Received: 23 February 2020 | Revised: 27 February 2020 | Accepted: 27 February 2020 | Published online: 16 April 2020

DOI: 10.1002/ctm2.2

CLINICAL AND TRANSLATIONAL MEDICINE Open Access

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REVIEW

Molecular regulation and clinical significance of caveolin-1 methylation in chronic lung diseases

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Abstract

Chronic lung diseases represent a largely global burden whose pathogenesis remains largely unknown. Research increasingly suggests that epigenetic modifications, especially DNA methylation, play a mechanistic role in chronic lung diseases. DNA methylation can affect gene expression and induce various diseases. Of the caveolae in plasma membrane of cell, caveolin-1 (Cav-1) is a crucial structural constituent involved in many important life activities. With the increasingly advanced progress of genome-wide methylation sequencing technologies, the important impact of Cav-1 DNA methylation has been discovered. The present review overviews the biological characters, functions, and structure of Cav-1; epigenetic modifications of Cav-1 in health and disease; expression and regulation of Cav-1 DNA methylation in the respiratory system and its significance; as well as clinical potential as disease-specific biomarker and targets for early diagnosis and therapy.

KEYWORDS

caveolae, caveolin-1, chronic lung diseases, epigenetic modification, methylation

1 | BACKGROUND

Caveolae are crucial in various cellular, physiological, and pathological processes, for example, cell proliferation, apoptosis, migration, differentiation, angiogenesis, tumorigenesis, and metastasis by special signal transduction, endocytosis, and transcytosis. Caveolae are a kind of flask-like invaginations in the plasma membrane. The constitute of caveolae includes caveolin, cavin (also named polymerase I and transcript release factor), lipids, transcription polymerase, as well as various ion channel proteins (Figure 1).[1] The caveolin family contains three subtypes: caveolin-1 (Cav-1), caveolin-2 (Cav-2), and caveolin-3 (Cav-3), of which Cav-1 is co-expressed primarily in many cells with Cav-2. Cav-2 is not essential in the formation of caveolae and can be located or expresses dependently on Cav-1.[2] Cav-3 is only specific to muscle cells.[3] Cav-1 is the major integral membrane protein for the assembly of caveolae in nonmuscle cells. Emerging evidence demonstrates that Cav-1 plays a positive or negative regulatory role in cell signaling transduction, which depends

Abbreviations: COPD, chronic obstructive pulmonary disease; CSD, caveolin scaffolding domain; EGF, epidermal growth factor; eNOS, endothelial nitric oxide synthase; IL-6, interleukin-6; IPF, idiopathic pulmonary fibrosis; LC3B, light chain 3B; MAP, mitogen-activated protein; PDGF, platelet-derived growth factor; TGF-β1, transforming growth factor-β1.

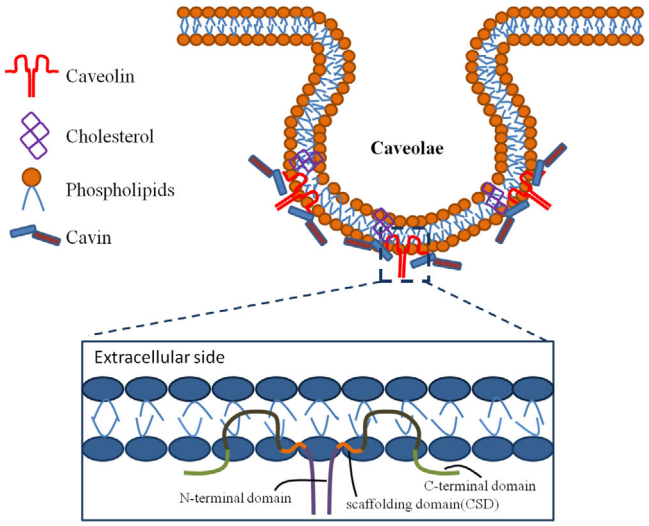

FIGURE 1 The structure of caveolae. Caveolae are a kind of flask-shaped invaginations in the plasma membrane, including caveolin, cavin (also named polymerase I and transcript release factor (PTRF)), lipids, and so on. Cav-1 is the major integral membrane protein for the assembly of caveolae. Cav-1 contains a highly conserved domain named caveolin scaffolding domain (CSD, acids 82-101). The CSD domain takes part in the interactions with signaling proteins and regulates signal transduction in various cellular processes

