Chapter 4
DDR Mouse Models

Kiyoshi Kano, Ken Takeshi Kusakabe, and Yasuo Kiso

4.1 Introduction

DDRs have been identified as collagen-binding receptors and subsequently confirmed as endogenous receptors, which regulate cell proliferation, cell adhesion, migration, as well as extracellular matrix remodeling, mainly in in vitro studies. DDR mouse models have been a powerful tool to elucidate the additional biological and physiological functions of DDRs associated with development and diseases by using phenotypic analyses.

4.2 Body Size

DDR1- and DDR2-knockout mice generated by gene targeting are viable; interestingly, their body size is smaller, which is one of distinguishing phenotypes of DDR loss. In addition, genetically modified DDR2 mice exhibit alterations in body size, including bone size, body weight, and fat volume. These mouse models suggest that DDRs play an important role in body growth and local and systemic development.

K. Kano (✉)
Laboratory of Developmental Biology, Joint Faculty of Veterinary Medicine, Yamaguchi University, Yamaguchi 753-8515, Japan

Laboratory of Veterinary Anatomy, Joint Faculty of Veterinary Medicine, Yamaguchi University, Yamaguchi 753-8515, Japan
e-mail: kanokiyo@yamaguchi-u.ac.jp

K.T. Kusakabe • Y. Kiso
Laboratory of Veterinary Anatomy, Joint Faculty of Veterinary Medicine, Yamaguchi University, Yamaguchi 753-8515, Japan

R. Fridman, P.H. Huang (eds.), *Discoidin Domain Receptors in Health and Disease*, DOI 10.1007/978-1-4939-6383-6_4

4.2.1 DDR1-Deficient Mice

DDR1 gene knockout mice have been produced using common gene targeting techniques. Heterozygous offspring have been crossed with mice on a 129/Sv, ICR, and C57BL/6 backgrounds, to generate inbred and outbred DDR1-deficient mice. DDR1-deficient homozygote mice are viable but smaller than their heterozygous littermates. DDR1-deficient female mice have a 35 % lower body weight, but DDR1-deficient male mice are only about 10 % smaller than wild-type mice, owing to an additional weight gain after puberty [1]. In both sexes, all organs are proportionately smaller. The skeletons of DDR1-deficient mice appear normal at 10 weeks of age, but a detailed analysis of DDR1-deficient mice revealed that the calcification of the fibula bone is reduced in the majority of cases. No difference in the extent of the zone of hypertrophic cartilage, chondrocyte proliferation rate, or chondrocyte apoptosis in the tibia has been observed between DDR1-deficient mice and wild-type mice. These data imply that hormonal changes affect bone and tissue growth in DDR1-deficient mice.

4.2.2 DDR2-Deficient Mice

DDR2-deficient mice have been produced by homologous recombination of a targeting vector into embryonic stem cells, to disrupt the DDR2 gene by replacing exon K1, encoding the ATP-binding region of the kinase domain, to a neomycin resistance cassette. Newborn DDR2-deficient mice do not have an abnormal appearance, but they gradually fail to develop, resulting in a proportionally smaller body size and reduced weight starting 10 days after birth. Adult mutant mice show a 30–40 % reduction in weight compared to heterozygous littermates in both sexes [2]. The length of the long bones of adult DDR2-deficient mice is reduced, and the axis skeleton and skull bones are also shorter. To elucidate the cellular mechanism underlying the reduced long bone growth in DDR2-deficient mice, chondrocyte proliferation, osteoblast differentiation, and the height of the growth plate have been analyzed. No difference was found in osteoblast differentiation, but both the chondrocyte proliferation and growth-plate height were found to be reduced in the metatarsals of DDR2-deficient mice at 2 weeks of age. The reduction of chondrocyte proliferation, and not cellular differentiation within the growth plate, is the major reason for the decreased bone size in DDR2-deficient mice. The evidence suggests that DDR2 contributes mainly to the regulation of chondrocyte proliferation as an extracellular matrix receptor in the skeletal structure.

4.2.3 Smallie: DDR2-Deficient Mutant Mice

Smallie (*slie*), a spontaneous, autosomal recessive mutation, which results in dwarfing and sterility, was discovered in a Jackson Laboratory colony [3]. *Slie* mutant mice arose spontaneously in a BKSChpLt(HRS)$^{Tg(Ins2\text{-}Cpe)\ 1LtCpe\mathit{fat}}$/LtJng line, and an unaffected

