

# Unraveling the Post-Translational Modifications and therapeutical approach in NSCLC pathogenesis

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## ABSTRACT

Non-Small Cell Lung Cancer (NSCLC) is the most prevalent kind of lung cancer with around 85% of total lung cancer cases. Despite vast therapies being available, the survival rate is low (5 year survival rate is 15%) making it essential to comprehend the mechanism for NSCLC cell survival and progression. The plethora of evidences suggests that the Post Translational Modification (PTM) such as phosphorylation, methylation, acetylation, glycosylation, ubiquitination and SUMOylation are involved in various types of cancer progression and metastasis including NSCLC. Indeed, an in-depth understanding of PTM associated with NSCLC biology will provide novel therapeutic targets and insight into the current sophisticated therapeutic paradigm. Herein, we reviewed the key PTMs, epigenetic modulation, PTMs crosstalk along with proteogenomics to analyze PTMs in NSCLC and also, highlighted how epi-miRNA, miRNA and PTM inhibitors are key modulators and serve as promising therapeutics.

## Abbreviations

NSCLC	Non-small cell lung cancer
PTM	Post-Translational Modification
NFκB	Nuclear factor-κB
BRAF	v-ras murine sarcoma viral oncogene homolog B1
ALK	Anaplastic lymphoma kinase
FOXO	Forkhead box O
GALNT2	Polypeptide N-Acetylgalactosaminyltransferase 2
HK2	Hexokinase 2
UBA2	Ubiquitin like modifier activating enzyme 2
AKT	Ak Strain Transforming/ Protein Kinase B
miRNA	MicroRNA
PDL1	Programmed Death Ligand 1
MTORC1	Mammalian target of rapamycin complex 1
EZH2	Enhancer of zeste homolog 2
MTUS1	Microtubule Associated Scaffold Protein 1
DAL-1	Differentially expressed in adenocarcinoma of the lung-1
SIRT1	Sirtuin1
PIASy	Protein Inhibitor of Activated STAT Y
Ubc9	Ubiquitin conjugating enzyme (E2)
ITGB3	Integrin Subunit Beta 3
MAP4K3	Mitogen-Activated Protein Kinase 3

HDAC1	Histone deacetylase 1
PFK1	Phosphofructokinase 1
DNMT3A	DNA (cytosine-5)-methyltransferase 3A
TIP60	60 KDa Tat-interactive protein
KRAS	Kristan rat sarcoma virus
TGIF2	TGF-β-induced factor homeobox 2
HMGCL	3-hydroxy-3-methylglutaryl-CoA lyase

## Introduction

Worldwide cancer is a major driver to mortality and a significant impediment to raising life expectancy. According to World Health Organisation (WHO) projection from 2019, the first and second most prevalent cause of death is cancer before 70 years of age in 112 of 183 countries. From the GLOBOCAN 2020, globally Lung cancer is the second most diagnosed cancer accounting for 11.4% and with an anticipated 1.8 million deaths (18%), lung cancer remained the most common type of cancer related death [1]. Approximately 85% of new lung cancer diagnoses are for non-small-cell lung cancer (NSCLC). Adenocarcinoma, squamous cell carcinoma, and large cell carcinoma are the three main histological subtypes of NSCLC [2]. Adenocarcinoma accounts for 40% and mostly found in lung's periphery, squamous cell carcinoma comprises around 25–30%, occurs in center of lung while large cell

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carcinoma comprises only 5–10%, mostly begins in the middle of the lungs, occasionally into surrounding lymph nodes, the chest wall, and distant organs [3]. Although therapies such as surgery, targeted therapy and immunotherapy are available, the development of drug resistance and the overall survival of NSCLC patients are poor. As a result, for investigating new possible therapeutic targets, it is necessary to deeply understand and comprehensively study the molecular mechanism involved in NSCLC.

Post-translational modifications (PTMs) are crucial for regulating the function of protein. Protein's physical and chemical characteristics, conformation, binding capability, and function are directly altered by the covalent binding of small molecular groups to their amino-acid side chains, which controls post-translational gene expression. It is reckoned that in the human body PTMs occur approximately in 50–90% of proteins. It is reported that PTMs are greater than 400 different types, and they influence many facets of how proteins work [4]. The same protein may undergo various PTMs, and PTMs involving various amino acid residues on the same protein are likely to have various consequences. The flexibility and diversity of functional control of proteins to take part in dynamic activities is substantially increased by the PTMs of different proteins [5]. Based on their functional specificity, PTM enzymes can be categorized into three subtypes; writers, which are responsible for adding the modifications to the substrates while Erasers, removes it and readers, recognizes and binds to modified protein to execute associated biological function [6]. It is notable that PTMs play a significant role in various cancer progression and immunological modulation, rendering them as key targets for the early identification and therapeutics for cancer treatment [7,8]. The PTMs can be enzymatic or non-enzymatic, depending on their biological function [9]. Enzymatic PTMs include phosphorylation, methylation acetylation, glycosylation, ubiquitination, SUMOylation, ADP-ribosylation, citrullination while

non-enzymatic PTMs include glycation, oxidation. PTMs have a significant impact on different hallmarks of cancer [10,11]. The different PTMs and its consequences on various processes of cancer were shown in Fig. 1.

It is also revealed that tumor microenvironment regulated by different PTMs. They also involved in crosstalk between TME and tumor cell including lung cancer cell and various immune cells [12]. It is noteworthy to investigate PTMs in NSCLC for novel therapeutic approaches as they are involved in various processes in cancer progression. Several recent studies have shown the importance of PTM. The recent literature suggested antigens that have undergone PTM give a new source of specificities for immune responses and their findings underline PTM-driven antigenicity principles that may have wide-ranging effects on T cell-mediated treatments for cancer and other diseases [13]. The abnormal activation of NLRP3 inflammasome has linked to numerous disease and different mechanism involved for its control regulation, including PTMs such as ubiquitination, phosphorylation which regulates NLRP3 inflammasome [14]. Considering impact of PTMs on functioning of proteins of several pathways, our review mainly focuses on PTMs which are implicated in NSCLC progression, the crosstalk between different PTMs and therapies such as inhibitors targeting PTMs. Further we have discussed how the miRNAs modulates PTMs specifically in NSCLC.

### Post translational modification

Recent numerous studies have uncovered important layers of regulation governing metabolic and cell signaling pathways, which PTMs influence in cancer. Herewith we are discussing key PTMs in NSCLC such as phosphorylation, glycosylation, SUMOylation, ubiquitination, acetylation and methylation. Their general mechanism or processes are