on the type of cells and signaling pathways. The changes in expression of Cav-1 may be vital on chronic lung diseases as a new target for the treatment. Our review aims at overviewing the biological characters, structure and function of Cav-1, epigenetic modifications of Cav-1 in health and disease, expression and regulation of Cav-1 in the respiratory system, Cav-1 methylation and significance in chronic lung diseases, as well as clinical potential as disease-specific biomarker and targets for early diagnosis and therapy.

1.1 | Structure and functions of Cav-1

Cav-1 consists of 178 amino acid residues with a highly conserved amphipathic region of caveolin scaffolding domain (CSD), which interacts with signaling proteins and regulates signal transduction through the entrapment of signaling partners.[4] Cav-1 monomers may form a disk-shaped oligomer with its carboxyl terminal part toward the center and insert into the plasma membrane by CSD and intramembrane domain, a second amphipathic helix.[5] CSD is dynamically allocated between fully helical or partly unstructured forms, which determine its accessibility.[6] The structure of Cav-1 is decided by its oligomerization state and the organization of other components in the caveolae like cavin or lipids. In caveolae, Cav-1 oligomers converge specific lipids such as cholesterol, phosphatidylinositol-4,5-bisphosphate, and phosphatidyl serine to aggregate cavin trimers. Caveolae are enriched in multiple lipids, some of which are highly important signaling molecules in cell membrane.[7] Cav-1 closely connects with cholesterol and sphingolipids. This connection cannot be separated at low temperatures by high concentrations of salts or nonionic surfactant detergents likes triton X-100.[8] Cav-1 can interact with cholesterol at a 1:1 stoichiometry through a sequence that matches to cholesterol recognition/interaction amino acid consensus domain.[9] Depletion of cholesterol causes a decrease in caveolae. Conversely, cholesterol supplementation will increase membrane cholesterol, and lead to a decrease in membrane fluidity and an increase in caveolae and Cav-1 number on the cell membrane.[10]

Cav-1 is encoded on 7q31.1 of human chromosome and critical in the formation of caveolae. Caveolae cannot be formed without Cav-1. Genetic deletion of Cav-1 caused the lack of caveolae.[11,12] The amino- and carboxy-terminal domains of Cav-1 are limited in the cytoplasmic surface of cell membrane and long putative hairpin intramembrane domain.[13] Cav-1 exists into two isoforms: Cav-1α and Cav-1β, having similar structures to CSD and an acetylated C-terminus.[14] The only difference between Cav-1α and Cav-1β structures is that the Cav-1α has an N-terminal 31 amino acids rather than Cav-1β.[15] Cav-1α and Cav-1β are produced from two distinct mRNAs. Full-length mRNA may produce the Cav-1α predominantly, but little Cav-1β. The Cav-1β was most partly generated from 5'-end variant mRNA.[16] The function of Cav-1 isoforms differs, for example, Cav-1α primarily expressed as an early marker for vasculogenesis during the development of lung blood vessels and in alveolar Type I cells in mature lungs.[17] Hyperexpression of Cav-1β may inhibit activation of the bone morphogenetic proteins pathway signaling, rather than Cav-1α.[14,18] In freeze-fracture immunoelectron microscopy, the α/β ratio in human fibroblasts is higher in the deep of caveolae than the shallow ones. The different ratio of Cav-1 isoforms in the deep and shallow of caveolae shows a unique molecular mechanism about the caveolae-shaped differentiation.[19]

