TABLE 5. CORRELATIONS OF MICROSTRUCTURAL PARAMETERS BY MDCT WITH AGE OR BODY WEIGHT

	Vs. age (years)		*Vs. body weight (kg)*	
	R^2	*p*	R^2	*p*
Microstructure parameters				
App BV/TV (%)	0.114	0.0005	0.029	NS
App Tb.N (1/mm^3)	0.122	0.005	0.147	0.0005
App Tb.Th (μm)	0.099	0.005	0.030	NS
App Tb.Sp (μm)	0.081	0.01	0.125	0.005
Structure model index	0.181	0.0001	0.048	0.05
Euler's number	0.185	0.0001	0.068	0.05
Fractal dimension	0.049	0.05	0.030	NS
Degree of anisotropy	0.005	NS	0.130	0.001
BMD				
Areal BMD by DXA (g/cm^2)	0.091	0.01	0.072	0.05
Volumetric BMD by MDCT (mg/cm^3)	0.118	0.005	0.001	NS

app BV/TV, apparent bone volume fraction; app Tb.N, apparent trabecular number; app Tb.Th, apparent trabecular thickness; app Tb.Sp, apparent trabecular separation; NS, not significant.

fracture.[21] A disadvantage of high-resolution MRI in vivo, however, is the relatively long acquisition time of up to 10 ± 20 minutes. A small FOV is required to obtain a high signal-to-noise ratio in the fast gradients with optimized coils. Because of these prerequisites and motion artifacts in the axial skeleton, application of high-resolution MRI is currently limited to peripheral sites such as phalanges, calcaneus, and distal radius.[19,20,25–27] Vertebral fracture is the most common osteoporotic fracture, and the presence of fracture indicates a greater risk for future fracture, independently of BMD.[28] The direct assessment of vertebral microstructure, as reported here, can be expected to provide greater sensitivity for assessing the risk of spinal fracture.

To our knowledge, there has been no report on in vivo analysis of microstructure by MDCT. Several specimen studies showed the capability to depict microstructure by MDCT in comparison with contact radiography[16] or μCT.[17] Although MDCT is the only available technique to analyze vertebral microstructure in vivo, high radiation exposure cannot be avoided. The radiation dose for DXA is small (0.08 ± 4.6 mSv). Fan beam DXA with increased resolution requires an increased radiation dose (6.7 ± 31 mSv), but this dose is smaller than that for QCT (25 ± 360 mSv).[29] In a study comparing radiation doses between single-detector row CT and MDCT, a 28% higher radiation dose was needed within the scanned volume for the latter at a constant noise level.[30] In this study, we evaluated the image quality of trabecular microstructure as a function of radiation exposure dose by using excised human vertebrae. Within the limits of clinically available radiation sets, MDCT at 300 mAs provided the best image quality and showed the highest correlation with the μCT data. As the reference level for CT examination of the abdomen (CTDIw) of adult patients is 35 mGy (according to European Commission 1999), the CTDIw for our current MDCT scanning at 300 mAs was 77.1 mGy. This radiation dose would be acceptable for a once-a-year study on postmenopausal women. On further advancement of the technology, a reduction in the radiation dose and higher resolution with a higher signal-to-noise ratio would be expected to make this method an even more useful diagnostic tool. In fact, the CTDIw for a new 16-detector row CT apparatus is 19.7 mGy, and advanced CT technology is expected to provide higher-resolution CT images. In the future, finite element analysis (FEA) may also be applied to 3D MDCT data for assessment of biomechanical properties,[31,32] and together this combination should provide a powerful tool for early evaluation of fracture risk.

In conclusion, 3D imaging of trabecular microstructure can be performed by using clinical MDCT at a high spatial resolution, and microstructure parameters derived from these images, especially those related to the shape of trabecular structures and connectivity, are more useful than spinal DXA for the assessment of fracture risk.

