Animal Models for Aging Bone

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Introduction

Age-related decline in bone mass is a universal phenomenon among laboratory mammals. Research on aging has been conducted using various models from yeast and nematode to mouse and non-human primates, and has rapidly progressed due to the recent development of forward and reverse genetics, as well as functional genomics. Some mouse models bearing artificially or naturally modified genes develop bone phenotypes with various pathologies. Among those mice, some are considered to be potent models for understanding the pathophysiology of age-related bone loss and osteoporosis in humans. Here, available models for the study of age-related bone loss and osteoporosis are introduced and discussed.

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Normally Aged Animal Models

Besides mice and rats, studies of osteoporosis in guinea pigs, rabbits, cats, dogs, and pigs have been reported. And while, compared to mice and rats, some of those evaluated are considered to be better models relatively to humans in terms of similarity in estrus cycles or Haversian remodeling, the number of studies is quite limited. Studies in non-human primates, such as monkeys, have been considered to be the best and most relevant in terms of human skeletal structure and metabolism [1–6]. While breeding cost and ethical considerations are the highest among the animal models, therapeutic trials in non-human primate models are considered the most informative relative to humans. An age-dependent bone loss in these animals has also been well described. On the other hand, primary screening of candidate anti-osteoporotic compounds has been tested more often in rat than mouse models, probably because they have relatively more bone mass and an overall better response to ovariectomy (OVX). As observed in humans, decreased bone marrow cellularity and increased adiposity, as well as an age-related decline in bone mass, are apparent in rodent models of aging (Fig. 6.1). Laboratory mice usually live for 2–3 years and show a peak bone mass at 4–8 months of age followed by declining bone mass as they age.

A popular laboratory mouse strain, C57BL/6, develops a senile osteoporosis-like bone phenotype with decreased bone mass and quality [7–14]. Both

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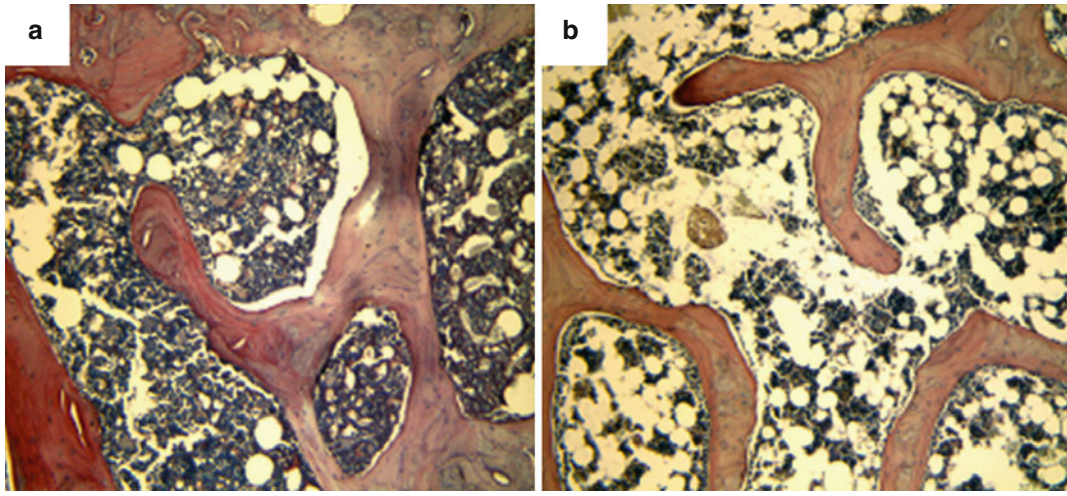

Fig. 6.1 A comparison of bone marrow between young and old rats. The figure shows remarkably higher levels of bone marrow fat (*white areas*) in a 24-month-old rat (**b**) as compared with a 4- month-old rat (**a**). In addition, the trabecular thickness is reduced in the old rat, as is the amount of hematopoietic tissue (Adapted from Duque [119])