heterozygous sibling was crossed with a C57BLKS/J mouse to produce *slie* carriers, because the homozygous mutants were infertile. To briefly review this colony, the fat mutation arose spontaneously in *Cpe* in the HRS inbred mouse strain, and this mutation was transferred to the C57BLKS/J strain through congenic backcrossing experiments in the Jackson Laboratory of Drs. Dorothy Chapman (Chp) and Ed Leiter (Lt). In addition, these mice carry the Cpe^{fat} transgene, under the control of the Ins2 promoter, which was generated by Dr. Leiter. The *slie* mutation spontaneously arose in a colony in the laboratory of Dr. Jurgen K. Naggert (Jng), in mice provided by Dr. Leiter. Selective breeding experiments have demonstrated that the dwarfing phenotype is independent of the presence of the Cpe^{fat} mutation or the transgene Ins2-Cpe. Both Cpe^{fat} and Ins2-Cpe have been removed from the *slie* colony through selective breeding. To determine the chromosomal location of *slie*, F1 progeny mice were generated by in vitro fertilization of BKS(HRS)-*slie*/Jng homozygotes to NOD.NON-$H2^{nb1}$/LtJ mice. The heterozygous F1 mice, which did not exhibit dwarfism, were intercrossed for the initial linkage mapping study. The resultant F2 progeny mice were weighed at 10 weeks of age to identify the affected animals. The fine structure genetic map encompasses an estimated 1.94 Mb of physical region on Chromosome 1, which contains the *slie* mutation. According to annotations in the *Mus musculus* genome assembly, the *slie* critical region contains 23 gene loci. Among these candidates, the relative levels of *Ddr2* gene expression were quite low in *slie* mutants. Whereas *Ddr2* expression was readily detected in wild-type mice, exons 1–17 of *Ddr2* did not amplify from the *slie* mutant genomic DNA by PCR. A genomic sequencing analysis detected a 150 kb deletion that extends into the *Ddr2* gene transcript. *Slie* homozygous mutant mice have skeletal abnormalities that include craniofacial deformities, such as protuberant eyes and snub noses (Fig. 4.1). After weaning, their weight gain is slower and they lack the juvenile growth spurt observed in control littermates. Whereas the total body mass is reduced in *slie* homozygotes, as assessed by dual X-ray absorptiometry, the percentage of body fat is significantly reduced with an accompanying increase in lean muscle mass, these mice compared to wild-type mice at 5–6 months of age. Similarly, the bone mineral content, but not the density, is reduced in *slie* homozygotes compared to wild-type mice. The results suggest that the absence of DDR2 leads to a growth retardation phenotype in *slie* mice, as seen in DDR2-deficient mice.

4.2.4 Dominant-Negative DDR2 Transgenic Mice

To investigate the molecular role of DDR2 in endochondral cellular proliferation in vivo, a transgenic mouse was created, in which the expression of the dominant-negative DDR2 protein is induced, to evaluate the role of DDR2 in cellular proliferation [4]. The dominant-negative DDR2 protein was made from a kinase-dead DDR2 mutant (KD-DDR2), which is a truncated form lacking the kinase domain but retaining the extracellular and transmembrane domains. Transgenic mice were produced by microinjecting several hundred molecules of the DNA fragment into the pronuclei of fertilized eggs from F1 hybrid mice (C57BL/6×DBA).

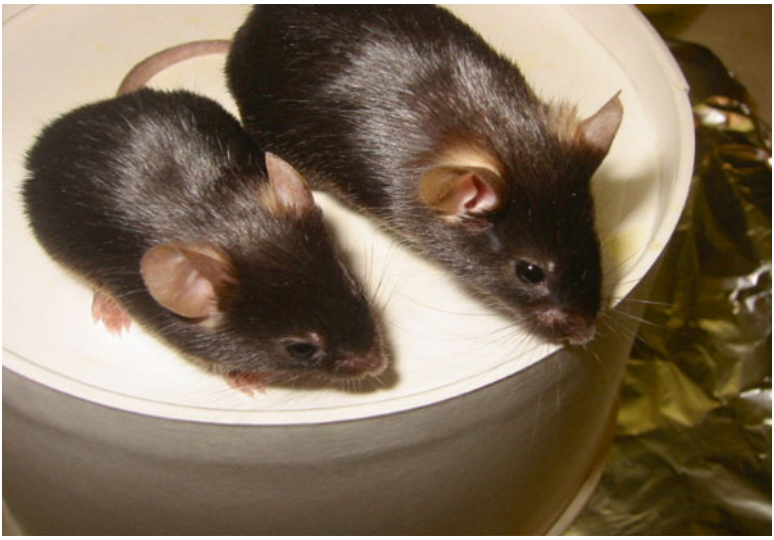

Fig. 4.1 Four-month-old wild-type and *slie* mutant mice (*left*)

Unexpectedly, the body size and the skeleton length of KD-DDR2 overexpressed mice were not significantly different compared to the littermates. On the other hand, whereas the layer of hypertrophic chondrocytes in KD-DDR2 transgenic mice was not significantly thicker than that of normal littermates, the layer of proliferative chondrocytes in KD-DDR2 transgenic mice was significantly thicker than that of normal littermates. These data indicate that DDR2 plays an important role in endochondral ossification, but neither a critical nor an essential role in total body and skeleton size determination. The greater thickness of the proliferative chondrocyte layer in KD-DDR2 transgenic mice might be an anomaly in the reduction of chondrocyte proliferation in DDR2-deficient mice [2]. This difference might stem from the regional and functional differences in DDR2 shortage; these differences suggest that DDR2 plays various roles under different conditions, either systemically or locally.