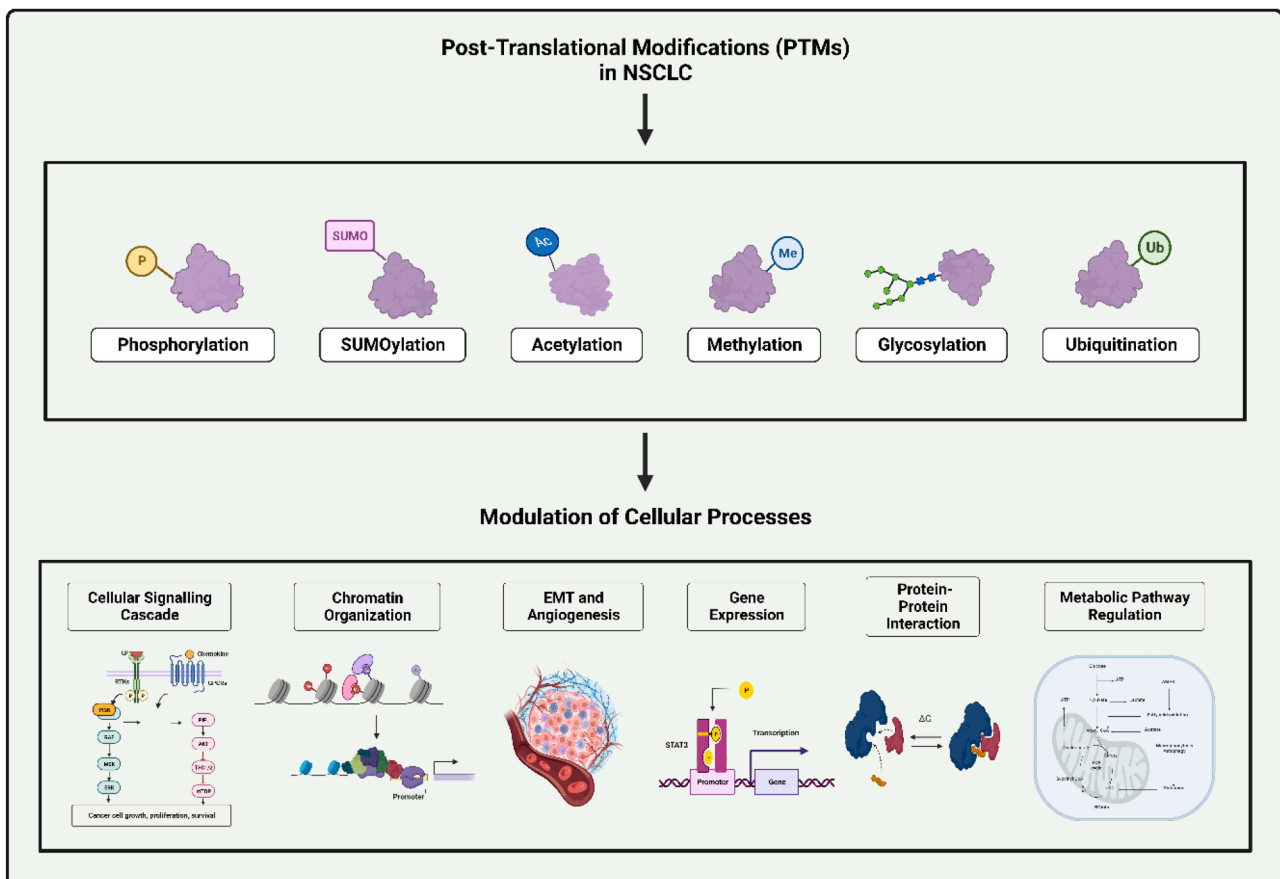


Fig. 1. Post-translational modification in NSCLC and its effect on cellular processes.

shown in Fig. 2.

### Phosphorylation

Protein phosphorylation is the most prevalent and significant PTM process. Almost all cellular activities, including cell division, protein breakdown, signal transduction, gene expression control, and protein interaction, are phosphorylated, making up around 30% of the human

proteome. Nevertheless, alterations at the region of protein phosphorylation have the potential to cause and promote the growth of a malignant tumor. As enzymes are proteins, faulty enzymes may result in aberrant cell life processes, which could therefore promote the development of cancer. Many phosphorylation pathways, such as MAPK, PI3K/Akt, tyrosine kinase, the cadherin-catenin complex, cyclin-dependent kinase, the NF-kappaB and IkappaB proteins, TGF-signaling, etc., are crucial in the development of cancer [15,16].

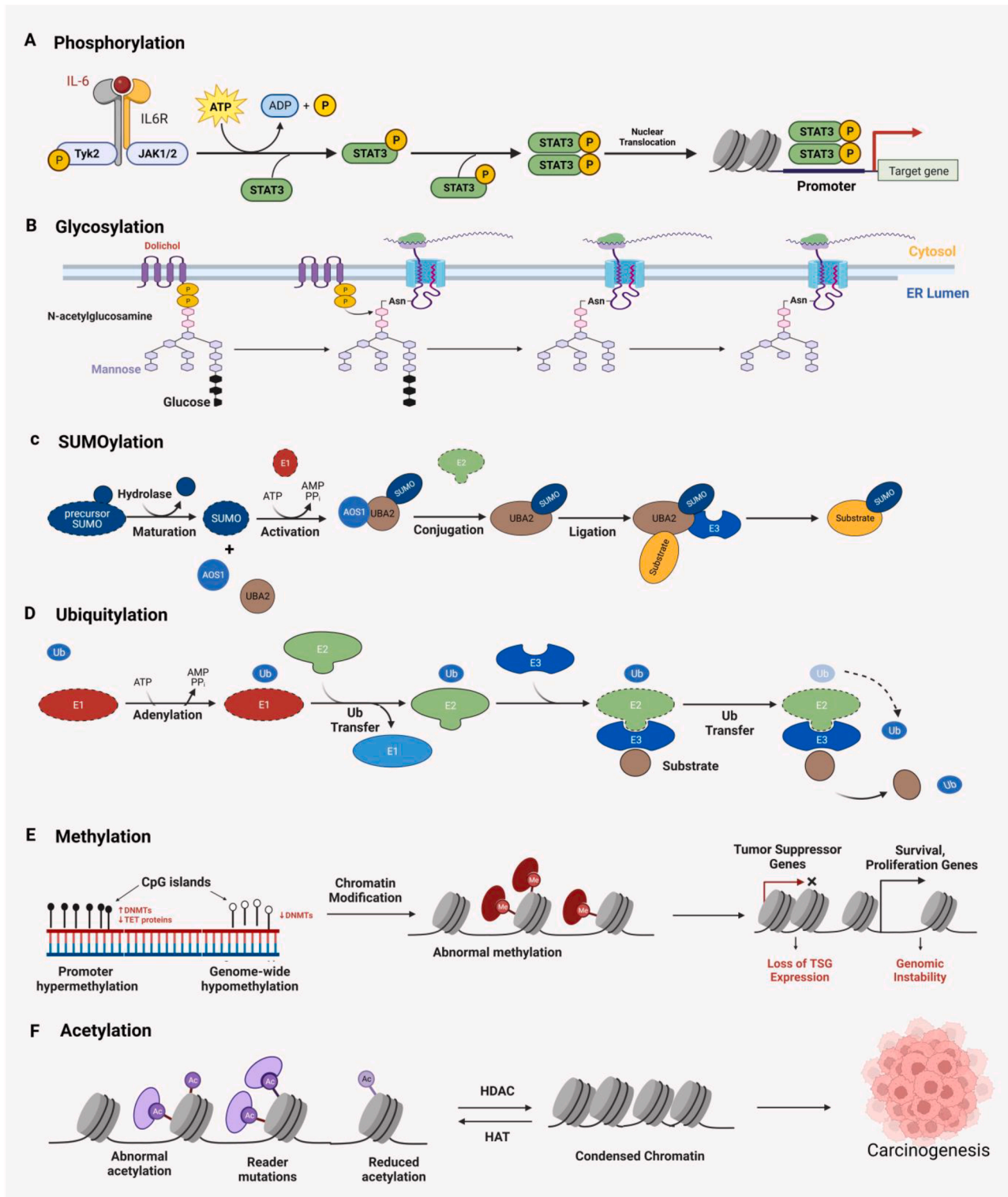


Fig. 2. The mechanism of different post translational modification (PTM).