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Ataxia telangiectasia mutated (*Atm*) knockout mice as a model of osteopenia due to impaired bone formation

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Abstract

ATM is a member of the PI-3 kinase protein family, encoded by the gene, *ATM*, responsible for ataxia telangiectasia (AT). AT is recognized as a genomic instability syndrome, sharing accelerated senescence symptoms in human and mouse. Here, we present evidence that the bone phenotype of *Atm* knockout (AtmKO) mice is similar to that observed in disuse and/or aging syndromes. A significant decrease in 3-dimensional bone volume fraction (BV/TV) of the fifth lumbar vertebra was observed in AtmKO mice by μCT, compared with heterozygous control mice at 10 weeks of age. Bone histomorphometry revealed that both BFR/BS and Oc.S/BS were significantly decreased in KO mice. To determine the cellular basis of this bone phenotype, we employed in vitro osteoclastogenesis and colony formation assays using bone marrow cells derived from KO and control mice. There was no difference in osteoclast formation in ex vivo cultures. CFU-F was markedly reduced in AtmKO-derived cultures compared with control mice, whereas differentiation of calvaria-derived osteoblasts did not differ between the genotypes. Furthermore, expression levels of IGF1R were significantly decreased, and p38 was aberrantly phosphorylated in marrow stromal cells from AtmKO mice. These results indicate that the pathogenesis of the osteopenic phenotype in AtmKO mice is similar to that of disuse and/or aging syndromes and is caused, at least in part, by a stem cell defect due to lack of IGF signaling.

Keywords: Premature aging syndrome; Animal models; Mesenchymal stem cells; Knockout

Introduction

Much progress has been achieved in our understanding of the pathogenesis of postmenopausal osteoporosis, characterized by accelerated bone resorption [1,2]. On the other hand, the pathophysiology of senile osteoporosis, caused mainly by declining bone-forming capacity, remains an enigma due to the lack of suitable animal models. Although aged (2 to 3 years old) animals are good candidates, natural aging is a complex phenomenon involving a plethora of factors and is difficult to dissect at the molecular level. Recently, genetically engineered mouse models for studying aging and age-related disorders have been developed [3]. Since they are caused by single gene mutations, they provide valuable tools for studying the pathogenesis of senile osteoporosis at the molecular level. For example, osteopenia with reduced bone formation and resorption has been reported in *klotho* (*kl/kl*) and mutant p53-expressing mice [4,5].

Ataxia telangiectasia (AT) is a human premature aging syndrome characterized by neurodegeneration, immune defects, tumor formation, hypersensitivity to ionizing radiation, and genomic instability [6]. The responsible gene, *ATM* (for AT mutated), is a large protein kinase that belongs

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to the PI-3 kinase family [10,11]. ATM functions in DNA damage checkpoint and oxidative stress responses, thereby playing a central role in the maintenance of genome stability. Mouse models for AT have been generated by knockout of the mouse Atm gene [7–9]. AtmKO mice exhibit radiosensitivity, genomic instability, growth retardation, and lymphoma, recapitulating main features of human AT [7–9]. The bone phenotype, however, has not been defined. Interestingly, mice deficient in Abl, a downstream protein kinase effector of ATM, exhibit an osteopenic phenotype with reduced bone formation [27]. We report here that AtmKO mice show osteopenia as early as 10 weeks of age, when growth retardation is not apparent. Histomorphometric and biochemical analyses revealed impaired bone formation, which may be caused by limited proliferative potential of osteogenic progenitors. Thus, AtmKO mice may provide a suitable model for studying senile osteoporosis.

Materials and methods

Mice

AtmKO mice (129/SvEv-*Atm*^{tm1Awb}) were obtained from Jackson Labs (Bar Harbor, Maine, USA). All generations were from matings of heterozygous parents. Genotyping was performed as described [9,12] with the exception of the primers used to detect the wild type allele. The primer sequences used for genotyping were as follows:

oIMR640, 5′-GCTGCCATACTTGATCAATG-3′
oIMR641, 5′-TCCGAATTTGCAGGAGTTG-3′.

The sequences used were recommended by Jackson Labs. All animal experiments were approved in advance by the Ethics Review Committee for Animal Experimentation of the National Institute for Longevity Sciences and the National Center for Geriatrics and Gerontology.

