Spatiotemporal expression of Prdm genes during *Xenopus* development

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Received: 5 January 2015 / Accepted: 16 January 2015 / Published online: 19 February 2015
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Abstract Epigenetic regulation is known to be important in embryonic development, cell differentiation and regulation of cancer cells. Molecular mechanisms of epigenetic modification have DNA methylation and histone tail modification such as acetylation, phosphorylation and ubiquitination. Until now, many kinds of enzymes that modify histone tail with various functional groups have been reported and regulate the epigenetic state of genes. Among them, Prdm genes were identified as histone methyltransferase. Prdm genes are characterized by an N-terminal PR/SET domain and C-terminal some zinc finger domains and therefore they are considered to have both DNA-binding ability and methylation activity. Among vertebrate, fifteen members are estimated to belong to Prdm genes family. Even though Prdm genes are thought to play important roles for cell fate determination and cell differentiation, there is an incomplete understanding of their expression and

functions in early development. Here, we report that Prdm genes exhibit dynamic expression pattern in *Xenopus* embryogenesis. By whole mount in situ hybridization analysis, we show that Prdm genes are expressed in spatially localized manners in embryo and all of Prdm genes are expressed in neural cells in developing central nervous systems. Our study suggests that Prdm genes may be new candidates to function in neural cell differentiation.

Keywords Epigenetics · Neural development · Prdm · *Xenopus* embryos

Introduction

Vertebrate development occurs as a series of cell fate determination and cell differentiation events and those are regulated by various secreted signaling molecules and transcription factors. Recently, epigenetic regulation has been reported that it is important in embryonic development, cell differentiation and regulation of cancer cells. Molecular mechanisms of epigenetic modification have DNA methylation and histone modification such as acetylation, phosphorylation and ubiquitination.

For example, histone acetylation is well correlated with the condition of gene expression. The acetylation level is regulated in a dynamic manner. Histone acetylation is catalyzed by HATs (histone

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acetyltransferase) and HDAC (histone deacetylase). HATs are classified into five groups, such as GNAT (GCN5-related *N*-acetyltransferase), MYST (MOZ, Ybf/Sas3, Sas2 and Tip60) and P300/CBP, and recently Camello is added as new group (Karmodiya et al. 2014). HDAC are also categorized into five groups; class 1, class 2a, class 2b, class 3 and class 4 by sequence homology and subcellular localization (Wang et al. 2014). Usually, HATs and HDAC are involved in a multisubunit and multifunctional complex and act as transcriptional activator and repressor, respectively.

Histone methylation is also reported as regulation system for gene expression level. Enzyme which catalyze protein methylation, has been first reported by study of SUV39H, which suppress SU(VAR)3–9 expression in position effect variegation of *Drosophila*. SUV39H contains the evolutionally conserved SET [Su(var)3–9, enhancer of zest (EZ), trithorax (TRX)] domain which consists of 130 amino residues (Aagaard et al. 1999). The SET domain is a catalytic domain conserved among lysine methyltransferases proteins and more than 60 SET protein genes have been identified in various organisms (Jenuwein et al. 1998; Trievel et al. 2002; Qian and Zhou 2006). SET proteins are known to function in cell development, for examples, Ezh2 which is a member of PcG regulates myogenesis by histone H3-K27 methylation (O'Carroll et al. 2001; Caretti et al. 2004). Mll and Mll2 are involved in TrxG sustain Hox genes regulation in normal development (Yu et al. 1998; Glaser et al. 2006).

Prdm genes family is one of sub-family of SET genes (Kim et al. 2003; Pinheiro et al. 2012; Eom et al. 2009; Wu et al. 2008; Hayashi et al. 2005). Prdm genes are characterized by an N-terminal PR/SET domain and C-terminal zinc finger domains (Fumasoni et al. 2007) and therefore they are considered to have both DNA-binding ability and methylation activity. Prdm family genes have been reported to control cell proliferation in cancer (Nishikawa et al. 2007; Tam et al. 2006) and in normal development (Davis et al. 2006). Moreover, they act to determine cell fate. For example, Prdm16 controls the switch between skeletal muscle and brown fat in mice (Seale et al. 2008) and hamlet decides neuron class in *Drosophila* (Moore et al. 2002). Prdm proteins interact with the other chromatin modifiers. Prdm1 and Prmt5 form complex and shows H2R3 and H4R3 di-methylation activity in mouse germ cell (Ancelin et al. 2006). Also, Prdm6

acts as an epigenetic regulator by cooperating with class 1 HDAC and G9a in mouse skeletal muscle (Davis et al. 2006). Prdm gene family consists of fifteen members and even though Prdm genes are thought to play important roles for cell fate determination and cell differentiation, there is an incomplete understanding of their expression and functions in early development.

