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Dynamic expression pattern of *Pde4d* and its relationship with CpG methylation in the promoter during mouse embryo development

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ABSTRACT

Mouse *Pde4d* is located on chromosome 13 and serves many functions in important physiological processes involving cyclic adenosine monophosphate. In this study, imprinting analysis indicated that *Pde4d* exhibits a dynamic and specific allelic expression pattern during embryo development. This showed paternal-origin sex bias in embryonic day 9.5 (E9.5) whole embryos and placenta, and biallelic expression in the major embryonic organs and placenta at E15.5. *In situ* hybridization determined the spatiotemporal expression pattern of *Pde4d* in mouse embryos from the mid- to late-embryonic stages. This demonstrated that *Pde4d* was widely expressed in the neural tissues, including the forebrain, midbrain, hindbrain, and neural tube, at the mid-embryonic stage. By the late-embryonic stage, *Pde4d* was extensively detected throughout the developing organism, including in the liver, brain, lung, kidney, and tongue. In addition, methylation analyses indicated that tissue-specific CpG methylation of the *Pde4d* promoter was correlated with *Pde4d* mRNA expression in major E15.5 tissues. Furthermore, stage-specific CpG methylation of the *Pde4d* promoter was associated with gene expression in the liver at three developmental stages. Our results suggest that *Pde4d* might serve specific biological functions in regulating the development process of the mouse embryo, and that CpG methylation of the *Pde4d* promoter may play an important role in regulating *Pde4d* at a transcriptional level.

1. Introduction

The mouse *Pde4d* gene spans a 1.3 Mb region on chromosome 13 and contains 17 exons (Fig. 1A). The gene product of *Pde4d* is phosphodiesterase (PDE)4D. PDE4D belongs to a large superfamily of PDEs, which are enzymes that hydrolyze second-messenger cyclic adenosine monophosphate (cAMP) to play an important role in cell signaling. In mammals, the PDE family is grouped into 11 different subfamilies (PDE1–PDE11) [1], and some specific families have been associated with various disorders. The PDE4 family is encoded by four genes (designated A though D), which generate more than 20 spliced variants through the activity of different promoters [2]. Transcriptome sequencing of the genome has revealed *Pde4d* to be a special imprinted gene that exhibits paternal-origin sex bias in the embryonic day 9.5 (E9.5) embryo [3]. The expression of imprinted genes is dependent on the sex of the parent of origin, and imprinted genes have important roles in fetal growth and development [4–6]. Therefore, it is possible that *Pde4d* might play a role in regulating the process of embryo development. For instance, *Pde4d* has been determined to be a proliferation-promoting factor in prostate cancer [7] and has also been associated with atherosclerotic stroke [8]. In addition, *Pde4d*$^{-/-}$ mice exhibit delayed growth and reduced viability and female fertility, suggesting that *Pde4d* plays a critical role in regulating growth and fertility [9].

Modification of DNA cytosine nucleotides by methylation is a stable epigenetic mechanism that has an impact on gene expression by affecting the accessibility of transcription factor complexes and chromatin structures at regional and genome-wide [10]. Previous studies have found tissue-specific differentially methylated regions at promoter sequences [11,12] and indicated a possible role for these regions in regulating transcription and development [13,14].

In this study, we show dynamic imprinting patterns of *Pde4d* and a spatiotemporal expression profile of *Pde4d* mRNA during mouse embryo development, which suggest that *Pde4d* might serve important biological roles in regulating the development process of the mouse embryo. Methylation analyses revealed that tissue-specific CpG methylation of the *Pde4d* promoter was correlated with *Pde4d* mRNA expression in major tissues at