Bone morphological and histomorphometric analyses

Lumbar vertebrae and tibias were obtained from 6-, 10-, and 14-week-old female and male mice and subjected to morphological analyses ($n = 3 \sim 6$). Microcomputed tomography (μCT) and bone histomorphometry of the vertebrae and tibias, respectively, doubly labeled by calcein, were performed as described previously [13].

CFU assay

Colony forming unit (CFU) assays were conducted according to Jilka et al. [14]. Briefly, bone marrow cells were obtained from femurs or tibias and seeded at 1.5×10^6 (for CFU-F/CFU-ALP) or 2.5×10^6 (for CFU-Ob or CFU-Adip) cells/well in a 6-well plate. For CFU-F and CFU-Ob, the marrow cell cultures were maintained in phenol red-free αMEM containing 15% FCS and 1 mM Asc-2-P; one-half of the medium was replaced every 5 days. For CFU-Adip, the cultures were maintained in phenol red-free αMEM (Invitrogen, Carlsbad, California, USA) containing 15% FCS and MDI (0.5 mM methylisobutylxanthine, 1 μM dexamethasone, 1 μg/ml insulin) for 25–28 days. The cells were cultured for 10 days and then stained for ALP and counterstained with hematoxylin. Colonies of cells containing a minimum of 20 cells were designated as CFU-F, and those positive for ALP activity as CFU-ALP. For CFU-Ob, the cells were maintained for 25–28 days, fixed in 50% ethanol and 18% formaldehyde, and then stained using 2% Alizarin Red. The oil drops in the adipocytic cells were stained by Oil Red-O.

Osteoblastic cell cultures

Osteoblastic cells were isolated from calvarias of neonatal (P2–P3) AtmKO or wild-type mice following the protocol described by Jochum et al. [15] with minor modifications. For alkaline phosphatase (ALP) staining, osteoblastic cells were cultured in 24-well tissue culture plates. After reaching confluency, medium was supplemented with 60 μg/ml ascorbic acid and 10 nM dexamethasone and cultured for 7 more days. ALP staining was performed using a leukocyte alkaline phosphatase staining kit (SIGMA Diagnostics, St. Louis, USA). For ALP activity measurement, cells from the same conditions described above were washed with PBS and sonicated in RIPA buffer (50 mM Tris–HCl (pH 7.5) containing 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate). ALP activity in the lysate was measured using an ALP activity measurement kit (Wako Pure Chemical Industries, Osaka, Japan). The protein content was determined using BCA protein assay reagent (Pierce Chemical Co., Rockford, Illinois, USA).

M-CSF-dependent cell proliferation assay

Bone marrow cells were isolated from AtmKO or control mice, and the erythrocytes in the collected cells were depleted by standard ammonium-chloride lysis. To assess M-CSF-dependent proliferative response, cells (3×10^4) were cultured in flat-bottom 96-well plates for 2 days with various amounts of M-CSF (R&D Systems, Minneapolis, USA). Cultures were pulsed for 24 h with 1 μCi/well of [^{3}H]thymidine, harvested on glass-fiber filters, and the incorporated radioactivity was determined using a beta counter.

In vitro osteoclastogenesis assay

Bone marrow cells were isolated from AtmKO or control mice and incubated in tissue culture plates with αMEM containing 10% FCS. After 4 h in culture, nonadherent cells

were collected and counted. 5×10^5 cells were cultured for 3 days in 24-well tissue culture plates with 10 ng/ml M-CSF and for an additional 3 days in the presence of both 10 ng/ml M-CSF and 10 ng/ml RANKL (R&D Systems). Adherent cells were then fixed with an acetone–citrate–formalin solution (65:27:7) and stained for TRAP using a leukocyte acid phosphatase kit (SIGMA Diagnostics). The number of TRAP-positive multinucleated cells containing more than three nuclei was counted as osteoclasts.

