Genes & Diseases (2023) 10, 2383–2392

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.keaipublishing.com/en/journals/genes-diseases

REVIEW ARTICLE

Coactivator-associated arginine methyltransferase 1: A versatile player in cell differentiation and development

Zhongrui Ma [a,b,1], Xinxing Lyu [a,c,1], Ning Qin [c], Haoyu Liu [c], Mengrui Zhang [c], Yongchao Lai [a], Bo Dong [d], Peiyuan Lu [a,b,c,*]

[a] *Medical Research Center, The First Affiliated Hospital of Shandong First Medical University, Jinan, Shandong 250014, China*
[b] *Department of Immunology, Medical Science and Technology Innovation Center, Shandong First Medical University & Shandong Academy of Medical Sciences, Jinan, Shandong 250117, China*
[c] *Department of Biochemistry and Molecular Biology, School of Basic Medicine, Shandong First Medical University & Shandong Academy of Medical Sciences, Jinan, Shandong 250117, China*
[d] *Department of Cardiology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, Shandong 250021, China*

Received 24 February 2022; received in revised form 19 April 2022; accepted 11 May 2022
Available online 3 June 2022

KEYWORDS
Arginine methylation;
CARM1;
Cell differentiation;
Development;
Epigenetic regulation

Abstract Protein arginine methylation is a common post-translational modification involved in the regulation of various cellular functions. Coactivator-associated arginine methyltransferase 1 (CARM1) is a protein arginine methyltransferase that asymmetrically dimethylates histone H3 and non-histone proteins to regulate gene transcription. CARM1 has been found to play important roles in cell differentiation and development, cell cycle progression, autophagy, metabolism, pre-mRNA splicing and transportation, and DNA replication. In this review, we describe the molecular characteristics of CARM1 and summarize its roles in the regulation of cell differentiation and development in mammals.

* Corresponding author. Medical Research Center, the First Affiliated Hospital of Shandong First Medical University, Jinan, Shandong 250014, China.
E-mail address: pylu@sdfmu.edu.cn (P. Lu).
Peer review under responsibility of Chongqing Medical University.
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.gendis.2022.05.021

Introduction

Post-translational modifications (PTMs) of proteins play significant roles in signal transduction and epigenetic regulation of gene expression. The nitrogen atoms of an arginine residue within a protein can be covalently linked to methyl groups, a process termed protein arginine methylation. Protein arginine methylation is catalyzed by the nine members of the protein arginine methyltransferase (PRMT) family.[1] PRMTs catalyze the transfer of a methyl group from S-adenosyl-L-methionine to the guanidino nitrogen atoms of an arginine residue within a protein, resulting in the formation of methylarginine and S-adenosyl-L-homocysteine.[2] There are three forms of methylarginine identified in eukaryotes: monomethylarginine (MMA), asymmetric dimethylarginine (aDMA), and symmetric dimethylarginine (sDMA)[3–6] (Fig. 1). Protein arginine methylation is a key PTM that is very common in mammals.[7] It is involved in regulating diverse important biological processes, including transcriptional regulation, pre-mRNA processing, signal transduction, DNA damage response, mRNA translation, and cell fate decision.[3–6]

Coactivator-associated arginine methyltransferase 1 (CARM1, also known as PRMT4) is a PRMT that was discovered as a transcriptional coactivator of nuclear receptors (NRs) by Stallcup and colleagues in 1999.[8] CARM1 can asymmetrically dimethylate R17, R26, and R42 of histone H3 to generate the H3R17me2a, H3R26me2a, and H3R42me2a marks, which are associated with activation of transcription.[4,5,9] It also regulates transcription by catalyzing arginine methylation of non-histone proteins, leading to both enhanced and repressed transcriptional activities of various transcription factors. For example, methylation of CBP at R742 by CARM1 enhances GRIP-1- and steroid hormone-induced gene activation,[10] whereas methylation of CBP at R600 in the KIX domain by CARM1 represses the transcriptional activity of CREB.[11] This dual function of CARM1 in transcriptional regulation implicates that it could play a role in multiple cellular functions. Indeed, CARM1 is found to be involved in cell cycle progression,[12] autophagy,[13–16] metabolism,[17–20] DNA damage response,[21] pre-mRNA splicing,[22–24] and DNA replication.[25] Recently, more and more studies have shown that CARM1 is also a critical regulator of cell differentiation and development. In this review, we will first describe the structure of CARM1 and regulation of its activity by PTMs, then we will discuss its roles in mammalian cell differentiation and embryonic development.

Structure of CARM1 and regulation of its activities by PTMs

The human, mouse, and rat *CARM1* genes all contain 16 exons. Five alternatively spliced variants of *Carm1* have been identified in rats.[22,26] Variant 1 is the full-length version of *Carm1*, which is composed of all the 16 exons. The isoform encoded by variant 1 is termed CARM1FL. Variant 2 retains intron 15 in addition to the 16 exons. Variant 3 contains introns 14, but lacks exons 15 and 16. Variant 4 excludes exon 15 and encodes the isoform termed CARM1ΔE15. Variant 5 retains a part of intron 15 in addition to the 16 exons.[22,26] Only two alternatively spliced variants of *CARM1* have been identified in human and mice. They encode CARM1FL and CARM1ΔE15 respectively.[27,28] CARM1ΔE15 is the dominant form in most human tissues, whereas CARM1FL seems to be the major isoform only in brain, heart, skeletal muscle, and testis.[27] The specific functions of all these CARM1 isoforms are still not well understood and need to be clarified in the future.

CARM1 is composed of an N-terminal domain, a C-terminal domain, and a central PRMT catalytic core[29–31] (Fig. 2). The N-terminal domain has a pleckstrin homology (PH) domain, which is a scaffold frequently found to mediate protein–protein interactions in a large variety of biological processes.[30] It is involved in substrate recognition and therefore indispensable for substrate methylation.[32]