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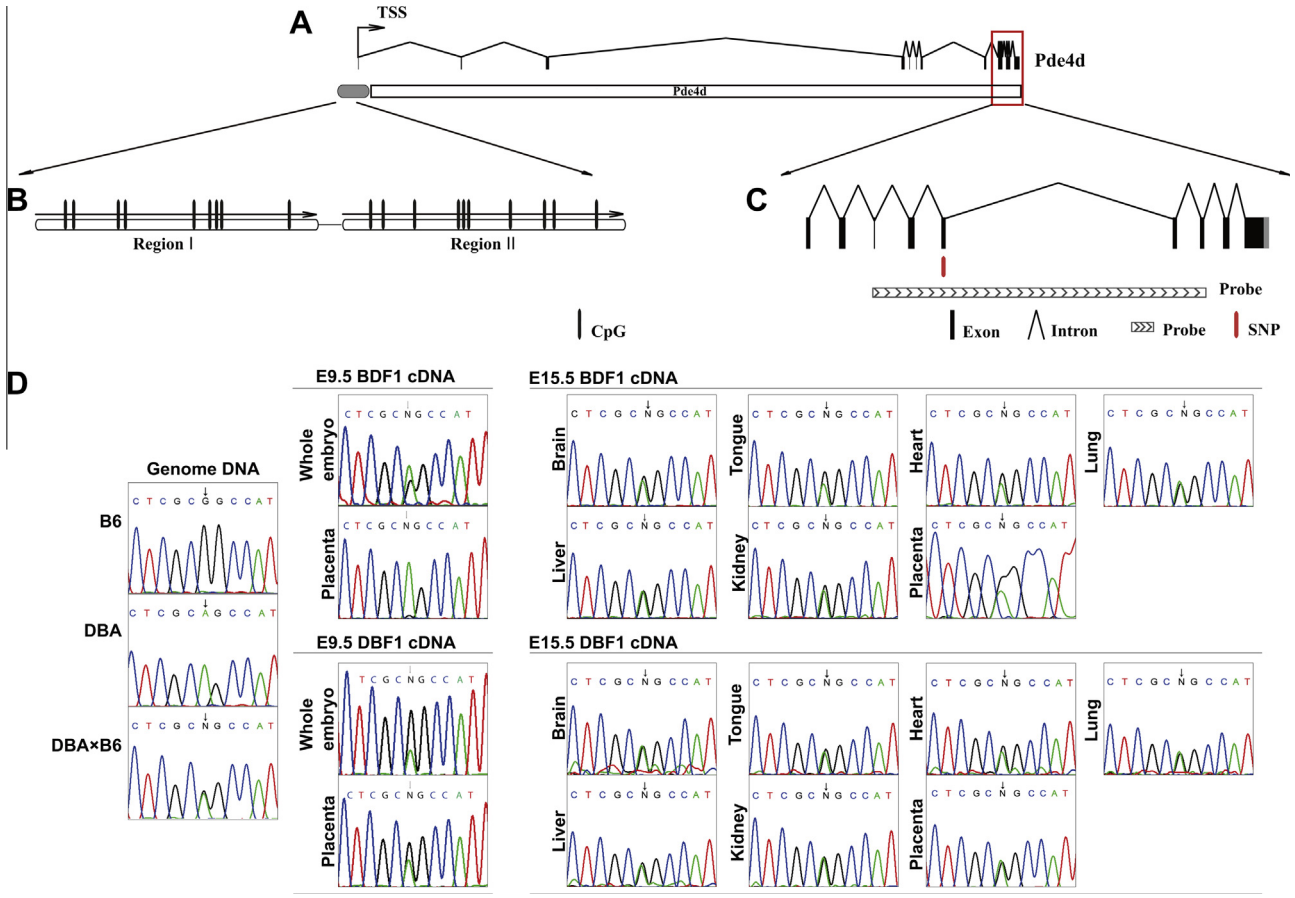

Fig. 1. Genomic structure of the *Pde4d* gene region and imprinting analysis of *Pde4d* in mouse embryo and placenta. (A) Schematic represents the overview of the structure of *Pde4d* gene. Vertical line indicates the exon, broken line indicates the intron, the grey box indicates the putative regulatory region. (B) Magnification view of putative regulatory region. Vertical line indicates the CpG site within the two regions. (C) Magnification view of region within exon 9 to exon 17. Black vertical line indicates the exon, broken line indicates the intron, red vertical line indicated the position of the SNP. Arrowed box indicates the position of RNA probe. (D) Sequencing of genomic DNA from E15.5 C57BL/6 and DBA embryos and F1 offspring demonstrate the F1 offspring is heterozygous for polymorphism G/A. Sequencing of cDNA from E9.5 F1 offspring (B × D and D × B) demonstrates paternally biased expression of *Pde4d* in whole embryo and placenta. Sequencing of cDNA from E15.5 F1 offspring (B × D and D × B) demonstrated biallelic expression of *Pde4d* in multiple tissues. The position of SNP used to determine allelic expression is indicated by the arrows. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

E15.5. In addition, a significant negative correlation between stage-specific CpG methylation of the *Pde4d* promoter and gene expression in the liver at three developmental stages was observed.

2. Materials and methods

2.1. Mice and cell lines

DBA and C57BL/6 (B6) mice were bred, and the F1 progeny (DBF1 and BDF1) obtained underwent further reciprocal crosses. Females were checked the following morning for vaginal plugs; their presence was considered as E0.5. The above-described mice were used as the source of total RNA and embryos at different developmental stages. All animal experiments carried out in this study were approved by the *Rules for Experiments on Animals* published by the Chinese government.

2.2. Whole mounts and sections for in situ hybridization

Fresh E9.5 B6 mouse embryos were fixed immediately in 4% paraformaldehyde for whole-mount *in situ* hybridization. Section *in situ* hybridization was performed on fixed B6 mouse embryos harvested from E12.5 to E15.5. Sections were obtained from mouse embryos, fixed in 4% paraformaldehyde, and embedded in paraffin wax. The RT-PCR products of *Pde4d* were cloned using pBluescript II KS(+) (Stratagene, La Jolla, CA, USA), and the recombinant plasmids were used as the template to transcribe antisense single-stranded RNA probes for *Pde4d in vitro* using an RNA labeling kit according to the manufacturer's instructions (Roche Diagnostics, Mannheim, Germany) (Fig. 1C). An alkaline phosphatase-coupled antibody to digoxigenin was used to detect the hybridized probe's signal according to the manufacturer's instructions (Roche Diagnostics).