Several studies have shown that the PI3K-Akt-mTOR pathway is abnormally activated often in NSCLC, with phosphorylated Akt being overexpressed by 50–70% causing anomalous cell proliferation. This phosphorylated AKT further activates downstream signaling such as mTOR, MDM2. Homeostasis has been maintained via phosphates such as PTEN and PHLPP which convert PIP3 to PIP2 and dephosphorylates AKT respectively [17]. Significant percentages of NSCLC patients have also been shown to exhibit upregulated mTOR pathway. S6K and 4E-BP1, which are present downstream of mTOR activation, have also been found in up to 58% and 25% of NSCLC specimens, respectively, with adenocarcinoma having a higher incidence [18]. Inhibiting FOXO1, FOXO3, and FOXO4, which could facilitate the transition from the G1 phase to the S phase, AKT plays a crucial part in interrupting the inhibitory process that prevents progression of cell cycle [19]. Many phosphorylation and dephosphorylation events that either activate or inhibit kinase activity modulate the cyclin/CDK complex [20]. Cyclin B1 is found in high expression in NSCLC. The WEE1 kinases control the phosphorylation process, which in turn regulates cdc2 activity. However, lung cancers have been found to lower the expression of WEE1 which promotes its progression [21]. The study suggests phosphorylation of nucleolin promoted A549 cells proliferation and migration [22]. Moreover, the study showed that pristimerin administration greatly inhibited the phosphorylation of VEGFR2, Akt, and Erk by Sonic hedgehog in endothelial cells. Similarly, administration of pristimerin in NCI-H1299 xenografts inhibits angiogenesis [23]. RKIP is lower in NSCLC patients, which causes STAT3 phosphorylation leading to A549 cells and NCI-H1299 metastasis [24]. Both in vitro and in vivo, the proliferation, migration, and metastasis of lung cancer A549 cells were stimulated by phosphorylation at S308 and S310 in CAP1, while dephosphorylation showed opposite results. Also, The EMT can be facilitated by CAP1 phosphorylation [25]. The recent study demonstrates the new role of TGIF2 phosphorylation in LUAD which promotes EMT and metastasis and these p-TGIF2 may be a possible therapeutic target to prevent LUAD metastasis [26]. The IKK $\beta$  phosphorylates HMGL leading to its degradation through NEDD4 in lung cancer [27].

### Glycosylation

Almost half of all protein modifications, or PTMs, are caused by glycosylation. It is crucial for controlling cellular activities such as protein transport and structure, cell metabolism, and extracellular connections. Glycosylation is the process whereby glycosyltransferases link sugar moieties or glycans, to certain proteins. There are different kinds of glycosylation; N-glycosylation and O-glycosylation are the two most prevalent types. Due to the variety of glycan structures and their glycosylation sites, this PTM is one of the most complex PTMs [28]. GALNTs modulates the early stages of O-glycosylation of mucins. Many solid tumors including NSCLC showed dysregulated GALNT2 expression. The upregulated GALNT2 expression is associated with a lower chance of recovery in NSCLC patients. This is shown to be essential for proliferation, migration and invasion of NSCLC cells (A549, H1975) in both loss-of-function and gain-of-function assays. It was also able to control apoptosis and the course of the cell cycle. Furthermore, GALNT2 promoted tumorigenesis in BALB/c nude mice [29]. The majority of transmembrane proteins are altered by N-glycosylation, and it has long been understood how crucial this modification is for cancer cells to metastasize. The finding suggests that at Asn291, TIM-4 showed N-glycosylation modification contributing towards NSCLC metastasis in vitro and in vivo [30]. The study provides evidence about significantly different levels of protein abundance and glycosylation of proteins involved migration and resistance to drugs using combined proteomic and N-glycoproteomic techniques to thoroughly describe cisplatin resistance in NSCLC cells related membrane proteins. Enhancing the high-mannose or sialylated form of glycosylation increased resistance to cisplatin, whilst inhibiting N-glycosylation decreased resistance, indicating that a crucial glycosylation type correlates to cisplatin resistance

[31]. The O-GlcNAcylation is modulate by two enzymes namely OGT (O-GlcNAc transferase) and OGA (O-GlcNAcase). Weng mi et al. demonstrated using immunohistochemistry that OGT expression is higher in lung tumor. Further findings from this study showed that inhibition of OGT enzyme in NSCLC cell lines decreases O-GlcNAcylation. This decrease significantly affects two hallmarks in nslc such as invasion and anchorage independent growth, these findings collectively imply that O-GlcNAcylation may contribute to the growth of lung cancer [32]. Certain glycolytic enzymes may be O-GlcNAc glycosylated, which could help control glycolysis and which in lung cancer cause distinct metabolic states. The study showed increasing O-GlcNAc levels in human NSCLC cell lines greatly reduces the activity of phosphofructokinase 1 (PFK1), a key regulatory enzyme that regulates the flux along the glycolytic pathway [33]. Higher expression of HK2 is observed in cancer cells and it is also found to contribute to tumorigenesis in kras-driven lung mice models while silencing HK2 reverses the results both in vivo and in vitro [34]. Identification of N-linked glycosylation of proteins also provide new approach as biomarker. The small extracellular vesicles with N-glycosylation in secretome of lung cancer reflects lung cancer histology [35].

### SUMOylation

SUMOylation is a PTM in which a target protein links to a small ubiquitin-like modifier (SUMO) covalently and permanently. The four different isoforms that have been identified in mammals are SUMO1, 2, 3, and 4. These 12 kDa proteins have a three-dimensional structure that is similar to ubiquitin. Unlike ubiquitin, SUMO proteins have a 10–25 amino acid tail in their N-terminal domains [36–38]. SUMOylation is catalysed by an enzyme cascade that is comparable to that of protein ubiquitination. The C-terminal glycine of SUMO and Cys173 of UBA2 form a thioester bond under the guidance of the SUMO-activating enzyme SAE, a heterodimer made up of SAE1 and UBA2. This thioester link initiates the SUMOylation cascade. SUMO then moves to UBC9, the only E2 enzyme it possesses, where it catalyses cysteine residues. With the aid of SUMO E3 ligases, UBC9 facilitates the formation of an isopeptide link between SUMO and a target lysine on the substrate [39–42]. The balance between SUMOylation and deSUMOylation is crucial for cell proliferation and genome stability. The deSUMOylation is governed by specific SUMO-specific proteases (SENP). SENPs includes SENP1, SENP2, SENP3, SENP5, SENP6 and SENP7, for every SENP there are specified SUMO proteins [43]. The SUMOylation process is vitally implicated in carcinogenesis, certainly in many cancers the abnormality in SUMOylation enzymes and also in target proteins SUMOylation linked to different cancers [44].

The progression of various cancers, including NSCLC, is caused by SUMO1. Around 74.23% NSCLC patients showed higher expression of SUMO1 correlated with poor prognosis and metastasis in NSCLC [45]. The SENP2 are found to be upregulated in lung cancer and associated with poor patient survival [46]. The activation of SIRT1 block EMT in A549 cells injected nude mice, but the hypoxia inhibits SIRT1 transcription through SUMOylation, while inhibition of SUMOylation could prevent lung cancer metastasis [47]. Similarly, Pei Fang Hung et al., reported that hypoxia induces slug SUMOylation via interacting with Ubc9 and PIASy causing slug's transcriptional repression activity by engaging HDAC1 ultimately affecting its subsequent slug target genes and promoting metastasis in lung cancer [48]. The findings suggest that PIAS3 depletion contributes to the pathology of squamous lung cancer and suggest that increasing PIAS3 expression may be used as a single treatment target to reduce signaling from the several receptor of RTK that have been discovered to be increased in squamous lung cancer [49].

The CBX4 is a SUMO E3 ligase which governs the BMI-1 sumoylation, contributing to metastasis as well as proliferation in the xenograft mice model of NCI-H460 and A549 cells [50].

## Ubiquitination

The ubiquitination is a complex and tightly controlled process which involves the attachment of ubiquitin to substrate protein at one or more lysine residues. The process is regulated by three enzymes namely E1, E2 and E3. E1 is ubiquitin activating enzymes, E2 is ubiquitin conjugating enzymes and E3 is ubiquitin ligases. The ubiquitinated protein is introduced into the 26S proteasome where it breaks down into small peptides which are then subsequently degraded by the lysosome to produce free amino acids. In addition to ubiquitination, deubiquitination involves removing ubiquitin from the target protein, also impacting ubiquitinated proteins levels in a cell. A group of enzymes namely deubiquitinating enzymes (DUBs) regulate the tightly controlled process of deubiquitination [51]. By controlling the activity as well as degradation of proteins that promote or repress tumor growth, ubiquitination has an important function in tumorigenesis. Due to ubiquitination's role in various cellular functions, failing to maintain strict control of it promotes cancer progression. Cell cycle transition is primarily driven by CDKs, and their activities are regulated by the cyclins, CDK inhibitors, also other kinases and phosphatases ubiquitination [52]. Mono-ubiquitination or poly-ubiquitination means the association of a single ubiquitin or poly ubiquitin chain to the target protein respectively.

