Chapter

p300/CBP Methylation is Involved in the Potential Carcinogenic Mechanism of Lung Cancer

Yu Zhang, Wei Shen, Jin Zou and Shibo Ying

Abstract

p300/CBP is involved in the expression of a wide range of genes, both as a histone acetyltransferase (HAT) and as a coactivator of transcription factors. p300/CBP is the specific substrate of CARM1, and its KIX domain and GBD domain are the main sites methylated by arginine methyltransferase 4 (PRMT4/CARM1). p300/CBP plays an important role in lung cancer, which is a cell cycle disease. More importantly, the methylation of p300/CBP by CARM1 affects the progression of lung cancer through the cAMP-PKA pathway, p53 pathway and ER pathway. The structure, function, methylation modification sites, methylation-related enzymes, genes associated with lung cancer and the possible mechanisms of p300/CBP action are reviewed.

Keywords: p300/CBP, methylation, CARM1, signal transduction pathway, EMT, lung cancer

1. Introduction to the p300/CBP protein

1.1 Structure and function of p300/CBP

The p300/CBP molecule has at least eight functional domains. The nuclear receptor scope (RID) mainly interacts with the nuclear receptor and has a cysteine-histidine-rich domain (a CH domain), namely, the CH1 domain, also known as transcription articulation zinc finger domain 1 (TAZ1). The CH2 domain in RID includes the RING and PHD domains. The RING domain is an E3 ligase that mediates the transfer of ubiquitin to substrates by binding an E2 ubiquitin binding enzyme [1]. The PHD domain is a zinc finger domain that identifies the methylated state of histones. The CH3 domain includes ZZ ZZ-type zinc finger domain (ZZ) and transcription cohesive device zinc finger domain 2 (TAZ2). The HAT domain of p300/CBP is the structural basis of the transcription complex bridge formed by multiple transcription factors and functions as an acetylase. The structural failure of RING-HAT connections or RING domain, and particularly the loss of the RING domain, usually results in significant increases in the automatic acetylation of p300/CBP and in the acetylation of p53 [2]. The bromine domain binds to acetylated histones and transcription factors in nucleosomes. The deletion or mutation of the bromine domain does not eliminate HAT domain activity but interferes with substrate targeting and transcriptional activity [3], and bromine domain inhibitors have been shown to reduce the expression of G protein signal regulators (RGS4) [4]. The KIX domain is a CREB-binding site and the main motif

modified by CARM1 methylation. Steroidal hormone receptor coactivator 1 (SID) mainly mediates protein-protein interactions, and many cell and viral proteins bind to this region, which is also the domain of the srC-1 interaction [5]. In glutamine- and proline-rich domains in the N-terminus and C-terminus have transactivation domains (TA domains), and their main function is activating transcription.

1.2 Major methylation sites of p300/CBP and related enzymes

As early as 2005, it was reported that three arginine methylation sites were found in the GBD domain (GRIP1-binding domain) in the C-terminus of p300, Arg-2056; Arg-2088; and Arg-2142. Among these residues, Arg-2142 is the most important site, and the methylation of Arg-2142 strongly inhibits the interaction between p300 and GRIP1 [6]. In subsequent studies, the importance of Arg-754 methylation in the p300·KIX domain in the cell response to DNA damage was gradually discovered, and Arg-754 in CBP is analogous to one of the three arginine residues in p300 mentioned above. The KIX domain of p300/CBP is not only the binding site of CREB but also the main site of CARM1 methylation modification, and studies have confirmed that CARM1 can be methylated to modify p300/CBP molecules both *in vivo* and *in vitro*, with Arg-754 being the main site for CARM1 methylation. It has been found that the methylation of Arg-754 can recruit the p53-binding region of BRCA1 to the p21 promoter, initiating the activation of p53 and, subsequently, p21 in response to DNA damage [7].

It has also been reported that the methylation site of CBP·KIX, Arg-580, is highly methylated by CARM1 *in vitro*, and Arg-600 of the CBP·KIX domain (equivalent to Arg-580 of p300·KIX) is located on the outer surface of the KIX-KID complex. Its methylation blocks the activation of CREB by blocking the interaction between KIX and the CREB kinase-induced domain (KID) [8]. In addition, CBP protein residues Arg714, Arg742 and Arg768 are the main methylation sites of CARM1 *in vitro*, and R742 is the main methylation site of CARM1 *in vivo* [9].

