Center for Experimental Medicine and Systems Biology

Laboratory of Molecular Pathogenesis
システム疾患モデル研究センター分子病態研究分野

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Recent development of transgenic techniques has made it possible to directly analyze the functions of a particular gene in a living animal. These techniques have also made it possible to produce various animal disease models as well as tools to analyze them. Immune disorders and infectious diseases are our major concerns, and we are attempting to produce transgenic mouse models for these diseases.

1. Studies on rheumatoid arthritis models

Shinobu Saijo, Noriyuki Fujikado, Toshimasa Kusaka, Satoshi Ikeda, Aoi Akitsu, Soo-Hyun Chung, Sun-Ji Park, and Yoichiro Iwakura

Rheumatoid arthritis (RA) is one of the most serious medical problems worldwide with approximately 1% of the people in the world affected. The disease is autoimmune in nature and characterized by chronic inflammation of the synovial tissues in multiple joints that leads to joint destruction. High levels of inflammatory cytokine expression in the joints are a characteristic of the disease, although the pathogenesis has not been elucidated completely. We have been studying the pathogenesis of the disease using two arthritis models that we originally developed. One is HTLV-I transgenic mice (Iwakura et al., *Science*, 1991) and the other is IL-1 receptor antagonist-deficient mice (Horai et al., *J. Exp. Med*., 2000). Both of these models develop autoimmunity and chronic inflammatory arthropathy closely resembling RA in humans.

To identify genes involved in the pathogenesis of arthritis, we analyzed the gene expression profiles of these animal models by using high-density oligonucleotide arrays. We extracted 554 genes which expression significantly changed in both models. Then, each of these commonly changed genes was mapped into the whole genome in a scale of the 1-megabase pairs. We found that the transcriptome map of these genes formed clusters including the major histocompatibility complex class I and class II genes, complement genes, and chemokine genes, which are well known to be involved in the pathogenesis of RA at the effector phase. By searching for such clusters, we could detect genes with marginal expression changes, including several genes whose involvement in the arthritis pathogenesis has previously not known. We have also searched for pathogenesis-related genes by analyzing the effect of genetic backgrounds on the arthritis development. We have identified several genes which are involved in the BALB/c strain-specific development of arthritis in these models. We are now analyzing the roles of these genes in the pathogenesis of arthritis and autoimmunity by generating the knockout mice of

these genes.

2. Studies on DCIR that maintains the homeostasis of the immune system and bone metabolism

Noriyuki Fujikado, Kazusuke Shimamori, Hiroko Manaka, Guangyu Ma, Akimasa Seno, Tomonori Kaifu, Shinobu Saijo, and Yoichiro Iwakura

The dendritic cell immunoreceptor (official gene symbol *Clec4a2*, called *Dcir* here) is a C-type lectin receptor expressed mainly in dendritic cells (DCs) that has a carbohydrate recognition domain in its extracellular portion and an immunoreceptor tyrosine-based inhibitory motif, which transduces negative signals into cells, in its cytoplasmic portion. We found that *Dcir* expression was enhanced in the joints of two mouse RA models. Because the structural characteristics of DCIR suggest that it may have an immune regulatory role, and because autoimmune-related genes are mapped to the *DCIR* locus in humans, we generated $Dcir^{-/-}$ mice to learn more about the pathological roles of this molecule. Recently, we showed that $Dcir^{-/-}$ mice spontaneously develop autoimmune enthesitis and sialadenitis, and showed a markedly exacerbated response to collagen-induced arthritis (CIA), allergic airway hyperresponsiveness (AHR) and experimental allergic encephalomyelitis (EAE) due to overexpansion of DCs, suggesting that DCIR has a crucial role in maintaining the homeostasis of the immune system (Fujikado et al., *Nat. Med*., 2008). Since these mice also develop ankylosing arthropathy, we analyzed the roles of this molecule in bone metabolism. We found that the osteoclastgenesis was enhanced in $Dcir^{-/-}$ mice due to GM-CSF-dependent expansion of osteoclast (OC) precursor pool, and *Dcir* also regulated osteoblast (OB) functions. The bone mass and bone turnover rate were increased in young $Dcir^{-/-}$ mice, due to the activation of both OCs and OBs. Thus, *Dcir* also regulates not only pathogenic bone degradation but also physiological bone metabolism. These findings may provide a clue for the treatment of several autoimmune and bone metabolic diseases.