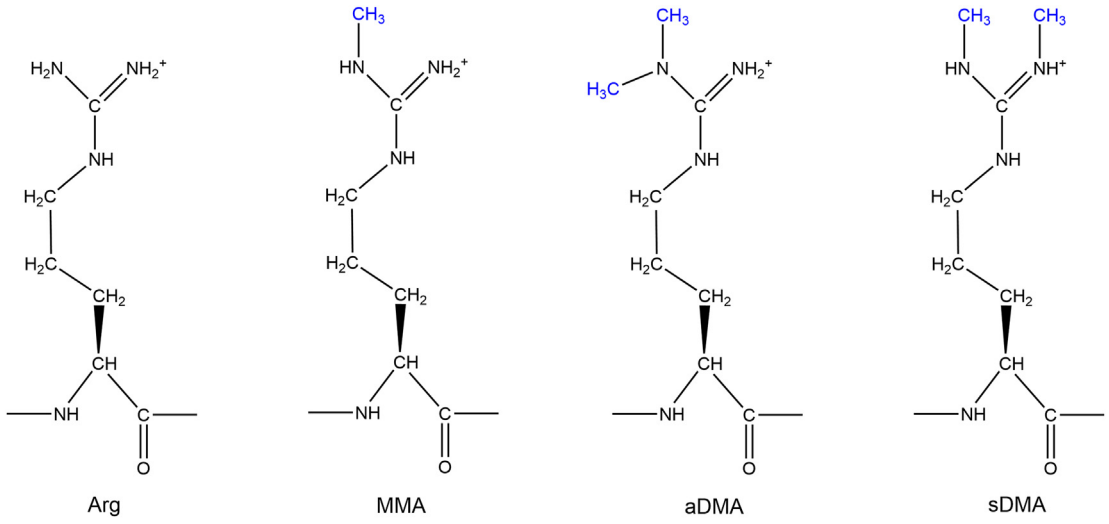

Figure 1 Types of methylation on arginine residues. PRMTs can transfer a methyl group from S-adenosyl-L-methionine to the guanidino nitrogen atoms of an arginine (Arg) residue and result in the formation of a monomethylarginine (MMA), asymmetric dimethylarginine (aDMA), or symmetric dimethylarginine (sDMA) residue in a protein.

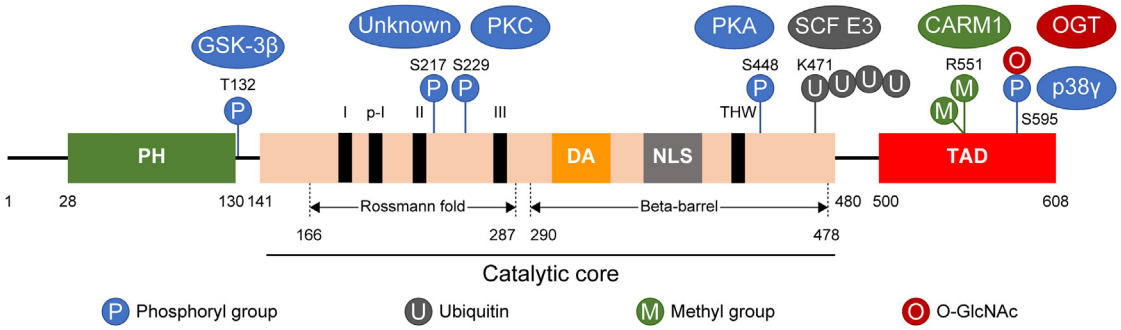

Figure 2 Schematic diagram of the structure and PTMs of CARM1. CARM1 is composed of an N-terminal domain, a C-terminal domain, and a central PRMT catalytic core. The N-terminal domain has a pleckstrin homology (PH) domain and is involved in substrate recognition. The C-terminal domain possesses a transcriptional activation domain (TAD) and is necessary for CARM1 to act as a transcriptional coactivator. The central PRMT catalytic core is composed of a Rossmann fold and a beta-barrel. It contains the five conserved signature PRMT motifs. There is also a dimerization arm (DA) and a nuclear localization sequence (NLS) in the central catalytic core. The PTMs of CARM1 include phosphorylation, ubiquitination, methylation, and O-GlcNAcylation. The enzymes that catalyze these PTMs are also shown in this schematic diagram. Phosphorylation of S572 of CARM1ΔE15 is labelled to S595 of CARM1FL that is corresponding to it. O-GlcNAcylation of S598, T601, and T603 is not displayed due to space limitation. I, motif I; p-I, motif post-I; II, motif II; III, motif III; THW, THW loop; SCF E3, SKP2-containing SCF E3 ubiquitin ligase complex; OGT, O-GlcNAc transferase.

The C-terminal domain possesses a strong autonomous transcriptional activation domain (TAD), which is necessary for CARM1 to act as a transcriptional coactivator of NRs.[29] The central PRMT catalytic core is composed of a Rossmann fold and a beta-barrel. It is essential for substrate binding and methyltransferase activity of CARM1.[29,31] The catalytic core contains the five conserved signature PRMT motifs: motif I (VLD/EVGXGXG), motif post-I (V/IXG/AXD/E), motif II (F/I/VDI/L/K), motif III (LR/KXXG), and THW loop (THWXQ).[1,3,33,34] There is also a dimerization arm in the central catalytic core. CARM1 forms dimers through this dimerization arm *in vivo*,[29–31] and just like other PRMTs,[35] dimerization seems to be essential for its methyltransferase activity.[36] A CARM1 mutant that fails to form dimers shows reduced methyltransferase activity in *in vitro* methylation assays.[36] In addition, the central catalytic core has a nuclear localization sequence (NLS), which is necessary for the nuclear localization of CARM1.[20]

CARM1 catalyzes arginine methylation of proteins, but itself is also subjected to multiple PTMs, including phosphorylation,[28,36–40] O-GlcNAcylation,[41–43] ubiquitination,[13,14,44] and methylation[24,27](Fig. 2). These modifications regulate the methyltransferase activity, dimerization, cellular localization, substrate specificity, and stability of CARM1 (Table 1). PTMs of CARM1 play important roles in various biological processes. S217 (in this article, all the amino acid residues of CARM1 are numbered according to mouse and rat CARM1FL unless specifically indicated) phosphorylation and S229 phosphorylation of CARM1 both occur during mitosis, indicating that these two PTMs may be involved in cell cycle regulation.[36,37] Decreased O-GlcNAcylation level of CARM1 in M phase is required for correct chromosomal segregation during mitosis.[42] K471 ubiquitination of CARM1 by the SKP2-containing SKP1-cullin 1-F-box protein (SCF) E3 ubiquitin ligase complex plays an important role in the regulation of autophagy.[13,14] R551 dimethylation of CARM1 via an automethylation mechanism is essential for it to act as a transcriptional coactivator of ERα and regulate pre-mRNA splicing as well.[24,27]

Roles of CARM1 in cell differentiation and development

CARM1 plays critical roles in cell differentiation and embryonic development. Unlike PRMT1 or PRMT5,[45,46] *Carm1* knockout does not result in complete embryonic lethality in mice, but the neonates die shortly after birth.[47] *Carm1*$^{-/-}$ embryos are smaller than their wild-type littermates. They also display retarded endochondral ossification and reduced chondrocyte proliferation,[48] impaired hematopoiesis and decreased thymopoiesis,[49,50] deficient adipocyte differentiation,[51] and abnormal differentiation of pulmonary epithelial cells,[47,52] suggesting that CARM1 is essential for these embryonic developmental events. Recently, CARM1 is also found to regulate early embryonic development via biasing cell fate in pre-implantation mouse embryos.[53–57] In addition, CARM1 is required for skeletal muscle differentiation[58,59] and spermiogenesis[60] in adult mice. Here, we will describe in detail the roles of CARM1 in early embryonic development, T cells development and hematopoiesis, lung morphogenesis, skeletal muscle differentiation as well as spermiogenesis. The substrates, methylated arginine residues, and effects of arginine methylation during these processes are outlined in Table 2.

