General Introduction

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Cancer is a complex and multifaceted disease characterized by the uncontrolled growth and spread of abnormal cells in body[1]. It is the second leading cause of death globally, demanding extensive scientific research for improved understanding and therapeutic interventions[2]. The etiology of cancer involves various genetic, environmental, and lifestyle factors, leading to cellular alterations and genomic instability[3–7]. Numerous studies have elucidated the molecular mechanisms underlying cancer development and progression, including genetic mutations, dysregulated signaling pathways, and immune evasion[8,9]. The emergence of sophisticated tools has provided researchers with the means to deeply explore the complex molecular aspects of cancer, revealing new insights into its details and opening doors for novel therapeutic possibilities. Continuous and dedicated research efforts are crucial in advancing cancer prevention, early detection, and personalized treatment protocols. By unraveling the intricate molecular characteristics of various cancer subtypes, researchers can facilitate the early identification and monitoring of individuals at a higher risk, thereby enhancing the effectiveness of interventions[10]. The pursuit of customized treatment strategies has the potential to bring about a revolutionary change in cancer therapy. Adapting interventions based on an individual's genetic composition and the specific molecular profile of their tumor holds the promise of achieving more accurate and efficient therapeutic results.

Hallmarks of Cancer

The hallmarks of cancer encompass a set of fundamental characteristics that collectively drive the initiation, progression, and metastasis of tumors. These hallmarks provide a comprehensive framework for understanding the underlying biological mechanisms that contribute to the development and behavior of cancer cells. By elucidating these hallmarks, researchers and clinicians gain insights into the complex nature of cancer and identify potential targets for therapeutic interventions. Hanahan and Weinberg (2000) introduced the hallmarks of cancer: sustained proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis[11]. These hallmarks reflect the fundamental changes in cellular processes that distinguish cancer cells from normal cells.

In cancer cells, the growth factor and activity of their receptors play a crucial role in sustaining continuous proliferation and self-sufficiency in growth signaling [12]. Evading growth suppressors or insensitivity to anti-growth signals refers to disruption of mechanisms that normally inhibit cell division, allowing cancer cells to bypass control checkpoints and proliferate uncontrollably[13]. Evading apoptosis or resistance to cell death, involves the evasion of programmed cell death mechanisms, such as apoptosis, enabling cancer cells to survive and accumulate genetic

alterations[14]. Limitless replicative potential or induction of replicative immortality involves the activation of telomerase or alternative lengthening of telomeres, allowing cancer cells to divide indefinitely[15]. Sustained angiogenesis, which is the formation of new blood vessels, is crucial for tumor growth and metastasis, as it supplies oxygen and nutrients to the expanding tumor mass. The initiation of invasion and metastasis allows cancer cells to infiltrate nearby tissues, enter the bloodstream or lymphatic system, and form secondary tumors in distant locations[16,17]. Additional hallmarks have been proposed, including reprogramming energy metabolism[18,19], evading immune destruction[20], and tumor-promoting inflammation[21,22], as well as proteotoxic stress[23]. Understanding the hallmarks of cancer provides a foundation for developing novel therapeutic strategies that specifically target these key features. Combination therapies have shown promising results in clinical trials by targeting multiple hallmarks simultaneously, offering new hope for cancer patients.

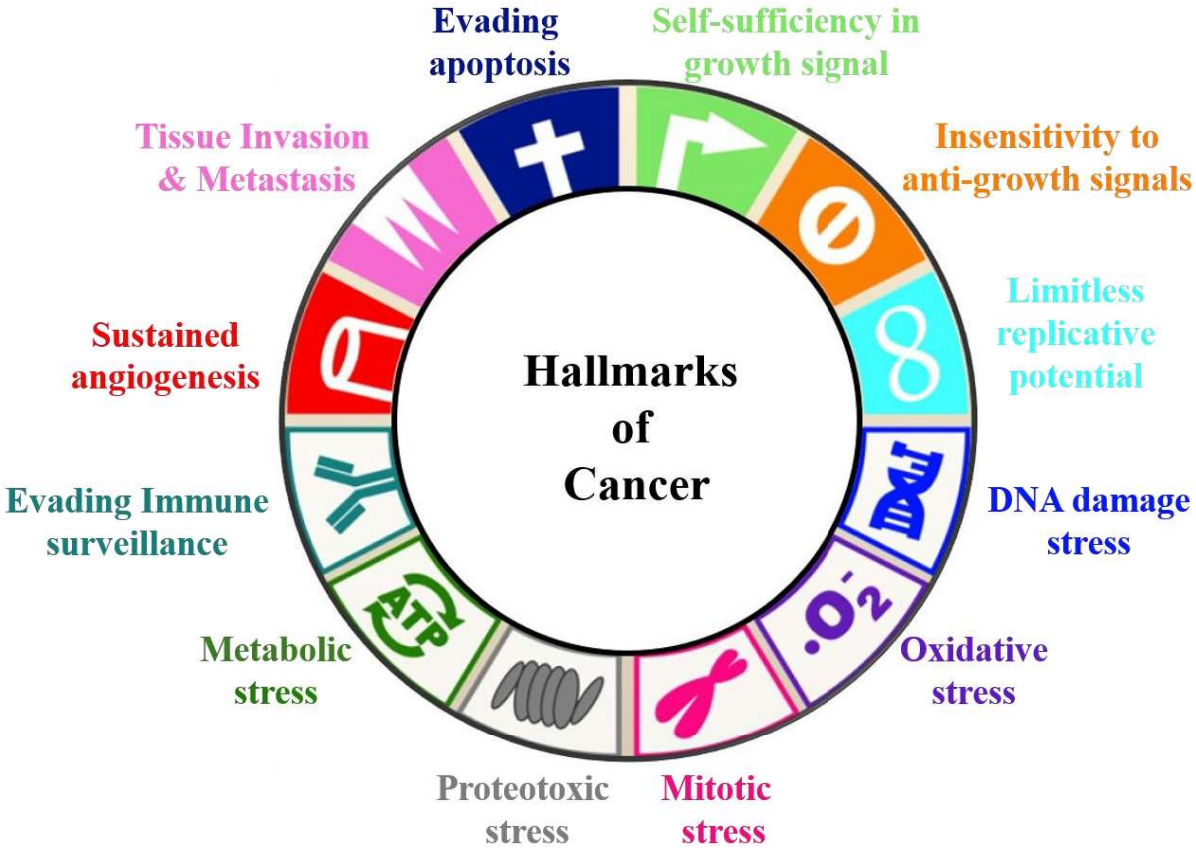

Figure A: The image illustrates the common hallmarks of cancer (Luo J *et al.*, 2009 [24]).

Lung Cancer: A growing global problem

Lung cancer remains a major global health concern, accounting for a significant proportion of cancer-related morbidity and mortality. It accounts for the majority of cancer deaths (18.0% of all cancer fatalities)[25,26]. Non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) are the two most common types of lung cancer [27–33].

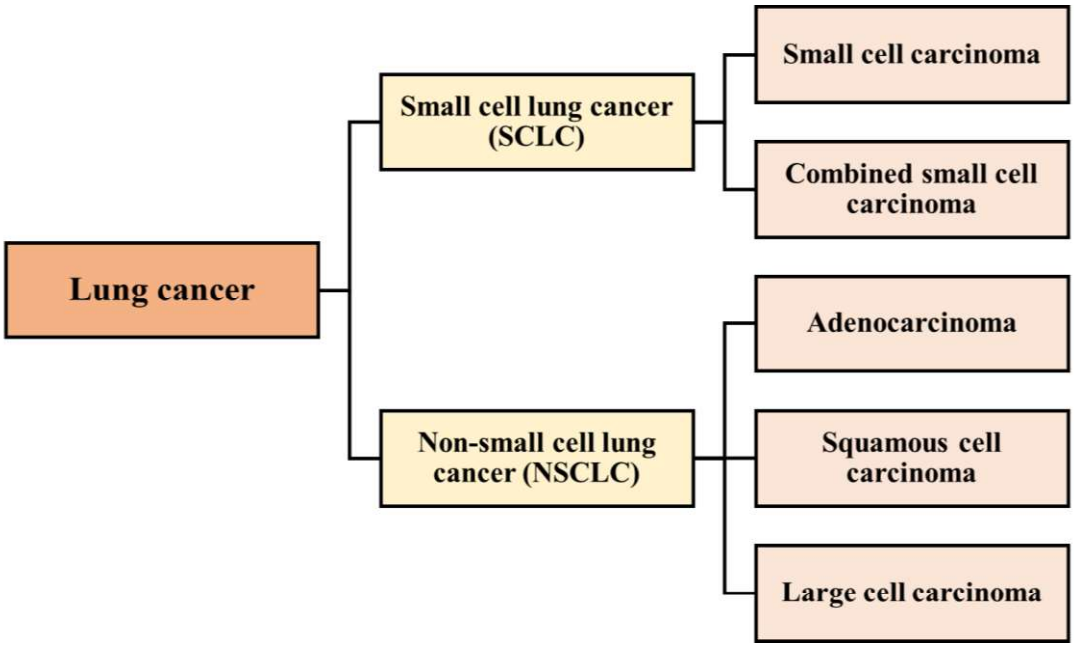

Figure B: Classification of lung cancer according to histological characteristics.