Here, we report that Prdm genes exhibit dynamic expression pattern in *Xenopus* embryogenesis. By whole mount in situ hybridization analysis, we show that Prdm genes were expressed in spatially localized manners in embryo and all of Prdm genes were expressed in developing central nervous systems (CNS). Our studies suggest that Prdm genes may be new candidates to function in neural cell differentiation.

Materials and methods

Xenopus embryo culture and RT-PCR

Xenopus laevis (*X. laevis*) embryos were obtained by artificial fertilization and were cultured in 0.1 % Steinberg's solution at 21 °C. The embryos were staged according to Nieukoop and Faber (1994). Total RNA was extracted from whole embryos using TRIzol[®] reagent (Life Technologies, Carlsbad, CA, USA). cDNA was synthesized with High Capacity cDNA Reverse Transcription Kits (Applied Biosystems, South San Francisco, CA, USA). Primers used for PCR is Table 1. Identification of *X. laevis* Prdm gene have not been completed, so Prdm3, 5, 6, 8, 10, 11, 13, 14, 15 and 16 were amplified by degenerate primers designed by *X. tropicalis* Prdm gene sequences.

Whole mount in situ hybridization

Whole mount in situ hybridizations were performed based on routine method using digoxigenin-labeled antisense RNA probe (Jones and Smith 1999). The fragments for Prdm 1–16 were amplified using cDNA as a template and cloned into pGEM[®]-T vector (Promega, Madison, WI, USA). The DIG probes were synthesized by T7 and SP6 RNA polymerase using pGEM[®]-T constructs as templates. These probes were hybridized to *Xenopus* embryos which were fixed in 10 % formaldehyde, 0.1 M MOPS, pH7.4, 2 mM EGTA, 1 mM MgSO₄ (MEMFA) for 30 to 60 minutes

Table 1 Primer sequence for RT-PCR

Gene name	Nucleotide Sequence (5'–3')
Prdm1	F: ATGAAAATGGATATGGAAGGAATTGATATG R: CTGAATTGGTGAATGCATCAGATAATTCTG
Prdm2	F: ATGTGGGAGGTATACTACCCAAACCTTGATGGATG R: AAATATTTTCTTACAGTATTTACAGGGATGCATCTC
Prdm3	F: ATGCCAGACCAGCGNACTTTNATGTCAGCAATHGARAAYATG R: GTGNAGGGATTCTGATCAGANAGNGANAGCATCAT
Prdm4	F: AGTGATAGTCACGAGCTGGACGCTGCGATTACTATG R: GGAGCTGAAGGAGTGAGAGCACATTGAGCA
Prdm5	F: GTGGATGAAGGTATAGACAACAGRTRATGTGG R: CTCACATGTGAAGGGGCGCTTTTCTGAATGNGTDATCATRTG
Prdm6	F: GAGCCTAGTAAGTCAAGCTGGATGAGRTAYGTNCGNTG R: CATCCTCTGATGTCGACTCAGCTGGGTNGCYTGNNGTRAA
Prdm8	F: AAGGACAACAGAGAGTTGGAGCCTCGNAAYACNATG R: CATTGCATATTCCTTTTTGTGGTGNGAYCTCAT
Prdm9	F: CAGATGTCGCAGGAGTCTTGT R: TGTGAAGTGTCTCCACACTC
Prdm10	F: TGTAAGTGGATGATGTTTGTACGNCCNGCNCA R: GAATCCCATCATACACACNCGCAYTTTAAACAT
Prdm11	F: ATGCACCCGGAAAAGACGGAGGAAATGTGCAGRAAYATG R: ATCGAACACTTGACACGCCTTACRAACAT
Prdm12	F: ATGATGGGCTCGGTGCTG R: CAGCTGTGAGTAGGCGCT
Prdm13	F: GGAGAGGAGCGNTACATCTGTTGGTAYTGTYGG R: CTCGCTTGCTATCTCAGANAGCTGRTGRCRTTGTCAT
Prdm14	F: ATGAACATACTNGCCACTCCNCAYGARATG R: GTGGACCCTCATGTGCTTGTTCAGGCTNGANGAYTGNGA
Prdm15	F: GCTGCTGAACCTGAAAAAGACCTGAACCCNAGYTCNTCN R: CCGGTGATGCTCCAGCATTACATCCCTCCTRTARAANAGYTT
Prdm16	F: CCTCTTCTTAAAAAGCCCTTTGAACCATAACGNGARGCNAAR R: AATGGCCGACATCTGTGGGTGAAAAAGNAGNGGNGANGG
Odc	F: GCGGGCAAAGGAGCTTAATG R: TAACGCCAGAATCTGCTGGG

