

Review

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Review

Exploring the Role of CBX3 as Potential Therapeutic Target in Lung Cancer

Muhammad Aamir Wahab ¹, Nunzio Del Gaudio ¹, Biagio Gargiulo ¹, Vincenzo Quagliariello ², Nicola Maurea ², Angela Nebbioso ^{1,3}, Lucia Altucci ^{1,3,4,5,*} and Mariarosaria Conte ^{1,*}

¹ Department of Precision Medicine, University of Campania "Luigi Vanvitelli", 80138 Naples, Italy; muhammadaamir.wahab@unicampania.it (M.A.W.); nunzio.delgaudio@unicampania.it (N.D.G.); Biagio.gargiulo@unicampania.it (B.G.); angela.nebbioso@unicampania.it (A.N.)

² Division of Cardiology, Istituto Nazionale Tumori-IRCCS-Fondazione G. Pascale, 80131 Naples, Italy; quagliariello.enzo@gmail.com (V.Q.); n.maurea@istitutotumori.na.it (N.M)

³ Biogem Institute of Molecular and Genetic Biology, 83031 Ariano Irpino, Italy

⁴ Institute of Endocrinology and Oncology "Gaetano Salvatore" (IEOS), 80131 Naples, Italy

⁵ Program of Medical Epigenetics, Vanvitelli Hospital, 80138 Naples, Italy

* Correspondence: lucia.altucci@unicampania.it (L.A.); mariarosaria.conte@unicampania.it (M.C.)

Simple Summary: Lung cancer is a major cause of cancer-related deaths in developed nations. Factors such as unhealthy lifestyle choices, particularly smoking, contribute to the development of this disease. Epigenetic abnormalities greatly affect gene expression and disrupt important cellular signaling pathways that are responsible for the proper growth, regulation, and functioning of cells. In the case of lung cancer, specifically non-small cell lung cancer (NSCLC), CBX3 acts as an epigenetic oncoprotein, promoting the growth and progression of tumors. Numerous studies have shown that CBX3 is overexpressed in NSCLC and is associated with a poor prognosis. It interacts with key oncogenic pathways leading to increased proliferation, reduced apoptosis, and enhanced resistance to therapy. Further research on the mechanisms and functions of CBX3 holds the potential to reveal new insights into the development of the disease and uncover novel therapeutic opportunities.

Abstract: Epigenetic changes regulate gene expression through histone modifications, chromatin remodeling and proteins interpreter of these modifications. The PRC1 and PRC2 complexes shape gene repression via histone modifications. Specifically, the CBX protein family aids PRC1 recruitment to chromatin, impacting the progressive multistep process driving chromatin silencing. Among family members, CBX3 is a complex protein involved in aberrant epigenetic mechanisms that drive lung cancer progression. CBX3 promotes lung tumorigenesis by interacting with key pathways such as PI3K/AKT, Ras/KRAS, Wnt/ β -catenin, MAPK, Notch, and p53, leading to increased proliferation, inhibition of apoptosis, and enhanced resistance to therapy. Given our current lack of knowledge, additional research is required to uncover the intricate mechanisms underlying CBX3 activity, as well as its involvement in molecular pathways and its potential biomarker evaluation. Specifically, the dissimilar roles of CBX3 could be reexamined in order to gain a greater insight into lung cancer pathogenesis. This review aims to provide a clear overview of the context-related molecular profile of CBX3, which could be useful for addressing clinical challenges and developing novel targeted therapies based on personalized medicine.

Keywords: CBX3/ HP1 γ ; lung cancer; epigenetics; chromatin and signaling pathways

1. Introduction

Modifications associated with chromatin structure and function, known as epigenetic changes, are responsible for the activation and repression of genes, thereby impacting the synthesis and