Ubiquitin/proteasome system (UPS) is a machinery used for protein degradation, regulates various processes in cells. Yiting Tang et al. investigated the scenario of ubiquitin in NSCLC, observed upregulated ubiquitin in around 62 NSCLC patients, further its knockdown in H1299 cells reduces proliferation and increases radiosensitivity of H1299 cells. Similar results observed in xenograft BALB/c nude mice [53]. One of the members of the USP family is USP10, particularly targeting this may increase susceptibility of NSCLC patients with mutant P53 to the platinum based therapy [54]. The EGFR-driven progression of NSCLC is suppressed by the F-box protein FBXL2 (E3 ubiquitin ligase), which specifically targets EGFR and also EGFR TKI-resistant mutations for proteasome-mediated destruction [55]. Siah1 is a ubiquitin ligase which stabilizes Notch through ubiquitination, causing AKT phosphorylation leading to A549 and H1299 proliferation [56]. The FBXO22 ubiquitinates LKB1 at lys63 residue implicated in inhibition of LKB1, further both in vivo and in vitro study showed growth of NSCLC via overexpressing FBXO22 [57]. Studies suggest that DUBs regulate the cell cycle progression in NSCLC via stabilizing cyclin A through deubiquitination. Deletion of DUB3 leads to decrease in Cyclin A expression while reverse results were found in overexpression of DUB3 [58]. By removing the PD-L1 polyubiquitin chain and inhibiting its degradation, USP5 raises PD-L1 protein levels. As a result, USP5 might be a new biomarker to predict the expression of PD-L1 on NSCLC cells [59]. OTUD3 deubiquitylates GRP78 leading to lung cancer [60].

## Epigenetic paradigm

It is well established that accretion of genetic and epigenetic defects causes a cancer that results in normal cells transformation towards malignant cells. This process develops tumor in a distinct way that determining the changes that generate, maintain, and involve in disease spread is an incredible challenge. The word 'epigenetics' refers to mitotically inheritable alterations in gene expression but that does not involve alteration of DNA sequence. This is accomplished through methylation of DNA at CpG dinucleotides and it is also extended to histone modifications [61]. Advances in next-generation sequencing have resulted in the discovery of several types of noncoding RNAs which from yeast to man acts as essential regulators of epigenetic modifications through chromatin-dependent regulation [62].

Non coding RNA such as miRNA and also lncRNA found to act as epigenetic modifiers [63]. Notably, as epigenetic modulators, miRNAs change the protein expression of target mRNAs without changing the gene sequences. Furthermore, miRNAs can be influenced by epigenetic

alterations such as DNA methylation, RNA modification, and also histone modifications. The reciprocal effects of miRNAs and the epigenetic process appear to establish a feedback loop of miRNA-epigenetic, which has a significant impact on gene expression. Thus, the disruption of this loop impairs biological processes contributing to various diseases [64]. Moreover, evidence suggests epi-miRNA role in cancer progression, while we will be discussing in detail about epigenetic regulation of miRNA along with its role as epigenetic regulators in NSCLC in further sections. PTMs falls under epigenetic regulation are acetylation and methylation which are discuss as follows

## Acetylation

Histone acetyltransferase (HAT) and histone deacetylase (HDAC) activities control the acetylation of proteins. The lysine residues in histones and other proteins are acetylated and deacetylated by the HAT and HDAC enzymes, respectively [65]. Members of the various classes of HATs and HDACs target various histones and other proteins and bind to certain lysine residues to carry out particular tasks. The primary proteins that make up chromatin are histones, which act as the "spools" around which DNA is arranged. Histone acetylation changes the chromatin's structure into a more "relaxed" state, facilitating greater transcription factor accessibility to DNA [66]. Deacetylation, on the other hand, causes transcriptional deactivation by entangling DNA more closely with histones and limiting transcription factors access to the DNA. By modulating the proteins' stability, acetylation may be able to directly or indirectly impact the functions of proteins. Independent of histone acetylation, the acetylation of transcription factors and nuclear co-factors may control transcriptional activity [67].

The acetylation of EZH2 (Enhancer of zeste homolog 2) at lysine 348 residue due to acetyltransferase PCAF (P300/CBP-associated factor) overall impacts on lung adenocarcinoma migration as this acetylated EZH2 affects its phosphorylation and stability [68]. Authors reported in A549 cells, ABCE1 acetylation and Tip60 are upregulated. They knockdown Tip60 using siRNA and found that Tip60 downregulation reduces the ABCE1 acetylation that ultimately affects cell proliferation, invasion and migration in vitro and in vivo [69]. In KRAS-driven lung adenocarcinoma, HDAC10 reported to regulates tumor stem cell characteristics and Hdac10 knockout mice developed early-onset lung adenocarcinomas more quickly, had more macrophages infiltrate the tumor microenvironment, and lived shorter [70]. SIRT1/2 are deacetylase that regulates cancer associated genes. Patients with NSCLC showed high expression of SIRT1/2 correlates with poor prognosis [71]. Ning Mu et al., demonstrate that in NSCLC cells, SIRT1/2 inhibition via salermide or siRNA promotes pro-survival autophagy through the acetylation of HSPA5 with followed by ATF4 and DDIT4 activation which inhibits mTOR signaling [72]. SIRT3 modulates the acetyl and pyruvate dehydrogenase complex, which is important for sustaining mitochondrial activity and for producing ATP (PDH). Melatonin alters the expression of Sirt3 and deacetylates PDH to improve complexes I and IV activity, reversing the Warburg effect and inhibiting lung cancer development through both in vivo and in vitro studies [73].

Meanwhile, The study also supported SIRT3 to prevent lung cancer cells from developing cisplatin resistance. The overexpression of SIRT3 reduces acetylated FOXO3 level leading to increase in FOXO3a/CDT1 axis expression causing cell apoptosis and makes lung cancer cells more susceptible to cisplatin [74]. In NSCLC, SIRT4 acts as a tumor suppressor leading to inhibition of invasion, migration, cell cycle progression and proliferation [75].

## Methylation

Protein methylation is the addition of a methyl group to the lysine or arginine amino acid of protein by the enzyme Methyltransferase, depending on which residue methyl group will be added named as lysine methyltransferases or arginine methyltransferases which are regulated

with demethylase. Demethylase removes the methyl group from the protein. The methylation process is involved in epigenetic modification which ultimately shapes cancer biology. The Epigenetics events includes DNA methylation, the addition of methyl group to the cystine base of DNA catalyzed the enzyme DNA methyltransferase (DNMT), which leads euchromatin (less condensed) to heterochromatin (Highly condensed) conversion [76]. The DNMT family consists of the following five members such as DNMT1, DNMT3a, DNMT3b, and also DNMT3L [77]. DNA methylation on the tumor-suppressor gene promoter, leads transcriptional repression of these genes in cancer. The Histone modification involves various enzymes which act on histones, histone methyltransferase (HMT), add methyl group while histone demethylase (HDM) removes it. However, addition of acetyl group by histone acetyltransferase (HAT) and removal by histone deacetyltransferase (HDAC) [78].