2.3. Imprinted expression analyses

DNA polymorphisms between DBA and B6 mice were detected using single-nucleotide polymorphism (SNP) analysis. The expressed alleles of *Pde4d* were detected from the SNP data obtained by direct sequencing of the RT-PCR products. Specific primers were designed for allelic expression analyses (Supplementary Table 1).

2.4. Methylation analysis by bisulfite sequencing

Stable bisulfite-converted genomic DNA was obtained using the EZ DNA methylation kit according to the manufacturer's instructions (Zymo Research, Orange, CA, USA). Two primer sets

were designed to amplify the region of interest (Supplementary Table 1). All PCR products were purified and cloned into pBluescript II KS(+) (Stratagene), following which the individual clones were sequenced.

2.5. Quantification of mRNA by qRT-PCR

Total RNA was treated with DNase I (Invitrogen, Eugene, OR, USA), then reverse-transcribed using M-MLV reverse transcriptase (Promega, San Luis Obispo, CA, USA) and oligo(dT)$_{12-18}$ primer (TaKaRa, Dalian, Japan) according to the manufacturers' instructions. qRT-PCR with SYBR green detection was performed using an ABI 7500 Real-Time PCR system (Applied Biosystems, Carlsbad, CA, USA). Thermal cycling conditions consisted of an initial denaturation step at 95 °C for 10 min, and 40 cycles at 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s. The mRNA expression levels of *Pde4d* were normalized to *Gapdh*. Experiments were carried out in triplicate for each data point.

2.6. Treating cells with inhibitors of DNA methylation

NIH/3T3 cells were treated with 5-Aza-CdR (Sigma–Aldrich, St. Louis, MO, USA). Cells were grown under standard conditions with fresh medium containing the drug, and were treated as previously described [15,16].

3. Results

3.1. Pde4d showed paternally biased expression at E9.5 and biallelic expression at E15.5

To verify allele-specific *Pde4d* expression in the mouse embryo, PCR products were sequenced and one polymorphic site at exon13 (G/A, dbSNP: rs29909890) was used to distinguish monoallelic or biallelic expression. PCR and RT-PCR products were sequenced to analyze the allele-specific expression. At E9.5, *Pde4d* showed paternally biased expression in the whole embryo and placenta. At E15.5, all of the major organs showed biallelic expression (Fig. 1D). Thus, *Pde4d* showed paternal-origin sex bias at E9.5 but biallelic expression at E15.5 (Table 1).

3.2. Quantitative expression analyses of Pde4d in whole embryos during embryogenesis

qRT-PCR was performed to determine whether *Pde4d* expression was altered at various developmental stages of the embryo. Comparing the expression level of *Pde4d* at four developmental stages, the expression level of *Pde4d* was increased from E9.5 to E15.5, followed by a decreased trend from E15.5 to E18.5. Thus, *Pde4d* showed the highest expression level at E15.5 (Fig. 2A).

Table 1
Allele specific expression of *Pde4d* during different embryonic stages.

Embryonic stage	Tissue	Allele specific expression
E9.5	Whole embryo	Paternally-biased
	Placenta	Paternally-biased
E15.5	Brain	Biallelic
	Tongue	Biallelic
	Heart	Biallelic
	Lung	Biallelic
	Liver	Biallelic
	Kidney	Biallelic
	Placenta	Biallelic

3.3. .Pde4d expression and location in middle and late stages of mouse embryo development

To gain further insight into the spatial and temporal expression patterns of *Pde4d* during embryo development, whole mount embryos and mouse embryo sections were analyzed by *in situ* hybridization. This revealed histological details of *Pde4d* expression in the developing mouse embryo. On E9.5, the most prominent expression was observed in the developing brain, including the forebrain, midbrain, and hindbrain (Fig. 2B); moreover, *Pde4d* expression could be detected in the neural tube and otic vesicle. Prominent expression in these areas was also seen at E11.5. In addition, *Pde4d* expression was also detected in the somite, hind limb bud, and tail bud at E11.5 (Fig. 2B).

With the development of the mouse embryo, *Pde4d* was found to be ubiquitously expressed in major organs such as the brain, tongue, lung, liver, and kidney from E12.5 to E15.5. At these stages, we found *Pde4d* to be highly expressed in the liver and developing brain, including the forebrain, midbrain and cerebellum. In addition, *Pde4d* was observed in major developmental spinal column, although there was only low *Pde4d* expression in the heart (Fig. 2C). These results suggest that *Pde4d* participates in the development and formation of the major organs.

3.4. Methylation and Pde4d mRNA expression in major E15.5 tissues

Considering the high level of *Pde4d* expression at E15.5, quantitative gene expression analysis was performed by qRT-PCR to obtain further insight into the *Pde4d* mRNA expression level in major developing tissues at this stage. The liver showed the highest levels of *Pde4d* mRNA expression among six major organs: brain, tongue, heart, lung, liver and kidney. In addition, *Pde4d* was highly expressed in the brain and sustained expression was observed in the tongue, lung, and kidney. Comparing the five organs, the lowest level of *Pde4d* expression was seen in the heart (Fig. 3A).