3. Studies on the roles of IL-17 family genes in inflammation and host defense against bacteria

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IL-17A is a proinflammatory cytokine that activates T cells and other immune cells to produce a variety of cytokines, chemokines, and cell adhesion molecules. This cytokine is augmented in the sera and/or tissues of patients with contact dermatitis, asthma, and rheumatoid arthritis. We previously demonstrated that IL-17 A is involved in the development of autoimmune arthritis, contact, delayed, and airway hypersensitivity, and experimental autoimmune encephalomyelitis in mice (Nakae et al., *Immunity*, 2002; Nakae et al., *PNAS*, 2003, Nakae et al., *J. Immunol*., 2003; Komiyama et al., *J. Immunol*., 2006). Recently, it was shown that IL-17A is produced by a newly discovered CD4+ T cell subset, Th17. Now, many diseases that were thought to be induced by Th1 or Th2 are proven to be induced by Th17. We are now analyzing the roles of IL-17A and its family molecules in inflammatory diseases and host defense against infection. IL-17F is a member of the IL-17 family and has the highest amino acid sequence homology to IL-17 A. Although IL-17F is produced by Th17 cells and binds the same receptor, the functional roles of this molecule remain largely unknown. To distinguish the roles of IL-17A and IL-17F, in allergy, autoimmunity, and infection, we generated $Il17a-/-$, $Il17f-/-$ and $Il17a/f-/-$ mice and demonstrated that only IL-17A, but not IL-17F, is involved in the development of delayed-type and contact hypersensitivities, and autoimmune encephalomyelitis. Similarly, IL-17A played more important roles than IL-17F in the development of collagen-induced arthritis and autoimmune arthritis in $Il1rn-/-$ mice. In contrast, both IL-17F and IL-17A were involved in the host defense against mucoepithelial infection by *Staphylococcus aureus* and *Citrobacter rodentium*. IL-17A was produced mainly in T cells, whereas IL-17F was produced in T cells, innate immune cells, and epithelial cells. Although only IL-17A efficiently induced cytokines in macrophages, both cytokines activated epithelial innate immune responses. These observations indicate that IL-17A and IL-17F have overlapping yet distinct roles in host immune and defense mechanisms.

Although several lines of evidence suggest that IL-17A is not involved in the host defense against intracellular bacteria which induce robust Th1-, but not Th17-, type response, it is still unknown whether IL-17F plays any protective

role against these bacteria. To elucidate the roles of IL-17F in the host defense against intracellular bacteria, we infected wide-type, *Il17a*−/−, *Il17f*−/− and *Il17a/f*−/− mice with these bacteria, and now we are analyzing the bacterial burdens and Th cell responses in lymphoid or non-lymphoid organs.

4. Studies on C-type lectins in the host defense against fungal infection

Shinobu Saijo, Satoshi Ikeda, Keiko Yamabe, Shigeru Kakuta, Harumichi Ishigame, Aoi Akitsu, Noriyuki Fujikado, Toshimasa Kusaka, Sachiko Kubo, Soo-hyun Chung, Ryohei Komatsu, Noriko Miura[3], Yoshiyuki Adachi[3], Naohito Ohno[3], Kazutoshi Shibuya[4], Natsuo Yamamoto[5], Kazuyoshi Kawakami[6], Sho Yamasaki[7], Takashi Saito[7,8], Shizuo Akira[9], and Yoichiro Iwakura

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C-type lectins comprise large family members, which are characterized by extra cellular carbohydrate recognition domains (CRDs). They bind sugar chains in a calcium dependent manner. Some C-type lectins have endogenous ligands, while other C-type lectins bind pathogen-associated molecular patterns. Some of them recognize both as their lignads. The cytoplasmic tails of the C-type lectins are diverse and contain several conserved motifs that are important for antigen uptake or potential signaling. Although the C-type lectins in NK cells are relatively well studied, the roles of these molecules in DCs or macrophages remain to be elucidated. Dectin-1 has an ITAM which is well known for transducing activation signals in T cells and B cells, but it is not known if ITAMs in DCs are also functional. Thus, we have generated Dectin-1 and Dectin-2 deficient mice to study the roles of C-type lectins in DCs.