2. Expression of p300/CBP and CARM1 in lung cancer

2.1 The role of high p300/CBP expression in lung cancer tissues

Highly expressed p300 significantly enhances the ability of cancer cells to invade and migrate in non-small cell lung cancer (NSCLC). If the p300 gene is knocked out, the invasion and invasion ability of the cancer cells is significantly reduced, which may be related to the increased ZEB1 activity caused by the formation of the p300-Smad complex and further induction of the EMT [10]. CBP is highly expressed in lung cancer cells and tumor tissues. CBP acetylation is associated with cleavage and polyadenylation specificity factor subunit 4 (CPSF4) in the gene promoter region and synergically regulates downstream gene transcription and tumor cell proliferation. This association between CBP and the CPSF4 and its synergistic effect on the activation of human telomerase reverse transcriptase (hTERT) expression may contribute to the involvement of CBP in the mechanism promoting lung cancer growth [11, 12].

2.2 CARM1 is highly expressed in lung cancer tissues

Silencing CARM1 expression significantly reduced the apoptosis rate of lung cancer cells and significantly promoted the migration of lung cancer cells, while the overexpression of CARM1 significantly increased the apoptosis rate of lung cancer

cells and reduced the migration of lung cancer cells, suggesting that CARM1 may attenuate the development of lung cancer [13]. Notably, a recent study found that the overexpression of CARM1 leads to methylated H3R17me2a and H3R26me2a at the core promoter region of the gene encoding the cell cycle protein E2 (CCNE2), activating CCNE2 expression at the height of the cell cycle, which facilitated the G1/S phase transition and promoted cell proliferation and colony formation. The overexpression of CCNE2 is often observed in lung cancer tissues, and the tumor-promoting effect of CARM1 in NSCLC has been shown by *in vitro* experiments [14].

Since CARM1 and PRMT1 are highly expressed in lung cancer tissues and expressed at low levels in normal tissues, CARM1 distribution was significant. Increased keratin expression and neurometalloprotein B receptor (NMBR) expression were observed in CARM1-knockout cancer cells, demonstrating that CARM1 expression is associated with lung cancer differentiation and staging [15].

3. Carcinogenic mechanism involves p300/CBP methylation

3.1 p53 pathway and $p21^{WAF1/CIP1}$ activation mechanism

p21 is a member of the KIP/CIP family of cyclin-dependent kinase (Cdk) inhibitors (CKIs) and inhibits the action of all Cdk complexes throughout the cell cycle by occupying the ATP-binding site of the Cdk complex; therefore, it is also known as cyclin inhibitory protein 1 ($p21^{WAF1/CIP1}$).

When DNA is damaged, the expression of the p53 gene is induced, and then, p53 induces $p21^{WAF1/CIP1}$ expression. The methylation of Arg-754 in the P300·KIX domain is essential for p53 activation of $p21^{WAF1/CIP1}$. First, p53 recruits CARM1-methylated p300 before it recruits BRCA1 and then mediates the interaction between the p21 promoter and the p53-binding region to initiate the activation of p53 and of p21 [7]. Activated $P21^{WAF1/CIP1}$, cyclin, Cdk and proliferating cell nuclear antigen (PCNA) combine to form the cyclin-Cdk-p21-PCNA tetramer complex, which prevents the cell proliferation signal from effectively being transmitted, and the damaged cell is arrested in the G_1 phase, inducing transcription of the DNA repair gene *GADD45*. If a damaged cell remains stagnated in the G_1 phase, p53 is induced to activate the apoptotic gene Bax and thus initiate cell apoptosis (**Figure 1a**). It has also been reported that the expression of $p21^{WAF1/CIP1}$ was directly upregulated p300/CBP-induced acetylation of KLF6 [16]. Peroxisomal proliferative factor receptor γ (PPAR-γ) also directly upregulated $p21^{WAF1/CIP1}$ expression in lung cancer cells.