Y = CT (meaning, Y can be C or T), R = AG, H = ACT, D = AGT, N = ACGT
F, forward primer; R, reverse primer

at room temperature. The color reaction was carried out using precipitating BM purple AP substrate (Roche, Mannheim, Germany).

Results

Temporal expression pattern of Prdm genes during development

First, we analyzed the temporal expression pattern of Prdm genes in *Xenopus* embryos. We extracted total RNAs from ten developmental stages, 6–7,

10–10.5, 12.5, 16–17, 19–20, 23–24, 26–27, 29/30–31, 34–35 and 37, and analyzed by RT-PCR method. Odc (ornithine decarboxylase) was used for internal control gene. As shown in Fig. 1, all of Prdm genes were expressed in early *Xenopus* embryos. Moreover, Prdm1, 2, 4, 9, 11 and 15 were observed at stage 6–7. Because transcription in embryos is not started until stage 8, it shows Prdm1, 2, 4, 9, 11 and 15 might existed as maternally inherited mRNAs and seem to function at very early phase of development. These results indicate most of Prdm genes are expressed and function in early embryogenesis.

Fig. 1 The temporal expression pattern of Prdm1–16 during development. RT-PCR analysis was carried out at stage 6–7, 10–10.5, 12.5, 16–17, 19–20, 23–24, 26–27, 29/30–31, 34–35 and 37 embryos. Total RNAs were isolated from whole embryos and that samples got converted to cDNA by reverse transcription reaction. ODC (ornithine decarboxylase) was used as control

Spatial expression pattern of Prdm genes during development

Next, we examined spatial expression pattern of Prdm genes in embryos at stage 10–40 by whole mount in situ hybridization (Fig. 2). Here, we described the expression regions of each Prdm gene member in detail.

Prdm1 started to be expressed in animal pole at stage 10.5 and then in branchia and somite at stage 21. At stage 26, its expression was observed in eyes and ears.

Prdm2 expression was observed in whole region at stage 13, which may be the maternally inherited RNA, and it was restricted in eyes and notochord regions as the development progress. At stage 37/38, Prdm2 was

Fig. 2 The spatial expression pattern of Prdm1–16 during development. *Xenopus* embryos were sampled at various stages and fixed with MEMFA (0.1 M MOPS, 2 mM EGTA, 1 mM magnesium sulfate, 10 % formaldehyde buffer). Digoxigenin-labeled antisense RNA probes were hybridized to embryos. The color reaction was carried out using precipitating BM purple AP substrate

expressed strongly in head region including eyes and notochord.

Prdm3 was expressed in notochord at stage 13 and in branchia and kidney at stage 21. As development progressed, Prdm3 expression was restricted in end-brain, midbrain, ventricular zone, eyes and nose at stage 21. It is consistent with the report in which Prdm3 was expressed in pronephron, forebrain, mid-brain and hindbrain in *Xenopus* embryo (Van Campenhout et al. 2006).

Prdm4 was expressed in whole region at gastrula stage, and at stage 27, the expression was restricted in eyes and notochord. Moreover, at stage 31, its expression was localized in branchia, nose, interbrain, midbrain and ventricular zone. In mouse development, Prdm4 was observed in embryonic cortex region (Chittka et al. 2012), thereby, *Xenopus* Prdm4 may function as well as mouse one.

Prdm5 was expressed in whole region at stage 13 and the expression was restricted in notochord as stage progressed. At stage 29/30, Prdm5 was expressed in nose.

Prdm6 was expressed in notochord at stage from 17 to 21 and then in hindbrain at stage 26. At later stages, the expression was clearly detected in ventricular zone and notochord at stage 29/30 and in eyes and notochord at stage 39. Mouse Prdm6 is known as PRISM (PR domain in smooth muscle) (Davis et al. 2006) and reported to act in post-mitotic neurons (Kinameri et al. 2008).