We have shown that Dectin-1 is a specific receptor for β-glucans, one of the fungal cell wall components, to produce cytokines. On the other hand, Dectin-2 recognizes α-mannans, another fungal cell wall component, and Dectin-2-deficient DCs had virtually no fungal α-mannan-induced cytokine production and Dectin-2 signaling induced cytokines through an FcRγ and Syk-CARD9-NF-κB-dependent pathway without involvement of MAPKs. In vivo, Dectin-1 deficient mice were more susceptible to pneumocystis infection, but not candida infection, compared to wild-type mice. On the other hand, Dectin-2 deficient mice were more susceptible to candida infection. Interestingly, we found that the cytokine production including IL-1β and IL-23 induced by the yeast form was completely dependent on Dectin-2, but that by hyphae depended only partially. Both yeasts and hyphae preferentially induced Th17 differentiation, in which Dectin-2, but not Dectin-1, was mainly involved. Because IL-17A-, but not IL-17F-, deficient mice were highly susceptible to systemic candida infection, it was suggested that Dectin-2 plays an important role in host defense against *C. albicans* by inducing Th17 differentiation.

5. Study on rheumatoid arthritis-related-gene-knockout mice

Toshimasa Kusaka, Masanori Murayama, Akihiko Mizoroki, Kazuma Ariyama, Soichiro Shimizu, Kenji Shimizu, Tetsuo Hirano, Akira Ohta, Sun-Ji Park, Shinobu Saijo, Noriyuki Fujikado, Shigeru Kakuta, and Yoichiro Iwakura

Rheumatoid arthritis (RA) is a systemic autoimmune disease with both genetic and environmental factors contributing to its etiology. Animal models provide a powerful tool to study genes involved in this complex disease and to gain insights into the pathogenic mechanisms.

We have established two different arthritis model mice, human T-cell leukemia virus type I-transgenic mice (HTLV-I Tg) and the Interleukin-1 receptor antagonist deficient mice. These mice develop autoimmunity and joint-specific inflammation that closely resembles human RA. BALB/cA strain mice were highly susceptible to develop arthritis in these models, whereas C57 BL/6J strain mice were resistant. Since genetic factors have been suggested to be involved in the development of arthritis, we performed linkage-analysis using BALB/c-HTLV-I Tg backcross progenies into C57BL/6J, and identified several RA-related gene locus that affected the strain specificity of the arthritis susceptibility. Furthermore, we analyzed the gene expression profiles of these animal models by using high-

density oligonucleotide arrays, and identified genes in the RA-related gene locus which are overexpressed in the affected joints.

Among these genes, we are now analyzing the functions of two cytokine receptors and three novel genes, "*Tora*", which is expressed in T cells and B cells, and "*Kusa*" and "*Shimi*", which are expressed in dendritic cells and macrophages. Because the physiological roles of these genes have not been reported, we generated gene targeted mice for these genes. Both *Tora*$^{-/-}$, *Kusa*$^{-/-}$, and *Shimi*$^{-/-}$ mice were fertile, were born at the expected mendelian ratios, and showed no obvious phenotypic abnormalities. We are now analyzing the function of these genes.

6. Generation of AIDS disease models and analysis of the pathogenesis using animal models

Motohiko Kadoki, Takuya Tada, Haruka Hirosaki, and Yoichiro Iwakura

Studies to elucidate AIDS pathogenesis and to develop therapeutic drugs and vaccines have been hampered by the lack of appropriate small animal models, because small animals like mice are resistant against HIV-1 infection due to the difference of the structure of some host factors necessary for HIV-1 replication. As primate models are difficult to handle, expensive and require expensive facilities, genetically not well characterized, often infected with other pathogens, and ethically less acceptable, we are trying to generate mouse models for AIDS. We are taking two approaches to generate AIDS models; one is HIV-1 carrier models which carry the HIV-1 genome as a transgene (Iwakura et al., *AIDS*, 1992) and the other is HIV-1 susceptible models in which all the host factors involved in the species barrier are humanized.