In NSCLC, EZH2 a methyltransferase adds three methyl group on lysine 27 of histone H3 (H3K27me3) leading to silencing of PRC2 genes. Thus, 3-deazaneplanocin A an inhibitor of EZH2, could directly reduce the NSCLC proliferation. The study also shown that combination of 3-deazaneplanocin A and SAHA inhibits the NSCLC tumor in vivo H1975 xenograft mice model [79]. SMYD3 is a histone lysine methyltransferase enzyme which methylates MAP3K2 at lysine 260, causing activation of Ras signaling in lung adenocarcinoma while ShRNA mediated decrease in MAP3K2 showed decrease proliferation in A549 and LKR10 cell lines [80]. Another study suggests aberrant histone methylation found in NSCLC. Authors used Publicly available databases TCGA and cBioportal to analyze the methyltransferases and demethylases expression data of NSCLC patients and predicted genes associated with histone methylation could be used as potential prognostic indicators or treatment targets for NSCLC [81]. Several studies have evidenced the correlation of smoker and DNA methylation in lung cancer development and metastasis [82,83], also found the several methylation marker in lung cancer [84,85]. In HCC827 cell line, upregulated DNMT1 inhibits hMLH1 and hMSH2 expression through increasing their promoter methylation which in turn promotes proliferation. Thus knockdown of DNMT1 showed reduced proliferation in HCC827 cell line and xenograft mice model [86]. The increased methylation of the MTUS1 promoter decreases MTUS1 expression in NSCLC [87]. The study reported that DAL-1 protein expression is crucial for NSCLC metastasis and loss of DAL-1 is linked to the methylation of its promoter [88].

### Post translational modification crosstalk in NSCLC

Several studies have provided evidence regarding crosstalk between various PTM [89,90]. PTM crosstalk is mainly classified as intra-protein (alteration within the same protein) and inter-protein (alteration in two different proteins) PTM crosstalk [91]. Further there are positive as well as negative crosstalk which regulates two PTMs interaction. Positive crosstalk occurs when one PTM acts as a signal for the insertion or deletion of a different PTM and also provides a recognition site for associated protein which performs the task of a different alteration. However, negative crosstalk can be further broken down into direct rivalry between two PTMs, also indirect impacts when the first PTM hides the second PTM's recognition site [92,93]. In epigenetic modification, The PTM crosstalk on class IIa HDACs leads to synergistic outcomes [94]. It is well known that PTMs interact with one another to produce complex phenotypic results [95]. Many studies indicated that the interaction of PTMs might regulate various diseases including tumorigenesis [96]. However, our understanding of the crosstalk of PTMs has significantly increased because of the advent of mass spectrometry (MS) which is a highly sensitive technique for proteomics. Large-scale PTM crosstalk has also been extensively identified using computational methods [97]. Here we have reviewed some PTM crosstalks with respect to NSCLC. The study showed, EGFR mutant cell mediates phosphorylation at S256 of Mig6 and causes its inactivation. These inactivated

Mig6 further decreases ubiquitination, ultimately increasing the EGFR stability, which encourages lung cancer pathogenesis [99]. The O-GlcNAcylation and Ubiquitination involved in cisplatin resistance in lung cancer. Sujit Luanpitpong et al. reported that P53 becomes unstable as a result of O-GlcNAcylation of cisplatin induced p53, which encourages ubiquitin-mediated proteasomal destruction. On the other hand, O-GlcNAcylation of c-Myc prevents it from being ubiquitinated and degraded, ultimately influencing drug resistance in lung cancer cells [100]. The p300 is histone acetyltransferase, phosphorylation of p300 at S1038 and S2093 causes its degradation while inhibiting these two sites means p300 phosphorylation could acetylated histone H3 reduces lung cancer progression [101]. The overexpression of PTPRB which is a protein tyrosine phosphatase in A549 and H1299 showed decreased proliferation while knockdown of this enhances phosphorylation of Src leading invasion and proliferation in NSCLC cells [102]. The study showed that when growth factors are stimulated, SETDB1 methylates Akt at position K64, which is a critical first step in the resultant K63-linked ubiquitination of Akt. This process involves bringing JMJD2A and E3 ligases to an Akt complex, which recruits cell membranes and causes phosphorylation at T308 and activates Akt [103]. Despite the actions, PTM crosstalk can coordinate complex interactions of PTMs to regulate the functionalities, signal transduction pathways and modulation of protein networks in oncogenesis.

### Proteogenomics: strategy to analyze PTM

Omics technologies are intended primarily for the thorough identification of genes, RNAs, proteins, metabolites, and quantitative aspects of medical imaging (radiomics), which are used widely in both fundamental research and the clinical cancer treatment. Genomic and transcriptome analyses based on next-generation sequencing (NGS) enable the identification of genes that are differentially expressed, which promote and sustain carcinogenesis as well as a better knowledge of the organization of the cancer genome. Furthermore genome profiling may help identify various molecular subtypes and patient classification, which is essential for specifically personalized treatment. Technologies including NMR, HPLC and MS are frequently utilised to find new biomarkers and therapeutic targets from the cancer proteomics and metabolomic data [104]. The proteomic analysis provide information regarding the proteins variants its spatial conformation, PTM along with sequence of amino acids and splicing copy number. Proteogenomics is one of the most well-established areas of study of multi-omics, and the underlying technologies include next-generation sequencing (NGS) along with mass spectrometry (MS). Even though proteomics and genomes are closely related, they have remained rather isolated. The barriers were not broken down until recently, and the blending of proteomics and genomics gave rise to the new area of "proteogenomics" [105]. Proteogenomics has potential to discover and also to quantitate PTMs which are involved in cancer formation. Basically in NGS data modalities include, whole genome sequencing (WGS), whole exome sequencing (WES) and transcriptomics which used to decode the entire genome of an organism, only exon region and RNA sequencing respectively. Whereas in proteomics top down and bottom up methods are used. The shotgun method is a bottom up approach where first peptides resulted from protein digestion separated using liquid chromatography then enters in MS for analysis while top down method analyze entire proteins directly, enabling for the accurate and thorough characterization of a variety of proteoforms, including PTMs, RNA splice variants, and protein mutations resulting from genetic polymorphisms. The proteogenomics used to identify genetic variation in the amino acid sequence of protein and also protein variants [106]. The proteogenomics workflow contains integrated NGS and Proteomics which is shown in Fig. 3. If a reference genome is available then it can be utilised as a template for aligning NGS data from various modalities of sequencing. Finally, Proteogenomics uses database search engines to search a customized protein FASTA, which contains results obtained