4.2.5 DDR2-Overexpressing Transgenic Mice

What happens when DDRs are overexpressed in mice? To investigate the systemic role of DDR2 in body size regulation, a transgenic mouse in which the DDR2 protein is overexpressed was produced, then screened for abnormalities using a systematic mouse abnormality screening system [5]. Transgenic mice were produced by microinjecting several 100 molecules of the DNA fragment into the pronuclei of fertilized eggs from F1 hybrid mice (C57BL/6×DBA), as described above. The transgenic mice were screened for abnormalities using the "Japan Mouse Clinic,"

a systematic mouse abnormality screening established and performed at the RIKEN BioResource Center (http://www.brc.riken.jp/lab/jmc/mouse_clinic/en/index.html). The modified-SHIPRA screen revealed that only the parameter of body size was significantly different among the genotypes. The body length was significantly increased, whereas the body weight was significantly decreased, in the DDR2-overexpressing transgenic mice compared to wild-type controls. In the transgenic mice, the epididymal fat pads were also significantly smaller than the normal littermate mice. This finding suggests that the decrease in body weight is correlated with the decrease in fat tissues and in cholesterol in transgenic mice. Clinical biochemical tests have revealed that the level of leptin, which is secreted from fat and known as a regulator of appetite and body weight [6–9], is higher in the transgenic mice compared to their wild-type littermates. These data suggest that an excess of DDR2 results in an increase of leptin in adipocyte cells, and ultimately affects the whole body weight in DDR2 transgenic mice. These data suggest that DDR2 plays a systemic role in the regulation of body size by affecting skeletal formation and fat metabolism.

4.3 Reproduction

Both DDR1- and DDR2-deficient mice exhibit infertility with various abnormalities, including anomalies in reproductive functions to a varying degree, which imply that DDRs might have unique functions to sustain the normal reproductive system.

4.3.1 Implantation in DDR1-Deficient Mice

DDR1-deficient female mice generally fail to mate to either mutant or wild-type control male mice [1]. Although virginal plugs have appeared in almost all female mice after mating, only 20 % of DDR1-deficient female mice are able to give birth. A normal number of blastocysts have been found in the uterus of DDR-deficient female mice before implantation at 3.5 days postcoitum (d. p. c.), but a swollen decidua was not found at 4.5 d. p. c. Considering that DDR1-deficient blastocysts transferred into the uterus of pseudopregnant wild-type mice progress to normal litters, DDR1-deficient female mice might have a maternal deficiency that affects the implantation process. Proteases such as those of the MMP family are essential for implantation to remodel the extracellular matrix of the maternal uterine stroma. DDR1 was originally localized to the decidua in the pre- and postimplantation uterus, where the DDR1 ligands, including type VI collagen, are specifically expressed in the uterine epithelium around the implantation region. DDR1 might play an important role in reconstruction throughout MMP production in the peri-implantation adhesion between the uterine wall and the blastocyst.

4.3.2 Mammary Glands in DDR1-Deficient Mice

All of the pups from DDR1-deficient mice have a nutritional deficiency 1 day after birth and die within a few days, even if they have been nursed by their mother with no milk spot in their stomach [1]. When pups delivered from DDR1-deficient mice were transferred to wild-type normal foster mothers shortly after birth, they were able to grow normally. In the mammary glands of DDR1-deficient mice, the alveolar structure is much more condensed, and fewer lipid vesicles are observed during the late gestation period, compared to wild-type female mice. After delivery, the adipose tissues generally disappear and are replaced by alveolar structures filled with milk in control female mice. However, the mammary glands of DDR1-deficient mice largely consist of adipocytes, and the alveoli are predominantly condensed. The alveoli and epithelium of DDR1-deficient mice start to collapse and regress at day 2 after birth. In the developing mammary glands of DDR1-deficient virgin mice, the outgrowth of the mammary ducts is delayed, but the primary ducts and the terminal end buds continue to proliferate, resulting in an increase in the number of alveolar ducts at 3 weeks of age. Although the fat pads are filled with epithelial ducts, the number and diameter of the ducts are largely increased, and the extracellular matrix deposition is substantially increased in the developing mammary glands of mature DDR1-deficient mice. The abnormal development of the mammary glands may be the reason why DDR1-deficient mice secrete very little milk, although the milk protein transcripts were detected at normal levels. These results suggest that DDR1 might be a crucial factor in the regulation of ductal development in the mammary glands.

4.3.3 Ovaries in Smallie Mice

It is not known whether the reproductive phenotypes observed in *slie* mutant mice are present in DDR2-deficient mice, because similar studies of the reproductive system have not been reported in DDR2-deficient mice. This is because matings between male and female *slie* mutants have failed to produce offspring. Homozygous *slie* mutant females mated to either homozygous *slie* or wild-type males have resulted in no pregnancies or live pups [3]. DDR2 is expressed in the ovaries of adult wild-type female mice in the interstitial and thecal cells, but not in the cumulus cells, the inner layer of granulosa cells, or the oocytes. In ovaries from *slie* mutant mice, the foremost difference was the striking absence of corpora lutea, in contrast to wild-type mice (Fig. 4.2). Conversely, no significant deviation was found in the amount of preantral, antral, and Graffian follicles at these ages. The basal levels and the gene expression of the pituitary and hypothalamic hormones, as well as the gene expression of the hypothalamic releasing hormones, are not significantly different between *slie* and wild-type mice. The administration of exogenous gonadotropins induced follicular growth at the Graffian stage, but it