Phosphatidylserine was accumulated on the cytoplasmic surface of the plasma membrane related to caveolae and the function of Cav-1.[20] Cav-1 is phosphorylated on tyrosine-14 in response to stimulation, responsible for various biological processes covering signal transduction and regulation in caveolae.[21] The phosphorylation of Cav-1 at Tyr14 can be regulated in posttranslational level to contribute to the pathogenesis of lung diseases.[22] Changes of the phosphorylation of Cav-1 may be the direction of targeted therapy. Phosphorylation of Cav-1 is a necessary process to enhance the interaction with endothelial nitric oxide synthase (eNOS)[23] and regulate nanoclustering of isotype-specific B-cell antigen receptors.[24] Curcumin prevented kidney injury in diabetic nephropathy by inhibiting phosphorylation of Cav-1.[25] Lipopolysaccharide (LPS)-induced phosphorylation of

Cav-1-enhanced microvascular permeability.[26] Various important processes are affected by Cav-1. Cav-1 gathering with other signal-sensing molecules can be activated by appropriate stimulation. Many growth factors, signaling receptors, kinases, enzymes, and other signaling regulators are clustered in caveolae.[27] Cav-1 can interact with regulatory factors of signaling pathways, such as Akt,[28] Src kinases,[29] Rab5 small GTPases,[30] and is also involved in maintenance of the immune system. Cav-1 is closely associated with eNOS, which is mainly reflected in the co-localization and the dynamic functional regulation.[31] In endothelial cells, eNOS directly binds with the CSD of Cav-1 and co-expressed with it at a special ratio.[23] On the one hand, Cav-1 regulates eNOS expression level and inhibits its activity when activated, and, on the other hand, sustained eNOS-derived NO production leads to the degradation of Cav-1.[31] The association between Cav-1 and eNOS was crucial in vascular homeostasis when confronted with oxidative stress, which was found in several disease states including atherosclerosis, diabetes, and myocardial infarction.[32,33] Epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) receptors were transiently associated with Cav-1 in the presence of ligand. Overexpressed Cav-1 suppressed p42/44 mitogen-activated protein (MAP) kinase activation and cell proliferation induced by EGF and PDGF.[34,35] The p42/44 MAP kinase could be activated in the absence of Cav-1, leading to cardiac hypertrophy.[36] Cav-1 regulates the distribution of nanoclusters of isotype-specific B-cell antigen receptors in B cell plasma membrane, a multiprotein complex, which plays an important role in the development, proliferation, and activation of B cells, to prevent B-cell-induced autoimmunity.[24] Cav-1 contributes to mitochondrial fatty acid catabolism and respiration through modulating mitochondrial cholesterol levels, stimulating peroxisome proliferator-activated receptor α–dependent fatty acid oxidation and enhancing ketogenesis production.[37,38] Cav-1 affects the regulation of glycolytic activities. Isoflurane inhibits cell apoptosis through increasing glycolysis in a Cav-1-dependent mechanism.[39] Cav-1 also plays part roles in the regulation of apoptosis. Microtubule-associated protein 1 light chain 3B (LC3B), as a vital regulator of autophagic and apoptotic signaling cascades, requires Cav-1 to form a complex with the death receptor Fas to regulate apoptosis.[40] The absence of Cav-1 caused various disorders related to normal life activities, leading to diseases.[41] Cav-1 knockout mice have multiple functional disorders including hyperglycaemia, lipidosis, and dysfunction in vascular permeability.[12,42-44] Cav-1 mutation leads to severely lipodystrophic diabetes.[45] Caveolae as the multifunctional organelle is important for the regulation of various cellular functions. Understanding of caveolae is useful to design therapies for caveolae-associated diseases

1.2 | Cav-1 in the respiratory system

Caveolae and their vital constituent Cav-1 play complex and significant roles in respiratory system. Within the lung, caveolae are widely present in airway or alveolar epithelium, airway or pulmonary artery smooth muscle, pulmonary endothelium, fibroblasts, and immune cells.[46] Thus, the widespread presence of caveolae raises the controllability of themselves and Cav-1 in lung disease states and can in turn influence the pathophysiology. The changes in Cav-1 expression lead to a series of function and morphological dysfunction in the respiratory system (Figure 2).