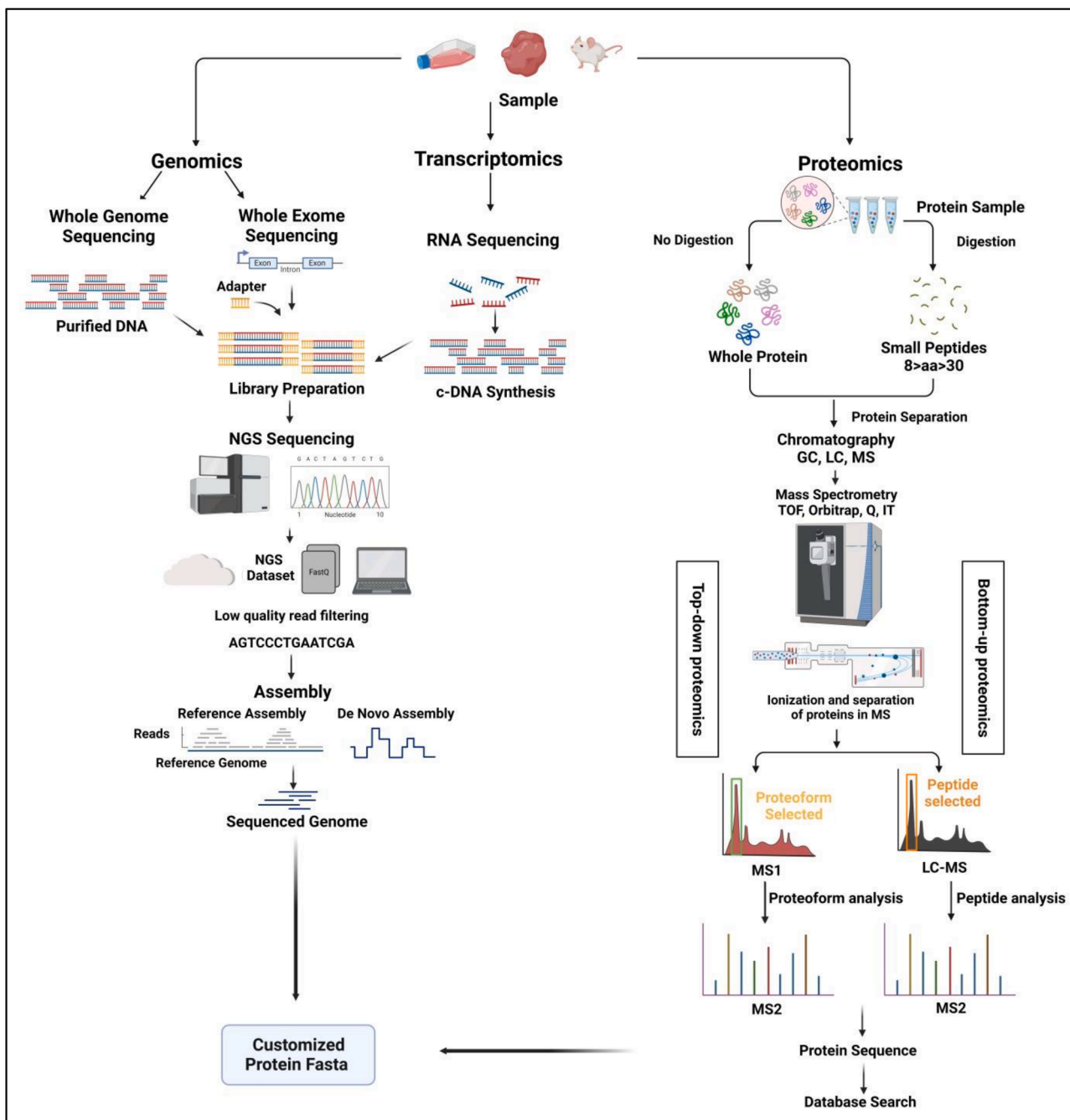


Fig. 3. Workflow of proteogenomics.

from various NGS platforms such as genome variants, transcripts variants along with proteomics data obtained from top-down or bottom up methods means Similar to proteomics, proteogenomics infers the identity of proteins from a collection of peptide-to-spectra matches through associating a peptide sequences with fragment ion mass spectra. The various tools are used in Proteogenomics such as Peptide Sequence Match Evaluator Tool, MSProGene, NextSearch, PGA Utility, proBAMtools, proBAMr, proBAMSuite, ProteogenomicsViewer, ProteoAnnotator, PoGo, Splicify [105]. The combined study of RNA seq proteogenomics with G-PTM leads to identification of various variant peptides [107]. Another study on colon cancer using proteogenomics infers novel therapeutic targets [108]. Moreover the findings through proteogenomics also helps in immunotherapy in cancer such as EGFR-mutant lung cancers and melanoma [109]. Proteogenomics is still

in growing phase, despite the fact that the proteogenomic study of cancer collectively represents a significant scientific success accompanied by a tremendous amount of molecular data [110]. Proteogenomics will ultimately play a larger role in cancer diagnosis and treatment as these methods and technologies advances, offering precisely targeted care for each patient while combining genomic and proteomic data to create a comprehensive picture of that patient's cancer [111].

### Therapeutic approach for targeting PTM in NSCLC

#### Phosphorylation inhibitors

There have long been tyrosine kinase inhibitors regarded as effective treatments for NSCLC and superior tailored drugs. There are many drugs

that target the EGFR, including gefitinib, erlotinib (first generation drug), cetuximab and panitumumab (monoclonal antibody). A multi-kinase inhibitor, sorafenib targets the angiogenesis and proliferation of NSCLC and with NSCLC preclinical models sorafenib along with other drugs such as Gefitinib, vinorelbine and cisplatin, showed the effectiveness, acceptability and tolerability [112]. It has been proven that PI3K, RAS and AKT suppression makes cancer cell lines more radio-sensitive [113]. The activation of mTORC1 implicated in EMT in NSCLC. Yu chen et al. showed that Everolimus is an mTORC1 inhibitor, increasing radiosensitivity in NCI-H661 NSCLC cells through altering EMT [114]. Inhibitors such as dabrafenib, encorafenib and vemurafenib (BRAF inhibitors) [115], Abemaciclib (CDK4/6 inhibitors) [116], Lorlatinib (ALK inhibitors) [117] has been reported. The various PTMs and its inhibitor drug are given in Table 1.

### Glycosylation inhibitors

The study has mentioned the combination of glycosylation inhibitors with chemotherapeutic drug in lung cancer, more specifically galectin inhibitor, GR-MD-02 with Pembrolizumab and GM-CT-01 with 5-Fluorouracil [118]. Another inhibitor is SGN-2FF which is fucosylation inhibitor but the clinical trial with these has been terminated due to adverse effects (ClinicalTrials.gov Identifier: NCT02952989). Further review article includes sialidase inhibitor, Sialyltransferase inhibitor, Fucosylation inhibitor on lung cancer cell lines [119].

### Ubiquitination and SUMOylation inhibitors

A number of NSCLC models have been evaluated in vitro and in vivo with bortezomib which is the first proteasome inhibitor licensed by the FDA and it exhibits an active action against NSCLC cells [120]. Another study showed TAK-246 as UAE inhibitor showed antitumor effect in CDX PHTX-132Lu NSCLC mice model [121]. The potent and selectively reversible inhibitors are pimozone and GW7647 which acts on USP1/UAF1 complex in NSCLC [122]. Other proteasome inhibitor is b-AP15 revealed reduction in tumor progression in lung cancer mice model [123]. The Findings suggest that TAK-981 is a First SAE Inhibitor for Cancer Therapy [124]. Several natural products also known to inhibit SUMOylation, including davidiin which reduces NCI-H460 lung cancer cells proliferation (GI50 16.4  $\mu$ M) [125].

### Acetylation inhibitors

In NSCLC, Entinostat as HDAC inhibitor has a potential to act as anticancer drug [126]. Although The NSCLC patients who had previously received anti-PD-(L)1 therapy, the combination of Entinostat and

pembrolizumab drug did not meet the primary response rate goal but it showed a clinically significant benefit (9% patients) with no side effects with either of drug [127]. The combination of two PTM inhibitor showed significant effect in NSCLC. Gabriele Greve et al. reported the combination of HDAC inhibitor and RTK inhibitors in NSCLC. They were able to demonstrate in this study that the combination of panobinostat (HDAC inhibitor) with erlotinib (RTK inhibitor) does cause additional anti-proliferative effects on HCC827, A549 [128]. Similarly Gefitinib and vorinostat together effectively inhibit signaling pathways namely IGF-1R/AKT, which in turn reduced cell proliferation and significantly triggered apoptosis. Gefitinib and vorinostat both significantly reduced tumor development in mice bearing lung adenocarcinoma tumor xenografts [129]. Mengmeng Niu et al. discovered nebevivolol which is a therapeutically employed small molecule inhibitor, which activates the expression FBXL2 to prevent EGFR mediated NSCLC growth and this drug along with osimertinib or Grp94-inhibitor-1 significantly inhibits the growth of NSCLC with osimertinib-resistant [55].

### Methylation inhibitors

The most research has been done on DNA methyltransferase inhibitors (DNMTis). Research has revealed that one of the important targets of tumor epigenetic therapies is DNMT [130]. 5-Azacytidine (5-AzaC) and 5-Aza-2'-deoxycytidine (5-Aza-CdR) analog of 5-AzaC are DNMT inhibitors used in lung cancer [131]. In lung cancer, histone methyltransferase such as MLL2, G9a, EZH2, SMYD2, SETD2, WHSC1L1, DOT1L, SETD8/PRSET7, SUV4-20H1/2, PRMTs and histone demethylase such as KDM1A, LSD1, KDM2A, KDM3A, KDM4A, KDM4C, KDM4D, KDM5A, KDM6A, JMJD6 and PAD4 plays function which make them as important target in treatments. It is reported that inhibitors of EZH2 have therapeutic potentials. It is found that 3-deazaneplanocin-A (DZNep) which is a inhibitor of EZH2, via using MTT assay shown that DZNep administration in the NSCLC cell lines namely A549, H1975, H1299 and PC-3 led to a dose-dependent inhibition with an IC50 range of 0.08 to 0.24  $\mu$ M [132]. Another more specific EZH2 inhibitor is GSK2816126, which showed dose dependent inhibition in the A549 cell line and also inhibits angiogenesis both in vitro and in chick embryo [133]. EPZ-6438 along with CDDP has shown on A549 cells [134]. However, EZH2 inhibition made lung cancers more sensitive to other inhibitors. For instance, in the case of BRG1 and EGFR mutant lung cancer, either administration of DZNep or GSK126 enhanced the anti-tumor activity of the TopoII inhibitor doxorubicin, indicating a potential for the combination of conventional chemotherapeutic drugs and EZH2 inhibitors in NSCLC [135].