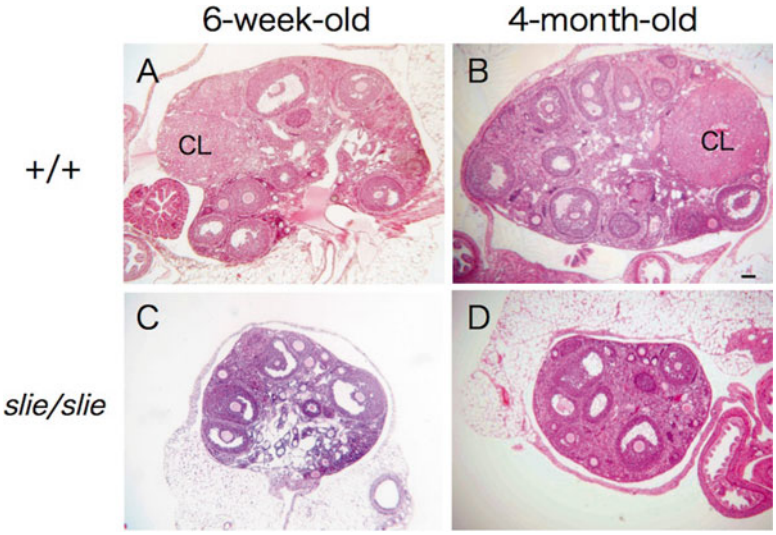

Fig. 4.2 Anovulation in female *slie* mutants. Six-week-old and 4-month-old wild-type (**a**, **c**) and mutant (**b**, **d**) ovary sections, stained with hematoxylin and eosin, depicting follicular development, CL, corpus lutea, scale bar, 50 μm

failed to alter the anovulatory phenotype of *slie* homozygotes, as the full luteinization of the thecal cells did not occur and no corpora lutea was formed. These results suggest that the absence of corpora lutea is caused by an inability to respond to gonadotrophic signals in *slie* mutant ovaries. Following exogenous gonadotropin administration, both female and male *slie* and wild-type mice secreted estradiol, progesterone, and testosterone, respectively, in response to the exogenous gonadotropins, but the circulating steroid levels in adult *slie* mutants were lower than in the adult wild-type mice.

A systems biology approach was performed to identify the biological networks affected by the *slie* mutation in ovaries, using a microarray analysis [10]. A transcriptome analysis indicated several altered gene categories in *slie* mutants, including gonadal development, ovulation, anti-apoptosis, and steroid hormones. DDR2 signaling pathways did not activate *Mmp* genes, as no difference in their expression was found in *slie* mutants compared to wild-type mice. In hormonal signaling pathways involved in ovulation, the activity of the luteinizing hormone/choriogonadotropin (LH) receptor was decreased in the somatic cells, but not in the oocytes of *slie* mutants, resulting in an intrinsic defect in germ cells. The overlapping spatial and temporal expression of DDR2 and the LH receptor may permit their mutual coregulation during specific periods of ovulation. A lack of DDR2 signaling in *slie* mutants most likely triggers anovulation, by altering the expression of the LH receptor. Considering that *slie* mutant oocytes are competent to complete meiosis and fertilization in vitro, in spite of the ovulation of significantly

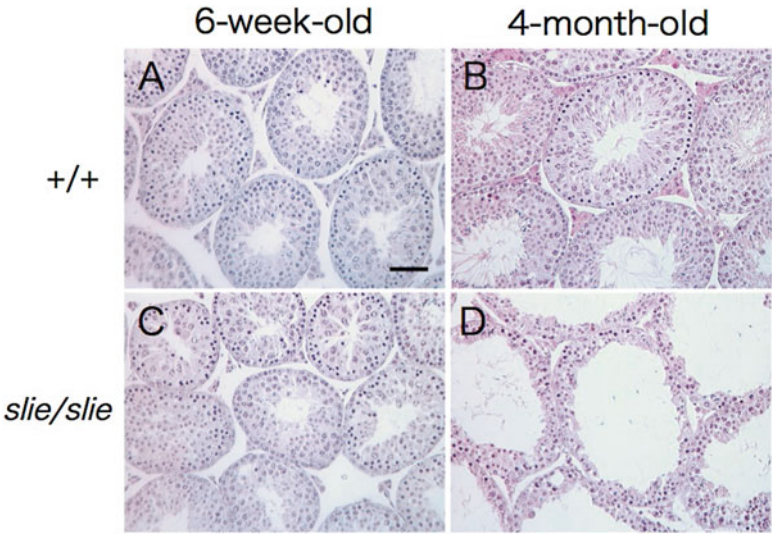

Fig. 4.3 Gonadal abnormalities in male s*lie* mutant mice. Cross sections of testes from 6-week-old and 4-month old wild-type (**a**, **b**) and *slie* mutant (**c**, **d**) mice, stained with hematoxylin and eosin, scale bar, 50 μm

fewer oocytes, these data identify DDR2 as a novel critical player in ovarian function, which acts upon classical endocrine pathways and LH signaling in somatic rather than in germline cells.

4.3.4 Testes in Smallie Mice

In homozygous *slie* males, fewer spermatids, along with atrophy of the spermatogonia, Sertoli and Leydig cells are observed (Fig. 4.3) [11]. The loss of DDR2 results in a progressive increase in apoptosis in spermatogenic cells, through the process of spermatogenesis. As described above, the absence of DDR2 induces a reduction in LH receptor expression in *slie* mutant females. This result suggests a correlation between DDR2 and the LH receptor in female gonads. In males, the localization of DDR2 and the LH receptor might reflect their interaction in interstitial Leydig cells during spermatogenesis, as in female gonads. Older Leydig cells in *slie* mutant mice fail to express the LH receptor, thereby triggering reductions in the expression levels of specific steroidogenic enzyme genes, and ultimately the inability to transduce signals from LH in the testes. DDR2 might be a survival and maintenance factor required for the viability of spermatogenic cells. These results suggest that DDR2 signaling also plays a critical role in the maintenance of male spermatogenesis throughout the LH signaling pathway.