production of specific proteins within cells and with variable expression patterns [1–3]. Gene transcription can be reversibly modulated via chromatin remodeling, which regulates the accessibility of promoter and enhancer regions to regulatory proteins. The remodeling process is controlled by more than 300 proteins and enzymes, which recognize, add, or remove chemical moieties from histone proteins. Such modifications, including acetylation, methylation, ubiquitination, and phosphorylation, occur at various lysine, arginine, and serine/threonine residues. The cumulative effect of these post-translational modifications results in the formation of a “histone code” [4–7]. Importantly, the biological significance of these histone modifications is deciphered by a diverse group of histone code *readers*. Reader proteins with specialized domains identify and interpret histone and DNA chemical modifications. These specialized enzymes include histone and DNA readers. Histone readers containing chromodomains, bromodomains, and plant homeodomain zinc fingers recognize and interpret histone modifications, while DNA readers that possess methyl-CpG-binding domains recognize specific alterations in DNA [8–10]. Additionally, RNA-binding proteins recognize and interpret modifications in RNA, such as 5-methylcytosine and N6-methyladenosine which affect gene expression [8,11]. Various enzymes, known as epigenetic writers and erasers, regulate the dynamic of epigenetic modifications. Specifically, the writers introduce chemical modifications to histones and DNA [1]. These include histone and DNA methyltransferases, histone acetyltransferases, ubiquitin ligases, and histone kinases. While erasers remove epigenetic modifications including histone deacetylases and demethylases, phosphatases, deubiquitinating enzymes, and ten-eleven translocations [9]. These activities collectively regulate gene expression and cellular homeostasis [12,13]. The epigenetic regulatory complex known as Polycomb repressive complex (PRC) 1, is a chromatin-modifying complex that is responsible for keeping the chromatin in a repressed state by mono-ubiquitinating histone H2A, thus restricting target gene transcription [14,15]. PRC1 is completed via its association with the Chromobox (CBX) family members, which are responsible for its recruitment to chromatin whereas the RING1a/b subunit represent the catalytic subunits of the complex [16]. Likewise, the PRC2 is a multi-subunit protein complex that cooperates with PRC1 playing a vital role in the epigenetic regulation of gene expression. The principal elements of PRC2 include the catalytic subunit enhancer of zeste homolog 1/2 (EZH1/2), which is able to methylate histones, as well as embryonic ectoderm development, suppressor of zeste 12 protein homolog and retinoblastoma-binding protein (RBBP) 4/7 [17–19]. In various cancers, the function of PRC2 is disrupted as a result of mutations or changes in the expression of its components. This phenomenon contributes to tumor development by activating tumor growth-suppressing genes or by triggering pathways that promote the growth of cancer cells [20–22]. PRC2 has therefore become a highly promising target for therapeutic intervention. The CBX family is a large group of proteins, comprising eight members, crucial in transcriptional repression and memory [23] due to the presence of a single N-terminal chromodomain [24]. The CBX family can be classified into two distinct groups. The first group, the Polycomb group (PcG), consists of CBX2, CBX4, CBX6, CBX7, and CBX8, all of which contain a C-terminal Polycomb repressor box and a conserved N-terminal chromodomain. The second group, the Heterochromatin Protein 1 (HP1) group, comprises CBX1, CBX3, and CBX5, characterized by an N-terminal chromodomain and a Chromoshadow domain associated with HP1. CBX family proteins facilitate the recruitment of PRC1 to chromatin, thus playing a crucial role in the initiation, growth, and development of tumors by suppressing the differentiation of cancer stem cells and promoting their self-renewal [24,25]. In non-small cell lung cancer (NSCLC), CBX3 has emerged as a promising prognostic biomarker. Higher CBX3 expression levels have been linked to the control of cell cycle progression and its potential impact on the PI3K/ AKT and Ras signaling pathways [26,27]. However, the precise mechanisms by which CBX3 contributes to the development and progression of lung cancer, including its involvement in different signaling pathways, have not been thoroughly examined and require additional research. Currently, the literature does not provide sufficient information to fully understand the involvement of CBX3 in these diseases, underlining the need to develop more elaborate and precise studies to obtain a greater insight into the function of CBX3 in molecular pathways. This review aims to give a detailed overview of the therapeutic

potential of CBX3. We describe the various pathways involving CBX3, highlighting its differential mechanisms of action as well as its significance as a potential therapeutic biomarker in lung cancer.

2. CBX3/HP1 γ Protein in Cancer Proliferation

The HP1 family in mammals consists of three distinct yet remarkably conserved non-histone homologs, namely CBX1/HP1 β , CBX3/HP1 γ , and CBX5/HP1 α [28,29]. The chromodomain proteins of HP1 and Polycomb group (PcG) exhibit a significant degree of amino acid sequence similarity, with over 60% identity [30]. The criticality of the CHD of HP1 lies in its association with chromatin, which is facilitated by the specific interaction between the CHD and histone H3 lysine K9 di/trimethylation (H3K9me2/3). The strength of the binding affinity between the CHD and H3K9me2/3 was found to be directly proportional to the higher levels of H3K9me2/3 [31,32]. The carboxyl-terminal region of the HP1 protein family contains a second conserved domain known as the chromo shadow domain (CSD) (Figure 1) [33]. Although the general architecture of the CSD resembles that of the CHD, these domains exhibit distinct functionalities. The CSD functions primarily as a dimeric domain, therefore HP1 proteins readily form homodimers and heterodimers via their CSDs [32,34,35]. As regards CBX3, its principal function is the establishment of heterochromatin, which represents the condensed state of chromatin. Within the chromatin structure, the “co-packed state” corresponding to heterochromatin is associated with gene transcriptional inactivation and/or gene silencing. Transcriptional inactivation is mediated by the binding of the CBX3 protein to regions of DNA that have undergone methylation at histone H3 lysine K9 (H3K9) via a positive feedback loop [36]. CBX3 is able to recognize and bind the H3K9me2 and H3K9me3 marks. Subsequently, these modifications facilitate the recruitment of the H3K9 methyltransferase known as histone-lysine N-methyltransferase SUV39H1 to methylate neighboring H3K9 residues [37]. The diffusion of H3K9me3 marks is concomitant with the recruitment of multiple proteins, which elicit chromatin compaction and transcriptional repression by sequestering genes, rendering them transcriptionally inactive [38–40].