One of the key problems in future drug discovery is a better knowledge of cancer's dependence on PTMs and the ability to predict the chemicals and pathways connected thereto. Overall, new and under-researched medications exhibit strong therapeutic response; nevertheless, resistance to developing drugs is also rising. Another difficulty facing scientists is this new resistance. Comprehending resistance mechanisms will help in better understand how cancer develops and open the door to the development of next-generation treatments with extreme selectivity. To confirm the efficacy of novel medications, numerous clinical trials will be necessary. Although the various inhibitors are currently in used and some are in clinical trial but drug resistance is one of the major issues which need to be resolved. The possible therapeutical approach to solve these problems is via PTMs regulating miRNAs which are discuss in below section.

### miRNA as a regulator in NSCLC progression

miRNA are conserved small noncoding RNA usually of 22 nucleotides which do not contain any protein encoding sequences. They specifically bind to 3'UTR of target mRNA and regulate its expression. Various evidences suggest that the dysregulation of miRNAs contributes towards hallmarks of cancer via acting as tumor suppressor or oncogene

**Table 1**

The list of PTMs and its inhibitor drug in NSCLC.

PTM	Target	Drug
Phosphorylation	EGFR	Gefitinib, erlotinib, cetuximab, panitumumab
	RAF	Sorafenib
	mTORC1	Everolimus
	BRAF	Dabrafenib, encorafenib, vemurafenib
	ALK	Lorlatinib
	CDK4/6	Abemaciclib
Glycosylation	Galectin	GR-MD-02
	Galectin	GM-CT-01
Ubiquitination	proteasome	bortezomib
	UAE	TAK-246
	USP1/UAF1 complex	Pimozone, GW7647
Acetylation	HDAC	Entinostat, panobinostat, vorinostat
Methylation	DNMT	5-Azacytidine (5-AzaC)
	EZH2	3-deazaneplanocin-A (DZNep), GSK2816126

miRNA. Our recent review has mentioned the significant role of different miRNA in NSCLC tumor microenvironment and autophagy, along with synthetic biology as a novel therapeutic approach [136]. The MRX34 (miR-34a), is the first miRNA based approach for cancer treatment which entered in phase1 clinical trials, but the trial was terminated early due to some serious adverse effects in patients [137]. miRNA-16 mimic (MesomiR-1) along with Antibody targeting EGFR, both are a part of TargomiR, completed a phase 1 clinical trial in patients with mesothelioma and lung cancer [138]. Here we are discussing some miRNA as a modulator of PTM in NSCLC as shown in Fig. 4.

As mentioned earlier epi-miRNA are miRNAs which regulate various enzymes of epigenetic modification. The dysregulated miRNA expression in cancer has been associated with genetic changes in miRNA genomic regions, transcription factor (TF) controlling miRNA expression, and defects in biogenesis. Nevertheless, epigenetic changes (such as DNA methylation and histone modifications) are the most common

causes of miRNA dysregulation in cancer as they are important entities of post translational modification [139]. Many cancers, including NSCLC, typically exhibit abnormal CpG methylation in miR-34a promoter region, which lowers miR-34a expression [140]. The hypermethylation of miR-124a and miR-200c promoter region decreases their expression affecting the NSCLC progression [141,142]. In contrast, lung adenocarcinomas have hypomethylated let-7a-3 promoter regions, which leads to increased let-7a-3 expression and therefore improved tumor phenotypic alterations in lung cancer [143]. The study demonstrated that miR-224 promotes tumor growth both in H1299, H1573, and H460 cell lines and nude mice. However they found that miR-224 downregulates SMAD4 and TNF-induced protein 1 expression, while the hypomethylation in miR-224 promoter region leads to upregulation of miR-224 promoting its oncogenic role in NSCLC progression [144]. Similar to DNA methylation Histone methylation is also involved in cancer formation and progression. Authors found that KDM5B which is a

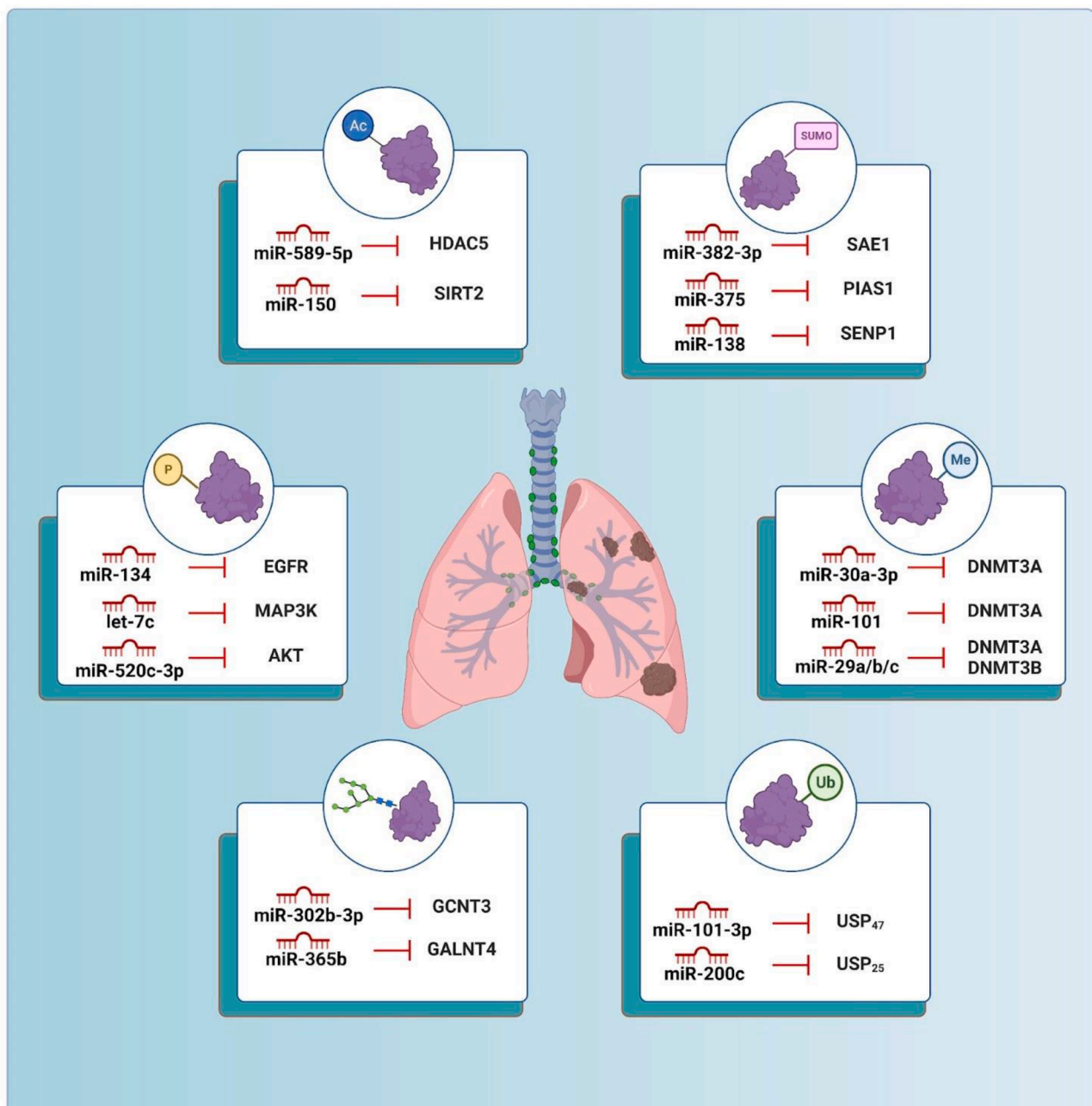


Fig. 4. miRNAs modulating different PTMs in NSCLC.