Cav-1 has a range of functions and effects, many of which are harmful, but some may also promote health. In addition, Cav-1 is found in a variety of cells and has different roles in these cell types. Therefore, it needs to be studied separately in cell culture and expression analysis and animal disease models.[47] The regulation of Cav-1 is multifunctional in chronic lung diseases. In most of the lung diseases, the expression of Cav-1 is lower compared to normal conditions. Complete loss of caveolae and Cav-1 in airways and vasculature is thought to occur in inflammatory lung diseases such as chronic obstructive pulmonary disease (COPD), asthma, and inflammation-induced lung injury.[48] Downregulation of Cav-1 may be related to pulmonary fibrosis due to increased extracellular matrix production, hypercellularity, inflammation, and dysfunction of epithelial barrier.[49] Cav-1-knockout mice enhanced the severity of transforming growth factor-β1 (TGF-β1)-induced oxidative stress, inflammation, and fibrosis.[50] Deletion of Cav-1 in mice also developed pulmonary hypertension, myocardial hypertrophy, and alveolar cell hyperproliferation through the activation of p42/44 MAP kinases.[51] Cav-1 may regulate pulmonary vascular homeostasis through influencing endothelial angiotensin-1 converting enzyme expression and activity, of which reduced expression of Cav-1 leads to abnormal pulmonary vascular development.[52] COPD is a type of emphysema and/or chronic bronchitis characterized by airflow obstruction. Chronic bronchitis is inflammation that occurs on the inner wall of airway. Emphysema is related to the destruction of the alveoli cells. In addition, oxidative stress, apoptosis, and aging are all involved in COPD. Cav-1 regulates these processes. For example, loss of Cav-1 is related to the deficiency of elastic fibers in the lung from the damaged parenchyma of COPD patients.[53] The expression of Cav-1 is required in lung fibroblasts and emphysema aging induced by smoking.[54] The imbalance of Th17/Treg cells was crucial in the pathogenesis of COPD. Cav-1 is related to the homeostasis of Th17/Treg cells in respiratory inflammation.[55] Downregulation of Cav-1 was accompanied by an increase in Treg and decrease in Th17 expression. These results indicate that Cav-1 plays a pivotal role in the occurrence and development of COPD. Cav-1 was