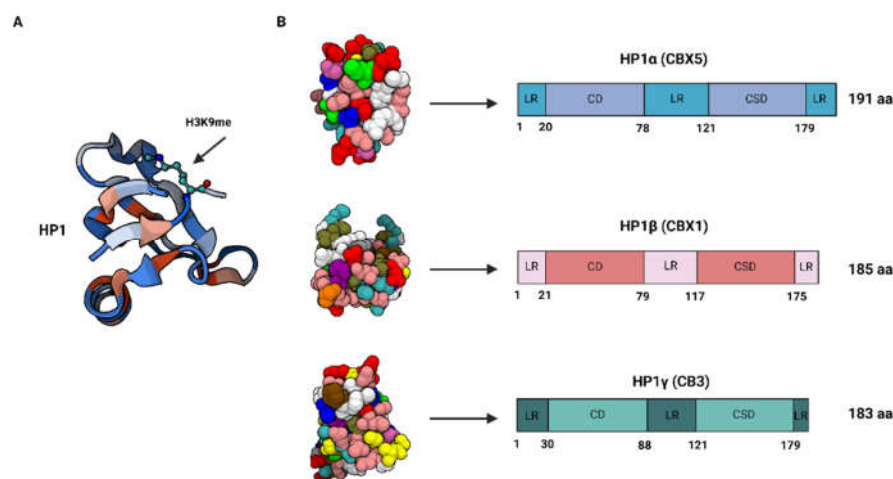


Figure 1. A. Ribbon diagram showing the chromodomain of HP1 complexed with histone H3 tail containing monomethyl lysine 9. B. Crystal structure of the HP1 α , HP1 β , HP1 γ chromo shadow domains (left); schematic representation of HP1 isoform proteins (right). LR = linker region; CD = chromodomain; CSD = chromoshadow domain.

CBX3, rendered as a soluble nuclear protein and HP1 family member, is encoded by the *CBX3* gene and is localized on chromosome 7p15.2 [41]. In addition, at subcellular level, it is localized to the nucleoplasm and nuclear bodies. CBX3 links methylation marks to RNA splicing, DNA repair, and transcriptional silencing resulting involved in various cellular processes, such as gene regulation,

DNA repair, and telomere function [42,43]. Importantly CBX3 is regarded as a multifaceted crystal-structured protein in humans that also has a function in transcriptional inhibition and activation, cell growth and differentiation, and epigenetic modifications [44,45]. The CBX3 chromodomain recognizes and binds with non-histone and histone methylated peptides and, based on comparable affinities, also binds with H1K26, H3K9, and G9aK185 methylated peptides [36]. The binding of the CBX3 chromodomain to methylated histones occurs via a conserved mechanism, which is enhanced by the chromodomain ARKS/T motif, allowing the chromodomain to recognize and specifically bind to methylated histones [43,46]. CBX3 also interacts with non-histone proteins, including PIM1, CBX5, CBX1, Ki-67, and Lamin B receptor, controlling gene expression [43]. CBX3 interacts directly with active genes, particularly within gene bodies, and facilitates the process of transcriptional elongation and RNA processing. It is also involved in recruiting splicing factors to enable efficient co-transcriptional splicing [47]. CBX3 interacts with the E2F1 transcription factor, a key player in regulating the cell cycle. Several studies report that cellular proliferation is enhanced by the increase in E2F1 transcriptional activity mediated by CBX3. E2F1 selectively directs its binding toward genes that encode proteins responsible for the regulation of cell cycle progression during transition from G1 to S phase, including cyclin D1 and CDK4 [48,49]. CBX3 is able to interact with the tumor suppressor protein p53, impeding its transcriptional activity and resulting in apoptosis reduction and a concomitant increase in cell survival. The primary role of p53 under cellular stress, such as oncogenic activation and DNA damage, is to induce apoptosis regulated by pro-apoptotic genes such as BCL2 Associated X, Apoptosis Regulator (*BAX*) and phorbol-12-myristate-13-acetate-induced protein 1 (*PMAIP1*, also known as *NOXA*), as well as transcriptional activation of p53 upregulated modulator of apoptosis (*PUMA*) [50]. CBX3 was found able to indirectly enhance transcriptional activation of genes involved in DNA repair, including *RAD51* and breast cancer gene 1 (*BRCA1*). *RAD51* plays a critical role in repairing DNA double-strand breaks (DSBs) via homologous recombination (HR) [51,52]. A recent study described an association between mutations in the *BRCA1* protein, which has a role in HR repair, and a higher susceptibility to breast and ovarian cancers [53]. The interface between CBX3 and E2F1 enhances the expression of *RAD51* and *BRCA1*, resulting in increased HR repair and resistance to chemotherapy. CBX3 also interacts with other DNA repair proteins, such as PARP1 and Ku70, which participate in the repair of DSBs through non-homologous end joining [54]. Through its interactions, CBX3 plays a crucial role in multiple DNA repair pathways either directly or indirectly by recruiting DNA repair proteins, thereby maintaining genomic stability [55–57].

