

MOLECULAR MECHANISM OF LUNG CANCER: DYSREGULATION OF PROTO-ONCOGENE AND TUMOR SUPPRESSOR GENE

Prerona Dutta,¹ Priyankar Pal^{2*}

¹Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, Kolkata 700121

^{2*}Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, Kolkata 700121

*Corresponding Author at: Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, Malir Math, Kolkata, Bara Kanthalia, West Bengal 700121

*Corresponding Email: priyankarpal97@gmail.com

ABSTRACT

Lung cancer remains a critical public health challenge, representing the leading cause of cancer-related mortality worldwide. Lung cancer accounts for approximately 20% of all cancer deaths, translating to around 1.6 million fatalities annually. The two primary classifications of lung cancer are small-cell lung carcinoma (SCLC) and non-small-cell lung carcinoma (NSCLC), with NSCLC comprising approximately 85% of cases. Key driver mutations in genes such as EGFR, KRAS, MET, HER2, BRAF, ROS1 and NTRK have been identified as pivotal in the development of NSCLC. In addition to genetic alterations, various signaling pathways such as PI3K/AKT/mTOR and MAPK contribute significantly to tumor growth and survival mechanisms in lung squamous cell carcinoma. Through altering chromatin structures and the specific expression of genes, epigenetic modifications in lung cancer play a significant role in cell transformation. These include DNA methylation, histone and chromatin protein modification, and micro-RNA, all of which suppress tumor suppressor genes while promoting the expression of oncogenes. Understanding the molecular mechanisms

behind lung cancer presents significant opportunities for enhancing early detection and developing targeted therapies.

Keywords: *Epigenetic changes, Lung cancer, Proto-oncogene, Tumor suppressor gene*

Introduction:

Lung cancer is a complex disease characterized by uncontrolled cell growth in lung tissues, primarily driven by genetic and environmental factors. With an estimated death toll of over 160,000 in 2006, lung cancer is the most common cause of cancer-related fatalities in the US for both sexes. Surgical resection, platinum-based doublet chemotherapy, and radiation therapy, either separately or in combination, are currently considered conventional treatments. Sadly, the disease is rarely cured by these treatments, and the 5-year survival rate is still only 15% overall (Jemal A et al. 2006).

The main causative agent for lung cancer is tobacco smoking. There are over 60 carcinogens in tobacco smoke, and over 20 of them are closely linked to the development of lung cancer. The most well-known of these substances are the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and the polycyclic aromatic hydrocarbons, both of which cause genetic alterations by forming DNA adducts (Hecht SS et al. 1999). According to the epidemiology studies, smokers have a 14-fold increased risk of lung cancer compared to non-smokers, only 11% of heavy smokers will ever get the disease (Amos CI et al. 1999).

The molecular mechanisms underlying lung cancer involve a series of alterations in cellular processes, including mutations, changes in gene expression, and epigenetic modifications that

can lead to tumorigenesis. Key genetic mutations in lung cancer include those in the epidermal growth factor receptor (EGFR), KRAS, and anaplastic lymphoma kinase (ALK) genes. These mutations can activate signaling pathways that promote cell proliferation and survival, contributing to the aggressive nature of lung cancer. In addition to genetic mutations, the tumor microenviron-

ment, which includes surrounding cells, blood vessels, and immune elements, play a significant role in lung cancer progression. The interaction between cancer cells and their micro environment can lead to enhanced tumor growth, metastasis, and resistance to therapies.

Based on this, to logical type, lung cancer is categorized. Small cell lung cancer (SCLC) makes up 15–20% of lung cancer patients, whereas non-small cell lung cancer (NSCLC) makes up the remaining 80–85% (Pikor LA et al. 2013). It is believed that neuroendocrine cells are the source of SCLC, which mainly arises from the central airways. Adenocarcinoma (LUAD), squamous cell carcinoma (LUSC), and large cell carcinoma (LCC) are the three main histological subtypes that make up non-small cell lung cancer (NSCLC). The most prevalent is LUAD, which makes up about 40% of all lung cases. LUAD typically arises from glandular epithelium, from bronchioalveolar stem cells, club (Clara) cells or type II pneumocytes in the lung periphery. LUAD is also the predominant subtype that arises in patients who have never smoked (Chen Z et al. 2014). About 20% of all instances of lung cancer are LUSC, which mostly occurs in the central airways and segmental bronchi and is closely correlated with a history of smoking. LCC is categorized as a tumor that lacks the common characteristics of SCLC, LUAD, or LUSC and can develop anywhere in the lung (Pikor LA et al. 2013).