trabecular and cortical bones suffer dynamic changes upon aging in these mice. Two studies [7, 8] have assessed the age-related changes in the bone of C57BL/6 J male mice. Mice were aged between 4 and 104 weeks. In young mice, rapid growth was marked by substantial increases in bone size, mineral mass, and mechanical properties. Maturity occurred between 12 and 42 weeks of age with the maintenance of bone mass and mechanical properties. From peak levels, mice aged for 104 weeks experienced decreased whole femur mass (12.1 and 18.6 % for dry and ash mass, respectively), percentage mineralization (7.4 %), diminished whole bone stiffness (29.2 %), energy to fracture (51.8 %), and decreased cortical thickness (20.1 %). Indices of surface-based formation decreased rapidly while the periosteal perimeter and, consequently, the cross-sectional moments of inertia continued to increase through 104 weeks, thus maintaining structural properties. This compensated for cortical thinning and increased brittleness due to decreased mineralization and stiffness. The shape of the mid-diaphysis became increasingly less elliptical in aged mice, and endocortical resorption and evidence of subsequent formation were present in 20–50 % of femurs aged $\geq$ 78 weeks. This, combined with the appearance of excessive endocortical resorption after 52 weeks, indicated a shift in normal mechanisms regulating bone shape and location, and was suggestive of remodeling. The authors concluded that the pattern of bone loss at the femoral mid-diaphysis was markedly similar to that seen in cortical bone in the human femoral neck in age-related osteoporosis.

Interestingly, expression of RANKL, also known as osteoclast differentiation factor, is increased upon aging and correlates with cancellous bone volume [9, 10]. On the other hand, bone marrow hematopoiesis is often affected by aging [11–14]. C57BL/6 is known to develop clonal B cell expansion and lymphoma frequently in this aging mouse strain [13, 14], suggesting that age-related, strain-specific hematopoietic disorganization, such as lymphoma, largely affects bone metabolism and bone resorption in particular. Furthermore, in another common strain, BALB/c, osteogenic stem cells from 24-month-old mice exhibit a decrease in proliferative potential upon aging [11]. It is suggested that the age-related bone loss in this model is caused by decreased osteogenic potential due to both quantitative and qualitative declines, especially in stem cell function (Figs. 6.2, 6.3, and 6.4).

In mature rats (from 8 to 36 months of age), the only change reported in bone structure is an increase in the cross-sectional moment of inertia (distribution of the bone around the central axis), due to the expansion of the outer diameter