3. CBX3 as a Multiplayer in Lung Cancer Progression

CBX3 has been found dysregulated showing an abnormal expression profile in various cancers. Expression levels of this gene are increased in several cancer types including gastric, prostate, breast, colorectal, and lung cancers. CBX3 expression is also dysregulated in osteosarcoma and hepatocellular carcinoma [44,54,58,59]. Conversely, expression levels of CBX3 are lower in colorectal cancer low-grade adenomas, hyperplastic and mucosal polyps [54]. Among the three HP1 proteins, CBX3 is the histone reader protein that is highly expressed in lung adenocarcinoma (LUAD). The expression level of messenger RNA (mRNA) encoding CBX3 exhibits a positive correlation with size of tumors, occurrence of lymph node metastasis, and unfavorable prognosis in LUAD patients. Interestingly, the *in vivo* inhibition of CBX3 results in a reduction of tumor size and an extension of the survival period in mice with KRAS^{G12D}-induced LUAD [60]. Increased expression of CBX3 is correlated with an unfavorable prognosis in NSCLC and LUAD [61,62] through mechanisms involving the promotion of tumor proliferation via regulatory pathways of signal transduction affecting the cell cycle, notably G1/S phase transition and the p53 pathway [27]. The prognostic value of CBX3 is further supported by its association with tumor diameter and lymph node metastasis, suggesting its involvement in tumor growth and metastasis [27]. The therapeutic potential of targeting CBX3 in lung cancer is underscored by its overexpression in NSCLC and its association with epigenetic modifications and cell differentiation [44]. The oncogenic role of CBX3 is also highlighted by the observation that CBX3 and H3K9me3 levels are increased in NSCLC tumor-

initiating cells, where they inhibit DNA damage responses to antineoplastic agents [61]. The expression of CBX3 is markedly increased in LUAD tissues of smokers compared to non-smokers and it is also associated with unfavorable prognosis and advanced disease stage [63]. Interestingly, a study show that cigarette smoke causes an increase in CBX3 expression by promoting binding of the transcription factor YBX1 to the CBX3 promoter [44]. High CBX3 protein levels also enhance the growth, invasion, and spread of LUAD cells by controlling the cell cycle progression and activating Rho GTPases [64]. Notably, elevated expression of CBX3 in lung cancers linked to smoking is often caused by genetic changes, such as an increase in the number of copies of the gene, as well as in epigenetic dysregulation [65]. In smokers, it has also been observed that CBX3 interacts with tripartite motif-containing (TRIM) 28, TRIM24, and RBBP4 to create a repressor complex. This complex binds to the Rho GTPase-activating protein 24 (ARHGAP24) promoter and inhibits its transcription. Reducing levels of ARHGAP24 results in the overexpression of active Ras-related C3 botulinum toxin substrate 1 (RAC1) that in turn, triggers signaling pathways (see also Subsection 4.6) promoting the advancement of LUAD [80]. Outside the CBX3/ARHGAP24/RAC1 axis, CBX3 can also facilitate smoking-induced LUAD by inhibiting the tumor suppressor FBP1 and controlling glycolysis [63]. Intriguingly, CBX3 can also enhances the development of lung tumors by suppressing the transcriptional activity of nuclear receptor co-repressor 2 (NCOR2) and zinc finger and BTB domain-containing 7A (ZBTB7A). These transcriptional regulators have an impact on cell proliferation and migration [66]. Further, the expression of CBX3 triggers the development of stem cell-like characteristics in lung tumors, enhancing the presence of markers associated with cancer stem cells and targets of the oncogenic transcription factor c-Myc [67]. Mechanistically, CBX3 plays a crucial role in suppressing target genes through chromatin remodeling, leading to abnormal cell development and the inhibition of differentiation pathways [44,67]. Due to its cancer-causing properties, CBX3 shows potential as a reliable predictive biomarker and a possible target for treatment in NSCLC associated with smoking. Manipulating the expression or activity of CBX3 could potentially limit the development of lung tumors and improve patient prognosis. Studies suggest that exposure to cigarette smoke can lead to specific alterations in the histone organization of lung cells, and that these changes can affect how transcription factors bind to promoters of genes, including CBX3 [63,68]. Although there is no direct evidence to support this hypothesis, it is suggested that the transcription factor NF- κ B, known to be activated by cigarette smoke, can potentially regulate the expression of target genes such as CBX3 [69,70].

