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Protein arginine Methyltransferase 8 gene is expressed in pluripotent stem cells and its expression is modulated by the transcription factor Sox2

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ABSTRACT

Addition of methyl groups to arginine residues is catalyzed by a group of enzymes called Protein Arginine Methyltransferases (Prmt). Although Prmt1 is essential in development, its paralogue Prmt8 has been poorly studied. This gene was reported to be expressed in nervous system and involved in neurogenesis. In this work, we found that Prmt8 is expressed in mouse embryonic stem cells (ESC) and in induced pluripotent stem cells, and modulated along differentiation to neural precursor cells. We found that Prmt8 promoter activity is induced by the pluripotency transcription factors Oct4, Sox2 and Nanog. Moreover, endogenous Prmt8 mRNA levels were reduced in ESC transfected with Sox2 shRNA vector. As a whole, our results indicate that Prmt8 is expressed in pluripotent stem cells and its transcription is modulated by pluripotency transcription factors. These findings suggest that besides its known function in nervous system, Prmt8 could play a role in pluripotent stem cells.

1. Introduction

Embryonic Stem Cells (ESC) are derived from the inner cell mass of blastocysts and are able to self-renew indefinitely in culture and to give rise to cells from the three germ layers, property known as pluripotency. There are three main transcription factors that govern the pluripotent state: Oct4, Sox2 and Nanog. They are simultaneously recruited to promoter regions of multiple genes throughout the genome inducing the expression of those that promote pluripotency and inhibiting the ones that lead to differentiation [1,2]. Moreover, forced expression of these factors reprograms terminally differentiated cells into pluripotent cells named induced pluripotent stem cells (iPSC) [3–5]. ESC can be maintained in undifferentiated state indefinitely, whereas *in vivo*, as development progresses, these cells leave their pluripotent state to follow diverse differentiation programs. The beginning of such

Abbreviations: Embryonic stem cells, ESC; induced pluripotent stem cells, iPSC; Mouse embyonic fibroblasts, MEFs; Protein arginine methyltransferase, Prmt; short hairpin RNA, shRNA.

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programs depends largely on the regulation of the expression of tissue-specific genes and changes in global chromatin organization and in specific *loci*. Hence, understanding what genes are involved and how they are regulated is crucial to unravel the process of cell differentiation. There is also increasing evidence that links the chromatin structure of marker genes of the undifferentiated state with the maintenance of pluripotency.

In addition to modulation of gene expression, posttranslational modification of proteins constitutes a major mechanism of cell identity specification. Although phosphorylation remains as the best studied and understood modification [6], methylation of proteins is emerging as a key controlling element in protein function. In particular, the modification of arginine side chain guanidino groups is quantitatively one of the most extensive protein methylation reactions in mammalian cells [7]. Arginine is a positively charged aminoacid known to mediate hydrogen bonding and amino–aromatic interactions. The nitrogen atoms of this aminoacid can be post-translationally modified to contain methyl groups. Protein arginine methylation results in the addition of one or two methyl groups to the guanidine nitrogens of arginine [8]. In mammalian cells there are three forms of methylated arginine residues: ω-NG, monomethylarginines (MMA); ω-NG,NG-asymmetric dimethylarginines (ADMA); and ω-NG,N'G-symmetric dimethylarginines (SDMA) being ADMA the most prevalent form [9].

Addition of methyl groups to arginine residues is catalyzed by a group of enzymes called Protein Arginine Methyltransferases (Prmt). Prmt family consists of nine members, classified as type I, type II or type III according to their capacity of catalyzing the formation of ADMA, SDMA or MMA, respectively. Prmt1, 2, 3, 4, 6 and 8 belong to type I, whereas Prmt5 and 9 belong to type II. In addition, type II and III activities have been observed for Prmt7. Prmt1 is responsible for at least 85% of all arginine methylation reactions in the cell and is ubiquitously expressed in mammalian cells [10]. It has been proven to be an essential enzyme during development, since embryos from Prmt1−/− knock-out mice die shortly after implantation. However, it is dispensable for basic cellular reactions such as gene expression and DNA replication, since ESC from such embryos are viable under cell culture conditions [11]. Prmt8 shares over 80% of aminoacid sequence homology with Prmt1. Mainly, Prmt8 is 33 aminoacids longer in its amino-terminal region, and harbors a glycine that can be myristoylated. Whether this modification occurs and targets this protein to plasmatic membrane [12] or not [13] is still a matter of debate. All type-I PRMTs adopt head-to-tail homodimeric architecture, essential for PRMT activity [14,15]. However, the reported crystal structure of Prmt8 showed a tetrameric structure in which two PRMT8 dimers are held together [16]. The authors proposed both a homo-tetrameric architecture and a hetero-tetramer model for inter-member interactions [16]. Recently, it was demonstrated that human PRMT8 forms an octamer in solution, and that this structure is necessary for plasma membrane localization and efficient methyltransferase activity [17]. It was also reported that the N-terminal domain may regulate Prmt8 activity [18,19].

