have been previously reported [17]. FES was measured at baseline and at 12 months. FES is based on the operational definition of fear as 'low perceived self-confidence at avoiding falls during essential, relatively nonhazardous activities'. Briefly, participants were asked how concerned they were about the possibility of falling while performing 10 different activities on a 4-category scale from 1 (not at all concerned) to 4 (very concerned). If participants indicated that they did not perform or were unable to perform the activity, they were encouraged to respond hypothetically. FES emphasises mainly indoor, home-based activities.

Required sample size

We designed the effect size of the current study to be 0.4. With a significance level of 0.05, a power of 80%, and a moderate effect size (0.4), a minimum of 100 participants were needed in both the intervention and control groups. Accounting for a potential 20% attrition rate, a total of 240 participants were recruited for this study, which was deemed large enough to detect statistically significant differences.

Statistical analysis

We analysed the effects of resistance training on all outcome measures using a mixed 2 (group: robust and frail groups) × 2 (time: pre-intervention, post-intervention) ANOVA. A 0.05 type 1 error rate was chosen *a priori* to indicate statistical significance. A *post hoc* paired *t*-test for within-group comparisons was performed to compare each dependent variable. The Bonferroni procedure was used to adjust the type 1 error rate of each analysis to 0.025 (0.05/2) as an indication of statistical significance to guarantee an overall type 1 error rate of 0.05. Data were entered and analysed using the Statistical Package for Social Science (Windows version 18.0).

Results

We screened 412 elderly and enrolled 337 (81.8%) who met the inclusion criteria for the trial and agreed to participate (Figure 1A). Most of the elderly who did not meet the inclusion criteria ($n = 66$) were excluded because they had exercised regularly for 6 months prior to the screening. Nine people who might have been eligible for the study declined after telephone screening. Of the 337 individuals who were enrolled in this study, 307 (91.1%) completed the 12-month

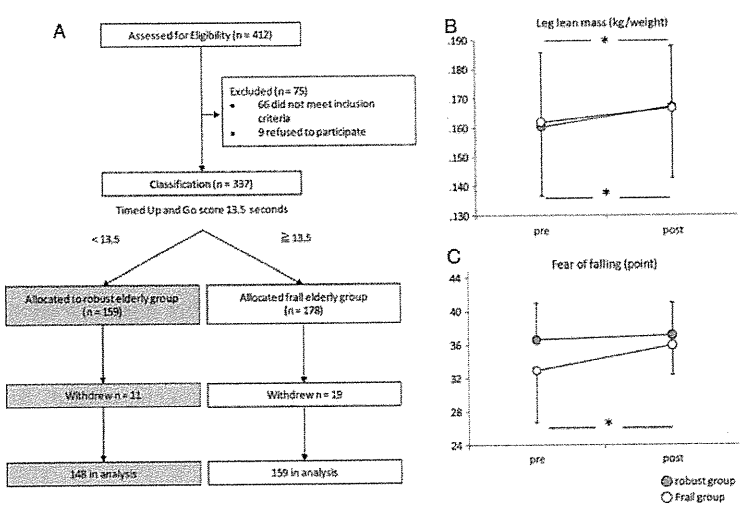

Figure 1. (A) Flow chart showing the disposition of participants throughout the trial. (B) LLM after resistance training in the robust and frail groups was significantly increased from baseline ($P < 0.05$). (C) The frail group had significantly greater improvements in fear of falling ($P < 0.025$).

intervention along with the second interview and the tests at the end of the study. Among them 148 in the robust group (93%) and 159 in the frail group (89%) completed the study.

All 100 scheduled intervention sessions were completed. The median relative adherence was 92% (25–75th percentile, 85–95%) for the robust group and 92% (85–95%) for the frail group. No health problems, such as cardiovascular and musculoskeletal complications, occurred during the training sessions or testing. Minor problems were observed in both groups such as aching muscles after the first training session and fatigue. All the problems were managed easily by adjustment of the intervention and were improved during subsequent interventions.

Effect of the resistance training on outcome measures

LLM after resistance training in the robust and frail groups was significantly increased from the baseline ($P < 0.05$) (Table 1, Figure 1B). Pre- and post-intervention group statistics and group × time interactions are summarised in Table 1. A statistically significant group × time interaction was observed for TUG, FR and fear of falling ($P < 0.05$) (Figure 1C). Bonferroni-corrected paired-sample t-tests demonstrated a significant effect of the resistance training on TUG, FR and fear of falling in the frail group ($P < 0.025$).