Early embryonic development

CARM1 regulates cell fate determination during early embryonic development. After fertilization, the first cell fate bias occurs in the two-cell stage.[61] This bifurcates the totipotent zygote into the inner cell mass (ICM), which contributes to the fetus, and the trophectoderm (TE), which contributes to the placenta. CARM1 is required for this first cell lineage segregation.[56,57] It interacts with *LincGET*, an endogenous retrovirus-associate lncRNA, through its TAD in the two-to four-cell mouse embryos.[56] This interaction localizes CARM1 to paraspeckles,[57] which are membrane-free dynamic structures working as open

Table 1 PTMs of CARM1.

PTM type	PTM site	Enzyme	Effects on CARM1	References
Phosphorylation	T132	GSK-3β	Protects it from ubiquitin-mediated proteasomal degradation in lung epithelial cells	40
	S217	unknown	Abolishes its methyltransferase activity by inhibiting SAM binding. Impairs its nuclear localization	37
	S229	PKC	Abolishes its methyltransferase activity by inhibiting SAM binding. Interferes with its dimerization	36,39
	S448	PKA	Is necessary and sufficient for it to bind directly to the unliganded hormone-binding domain of ERα	38
	S572 of CARM1ΔE15	p38γ/MAPK12	Disrupts its nuclear localization	28
O-GlcNAcylation	S595, S598, T601, and T603	O-GlcNAc transferase	Alters its substrate specificity	41—43
Ubiquitination	K471	SKP2-containing SCF E3 ubiquitin ligase complex	Controls its nuclear protein level	13,14
Methylation	R551	CARM1	Is essential for it to act as a transcriptional coactivator of ERα and regulate pre-mRNA splicing	24,27

systems as their components exchange with freely diffusing molecules in the nucleoplasm.[62] *LincGET*/CARM1 complexes are asymmetrically distributed between the blastomeres of the two-to four-cell mouse embryos, which results in asymmetric levels of H3R26me2a as well as the expression of the ICM-specific genes *Sox2*, *Nanog*, and *Sox21*. Thus, the blastomere containing more *LincGET*/CARM1 complexes is biased toward an ICM cell fate.[56]

CARM1 also contributes to cell fate determination in the four-cell mouse embryos through a similar mechanism. CARM1 is heterogeneously distributed between the blastomeres of the four-cell mouse embryos,[53,63] which is regulated by microRNA (miRNA) miR-181a.[63] This leads to different levels of H3R26me2a as well as heterogeneous expression of the ICM-specific genes *Sox2*, *Nanog*, and *Sox21* in the blastomeres of the four-cell mouse embryos.[53,54] Because Sox21 inhibits the expression of the TE master gene *Cdx2*, *Cdx2* is also heterogeneously expressed in the blastomeres of the four-cell mouse embryos.[54] Thus, this heterogeneous gene expression profile biases cell fate decisions at the four-cell stage. Very interestingly, CARM1-mediated H3R26 dimethylation leads to long-lived DNA binding of Sox2 but not Oct4 in the four-cell mouse embryos,[55] although Oct4 collaboratively activates *Sox21* transcription with Sox2.[64] H3R26me2a-induced long-lived DNA binding of Oct4 also occurs at the eight-cell stage.[55]

Table 2 Roles of CARM1 in cell differentiation and development.

Process	Substrate	Methylated arginine residues	Effects of arginine methylation	References
Early embryonic development	Histone H3	R17, R26	Transcriptional activation	53,54,55,56,57,63,65
	BAF155	Not determined	Decreased stability of the SWI/SNF complex	66
T cells development and Hematopoiesis	Not determined	—	Proper cellularity and survival of hematopoietic progenitor cells	49,50
Lung morphogenesis	Not determined	—	Proper expression of *Aqp1*, *Aqp5*, *Gadd45g*, *Scn3b*, *and Nkd1*	52
Skeletal muscle differentiation	Pax7	R10, R13, R22, R37	Activation of *Myf5* expression	59
	Histone H3	R17	Activation of the expression of late myogenic genes and myogenic microRNAs	75,76
Spermiogenesis	p300	R2142	Impaired formation of p300-ACT-CREMτ complex	60

In addition to H3R26 dimethylation, it seems that CARM1-mediated H3R17 dimethylation is also involved in the regulation of early mouse embryonic development.[53,65] Moreover, CARM1 regulates cell fate determination in the pre-implantation mouse embryos via methylating the SWI/SNF chromatin remodeling complex component BAF155, a major SWI/SNF complex subunit that regulates its assembly. The SWI/SNF complex represses the expression of the pluripotency-related gene *Nanog* in the TE lineage. CARM1-mediated arginine methylation of BAF155 decreases the stability of the SWI/SNF complex and thus increases the expression of *Nanog* in the pluripotent epiblast lineage.[66]

In summary, CARM1 ensures proper lineage allocation via methylation of histone H3 and other factors at their arginine residues to regulate the gene expression program for proper cell fate determination during early embryonic development (Fig. 3).

T cells development and hematopoiesis

CARM1 is required for hematopoiesis and T cell differentiation during embryonic development.[49,50] Compared with their wild type littermates, *Carm*$^{-/-}$ mouse embryos exhibit a significant reduction in thymocyte cellularity and a decreased proportion of double negative thymocytes due to a partial arrest in the development of the early thymocyte progenitors.[49] Reduced thymopoiesis in *Carm*$^{-/-}$ mouse embryos is due to a defect in the fetal hematopoietic compartment rather than in the thymic stroma.[50] CARM1 functions cell-intrinsically to regulate the activity and cellularity of hematopoietic progenitor cells in the fetal liver and bone marrow. CARM1 deficiency remarkably reduces cellularity of hematopoietic progenitors in the embryonic bone marrow, and *Carm1*$^{-/-}$ fetal liver cells show a deficit in the ability to contribute to hematopoiesis.[50] Moreover, CARM1 is required for the survival of hematopoietic progenitors, particularly at the earliest stages of T cell differentiation.[50] Taken together, CARM1 is a key regulator of hematopoiesis and T cell development that affects multiple lineages at different stages of differentiation during embryonic development. However, CARM1 is not indispensable for hematopoiesis in adult mice. Conditional knockout of *Carm1* in adult mice has little effect on hematopoiesis.[67] Furthermore, CARM1 plays essential roles in myeloid leukemogenesis. Loss of CARM1 strongly impairs both the initiation and maintenance of acute myeloid leukemia (AML) by inhibiting cell-cycle progression, promoting