Immunoblot analysis

Adherent cells from bone marrow of AtmKO or control mice were lysed in RIPA buffer containing 2 mM phenyl–methyl–sulfonyl fluoride, 10 mM sodium fluoride, 2 mM sodium vanadate, and proteinase inhibitor cocktail (Complete™, Roche Diagnostics, GmbH Mannheim, Germany). The cell lysates (15 μg each of protein) were subjected to SDS-PAGE, transferred, and then detected with antibodies using ECL-Plus (Amersham-Pharmacia). Anti-p38 (sc-535), anti-IGF1R (sc-713), and anti-c-Abl (sc-131) antibodies were purchased from Santa Cruz Biotechnology, California, USA. Anti-phosphorylated p38 (9211) and anti-tubulin (T5168) were obtained from Cell Signaling Technology (Beverly, Massachusetts, USA) and SIGMA, respectively.

IGF-dependent cell proliferation assay

Bone marrow cells were obtained as described above. The adherent cells were harvested and replated at a density of 5×10^4 cells/well in 96-well plates. After 24-h serum deprivation, the cultures were labeled with [^{3}H]thymidine with or without human recombinant IGF-I (10 ng/ml; Invitrogen). The incorporated [^{3}H] was measured as described.

Statistical analysis

Data are presented as mean ± SD. All data were analyzed by Student's *t* test. Statistical significance was considered at $P < 0.05$, unless otherwise indicated.

Results

Osteopenia due to impaired bone formation in AtmKO mice

To characterize the bone phenotype of Atm homozygous knockout mice with heterozygous littermates as controls, μCT scanning technique was employed. We analyzed 6-, 10-, and 14-week-old animals. At 6 weeks of age, there was no significant decrease in bone mass of AtmKO mice (data not shown). Since ovarian defect, which was possible to affect bone metabolism, was observed in female KO mice [7–9], we focused in the structural data from male animals. As shown in Figs. 1A and B, 3-dimensional bone volume (BV/TV) of the lumbar vertebrae was markedly decreased in AtmKO mice at 10 weeks of age. At this age, there is no significant difference in body weight between AtmKO and littermate control mice (24.8 ± 1.6 versus 21.5 ± 2.4 g). The trabecular number (Fig. 1C) and thickness (Fig. 1D) were also reduced in AtmKO mice compared to control. The difference between KO and control mice was more pronounced at age of 14 weeks, when some KO animals became cachexic.

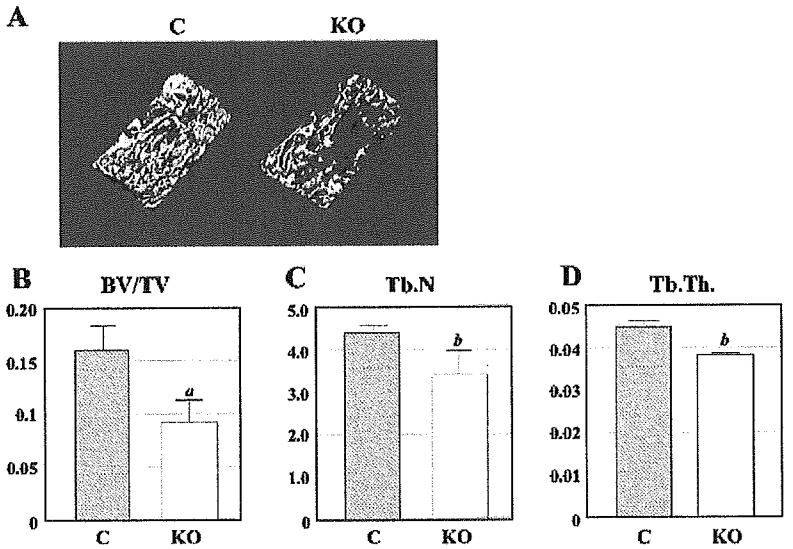

Fig. 1. Micro-CT analysis of lumber vertebrae of AtmKO mice. The fifth lumbar vertebra from heterozygous (C) and knockout (KO) mice were subjected to μCT analysis at 10 weeks of age. (A) Representative 3D images of trabecular architectures are shown. Note that the osteopenic phenotype was observed in knockout mice. (B) Trabecular bone volume fraction (BV/TV); (C) trabecular number (Tb.N); (D) trabecular thickness (Tb.Th.). The data were obtained in male mice. $^aP < 0.005$ and $^bP < 0.05$, significantly different from the respective heterozygous control group.