According to the American Cancer Society (ACS), NSCLC accounts for 80 to 85 percent of lung cancer diagnoses, whereas SCLC accounts for 10 to 15 percent of occurrences. The three subtypes of NSCLC that are characterized histologically are adenocarcinoma, squamous cell carcinoma, and large cell carcinoma[34]. Combined small cell lung cancer (CSCLC) is a variant of SCLC that encompasses elements of both SCLC and NSCLC[33,35]. Subtypes differ in their origin of cell, location within the lung, and development pattern despite having many biological characteristics, indicating they are separate illnesses that evolve through diverse molecular pathways.

The Globocan database (2020) highlights that lung cancer ranks second globally in terms of new cases (11.4%), following breast cancer (11.7%), while it stands as the leading cause of cancer-related mortality (18%). Across all ages and genders, the estimated prevalence of lung cancer cases was 5.2% in 2020. In India, lung cancer ranks fourth in new cases (5.5%), following breast, lip & oral cavity, and cervix uteri cancers. Similarly, it holds fourth in mortality (7.8%), trailing breast, cervix uteri, and lip & oral cavity cancers[26].

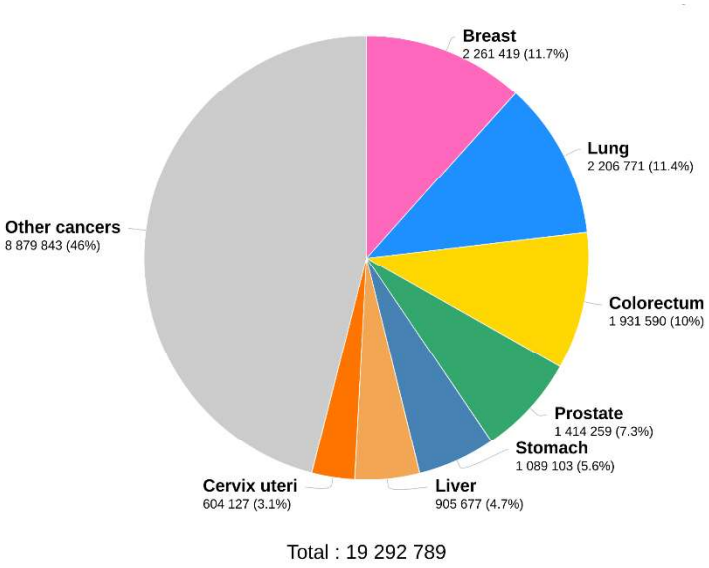

Figure C: Global incidence of new cancer cases across all ages and genders in 2020 (Globocan 2020).

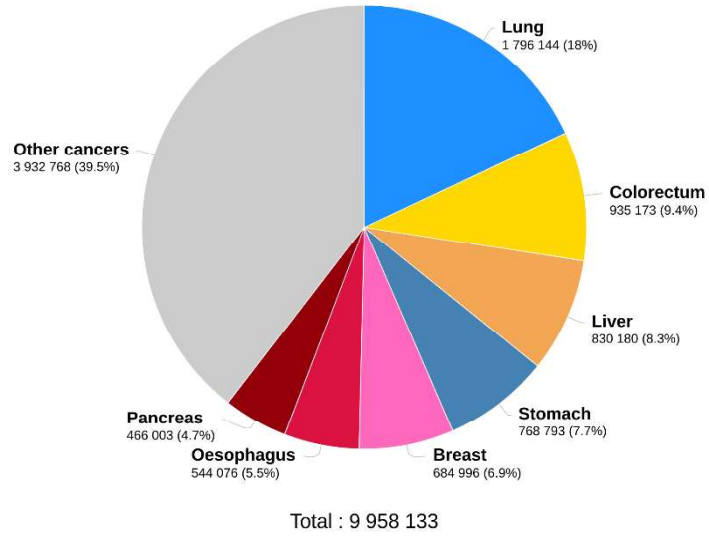

Figure D: Global incidence of cancer death across all ages and genders in 2020 (Globocan 2020).

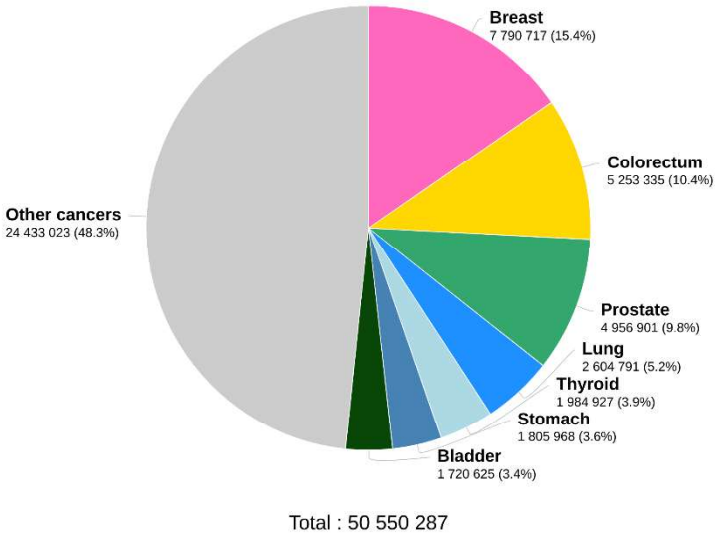

Figure E: Global incidence of prevalent cases (5years) across all ages and genders in 2020 (Globocan 2020).

Etiology of Lung Cancer

It is well-recognized that lung cancer arises due to the interplay of genetic and environmental factors, making it a complex and multifactorial disease. Understanding the causes of lung cancer is crucial for developing effective prevention strategies and targeted interventions. Tobacco smoke, particularly cigarette smoking, is the leading cause of lung cancer. Numerous epidemiological studies have consistently demonstrated the strong association between smoking and lung cancer risk[36–38]. Cigarette smoke contains numerous carcinogens and toxic compounds, such as polycyclic aromatic hydrocarbons (PAHs), aromatic amines, nitrosamines, and reactive oxygen species (ROS), which can induce DNA damage and genetic alterations in lung cells[39,40]. These genetic changes, including mutations in tumor suppressor genes and oncogenes, contribute to the initiation and progression of lung cancer. Furthermore, exposure to environmental carcinogens and occupational hazards is linked to an increased risk of lung cancer. Radon, a naturally occurring radioactive gas, is a recognized cause of lung cancer, particularly in areas with high levels of radon concentration [41,42].

Occupational exposures, such as asbestos, silica, arsenic, chromium, nickel, and PAHs, have been identified as significant risk factors for lung cancer development[43,44]. These substances can penetrate the respiratory system and induce chronic inflammation, oxidative stress, and DNA damage, leading to carcinogenesis. Genetic susceptibility plays a crucial role in lung cancer etiology. Certain inherited genetic variants can modify an individual's susceptibility to lung cancer, either independently or in combination with environmental exposures. For example, variations in genes involved in detoxification pathways, DNA repair mechanisms, and the metabolism of carcinogens have been associated with lung cancer risk[45]. Understanding these genetic factors can provide valuable insights into individual susceptibility and personalized risk assessment.

Recent research has also implicated air pollution as a significant contributor to lung cancer. Ambient air pollutants, such as particulate matter (PM), nitrogen oxides (NOx), volatile organic compounds (VOCs), and PAHs, have been associated with increased lung cancer incidence and mortality[46,47]. Understanding the underlying mechanisms and interactions between these factors can guide effective prevention strategies and targeted interventions to reduce the burden of lung cancer.

Genetic alteration as a risk for lung cancer

Lung cancer is a complex and heterogeneous disease characterized by a wide spectrum of genetic mutations. These mutations contribute to the development and progression of lung tumors, affecting their behavior, treatment response, and patient outcomes. Understanding the diverse landscape of genetic mutations in lung cancer is crucial for advancing precision medicine approaches and developing targeted therapies. The causes of lung cancer by oncogene activation, tumor suppressor inactivation, and accumulative gene damage by several mechanisms, such as abnormal expression,

genetic mutation, promoter methylation, and post-transcription and translation, are involved in many stages of tumor development as well as metastasis [48–55]. Over 50% of lung adenocarcinomas have somatic mutations in the five major oncogenes, KRAS, EGFR, ALK, ERBB2, and BRAF[56–60]. Inherited genetic variants also influence lung cancer susceptibility. Genome-wide association studies (GWAS) have identified common genetic variants associated with lung cancer risk, providing insights into biological pathways involved in disease development[61]. Variants in genes related to DNA repair, inflammation, metabolism, and xenobiotic detoxification have been implicated in lung cancer susceptibility, reflecting the interplay between genetic susceptibility and environmental exposures. Understanding these genetic factors can facilitate risk assessment, screening, and prevention strategies for individuals at higher risk of developing lung cancer[62,63]. Genomic instability, a hallmark of cancer, is often pronounced in lung tumors. Chromosomal aberrations, such as copy number alterations, chromosomal rearrangements, and chromothripsis, contribute to genomic instability and tumor heterogeneity [64]. These alterations disrupt the integrity of the genome, leading to the dysregulation of critical cellular processes and promoting oncogenesis. Epigenetic alterations also play a significant role in lung cancer development. DNA methylation, histone modifications, and non-coding RNA dysregulation can modulate gene expression patterns, leading to altered cellular phenotypes and promoting tumor initiation and progression[65]. Epigenetic alterations can result from both genetic and environmental factors, highlighting their intertwined relationship in lung cancer etiology.