The different interactors of CBX3, their molecular mechanisms, and the different pathways involved in lung cancer are listed in **Table 1**.

Table 1. CBX3 interactors, their effects on lung cancer and pathways involvement via differential mechanisms of action.

Interactor	Type of Interaction	Effect on Lung Cancer	Pathway Involvement	Mechanism of Action
EGFR/RAC1	Genetic Interaction	Co-amplification with CBX3 is associated with lung adenocarcinoma proliferation and poor prognosis	Not Specified	Increase in CBX3 mRNA leads to increased EGFR/RAC1 protein levels, promoting cancer cell proliferation [71]
CDK6/P21	Transcriptional Regulation	CBX3 inhibits transcription of negative cell cycle regulators, promoting colorectal cancer cell proliferation; similar mechanisms may be involved in lung cancer	Cell Cycle Regulation	CBX3 is able to inhibit transcription of CDK6 and p21, promoting cell proliferation [72]

CBX Molecular Family (CBX1/2/3/5/7)	Gene Expression	CBX3/5 expression is associated with poor prognosis in lung adenocarcinoma, while CBX7 shows the opposite effect	Tumorigenesis and Immune Infiltration	Differential expression of CBX family members affects tumor progression and immune response [65]
PI3K/AKT Pathway	Activation	Although the study is on renal carcinoma, similar activation by CBX3 may occur in lung cancer, promoting metastasis and invasion	PI3K/AKT Pathway	CBX3 promotes cancer progression through PI3K/AKT activation, which regulates cell metastasis and invasion [73]
ARHGAP24	Suppression	Smoking-associated upregulation of CBX3 suppresses ARHGAP24, activating RAC1 signaling and promoting tumor progression in lung adenocarcinoma	RAC1 Signaling	CBX3 overexpression leads to suppression of ARHGAP24, activating RAC1 and promoting tumor progression [63]
NCOR2	Regulation	In ovarian cancer, CBX3 inhibits NCOR2, affecting p53/p21-mediated glucose metabolism; similar effects may occur in lung cancer	Glucose Metabolism	CBX3 inhibits NCOR2, affecting p53/p21-mediated pathways and potentially promoting cancer metabolism [74]
Immune System	Immunological Biomarker	CBX3 expression is related to immune cell infiltration and may serve as an immunological and prognostic biomarker in various cancers, including lung cancer	Immune Response	CBX3 expression influences immune cell infiltration and tumor immunity, which varies based on tumor type [72]
Transcriptome/Metabolome	Biomarker Association	Possible association between CBX3 expression and transcriptome/metabolome changes in cancers, including lung cancer	Various Pathways	CBX3 expression may be linked to changes in the transcriptome and metabolome, affecting multiple cancer-related pathways [75]

4. Involvement of CBX3 in Pathways Leading to Lung Cancer

Molecular pathways involved in the regulation of cell cycle, differentiation, death, and signaling are known to be altered in processes of tumorigenesis. To date, major efforts have been made to discover new cancer driver genes and unravel the molecular mechanisms in which they are involved by combining scientific data, such as multi-omics data, and knowledge obtained from the literature. For example, therapies based on molecular targets have transformed anticancer treatment approaches through personalized and/or precision medicine strategies. Based on this premise, CBX3 has been found implicated in a broad spectrum of human cancers, including NSCLC [27,71]. Remarkably, CBX3 is involved in several signaling pathways, critical for cell survival, proliferation, and differentiation, and plays a dominant role in lung cancer. However, further research is needed to better elucidate these pathways and deeper explore the potential of CBX3 as a therapeutic target.

The following subsections describe the role of CBX3 in key cancer-associated pathways aim to shedding light its mechanistic role in lung cancer progression. The involvement of CBX3 in crucial lung cancer networks is schematically illustrated in **Figure 2**.