Currently, it is essential to carry out a suitable histological categorization, choose each patient for a treatment plan based on a thorough examination of the molecular tumor characteristics, and assess predictive factors (Travis WD et al. 2013). Understanding the intricate molecular mechanisms of lung cancer is also very crucial for developing targeted therapies and improving patient outcomes. Insights into these mechanisms pave the way for innovative treatment strategies, including the use of targeted therapies and immunotherapies that can specifically address the unique molecular profiles of lung cancer.

ABNORMALITIES IN PROTO-ONCOGENES MEDIATED PATHWAYS EGFR

Family- Epidermal growth factor receptor (EGFR) is a transmembrane tyrosine kinase receptor that plays a central role in regulating cell division and death. It belongs to the HER family of receptors, which includes EGFR (HER1/ ErbB1), ERBB2 (HER2/neu), ERBB3 (HER3), and ERBB4 (HER4). The epidermal growth factor receptor is the archetypal member of this family. A transmembrane segment, an intracellular TK domain, and an extracellular ligand-binding domain make up an ERBB receptor. A regulatory C-terminal section comes after the intracellular TK domain. The TK domain contains the highest sequence homologies (59–81% identity) of the four genes (Sharma *et al.* 2007). When ERBB receptors attach to ligands, they either homodimerize or heterodimerize, which triggers intrinsic kinase activities that start intracellular signaling transduction cascades, such as the MAPK in a sequential way. In about 30% of NSCLCs, particularly adenocarcinomas, ERBB2 (HER2/neu) is strongly expressed (Weiner *et al.* 1990). According to transfection studies in immortalized human bronchial

epithelial cells, ERBB2 overexpression plays a role in tumorigenicity (Noguchi *et al.* 1993). Mutations in the genes involved in the EGFR signaling pathway have been discovered in NSCLC (and extremely rarely in other tumors). EGFR and KRAS mutations have also been found in approximately 10–30% of NSCLCs, depending on the region (Shigematsu *et al.* 2006). The most common RAS gene activation in lung cancer is KRAS, which is typically caused by mutations at codon 12 but sometimes occasionally at codons 13 and 61. The downstream KRAS gene codes for a tiny guanosine-5'-triphosphate-binding protein. It is the most common oncogene known to be active in human malignancies, and missense mutations in many of these cancers usually activate it. About 20% of non-small cell lung cancers have KRAS mutations, particularly in ADC and smokers. There is mutual exclusion between EGFR and KRAS mutations, according to a number of studies that examined the presence of both mutations in the same tumors. Both KRAS and BRAF are members of the EGFR family

signaling cascade because they link to each other. However, compared to KRAS mutations, BRAF mutations are seen in lung cancer far less frequently (0–3%) (Dhomen N et al. 2007).

PI3k-Inlungcarcinogenesis, the PI3K/ AKT/ m TOR path way is dysregulated, and it has been linked to advanced illness (stage III) and high-grade tumors (G3–G4) (Scrima M et al. 2012). PI3K signaling inhibitors have therefore been proposed as possible treatment agents for NSCLC. Deregulation of this pathway can be caused by a number of processes, such as PIK3CA amplification, activation of tyrosine kinases upstream of PI3K, mutations in KRAS, PI3K, or AKT, or loss of negative regulation by the tumor suppressor gene PTEN. According to reports, 3–10% of SQCLC and 0–2.7% of ADK have PIK3CA activating mutations in exons 9 (E545K) and 20 (H1047R), which affect the protein's helix binding domain and catalytic subunit, respectively (Stjernstrom A et al. 2014). Interestingly, EGFR, KRAS, and ALK mutations may coincide with PIK3CA mutations in ADK (Chaffa JE et al. 2012). The loss of PTEN function may be caused by mutations, deletions or transcriptional repression via promoter hypermethylation. PTEN loss and PTEN mutations are reported in 8–59% and 3–10% of SQCLC, and 4–46% and 2–5% of ADK, respectively (Scrima M et al. 2012).

MYC family- Nuclear proto-oncogene products like MYC are eventually activated by RAS signaling, and upon heterodimerization, these products transcriptionally activate downstream genes that promote cell growth. MYC, MYCN, and MYCL (which was first identified from a lung cancer cell line) are members of the Myc family. While the others often exclusively affect SCLC, MYC is the most commonly activated and affects both SCLC and NSCLC. Overexpression of proteins is the result of either transcriptional dysregulation or gene amplification (~20–115 copies per cell). About 15% to 30% of SCLC and less often NSCLC have amplified MYC genes. Specific to neuroendocrine lung tumors, such as large cell neuroendocrine carcinoma (LCNEC) and small cell lung cancer (SCLC), L- and N-MYC amplification occurs. A high degree of transcription causes MYC protein overexpression more often than amplification (Gazzeri S et al. 2004).