Bone histomorphometric analysis of the proximal tibia showed that bone formation rate (BFR/BS) and the osteoblast surface (Ob.S/BS) were significantly decreased in both female and male AtmKO mice (Fig. 2). The osteoclast surface (Oc.S/BS) was also reduced in the KO mice, suggesting that both bone formation and resorption were suppressed in AtmKO mice and that the observed osteopenic phenotype was caused mainly by impaired bone formation.

Cellular basis of impaired bone formation

To determine whether impaired bone formation was due to a cell autonomous defect, in vitro osteoblastogenesis assays were performed. The number of CFU-F (Fig. 3A), CFU-ALP (Fig. 3B), and CFU-OB (data not shown) were significantly reduced in the bone marrow of AtmKO mice. The decrease in CFU-F, which reflects the number and/or proliferative potential of mesenchymal progenitors, suggested that the defect was not specific to the osteoblastic lineage but involved other lineages as well. In fact, the number of CFU-adipocyte was also reduced (data not shown). We performed osteoblastogenesis assays in the presence of *N*-acetyl cysteine (NAC) to reduce oxidative stress, which often affects cell proliferation and induces senescence of primary cultured murine cells. As shown in Figs. 3A and B, supplementation with NAC increased the number of CFU-F and CFU-ALP in bone marrow cultures from control mice, but not in cultures from AtmKO mice. Notably, the ratio of CFU-ALP to CFU-F was not markedly altered between the genotypes, suggesting that differentiation, especially the ability to differentiate into osteoblasts, was not impaired in AtmKO mice.

To address the question whether the absence of Atm affected the differentiation process, osteoblasts were isolated from calvarias of homozygous and heterozygous knockout mice and cultured in vitro. As shown in Fig. 3C, calvaria-derived osteoblastic cells differentiated at comparable levels, suggesting that the differentiation potential of osteoblasts was not impaired in AtmKO mice. Together, with results from the CFU assays, these data collectively suggested that the impaired bone formation was not due to a defect in the differentiation program of osteoblasts per se but to the proliferative potential of mesenchymal progenitors.

As for the hematopoietic lineage, M-CSF-dependent proliferation of bone marrow cells from AtmKO mice did not differ from that of control mice (Fig. 4A). When osteoclastogenic potential was assessed in bone marrow cultures with M-CSF and RANKL, neither the number of TRAP-positive multinucleated cells formed (Fig. 4B) nor their morphology (Fig. 4C) differed between the genotypes.

Reduced IGF1 receptor with activation of p38 in Atm-deficient marrow stromal cells

To gain some insight into the molecular mechanism underlying the impaired proliferation of mesenchymal progenitors in AtmKO mice, the expression of molecules involved in ATM signaling was analyzed by immunoblotting. As shown in Fig. 5 (right lane), the level of phosphorylated p38MAPK (P-p38) was elevated in marrow stromal cells derived from AtmKO mice, while the total amount of p38 in the experimental and control cultures did not differ. By contrast, the expression of IGF-I receptor (IGF1R) was markedly reduced in AtmKO-derived marrow stromal cells compared with control cells (Fig. 5A). The level of Abl, a downstream effector molecule involved in the ATM pathway, did not differ between the genotypes. Next, we determined whether the IGF response was impaired in cells derived from AtmKO mice. As shown in Fig. 5B, control cells responded to IGF with increased proliferation, whereas the response to IGF was severely impaired in the stromal cells from AtmKO mice, suggesting that a decline in the expression level of IGF1R causes IGF-dependent proliferation in AtmKO mice.