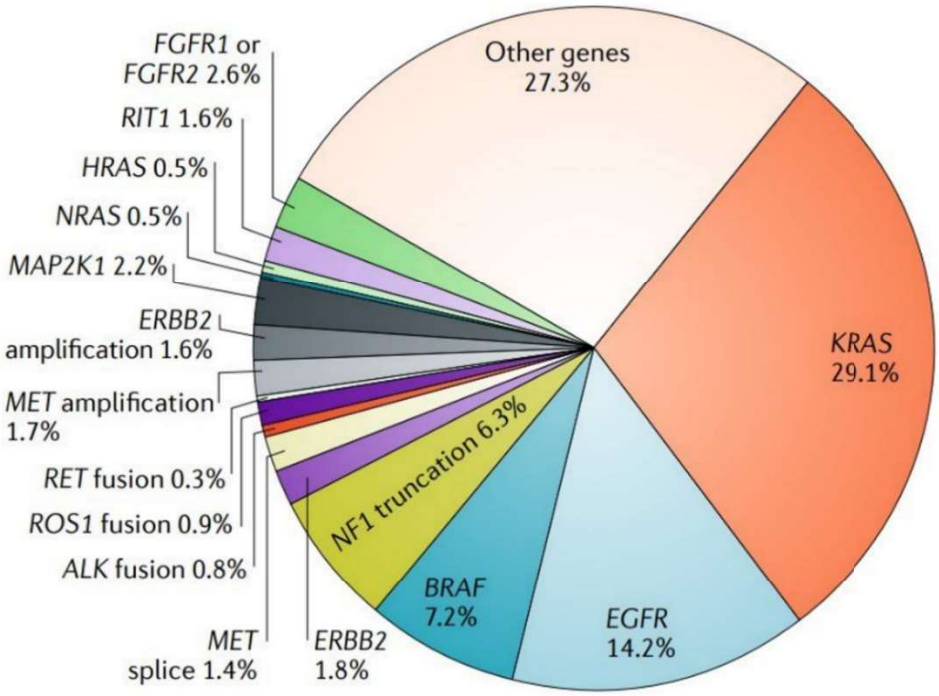

Figure F: The frequencies of common oncogenic driver mutations in early stage of NSCLC (Skoulidis *et al.*, 2019[48]).

As mentioned earlier, lung cancer can also arise as a result of tumor suppressor gene inactivation. Targeted therapeutics for tumor suppressor genes are more difficult than for oncogenes. Strategies include focusing on the activated genes that are downstream of a tumor suppressor gene that has been rendered inactive or finding synthetic lethal interactions are being investigated. Several tumor suppressors, including those already known to be engaged in lung adenocarcinoma, including TP53, STK11, CDKN2A, NF1, ATM, APC, and RB1, were dramatically mutated[56,59,60].

Somatic mutations in oncogenes and tumor suppressor genes are key genetic alterations in lung cancer. Oncogenes, such as EGFR, KRAS, ALK, and ROS1, can become activated through mutations or gene fusions, leading to aberrant signaling pathways and uncontrolled cell growth[66,67]. These genetic changes promote cell proliferation, survival, and resistance to apoptosis, driving tumor formation. These alterations contribute to the acquisition of hallmark characteristics and aggressive behavior of lung tumors. One of the most prevalent mutations in lung cancer is the epidermal growth factor receptor (EGFR) gene mutation. EGFR mutations, particularly exon 19 deletions and the L858R point mutation in exon 21, are frequently observed in non-small cell lung cancer (NSCLC) patients, especially in those who have never smoked[68,69]. These mutations lead to constitutive activation of the EGFR pathway, promoting uncontrolled cell proliferation and survival. Targeted therapies, such as EGFR tyrosine kinase inhibitors (TKIs), have shown remarkable clinical efficacy in patients harboring EGFR mutations [70,71]. However, acquired resistance mechanisms, such as the T790M secondary mutation, pose challenges to long-term treatment effectiveness[72,73]. Another notable mutation in lung cancer is the mutation in the Kirsten rat sarcoma viral oncogene homolog (KRAS) gene. KRAS mutations are frequently observed in NSCLC, particularly in adenocarcinoma, and are associated with poor prognosis and resistance to targeted therapies[74–76]. KRAS mutations result in constitutive activation of downstream signaling pathways, such as the mitogen-activated protein kinase (MAPK) pathway, promoting cell proliferation and survival. Targeting KRAS has been a longstanding challenge; however, recent breakthroughs in the development of KRAS-specific inhibitors offer new hope for effective targeted therapy[77,78].

Mutations in the BRAF gene, encoding the V-Raf murine sarcoma viral oncogene homolog B (BRAF) kinase, play a pivotal role in cellular signaling, growth, and survival. In NSCLC, BRAF mutations, occur predominantly in never-smokers, women, and aggressive histological types. BRAF mutations are relatively rare, occurring in approximately 3-5% of non-small-cell lung cancer (NSCLC), primarily in the adenocarcinoma subtype. These mutations are categorized into three classes, with the most common being V600 (class 1), while the less common mutations fall into non-V600 categories (classes 2 and 3)[79–81]. Anaplastic lymphoma kinase (ALK) gene rearrangements represent another important mutation in lung cancer. ALK rearrangements occur in a subset of NSCLC patients, typically in younger individuals with a never-smoker history[82]. ALK fusions result in the

constitutive activation of the ALK kinase domain, driving oncogenic signaling. ALK inhibitors, such as Crizotinib, have shown significant clinical responses in patients with ALK-positive lung cancer[83]. However, acquired resistance mechanisms, such as secondary ALK mutations or bypass signaling activation, limit the long-term efficacy of ALK inhibitors[84]. Furthermore, other genetic alterations, including ROS1 rearrangements, MET amplifications, RET fusions, and HER2 mutations, have been identified in subsets of lung cancer patients, highlighting the molecular heterogeneity of the disease[85–88]. These alterations have shown sensitivity to targeted therapies, leading to improved treatment outcomes in specific patient populations.

Recent advances in genomic technologies have enabled the characterization of lung cancer at unprecedented resolution, unveiling the landscape of genetic alterations and their clinical implications. Comprehensive genomic profiling has identified rare driver mutations and actionable alterations that can guide targeted therapies [89,90]. The identification of specific genetic alterations in lung cancer has paved the way for personalized medicine approaches, improving treatment outcomes and prognosis.

An interruption in the cellular proteostasis network can occur due to a range of factors, such as genetic mutations, environmental stressors, and the natural aging process. This disruption leads to the buildup of misfolded or impaired proteins within the cells. The resulting condition, known as proteotoxic stress, emerges when there is an inequilibrium between the production of misfolded or damaged proteins and the cell's ability to effectively fold, repair, or degrade these proteins using its molecular machinery. As a consequence, protein aggregates gather and the harmonious balance within the cell gets disturbed. This process culminates in cellular malfunction and the onset of various diseases[23]. Proteotoxic stress has emerged as a key contributing factor in the pathogenesis of various neurodegenerative disorders and age-related diseases. Morimoto *et al.* (2008) provided a comprehensive overview of proteotoxic stress and its implications in human diseases[91]. It highlights the role of misfolded proteins in initiating cellular stress responses and the activation of stress-inducible molecular chaperones and proteolytic systems. These stress responses, collectively known as the proteostasis network, aim to restore protein homeostasis and promote cellular survival. Under normal conditions, the cellular proteostasis network maintains protein quality control through protein folding, refolding, and degradation processes[92]. Proteotoxic stress is particularly relevant in neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and Huntington's disease[93]. In these conditions, specific proteins associated with the respective diseases, such as amyloid-beta, alpha-synuclein, and huntingtin, adopt abnormal conformations and aggregate within neurons, leading to neuronal dysfunction and cell death. Understanding the underlying mechanisms of proteotoxic stress and its consequences has opened up new avenues for therapeutic interventions. Strategies aimed at enhancing the cellular protein quality control machinery, promoting protein

refolding, or facilitating protein degradation pathways have shown promise in preclinical studies and hold potential for the development of novel therapies to combat proteotoxicity-associated diseases[94–96].

Protein degradation machinery

To counteract proteotoxic stress, cells have evolved intricate protein degradation pathways that facilitate the removal of abnormal proteins and maintain protein quality control. These degradation pathways, such as the ubiquitin-proteasome system and autophagy-lysosome pathway, play crucial roles in maintaining cellular proteostasis and preventing the buildup of toxic protein aggregates[97,98].

Autophagy is an intracellular degradation pathway that targets bulk cytoplasmic components, including long-lived proteins and organelles. It involves the formation of double-membrane vesicles called autophagosomes, which sequester cellular cargo and fuse with lysosomes to form autolysosomes, where degradation occurs[99]. Autophagy serves as a crucial mechanism for the removal of damaged organelles, protein aggregates, and pathogens. The impairment of autophagy has been implicated in the pathogenesis of various diseases, including neurodegenerative disorders and cancer[100]. Within mammalian cells, three main forms of autophagy exist microautophagy, macroautophagy, and chaperone-mediated autophagy (CMA). Microautophagy is a mechanism through which cellular materials are internalized by lysosomes via the deformation of lysosomal membranes. This allows lysosomes to engulf soluble contents, contributing to cellular maintenance[101,102].