In the course of the development of AIDS, bacterial infection causes deleterious effects on the progression of the disease; bacterial lipopolysaccharides (LPS) in the circulation activate immune cells, resulting in the acceleration of HIV replication. However, the precise HIV activation mechanisms in infected hosts remain largely unknown. Previously, we generated transgenic mice carrying the HIV-1 genome and showed that LPS induces the activation of HIV-1 in splenocytes through the induction of TNF and IL-1, although similarly induced IFN-γ and IL-6 are not involved (Iwakura et al., *AIDS*, 1992; Tanaka et al., *AIDS*, 2000; 2003). This year, we analyzed the mechanisms of HIV-1 activation in macrophages using these HIV-1 transgenic mice, because macrophages are one of the major reservoirs of HIV-1. In contrast to splenocytes, direct TLR4 signaling rather than TLR-induced pro-inflammatory cytokines was responsible for the LPS-induced activation of HIV-1 in macrophages, because the time course of HIV-1 activation was earlier than that observed in splenocytes and TNF neutralization did not inhibit the activation. p38 MAPK and NF-κB activation, but neither ERK nor JNK activation, were required for the activation, because only inhibitors for p38 MAPK and NF-κB suppressed activation of HIV-1. Furthermore, we showed that MyD88, rather than TRIF, was required as an adaptor molecule for this activation using *MyD88*$^{-/-}$ mice and Dynasore, a specific inhibitor for TRIF, and siRNAs specific for *MyD88* and *Trif*. These observations suggest that suppression of these molecules, which are involved in the TLR4-MyD88 pathway and the downstream p38 MAPK and NF-κB pathways, should be beneficial to prevent development of AIDS in HIV-1 infected people.

7. Gene trap screening of membrane proteins involved in the regulation of pluripotent stem cell differentiation

Hiroaki Okae, and Yoichiro Iwakura

The pluripotent stem cells can give rise to all types of cells seen in the adulthood. Although many cell fates are determined by cell-cell interactions, the molecular mechanisms are not fully understood. Thus, we analyzed the roles of membrane proteins in the regulation of pluripotent stem cell differentiation.

A combination of both signal sequence trap and *in vitro* differentiation of ES cells was used to identify genes that are involved in these processes. Signal sequence trap is a method to specifically disrupt genes that have signal sequences. By using these methods, 26 genes that were highly expressed in ES cells and encoded membrane bound proteins were disrupted in ES cells. From these ES cells, we have established 3 KO mouse lines.

Among three KO lines produced in this study, *Gβ1* KO mouse line was selected for further analysis because these mice died early in pregnancy. Heterotrimeric G proteins are well known for their roles in signal transduction downstream of G protein-coupled receptors (GPCRs), and both Gα subunits and tightly associated Gβγ subunits regulate downstream effector molecules. Compared to Gα subunits, the physiological roles of individual Gβ and Gγ subunits are poorly understood. *Gβ1* gene encodes one of heterotrimeric G protein β subunits and is highly expressed in pluripotent stem cells

and neural progenitor cells. Using *Gβ1* KO mice, we found that *Gβ1* is required for neural tube closure, neural progenitor cell proliferation, and neonatal development. About 40% $G\beta1^{-/-}$ embryos developed neural tube defects (NTDs) and abnormal actin organization was observed in the basal side of neuroepithelium. In addition, $G\beta1^{-/-}$ embryos without NTDs showed microencephaly and died within 2 days after birth. GPCR agonist-induced ERK phosphorylation, cell proliferation, and cell spreading, which were all found to be regulated by Gαi and Gβγ signaling, were abnormal in $G\beta1^{-/-}$ neural progenitor cells. These data indicate that *Gβ1* is required for normal embryonic neurogenesis.

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Center for Experimental Medicine and Systems Biology

Laboratory of Developmental Genetics
システム疾患モデル研究センター　発生工学研究分野

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Gene targeting technology has revealed many aspects of gene functions in vivo. Knock out mice offer the opportunities of not only analyzing the complex gene functions in vivo, but also presenting various human disease models, where new therapeutic approaches can be explored. To allow more detailed dissection of gene function, we introduce a point mutation or disrupt genes in certain lineages (or stages) using Cre-loxP system, a method of conditional gene targeting. In the process of analyzing knock out mice, we have isolated spontaneous mutant mice which develop chylous ascites and edematous limbs. In order to understand the mechanism of lymphatic development and functions in more detail, we are also generating various knock-out/knock-in mouse lines including a conditional knock out mouse. ES cells, which are used for gene targeting, are defined as the cells that have the abilities of self-renewal and pluripotency. We are particularly interested in the neural differentiation of ES cells, aiming to understand the molecular mechanism of the maintenance of stemness and neural differentiation and to advance towards cell therapy of the damaged or degenerating nervous system.