Discussion

In the present study, we demonstrate that AtmKO mice show reduced bone formation due to a proliferative defect in

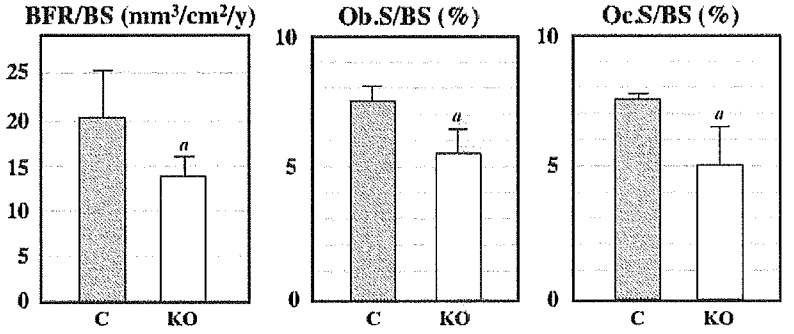

Fig. 2. Bone histomorphometric analysis of AtmKO mice. Decreases in bone formation rate (BFR/BS, left), osteoblast surface (Ob.S/BS, center), and osteoclast surface (Oc.S/BS, right) were measured from the proximal tibias of male AtmKO (KO) mice and compared with heterozygous (C) littermate controls. Data expressed are the means ± SD. $^{a}P < 0.05$, significantly different from the respective heterozygous control group.

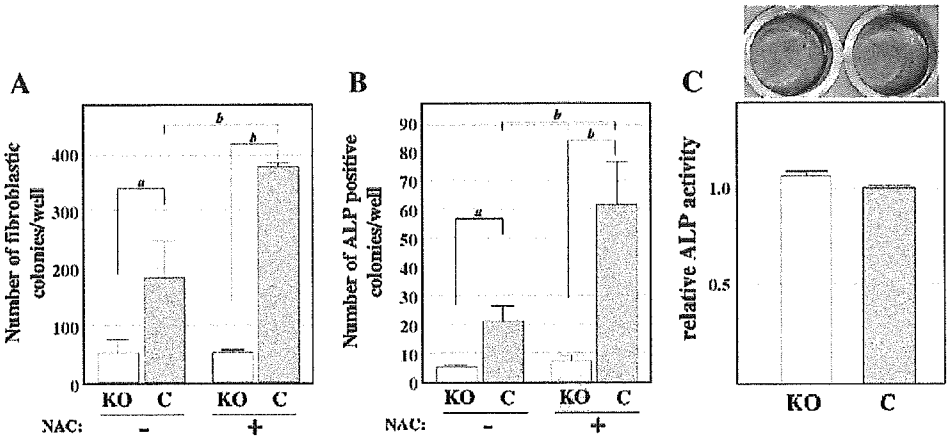

Fig. 3. Colony formation assay of bone marrow cells and calvarial osteoblast differentiation. (A) CFU-F in bone marrow cells derived from AtmKO (KO) or littermate wild-type or heterozygous control (C) mice ($n = 3\sim5$) are shown. (B) CFU-ALP. Note the significant reductions in both CFU-F and CFU-ALP ($P < 0.05$, KO versus control). *N*-acetyl cysteine (NAC, 10 mM) improved the numbers of CFU-F and CFU-ALP in control but not in cells from AtmKO mice. (C, upper panel) A representative photograph of the wells stained for ALP activity. Lower panel, relative ALP of calvarial cell cultures. No significant difference in morphology or ALP activity was observed between knockout (KO) and controls (C). Data represent the means ± SD. $^{a}P < 0.05$ and $^{b}P < 0.005$, significantly different from the respective control group. No significant difference was detected in the presence or absence of NAC in KO cells either in CFU-F or CFU-ALP assays.