In contrast to microautophagy and chaperone-mediated autophagy, macroautophagy exhibits distinctive attributes in its sequestration mechanism. Within this process, the cell initiates the formation of a bilayer-membrane sequestering entity termed the phagophore, which subsequently matures into an autophagosome. Following its delivery to the lysosome, the enclosed cargo undergoes degradation, resulting in the release of macromolecules back into the cytosol for subsequent reuse[103,104]. Chaperone-mediated autophagy (CMA), a distinct autophagic pathway identified only in mammalian cells, stands apart from macroautophagy and microautophagy by its selectivity. Unlike the other forms that can engulf various cellular materials, CMA is precise, targeting substrates with a specific pentapeptide motif Lys-Phe-Glu-Arg-Gln (KFERQ)[105]. CMA involves the transport of proteins to the lysosome using the chaperone known as heat shock cognate 71 kDa protein (HSC70), along with co-chaperone proteins. Subsequently, these proteins are taken in by the lysosomal-associated membrane protein 2A (LAMP2A) receptor located on the lysosomal membrane for degradation[106,107]. This mechanism is responsible for breaking down a diverse array of substrate

proteins, encompassing glycolytic enzymes, transcription factors, calcium-binding proteins, and more[108].

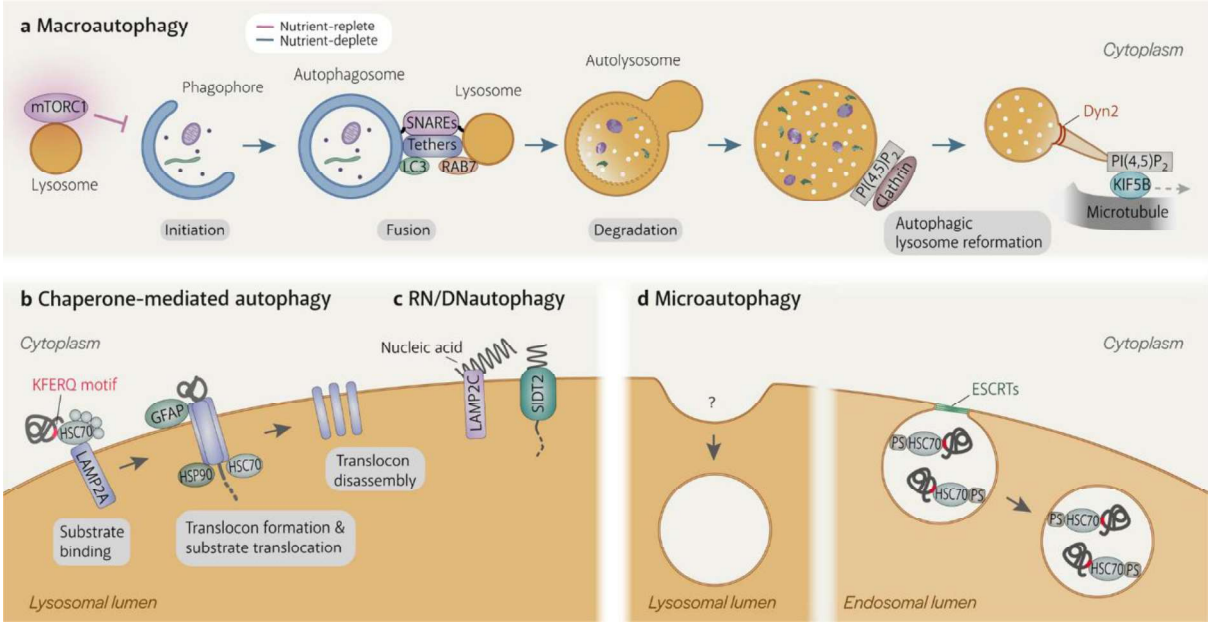

Figure G: Mechanism and function of autophagy processes (Yim *et al.*, 2020 [109]).

A considerable portion of intracellular proteins undergo degradation through the ubiquitin-proteasome pathway (UPP) subsequent to ubiquitin tagging [110]. Consequently, the UPP is a pivotal regulatory modification mechanism that intricately governs essential cellular processes such as cell cycle progression, signal transduction cascades, DNA damage response, apoptotic pathways, and immune responses[111]. The Ubiquitin-Proteasome System (UPS) plays a critical role in the elimination of short-lived and regulatory proteins, as well as the clearance of misfolded or damaged proteins. Dysregulation of the UPS has been implicated in the pathogenesis of neurodegenerative diseases, including Alzheimer's disease and Parkinson's disease[112].

Ubiquitin-Proteasome System (UPS):

Ubiquitin, initially termed 'ubiquitous immunopoietic polypeptide' (UBIP) in 1975, is a conserved protein consisting of 76 amino acids and is present across all eukaryotic cells [113,114]. The process of ubiquitination (also recognized as ubiquitylation or ubiquitinylation) differs from lysosomal protein breakdown and is ATP-dependent, functioning as a means of protein degradation [115]. This intricate process was unveiled in 1983 as a cascade enzymatic reaction characterized by three fundamental steps. Firstly, ubiquitin undergoes activation via an E1-activating enzyme, leading to the formation of a thioester linkage with the catalytic cysteine. Subsequently, ubiquitin is translocated to an E2 conjugating enzyme through a trans-esterification reaction. The third and final step involves the transfer of ubiquitin from the charged E2 enzyme to the intended substrate with the assistance of an E3 ligase enzyme. This E3 ligase facilitates the creation of an isopeptide bond between the substrate's

lysine residue and the C-terminal glycine of ubiquitin [116]. Ultimately, this covalent attachment of ubiquitin molecules to target proteins, mark them for recognition and subsequent degradation by the 26S proteasome[117].

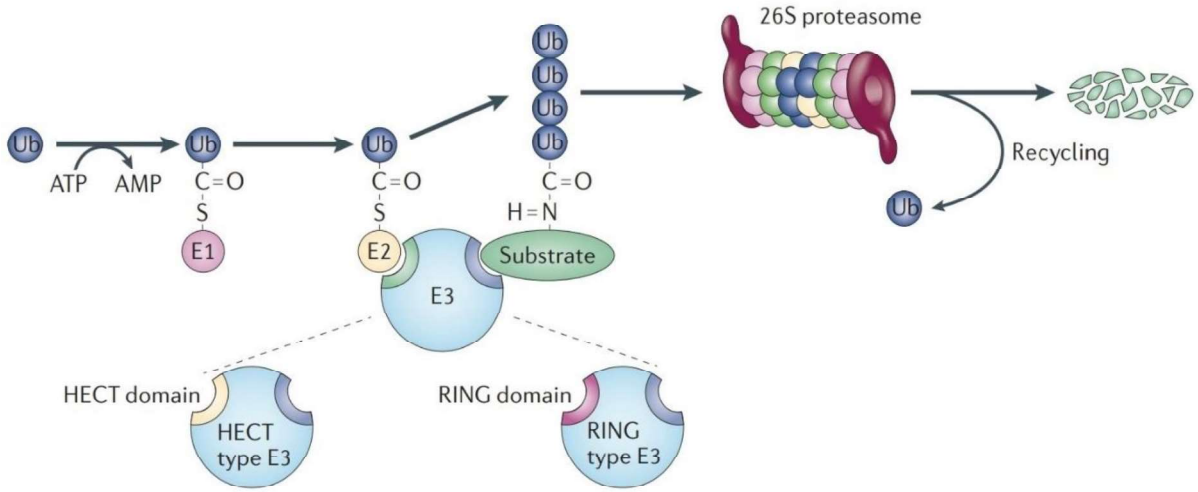

Figure H: An overview of the ubiquitin-proteasome system (Hatakeyama S *et al.*, 2011[55]).

Both the UPS and autophagy-lysosome pathways are tightly interconnected and work synergistically to maintain protein homeostasis and prevent the accumulation of toxic protein aggregates. They are regulated by a complex network of molecular chaperones, ubiquitin ligases, and adaptor proteins that coordinate the recognition, ubiquitination, and degradation of abnormal proteins. Disruption of these degradation pathways can lead to the buildup of misfolded proteins and contribute to disease pathology. Understanding the intricate interplay between protein degradation pathways and proteotoxic stress is crucial for developing therapeutic strategies aimed at restoring proteostasis and ameliorating disease-associated protein aggregation. Pharmacological modulation of these degradation pathways or the enhancement of cellular clearance mechanisms holds great promise for the treatment of proteotoxic stress-related disorders[97,118,119]. The therapeutic potential of targeting proteasome-mediated degradation was effectively demonstrated in 2003 when the FDA approved the proteasome inhibitor bortezomib (Velcade®) for treating relapsed or refractory multiple myeloma [120]. However, a pivotal shift in ubiquitin-based drug discovery emerged earlier, around 2001, with the inception of PROteolysis TArgeting Chimeras (PROTACs). In this novel approach, the $SCF^{\beta\text{-}TRCP}$ E3 ubiquitin ligase was harnessed to facilitate the targeted protein degradation (TPD) of methionine aminopeptidase-2 (MetAp-2) [121,122].

- **E3 Ubiquitin ligases**

Ubiquitination stands as a significant post-translational modification, intricately involved in protein degradation and a spectrum of cellular processes. Over more than twenty years, it has

held a focal point within the biotech and biopharmaceutical sectors[95,96,121]. E3 ligases play a pivotal role in cancer, acting as either promoters or suppressors. Their specificity in cancer pathways has led to the development of targeted compounds for cancer therapy. These ligases influence key cancer hallmarks such as sustained proliferation, immune evasion, inflammation, and apoptosis evasion. Small compounds targeting E3 ligases hold promise for cancer treatment, highlighting their significance in potential therapeutic strategies[123].