Considering the differential expression of *Pde4d* between the liver and other organs at E15.5, we suspected that there might be a differentially methylated regulatory region for *Pde4d*, and that differential methylation of CpG sites in this region might explain the differential mRNA expression. To verify this hypothesis, we studied two regions of the *Pde4d* gene covering 1000 bp, containing the transcription start site, 500 bp upstream of the transcription start site as the predicted promoter (region I), and exon 1 to intron 1 as the putative regulatory region (region II) (Fig. 1B). Accordingly, we examined the methylation status of nine CpGs within region I and 10 CpGs within region II in the major organs. For region I, hypomethylation was observed in the liver and hypermethylation in other tissues; region II displayed a similar hypermethylation status in all of the major tissues (Fig. 3B). Taken together, these findings indicate that tissue-specific CpG methylation of region I may be associated with *Pde4d* mRNA expression in major tissues at E15.5.

3.5. Methylation and Pde4d mRNA expression in the liver at three developmental stages

Based on the association between the hypomethylation of region I and high *Pde4d* mRNA expression in the liver at E15.5, we investigated whether methylation of regions I or II was associated with developmental-stage-specific gene expression in the liver. With this in mind, we assessed *Pde4d* mRNA expression and methylation in the liver at three developmental stages: E12.5, E15.5, and E18.5. qRT-PCR analyses indicated that *Pde4d* was highly expressed in the E15.5 liver but weakly expressed in the E12.5 liver, and expressed at a moderate level in the E18.5 liver compared with the E12.5 and E15.5 liver (Fig. 4A). Methylation

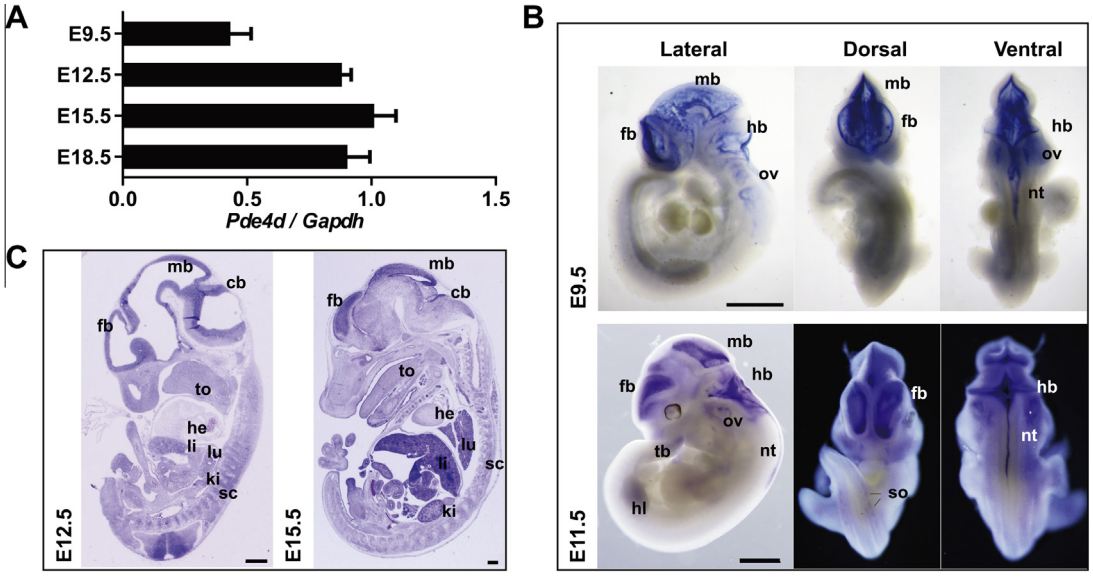

Fig. 2. Spatiotemporal expression pattern of *Pde4d* during mouse embryogenesis. (A) Total RNA was obtained from four mouse embryo development stages (E9.5, E12.5, E15.5 and E18.5). All qRT-PCR data were normalized to the level of *Gapdh*. Data are given as mean values ± SEM of 3 independent experiments. (B) Expression of *Pde4d* in E9.5 and E11.5 embryo by whole mount *in situ* hybridization. Figure annotation was as follows: fb: forebrain, hb: hindbrain, hl: hindlimb bud, mb: midbrain, so: somite, tb: tail bud. (C) Sagittal sections demonstrating widespread expression of *Pde4d* in the E12.5 and E15.5 major developmental tissues by *in situ* hybridization. Scale bar = 100 μm. Figure annotation was as follows: cb: cerebellum, fb: forebrain, he: heart, ki: kidney, li: liver, lu: lung, mb: midbrain, nt: neural tube, ov: otic vesicle, so: somite, sc: spinal column, to: tongue.