ABNORMALITIES IN TUMOURS UP PRESS ORGENSE PATHWAYS

The p53 path way-In the face of carcinogens, UV radiation, and γ -induced DNA damage, p53 preserves genomic integrity. p53, a sequence-specific transcription factor that controls downstream genes like p21, MDM2, GADD45, and BAX, rapidly in response to

DNA damage or hypoxia. This helps to control the G1/S cell cycle transition, G2/M DNA damage checkpoint, and apoptosis. The accumulation of several mutations and the eventual development of a cancer cell are made possible by p53 dysfunction, which permits genetically damaged cells to survive inappropriately. p53 is essential for lung cancer; 75% or more of SCLC and roughly 50% of NSCLC have mutation in activation of the main allele, and the chromosome 17p13 locus is commonly hemizygously deleted. The G-T conversions anticipated of tobacco smoke carcinogens are mostly responsible for the 42, 52, 53 p53 alterations in lung cancers that correlate with cigarette smoking (Greenblatt MS et al 1994). Research has indicated that 40% to 70% of SCLCs and 40% to 60% of NSCLCs (squamous cell carcinomas higher than adenocarcinomas) exhibit aberrant p53 expression by immunohistochemistry (Brambilla E et al. 1996).

p16-cyclin D1-CDK4-Rb path way-

The retinoblastoma, Rb gene- The Rb gene, which activates the cyclin-dependent kinase (CDK) inhibitor p21, is the downstream effector of p53-mediated G1 arrest and was the first tumor suppressor gene to be identified. The G1 checkpoint function of Rb is closely related to its phosphorylation status; in contrast, hypophosphorylated Rb binds the transcription factor E2F1 to sustain a cell cycle arrest in G0-G1. Rb phosphorylation is another method of Rb deactivation. When CDK4-6, cyclin D, and CDK2-cyclin E phosphorylate Rb, the transcription factor E2F1 is released, causing the G1-S transition. Therefore, the G1 checkpoint control is disrupted by both the often-observed hyperphosphorylation of Rb in NSCLC and the absence of Rb protein, which is the most common escape strategy from the G1 checkpoint in SCLC.

Less than 15% of NSCLC and more than 70% of high-grade neuroendocrine LCNEC and SCLC tumors, respectively, had loss of Rb protein (Ding L et al. 2008).

Cyclin D1 and CDK4- Cyclin D1 stimulates CDK4 to phosphorylate RB, which in turn suppresses its activity. There fore, another mechanism for interfering with the p16-cyclin D1- CDK4-RB path way besides RB mutation is cyclin D1 over expression. Between 25% and 47% of initial NSCLC shave over expressed cyclin D1, which has occasionally been linked to a poor prognos, is (Betticher DC et al. 1996). Whena cyclin D1 antisense construct is transfected into lung cancer cell lines, it destabilizes RB and slows development by preventing any tumor- related cyclin D1 synthesis (Driscoll B et al 1996).

p16- Many lung tumors have chromosome 9p allele deletion (Merlo A et al. 1994). In lung cancer, p16 (p16 INK4 CDKN2), which is located at 9 p21, frequently experien cesmutation and allele loss. Disrupting the p16-cyclin D1-CDK4-RB cell cycle control pathway can also be accomplished via p16 activation, since p16 controls RB function by suppressing CDK4 and CDK6 kinase activity. NSCLC frequently exhibits p16 abnormal ities; although SCLC rarely does (RB mutations typically affect the pathway). 10–40% of NSCLCs have homozygous deletion or point mutations. Loss of p16 expression from the remaining allele may also result from hyper methy lationin the 5'Cp Gisland of the maintained allele (Otterson GAetal.1995).

EPIGENETICC HANGESIN LUNG CANCER

Epigenetic changes in lung cancer refer to alterations in gene expression that do not involve changes in the under lying DNA sequence. These changes can affect various cellular processes, including cellgrowth, differentiation, and apoptosis and are known to play a keyrole in the Development and progression of lung cancer. Some of the common epigenetic changes observe dinlung cancer include DNA methy lation, His tone modifications, Non-coding RNAs, Chromatin remodelling etc.