1. Polypyrimidine tract-binding protein is essential for early mouse development and embryonic stem cell proliferation.

Masaki Shibayama, Satona Ohno, Takashi Osaka, Reiko Sakamoto, Akinori Tokunaga, Yuhki Nakatake, Mitsuharu Sato and Nobuaki Yoshida.

Polypyrimidine tract-binding protein (PTB) is a widely expressed RNA-binding protein with multiple roles in RNA processing, including the splicing of alternative exons, mRNA stability, mRNA localization, and internal ribosome entry site-dependent translation of cellular and viral RNA. Although it has been reported that increased expression of PTB is correlated with cancer cell growth, the role of PTB in mammalian development is still unclear. Here, we report that a homozygous mutation in the mouse Ptb gene causes embryonic lethality shortly after implantation. We also established Ptb(−/−) embryonic stem (ES) cell lines and found that these mutant cells exhibited severe defects in cell proliferation without aberrant differentiation in vitro or in vivo. Furthermore, cell cycle analysis and a cell synchronization assay revealed that Ptb(−/−) ES cells have a prolonged G(2)/M phase. Thus, our data indicate that PTB is essential for early mouse development and ES cell proliferation.

2. Regulation of protein Citrullination through p53/PADI4 network in DNA damage response.

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Upon a wide range of cellular stresses, p53 is activated and inhibits malignant transformation through the transcriptional regulation of its target genes related to apoptosis, cell cycle arrest, and DNA repair. However, its involvement in posttranslational modifications of proteins has not yet been well characterized. Here, we report the novel role of p53 in the regulation of protein citrullination. p53 transactivated peptidylarginine deiminase type 4 (PADI4) through an intronic p53-binding site. The PADI4 gene encodes an enzyme catalyzing the citrullination of arginine residues in proteins, and ectopic expression of p53 or PADI4 induced protein citrullination. In addition, various proteins were citrullinated in response to DNA damage, but knockdown of PADI4 or p53 remarkably inhibited their citrullination, indicating the regulation of protein citrullination in a p53/PADI4-dependent manner. We found that PADI4 citrullinated the histone chaperone protein, nucleophosmin (NPM1), at the arginine 197 residue in vivo under physiologic conditions. Citrullination of NPM1 by PADI4 resulted in its translocation from the nucleoli to the nucleoplasm, whereas PADI4 did not alter the localization of mutant NPM1 (R197K). Furthermore, ectopic expression of PADI4 inhibited tumor cell growth, and concordantly, the knockdown of PADI4 attenuated p53-mediated growth-inhibitory activity, demonstrating the significance of PADI4-mediated protein citrullination in the p53 signaling pathway.

3. XEDAR as a putative colorectal tumor suppressor that mediates p53-regulated anoikis pathway.

Chizu Tanikawa[1], Yoichi Furukawa[3], Nobuaki Yoshida, Hirofumi Arakawa[4], Yusuke Nakamura[1] and Koichi Matsuda[1]: [3]Division of Clinical Genome Research, The University of Tokyo, [4]Cancer Medicine and Biophysics Division, National Cancer Center Research Institute.

Colorectal cancers with mutations in the p53 gene have an invasive property, but its underlying mechanism is not fully understood. Through the screening of two data sets of the genome-wide expression profile, one for p53-introduced cells and the other for the numbers of cancer tissues, we report here X-linked ectodermal dysplasia receptor (XEDAR), a member of the TNFR superfamily, as a novel p53 target that has a crucial role in colorectal carcinogenesis. p53 upregulated XEDAR expression through two p53-binding sites within intron 1 of the XEDAR gene. We also found a significant correlation between decreased XEDAR expressions and p53 gene mutations in breast and lung cancer cell lines ($P=0.0043$ and $P=0.0122$, respectively). Furthermore, promoter hypermethylation of the XEDAR gene was detected in 20 of 20 colorectal cancer cell lines (100%) and in 6 of 12 colorectal cancer tissues (50%), respectively. Thus, the XEDAR expression was suppressed to $<25\%$ of surrounding normal tissues in 12 of 18 colorectal cancer tissues (66.7%) due to either its epigenetic alterations and/or p53 mutations. We also found that XEDAR interacted with and subsequently caused the accumulation of FAS protein, another member of p53-inducible TNFR. Moreover, XEDAR negatively regulated FAK, a central component of focal adhesion. As a result, inactivation of XEDAR resulted in the enhancement of cell adhesion and spreading, as well as resistance to p53-induced apoptosis. Taken together, our findings showed that XEDAR is a putative tumor suppressor that could prevent malignant transformation and tumor progression by regulating apoptosis and anoikis.