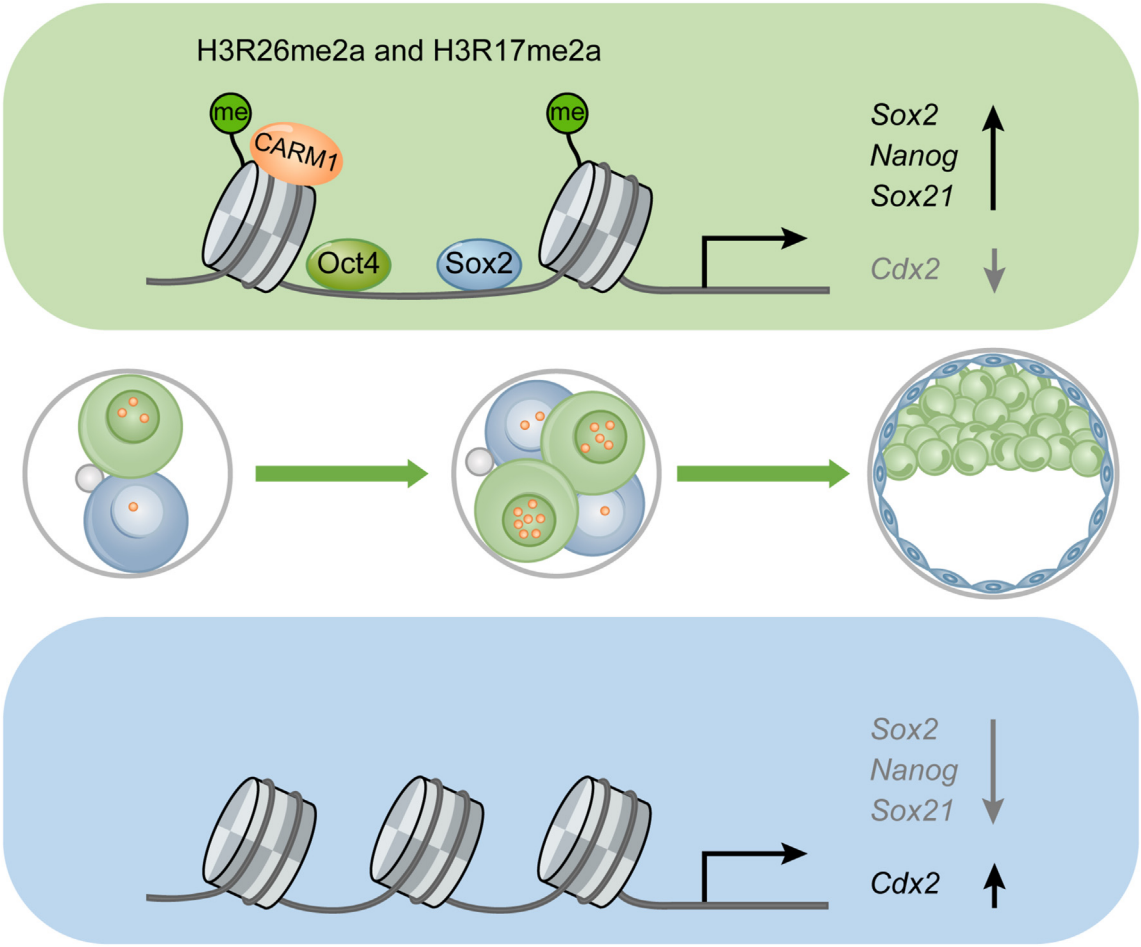

Figure 3 Regulation of early embryonic development by CARM1. CARM1 is heterogeneously distributed between the blastomeres of the two-cell and four-cell mouse embryos, which leads to different levels of H3R26me2a and H3R17me2a as well as heterogeneous expression of the ICM-specific genes *Sox2*, *Nanog*, and *Sox21* and the TE master gene *Cdx2* in these cells. This heterogeneous gene expression profile biases cell fate decisions at the two-cell and four-cell stage.

myeloid differentiation, and inducing apoptosis in AML cells, suggesting that CARM1 may be an effective therapeutic target for AML.[67] However, the exact mechanisms by which CARM1 regulates hematopoiesis and T cell differentiation as well as myeloid leukemogenesis are not clear and need to be clarified. It will be necessary to identify the substrates of CARM1 and the involved genes during these processes to answer these questions.

Lung morphogenesis

The lungs of the *Carm1*$^{-/-}$ neonates failed to inflate with air after birth, and their alveolar air spaces were smaller compared with their wild-type littermates,[47] suggesting that CARM1 is required for normal lung morphogenesis.

Development of the lung is tightly regulated by a cascade of signaling pathways and various transcription factors.[68,69] At the late stage of embryogenesis, bipotent alveolar progenitor cells of the distal lung differentiate into pulmonary epithelial alveolar type I (AT1) and alveolar type II (AT2) cells.[70] AT1 cells are large squamous cells that coordinate the air exchange of capillaries in the distal lung. AT2 cells are smaller cuboidal cells located in the alveolar sacs, which produce surfactant to reduce their surface tension in order that they can be filled with air. CARM1 plays an important role in regulating the proliferation and differentiation of pulmonary epithelial cells.[52] Hyperproliferation of AT2 cells occurs in the lung of *Carm1*$^{-/-}$ mice, but differentiation of AT1 cells is blocked in the absence of CARM1, resulting in smaller alveolar air spaces in the distal lung.[52] CARM1 controls proliferation and differentiation of pulmonary epithelial cells through regulating the expression of the genes crucial for cell cycle regulation and AT1 differentiation, including *Aqp1*, *Aqp5*, *Gadd45g*, *Scn3b*, and *Nkd1*.[52] Further investigations are needed to identify the substrates of CARM1 in alveolar progenitor cells to elucidate the molecular mechanisms by which CARM1 regulates proper expression of these genes during lung morphogenesis.

Skeletal muscle differentiation

CARM1 is an important regulator of asymmetric satellite stem cell divisions in skeletal muscle.[59] Satellite stem cells are a small subset of satellite cells that are located beneath the basal lamina surrounding myofibers. They are essential for the growth and regeneration of skeletal muscle.[71,72] Satellite stem cells can expand their number through symmetric divisions and give rise to committed myogenic progenitors through asymmetric divisions.[73–75] During asymmetric satellite stem cell divisions, CARM1 regulates satellite stem cell entry into the myogenic program via epigenetic activation of the myogenic determination factor gene *Myf5*, a target gene of the transcription factor Pax7, in the committed satellite myogenic cells.[59] CARM1 specifically methylates the multiple arginine residues in the N-terminus of the transcription factor Pax7. Methylated Pax7 directly binds the C-terminal cleavage forms of the trithorax proteins MLL1/2 and recruits the MLL histone H3K4 methyltransferase complex to the enhancers and proximal promoter of *Myf5*, resulting in increased H3K4me3 levels at these loci and *de novo* activation of *Myf5* transcription in the committed satellite myogenic cells following asymmetric satellite stem cell divisions.[59] Thus, methylation of Pax7 by CARM1 acts as a switch that activates the expression of *Myf5* during the satellite stem cell asymmetric divisions and entry into the myogenic program (Fig. 4).