Among the Tripartite Motif (TRIM) family proteins, distinguished by their established E3 ubiquitin ligase activities, a vast array of cellular, physiological, and pathophysiological processes are encompassed, spanning from cancer to rare genetic disorders[50,124]. The TRIM family plays a significant role in cancer initiation, progression, and resistance to therapy. Members of the TRIM family exhibit both oncogenic and tumor-suppressive functions in various human cancer types[125–128]. These proteins also hold promise as potential biomarkers for cancer diagnosis and prognosis. The mechanisms underlying the involvement of TRIM family members in tumorigenesis and cancer development include interactions with dysregulated signaling pathways such as JAK/STAT, PI3K/AKT, TGF-β, NF-κB, Wnt/β-catenin, and p53 hub. The intricate interplay between TRIM proteins and these signaling pathways contributes to their impact on cancer-related processes[129].

While recent times have seen numerous E3 ubiquitin ligases gain prominence as favored selections for major pharmaceutical companies and burgeoning biotech startups, the recognition of TRIM E3 ubiquitin ligases in the domain of drug discovery seems comparatively limited. These innovations have left a lasting impact on the biopharmaceutical community, influencing established biotech giants as well as emerging startups. Furthermore, an exploration into the potential trajectory of TRIM family proteins in the realm of E3 ubiquitin ligase-centered drug discovery will be undertaken[130].

This departure from conventional 'inhibition' to 'inducing degradation' through E3 ligases laid fertile ground for a contemporary biopharmaceutical theme – 'drugging the undruggable' [131]. This innovation is escorted in a new era characterized by startups and technological advancements inspired by ubiquitin-related concepts. Today, degradation has emerged as the preferred strategy over mere inhibition. E3 ligases, constituting the largest and most diverse protein family within the ubiquitin pathway with approximately 700 members (comprising around 5% of the human genome), have taken the forefront in the realm of drug discovery [131,132].

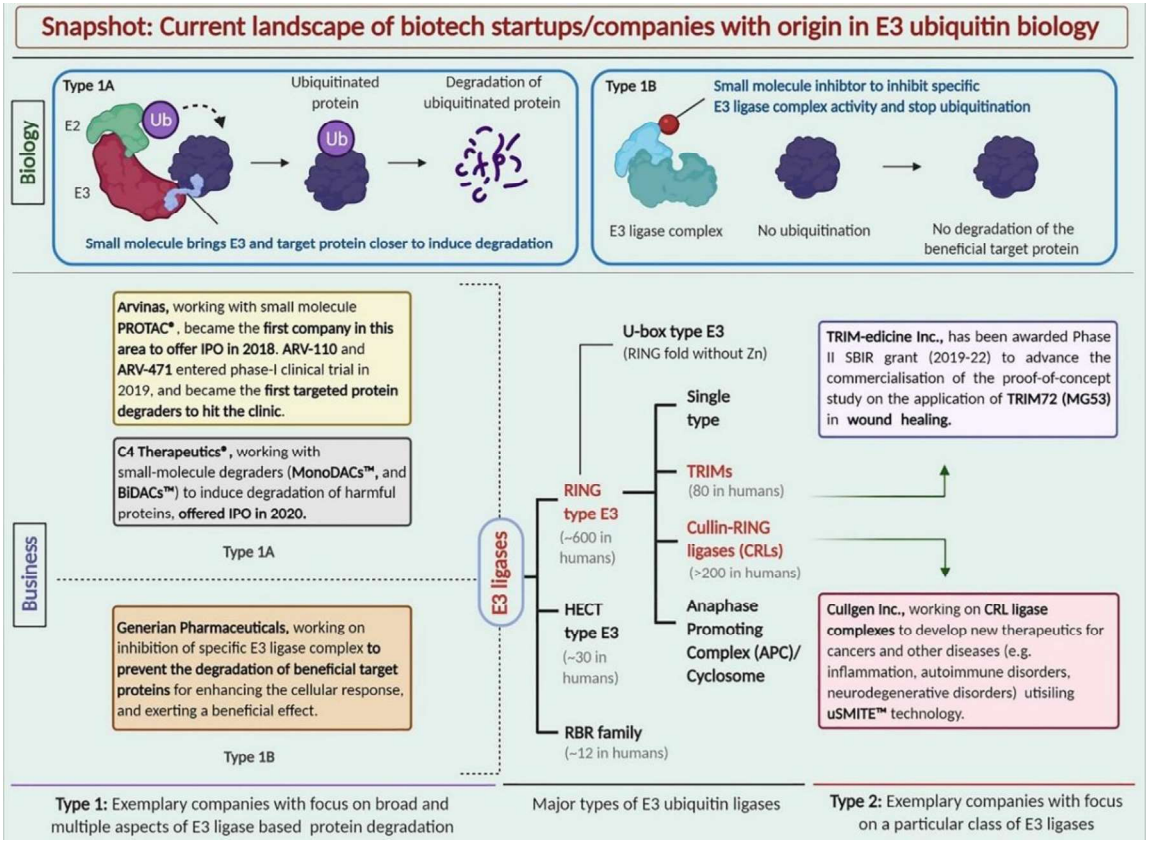

Figure I: Types of E3 ubiquitin ligases and the current landscape of biotech startups with their origin in E3 ubiquitin biology. Schematic representation of exemplary startups working with different approaches in the space of E3 ubiquitin drug discovery (Bhaduri U *et al.*, 2021[130]).

- **TRIM** (**TRI**partite **M**otif)

TRIM Proteins are composed of three conserved zinc-binding domains in the N-terminal part of the protein that is RING (really interesting new gene) finger domain, one or two B-box domains, and Coiled-coil domain (RBCC Domain) and a different C-terminal domain, although some TRIMs do not have RING Finger domain [133–136]. There are now more than 80 different TRIM proteins that have been identified in humans.

General TRIM protein's structure and functions:

A) N-Terminal domains:

A.1) RING (Really Interesting New Gene) Finger Domain

A.2) One or two B-box domains

A.3) Coiled-coil domain

B) C-terminal domains

A) N-Terminal domain:

A.1) RING (Really Interesting New Gene) Finger Domain:

Almost all TRIMs with the RING-Finger domain with a cysteine-rich motif ($Cys_3HisCys_4$ or $Cys\text{-}X_2$-

Cys-X_{9-39}-Cys-X_{1-3}-His-X_{2-3}-Cys-X_2-Cys-X_{4-48}-Cys-X_2-Cys, where X is any amino acid) that 40 to 60 residues facilitate binding with two zinc atoms, where four cysteines bind a one zinc ion and three cysteines and one histidine residue binds with another zinc ion in a cross-brace manner; therefore, it is known as a zinc-binding motif [135,137].

Many TRIM proteins family members function as E3 ubiquitin ligases because of the presence of the RING-finger domain for ubiquitylation in the process of post-translational modification that helps in protein degradation [138].

A.2) B-box domains:

Type 1 and 2 B-box domains are present in more than 1,500 proteins from all multicellular species and some unicellular eukaryotes [139]. Some TRIM proteins have both type 1 and type 2 B-box domains, while others have only type 2 B-box domains adjoining their RING domain. These two types of B-boxes have little sequence similarity in terms of the presence of seven or eight zinc-binding residues at different locations in both types. In some cases, B-box domains are also present in proteins that do not have the RING finger domain or coiled-coil domains [136,140,141].

Type 1 B-box contain Cys-X_2-Cys-X_{7-10}-Cys-X_2-Cys-X_{4-5}-Cys-X_2-Cys-His-X_{3-6}-His-X_{2-8}-His (Cys_5(Cys/His) His_2) and **Type 2 B-box** contain Cys-X_{2-4}-Cys/His-X_{7-10}-Cys-X_7-Cys-X_2-Cys-X_{-3-6}-His-X_{2-8}-His (Cys(Cys/His)C_3His_2), (where X is any amino acid) [136,141,142].

Type 1 and 2 B-box domains are not homologous in their zinc-binding motifs, but their secondary and tertiary structures are very similar. Many B-box 2-containing proteins are involved in the ubiquitylation process by regulating the function of the RING-finger domain because it can multimerize and interact with other proteins, while the B-box 1 domain enhances the activity for substrate recognition and ubiquitin ligase activity [142,143]. B-box domains function as a protein-protein interactive motif and some TRIMs, which may be important for TRIM oligomerization[144–146].

A.3) Coiled-coil domain:

Recent studies proved that TRIM proteins form a homodimer and, in some cases, form a heterodimer in an anti-parallel manner by their coiled-coil domain, which is responsible for the ubiquitylation of substrates via separation of two catalytic RING domains [143,147–150]. In TRIMs, this domain of coiled coils domain can bind with other coiled-coil domains and mediate homo-oligomeric and hetero-oligomeric interactions and this domain protein oligomerization, while deletion of this domain prevents TRIM self-associations [134,151].

B) C-terminal domain:

Based on variable domains, human TRIM protein family members are divided into different C-I to C-XI subfamilies (Fig. J). Different motifs are present alone or in combination with other, like NHL (NCL-1/HT2A/LIN-41 repeat), MATH (meprin and tumor necrosis factor receptor-associated factor homology), FIL (filamin-type IG domain, B30.2-like/RFP (Ret finger protein)/SPRY (SplA and ryanodine receptor) (the largest subgroup in humans), ARF (ADP-ribosylation factor), PHD (plant homeodomain finger), COS (C-terminal subgroup one signature), BROMO domains, ACID (Acid-rich region), TM (transmembrane domain) and FN3 (fibronectin type III) [134,141,151–154].

As a result, TRIM proteins act as E3 ubiquitin ligases. Through their CC domain, TRIM proteins form homodimers in an anti-parallel arrangement, indicating that the two catalytic RING domains, separated by the extended CC domain, collaborate in the process of ubiquitinating substrates[150]. The TRIM protein family is associated with various pathophysiological processes such as cell proliferation, DNA repair, signal transduction, and transcription [155–159].