Taken together, these results obtained from the study of the *slie* mutant reproductive system suggest that the absence of DDR2 leads to gonadal dysfunction, due to peripheral defects in the hormone response pathways.

4.4 Skin Wound Healing

The wound-healing response depends on the regulated linkage between recruited fibroblasts and the collagenous extracellular matrix. DDR2 expression is upregulated in wound healing following cutaneous burns [12]. In a skin wound-healing model, DDR2-deficient mice exhibit a reduced proliferative response in epidermal fibroblasts and keratinocytes compared to wild-type littermates [2]. Closure of the skin burn wounds is also significantly delayed in DDR2-deficient mice compared to wild-type mice [13]. All of the skin wound healing indices, that is, the expression of α-smooth muscle actin, the MMP2 activity, the tensile strength, and several collagen levels were significantly decreased in skin tissue extracts from DDR2-deficient mice following a cutaneous wound compared to wild-type littermates. This suggests the defective recruitment of skin fibroblasts in the skin of DDR2-deficient mice [14].

These results indicate that the study of DDR2-deficient mice provides a suitable model to elucidate the role of DDR2 in modulating the proliferative response in situations associated with extreme matrix deposition and degradation, such as a tissue injury, where the extracellular matrix provides a signal to increase proliferation.

4.5 Osteoarthritis

Osteoarthritis is a weakening disease that results from the progressive loss of articular cartilage. Mutations in type IX and XI collagen have resulted in early-onset osteoarthritis with a wide spectrum of osteochondrodysplasia in human genetic studies. The chondrocytes in the articular cartilage are affected by extracellular interactions such as cell–matrix interactions, as well as interactions between integrins and DDRs, rather than by direct intercellular communication [15]. Along with single gene-targeting models, double gene-targeting mouse models with a collagen deficiency, which represent an osteoarthritis model, as well as DDR deficiency models, are suitable to study the function of DDR in the pathogenesis of osteoarthritis.

4.5.1 Osteoarthritis in DDR2-Deficient Mice

DDR2 expression is increased in the chondrocytes of the articular cartilage of knee joints in mice that have developed osteoarthritis as a result of a heterozygous mutation in type XI collagen [16, 17], suggesting that DDR2 might be correlated with the

disease. The expression and activity of matrix metalloproteinase 13 (MMP-13) is also increased in the knee cartilage of mutant mice. The upregulation of MMP-13 might be one of the common events in osteoarthritis progression, because the deletion of MMP-13 results in a delay in osteoarthritis progression.

To elucidate the function of DDR2 in articular cartilage, double-heterozygous (type XI collagen- and DDR2-deficient) mutant mice were generated [18]. Heterozygous mutant DDR2 mice appear normal in size, but homozygous mutant mice are inappropriate for the study of osteoarthritis because the phenotypes of DDR2-deficient mice include dwarfism and a reduction in chondrocyte proliferation in the cartilage growth plate. The double heterozygous mutation in mice has shown that the rate of osteoarthritis progression, which is caused by a type XI collagen insufficiency, is considerably delayed in the mouse knee joint. A microsurgery was able to mechanically mimic osteoarthritis. The progression toward osteoarthritis was also considerably delayed in heterozygous DDR2-deficient mice compared to their wild-type littermates, following a surgery on the knee joint between the medial meniscus and the anterior tibial plateau. These results suggest that reduction of DDR2 might play a positive role in the attenuation of the degeneration of osteoarthritis of the knee joint that has been induced by a lack of type XI collagen or by surgical destabilization of the medial meniscus.

4.5.2 Osteoarthritis in DDR1-Deficient Mice

Contrary to DDR2, DDR1 has been suggested to play a protective role in osteoarthritis. DDR1-deficient mice show several features typical of osteoarthritis pathogenesis, including surface fissures, loss of proteoglycans, chondrocyte cluster formation, collagen type I upregulation, and atypical collagen fibril arrangements in the temporomandibular joint [19]. The frequency of osteoarthritis degeneration is also increased at a younger age in this model, compared to other mouse models previously used to study temporomandibular disorders in DDR1-deficient mice. An analysis of a three-dimensional reconstruction of the mandibular condyles in DDR1-deficient mice indicated a scabrous surface of the subchondral bone and flattened mandibular condyles, which are typical alterations in temporomandibular disorders. An ultrastructural analysis revealed that the collagen fiber arrangement was altered in the superficial layer of DDR1-deficient mice, compared to the parallel fiber alignment observed in the wild-type littermates. A microarray analysis of cartilage tissue samples from the mandibles of DDR1-deficient mice and wild-type littermates showed that the overall changes in gene expression resemble the typical pattern of osteoarthritis. Interestingly, DDR1-null chondrocytes induced the compensatory expression of DDR2, which is linked to the upregulated expression of MMP13 and to articular degeneration.

Although DDR1-deficient mice exhibit osteoarthritis of the temporomandibular joints at a young age, no symptom of osteoarthritis has ever been observed in the knee joints of double-heterozygous (type XI collagen and DDR2-deficient) mutant

mice at this stage. The differences in the structural or embryonic origin between the two joints might help in the phenotypical identification.

These data suggest that DDR1-deficient mice represent a good animal model, which contributes to the study of osteoarthritis in the temporomandibular disorders in vivo, and which can supply new treatment options for the disease.

Taken together, these findings from the study of osteoarthritis in DDR1- and DDR2-deficient mice suggest that DDR2 therapy provides the best treatment among members of the DDR family to cure the various symptoms of osteoarthritis, either with a specific tyrosine kinase inhibitor or with a molecule that prevents the binding to collagen type II.