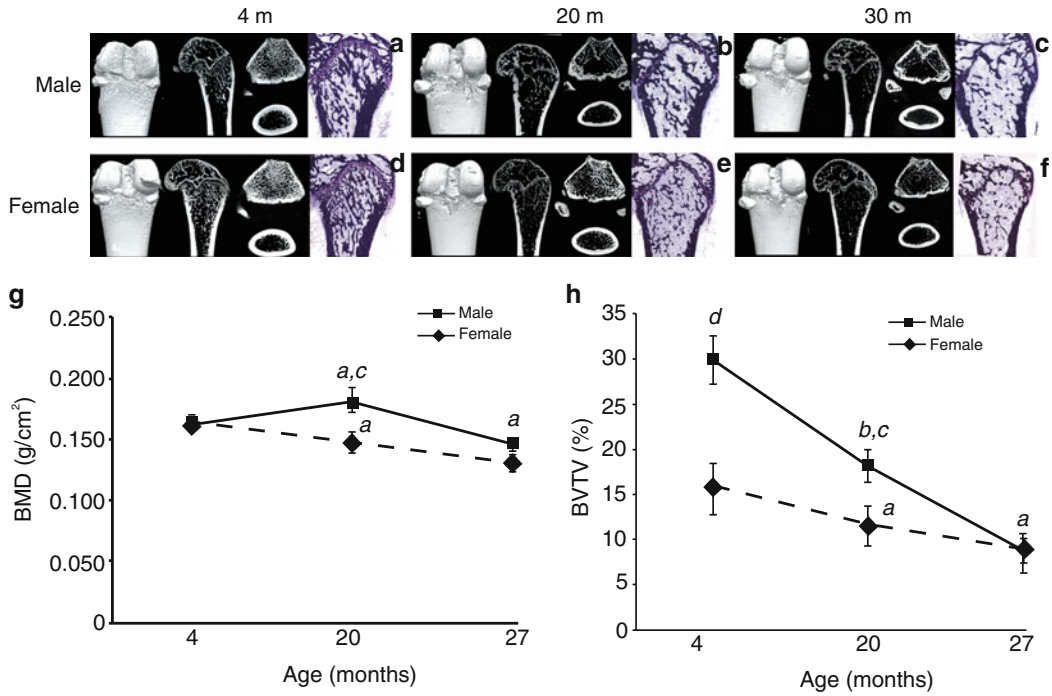

Fig. 6.2 MicroCT analysis (**a**–**f**) to evaluate bone structure and sections of undecalcified bone stained with von Kossa (**a**–**f**, *right panels*) (magnification × 10) to evaluate mineralized tissue (*black*) and fat volume (*white*). The figure shows three-dimensional images of the trabecular bone and coss-sectional images of the cortical bone from rats aged 4, 20 and 30 months (**a**–**f**). The loss in bone volume, the reduction in both trabecular bone and cortical thickness and the increasing cortical porosity with age are visually apparent. Age-related changes in bone mineral density (*BMD*) (**g**) and bone volume/trabecular volume (*BV/TV*) (**h**) showed a significant decline in both groups matching similar levels of bone mass and bone quality at 30 months of age. $^{a}p<0.01$, $^{b}p<0.001$ compared with 4 months, one-way ANOVA and Dunnett's test; $^{c}p<0.01$, $^{d}p<0.001$ males vs. females (From Duque et al. [19])

(periosteal deposition) of their bones, with a thinning of the cortical walls (endosteal resorption) [15]. Other study [16, 17], performed in trabecular bones in proximal tibias of 23-month-old and 5-month-old rats, found that mineral density, bone volume fraction, and trabecular number were significantly reduced in the aged rats compared with the younger rats. In addition, serum markers of bone formation were also reduced in the older rats [17, 18]. Interestingly, a model of "healthy-aging" rats known as LOU rats show the typical features of age-related osteoporosis – including high levels of bone marrow fat – despite their longer life span and low prevalence of diabetes and cancer [19].

Overall, the "normally aged" animal model of osteoporosis has the advantage of closely mimicking the age-related changes in bone. However, disadvantages include the high cost of maintaining a normally aging colony, the variability between different strains in terms of peak of bone mass and levels of bone turnover, and the fact that steroid hormones could play an important role in the levels of bone turnover during the post-menopausal months, which also vary from strain to strain.

Genetic Manipulation and Accelerated Aging

Development of genetic approaches to mimic osteoporosis is becoming a common practice worldwide. Genetic manipulation has been instrumental to our rapidly expanding knowledge of the molecular and cellular mechanisms underlying both normal and pathological bone biology. This methodology, which is more easily applied