Wang Y. *et al.* (2015) have conducted research that highlights the regulatory roles of TRIMs in multiple signaling pathways mediated by interferon response and inflammation [160]. It regulates various cellular processes, including protein quality control, intracellular signaling, innate immunity, inflammation, transcription, autophagy, cell metabolism, developmental processes, chromatin modification, carcinogenesis, and many others [55,126,134,149,154,161–164]. Alterations in TRIM genes are responsible for several human diseases, such as genetic diseases, cardiovascular diseases, metabolic diseases, developmental disorders, neurodegenerative diseases, infectious diseases, and cancer [50]. TRIM proteins have been implicated in several diseases, even in neuropsychiatric disorders such as multiple sclerosis (MS) [165], where susceptibility to MS was linked to single nucleotide polymorphisms (SNPs) in TRIM10, TRIM15, TRIM26, TRIM39, and TRIM40 genes. In schizophrenia patients, TRIM3 and TRIM26 were seen to be upregulated in the post-mortem dorsolateral prefrontal cortex as compared to healthy individuals [166].

It was also reported that the pro-inflammatory gene variant TRIM20 is associated with the age onset of Alzheimer's disease (AD) [167]. TRIM proteins were also linked to developmental diseases, as in Opitz syndrome (OS). OS is a genetic disorder characterized by midline defects such as cleft lip, cleft palate, and laryngotracheal abnormalities. X-linked OS is caused by at least loss-of-function mutations of TRIM18, and the mutations are dispersed throughout the gene [168]. TRIM50 is possibly involved in Williams-Bauren syndrome (WBS) – a multisystemic disorder characterized by distinctive facial features and cardiovascular, endocrine, and neurodevelopmental abnormalities [169].

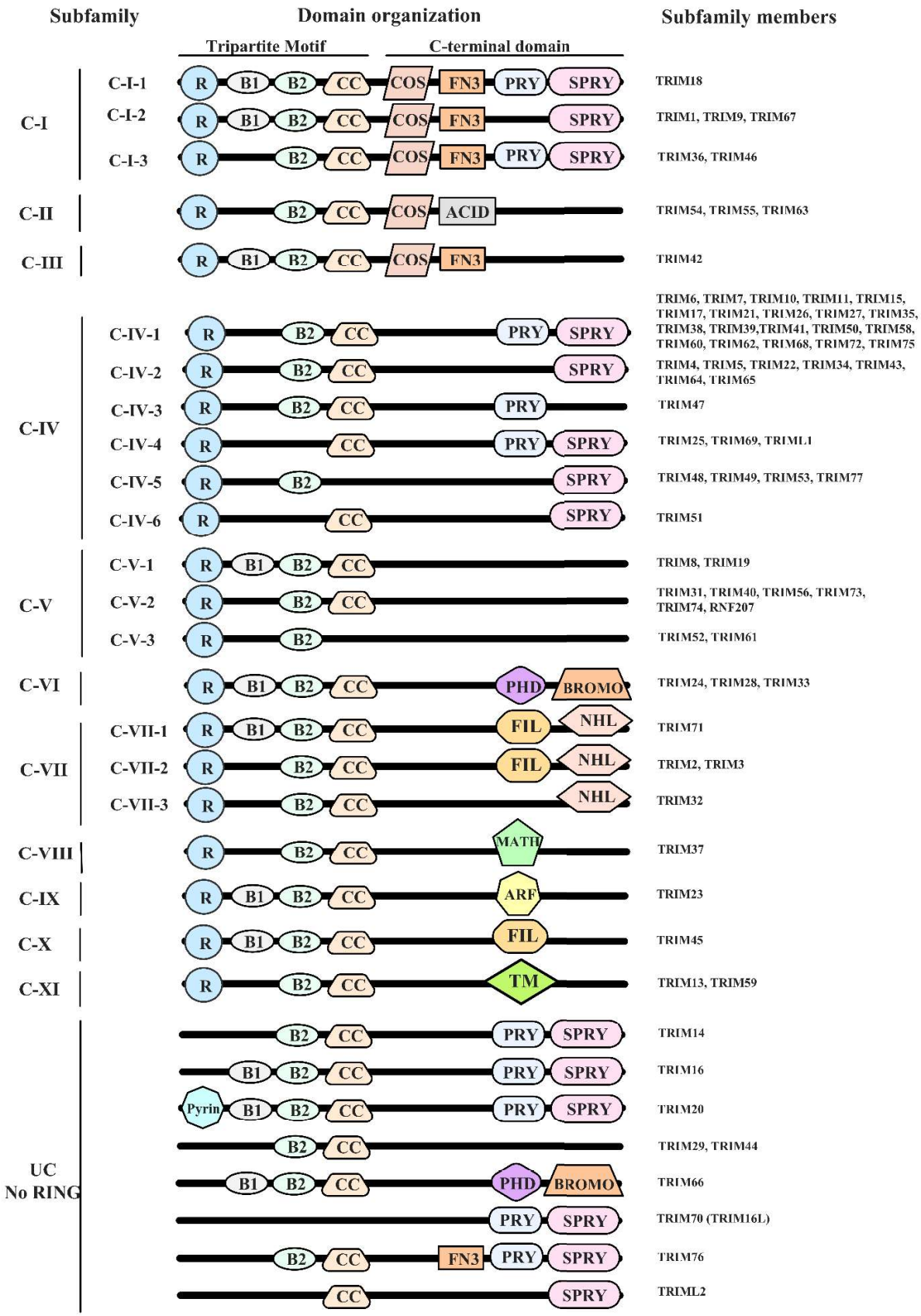

Figure J: TRIMs classification with different subfamilies.

TRIM3 gene was revealed to have lower methylation levels in obesity as seen in a genome-wide methylation analysis using peripheral blood leukocytes[170]. Genomic studies from acute myocardial infarction (AMI) patients have shown that the TRIM20 gene likely increases the risk of AMI [171]. TRIM63 plays an important role in the regulation of cardiac hypertrophy and also in atrophy conditions of muscle tissue [172]. Thus, TRIM proteins were also seen to be a part of several cardiovascular and metabolic diseases. TRIM proteins are also seen to influence the pathogenesis of infectious diseases. Rhesus monkey TRIM5α has been identified as an essential factor for the restriction of the Human immunodeficiency virus type I (HIV-1) in Old World monkeys [173]. TRIM22 functions as a restriction factor of HIV through the inhibition of long terminal repeat (LTR) promoter-driven transcription [174]. TRIM proteins may cause diseases or modify symptoms of diseases by modulating the type of polyubiquitin chain of their substrates.

Role of TRIM proteins and Cancer

TRIM proteins have emerged as key regulators in various cellular processes, including innate immunity, antiviral defense, cellular signaling, and carcinogenesis. The dysregulation of TRIM proteins has been implicated in the development and progression of cancer, highlighting their significance in tumorigenesis. Hatakeyama (2017) provides the role of TRIM proteins in cancer and their diverse functions in modulating tumor biology [154]. It elucidates the molecular mechanisms by which specific TRIM proteins act as either tumor suppressors or oncogenes, exerting control over critical cellular processes involved in cell growth, apoptosis, DNA repair, and cell migration. TRIM proteins exhibit diverse functions in cancer by interacting with various signaling pathways. For instance, TRIM29, also known as ATDC (ataxia telangiectasia group D complementing), has been implicated as an oncogene in multiple cancer types, promoting cell survival, proliferation, and metastasis through its interactions with signaling molecules such as nuclear factor kappa B (NF-κB) and beta-catenin[175]. Notably, TRIM19, which is encoded by the promyelocytic leukemia (PML) gene, displays a specific involvement in the t(15;17) translocation observed in acute promyelocytic leukemia (APL). This translocation event leads to the formation of a fusion protein between PML and retinoic acid receptor-α (RARα), thereby influencing the development of APL[55,176–178]. In addition to their direct involvement in tumorigenesis, several TRIM proteins, including TRIM19, TRIM24, TRIM25 (also known as EFP), and TRIM68, exert regulatory control over the activation of nuclear receptors such as RARα and hormone receptors. Consequently, these TRIM proteins play critical roles in the progression of leukemia and the development of breast and prostate cancers. Moreover, TRIM13, TRIM19, TRIM24, TRIM28, and TRIM29 can modulate the stability or transcriptional activity of p53, a paramount tumor suppressor molecule with significant implications in the control of cancer development and progression[55].