mesenchymal progenitor cells, in addition to other age-related disorders observed in the human syndrome, AT, including neurodegeneration, immune defects, tumor formation, and genomic instability [7–9]. It has been suggested that, in mice as well as in humans, age-related decline in osteoblast number and function is caused by a decrease in number and proliferative potential of mesenchymal stem cells [16–19]. AtmKO mice recapitulate these cellular defects as early as 10 weeks of age. At this age, there are no signs of lymphoma or severe growth retardation in KO mice [7], suggesting that the bone phenotype is not caused by the secondary effects of those defects. The difference in bone mass between genotypes was more pronounced with age. The loss of bone mass associated with age may be accelerated by other abnormalities, such as growth retardation, lymphoma, immune defects, and cachexia. Importantly, we have shown in this study that the bone marrow cells exhibited a cell autonomous defect in proliferation. Therefore, it is suggested that the impairment in bone formation with a proliferative defect in bone marrow stem cells causes the osteopenic phenotype in AtmKO mice, although we cannot exclude the possibility that the defects such as T-lymphopenia and hypogonadism affected the bone phenotype of more aged mice. The microstructural alterations in the lumbar vertebrae of AtmKO mice are also similar to those observed in naturally aging C57BL/6J mice [20]. Thus, AtmKO mice may provide a suitable model to study the pathophysiology of senile osteoporosis without the necessity of raising them for 2 to 3 years.

Recently, Bonyadi et al. reported that Sca1 KO mice develop an age-dependent osteopenia [21]. Sca1 KO mice exhibit a decrease in the number of osteoprogenitors as well

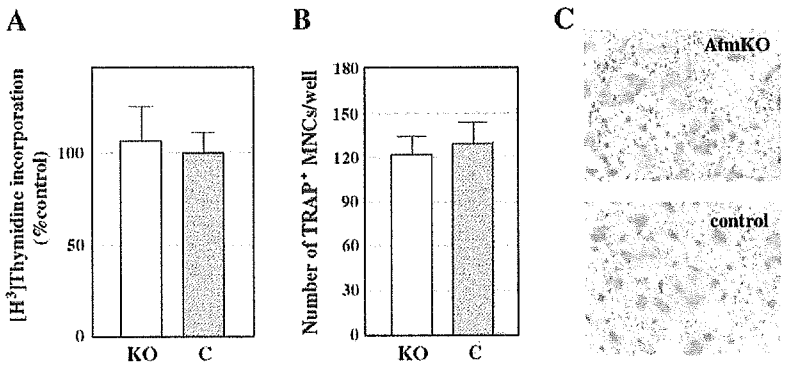

Fig. 4. M-CSF-dependent proliferation and in vitro osteoclastogenesis of bone marrow cells. (A) No obvious difference in M-CSF-dependent [^{3}H]thymidine incorporation into bone marrow cells was detected. (B) The number of multinucleated TRAP-positive cells derived from bone marrow cells in the presence of RANKL and M-CSF. No significant difference between the cells from AtmKO (KO) and littermate control (C) mice was observed. (C) TRAP staining of the in-vitro-generated multinucleated cells.

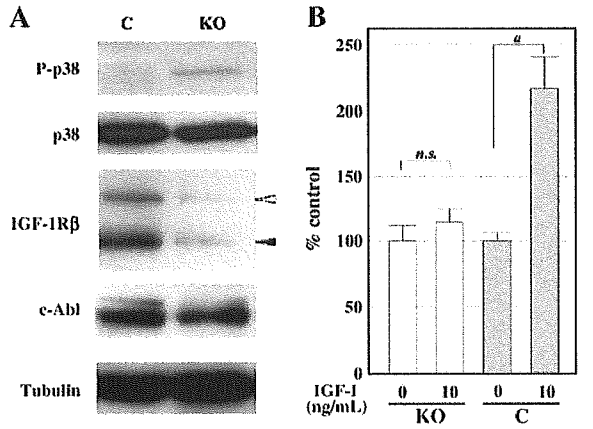

Fig. 5. Expression analysis of IGF-1 receptor in bone marrow cells. (A) Total cell lysates of bone marrow stromal cells from wild-type (C, left lane) or Atm knockout (KO, right lane) were subjected to immunoblot analyses. Top panel (P-p38), activated (phosphorylated) p38. Second panel (p38), total p38 MAP kinase. The activated p38 is only detected in AtmKO. These two panels were from a membrane blot. Third panel (IGF-IRβ), probed with antibody against the insulin-like growth factor receptor β subunit. The upper (open arrowhead) and lower (closed arrowhead) bands correspond to unprocessed and processed β subunit protein, respectively. Fourth panel (c-Abl) shows expression level of c-Abl protein. The bottom panel, probed with anti-tubulin antibody to control for protein loading in the last three panels (IGF-IRβ, c-Abl). (B) IGF-I response. Bone marrow stromal cells from Atm knockout (KO) or wild-type (C) were cultured with or without IGF-I. Incorporated thymidine was measured and displayed as fold change (%) compared to culture without IGF for each genotype. $^{a}P < 0.005$; n.s., not significant.