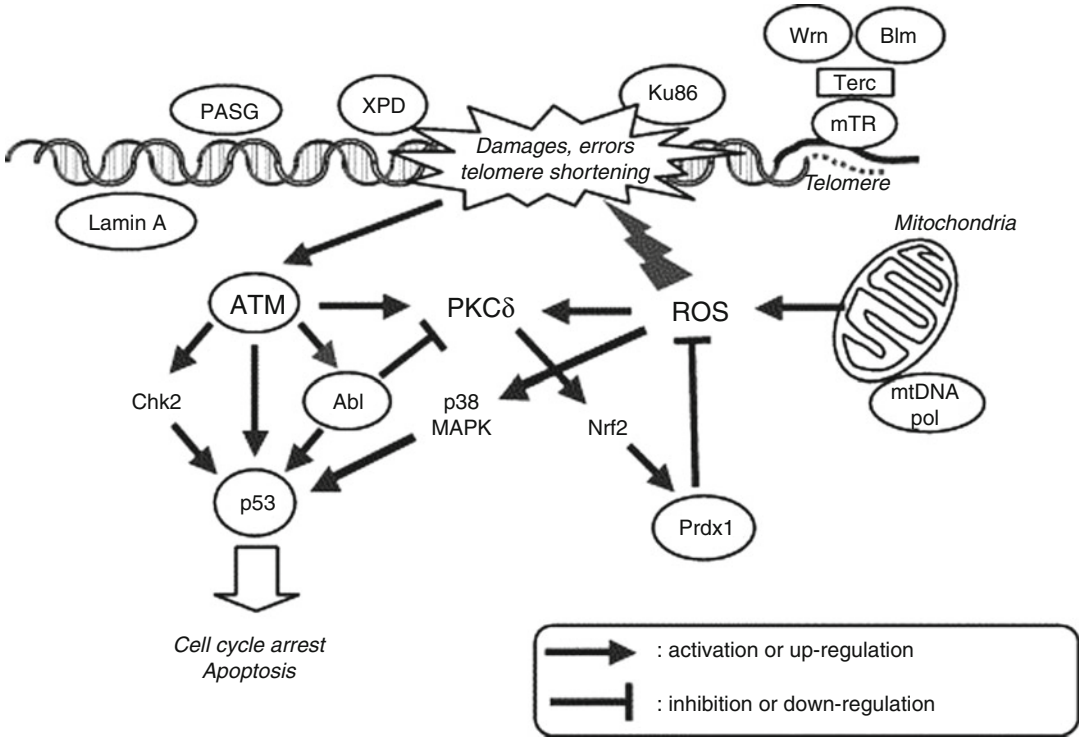

Fig. 6.3 Predicted pathways connecting the gene products responsible for the premature aging mutant phenotype. Most of the mouse models for premature aging described by now are caused by mutations in the genes involved in genomic integrity and subsequent cell cycle regulation. Errors and damage to the genome or telomere shortening, which also affects DNA integrity could, in theory, be detected and corrected. Mutations in the genes responsible for genomic stability cause accumulation of phenotypic abnormalities. Genomic disorganization activates cell cycle-regulating pathways involving checkpoint kinases and p53. Oxidative stress is among the triggers that elicit genomic instability via DNA damage. Elevation and excess of ROS affect downstream signaling, including PKCδ which subsequently stimulates the anti-ROS pathway, including transcriptional activation of Prdx1 (From Watanabe and Hishiya, Ref. [118]. With permission from Elsevier)

to mice, includes the targeted manipulation and ablation *in vivo* of one or several genes.

Some of the genetically modified mice recently developed by knockout or transgenic techniques show premature aging phenotypes. The clearest conclusion to be drawn from these models is that single gene mutations cause multiple aging phenotypes. This advantage is useful in defining the mechanisms regulating bone metabolism.

Amongst those mouse models, the senescence accelerated mice (SAM) have been established by Takeda et al., and accepted as suitable models for aging [20]. The SAM lines, derived from a mouse strain AKR/J, are divided into two classes; SAM-P lines exhibit an accelerated aging phenotype with shortened life-span, and SAM-R lines, which show a less accelerated phenotype than that of SAM-P. The aging phenotype of SAM-P lines becomes apparent at 6–8 months of age. Among the SAM lines, SAM-P6 has been demonstrated to be a correlative model for age-related osteoporosis in humans [21–24], and its bone phenotype has been well described. For example, Jilka et al. [21] demonstrated that the osteopenic phenotype in SAM-P6 is caused by reduced osteoblastogenesis and that their bone metabolism is resistant to gonadectomy. Furthermore, increased adipogenesis and myelopoiesis are observed in the bone marrow of these mice [23]. In addition, the long bones in SAM-P6 are longer but more fragile than controls [22]. This line is among the best studied as a model for age-related osteoporosis not only in terms of

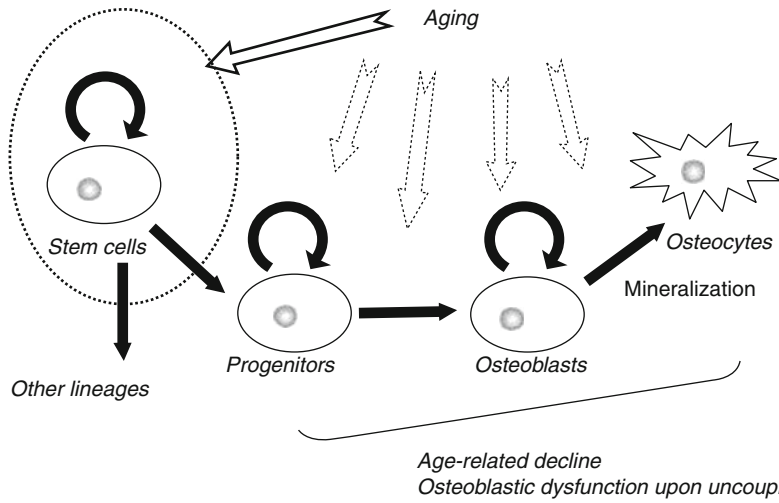

Fig. 6.4 Schematic presentation of osteogenesis and aging. Observations in naturally aged laboratory animals and mutant mice with aging phenotypes suggest that one of the keys to understanding aging and premature aging pathogenesis may be self-renewing stem cells. In these models, the pathway involving p53 (Fig. 6.3) upregulates the genes responsible for cell cycle arrest and/or apoptosis, lowering the regenerative potential necessary for homeostasis and tissue repair. While the mechanisms responsible for aging are largely unknown, the existing models suggest that there are common pathways, which may help in our understanding of the aging phenotype (From Watanabe and Hishiya, Ref. [118]. With permission from Elsevier)

skeletal morphology and pathology, but also in terms of its application for therapeutic-targeting experiments, such as drug testing and bone marrow transplantation [25–27]. Other numerous *in vivo* and *ex vivo* reports of SAM-P6 have been published whose observations are thought to be consistent between these aged mice and humans, but also include some controversial observations or interpretations, probably due to their complicated genetic backgrounds. Because the SAM strains are polygenic, the specific genetic factors accounting for their bone phenotype remain to be elucidated.