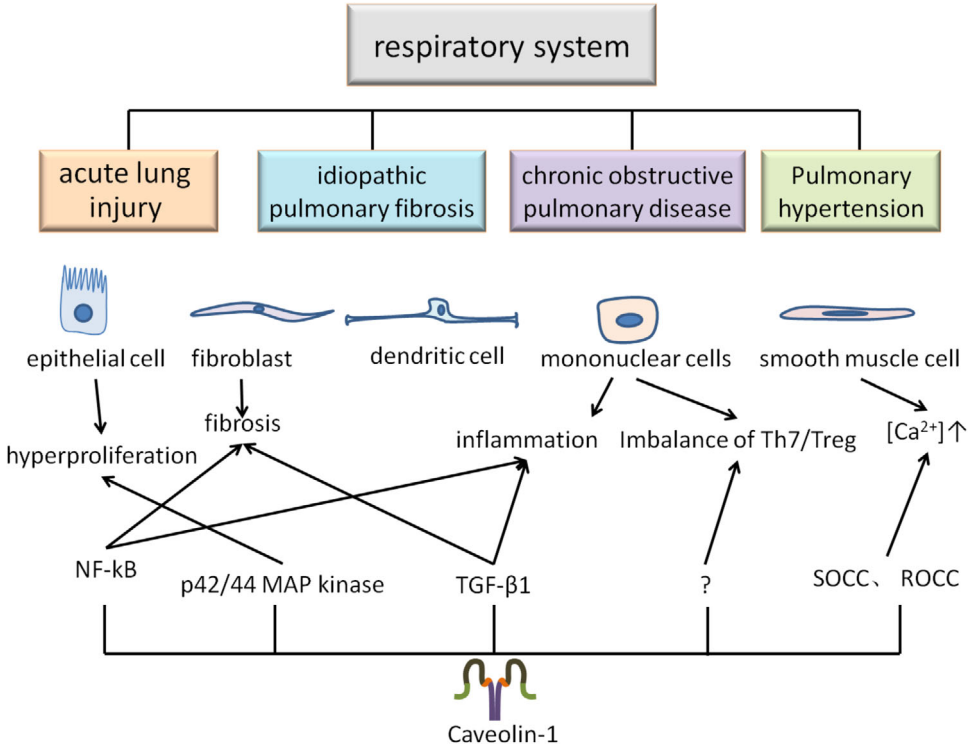

FIGURE 2 Cav-1 in the respiratory system. Caveolae widely exists in many cells of the respiratory system and are involved in various cellular activities. Cav-1 as the important structural proteins of caveolae is involved in a variety of signaling pathways, and the abnormal expression level of it will lead to the structural and functional dysfunction and induce the occurrence of diseases

found to be involved in the formation of pulmonary capillary leakage, pulmonary edema, and lung injury during acute inflammatory response.[56,57] Cav-1 deletion enhanced expression of the pro-inflammatory cytokines stimulated by LPS. The expression of Cav-1 was downregulated in peripheral monocytes or plasma harvested from patients with asthma or COPD along with pulmonary hypertension.[58,59] Fibrotic disorders are related to the abnormal accumulation of fibroblasts in tissues. TGF-β1 is the key modulator of fibrogenesis in various tissues and the essential regulator in myofibroblast differentiation, leading to the apoptosis-resistant phenotype by multiple signaling pathways. The suppression of Cav-1 contributes to fibroblast proliferation and apoptosis resistance through TGF-β1-associated pathway in the development of idiopathic pulmonary fibrosis (IPF).[60-62]

However, other studies suggested that the downregulated Cav-1 expression might reduce the severity of lung inflammation and vascular injury through activating polymorphonuclear neutrophils.[63] The role of Cav-1 in pulmonary arterial hypertension was verified in pulmonary arterial hypertension rat models, where Cav-1 activated signal transducers and activators of transcription 3 (STAT3) transcription factor[64] and regulated the bioavailability of NO.[65] Increased Cav-1 expression in pulmonary arterial hypertension enhanced agonist-induced contraction via modulation of receptor-operated calcium channels and store-operated calcium channels in pulmonary arteries, playing a vital role in disease pathology.[66] In lung cancer, Cav-1 plays both suppressive and promoting roles.[67] Downregulated Cav-1 expression of cancer-associated fibroblasts is observed in many aggressive cancers, indicating that Cav-1 may inhibit tumor cell growth and increase the production of α-smooth muscle actin, responsible for poor cancer outcomes.[68] However, degradation of Cav-1 can increase autophagy markers, such as cathepsin B (active form), lysosomal-associated membrane protein-1, LC3B, beclin 1, autophagy-related 16 like 1, (ATG16L1), and BCL2 interacting protein 3 (BNIP3), to increase autophagy of cancer cells.[69] Cav-1 also regulates cellular senescence, for example, senescent lung fibroblasts, contributing to the progression of lung cancer.[70,71] Cav-1 was identified to modulate the secretion of interleukin-6 (IL-6), which is an important factor in the microenvironment of tumor and the growth of cancer cells. Overexpression of Cav-1 may induce premature senescence. Senescent fibroblasts stimulate the growth of cancer cells by secretion of IL-6.[72]

1.3 | Epigenetic modifications of Cav-1 in healthy and diseased lungs

Epigenetics influence gene expression with changes in DNA sequences through two major mechanisms (Figure 3). Of