DNA Methylation - The most researched epigenetic regulation mechanism is DNA methylation. Various DNA methyl transferases (DNMTs) finish the methylation of the CpG island, which may result in gene silence. A methyl group is transferred from S-adenosyl-L- methionine to the CpG islands 5'-cytosine carbon by three active DNMTs (DNMT1, DNMT3a, and DNMT3b) (Forde PM et al. 1994). The pathophysiology of lung cancer is linked to DNMT over expression. Lung cancer patients may have elevated DNMT levels due to over expression of transcriptional activators, loss of micro RNAs that inhibit DNMTs, or compromised hsp 90 proteasomal DNMT break down. Smokers with lung cancer had higher levels of DNMT1 expression, most likely as a result of tobacco-specific nitrosamines that inhibit DNMT1 ubiquitination and degradation (Damiani LA et al. 2008). Additionally smoking-induced chronic inflammation and increased reactive oxygen species generation, leading to increased DNA methylation (O'Hagan H Metal.2011). Two cigarette carcinogens, methyl nitrosourea (MNU) and benzo (a) pyrenediolepoxide 1 (BPDE), were administered to immortalized normal human bronchial epithelial cells (HBECs) for a duration of 12 weeks. Following exposure to these carcinogens, cell transformation was avoided by stable knock down of DNMT1, but not DNMT3 (Merlo A et al. 1995).

Histone modifications- The chromosomal building blocks known as nucleosomes are made up of two molecules of each of the main histones H2A, H2B, H3, and H4. About 1.8 times, DNA encircles the octameric core of the nucleosome (Luger K et al. 1997). In contrast to healthy lung cells, lung cancer cells showed abnormal patterns of histone H4 modification, including loss of H4K20 trimethylation, hypoacetylation of H4K12/H4K16, and hyperacetylation of H4K5/H4K8. Their results point to H4K20me3 as a possible biomarker for the early identification and treatment of lung cancer and show significant role for histone H4 alterations (Van Den Broeck A et al.2008). Furthermore, the patterns of HAT and HDAC expression in tumor samples differ from those in normal samples, which may have therapeutic ramifications for prognostication, early tumor diagnosis, and the direction of epigenetic-targeted treatments (Ozdağ H et al. 2006).

The histone changes may also be influenced by respiratory epithelia exposed to cigarette smoke. Tobacco smoke contains nickel compounds, which cause histone deacetylation and enhance histone H3K9 dimethylation as well as smoking carcinogenic components such as nickel, chromate, and arsenite also cause H3K4 methylation (Zhou X et al. 2009).

MicroRNA- Through direct base-pairing interactions, miRNAs, a type of tiny (~22 nucleotides) nonprotein-encoding RNA molecules, modify the activity of particular messenger RNA targets to control gene expression. Over one-third of coding mRNAs are regulated by more than 1,000 miRs, each of which has the ability to control hundreds of target RNAs (81). Therefore, methylation-induced silencing of miR can significantly alter the initiation and spread of tumors (Cowland JB et al. 2007).

Conclusion and Future implications

The molecular mechanism underlying lung cancer continues to be an area of intense research, particularly given the disease's status as a leading cause of cancer-related mortality in the Western world. Recent advances have illuminated various pathways involved in lung carcinogenesis, revealing how genetic and environmental factors contribute to tumor heterogeneity and resistance to conventional therapies. This understanding is crucial as it lays the foundation for developing more effective treatment strategies tailored to individual patient profiles. The traditional one-size-fits-all approach based on disease stage is gradually giving way to precision medicine that considers the unique molecular characteristics of each tumor. As researchers delve deeper into the molecular underpinnings of lung cancer, several key implications for future clinical practice emerge. First, there is potential for early detection through identifying specific biomarkers associated with the onset of lung cancer. These biomarkers could facilitate the development of sensitive screening tools capable of detecting tumors at earlier stages when they are more amenable to treatment. Such advancements could significantly improve survival rates and quality of life for patients diagnosed with this aggressive form of cancer. Moreover, advancements in molecular classification

are paving the way for targeted therapies that exploit specific vulnerabilities within different subtypes of lung cancer. By integrating genomic data with treatment protocols, clinicians can develop personalized therapeutic regimens that enhance efficacy while minimizing adverse effects (Massion & Carbone, 2023). This shift toward individualized therapy represents a transformative approach in oncology that holds promise not just for lung cancer but also for other malignancies. Understanding the molecular mechanisms behind lung cancer presents significant opportunities for enhancing early detection and developing targeted therapies. As research progresses, it will be essential to translate these findings into clinical applications effectively. Future studies should focus on refining biomarker identification processes and optimizing therapeutic strategies based on tumor genomics. Ultimately, such endeavors will not only improve patient outcomes but may also provide insights applicable across various forms of cancer (Massion & Carbone et al. 2023).

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