4.6 Atherosclerosis

Atherosclerosis is a fibroinflammatory disease of the arterial wall. The responses of vascular smooth muscle cells during arterial wound repair are regulated by collagens as important signaling molecules. Following a mechanical vascular injury, the intimal thickening area was considerably attenuated in DDR1-deficient mice; however, there were no significant differences in the medial area, adventitial area, or external elastic lamina perimeter between DDR1-deficient and wild-type mice [20]. The density of the cells in the intima was almost twice as high in DDR1-deficient mice compared to wild-type mice, suggesting that matrix accumulation was decreased in the artery of the DDR1-deficient mice. The deposition of collagen fibrils in the injured arteries of the DDR1-deficient mice was substantially decreased compared to wild-type mice, as assessed using picrosirius red staining and polarized-light microscopy to visualize the collagen birefringence. These results suggest that DDR1 plays a critical role in the hyperplastic intima following an artery injury.

Double-targeted mouse with lipoprotein receptor-deficiency, which mimic atherosclerosis and DDR deficiency, also provide a good model to study the role of DDRs in the pathogenesis of atherosclerosis. Low-density lipoprotein receptor-deficient (*Ldlr*−/−) mice fed on an atherogenic diet displayed the development of complex atherosclerotic lesions, including the accumulation of lipids in vascular smooth muscle cells, macrophages, and extracellular matrix. Both DDR1 and DDR2 mRNA was expressed in *Ldlr*−/− mice [21]. Double-homozygous (DDR1 and low-density lipoprotein receptor-deficient, *Ddr1*−/−; *Ldlr*−/−) mutant mice fed an atherogenic diet exhibited drastically reduced atherosclerotic plaque development compared to *Ddr1*+/+ *Ldlr*−/− mice. Interestingly, the DDR1 expression level was absent in the plaque of *Ddr1*−/−; *Ldlr*−/−, but that of DDR2 was not different in the plaque between *Ddr1*+/+; *Ldlr*−/− and *Ddr1*−/−; *Ldlr*−/− mice.

The accelerated accumulation of fibrillar collagen and elastin in artherosclerotic plaques was observed in the *Ddr1*−/−; *Ldlr*−/− mice compared to the *Ddr1*+/+; *Ldlr*−/− mice. This was confirmed by the data on the expression of matrix synthesis molecules, procollagen and tropoelastin, which revealed that they were increased in *Ddr1*−/−; *Ldlr*−/− plaques at an early atherosclerotic stage. On the other hand, the

proteolytic activity in the plaque of *Ddr1*−/−; *Ldlr*−/− mice was decreased, which is related to the reduction in the macrophage content of the plaque, because macrophages are a major source of proteolytic enzymes, such as members of the MMP family, and cysteine and serine proteases, in the artherosclerotic plaques. The elimination of DDR1 substantially changed the plaque constitution, which was similar to the content of smooth muscles, but with less macrophage accumulation in *Ddr1*−/−; *Ldlr*−/− mice, which is consistent with the intact role of DDR1 in the mediation of macrophage recruitment, invasion and persistence, especially at an early stage in the artherosclerotic plaque.

A sex-mismatched bone marrow transplant was performed using *Ddr1*−/− ; *Ldlr*−/− mice and their *Ddr1*+/+; *Ldlr*−/− littermates, to investigate the independent role of DDR1 on bone marrow-derived cells, such as macrophages, during atherosclerosis [22]. Atherosclerotic plaques from chimeric mice with DDR1-deficient bone marrow (*Ddr1*−/−; *Ldlr*−/− to *Ddr1*+/+; *Ldlr*−/−; *Ddr1*−/− => *Ddr1*+/+) had remarkably smaller descending aortas compared to *Ddr1*+/+ ⇒ *Ddr1*+/+ mice, 12 weeks following the administration of an atherogenic diet. However, the matrix composition was similar in the two genotypes. The accumulation of bone marrow-derived macrophages was reduced in the aortic sinus lesions of *Ddr1*−/− ⇒ *Ddr1*+/+ mice, compared to *Ddr1*+/+ ⇒ *Ddr1*+/+ mice. By contrast, there was no effect on the number of cells of the host-derived resident vessel wall in bone marrow-specific DDR1-deficient mice, which suggests that the absence of DDR1 in bone marrow cells allows the attenuation of lesion growth, by regulating macrophage accumulation in the atherosclerotic plaques. The proportion of BrdU-positive bone marrow and circulating monocytes was comparable between *Ddr1*−/− ⇒ *Ddr1*+/+ and *Ddr1*+/+ ⇒ *Ddr1*+/+ mice, but the proportion of the ascending aortic area occupied by a fatty streak from a lesion was significantly decreased in size in *Ddr1*−/− ⇒ *Ddr1*+/+ mice compared to *Ddr1*+/+ ⇒ *Ddr1*+/+ mice, which suggests that DDR1 regulates the generation of a fatty streak, irrespective of the infiltration of monocytes and macrophages. These data provide support to the suggestion that DDR1 plays a critical role in promoting macrophage accumulation and lesion growth in early-stage atherogenesis.