CARM1 is also essential for the differentiation of skeletal muscle cells. It functions as a transcriptional coactivator of the transcription factors of the myocyte enhancer factor-2 (MEF2) family to activate myogenic gene expression.[58] Moreover, CARM1 is also required for the expression of late myogenic genes [76]and myogenic microRNAs.[77] It dimethylates H3R17 of the upstream regulatory regions of late myogenic genes and myogenic microRNAs to facilitate the binding of myogenin and the Brg1 ATPase of the SWI/SNF complex to these loci to activate their expression.[76,77]

Spermiogenesis

Although CARM1 is not required for spermatocyte development, it is essential for the late stage of haploid spermatid development.[60] CARM1 is highly expressed in the mouse testis, and its moves from cytoplasmic to nuclear during the spermatocyte to spermatid transition, suggesting an important role of CARM1 in the late stages of sperm development. Loss of CARM1 leads to a significant drop in sperm count as well as an accumulation of immature germ cells and aberrant sperms in the cauda epididymis. The anomalies in sperm cell morphology and structure include abnormal head formations, headless sperms, and sperms with a bent midpiece. Moreover, Loss of CARM1 causes a dramatic decrease in the motility of sperms.[60]

CARM1 regulates the late stages of spermiogenesis via repressing the transcriptional activity of the testis-specific transcription factor cAMP response element modulator tau (CREMτ),[60] which is a master regulator of the early stage of haploid spermatid development.[78] During the late stages of sperm development, CARM1 dimethylates R2142 in the GRIP1 binding domain (GBD) domain of p300 , which is a coactivator of CREMτ. This attenuates the interaction of p300 with activator of CREMτ in the testis (ACT), the mediator of the interaction between CREMτ and p300, resulting in an inhibition of the p300-ACT-CREMτ-mediated transcription program in the haploid spermatids.[60]

Conclusions and perspectives

CARM1 is a PRMT that functions as an important transcriptional regulator. It regulates transcription through asymmetric dimethylation of histone H3 as well as non-histone proteins. Moreover, CARM1 can both enhance and repress the transcriptional activities of various transcription factors. This dual functionality of CARM1 in transcriptional regulation makes it a critical regulator of cell differentiation and embryonic development. It not only regulates early embryonic development via biasing cell fate in the two-cell and four-cell mouse embryos, but also play essential roles in endochondral ossification and chondrocyte proliferation, hematopoiesis and thymopoiesis, adipocyte differentiation, lung morphogenesis, skeletal myogenesis, spermiogenesis, etc. The enzymatic activity seems to be required for these

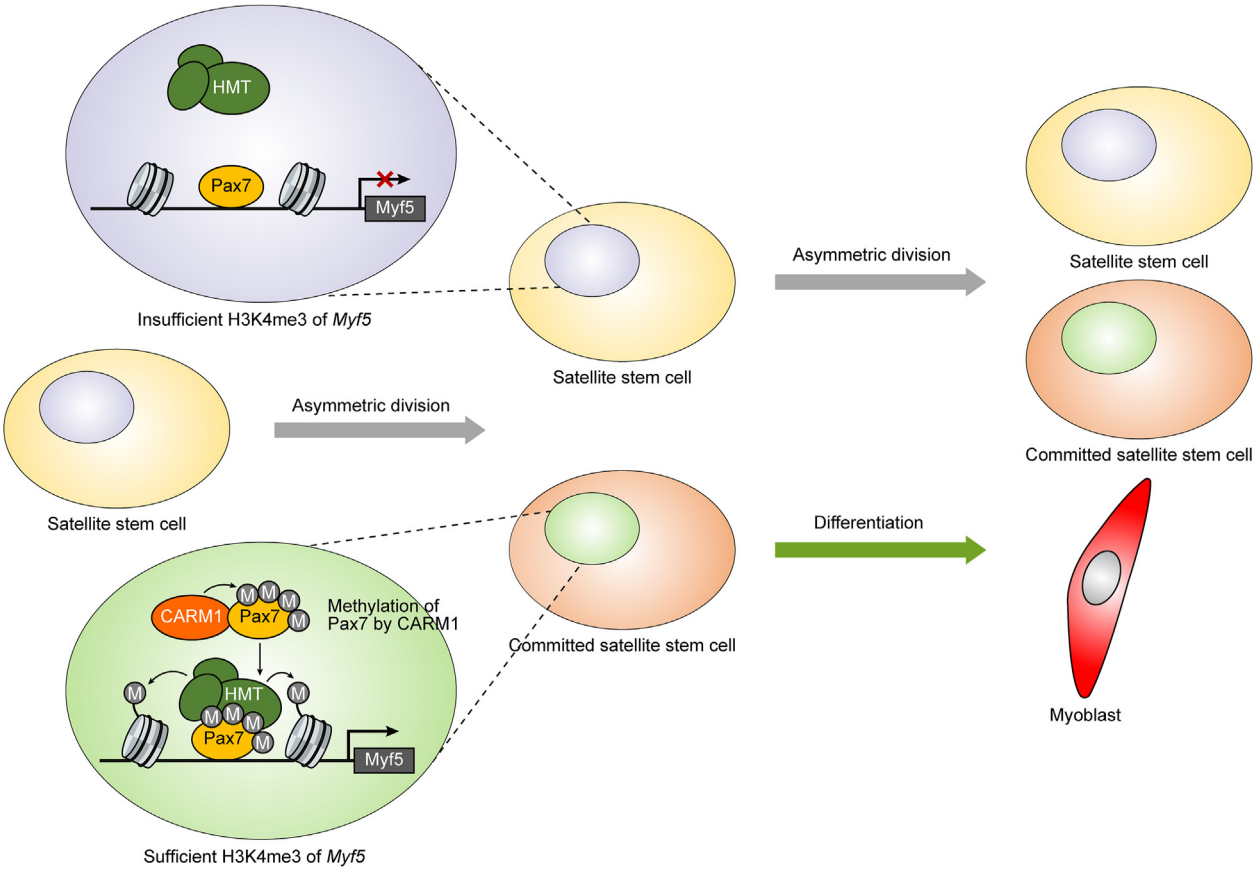

Figure 4 Regulation of asymmetric satellite stem cell divisions by CARM1. During asymmetric satellite stem cell divisions, CARM1 specifically methylates the multiple arginine residues in the N-terminus of the transcription factor Pax7 in the committed satellite myogenic cells. Methylated Pax7 directly binds the C-terminal cleavage forms of the trithorax proteins MLL1/2 and recruits the MLL histone H3K4 methyltransferase (HMT) complex to the enhancers and proximal promoter of the myogenic determination factor gene *Myf5*, resulting in increased H3K4me3 levels at these loci and *de novo* activation of *Myf5* transcription in the committed satellite myogenic cells. These committed satellite myogenic cells then differentiate into myoblasts.

functions of CARM1, because the enzyme-dead *Carm1* knockin mice have similar defects to those seen in their knockout counterparts.[79] Moreover, the two isoforms, CARM1FL and CARM1ΔE15, could play distinctive roles in cell differentiation and embryonic development due to their differential tissue distributions.[27] It will be of great importance to explore this area more comprehensively. In addition, more efforts are needed to identify new CARM1 substrates to better characterize its functions in cell differentiation and embryonic development, and it is necessary to clarify how CARM1-mediated arginine methylation is recognized and how the signals are transduced especially when the substrates are non-histone proteins.