Discussion

In this study, we showed that LLM was improved by the resistance training programme in both groups. However, the effect on physical function was limited to frail elderly defined by TUG. The role of muscle strength on physical function is supported by numerous cross-sectional studies that have shown a strong association between low muscle strength and decreased mobility in elderly [18]. On the

Table 1. Functional fitness items by group at pre- and post-intervention

	Robust group ($n = 148$)		E/S	P-value[a]	Frail group ($n = 159$)		E/S	P-value[a]	P-value[b]	F-value 1. Time effect 2. Group × Time
	Mean	SD			mean	SD				
Age, years	75.4	7.7			76.1	8.3			0.440	
Height, cm	157.7	10.1			156.7	9.1			0.266	
Weight, kg	58.2	11.1			56.8	10.9			0.280	
Gender, female n (%)	74 (50.0%)				82 (51.5%)				0.436	
Fall incidence, n (%)	48 (32.4%)				77 (48.4%)				0.003	
Leg lean mass, kg/weight										
Pre	0.160	0.024	0.39	<0.001	0.162	0.024	0.27	0.002	0.448	32.1**
Post	0.167	0.024			0.167	0.021				1.1
Percent change, %	0.05	0.09			0.04	0.11				
Walking time, s										
Pre	10.0	1.9	0.11	0.294	16.1	3.8	0.16	0.130	0.017	1.1
Post	10.2	2.1			15.5	4.1				3.6
Percent change, %	0.3	15.5			−7.7	27.5				
Timed up and go test, sec										
Pre	9.9	1.8	0.09	0.374	17.4	3.0	0.32	0.004	0.002	6.1*
Post	10.1	2.5			16.1	3.9				10.5**
Percent change, %	0.9	18.1			−14.5	37.6				
One leg standing, s										
Pre	9.8	11.8	0.06	0.567	1.7	1.9	0.16	0.160	0.987	0.1
post	9.2	13.9			2.6	5.4				1.4
Percent change, %	−47.3	173.4			46.8	248.3				
Functional reach, cm										
Pre	23.5	5.9	0.01	0.948	18.0	5.6	0.46	<0.001	0.029	7.5**
Post	23.4	5.9			20.9	6.8				8.0**
Percent change, %	−7.2	46.4			23.6	48.1				
Five chair stand, s										
Pre	11.2	3.2	0.07	0.498	16.8	5.2	0.17	0.144	0.004	1.6
Post	11.5	4.7			15.1	8.6				3.1
Percent change, %	5.0	31.3			−29.9	72.8				
Fear of falling, points										
Pre	36.6	4.4	0.18	0.081	32.9	6.2	0.51	<0.001	<0.001	26.2**
Post	37.1	3.9			35.9	3.5				15.4**
Percent change, %	1.5	7.3			12.9	23.3				

E/S, effect size.

[a]As calculated by comparing pre- and post-intervention.

[b]As calculated by group comparison.

*$P < 0.05$.

**$P < 0.01$.

other hand, muscle strength does not depend solely on muscle mass, and the relationship between strength and mass is not linear [19]. Rantanen *et al.* reported that the relationship between muscle strength and physical disability in older adults is non-linear [20]. The discrepancy between these results may stem from the heterogeneity of subjects. In this study, we stratified subjects into robust and fail elderly groups. In frail elderly, the 50-week resistance training programme was effective for the improvement of LLM and physical performance. In contrast, there was no correlation between the change in LLM and physical performance in robust elderly undergoing the resistance training programme. These results suggested that our resistance training programme is not effective for the improvement of physical performance in robust elderly. Furthermore, resistance training improved muscle strength, but did not improve physical performance in the relatively healthy elderly [21]. On the other hand, in frail elderly, improvements in leg power, independent of strength, appear to make an important contribution to clinically meaningful improvements in physical performance [22].

Resistance training improved balance function, such as FR in frail elderly. Improved balance function with resistance training is hypothesised to occur by reduced motor-unit discharge variability [23]. However, SLS was not improved. These results suggested that balance improvement after power training may be explained, in part, by adaptations in force control. However, resistance training *per se* is not effective for balance function. For the improvement of balance function, it is useful to add not only the resistance training but also balance training, such as Tai Chi Chuan [24].

In addition to improving physical performance, the resistance training programme was effective for decreasing fear of falling, but only in the frail group. It is considered important to reduce fear of falling by targeting downstream factors such as physical functioning [25] or predictors of those factors [26]. Thus, our study has an important implication for the reduction in fear of falling in frail elderly.

There are several limitations to this study that warrant mention. First, although we used only TUG to define frailty, TUG may not be enough to define frailty. For example, the short physical performance battery evaluates balance, gait, strength and endurance by examining an individual's ability [27]. It has been recently recommended by an international working group to use a functional outcome measure in clinical trials in frail older adults [28]. Second, we did not measure muscle force. The relationship between LLM and muscle strength is still unclear and needs to be addressed in future studies. Third, no follow-up was conducted. Evidence regarding the long-term effect of exercise on fall prevention is limited, and, therefore, this issue also needs to be addressed. Finally, a control group was lacking. The participants in both groups may have had higher motivation and interest in health issues than the general elderly population.