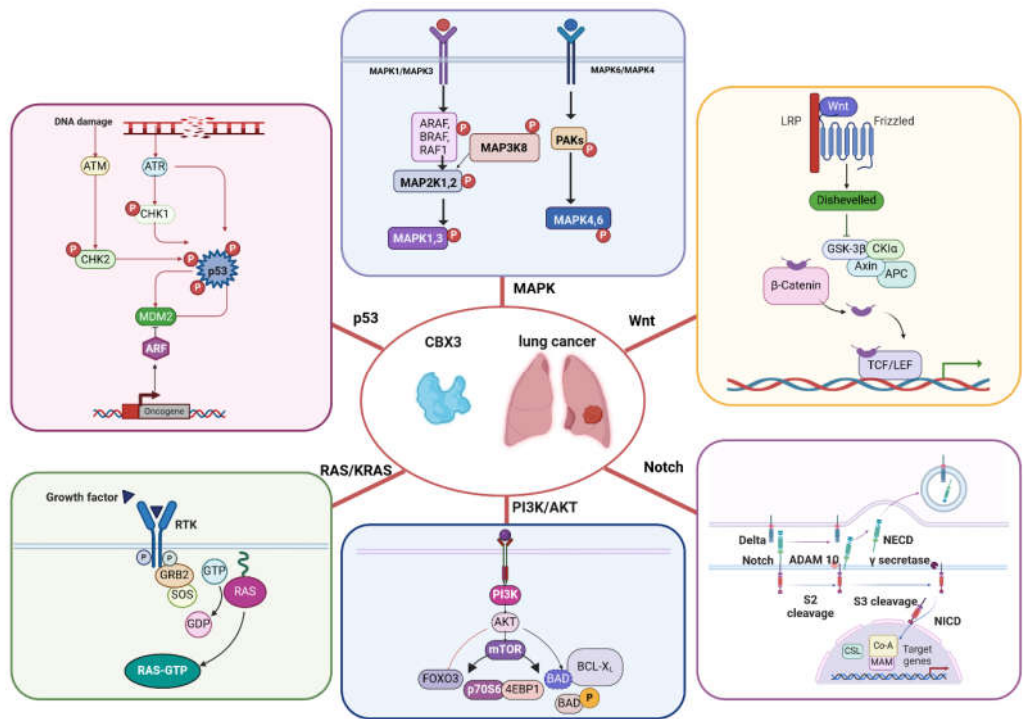


Figure 2. Cellular pathways directly or indirectly modulated by CBX3 in lung cancer.

4.1. Role of CBX3 in PI3K-AKT and (K)Ras Signaling Pathways

Recent studies describe a significant connection between CBX3 and the activation of the PI3K-AKT pathway. This pathway regulates essential cellular processes such as growth, survival, and metabolism [73]. The aberrant activation of the PI3K-AKT pathway is a well-known characteristic of tumor development. In this context, CBX3 was found to play a role in PI3K-AKT dysregulation by facilitating the phosphorylation and subsequent activation of AKT. CBX3 may act as an oncogenic driver and is potentially involved in the Ras signaling pathway, one of the most crucial molecular mechanisms inducing oncogenic transformation. Enrichment analysis seems to support this hypothesis, but further research is needed to establish the specific role of CBX3 in this pathway in lung cancer [76]. A functional relationship is known to exist between CBX3 and EGFR or RAC1 in different human cancers [71], potentially impacting these signaling pathways. CBX3 has a significant effect on the KRAS signaling pathway in lung cancer. Specifically, CBX3 preferentially interacts with EZH2 triggering the transcription inhibition of microRNAs (miRNAs) such as let-7b, miR-31, and miR-128b. This results in the upregulation of target genes, including *KRAS* and *MYC*, that stimulate the growth and survival of tumor cells [77]. CBX3 also promotes oncogenic KRAS signaling, activating downstream effector pathways such as MAPK/ERK and PI3K/AKT. This activation enhances cancer cell proliferation, invasion, and metastasis [78]. Of note, a strong association is known to exist between elevated CBX3 levels and unfavorable prognosis in individuals with LUAD harboring *KRAS* mutations [79]. These findings highlight the potential of CBX3 as both a prognostic biomarker and a therapeutic target. Its association with these critical oncogenic signaling pathways further supports the importance of gaining a better insight into its role in cancer progression [72].

4.2. Role of CBX3 in Notch Signaling Pathway

In the context of lung cancer, the involvement of CBX3 in the Notch signaling pathway is intricate and diverse, underscoring the complex interplay between chromatin organization and signaling pathways in cancer progression [80]. The Notch signaling pathway is a crucial cell communication system in determining cell fate. CBX3 is able to interfere with the functioning of this pathway, and its effect may vary depending on the specific cellular environment and cancer type [81]. The Notch signaling pathway is highly conserved and involves the interaction of Notch receptors with their ligands, leading to cleavage of the Notch intracellular domain (NICD) and its translocation to the nucleus, where it influences gene expression [82,83]. However, the involvement of CBX3 in lung cancer, and particularly its relationship with the Notch signaling pathway, remains relatively understudied and seems to present a more complex scenario. CBX3 directly interacts with the NICD, recruiting the co-repressor complex, including histone deacetylases and DNA methyltransferases, to the promoter regions of Notch3 target genes, such as *HES1* and *HEY1* [84]. This interaction leads to the epigenetic silencing of these genes through increased histone deacetylation and DNA methylation, ultimately resulting in the downregulation of the entire signaling pathway [84]. Thus, the chromatin remodeling may arise due to the ability of CBX3 to modify histones and change the chromatin state, consequently affecting the accessibility of Notch-responsive elements in the genome. In addition, since CBX3 has been linked to epigenetic changes such as H3K9me3, which plays a role in regulating the response to DNA damage, we speculate that it could potentially contribute to the resistance of tumor-initiating cells in NSCLC to antineoplastic drugs. This specific resistance is potentially conferred due to CBX3 binding to H3K9me3, resulting in the formation of transcriptionally repressive chromatin environments that can lead to the silencing of tumor suppressor genes in drug metabolism and efflux, contributing to drug resistance [61,85]. Interestingly, CBX3 expression has also been correlated to immune-related function regulation, also regulated by Notch signaling [86]. These interactions could additionally modulate the tumor microenvironment, supporting tumor growth as well as resistance to various therapies. In the case of NSCLC, CBX3 might indirectly favor drug resistance by influencing immune evasion and immune cell infiltration mechanisms [72].