The observed differences in bone metabolism resulting from the various genetic backgrounds of these different mouse strains have been quantified by QTL analyzes. For example, whereas C57BL/6 mice have a relatively low bone mineral density (BMD) and reduced bone mass, C3H/HeJ have high BMD and are resistant to bone loss in response to OVX [28, 29]. These studies indicate that usage of wild-type inbred strains of mice, as well as rats, need to be well-characterized and given strong consideration in studies of bone metabolism and pathophysiology.

Other mouse models mimicking the human progeroid syndromes have been reported [30–35]. These genetically modified mice develop multiple aging phenotypes and exhibit a shortened life span (Table 6.1). For example, Werner syndrome is caused by a loss-of-function mutation in *WRN*, encoding the RecQ family DNA helicase, which plays a role in genome stability including telomere maintenance [36]. Unexpectedly, knockout mice for the Wrn gene are essentially normal and exhibit no characteristics of premature aging [37]. Mice have long telomeres and relatively high telomerase activity, suggesting that the aging phenotype is latent in these mice and results from residual activity surrounding telomere maintenance. Evidently, double knockout mice for Wrn and Terc, which encodes the RNA component of telomerase activity, show a Werner-like phenotype with osteoporosis [38, 39]. RecQ like-4 (Recql4) is a gene mutated in a subset of Rothmund-Thomson syndrome, recognized as a premature aging syndrome [40, 41]. Although Reql4 null mice are embryonic lethal, targeted deletion of exon 13 results in a form of aging phenotype that includes osteopenia [42]. Yang et al.

Table 6.1 Genetically modified mice with premature aging phenotype and/or short life span

Gene	Function	Modification	Bone phenotype	Characterization of bone	Related human case
Atm	Cell cycle checkpoint	K0	Osteopenia	MicroCT, histological analysis, *ex vivo* cell culture	Ataxia telangiectasia
BubR1	Spindle assembly checkpoint	Hypomorph	Normal (kyphosis)	DXA	
DNA-PKcs	DNA repair	K0	Osteopenia	X-ray analysis	
Klotho	Hormone /growth factor stimulating, mineral metabolism	K0	Osteopenia	SXA, microCT, histological analysis, *ex vivo* cell culture	
Ku86	DNA repair, transcription	K0	(Not indicated)		
Lmna	Nuclear architecture	Knock-in	Osteopenia	DXA	Hutchinson-Gilford progeria syndrome
mTR	Telomere maintenance	K0	Normal[a]	X-ray analysis, histological analysis	
PASG	DNA methylation	Hypomorph	Osteopenia	X-ray analysis, histological analysis	
PolgA	Mitochondrial DNA replication	Knock -in	Osteoporosis	X-ray analysis	
Recql4	DNA replication and repair	K0	Osteopenia	X-ray analysis, histological analysis	Rothmund-Thomson syndrome
Sirt6	DNA repair	K0	Osteopenia	X-ray analysis, DXA	
TRp53	Cell cycle checkpoint	Deletion mutant	Osteopenia	X-ray analysis, histological analysis	
		Mutant Tg	Ostopenia	X-ray analysis	
		Short isoform Tg	Osteopenia	X-ray analysis, histological analysis	
XPD	DNA replication and repair	Knock-in	Osteoporosis	X-ray analysis, DXA	Xeroderma pigmentosum
Wrn/Terc	Telomere maintenance	Double K0	Osteopenia	MicroCT	Werner syndrome

[a]The phenotype was observed in the 6th generation from mTR knockout mouse mattings

showed that osteoprogenitors are significantly decreased in heterozygous Recql4 (±) mice compared to wild-type controls [43]. In addition, mutated Recql4 has also been reported in Baller-Gerold syndrome, a rare autosomal recessive disorder with radial aplasia/hypoplasia and craniosynostosis [44].

Recently, a gene encoding lamin A has been identified to be responsible for human progeria, Hutchinson-Gilford syndrome [45, 46]. Mice carrying an autosomal recessive point mutation in the lamin A gene, corresponding to that identified in humans, also develop a progeria-like phenotype with osteoporotic symptoms [47]. Interestingly, expression of lamin A/C in osteoblasts and chondrocytes of C57BL/6 mice is decreased in an age-related manner [48]. In addition, recent studies have reported that lamin A-deficient mice are osteoporotic [49, 50], show a low anabolic response to exercise [51], and have high levels of fat infiltration in muscle and bone [52].