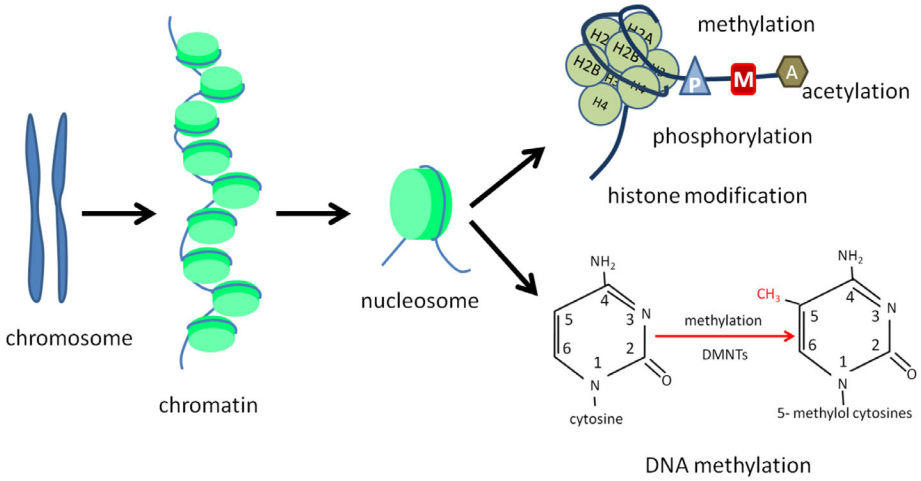

FIGURE 3 Two major epigenetics influence in gene expression. There are two major mechanisms involved in epigenetic regulation. One is the DNA methylation, changes on that could influence the gene expression level of gene. The other one is histone modification, which contains phosphorylation, methylation, and acetylation

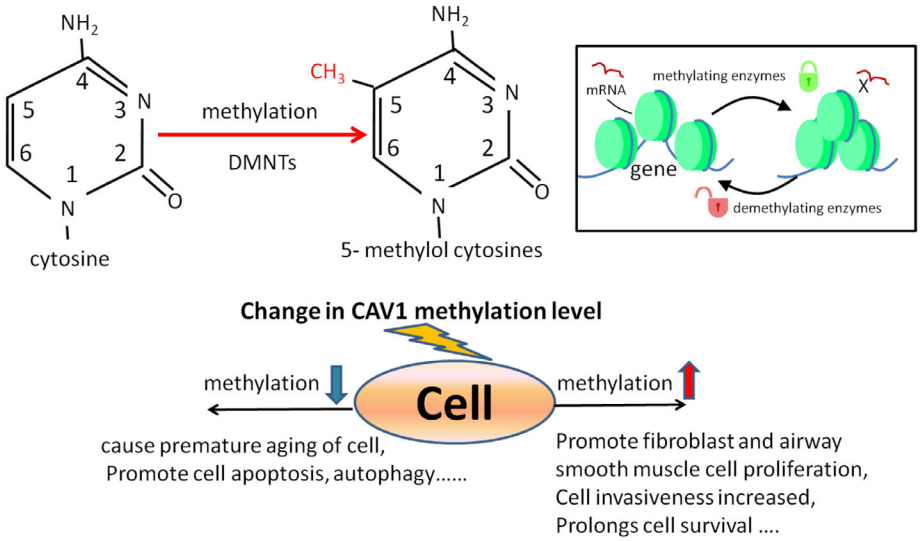

FIGURE 4 DNA methylation changes in *Cav-1* gene expression. Methylation refers to the process of catalyzing the transfer of methyl groups from active methyl compounds to other compounds. DNA methylation generally occurs at the CpG site of gene promoter region. DNA methylation is an important modification of genes, which could regulate the expression level of genes and is closely related to many diseases. It is one of the crucial researches on epigenetic regulation

those, DNA methylation involves methylation of gene promoter regions (Figure 4), whereas the histone modification is related to the structural changes of chromatin. Changes in epigenetic regulation can be restored by using some chemical agents.[73] DNA promoter hypermethylation is induced by the modification of cytosine residues in the CpG dinucleotides to constitute 5-methylcytosine via covalent addition of methyl group with DNA methyltransferase. CpG dinucleotides are disproportionally distributed in mammals. The CpG islands (CpGi) DNA within the gene promoters is a short sequence with high densities of CpG dinucleotides. The promoter region of genes with methylated CpGi is transcriptionally inactive due to the inhibitory role of methyl groups in transcriptional elements via accessing the promoter region.[73,74] The downregulation of Cav-1 in various diseases is caused by the methylation at the *Cav-1* coding genes CAV1 promoter region.