This is the first study to demonstrate that the effects of a resistance training programme on physical performance differed according to the level of physical well-being. Future work should determine whether tailor-made interventions can effectively improve physical function in both robust and frail elderly.

Key points

- The current trial compared the effects of resistance training between robust and frail elderly on skeletal muscle mass, physical performance and fear of falling.
- Skeletal muscle mass after resistance training was significantly increased from the baseline in both groups.
- The resistance training programme was more effective for the improvement of physical performance and fear of falling in frail elderly than in robust elderly.

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Conflicts of interest

None declared.

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Transient ischaemic attack, vascular risk factors and cognitive impairment: a case–controlled study

SIR—Cognitive impairment, especially difficulties with temporal orientation and verbal recall, is associated with the increasing number and severity of vascular risk factors (VRFs) such as hypertension and diabetes [1–3] which can result in an associated impairment of the cerebral microcirculation causing white matter volume changes linked to large artery stiffness [4, 5]. These cognitive deficits can be detected by using simple standard screening tools [6] such as the Mini Mental State Examination [7], Montreal Cognitive Assessment (MoCA) [8] and the DemTec [9], and have been shown to be related to the development of both subclinical (mild) or established vascular disorders [7–12].

However, our understanding of the relation between transient ischaemic attacks (TIAs) and cognitive status is incomplete. We hypothesised that subjects with newly diagnosed TIA would have evidence of an associated mild cognitive impairment; this being a manifestation of the same pathological process underlying the pathogenesis of the vascular event being initiated and accelerated by VRFs. The aims of the current study were, therefore, (i) to examine whether patients with first ever TIA and no history of stroke have evidence of cognitive impairment and, if so, whether the extent of the impairment was greater than expected compared with an age-, sex-matched control populations without VRFs and (ii) to determine which VRFs are associated with cognitive impairment.

Methodology

We conducted a case–controlled study between August and November 2008 in a University Hospital in UK (catchment population 750,000). Cases were defined as those patients with first ever TIA aged ≥45 years, assessed in a

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Prostaglandin E2 receptor type 2-selective agonist prevents the degeneration of articular cartilage in rabbit knees with traumatic instability

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Abstract

Introduction: Osteoarthritis (OA) is a common cause of disability in older adults. We have previously reported that an agonist for subtypes EP2 of the prostaglandin E2 receptor (an EP2 agonist) promotes the regeneration of chondral and osteochondral defects. The purpose of the current study is to analyze the effect of this agonist on articular cartilage in a model of traumatic degeneration.

Methods: The model of traumatic degeneration was established through transection of the anterior cruciate ligament and partial resection of the medial meniscus of the rabbits. Rabbits were divided into 5 groups; G-S (sham operation), G-C (no further treatment), G-0, G-80, and G-400 (single intra-articular administration of gelatin hydrogel containing 0, 80, and 400 μg of the specific EP2 agonist, ONO-8815Ly, respectively). Degeneration of the articular cartilage was evaluated at 2 or 12 weeks after the operation.

Results: ONO-8815Ly prevented cartilage degeneration at 2 weeks, which was associated with the inhibition of matrix metalloproteinase-13 (MMP-13) expression. The effect of ONO-8815Ly failed to last, and no effects were observed at 12 weeks after the operation.

Conclusions: Stimulation of prostaglandin E2 (PGE2) via EP2 prevents degeneration of the articular cartilage during the early stages. With a system to deliver it long term, the EP2 agonist could be a new therapeutic tool for OA.

Keywords: prostaglandin E2, EP_2, ONO-8815Ly, osteoarthritis, ACLMT

Introduction

Osteoarthritis (OA) is the single most common cause of disability in older adults [1]. It is a complex process involving a combination of cartilage degradation, repair, and inflammation. However, its pathogenesis is not yet fully understood [2]. Articular cartilage is composed of chondrocytes, and an extensive extracellular matrix (ECM). The major ECM components are type II collagen and aggrecan. In normal cartilage, catabolic and anabolic activities are in dynamic equilibrium. Chondrocytes can produce several catabolic cytokines such as IL-1 and TNF-α, which in turn induce the production of proteinases including matrix metalloproteinases (MMPs) and disintegrin-like and metalloproteinase with thrombospondin, that lead to the destruction of the matrix network [3,4]. Among the MMPs, MMP-13 (collagenase 3) plays a particularly important role in causing OA [5]. Indeed, transgenic mice carrying an inducible human *MMP-13* gene develop pathological changes similar to those observed in human OA patients, when the transgene is expressed in articular cartilages of postnatal mice [6]. Moreover, inhibitors of MMP-13 prevent the degradation of articular cartilage [5,7]. Chondrocytes also produce anabolic cytokines such as the bone morphogenetic protein family members and insulin-like growth factor-1 (IGF-1), which induce the synthesis of collagen and initiate the proliferation of chondrocytes [3]. A disruption of

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