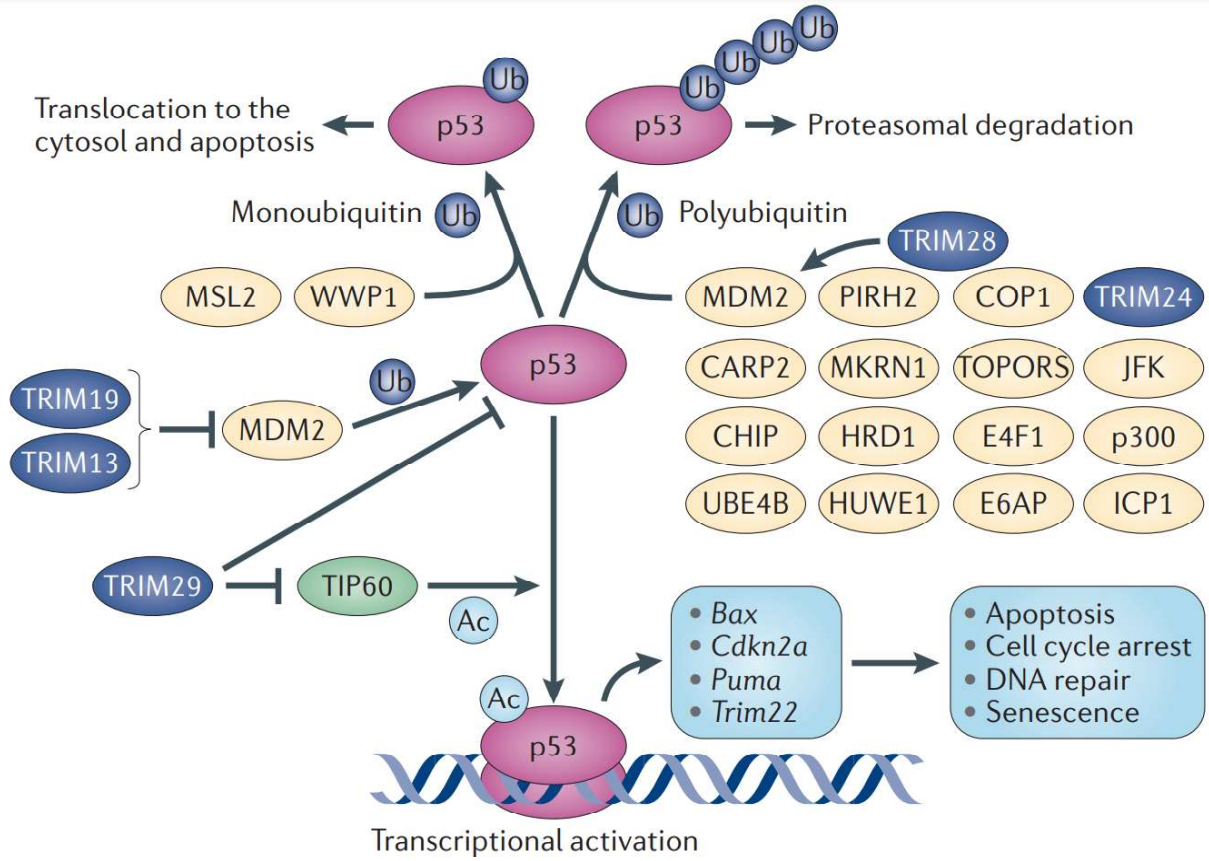

Figure K: Role of TRIM proteins in the regulation of p53 (Hatakeyama S. *et al.,* 2011[55]).

It was interesting to note that certain TRIM genes exhibit tumor-inhibiting properties. The expression of these tumour suppressor TRIMs is relatively low in cancers and thus inducing cancer progression. In other words, TRIM proteins positively or negatively regulate oncogenesis and tumour progression by affecting pathways such as cell proliferation, DNA repair, and apoptosis. For example, TRIM24 can also function as a potent liver-specific tumour suppressor by attenuating RARα-mediated transcription in mice [179]. TRIM27 is another TRIM gene that is reported to function to either enhance or suppress tumour development. It can function as an oncogene [180] and also induces apoptosis through a mechanism that involves JUN N-terminal kinase [181]. TRIM proteins have been identified as key regulators of EMT through various signaling pathways. For instance, TRIM50 acts as a tumor suppressor by directly targeting snail and reversing EMT in hepatocellular carcinoma and pancreatic cancer[182,183]. TRIM58, a tumor suppressor, plays a crucial role in inhibiting tumorigenesis and progression by modulating epithelial-to-mesenchymal transition (EMT) through the Wnt-β-catenin signaling pathway. Suppression of TRIM58 expression may serve as a potential marker for early detection of colorectal cancer (CRC), highlighting its clinical significance in CRC management and prognosis[184]. Many other TRIMs are also implicated in EMT modulation[129]. Further research is needed to elucidate the detailed mechanisms underlying the involvement of TRIM proteins in the progression of cancer. Furthermore, TRIM proteins are involved in DNA damage response and repair. TRIM proteins such as TRIM24, TRIM28, TRIM33, and TRIM66 contribute to

the maintenance of genome integrity by participating in DNA repair pathways, such as homologous recombination and nucleotide excision repair[185]. Dysregulation of these TRIM proteins can compromise DNA repair mechanisms, leading to genomic instability and increased susceptibility to cancer development. Downregulation or loss of specific TRIM proteins has been associated with poor prognosis and advanced disease in various cancer types. For example, reduced expression of TRIM29 is correlated with poor survival outcomes in hepatocellular carcinoma patients [186]. Understanding the tumor-suppressive roles of TRIM proteins has important implications for cancer diagnosis, prognosis, and therapy. These proteins have the potential to serve as diagnostic and prognostic markers, aiding in the identification of high-risk patients and guiding treatment decisions. Moreover, targeting specific TRIM proteins or modulating their activity may represent a promising therapeutic approach to restore tumor suppressor functions and inhibit cancer progression.

The involvement of TRIM proteins in cancer has been extensively studied in multiple tumor types, and their specific roles in lung cancer are increasingly being recognized. The dysregulation of TRIM proteins has been implicated in the pathogenesis of lung cancer, highlighting their potential as diagnostic markers and therapeutic targets in this deadly disease. Zhan *et al.* (2021) provide a comprehensive analysis of the TRIM family proteins in lung cancer and their functional implications in tumor biology [128]. The study highlights the differential expression patterns and biological functions of specific TRIM proteins in lung cancer, shedding light on their potential as prognostic indicators and therapeutic candidates. Several TRIM proteins have been identified as important players in lung cancer. For example, TRIM29, also known as ATDC, is upregulated in lung adenocarcinoma and associated with poor patient prognosis. It promotes cell proliferation, migration, and invasion through interactions with various signaling pathways, including Wnt/β-catenin and NF-κB[175,187,188]. Similarly, TRIM24 has been implicated as an oncogene in lung cancer, promoting tumor growth and metastasis through its regulation of gene expression [189].

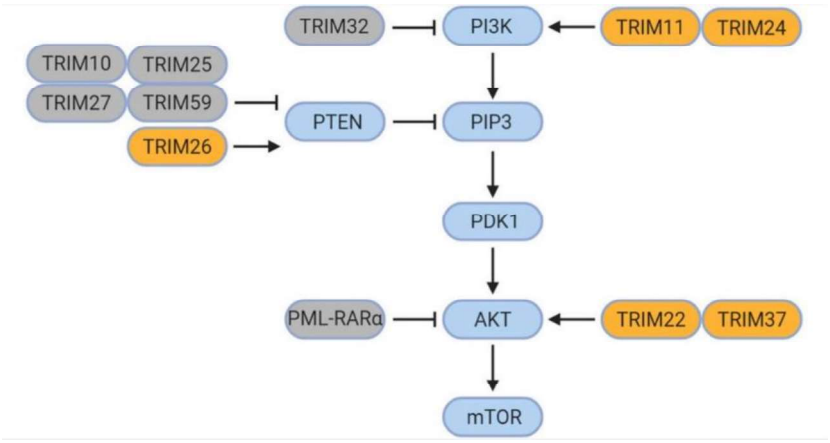

Figure L: TRIM proteins regulate key components of the PI3K/AKT pathway (Zhan W *et al.*, 2021[55]).

Understanding the precise roles of TRIM proteins in lung cancer provides valuable insights into the underlying molecular mechanisms of tumorigenesis and may have significant clinical implications. TRIM proteins hold promise as potential diagnostic and prognostic biomarkers, aiding in the early detection of lung cancer patients. Moreover, targeting specific TRIM proteins or modulating their activity may represent a promising therapeutic strategy for lung cancer treatment, either as single agents or in combination with existing therapies.

Different types of therapies used to treat Lung cancer

Lung cancer is a complex disease with diverse treatment options. The choice of therapy depends on factors such as tumor stage, histological subtype, patient characteristics, and underlying genetic alterations. A combination of therapies is often employed to achieve optimal outcomes. The management of lung cancer requires a multidisciplinary approach, including various therapeutic modalities. Surgery, radiation therapy, chemotherapy, targeted therapy, immunotherapy, and emerging strategies all play crucial roles in improving patient outcomes[190].

Individualized treatment decisions, considering tumor characteristics and patient factors, are essential for optimizing therapeutic efficacy. Ongoing research and clinical trials continue to expand the treatment options and improve the prognosis for lung cancer patients. Surgical resection remains the primary treatment option for early-stage non-small cell lung cancer (NSCLC) and certain cases of small cell lung cancer (SCLC). Studies have demonstrated that surgical removal of the tumor, along with appropriate lymph node dissection, significantly improves survival rates[191]. Minimally invasive techniques, such as video-assisted thoracic surgery (VATS) and robotic-assisted surgery, have shown comparable outcomes to open surgery, with reduced morbidity and improved patient recovery[192]. Radiation therapy, either alone or in combination with surgery and/or chemotherapy, plays a crucial role in the management of lung cancer. External beam radiation therapy (EBRT) is commonly employed, utilizing high-energy X-rays or protons to target and kill cancer cells. Advanced techniques, including intensity-modulated radiation therapy (IMRT) and stereotactic body radiation therapy (SBRT), have shown promising results in delivering precise radiation doses to the tumor while sparing surrounding healthy tissues [193]. Radiotherapy can be curative in early-stage disease and palliative in advanced stages to alleviate symptoms and improve quality of life[194]. Chemotherapy is a systemic treatment option that involves the administration of cytotoxic drugs to destroy cancer cells. Platinum-based doublet regimens, such as cisplatin and pemetrexed, are commonly used for advanced NSCLC, whereas etoposide combined with platinum agents is utilized in SCLC [195].