histone H3 lysine 4 (H3K4) demethylase induces EMT via inhibiting miR-200 family expression in lung cancer [145]. The numerous findings also revealed the miRNA as epigenetic modulators in NSCLC. The bioinformatics study revealed that miR-101 downregulates DNMT3A, thus overexpression of miR-101 showed reduction in promoter methylation of PTEN in A549 cell line inhibiting the progression of lung cancer [146]. However Another miR-30a-3p also targets DNMT3a in lung cancer [147]. Muller Fabbri et al. reported that miR-29 family targets DNA methyltransferase enzymes namely DNMT3A and DNMT3B in NSCLC. Restoration of miR-29 family (miR-29a, miR-29b, and miR-29c) resulted in reexpression of FHIT and WWOX (tumor suppressor genes) leading inhibition of tumorigenicity both in vitro and in vivo [148]. The study shown that abnormally high levels of HDAC5 were found in NSCLC and subsequent investigation revealed a negative correlation between HDAC5 and miR-589-5p in NSCLC patient sample. The crucial regulation of miR-589-5p/HDAC5 impacts on NSCLC tumorigenesis proved using in vitro and in vivo experiments [149]. SIRT2 is histone deacetylase which downregulated due to the overexpression of miR-150 in A549, H460, H1299 and H520, causes NSCLC proliferation [150]. Various miRNAs were found to regulate signaling cascade such as let7c targets ITGB3 and MAP4K3 in NSCLC. The reduced expression of let7c is associated with NSCLC metastasis [151]. However, miR-134 in A549 xenograft mice model showed reduced tumor size, Moreover miR-134 downregulated EGFR in A549 and H1299, H1975 and H520 cell lines thus suppresses NSCLC proliferation [152]. Our study showed miR-520c-3p could target AKT in NSCLC [153]. Several literatures mentioned various miRNA targeting ubiquitination processes in cancer. Jinyoung park et al. investigated the role of miR-101-3p in lung cancer, suggesting that miR-101-3p targets USP47 and thus regulates ubiquitination of RPL11 [154], while another miRNA, miR-200c downregulates USP25 in NSCLC acting as tumor suppressor miRNA [155].

The study revealed that miR-365b is downregulated in A549 and H1299 cell line of NSCLC, which regulates GALNT4 expression, thus increased expression of GALNT4 causes NSCLC proliferation [156]. Another enzyme GCNT3 also found to be downregulated by miR-302b-3p in NSCLC, implicate reduction in proliferation of NSCLC cell lines [157]. miR-138 found to downregulate the protease SENP1 and which is directly shown to have relation with radiosensitization in A549 cells [158]. In lung Adenocarcinoma, negative correlation within miR-375 and its 10 probable target including PIAS1 were observed [159]. Hua fang et al., suggested using dual-luciferase and also immunoblotting experiments that SAE1 which is important for SUMO-activating complex was discovered to be a direct target of miR-382-3p, these SAE1 overexpression causes SUMOylation of AKT followed by promoting the phosphorylation of AKT, ultimately activation of the AKT signaling pathway. Thus, SAE1 knockdown reversed the effects of miR-382-3p inhibition, which increased proliferation and decreased apoptosis in lung adenocarcinoma cell line (A549 and H1299) [160]. Another miR-708-5p reduces the onset, growth, and stemness of NSCLC by directly targeting the enzyme of DNA methylation DNMT3A [161], similarly recent study has mentioned miR-26a-5p also targets DNMT3A in NSCLC [162]. The histone deacetylase SIRT 7 is a direct target of miR-3666 which reduces NSCLC growth [163].

The study confirmed that miR-138-5p mimic transfection dramatically increased H1975 cells' sensitivity to gefitinib, which is consistent with the finding that miR-138-5p expression was 10 times lower after exposure to gefitinib for six months [164]. Wei ma et al., showed that miR-497 inhibits the expression of IGF-1R and reduces activation of the downstream AKT signaling pathway, which may increase NSCLC sensitivity to EGFR-TKIs and reverse gefitinib resistance [165]. The expression of miR-373 is regulated via treatment of suberoylanilide hydroxamic acid and Trichostatin A in NSCLC cell lines [166]. These literature evidences showed that miRNA along with inhibitors have impact on NSCLC progression. Although the regulatory role of miRNAs is a promising therapeutic approach but before miRNA treatments are made available in clinics, dosage issues need to be resolved because too

much miRNA mimics/antimiRs can exacerbate toxicities, unspecific immune responses, and off-target side effects.

### Future perspective

PTMs, which are involved in governing a variety of biological activities, most likely make up the most intricate and sensitive regulatory networks. Any changes or disruptions in these PTMs might cause a number of various diseases [167]. Understanding PTM mechanisms has helped researchers find novel biomarkers that are useful in clinical practice and has made it possible to apply focused therapeutic strategies for the treatment of cancer. Particular inhibitors are being designed, and testing them against the target protein may show that they are useful in preventing some types of cancer. Cancer research has primarily focused on targeting one or the other alteration because to the importance of crosstalk between various forms of PTMs. Means PTMs of target proteins should be systematically identified, together with their functional and molecular characterization, to help create novel approaches to drug discovery and therapy for cancer. One of the key problems in future drug discovery is a better knowledge of cancer's dependence on PTMs and the ability to predict the chemicals and pathways connected therewith [168].

The recent study has discussed various treatment modalities that emphasize on protein alterations that are misregulated in the development of lung cancer. The different levels of success, a number of therapeutic design approaches targeting particular kinases or phosphatases which are improperly regulated in lung cancer growth have been discovered in recent years. These therapeutic approaches, which showed good bioavailability and patient tolerability, consisted of adenoviral and lipid nanoparticle-based techniques. The PROTACs (proteolysis targeting chimeras) or DUBTACs (Deubiquitinase-targeting chimeras) strategy is another method that takes advantage of the ubiquitin framework to which patients responded appropriately, once optimizing the compound's delivery. All these approach have some advantages along with limitations. Thus better understanding and advancement are necessary for therapeutic approach targeting PTMs [169]. Our knowledge of the tumor microenvironment's multifaceted regulatory network may be expanded by further characterization of the functional effects of novel PTMs on immune regulation, which could also lead to new suggestions for enhancing the efficacy of immunotherapy. Although the drugs that target PTMs have shown to be rather effective in preclinical as well as clinical investigations due to the various and varied connections between tumor cell and TME regulation. However, there are still many obstacles and ambiguities to overcome before PTMs can be used in clinical practice [170].

### Conclusion

This review mainly highlighted various regulatory role of post translational modification in NSCLC progression and the functional consequences of epigenetic changes associated with miRNAs dysregulation. It is notably evident that miRNA acts a biomarker and therapeutic target in cancer. Moreover, miRNA expression modulates via epigenetic mechanism and miRNAs itself can regulate various enzymes of PTMs to maintain homeostasis. The PTM inhibitors along with miRNA have shown significant effect on drug resistance. Furthermore, the advancement in techniques for the identification of PTM and the deeper understanding of importance of PTM in NSCLC provides potential future prospects for the development of therapeutics with more specific and effective target, thus it will be the emerging method to enhance cancer early diagnosis and therapeutic treatment to target PTMs of important proteins or pathways.

### CRediT authorship contribution statement

**Pooja Gulhane:** Writing – original draft, Writing – review & editing.

**Shailza Singh:** Conceptualization, Writing – review & editing, Funding acquisition, Supervision.

## Declaration of Competing Interest

The authors potentially declares no conflict of interest

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