4.3. Role of CBX3 in Wnt Pathway

CBX3 regulates the Wnt/ β -catenin signaling pathway, essential for cell proliferation, differentiation, and tumorigenesis in several types of cancer, including lung cancer [87]. The mode of action of CBX3 in lung cancer involves its function as a transcriptional regulator. CBX3 is able to bind gene promoters and influence gene expression. A recent study showed that CBX3 plays a crucial role in the transcriptional regulation of non-structural maintenance of chromosomes condensin I complex subunit G (*NCAPG*). This regulation, in turn, leads to activation of the Wnt/ β -catenin signaling pathway. In colorectal cancer, activation of this pathway promotes cell proliferation and cell cycle progression, while inhibiting apoptosis. This mechanism likely operates in a similar manner in lung cancer, as the pathways involved in tumor formation are shared [88]. CBX3 overexpression has been linked to the advancement of lung adenocarcinoma via activation of the RAC1 pathway, a component of the Wnt signaling network [71,72]. Previous studies found a connection between CBX3 and cell cycle control [89]. Specifically, CBX3 was found to reduce the transition from G1 to S phase by influencing the activity of p21 [64,90]. This, in turn, may contribute to the growth of tumors. It is also reported that CBX3 might have an impact on tumor growth by engaging with cell cycle regulators [91,92]. To summarize, CBX3 functions as a transcriptional regulator in the Wnt/ β -catenin signaling pathway, impacting the advancement of lung cancer by controlling gene expression, facilitating cell cycle progression, and interacting with pathways such as RAC1. In this context, the overexpression of CBX3 gene is associated with a negative outlook, suggesting its potential role as a target for treatment and a useful indicator of lung cancer prognosis [64,88].

4.4. Role of CBX3 in p53 Pathway

The tumor suppressor p53, a transcription factor responsible for initiating cell cycle arrest, apoptosis, and DNA repair when cells undergo stress, is involved in the multiple mechanisms that

provide evidence supporting the role of CBX3 in lung cancer progression [93]. p53 activity is typically suppressed in normal cells by specific degradation regulated by the E3 ubiquitin ligase mouse double minute 2 homolog (MDM2) [93]. MDM2 primarily targets p53 via proteasomal degradation. In addition, its auto-ubiquitination activity does not directly affect MDM2 itself, but rather its interactions with other proteins [94]. It has been observed that CBX3 interacts with and potentially enhances the stability of MDM2 in lung cancer cells, inhibiting its auto-ubiquitination. However, the precise mechanism by which CBX3 enhances the stability of MDM2 is still unknown, and more research is required to elucidate this interaction [44,94]. CBX3 facilitates the survival and growth of lung cancer cells by indirectly impeding the activity of p53 through the MDM2 axis [95]. Additionally, CBX3 can directly suppress the expression of p53 target genes responsible for inhibiting growth, regardless of its impact on p53 protein levels [96]. Collectively, these findings show that CBX3 is able to bypass the tumor-suppressing effects of p53 and promote the development of lung tumors [97]. Thus, targeting the CBX3-MDM2-p53 pathway could potentially offer a novel therapeutic approach to restore the functionality of p53 in lung cancer.

4.5. Role of CBX3 in ErbB Pathway

The ErbB signaling pathway is crucial in controlling numerous cellular processes, such as differentiation, proliferation, migration, adhesion, and apoptosis [98]. This pathway is activated by EGF-like growth factor ligands' attachment to the outer part of ErbB receptors [99]. This causes the receptors to form dimers, which can be either homodimers or heterodimers with other members of the same family. The ErbB family consists of four members: ErbB1 (HER1), ErbB2 (HER2), ErbB3 (HER3), and ErbB4 (HER4) [100]. When the receptors dimerize, their inherent tyrosine kinase activity is triggered, leading to autophosphorylation and activating the downstream signaling cascades, including the MAPK, Akt, and JAK/STAT pathways [99,100]. Although the precise mechanism by which CBX3 contributes to lung cancer is far to be completely elucidated, some evidence points to its potential interaction with EGFR signaling. Intriguingly, a co-occurrence of positive CBX3 expression and EGFR mutations was found in NSCLC samples [62]. However, in the same study, the expression of CBX3 remained unaltered in EGFR mutant NSCLC cell lines treated with the EGFR inhibitor gefitinib [101], suggesting that the downstream signaling of EGFR may not influence expression of CBX3 [98]. CBX3 is overexpressed in NSCLC and is associated with an unfavorable prognosis. Nonetheless, further investigation is required to clarify its exact function in interacting with the ErbB/EGFR pathway during the advancement of lung cancer. Specifically, future studies should focus on determining whether CBX3 governs the expression of ErbB pathway members via epigenetic regulation, investigating the impact of EGFR signaling on CBX3 activity, and elucidating the mechanisms by which CBX3-mediated gene regulation enhances lung cancer development and survival.