Recent advancements have led to the development of immune checkpoint inhibitors (ICIs) in combination with chemotherapy, showing improved survival outcomes[196]. However, chemotherapy is associated with significant side effects, including hematological toxicities and gastrointestinal

complications. Targeted therapies aim to inhibit specific molecular targets that drive tumor growth and survival. In NSCLC, several genetic alterations, such as EGFR mutations and ALK rearrangements, have been identified as actionable targets. Tyrosine kinase inhibitors (TKIs), such as gefitinib and crizotinib, have demonstrated substantial clinical efficacy in patients with these specific alterations[83]. However, acquired resistance remains a challenge, necessitating the development of novel therapeutic strategies. Immunotherapy, particularly immune checkpoint inhibitors, has revolutionized the treatment landscape for advanced NSCLC. Agents targeting programmed death-ligand 1 (PD-L1) or programmed cell death protein 1 (PD-1), such as pembrolizumab have shown improved overall survival compared to chemotherapy in PD-L1-positive patients[197]. Immune-related adverse events are potential side effects associated with immunotherapy, which require close monitoring and management. Several emerging therapeutic strategies are being explored in lung cancer treatment. These include combination therapies involving targeted agents and immunotherapies, adoptive cell transfer (ACT) using chimeric antigen receptor (CAR) T cells, and novel molecular inhibitors targeting resistance mechanisms[198–200]. Early-stage clinical trials have shown promising results, providing avenues for future research and personalized treatment approaches.

Therapeutic potential of Interferons in immunotherapy

Interferons (IFNs) are a group of cytokines that are vital for regulating the immune system. They are classified into three major types: Type I (IFN-α, IFN-β, IFN-ε, IFN-κ, and IFN-ω), Type II (IFN-γ), and Type III (IFN-λ). The diverse immunomodulatory functions of interferons make them valuable tools in immunotherapy. Promising clinical studies have demonstrated the potential of interferons in the treatment of cancer, viral infections, and autoimmune disorders [201–203]. Interferons (IFNs) regulate immunity and exhibit anti-cancer effects across various cancers. IFN signaling aids cancer therapy success but can hinder eradication and enable immune escape. IFNs have a dual impact on the cancer immune response. Recent research demonstrates IFNs' influence on the cancer immunity cycle stages: antigen release, presentation, T cell activation, T cell infiltration, and cancer cell elimination. Additionally, ongoing clinical trials explore diverse interferon types in cancer treatment[204].

Lung cancer poses significant challenges due to its highly heterogeneous nature. In recent years, immunotherapy has emerged as a successful treatment approach for lung cancer, utilizing the body's immune system to target and eliminate cancer cells [205]. Interferons have shown remarkable efficacy in cancer immunotherapy, leading to cancer regression. Consequently, they have been recognized as excellent immunotherapeutic agents for cancer treatment. Growing evidence supports the synergistic effect of enhancing both endogenous and exogenous type I interferons on anti-tumor immune responses. The US FDA has approved interferons as the first human immunotherapeutic for cancer

[206]. This has prompted the development of clinical studies investigating novel targeted therapeutic approaches in combination with interferons [205,207].

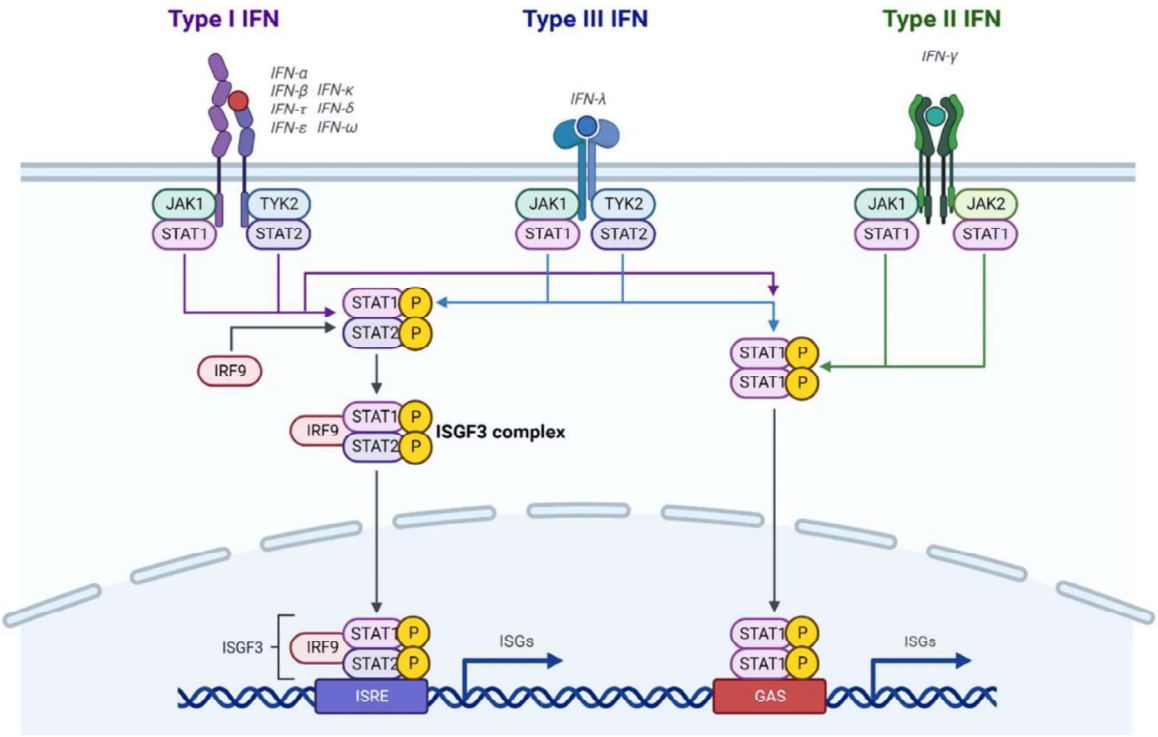

Figure M: Schematic diagram of signal transduction pathways for the three different types of IFNs (Park *et al.* 2022 [208]).

Interferon and TRIMs

TRIM proteins are a diverse family of molecules that serve various roles in innate immunity and antiviral responses. Multiple research studies have investigated the effects of IFNs on the expression and function of TRIM genes, shedding light on their significance in host-virus interactions. Research has shown that IFNs, both type I and type II, have a profound impact on the expression of TRIM genes. Extensive research conducted in human primary lymphocytes and monocyte-derived macrophages has shown that around 27 out of the 72 known human TRIM genes are responsive to interferons (IFNs)[209]. This sensitivity implies that IFNs play a pivotal role in regulating the expression of TRIM proteins, which, in turn, are involved in antiviral immune responses.

IFN-induced TRIM22, has proven effective in suppressing hepatitis B virus (HBV) gene expression and replication in hepatoma cells [210]. Moreover, TRIM56 has been identified as a host factor that restricts pestivirus infections, such as bovine viral diarrhea virus (BVDV). It is constitutively expressed in various tissues and is further upregulated in response to IFNs or viral infections[211]. An RNA virus infection triggers the movement of TRIM24 from the nucleus to the mitochondria in a process dependent on CRM1. Within the mitochondria, TRIM24 facilitates the K63-linked ubiquitination of TRAF3, leading to the activation of the downstream antiviral transcription factor

IRF3. This activated IRF3 subsequently transports into the nucleus to initiate the transcription of IFN-I genes[212]. Furthermore, Plasmacytoid dendritic cells (pDCs) are instrumental in producing large amounts of type I IFNs during viral infections. TRIM8 has been identified as a critical regulator of IFN regulatory factor 7 (IRF7) function. Maarifi *et al.* (2019) showed that TRIM8 safeguards phosphorylated IRF7 (pIRF7) from proteasomal degradation, ensuring the stability of pIRF7 and promoting the production of type I IFNs in pDCs[213].

Tripartite Morif 34 (TRIM34)

Our study focuses on TRIM34; an antiviral protein and it functions as a protective role in colon cancer. The major focus of this study is to investigate the role of TRIM34 in lung adenocarcinoma. TRIM34, also known as RING Finger Protein 21 (RNF21), is an interferon-inducible protein belonging to the ubiquitin E3 ligase family. It is upregulated by interferons, suggesting its potential role as an effector in the cellular response against viruses and in cancer therapy [214]. The other TRIM proteins, including TRIM6, TRIM22, and TRIM5 are seen to be closely related functionally to TRIM34 [215]. Orozco *et al.* (2009) reported that TRIM34 expression was highly correlated to copy number variants (CNVs) influencing gene expression and metabolic traits in mice [216]. In a recent study elucidating the genetics of Parkinson's disease, the signature of DNA methylation suggested that TRIM34 was one of the genes with multiple CpGs associated with epigenetic variations [217].

TRIM34 exhibits anti-viral properties and is found to be downregulated in colon cancer[218]. Therefore, we hypothesize that TRIM34 may function as a tumor suppressor gene. We propose that the upregulation of TRIM34 could potentially induce cancer regression. Understanding the functional roles and molecular mechanisms of TRIM34 in cancer provides insights into its potential clinical significance. TRIM34 holds promise as a diagnostic marker for cancer detection and prognosis prediction. Moreover, targeting TRIM34 or manipulating its downstream signaling pathways may represent a novel therapeutic strategy for cancer treatment.