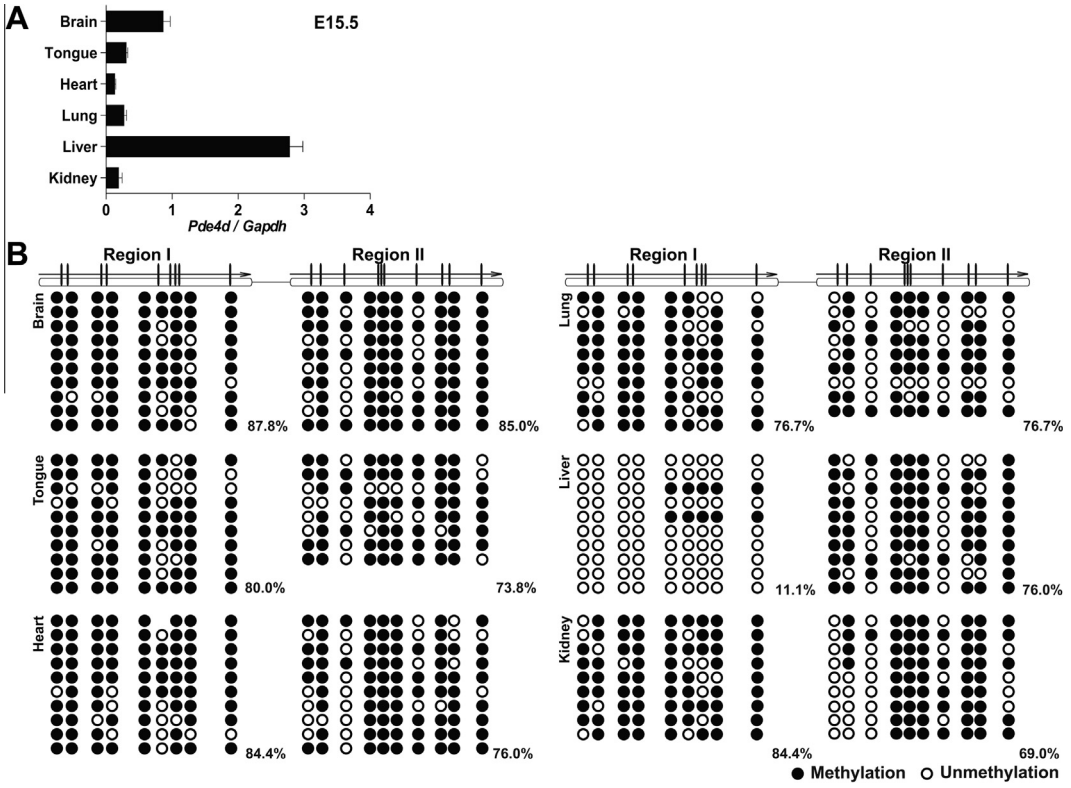

Fig. 3. Methylation and mRNA expression of *Pde4d* in major E15.5 tissues. (A) *Pde4d* mRNA expression level in major E15.5 tissues determined by qRT-PCR, all qRT-PCR data were normalized to the level of *Gapdh*. Data are given as mean values ± SEM of 3 independent experiments. (B) Vertical lines above indicated the position and numbering of CpG dinucleotides; CpG methylation status of region I and region II in various tissues were analyzed.

analyses indicated that region I was almost completely methylated in the E12.5 liver and almost completely unmethylated in the E15.5 liver, and partially unmethylated in the E18.5 liver. For region II, methylation analyses indicated that the region was almost completely methylated in the liver at all developmental stages (Fig. 4B). Taken together, these results indicate that