Cav-1 can be downregulated by the aberrant promoter methylation of CAV1 in the stage and may be crucial in the development of many cancers.[75,76] The DNA methyltransferases play key roles in CAV1 expression in different stages of many cancers.[77,78] The positive or negative effects of Cav-1 vary among a variety of aspects of tumor progression, due to the direct or indirect interaction of Cav-1 with effector

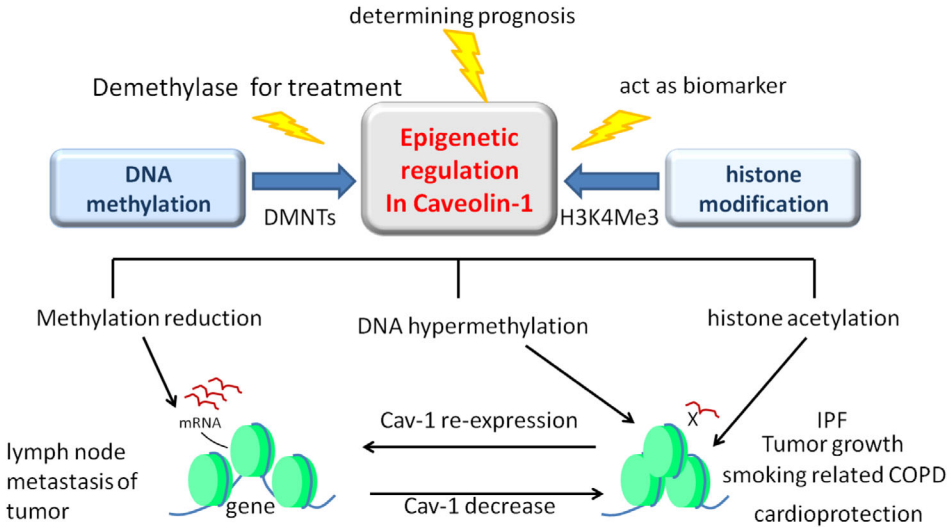

FIGURE 5 Epigenetic regulation changes in *Cav-1* gene expression. DNA methylation and histone modification are the two major mechanisms of *Cav-1* gene. Epigenetic regulation changes in *Cav-1* gene could act as diagnosis and prognosis biomarkers of various lung diseases. Application of demethylase can reverse the methylation degree changes in promoter region of *Cav-1* gene and induce the re-expression of Cav-1. The re-expression of Cav-1 makes therapeutic effect on some chronic lung diseases

molecules to affect caveolae's function.[79] The promoter CpG hypermethylation of CAV1 occurred at the onset of tumor development though a hypermethylated state remains in full-blown tumors.[80] However, the degree of methylation in metastatic foci and lymph nodes decreased to re-express those related genes. Those genes are most partly inactivated through changes in DNA methylation and reactivated in demethylation activity.[81] It was reported that the 5′ promoter of CAV1 was methylated in human breast cancer cells, whereas not in the normal human mammary epithelial cells.[82] Furthermore, hypermethylation in CAV1 promoter region is involved in the histopathological grading of the tumor[83] and with nodal metastasis, which is the most common form of metastasis pattern.[84] Although there were different epigenetic changes in Cav-1 among breast cancer subtypes, for example, CAV1 was overexpressed after being hypomethylated in inflammatory breast cancer.[85] In addition to cancers, there are many other diseases involving the regulation of Cav-1 methylation. Epigenetic regulation in Cav-1 could protect cardiac function from ischaemic injury as a potential mechanism of cardioprotection.[86] CAV1 deletion decreased expression of sirtuin1 in the ischemic preconditioning heart, which may affect DNA methylation across the genome and play a protective role in cardiac ischemia reperfusion injury.[86]