4. Phospholipase Cγ2 is necessary for separation of blood and lymphatic vasculature in mice.

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The lymphatic vasculature originates from the blood vasculature through a mechanism relying on Prox1 expression and VEGFC signalling, and is separated and kept separate from the blood vasculature in a Syk- and SLP76-dependent manner. However, the mechanism by which lymphatic vessels are separated from blood vessels is not known. To gain an understanding of the vascular partitioning, we searched for the affected gene in a spontaneous mouse mutant exhibiting blood-filled lymphatic vessels, and identified a null mutation of the Plcg2 gene, which encodes phospholipase Cgamma2 (PLCgamma2), by positional candidate cloning. The blood-lymph shunt observed in PLCgamma2-null mice was due to aberrant separation of blood and lymphatic vessels. A similar phenotype was observed in lethally irradiated wild-type mice reconstituted with PLCgamma2-null

bone marrow cells. These findings indicate that PLCgamma2 plays an essential role in initiating and maintaining the separation of the blood and lymphatic vasculature.

5. Role of polypyrimidine tract binding protein in cell cycle regulation.

Satona Ohno, Mitsuharu Sato, Masaki Shibayama and Nobuaki Yoshida.

PTB is a member of the heterogeneous nuclear ribonucleoprotein particle (hnRNP) family that binds to pyrimidine-rich sequences of RNAs. Our group has shown that a homozygous mutation in the mouse Ptb gene causes embryonic lethality shortly after implantation. Furthermore, we also established mouse embryonic stem (ES) cells with a homozygous disruption of *ptb* ($ptb^{-/-}$) represent severe defects in cell proliferation without aberrant differentiation. To reveal a cause of proliferation defect in $ptb^{-/-}$ ES cells, we analyzed the cell cycle progression of $ptb^{-/-}$ ES cells by flow cytometry. We found that G(2)/M phase is prolonged in $ptb^{-/-}$ ES cells. Thus, our data indicate that PTB is essential for early mouse development and ES cell proliferation.

Then we focused on the regulation of IRES-dependent translation, which is one of the functions of PTB involved in M phase regulation. We showed that the IRES activity of $CDK11^{p58}$, which is one of the M phase regulators, is higher in $ptb^{-/-}$ ES cell than that in $ptb^{+/+}$ ES cells. Furthermore, we found that PTB binds to $CDK11^{p58}$ IRES directly and represses the IRES-dependent translation of $CDK11^{p58}$ in ES cells.

These results suggest the importance of PTB in the progression and termination of M phase through the regulation of IRES-dependent translation and the regulation of IRES-dependent translation in ES cells is different from that in differentiated cells. Our finding would contribute to further understanding of the cell cycle regulation in ES cells.

6. Analysis of Fbxl10 function in mouse development.

Tsuyoshi Fukuda, Reiko Sakamoto, Mitsuharu sato and Nobuaki Yoshida.

Histone methylation has important roles in regulating transcription, genome integrity and epigenetic inheritance. Historically, methylated histone lysine residue has considered stable modification because of its thermodynamic stability. Recent study, however, demonstrated that methylation of histone lysine is removed by jumonji C (JmjC)-domain-containing protein.

Fbxl10 is a paralog of the first identified JmjC-domain-containing histone demethylase, Fbxl11, which targets histone 3 lysine 36 for demethylation. Although histone demethylase activity and the target genes of Fbxl10 have been identified so far, very little is known regarding the physiologic functions of Fbxl10.

To investigate biological functions of Fbxl10, we disrupted Fbxl10 gene in mouse embryonic stem cells and generated Fbxl10 knockout mice. Fbxl10 null mice show exencephaly during embryonic stage and die shortly after birth with moderate penetration rate.

Fbxl10 is dominantly expressed in neural progenitor cells and regulates proliferation and apoptosis of these cells. These results indicated that Fbxl10 has important roles in mouse neural development. Physiological roles of Fbxl10 in mouse embryogenesis are currently under investigation.

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