Because of its significant roles in cell differentiation and embryonic development, CARM1 is involved in a couple of human disorders, including cancer,[3,4,6,9] muscular atrophy,[28,80–82] diabetic nephropathy,[44,83,84] and senescence.[85–87] Understanding the functions of CARM1 in cell differentiation and embryonic development may shed light on the pathogenesis and therapies of these diseases. Indeed, some recently developed specific and potent CARM1 small-molecule inhibitors have been tested to treat a subset of cancers.[67,88–91] Hopefully they could become approved drugs for cancer treatment in the future.

Conflict of interests

The authors declare no conflict of interests.

Funding

This work was supported by Funding for Top-notch Personnel from Shandong First Medical University and Taishan Scholars Program (No. ts 2019097).

Acknowledgements

We thank Dr. Mike Powell at Emory University for proofreading the manuscript and Home for Researcher (www.home-for-researchers.com) for optimizing the figures.

References

1. Bedford MT, Clarke SG. Protein arginine methylation in mammals: who, what, and why. *Mol Cell*. 2009;33(1):1—13.

2. Lee HW, Kim S, Paik WK. S-adenosylmethionine: protein-arginine methyltransferase. Purification and mechanism of the enzyme. *Biochemistry*. 1977;16(1):78—85.
3. Yang Y, Bedford MT. Protein arginine methyltransferases and cancer. *Nat Rev Cancer*. 2013;13(1):37—50.
4. Blanc RS, Richard S. Arginine methylation: the coming of age. *Mol Cell*. 2017;65(1):8—24.
5. Guccione E, Richard S. The regulation, functions and clinical relevance of arginine methylation. *Nat Rev Mol Cell Biol*. 2019; 20(10):642—657.
6. Jarrold J, Davies CC. PRMTs and arginine methylation: cancer's best-kept secret? *Trends Mol Med*. 2019;25(11):993—1009.
7. Larsen SC, Sylvestersen KB, Mund A, et al. Proteome-wide analysis of arginine monomethylation reveals widespread occurrence in human cells. *Sci Signal*. 2016;9(443):rs9.
8. Chen D, Ma H, Hong H, et al. Regulation of transcription by a protein methyltransferase. *Science*. 1999;284(5423):2174—2177.
9. Suresh S, Huard S, Dubois T. CARM1/PRMT4: making its mark beyond its function as a transcriptional coactivator. *Trends Cell Biol*. 2021;31(5):402—417.
10. Chevillard-Briet M, Trouche D, Vandel L. Control of CBP co-activating activity by arginine methylation. *EMBO J*. 2002; 21(20):5457—5466.
11. Xu W, Chen H, Du K, et al. A transcriptional switch mediated by cofactor methylation. *Science*. 2001;294(5551):2507—2511.
12. El Messaoudi S, Fabbrizio E, Rodriguez C, et al. Coactivator-associated arginine methyltransferase 1 (CARM1) is a positive regulator of the Cyclin E1 gene. *Proc Natl Acad Sci U S A*. 2006; 103(36):13351—13356.
13. Shin HJ, Kim H, Oh S, et al. AMPK-SKP2-CARM1 signalling cascade in transcriptional regulation of autophagy. *Nature*. 2016;534(7608):553—557.
14. Li C, Yu L, Xue H, et al. Nuclear AMPK regulated CARM1 stabilization impacts autophagy in aged heart. *Biochem Biophys Res Commun*. 2017;486(2):398—405.
15. Wei X, Li X, Yan W, Zhang X, Sun Y, Zhang F. SKP2 promotes hepatocellular carcinoma progression through nuclear AMPK-SKP2-CARM1 signaling transcriptionally regulating nutrient-deprived autophagy induction. *Cell Physiol Biochem*. 2018; 47(6):2484—2497.
16. Yu YS, Shin HR, Kim D, et al. Pontin arginine methylation by CARM1 is crucial for epigenetic regulation of autophagy. *Nat Commun*. 2020;11(1):6297.
17. Wang YP, Zhou W, Wang J, et al. Arginine methylation of MDH1 by CARM1 inhibits glutamine metabolism and suppresses pancreatic cancer. *Mol Cell*. 2016;64(4):673—687.
18. Liu F, Ma F, Wang Y, et al. PKM2 methylation by CARM1 activates aerobic glycolysis to promote tumorigenesis. *Nat Cell Biol*. 2017;19(11):1358—1370.
19. Zhong XY, Yuan XM, Xu YY, et al. CARM1 methylates GAPDH to regulate glucose metabolism and is suppressed in liver cancer. *Cell Rep*. 2018;24(12):3207—3223.
20. Liu Y, Wang T, Ji YJ, et al. A C9orf72-CARM1 axis regulates lipid metabolism under glucose starvation-induced nutrient stress. *Genes Dev*. 2018;32(21—22):1380—1397.
21. Lee YH, Bedford MT, Stallcup MR. Regulated recruitment of tumor suppressor BRCA1 to the p21 gene by coactivator methylation. *Genes Dev*. 2011;25(2):176—188.
22. Ohkura N, Takahashi M, Yaguchi H, Nagamura Y, Tsukada T. Coactivator-associated arginine methyltransferase 1, CARM1, affects pre-mRNA splicing in an isoform-specific manner. *J Biol Chem*. 2005;280(32):28927—28935.
23. Cheng D, Côté J, Shaaban S, Bedford MT. The arginine methyltransferase CARM1 regulates the coupling of transcription and mRNA processing. *Mol Cell*. 2007;25(1):71—83.
24. Kuhn P, Chumanov R, Wang Y, Ge Y, Burgess RR, Xu W. Automethylation of CARM1 allows coupling of transcription and mRNA splicing. *Nucleic Acids Res*. 2011;39(7):2717—2726.
25. Genois MM, Gagné JP, Yasuhara T, et al. CARM1 regulates replication fork speed and stress response by stimulating PARP1. *Mol Cell*. 2021;81(4):784—800.
26. Gupta N, Madapura MP, Bhat UA, Rao MR. Mapping of post-translational modifications of transition proteins, TP1 and TP2, and identification of protein arginine methyltransferase 4 and lysine methyltransferase 7 as methyltransferase for TP2. *J Biol Chem*. 2015;290(19):12101—12122.
27. Wang L, Charoensuksai P, Watson NJ, et al. CARM1 automethylation is controlled at the level of alternative splicing. *Nucleic Acids Res*. 2013;41(14):6870—6880.
28. Chang NC, Sincennes MC, Chevalier FP, et al. The dystrophin glycoprotein complex regulates the epigenetic activation of muscle stem cell commitment. *Cell Stem Cell*. 2018;22(5):755—768.
29. Teyssier C, Chen D, Stallcup MR. Requirement for multiple domains of the protein arginine methyltransferase CARM1 in its transcriptional coactivator function. *J Biol Chem*. 2002; 277(48):46066—46072.
30. Troffer-Charlier N, Cura V, Hassenboehler P, Moras D, Cavarelli J. Functional insights from structures of coactivator-associated arginine methyltransferase 1 domains. *EMBO J*. 2007;26(20):4391—4401.
31. Yue WW, Hassler M, Roe SM, Thompson-Vale V, Pearl LH. Insights into histone code syntax from structural and biochemical studies of CARM1 methyltransferase. *EMBO J*. 2007;26(20): 4402—4412.
32. Shishkova E, Zeng H, Liu F, et al. Global mapping of CARM1 substrates defines enzyme specificity and substrate recognition. *Nat Commun*. 2017;8:15571.
33. Wei H, Mundade R, Lange KC, Lu T. Protein arginine methylation of non-histone proteins and its role in diseases. *Cell Cycle*. 2014;13(1):32—41.
34. Fuhrmann J, Clancy KW, Thompson PR. Chemical biology of protein arginine modifications in epigenetic regulation. *Chem Rev*. 2015;115(11):5413—5461.
35. Morales Y, Cáceres T, May K, Hevel JM. Biochemistry and regulation of the protein arginine methyltransferases (PRMTs). *Arch Biochem Biophys*. 2016;590:138—152.
36. Higashimoto K, Kuhn P, Desai D, Cheng X, Xu W. Phosphorylation-mediated inactivation of coactivator-associated arginine methyltransferase 1. *Proc Natl Acad Sci U S A*. 2007;104(30): 12318—12323.
37. Feng Q, He B, Jung SY, et al. Biochemical control of CARM1 enzymatic activity by phosphorylation. *J Biol Chem*. 2009; 284(52):36167—36174.
38. Carascossa S, Dudek P, Cenni B, Briand PA, Picard D. CARM1 mediates the ligand-independent and tamoxifen-resistant activation of the estrogen receptor alpha by cAMP. *Genes Dev*. 2010;24(7):708—719.
39. Lim CS, Alkon DL. Protein kinase C stimulates HuD-mediated mRNA stability and protein expression of neurotrophic factors and enhances dendritic maturation of hippocampal neurons in culture. *Hippocampus*. 2012;22(12):2303—2319.
40. Li X, Lai Y, Li J, Zou M, Zou C. Oxidative stress destabilizes protein arginine methyltransferase 4 via glycogen synthase kinase 3β to impede lung epithelial cell migration. *Am J Physiol Cell Physiol*. 2017;313(3):C285—C294.
41. Cheung WD, Sakabe K, Housley MP, Dias WB, Hart GW. O-linked beta-N-acetylglucosaminyltransferase substrate specificity is regulated by myosin phosphatase targeting and other interacting proteins. *J Biol Chem*. 2008;283(49): 33935—33941.
42. Sakabe K, Hart GW. O-GlcNAc transferase regulates mitotic chromatin dynamics. *J Biol Chem*. 2010;285(45):34460—34468.
43. Charoensuksai P, Kuhn P, Wang L, Sherer N, Xu W. O-GlcNAcylation of co-activator-associated arginine methyltransferase 1 regulates its protein substrate specificity. *Biochem J*. 2015; 466(3):587—599.