As another experiment of reverse-oriented transplantation, bone marrow from *Ddr1*+/+ ; *Ldlr*−/− mice was transplanted into *Ddr1*−/−; *Ldlr*−/− hosts, to generate chimeric mice with a deficiency in DDR1 specific to the cells of the resident vessel wall (*Ddr1*+/+ ⇒ *Ddr1*−/−). The atherosclerotic plaque size was considerably increased in the cells of the resident vessel wall, but the lipid content of the lesions was decreased in *Ddr1*+/+ ⇒ *Ddr1*−/− mice compared to *Ddr1*+/+ ⇒ *Ddr1*+/+ control mice, 12 weeks after having placed the mice on an atherogenic diet [23]. The extracellular matrix, comprising collagen, elastin, and proteoglycan, accumulated and the relative fibrous cap thickness was significantly increased in the plaques of *Ddr1*+/+ ⇒ *Ddr1*−/− mice, which suggests that the cellular composition of the lesions shifted toward increased numbers of vessel wall-derived smooth muscle cells compared to bone marrow-derived macrophages in the vessel wall. These data support the suggestion that DDR1 acts as a negative regulator of matrix turnover by attenuating the proliferation, migration, and matrix accumulation during atherogenesis on the resident vessel walls.

Arterial calcification within the intimal layer is also an aspect of advanced atherosclerotic disease, including the risk of coronary arterial disorders. It is known that type I collagen promotes vascular smooth muscle cell-mediated calcification. *Ddr1*−/−; *Ldlr*−/− mice exhibit a significant attenuation of artherosclerotic intimal calcification in the aortic arch, which is independent of the serum calcium concentration but correlated with decreased inflammation [24]. This result suggests that DDR1 is also an important positive mediator of artherosclerotic intimal calcification by promoting mineralization.

Taken together, these results suggest that the DDR1, *Ldlr*-deficiency double-targeting model may have advantages when it comes to analyzing the molecular mechanism underlying atherosclerotic disease. DDR1 plays an important role in regulating the pathology of atherogenic diseases by modulating the number of macrophages and a decrease in extracellular matrix deposition.

4.7 Fibrosis

4.7.1 Lung Fibrosis

DDR1 has been implicated in pulmonary fibrosis through its expression in the pulmonary epithelium and its increasing expression pattern in the bronchoalveolar lavage cells of patients with idiopathic pulmonary fibrosis disease. Following bleomycin administration, the ultrastructure of epithelial and endothelial cells of DDR1-deficient mice were highly normal, but wild-type littermates were substantially injured due to interalveolar thickening of the septa and a reduction of the alveolar space [25]. In the analysis of fibrosis following bleomycin administration, wild-type mice exhibited increased collagen-rich nodular deposition, which was not the case in the injured DDR1-deficient mice. Myofibroblast expansion was reduced in DDR1-deficient mice treated with bleomycin for 2 weeks, compared to wild-type mice. Cellular apoptosis and proliferation was significantly decreased in the pulmonary tissues of DDR1-deficient mice. Inflammation, which was assessed by a quantification of the number of lymphocytes and macrophages within the bronchoalveolar lavage, was decreased in DDR1-deficient mice compared to wild-type mice. These data suggest that DDR1 is a positive regulator in the development of lung inflammation and fibrosis.

4.7.2 Kidney Fibrosis

In the kidneys, DDR1 is found in the basolateral membranes of nephron segments in the tubules connecting to the renal papilla in normal rats and is upregulated within the glomeruli in remnant rats kidney, suggesting that DDR1 plays a crucial role in cell–matrix interactions and kidney injury [26]. DDR1-deficient mice are useful to understand the unique functions of DDR1 in renal fibrosis and inflammation.

Adult DDR1-deficient mice show no obvious signal of abnormal renal function, such as edema, weight loss, or abnormal water intake, but exhibit proteinuria in high-molecular-weight proteins and urinary acanthocytes [27]. Histological and immunohistochemical analyses have revealed modest abnormalities in kidney structure, including a minor increment of intracellular aerocysts in the tubular epithelial cells of DDR1-deficient mice. An electron microscopy analysis has revealed a localized, subepithelial, mushroom-like isodense thickening of the glomerular basement membrane in DDR1-deficient mice. Within this area, a partial loss of the slit diaphragm between the podocyte foot processes can be observed. These data support the suggestion that DDR1 has an important function in the synthesis of basement membrane proteins and in maintaining the slit diaphragm anchored to the podocyte and glomerular basement membrane structure.

In chronic renal failure, hypertension is initiated by inflammation that develops to increase the synthesis and accumulation of collagen within the renal tissue. Following exposure to angiotensin II, DDR1 expression is increased in the renal cortex and within the glomeruli in normal mice. Although the blood pressure response to the continuous administration of angiotensin II is similar to that in wild-type mice, the renal cortex lesions and the infiltration of macrophages and lymphocytes are drastically decreased in the hypertensive DDR1-deficient mice, compared to wild-type mice [28]. Following angiotensin II treatment for 4 weeks, the expression of collagen I and IV was also decreased in the renal vessels and glomeruli of DDR1-deficient mice, compared to wild-type mice. These data suggest that DDR1 might take part in promoting inflammation and fibrosis through the synthesis of collagen and by recruiting cytokines in renal fibrosis diseases. The induction of unilateral ureteral obstruction (UUO) also leads to renal perivascular and interstitial inflammation and fibrosis in normal mice, but these severe symptoms of fibrosis are alleviative, and proinflammatory cytokines are drastically decreased in DDR1-deficient mice, compared to wild-type mice [29]. A deficiency in DDR1, induced by the injection of alloimmune sheep nephrotoxic serum, also protects from the development of crescentic glomerulonephritis [30].