3.2 cAMP-PKA pathways

As one of the most common signaling pathways of G protein-coupled receptors, the cAMP-PKA pathway plays a very important role in the regulation of cell activity. Many signaling molecules, such as glucagon, adrenaline and corticotropin, are regulated by this pathway. Under normal physiological conditions, adenylate cyclase (AC) is activated after the ligand binds specifically to the receptor, and AC converts intracellular ATP into cAMP, which is an intracellular second messenger. cAMP activates cAMP-dependent protein kinase A (PKA), and the free C-subunit of PKA encounters a specific serine residue (Ser133) site in the kinase-induced domain of cAMP reactive element-binding protein (CREB) which is phosphorylated within the nucleus to recruit p300/CBP molecules [17]. Through p300/CBP acetylation, which promotes general transcription factors (such as TFIIB) binding with the target gene promoter, target gene expression is regulated. Activated CREB has a wide range of cytological effects, including *in vitro* participation in the regulation of

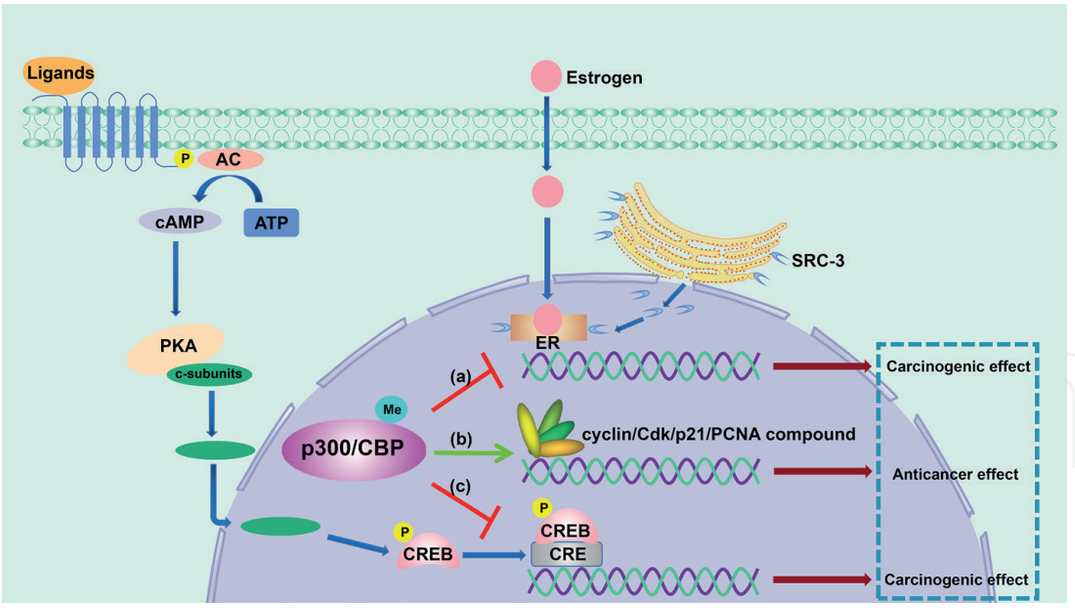

Figure 1.
a: p300/CBP methylation by CARM1 blocks the ER pathway by inhibiting the formation of core transcription complexes; b: P300/CBP methylation by CARM1 blocks cAMP-PKA-CREB activation; c: P300/CBP methylation by CARM1 is beneficial to p53-dependent p21 activation. ⊣ (red): inhibition; → (green): promotion. AC: adenylate cyclase; ATP: Adenosine triphosphate; cAMP: adenosine cyclophosphate; PKA: protein kinase A; SRC-3: steroid receptor coactivator-3; ER: estrogen receptor; Cdk: cyclin-dependent kinases; PCNA: proliferating cell nuclear antigen; CREB: cAMP-response element binding protein; CRE: cAMP response element; P: phosphorylation; Me: Methylation.

cell migration/invasion, cell proliferation, cell survival, Warburg effect induction, etc. It is involved in the immune response, tumorigenicity, vascular growth and tumor progression *in vivo* [18]. However, p300/CBP·KIX was modified by CARM1 methylation, which blocked the activation of CREB and induced apoptosis by preventing the combination of KIX and KID [8] (**Figure 1b**).

In addition, *LKB1* is the target of mutational inactivation in sporadic cancers, especially NSCLC. *LKB1* is mutated in approximately 20%-30% of NSCLC cases, making it the third most common genetic change site after *P53* and *K-RAS*. The inactivation of *LKB1* and subsequent activation of cyclic adenosine reactive element-binding protein (CREB)/CREB regulating transcription coactivator (CRTC) induced *LINC00473*. *LINC00473* is a nuclear gene that interacts with *NONO*, which is a component of the cyclic adenosine signaling pathway, to promote CRTC/CREB-mediated transcription. *LINC00473* is critical for maintaining the growth and survival of lung cancer cells [19]. Methylation of the p300/CBP·KIX domain blocks the activation of CREB and may also affect the expression of CRTC-mediated *LINC00473*, thereby blocking the progression of lung cancer.