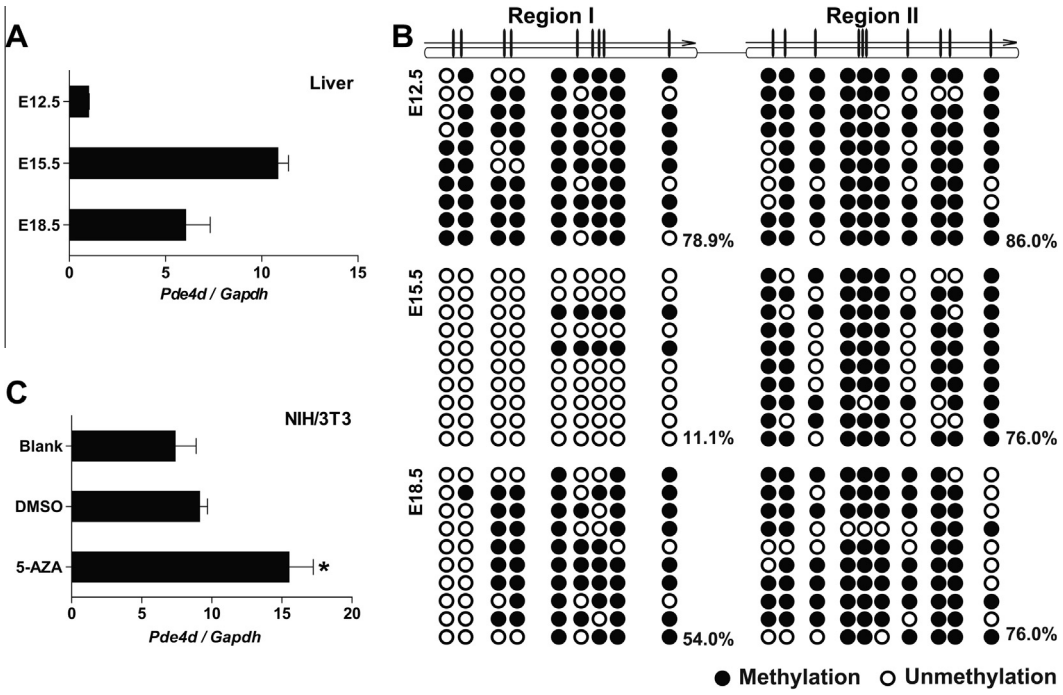

Fig. 4. Methylation and mRNA expression of *Pde4d* in liver at three developmental stages. (A) *Pde4d* mRNA expression level in liver at three developmental stages determined by qRT-PCR, all qRT-PCR data were normalized to the level of *Gapdh*. Data are given as mean values ± SEM of 3 independent experiments. (B) Vertical lines above indicated the position and numbering of CpG dinucleotides; CpG methylation status of region I and region II in liver at three developmental stages were analyzed. (C) Analysis of transcriptional change of *Pde4d* in NIH/3T3 treated 5-azadC by qRT-PCR. All qRT-PCR were normalized to the level of *Gapdh*. Data are given as mean values ± SEM of 3 independent experiments. A *P* value of less than 0.05 was considered statistically significant.

developmental-stage-specific CpG methylation of region I may be associated with differential *Pde4d* mRNA expression in the liver at three developmental stages.

4. Discussion

The present study investigated the imprinting status of *Pde4d* in the E15.5 mouse embryo, and demonstrated the *Pde4d* mRNA expression pattern during the middle and later periods of mouse embryogenesis. Furthermore, this work demonstrated that tissue- and developmental-stage-specific CpG methylation in the promoter region and differential CpG methylation in the promoter were associated with differential mRNA expression.

To date, more than 100 imprinted genes have been discovered in the mouse [17]. Most of these imprinted genes are clustered in chromosome regions, with most clusters located on chromosome 7. However, there are many new imprinted candidates in the genome that are not in gene clusters. High-throughput sequencing in a genome-wide screen for imprinting in the mouse has indicated that *Pde4d* exhibits paternal bias [3]; therefore, it is plausible that *Pde4d* is an imprinted gene with tissue- or developmental-stage-specific patterns of imprinting expression. No mouse data are available on the imprinting status of the *Pde4d* at other development stages. In this study, we found that *Pde4d* shows paternal-origin sex bias in E9.5 whole embryos and placentas, and biallelic expression in major E15.5 tissues. These results indicate that parentally biased *Pde4d* expression is altered with embryo development. Accordingly, we postulate that stage-specific allelic *Pde4d* expression is important to embryo development, just as other stage-specific imprinted genes have been reported to serve important functions in embryo development [18,19]. It is difficult to explain why *Pde4d* showed parentally biased expression in one stage but not in other stages of development, although we believe it is worth considering the mechanism of altering parentally biased expression.

Little is known about *Pde4d* expression patterns in mammalian development. The current study demonstrated the characteristics of *Pde4d* mRNA expression, with the results from *in situ* hybridization showing that *Pde4d* is highly expressed in the brain from E9.5 to E15.5. These findings are in general agreement with previous studies [20], which reported *Pde4d* expression in the brain. Because *Pde4d* has been found to play a critical role in the mediation of memory and hippocampal neurogenesis by cAMP/CREB signaling [21], we postulate that *Pde4d* might play a key role in brain development in mouse embryos. In addition, *Pde4d* was broadly detected in the developing lung from E12.5 to E15.5 by *in situ* hybridization, and qRT-PCR analysis of *Pde4d* gene expression in the lung at various fetal ages showed a gradually elevated pattern of expression. These findings are in general agreement with those from a previous study, which demonstrated steady-state and elevated levels of *Pde4d* mRNA at various fetal ages in rats [22]. Simultaneously, *Pde4d* was broadly detected in the liver, kidney, and tongue. As *Pde4d* is known to hydrolyze cAMP to have an important function in cell signaling [23], it is plausible that the presence of *Pde4d* in the above tissues might serve some functions in regulating the development of these tissues. Previous studies have provided evidence that decreased expression levels of *Pde4d* (via selective inhibitors) promote osteoblast differentiation in progenitor cells [24] and increase bone mass by inducing bone formation [25]. Therefore, detection of *Pde4d* mRNA in the spinal column suggests a possible role for *Pde4d* in bone development by regulating cAMP expression. In view of the above findings, it is likely that *Pde4d* plays a major role in regulating the developmental process of the mouse embryo. In the future, it will be necessary to identify these events to understand how these changes affect normal development.