Prdm8 was expressed in notochord at stage 23–27. Subsequently, at stage 31, it was expressed in midbrain and hindbrain. In development of mouse CNS including retina, spinal cord and endbrain, Prdm8 expression is temporally and spatially regulated in those cells (Komai et al. 2009) and it plays a role in neocortical development which morphological changes during late and terminal multipolar phases (Inoue et al. 2014).

Prdm9 expression was observed in notochord at stage 25 and in eyes and ears at stage 31. At stage 39, it was strongly expressed in midbrain region.

Prdm10 expression was not detected until stage 26. At stage 31, expression occurred in eyes, branchia,

Prdm1

Prdm2

Prdm3

Prdm4

Prdm5

Prdm6

Prdm8

Prdm9

Fig. 2 continued

interbrain, midbrain and the ventricular zone. Mouse Prdm10 was expressed in mesoderm and neural crest cell at E8.5 (Park and Kim 2010).

Prdm11 was not observed until stage 25. Its expression began in spinal cord and notochord at stage 27 and in the whole head and spinal cord at stage 39.

Prdm12 was expressed in neural crest cells at stage 13, in spinal cord at stage 21, and in forebrain, midbrain, ears, pronephron and nose at stage 24. Mouse Prdm12 is reported to be expressed similarly in spinal cord, forebrain and midbrain at fetal mouse E9.5 (Kinameri et al. 2008).

Prdm13 was expressed in neural crest cells and spinal cord at stage from 13 to 21. At stage 26, it was observed in eyes and hindbrain along with spinal cord and then in whole head and branchia at stage 40. Hanotel et al. (2014) indicated that Prdm13 suppresses neural cell differentiation to glutamatergic neuron by suppressing of Neurog2 in *Xenopus* embryo.

Prdm14 was expressed in spinal cord at stage 13 and in pharyngeal pouch at stage 23. At stage 27, it was expressed in notochord and at stage 37/38, strongly expressed in eyes and hindbrain. Prdm14 was reported to be expressed in mouse ICM (Yamaji et al. 2008) and to be essential for reacquisition of pluripotency in fibroblast (Chia et al. 2010).

Prdm15 was expressed in animal pole at stage 12 and in area of brain at stage 21. Moreover, the gene begun to be expressed in eyes, branchia, endbrain and ventricular zone at stage 28.

Expression of Prdm16 was observed in olfactory cord and pharyngeal pouch at stage 26. Moreover, Prdm16 begun to be expressed in branchia at stage 31, and the gene was strongly expressed in whole head and kidney at stage 39.

From these results, it was shown that Prdm genes are expressed in spatially localized manners in embryo and all of Prdm genes are expressed in developing CNS, suggesting that the genes of Prdm family function mainly in neural cell differentiation and proliferation through histone tail modification.

Discussion

Here, we showed that Prdm family genes were expressed in a tissue-specific manner in *Xenopus* embryos and moreover, all Prdm genes were expressed in neural region.

In mouse, expression analysis and functional analysis of some Prdm genes have been carried out. Prdm6, 8, 12, 13 and 16 were reported to be expressed in CNS (Kinameri et al. 2008). Yang and Shinkai (2013) showed that Prdm12 was expressed transiently in neural differentiation process of mouse embryonic carcinoma cells P19 and it was involved in neural cell differentiation by suppressing cell proliferation. Also, Prdm13 has been considered to switch from excitatory to inhibitory neuron differentiation in mouse brain through methylation activity for H3K9 and H3K27 (Chang et al. 2013). Also, the Prdm genes whose

expressions were observed in the tissues besides the CNS have been identified and examined in mouse embryos. For example, Prdm9 which has catalytic activity for H3K9 tri-methylation, was detected in germ cells in meiotic phase and postnatal testis (Hayashi et al. 2005). Prdm3, 11 and 16 were expressed in mouse hematopoietic stem cells and might serve as regulator for their self-replication, maintenance and function (Thoren et al. 2013). Also, Prdm15 has been observed in human Burkitt's B cell lymphoma (Giallourakis et al. 2013). In our analysis, we found also that *Xenopus* Prdm genes were expressed in tissues beside CNS in addition to expression in CNS. These indicate that Prdm genes may have regulative role for various cell differentiation.

Our and recent evidences showed that the Prdm genes have emerged as important factors in development and cell differentiation of CNS and the other tissues in vertebrates. Even though Prdm genes are estimated as not only protein modification enzyme but also DNA-binding protein, the targets for their binding has not been identified and hence, the molecular mechanisms of their functions have been not dissolved yet. The identification of their target gene might give a better understanding of the roles of Prdm genes in the animal development.

Acknowledgments This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan.

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