Furthermore, mice presenting with multiple aging phenotypes have also been reported. Null mutation of a gene, Ku86 (also known as Ku80), which plays roles in DNA repair and transcription exhibits a shortened life span and elicits a premature aging phenotype including osteopenia [53]. The aging phenotype has also been observed in mice lacking proliferation-associated SNF2-like gene (PASG), an SNF-like molecule that functions in DNA methylation [54]. Mutant mice show decreased BMD and a delay in the secondary ossification of the tibial epiphyses [54]. In addition to mutations in genes involved in genomic stability and nuclear organization, mice carrying mitochondrial DNA polymerase mutations that exclude a region responsible for its proofreading activity, also present the osteoporotic phenotype together with other premature aging symptoms [55]. A sir2/SIRT family of NAD-dependent histone deacetylases regulates life span. Knockout mice for Sirt6 exhibit genomic instability and an aging-like phenotype with osteopenia [56]; in particular decreased bone mass, now considered a hallmark of premature aging phenotypes. However, most observations of the skeletal phenotype were examined by X-ray analysis. The pathophysiology, including histology, of the bone phenotype in these models for premature aging, has not yet been fully described.

Errors in cell duplication, such as those miss programmed by the above-mentioned mutations can be detected and corrected by arresting the cell cycle. A system of cell cycle checkpoints has been shown to play a critical mechanistic role [57, 58]. Checkpoint kinase cascades are involved in DNA replication and other cell cycle events. ATM is a PI3K family kinase involved in DNA repair and oxidative response [59]. The gene encoding the protein kinase has been identified as a gene mutated in ataxia telangiectasia, recognized as one of the human premature aging syndromes [60]. Knockout mice for ATM exhibit a similar phenotype to the human disease, including hyper-radiosensitivity and ataxic defects [61–63]. It has been shown that the self- renewal capacity of hematopoietic stem cells in Atm knockout mice is significantly impaired with elevated reactive oxygen species (ROS), and that treatment with anti-oxidative agents rescues the bone marrow failure [64]. An osteopenic phenotype has also been observed in these knockout mice. Colony formation assays revealed that the phenotype was mainly caused by a proliferative defect in bone marrow mesenchymal stem cells or its progenitors [65].

Gain-of-function mutations in p53, a downstream effector of ATM kinase, also exhibit premature aging with an osteoporotic phenotype [66, 67]. Among them, p44 transgenic mice show a low progenitor turnover with significant decreases in osteoblast number and a slight reduction of osteoclasts [67]. Although further characterization of these models is required, these data suggest that the stem cell defect due to cell cycle arrest upon DNA damage or other cell cycle abnormalities, at least in part, may account for the decreased bone formation and subsequent osteopenia observed in these premature aging models.

In addition to stem cell defects in p53 and other checkpoint deficiencies, recent evidence indicates that p53 can directly regulate osteoblast differentiation [68]. Wang et al. [69] showed that mice lacking p53 exhibit increased bone mass due to accelerated osteoblast differentiation caused by elevated Osterix levels. Lengner et al. [68] examined osteoblast-specific ablation of Mdm2, a negative regulator of p53, and found reduced proliferation and decreased levels of Runx2 in the osteoblasts. Furthermore, they also described elevated Runx2 levels in p53-null osteoblasts, suggesting that p53 negatively regulates bone development and growth by inhibition of Runx2. Defects in osteoblast differentiation caused by dysregulation of Osterix was also recently reported in Atm knockout mice [70]. Thus, not only stem cell defects but also cell autonomous differentiation defects of osteoblasts are associated with the osteopenic phenotype in mouse models of premature aging.

Osteopenia Caused by Decrease in Bone Formation

Low turnover rates or uncoupling between bone resorption and formation in aged bones is often associated with a decline in osteoblast function [71]. Reduced bone formation is one of the features

observed in models for age-related osteoporosis. Some genes that play critical roles in bone formation have been described using genetically modified mice [72–75]. Several typical models are listed in Table 6.2. Sca1/Ly6A is a GPI-anchored membrane protein expressed in hematopoietic stem cells and a subset of bone marrow stromal cells [76, 77]. Whereas Sca1 knockout mice have normal bone development, the aged animals (15 months of age) show a significant bone loss [78]. Progenitor and differentiation assays of bone marrow cells in these mice reveal that decreased bone mass is caused by impaired self-renewal of mesenchymal progenitors. Stem cell defects in hematopoietic lineages have also been reported in Sca1 knockout mice [79]. Although multiple aging phenotypes in Sca1 knockout mice have been reported, this is a good model for age-related osteoporosis in humans, supporting the stem cell hypothesis in the pathogenesis of age-related osteoporosis [80].