Although regulation and cellular substrates of Prmt8 are poorly understood, it was reported that Prmt8 expression is restricted to the adult brain neurons of mice [13] and that it plays a critical role in embryonic and neural development in zebrafish [20]. Recently, it was reported that Prmt8, along with Prmt1, have key roles in neuronal differentiation as co-activators of retinoic acid in a differentiation model of mouse ESC to neurons [21].

Since the role and gene regulation of Prmts in ESC is still weakly studied, and Prmt1 seems to be necessary for embryonic development but not for ESC survival, we sought to study its paralogue Prmt8. Here we show that Prmt8 is expressed in mouse ESC and iPSC and is modulated along a neural progenitor differentiation protocol. Moreover, we found that Prmt8 promoter activity is induced by pluripotency transcription factors Oct4, Sox2 and Nanog and its mRNA was downregulated in ESC transfected with shRNA vector targeting Sox2.

2. Materials and methods

2.1. Cell culture and differentiation

R1 (ATCC) ESC line and 46C Sox1-GFP ESC line (a kind gift from Austin Smith) were cultured and differentiated as previously described [22–25] and [26], respectively. The iPSC-20 line was derived and validated previously by us and cultured as previously described [27]. NIH/3T3 cell line (ATCC) and mouse embryonic fibroblasts (MEFs) from embryonic day 13.5 were cultured in DMEM supplemented with 10% FBS (GIBCO) and antibiotics.

2.2. Qualitative and quantitative reverse transcription–polymerase chain reaction (RT-PCR and RT-qPCR)

RT-PCR and RT-qPCR were performed and analyzed as previously described [22,25]. Primers sequences are detailed in Ref. [25] except for Prmt8 (S1 Table).

2.3. Reporter vector construction, transfection and luciferase activity assay

To construct the reporter vector pPrmt8-Luc, a 1794 kpb fragment of the promoter region of Prmt8 was amplified by PCR from R1 ESC genomic DNA, and cloned into NheI and XhoI cloning sites in the pGL3-Basic vector (Promega) upstream of the Luciferase gene. The oligonucleotides are listed in S1 Table. Restriction enzymes were obtained from Promega. The construction was verified by sequencing. NIH/3T3 cells were co-transfected in 24-well plate with 100 ng of pPrmt8-Luc reporter and 0 (basal), 100, 200 or 400 ng of pMXs-Nanog, pMXs-Oct4, pMXs-Sox2 (Addgene) or the three pMXs vectors simultaneously, as indicated. Transfection and luciferase assay were carried out as previously described [25].

2.4. Downregulation of transcription factors by shRNA approach

R1 ESC cultured in standard medium on gelatin coated p60 plates, were transfected with 3 μg pLKO.1-puro derived vectors (Sigma), expressing shRNA targeting Nanog (SHCLND-XM_132755), Oct4 (SHCLND-NM_013633), Sox2 (SHCLND-NM_011443) or eGFP (SHC005), which was used as control vector. Transfection, selection and mRNA expression analyses were carried out as previously described [25].

2.5. Statistics and data analysis

Experimental results are presented as mean ± SEM. In all cases, statistical analysis were performed using randomized block design ANOVA for at least three biological replicates on *Infostat* software [28]. When necessary, data was transformed with $\log_{10}$. Residuals fitted normal distribution, assessed by the Shapiro–Wilks test, and homogeneity of variance, using the Levene test. Tukey-test was used for comparisons between means.