3.3 The estrogen receptor (ER) pathway

Many steroid hormone receptors are expressed on the surface of lung cancer cells, including estrogen receptor (ER). When ligands (such as estrogen) diffuse into cells or undergo in situ synthesis and when ERs are induced to form homologous or heterologous dimers, these dimers combine with nuclear DNA enhancer ERE estrogen response elements (EHRs) and recruit steroid receptor auxiliary activation factor-3 (SRC-3). The study found that the ER compound needs two SRC-3 to form an initial stable ERE/ERα/SRC-3a/SRC-3b/p300 core complex. This is a key step in establishing the core ER coactivator complex and recruiting the p300 protein to the ER genomic binding site [20].

In subsequent studies, sequential recruitment and transcriptional activation models of the coactivators of ER were proposed: each ER monomer recruits one SRC-3, two SRC-3 molecules work together to lock one p300 molecule safely into the ER complex, and then, histone H3 acetylation is initiated. Next, CARM1 binds to the complex, where it can easily methylate its substrates SRC-3, p300, and histones. Due to the proximity of SRC-3b to ER, SRC-3a and p300, the binding of CARM1 to the ER complex results in the release of SRC-3b, and CARM1 occupies the site vacated by SRC-3b. Second, the combination with CARM1 leads to further conformational changes of p300. This structural change caused by the sequential coactivator recruitment process further alters the activity of p300 acetyltransferase and the activity of CARM1 HMT on histone H3. The synergistic effect of CARM1 and p300 enhances the acetylation of histone H3K18 and the methylation of H3R17 and promotes the synergistic activation of target gene transcription [21].

After CARM1 methylation modifies p300, the interaction between SRC and p300 is inhibited [6]. CARM1 also methylates SRC-3 and destabilizes the SRC-3/CARM1 complex [22] (**Figure 1c**). Thus, the assembly of the core ER coactivator complex (ERE/ERα/SRC-3a/SRC-3b/p300) is destroyed, and the effect of the ER pathway is blocked.

4. Discussion

As a histone acetyltransferase, p300/CBP participate in various carcinogenic signal transduction pathways through its acetylation function. Methylated p300/CBP may selectively block the transcriptional activation of cAMP-PKA and steroid-dependent pathways [23], but after CARM1 methylation modifies p300/CBP, the transmission of signaling pathways is blocked in cancer, which seems to be conducive to the suppression of the transfer of signaling and the expression of signaling pathway components, such those in the ER pathway. The methylation of p300/CBP induced by inhibiting the interaction between SRC and p300 blocks the formation of the ERE/ERα/SRC-3/p300 core complex, resulting in the inhibition of cell proliferation and cell growth. In the p53-p21 pathway, p21 recruitment by p53 is mediated by p300/CBP that has been modified by CARM1 methylation, which results in cell cycle blockade and DNA repair. In the cAMP-PKA pathway, methylated p300/CBP blocks the activation of CREB, which in turn blocks the function of CREB and inhibits cell proliferation and migration. Moreover, we speculate that the high expression of p300/CBP in lung cancer tissue may be the result of CARM1 methylation of p300/CBP, mediating the activation of cancer suppression-related signaling pathways and blocking cancer-related signaling pathways. However, the high expression of CARM1 can promote CCNE2 activation and accelerate the progression of lung cancer through the methylation of histones. Therefore, inhibiting the histone methylation of CARM1 and increasing the methylation of p300/CBP are new ideas for novel targets and the treatment of lung cancer.

5. Conclusion

CARM1 may promote the apoptosis of cancer cells and inhibit the metastasis of cancer cells through the methylation of p300/CBP. The mechanism for inhibiting the occurrence of lung cancer may involve blocking the activation of oncogenic signaling pathways and mediating the activation of tumor suppressor signaling pathways.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (81973011), the Zhejiang Provincial Natural Science Foundation of China (LGF21H260010), Zhejiang Provincial Program for the Cultivation of High-level Innovative Health talents (2020), and the Science and Technology Project of Cixi City (CN2018023, CN2019031).

Conflict of interest

The authors declare no conflicts of interest.

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