DNA methylation has been found to be crucial for tissue-specific gene expression and globe silencing [26]. In addition, tissue-specific differential methylation in mouse promoter regions has been associated with tissue-specific gene expression [27]. In the present study, we found that the CpG methylation status of

the promoter was associated with differential mRNA expression in E15.5 liver versus other E15.5 tissues, implying that tissue-specific CpG methylation of the *Pde4d* promoter was correlated with differential mRNA expression within tissues. A previous report indicated that methylation changes during development are dynamic, and that the changes involve demethylation and methylation, and may occur at late-stage embryo development or even postnatally [28]. Also consistent with our results, we found that stage-specific CpG methylation of region I may be associated with differential *Pde4d* mRNA expression in the liver at three developmental stages; a significant negative correlation between CpG methylation of region I and gene expression was found. Therefore, it is plausible that differential mRNA expression in tissues is associated with differential DNA methylation of the *Pde4d* promoter region. In addition, we observed elevated *Pde4d* mRNA expression in cells treated with 5-Aza-CdR (Fig. 4C), further implying that the methylation status of the *Pde4d* promoter region might affect *Pde4d* expression. Accordingly, we suspect that the methylation status of the *Pde4d* promoter may be associated with *Pde4d* expression at a transcriptional level.

In summary, *Pde4d* showed parental-origin sex bias with developmental-stage specificity. *Pde4d* was ubiquitously expressed in the mouse embryo at various developmental stages, suggesting that this gene has important functions in regulating the development process of the mouse embryo. In addition, differential CpG methylation of the *Pde4d* promoter was correlated with differential mRNA expression, indicating that CpG methylation of the *Pde4d* promoter is essential for *Pde4d* mRNA expression.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.11.004.