Alport syndrome, which is caused by mutations in the COL4A3, 4 or 5 genes encoding type IV collagen α3/α4/α5-chains, is a chronic disease that is associated with proteinuria, hematuria, renal disorders, and deafness. COL4A3-deficient mice (*Col4a3*−/−) represent a model to examine renal degeneration in Alport syndrome. Double-homozygous (DDR1 and COL3A4-deficient, *Ddr1*−/−; *Col4a3*−/−) mutant mice show a prolonged lifespan, until they die from progressive renal fibrosis, compared to *Ddr1*+/+; *Col4a3*−/− mice [31]. Uremia is delayed and proteinuria is reduced in double-homozygous *Ddr1*−/−; *Col4a3*−/− mice. The kidneys of 9.5-week-old *Ddr1*+/+; *Col4a3*−/− mice are 33 % smaller than those of *Ddr1*−/−; *Col4a3*−/− mice. Severe glomerular, periglomerular, and tubulointerstitial fibrosis, observed in the kidneys of *Ddr1*+/+; *Col4a3*−/− mice are not found in those of *Ddr1*−/−; *Col4a3*−/− mice. An electron microscopy analysis has revealed that *Ddr1*+/+; *Col4a3*−/− mice exhibit a thickening and splitting of the glomerular basement membrane, which is a typical symptom of Alport syndrome, and the loss of the podocyte foot process. *Ddr1*−/−; *Col4a3*−/− mice, by contrast, show less severe phenotypes of the glomerular basement membrane and podocytes.

Extracellular matrix deposition of fibronectin and laminin was less pronounced in *Ddr1*−/−; *Col4a3*−/− mice compared to *Ddr1*+/+; *Col4a3*−/− mice. The expression levels of fibrotic, proliferative and inflammatory molecules are also decreased in *Ddr1*−/−; *Col4a3*−/− mice compared to *Ddr1*+/+; *Col4a3*−/− mice. A deficiency of DDR1 reduces the glomerular and tubulointerstitial matrix deposition and fibrosis, via the downregulation of the profibrotic cytokines TGF-β and CTGF, with a subsequent reduction in the tubulointerstitial infiltration of T-lymphocytes and macrophages. These data suggest that a deficiency in DDR1 relieves various Alport syndrome symptoms, such as renal fibrosis and inflammation, which indicates that DDR1 is an important collagen receptor in fibrosis and inflammation in the podocyte–matrix interaction.

Taken together, these results suggest that DDR1-deficient mice develop resistance to fibrosis and inflammation followed by macrophage infiltration, which in turn implies that DDR1 might play an important proinflammatory role in kidney damage.

4.7.3 Liver Fibrosis

Contrary to the positive role of DDR1 in fibrosis, DDR2 has been reported as an inhibitor of fibrosis in the liver [32]. The administration of carbon tetrachloride (CCl_4) increases collagen deposition and the extracellular matrix remodeling response, related to the altered recruitment of activated hepatic stellate cells in the livers of DDR2-deficient mice. Immunomodulatory and fibrogenic genes are upregulated, and chemotactic migration and proliferation is induced. However, the extracellular matrix remodeling is reduced in the hepatic stellate cells following a CCl_4 injection. DDR2 expression and phosphorylated DDR2 levels are raised in hepatic stellate cells following a CCl_4 injection. These data suggest that DDR2 is plays an important role in suppressing fibrosis in chronic injury of the normal liver.

4.8 Auditory Sensation

DDR1-deficient mice have an alternative phenotype that is associated with auditory sensation. DDR1-deficient mice develop a fundamental hearing loss by 2 months of age [33]. In normal mice, DDR1 is mainly expressed in the basal cells of the stria vascularis, type III fibrocytes, and in the cells lining the basilar membrane of the organ of Corti in the normal cochlea. In DDR1-deficient mice, a number of distinguishable alterations on the stria vascularis are observed, for example, the morphological alterations of the basal cells and the increase in electron-dense matrix deposition within the stria vascularis. These cytological alterations may be responsible for the hearing loss in DDR1-deficient mice. These results suggest that DDR1 might play an essential role in tissue composition maintenance and in regulating collagen deposition in the inner ear.

4.9 Heart Structure and Function

DDR2 is expressed in the mesenchymal cells throughout the body, and also in cardiac fibroblasts in the heart. To elucidate the role of DDR2 in heart function and structure, DDR2-deficient mice were produced by the homologous recombination of a targeting vector into embryonic stem cells [34]. The heart size in DDR2-null mice was reduced due to shorter cardiomyocytes, and not simply because of a reduced body size. The DDR2-null heart was physiologically affected, with alterations such as a lower heart rate, reduced contractility, and slower relaxation. The normalized cardiac collagen mass was not different between DDR2-null and normal mice, but the proportion of the collagen area in DDR2-null mice was significantly higher, which suggests that collagen density was reduced due to an altered cardiac fibroblast function. These results suggest that DDR2 plays an important role in collagen deposition to maintain the cardiac structure and function.

4.10 Conclusion

DDR mouse models shed light on the functional importance of the DDRs, such as their contribution to normal and homeostatic development, and highlight their enhancement or attenuation of diseases, including fibrosis, atherosclerosis, and osteoarthritis, in various conditions.

Studies on DDR mouse models might help elucidate the molecular functions of this receptor and help to develop molecular therapies for DDR-related diseases.

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