4.6. Role of CBX3 in MAPK Pathway

The MAPK/ERK pathway is a crucial signaling cascade that regulates cellular growth and survival [63]. CBX3 is able to inhibit transcription of the *ARHGAP24* gene by interacting with H3K9me3 and binding to proteins that modify the structure of chromatin, such as TRIM28 and TRIM24, at the *ARHGAP24* promoter. As a result, the expression of *ARHGAP24* is suppressed, leading to an increase in RAC1 activity. Subsequently, RAC1 stimulates receptor tyrosine kinases on the cell membrane, propagating signals through the conventional MAPK cascade involving MEK1/2, ERK1/2, Ras, and Raf. Ultimately, activated ERK1/2 migrates to the nucleus and phosphorylates many transcription factors that control the proliferation and survival of cancer cells [102]. CBX3 also impacts other pathways such as PI3K/AKT and Ras signaling, which converge on MAPK. For example, AKT is able to phosphorylate and inhibit Raf proteins, providing crosstalk between these pathways. Ras functions directly upstream of Raf in the MAPK cascade [102]. In conclusion, targeting the CBX3-MAPK network may represent a potential treatment strategy for controlling lung cancer caused by an overactive CBX3 and MAPK pathway.

5. Conclusions

Lung cancer is one of the leading causes of cancer-related deaths in industrialized countries due to the extremely variable contributing factors such as detrimental lifestyle behavior, including smoking [103]. Therefore, aberration of epigenetic mechanism significantly impacts on gene expression and divert cellular signaling pathways that are crucial for coordinating the correct growth, regulation, and functioning of cells. In lung cancer, and especially in NSCLC, CBX3 appears to act primarily as an oncoprotein, promoting tumor growth and progression. Multiple studies show that CBX3 is overexpressed in NSCLC [104] and correlates with poor prognosis [27]. Mechanistically, CBX3 interacts with key oncogenic pathways such as PI3K/AKT, Ras, Wnt/ β -catenin, and p53, thereby stimulating proliferation, inhibiting apoptosis, and increasing therapeutic resistance [81]. It may also contribute to a stem cell-like phenotype in lung cancer cells [105]. The upregulation of CBX3 in smoking-associated LUAD and its inhibition of tumor suppressors such as ARHGAP24 and NCOR2/ZBTB7A provide further evidence for its oncogenic role [63]. Carefully designed preclinical studies are essential before CBX3-based therapies can progress to the clinic. Additional mechanistic and functional studies of CBX3 hold the promise of uncovering new insights into disease pathogenesis and unveiling novel therapeutic opportunities. Thanks to the spread of increasingly advanced technologies such as those based on NGS techniques, it will be possible to extend studies aimed at clarifying the complex mechanisms that underlie the functioning of CBX3. Such studies could be particularly useful in the context of lung cancer, providing a more personalized molecular signature in high-risk patients.

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Abbreviations

ARHGAP24	rho GTPase Activating Protein 24
BAX	bcl2-associated X protein
BRCA1	breast cancer gene 1
CBX	chromobox
CHD	chromodomain
CSD	chromoshadow domain
DSBs	DNA double-strand breaks
DSBs	double-strand breaks
EGFR	epidermal growth factor receptor
EZH1/2	enhancer of zeste homologous 1/2
HR	homologous recombination
LUAD	lung adenocarcinoma

MDM2	mouse double minute 2 homolog
mRNA	messenger RNA
SMC	structural maintenance of chromosomes
NCAPG	non-SMC Condensin I Complex Subunit G
NICD	notch intracellular domain
NSCLC	non-small cell lung cancer
PI3K	phosphoinositide 3-kinase
PMAIP1	phorbol-12-myristate-13-acetate-induced protein 1
PRC1	polycomb (Pc) inhibitory complex 1
PRC2	polycomb Repressive Complex 2
PUMA p53	upregulated modulator of apoptosis
RAC1	active Ras-related C3 botulinum toxin substrate 1
RBBP4/7	retinoblastoma binding protein 4/7
STAT3	signal transducer and activator of transcription 3
TRIM	tripartite motif-containing
ZBTB7A	zinc finger and BTB domain containing 7A

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