References

[1] L. Liu, X. Xu, J. Li, X. Li, W. Sheng, Lentiviral-mediated shRNA silencing of PDE4D gene inhibits platelet-derived growth factor-induced proliferation and migration of rat aortic smooth muscle cells, Stroke Res. Treat. 2011 (2011) 534257.
[2] M. Conti, W. Richter, C. Mehats, G. Livera, J.Y. Park, C. Jin, Cyclic AMP-specific PDE4 phosphodiesterases as critical components of cyclic AMP signaling, J. Biol. Chem. 278 (2003) 5493–5496.
[3] T. Babak, B. Deveale, C. Armour, C. Raymond, M.A. Cleary, D. van der Kooy, J.M. Johnson, L.P. Lim, Global survey of genomic imprinting by transcriptome sequencing, Curr. Biol. 18 (2008) 1735–1741.
[4] S. Abu-Amero, D. Monk, S. Apostolidou, P. Stanier, G. Moore, Imprinted genes and their role in human fetal growth, Cytogenet. Genome Res. 113 (2006) 262–270.
[5] G. Kelsey, Genomic imprinting-roles and regulation in development, Endocr. Dev. 12 (2007) 99–112.
[6] W. Reik, J. Walter, Genomic imprinting: parental influence on the genome, Nat. Rev. Genet. 2 (2001) 21–32.
[7] E.P. Rahrmann, L.S. Collier, T.P. Knutson, M.E. Doyal, S.L. Kuslak, L.E. Green, R.L. Malinowski, L. Roethe, K. Akagi, M. Waknitz, W. Huang, D.A. Largaespada, P.C. Marker, Identification of PDE4D as a proliferation promoting factor in prostate cancer using a Sleeping Beauty transposon-based somatic mutagenesis screen, Cancer Res. 69 (2009) 4388–4397.
[8] S. Gretarsdottir, G. Thorleifsson, S.T. Reynisdottir, A. Manolescu, S. Jonsdottir, T. Jonsdottir, T. Gudmundsdottir, S.M. Bjarnadottir, O.B. Einarsson, H.M. Gudjonsdottir, M. Hawkins, G. Gudmundsson, H. Gudmundsdottir, H. Andrason, A.S. Gudmundsdottir, M. Sigurdardottir, T.T. Chou, J. Nahmias, S. Goss, S. Sveinbjornsdottir, E.M. Valdimarsson, F. Jakobsson, U. Agnarsson, V. Gudnason, G. Thorgeirsson, J. Fingerle, M. Gurney, D. Gudbjartsson, M.L. Frigge, A. Kong, K. Stefansson, J.R. Gulcher, The gene encoding phosphodiesterase 4D confers risk of ischemic stroke, Nat. Genet. 35 (2003) 131–138.
[9] S.L. Jin, F.J. Richard, W.P. Kuo, A.J. D'Ercole, M. Conti, Impaired growth and fertility of cAMP-specific phosphodiesterase PDE4D-deficient mice, Proc. Natl. Acad. Sci. USA 96 (1999) 11998–12003.
[10] R.J. Klose, A.P. Bird, Genomic DNA methylation: the mark and its mediators, Trends Biochem. Sci. 31 (2006) 89–97.
[11] R. Illingworth, A. Kerr, D. Desousa, H. Jorgensen, P. Ellis, J. Stalker, D. Jackson, C. Clee, R. Plumb, J. Rogers, S. Humphray, T. Cox, C. Langford, A. Bird, A novel CpG island set identifies tissue-specific methylation at developmental gene loci, PLoS Biol. 6 (2008) e22.
[12] R. Chatterjee, C. Vinson, CpG methylation recruits sequence specific transcription factors essential for tissue specific gene expression, Biochim. Biophys. Acta 2012 (1819) 763–770.
[13] A.K. Maunakea, R.P. Nagarajan, M. Bilenky, T.J. Ballinger, C. D'Souza, S.D. Fouse, B.E. Johnson, C. Hong, C. Nielsen, Y. Zhao, G. Turecki, A. Delaney, R. Varhol, N. Thiessen, K. Shchors, V.M. Heine, D.H. Rowitch, X. Xing, C. Fiore, M. Schillebeeckx, S.J. Jones, D. Haussler, M.A. Marra, M. Hirst, T. Wang, J.F. Costello, Conserved role of intragenic DNA methylation in regulating alternative promoters, Nature 466 (2010) 253–257.
[14] P. Liang, F. Song, S. Ghosh, E. Morien, M. Qin, S. Mahmood, K. Fujiwara, J. Igarashi, H. Nagase, W.A. Held, Genome-wide survey reveals dynamic widespread tissue-specific changes in DNA methylation during development, BMC Genomics 12 (2011) 231.
[15] J. Schultz, S.M. Ibrahim, J. Vera, M. Kunz, 14-3-3Sigma gene silencing during melanoma progression and its role in cell cycle control and cellular senescence, Mol. Cancer 8 (2009) 53.
[16] G. Egger, A.M. Aparicio, S.G. Escobar, P.A. Jones, Inhibition of histone deacetylation does not block resilencing of p16 after 5-aza-2′-deoxycytidine treatment, Cancer Res. 67 (2007) 346–353.
[17] C.M. Brideau, K.E. Eilertson, J.A. Hagarman, C.D. Bustamante, P.D. Soloway, Successful computational prediction of novel imprinted genes from epigenomic features, Mol. Cell. Biol. 30 (2010) 3357–3370.
[18] L. Deltour, X. Montagutelli, J.L. Guenet, J. Jami, A. Paldi, Tissue- and developmental stage-specific imprinting of the mouse proinsulin gene, Ins2, Dev. Biol. 168 (1995) 686–688.
[19] K.E. Latham, A.S. Doherty, C.D. Scott, R.M. Schultz, Igf2r and Igf2 gene expression in androgenetic, gynogenetic, and parthenogenetic preimplantation mouse embryos: absence of regulation by genomic imprinting, Genes Dev. 8 (1994) 290–299.
[20] J.A. Cherry, R.L. Davis, Cyclic AMP phosphodiesterases are localized in regions of the mouse brain associated with reinforcement, movement, and affect, J. Comp. Neurol. 407 (1999) 287–301.
[21] Y.F. Li, Y.F. Cheng, Y. Huang, M. Conti, S.P. Wilson, J.M. O'Donnell, H.T. Zhang, Phosphodiesterase-4D knock-out and RNA interference-mediated knock-down enhance memory and increase hippocampal neurogenesis via increased cAMP signaling, J. Neurosci. 31 (2011) 172–183.
[22] E. Lopez, P.H. Jarreau, E. Zana, M.L. Franco-Montoya, T. Schmitz, D. Evain-Brion, J. Bourbon, C. Delacourt, C. Mehats, Differential expression of cyclic nucleotide phosphodiesterases 4 in developing rat lung, Dev. Dyn. 239 (2010) 2470–2478.
[23] H. Liu, D. Palmer, S.L. Jimmo, D.G. Tilley, H.A. Dunkerley, S.C. Pang, D.H. Maurice, Expression of phosphodiesterase 4D (PDE4D) is regulated by both the cyclic AMP-dependent protein kinase and mitogen-activated protein kinase signaling pathways. A potential mechanism allowing for the coordinated regulation of PDE4D activity and expression in cells, J. Biol. Chem. 275 (2000) 26615–26624.
[24] S. Wakabayashi, T. Tsutsumimoto, S. Kawasaki, T. Kinoshita, H. Horiuchi, K. Takaoka, Involvement of phosphodiesterase isozymes in osteoblastic differentiation, J. Bone Miner. Res. 17 (2002) 249–256.
[25] R.H. Reneland, S. Mah, S. Kammerer, C.R. Hoyal, G. Marnellos, S.G. Wilson, P.N. Sambrook, T.D. Spector, M.R. Nelson, A. Braun, Association between a variation in the phosphodiesterase 4D gene and bone mineral density, BMC Med. Genet. 6 (2005) 9.
[26] E. Li, Chromatin modification and epigenetic reprogramming in mammalian development, Nat. Rev. Genet. 3 (2002) 662–673.
[27] S. Yagi, K. Hirabayashi, S. Sato, W. Li, Y. Takahashi, T. Hirakawa, G. Wu, N. Hattori, J. Ohgane, S. Tanaka, X.S. Liu, K. Shiota, DNA methylation profile of tissue-dependent and differentially methylated regions (T-DMRs) in mouse promoter regions demonstrating tissue-specific gene expression, Genome Res. 18 (2008) 1969–1978.
[28] F. Song, S. Mahmood, S. Ghosh, P. Liang, D.J. Smiraglia, H. Nagase, W.A. Held, Tissue specific differentially methylated regions (TDMR): changes in DNA methylation during development, Genomics 93 (2009) 130–139.