1.4 | Caveolin-1 methylation in chronic lung diseases

The epigenetic changes in Cav-1 may be a new target for the treatment of chronic lung diseases (Figure 5). Suppression of Cav-1 expression was related to the gene promoter hypermethylated in COPD as well as in IPF.[87] Compared with lung tissue in the COPD group and the nonsmoker group, the CpG sites of CAV1 in the COPD group were significantly hypermethylated.[88] DNA methylation is seriously disrupted because of cigarette smoking, responsible for a wide range of malignant and nonmalignant diseases progression. It is an important mechanism contributing to COPD pathology. Abnormal CAV1 methylation was a whole genome phenomenon in small airways of patients with COPD, altering gene expressions and pathway activities important to COPD.[87] Cav-1 methylation can be a powerful predictor in the stable stage of lung cancer, and a potential biomarker for taxane-based chemotherapy in lung cancers.[89] *Cav-1* gene methylation was related to overall survival of patients with lung cancer treated with taxane, although Cav-1 expression levels did not show significant difference.[89] Those effects of CAV1 promoter methylation in lung cancers seem to be cell and tissue specific. CAV1 could be a key molecule for lung cancer development. It plays quite different roles between small-cell lung cancer and nonsmall-cell lung cancer because the changes in CAV1 methylation may have opposite functions leading to either growth inhibition or growth promotion. For example, *CAV1* has been considered as a tumor suppressor gene in SCLC, whereas in NSCLC, *CAV1* acts as an oncogene and is responsible for survival and growth of tumor cells.[67] Other epigenetic mechanisms, such as histone modifications, were observed in chronic lung diseases, for example, Cav-1 expression was suppressed by the histone deacetylase inhibitor, trichostatin A.[90] Expression of Cav-1 was downregulated in IPF, when CAV1 was silenced through diminished

binding of the active histone mark histone H3 trimethyl Lys4 with its promoter region.[91] Combining with the evidences that Cav-1 expression is significantly reduced in a variety of chronic lung diseases, we suspect that epigenetic changes of Cav-1 may be a key pathological mechanism of chronic lung diseases.

The methylation of Cav-1 promoter region by DNA methyltransferases is reversible and can be a new direction for targeted treatment of diseases. Treatment with a DNA methyltransferase inhibitor in breast cancer cell lines leads to the re-expression of Cav-1 through demethylation of CpGi shores.[92] Treatment with 5-AZA, which may reverse DNA promoter hypermethylation, could cause Cav-1 re-expression and restoration in ovarian cancer-associated hypermethylation.[90] Hypermethylation in Cav-1 promoter region was reported in patients with colorectal cancer, whereas 5-AZA could inhibit colon cancer cell growth through the Cav-1 signal pathways.[93,94] Further, 5-AZA treatment in hepatoma cells also leads to upregulated Cav-1 expression.[95] DNA is not easily degraded, DNA methylation happens uniquely in the CpG-rich region and can be detected easily with a single pair of primers, or Cav-1 can be secreted into the plasma and detected. Therefore, the treatment of abnormal methylated DNA by methyltransferase inhibitors is feasible, which can trigger the re-expression of silenced genes, thereby improving the treatment efficiency.

2 | CONCLUSIONS

In conclusion, Cav-1 is important in healthy and diseased lungs, of which the suppression of Cav-1 expression and function may be associated with the pathogenesis of chronic lung disease. Cav-1, especially altered DNA methylation patterns in the promoter region, was associated with chronic lung diseases. Treatment with DNA methyltransferase inhibitor can activate Cav-1 through demethylation of CpGi shores as therapeutic potentials for lung diseases, although there still are a large number of challenges to be overcome to meet criteria of disease-specific biomarkers and targets to dynamically monitor disease severity, duration, stage, and response to therapy.[96-111] Understanding of Cav-1 may contribute to developing the new therapies. Further researches will be needed to clarify the role of CAV1 in the development of chronic lung disease and to determine whether CAV1 expression and/or promoter methylation could be used as an alternative of diagnostic biomarkers and therapeutic targets for chronic lung diseases in the early diagnosis and clinical treatment.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

FUNDING INFORMATION

National Key Research and Development Program of China; Grant Numbers: 2016YFC1304000 and 2016YFC1304003.

AUTHOR CONTRIBUTIONS

All the authors contributed to the writing of this review. All the authors read and approved the final manuscript.

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How to cite this article: Yan F, Su L, Chen X, Wang X, Gao H, Zeng Y. Molecular regulation and clinical significance of caveolin-1 methylation in chronic lung diseases. *Clin Transl Med*. 2020;10:151–160. https://doi.org/10.1002/ctm2.2