In addition, IRS1 is a major substrate of insulin receptor (IR) and IGF-1 receptor (IGF1R) that transduces signals by interacting with signaling molecules in a phosphorylation-dependent manner, which is expressed in osteoblasts but not in osteoclasts. IRS1 knockout mice exhibit low bone mass compared to wild-type controls, and cultured osteoblasts from the knockout mice are impaired in IGF-induced proliferation and differentiation, whereas BMP-induction is not altered [73]. Reduced osteoclast formation is then the result of defective osteoblasts, resulting in low turnover osteopenia [81].

Wnt signaling regulates bone mass through the osteoblastic lineage. It has been revealed that an autosomal recessive disorder, osteoporosis-pseudoglioma syndrome (OPPG), is caused by mutations in the gene encoding LRP5, a cell surface co-receptor for Wnt [82]. It has also been independently shown that Val171 mutation of LRP5 causes high bone density in humans [83]. These correlative findings indicate a role for the Wnt pathway in bone development and remodeling. Kato et al. generated mice deficient in Lrp5, and showed that Lrp5 knockout mice also develop osteopenia caused by reduced osteoblast proliferation and function [84]. A significant decrease in the number of bone marrow stromal progenitor cell (CFU-F) colonies was observed in the knockout mice. Inhibition of GSK3, a negative regulator of Wnt/β-catenin signaling stimulates osteoblastic differentiation of the progenitors [85, 86]. The ligands, such as Wnt10b, specifically activate the canonical pathway, and constitutively activate β-catenin-stimulated osteoblast differentiation [87]. These findings support the idea that the canonical pathway via β-catenin signaling of Wnt plays a role in the regulation of osteoblasts. It should be noted that the canonical pathway also inhibits adipogenic differentiation of progenitor cells [88], suggesting that the pathway is also important in lineage commitment between osteoblastic and adipogenic fates. This observation may be associated with age-related alterations of bone marrow, resulting in decreased bone formation and increased adipogenesis to what is described as "fatty marrow".

On the other hand, some models presenting with osteopenia exhibit defects in osteoblast differentiation. Mice lacking a transcriptional cofactor, *four and a half LIM domains 2 (Fhl2)*, also

Table 6.2 Osteopenic mice with altered bone formation

Gene	Phenotype (knockout)	Osteoprogenitor (incl. stem cells)	Number of osteoblasts	Number of osteoclasts	Ex vivo osteoblast differentiation
Kl (klotho)	Osteopenia	⇓	⇓	⇓	⇓
ly6a (Seal)	Osteopenia	n.d.	⇓	⇓	⇔
lrs1	Osteopenia	⇓	⇓	⇓	–
lrp5	Osteopenia	⇓	⇓	⇔	⇔
Fhl2	Osteopenia	n.d.	⇔	⇔	⇓
Abl1 (Abl)	Osteopenia	⇓	⇓	⇔	⇓
Lmna	Osteopenia	⇓	⇓	⇓	⇓

n.d. not described

present with a significant decrease in bone mass [89]. Although numbers of osteoblasts and osteoclasts were comparable to littermate controls, bone formation rate was markedly reduced. Furthermore, transgenic mice overexpressing Fhl2 in osteoblasts exhibited enhanced bone formation and increased bone mass. Fhl2 interacts with Runx2 to increase its transcriptional activity and stimulates osteoblast maturation, suggesting that the Fhl2 knockout is a unique model for osteopenia caused by osteoblast activation deficiency [90].