44. Kim D, Lim S, Park M, et al. Ubiquitination-dependent CARM1 degradation facilitates Notch1-mediated podocyte apoptosis in diabetic nephropathy. *Cell Signal*. 2014;26(9):1774—1782.
45. Pawlak MR, Scherer CA, Chen J, Roshon MJ, Ruley HE. Arginine N-methyltransferase 1 is required for early postimplantation mouse development, but cells deficient in the enzyme are viable. *Mol Cell Biol*. 2000;20(13):4859—4869.
46. Tee WW, Pardo M, Theunissen TW, et al. Prmt5 is essential for early mouse development and acts in the cytoplasm to maintain ES cell pluripotency. *Genes Dev*. 2010;24(24):2772—2777.
47. Yadav N, Lee J, Kim J, et al. Specific protein methylation defects and gene expression perturbations in coactivator-associated arginine methyltransferase 1-deficient mice. *Proc Natl Acad Sci U S A*. 2003;100(11):6464—6468.
48. Ito T, Yadav N, Lee J, et al. Arginine methyltransferase CARM1/PRMT4 regulates endochondral ossification. *BMC Dev Biol*. 2009;9:47.
49. Kim J, Lee J, Yadav N, et al. Loss of CARM1 results in hypomethylation of thymocyte cyclic AMP-regulated phosphoprotein and deregulated early T cell development. *J Biol Chem*. 2004;279(24):25339—25344.
50. Li J, Zhao Z, Carter C, Ehrlich LI, Bedford MT, Richie ER. Coactivator-associated arginine methyltransferase 1 regulates fetal hematopoiesis and thymocyte development. *J Immunol*. 2013;190(2):597—604.
51. Yadav N, Cheng D, Richard S, et al. CARM1 promotes adipocyte differentiation by coactivating PPARgamma. *EMBO Rep*. 2008;9(2):193—198.
52. O'Brien KB, Alberich-Jordà M, Yadav N, et al. CARM1 is required for proper control of proliferation and differentiation of pulmonary epithelial cells. *Development*. 2010;137(13):2147—2156.
53. Torres-Padilla ME, Parfitt DE, Kouzarides T, Zernicka-Goetz M. Histone arginine methylation regulates pluripotency in the early mouse embryo. *Nature*. 2007;445(7124):214—218.
54. Goolam M, Scialdone A, Graham SJL, et al. Heterogeneity in Oct4 and Sox2 targets biases cell fate in 4-cell mouse embryos. *Cell*. 2016;165(1):61—74.
55. White MD, Angiolini JF, Alvarez YD, et al. Long-lived binding of Sox2 to DNA predicts cell fate in the four-cell mouse embryo. *Cell*. 2016;165(1):75—87.
56. Wang J, Wang L, Feng G, et al. Asymmetric expression of LincGET biases cell fate in two-cell mouse embryos. *Cell*. 2018;175(7):1887—1901.
57. Hupalowska A, Jedrusik A, Zhu M, Bedford MT, Glover DM, Zernicka-Goetz M. CARM1 and paraspeckles regulate pre-implantation mouse embryo development. *Cell*. 2018;175(7):1902—1916.
58. Chen SL, Loffler KA, Chen D, Stallcup MR, Muscat GE. The coactivator-associated arginine methyltransferase is necessary for muscle differentiation: CARM1 coactivates myocyte enhancer factor-2. *J Biol Chem*. 2002;277(6):4324—4333.
59. Kawabe Y, Wang YX, McKinnell IW, Bedford MT, Rudnicki MA. Carm1 regulates Pax7 transcriptional activity through MLL1/2 recruitment during asymmetric satellite stem cell divisions. *Cell Stem Cell*. 2012;11(3):333—345.
60. Bao J, Rousseaux S, Shen J, Lin K, Lu Y, Bedford MT. The arginine methyltransferase CARM1 represses p300•ACT•CREMτ activity and is required for spermiogenesis. *Nucleic Acids Res*. 2018;46(9):4327—4343.
61. Shi J, Chen Q, Li X, et al. Dynamic transcriptional symmetry-breaking in pre-implantation mammalian embryo development revealed by single-cell RNA-seq. *Development*. 2015;142(20):3468—3477.
62. Mao YS, Sunwoo H, Zhang B, Spector DL. Direct visualization of the co-transcriptional assembly of a nuclear body by noncoding RNAs. *Nat Cell Biol*. 2011;13(1):95—101.
63. Sun H, Su J, Wu T, et al. CARM1 is heterogeneous in mouse four-cell embryo and important to blastocyst development. *Reproduction*. 2020;159(1):91—104.
64. Chakravarthy H, Ormsbee BD, Mallanna SK, Rizzino A. Rapid activation of the bivalent gene Sox21 requires displacement of multiple layers of gene-silencing machinery. *FASEB J*. 2011;25(1):206—218.
65. Yang G, Zhou C, Wang R, et al. Base-editing-mediated R17H substitution in histone H3 reveals methylation-dependent regulation of Yap signaling and early mouse embryo development. *Cell Rep*. 2019;26(2):302—312.
66. Panamarova M, Cox A, Wicher KB, et al. The BAF chromatin remodelling complex is an epigenetic regulator of lineage specification in the early mouse embryo. *Development*. 2016;143(8):1271—1283.
67. Greenblatt SM, Man N, Hamard PJ, et al. CARM1 is essential for myeloid leukemogenesis but dispensable for normal hematopoiesis. *Cancer Cell*. 2018;34(5):868.
68. Cardoso WV, Lü J. Regulation of early lung morphogenesis: questions, facts and controversies. *Development*. 2006;133(9):1611—1624.
69. Maeda Y, Davé V, Whitsett JA. Transcriptional control of lung morphogenesis. *Physiol Rev*. 2007;87(1):219—244.
70. Desai TJ, Brownfield DG, Krasnow MA. Alveolar progenitor and stem cells in lung development, renewal and cancer. *Nature*. 2014;507(7491):190—194.
71. Chargé SB, Rudnicki MA. Cellular and molecular regulation of muscle regeneration. *Physiol Rev*. 2004;84(1):209—238.
72. Dumont NA, Bentzinger CF, Sincennes MC, Rudnicki MA. Satellite cells and skeletal muscle regeneration. *Compr Physiol*. 2015;5(3):1027—1059.
73. Collins CA, Olsen I, Zammit PS, et al. Stem cell function, self-renewal, and behavioral heterogeneity of cells from the adult muscle satellite cell niche. *Cell*. 2005;122(2):289—301.
74. Montarras D, Morgan J, Collins C, et al. Direct isolation of satellite cells for skeletal muscle regeneration. *Science*. 2005;309(5743):2064—2067.
75. Kuang S, Kuroda K, Le Grand F, Rudnicki MA. Asymmetric self-renewal and commitment of satellite stem cells in muscle. *Cell*. 2007;129(5):999—1010.
76. Dacwag CS, Bedford MT, Sif S, Imbalzano AN. Distinct protein arginine methyltransferases promote ATP-dependent chromatin remodeling function at different stages of skeletal muscle differentiation. *Mol Cell Biol*. 2009;29(7):1909—1921.
77. Mallappa C, Hu YJ, Shamulailatpam P, Tae S, Sif S, Imbalzano AN. The expression of myogenic microRNAs indirectly requires protein arginine methyltransferase (Prmt)5 but directly requires Prmt4. *Nucleic Acids Res*. 2011;39(4):1243—1255.
78. Sassone-Corsi PCREM. A master-switch regulating the balance between differentiation and apoptosis in male germ cells. *Mol Reprod Dev*. 2000;56(2 Suppl):228—229.
79. Kim D, Lee J, Cheng D, et al. Enzymatic activity is required for the in vivo functions of CARM1. *J Biol Chem*. 2010;285(2):1147—1152.
80. Sanchez G, Bondy-Chorney E, Laframboise J, et al. A novel role for CARM1 in promoting nonsense-mediated mRNA decay: potential implications for spinal muscular atrophy. *Nucleic Acids Res*. 2016;44(6):2661—2676.
81. Liu Y, Li J, Shang Y, Guo Y, Li Z. CARM1 contributes to skeletal muscle wasting by mediating FoxO3 activity and promoting myofiber autophagy. *Exp Cell Res*. 2019;374(1):198—209.
82. Hu R, Wang MQ, Liu LY, et al. Calycosin inhibited autophagy and oxidative stress in chronic kidney disease skeletal muscle atrophy by regulating AMPK/SKP2/CARM1 signalling pathway. *J Cell Mol Med*. 2020;24(19):11084—11099.
83. Kim DI, Park MJ, Lim SK, et al. High-glucose-induced CARM1 expression regulates apoptosis of human retinal pigment