as CFU-F and have a non-cell autonomous defect in osteoclasts [21]. Although defects of osteoblasts and osteoclasts are similarly observed in AtmKO mice, the expression of Sca1 mRNA was not altered in AtmKO mice (data not shown). Additionally, the onset of the bone phenotype in Sca1 KO (~12 months of age) was very late, compared with that of AtmKO mice (~10 weeks). Thus, the impact of ATM deficiency on bone formation was more severe than that of Sca1 deficiency.

It has been reported that AtmKO mice showed reduced numbers of leukocytes in the circulation as well as bone marrow [7–9,22]. Although osteoclast surface was reduced in the bone of AtmKO mice, osteoclastogenic potential as well as M-CSF-dependent cell proliferation in vitro was normal, pointing to the involvement of impaired ability of the marrow environment to support proliferation/differentiation of osteoclast progenitors rather than cell autonomous dysfunction in the hematopoietic lineage. Concerning the osteoblastic lineage of AtmKO mice, the defect lies mainly in CFU-F, while the differentiation of osteoblasts was rather normal. Thus, it is tempting to speculate that multi-potent stem or progenitor cells are more sensitive to Atm deficiency than are differentiated cells. As we did not find any difference in the expression of Jagged-1 (data not shown), one of the key molecules that function in the bone marrow niche [23], the molecular defect in niche function of AtmKO mice remains to be clarified.

Chk2 is a checkpoint kinase that functions downstream of ATM and phosphorylates p53 to stabilize it [11,24]. Soft X-ray analysis of bones from Chk2 KO mice [25] revealed no alteration in bone density (AH et al., unpublished observation). It has been reported that the bones of mice lacking p53, the target of ATM and Chk2 kinases, were normal, although they were resistant to bone loss in response to unloading [26]. Thus, it is unlikely that the Chk2 pathway is involved in the impaired bone formation in AtmKO mice.

It has been reported that mice lacking Abl, another downstream kinase of ATM, exhibit an osteopenic phenotype due to reduced bone formation, which is caused mainly by a defect in osteoblast differentiation [27]. In AtmKO mice, by contrast, osteoblastic differentiation is normal. Li et al. have recently demonstrated distinct roles for ATM and Abl in the oxidative stress response and that ATM and Abl differentially regulate osteoblast expression of PKCδ [28]. We demonstrated that the improved colony formation following supplementation with NAC was diminished in AtmKO-derived cultures. Thus, the possibility exists that irreversible damage by oxidative stress had already altered the proliferative potential of AtmKO-derived progenitor cells or that an additional molecular defect(s), other than the oxidative stress response, is involved.

IGF plays important roles in bone growth and metabolism [29–31]. This has been supported by genetic approaches in mice [32–35]. *ATM* deficiency downregulates the transcription of the *IGF1R* gene [36], and we found, in fact, that the expression of IGF1R, as well as the responsiveness to IGF, was decreased in marrow stromal cells of AtmKO mice. These results indicate that defective IGF signaling may be involved in the impaired bone formation observed in AtmKO mice. In addition, since serum deprivation induces oxidative stress, due mainly to loss of IGF [37], the connection between IGF activity and oxidative stress may be an interesting relationship warranting further investigation.

In conclusion, we demonstrate that AtmKO mice present an osteopenic phenotype as early as 10 weeks, apparently due to decreased bone-marrow-derived mesenchymal progenitors. This decline in mesenchymal progenitors may be the result of decreased expression of IGF1R, an important regulator of proliferation. The AtmKO mouse model may be a useful tool for further studies of senile osteoporosis.

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