Furthermore, c-Abl, a downstream protein kinase of ATM, functions in DNA repair and oxidative stress response [91]. Mice deficient for the Abl gene also develop osteopenia with reduced bone formation [92]. *Ex vivo* assays of osteoclastogenesis were not affected, and the number of osteoclasts in the Abl-deficient mice was similar to that of wild-type controls. Whereas the number of progenitors in bone marrow is significantly decreased, the differentiation of osteoblasts from Abl knockout mice is also impaired [92]. Using osteoblast culture, distinct roles in the oxidative stress response between c-Abl and ATM, have been proposed [93]. Although decreased expression of peroxiredoxin 1 (Prdx1) due to down-regulation of PKCδ was observed upon arsenate-induced oxidative stress in osteoblasts from Atm knockout mice, expression of the redox protein, through the upregulation of PKCδ, was increased in the cells derived from Abl knockouts. The opposite roles in the oxidative stress response may cause similar bone phenotypes in the knockout mice of Abl and Atm genes through distinct mechanisms. Life-span shortening and age-related defects have been reported in mice lacking Prdx1 or MsrA, which encodes methionine sulfoxide reductase [94, 95]. Both genes play important roles in the oxidative stress response through anti-ROS activity. Whereas the bone phenotype in these mutant mice has not yet been described, it will be interesting to see the potential pathogenic phenotype in bone from these mice. Oxidative stress, such as that caused by ROS, often causes damages in DNA, suggesting that the genomic stability and oxidative stress response may share some common pathways in the aging phenotype. As mentioned with Atm mice, an antioxidant also partially rescues the perinatal lethality observed in Ku86 knockout mice [96]. In addition to DNA damage, ROS is important in signal transduction and pathogenesis of diseases as well. For example, anti-oxidative agents reverse insulin resistance in diabetic models [97, 98]. Although it remains unclear whether ROS targets are part of the mechanistic pathways affected by aging, management of ROS may be significantly implicated in osteoblast function and aging.

The Aging Phenotype and Defects in Mineral Metabolism

Other models for accelerated aging phenotypes, where the responsible genes are apparently not directly involved in genomic integrity also exist. Mice carrying hypomorphic mutations of the gene Klotho show multiple aging phenotypes [99]. In Klotho mice (kl/kl), both bone formation and resorption are reduced, indicating a low turnover of bone metabolism resembling human osteoporosis [100]. Although neither osteoblasts nor osteoclasts express the kl gene, *ex vivo* cultures of osteoblastogenesis and osteoclastogenesis show reduced differentiation independently in both lineages. In contrast to the canonical progeroid models, this is a unique model for age-related osteoporosis in humans. Indeed, the molecular functions of KL protein have been reported. The protein, which is structurally similar to β-glucosidase, possesses β-glucuronidase activity [101]. KL protein acts as a co-receptor for IGF and is also required for FGF23 signaling through FGFR1 [102–107]. FGF23 has been identified as a gene responsible for autosomal dominant hypophosphatemic rickets and is suggested to play an important role in phosphate metabolism as a hormone, a candidate for phosphatonin [104]. FGF23 knockout mice also exhibit a premature aging-like phenotype [105]. Interestingly, the mice have elevated serum levels of vitamin D and hyperphosphatemia, and a part of the aging phenotype was rescued by lowering the vitamin D levels [105–107]. It is therefore suggested that control of the phosphate-regulating system by FGF23-KL is associated with the aging phenotype including

osteoporosis. Notably, PHEX (phosphate-regulating gene with homology to endopeptidases on the X chromosome) is highly expressed in osteocytes [108–111], and declines with age as well as with post-OVX and mechanical unloading [112–114]. Conceivably, osteocytes may be implicated in phosphate metabolism and age-related osteoporosis. In fact, FGF signaling coordinately regulates mineralization-related genes in the osteoblast lineage, and that ERK signaling is essential for Dmp1 expression and osteocyte differentiation in vivo [115].

What Mouse Models Teach

It has been recently described that mice deficient for molecular clock genes, such as Per1/2, Cry1/2, and BMAL1, exhibit increased bone mass with elevated bone formation [116]. The clock components inhibit osteoblast proliferation triggered by CREB activation responding to signals from sympathetic neurons. In contrast, it has also been reported that BMAL1 knockout mice have impaired circadian rhythms and display a premature aging phenotype including decreased bone mass [117]. Although these apparently opposite observations might be due to age differences (increased bone mass at 2 months; decreased at 40 weeks of age, compared to wild-type controls), bone phenotype is largely affected by many factors including mobility. Thus, the same mouse can tell different stories. Whereas decreased bone mass is a major indication of the aging phenotype as mentioned, age-related structural and functional alterations are seen not only in bone but also in other tissues and organs as well. Age-related osteoporosis has been recognized as due to a combination of age-related changes in bone caused by bone cell dysfunction, age-related decline in mineral metabolism or hormonal regulation, and neuronal and/or gonadal dysregulation. Nevertheless, these models inform us of the molecular mechanisms involved in bone biology, especially the molecular and cellular basis of bone pathophysiology, and include the possibility that cell autonomous bone defects may be implicated, at least in part, in the pathogenesis of age-related osteoporosis. Furthermore, the described genetically defined models can be useful for elucidation of the underlying mechanisms in pharmacological and other therapeutic-targeting studies.

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