epithelial cells via histone 3 arginine 17 dimethylation: role in diabetic retinopathy. *Arch Biochem Biophys*. 2014;560:36—43.
84. Guo N, Nulahou A, Bu Q, et al. MiR-542-5p regulates the progression of diabetic retinopathy by targeting CARM1. *Acta Biochim Pol*. 2020;67(3):373—378.
85. Pang L, Tian H, Chang N, et al. Loss of CARM1 is linked to reduced HuR function in replicative senescence. *BMC Mol Biol*. 2013;14:15.
86. Sarker RS, John-Schuster G, Bohla A, et al. Coactivator-associated arginine methyltransferase-1 function in alveolar epithelial senescence and elastase-induced emphysema susceptibility. *Am J Respir Cell Mol Biol*. 2015;53(6):769—781.
87. Xu Z, Wu W, Shen F, Yu Y, Wang Y, Xiang C. Histone arginine methylation-mediated epigenetic regulation of discoidin domain receptor 2 controls the senescence of human bone marrow mesenchymal stem cells. *Stem Cells Int*. 2019;2019:7670316.
88. Veazey KJ, Cheng D, Lin K, et al. CARM1 inhibition reduces histone acetyltransferase activity causing synthetic lethality in CREBBP/EP300-mutated lymphomas. *Leukemia*. 2020;34(12):3269—3285.
89. Kumar S, Zeng Z, Bagati A, et al. CARM1 inhibition enables immunotherapy of resistant tumors by dual action on tumor cells and T cells. *Cancer Discov*. 2021;11(8):2050—2071.
90. Zhang Z, Guo Z, Xu X, et al. Structure-based discovery of potent CARM1 inhibitors for solid tumor and cancer immunology therapy. *J Med Chem*. 2021;64(22):16650—16674.
91. Inoue F, Sone K, Toyohara Y, et al. Histone arginine methyltransferase CARM1 selective inhibitor TP-064 induces apoptosis in endometrial cancer. *Biochem Biophys Res Commun*. 2022;601:123—128.