The Role of Tumor Suppressor Genes in Molecular and Biological Basis of Lung Cancer

Nikolaos Andreas Chrysanthakopoulos

Dental Surgeon (DDSc), Resident in Maxillofacial and Oral Surgery, 401 General Military Hospital of Athens, Oncologist (MSc), Specialized in Clinical Oncology, Cytology, Histopathology, Dept. of Pathological Anatomy, Medical School, University of Athens, Athens, Greece, PhD in Oncology (cand)

Abstract: Lung Cancer (LC) is the 5th leading cause of cancer-related death in males and females worldwide and, it is also responsible for a high proportion of morbidity and mortality as the 5-year survival is extremely poor. LC is characterized by a large variety of genetic alterations and mutations that occur at high frequency. Improvements in molecular and biological basis of LC may lead to customized and personalized treatment based on targeting specific cellular signaling pathways and genes. Signaling pathways and genes that could be involved in LC treatment include cellular signaling pathways which promote tumor's development, such as the Epidermal Growth Factor Receptor/Ras/Phosphatidylinositol 3-Kinase (EGFR/Ras/PI3-K) pathway, pathways which inhibit tumor's development, such as the p53/Rb/P14ARF, STK11 pathway, apoptotic promoting pathways such as the Bcl-2/Bax/Fas/FasL pathway, immortalization genes which regulate senescence/immortalization and include regulator genes and pathways, such as the cell cycle pRB/p53, cytoskeletal, interferon-related (IFN), insulin growth factor-related, MAP kinase and oxidative stress pathway, and, DNA repair defects and mutations. Epigenetic changes in LC also contribute strongly to cell transformation as they are able to modify the structure of chromatin and the specific gene expression and include DNA methylation, chromatin and histone modification, and micro-RNA, which are responsible for the silencing of tumor suppressor genes whereas enhancing oncogenes expression. The genetic and epigenetic pathways involved in lung tumorigenesis differ among LC histological types, and are tools for LC diagnosis, prognosis, clinical follow-up and targeted therapies. The current review, focuses on the presentation of molecular and biological events that are involved in LC development giving emphasis to tumor suppressor genes and signaling pathways that are implicated in LC development.

Keywords: Tumor Suppressor Genes, Signaling pathways, Mutations

1. INTRODUCTION

Lung cancer (LC) is the 2nd most common cancer diagnosis by gender, behind prostate cancer for males and breast cancer for females [1]. The most common age of LC diagnosis is 70 years, whereas in most cases the prognosis is extremely poor as 17.4% of people in the United States that were diagnosed with LC survive five years after the diagnosis [2]. It is also responsible for a high rate of morbidity and mortality as advanced LC has extremely poor prognosis, with a 5-year survival of only 5% [1].

LC pathogenesis has not been fully elucidated, whereas LC molecular and biological basis is complex and heterogeneous. LC development is a multistage process involving genetic alterations in DNA sequence, epigenetic modifications, activation of growth promoting pathways and inhibition of tumor suppressor pathways that result in DNA damage, contribute to cancer initiation, promotion and progression as regulate gene expression and cellular signaling pathways in the normal cells and transform normal lung epithelial cells into LC cells [3,4]. However, it remains unknown whether all lung epithelial cells or only a subset of those, such as lung epithelial stem cells or their immediate progenitors are susceptible to complete malignant transformation.

Moreover, whereas the tumor-initiating cells may have only a few mutations, as the tumor
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expands, cells may acquire additional mutations [5]. Previous researches have shown that those alterations usually occur early in malignant transformation [6, 7]. Those alterations lead to LC development and exhibit the classical hallmarks of cancer, namely self-sufficiency of growth signals, insensitivity to growth-inhibitory/antigrowth signals, limitless replicative potential, evasion of programmed cell death/apoptosis, tissue invasion, sustained angiogenesis, and metastasis [8].

LC is divided into two main types based on histological, clinical, and neuroendocrine characteristics, non-small cell LC (NSCLC) and small cell LC (SCLC), their prevalence is 80%-85% and 15%-20%, respectively. Moreover, NSCLC is also divided into other histological subtypes, such as adenocarcinoma (LADC), squamous carcinoma (SCC), large-cell carcinoma (LCC), including large-cell neuroendocrine LC (LCNEC), bronchoalveolar LC, and mixed histological types, such as adenosquamous carcinoma (ASQC) [9].

The main histological types and subtypes of LC have also differences in molecular basis. In particular, those molecular differences between NSCLC and SCLC types and among NSCLC subtypes are associated with oncogenic mutations, increased protein expression, gene amplification, tumor suppressing alterations which include mutations, deletion and loss of heterozygocity (LOH), loss of protein expression, tumor-acquired DNA methylation, chromosomal aberrations, and presence of telomerase activity. Various genes and cellular signaling pathways are responsible for the mentioned genetic alterations and are also implicated in cell functions such as growth, survival, differentiation, proliferation, programmed cell death, invasion, metastasis, etc., and include oncogenes (ONG) and tumor suppressor genes (TSG), such as BRAF, KRAS, MET, PIK3CA, EGFR, ErbB2/HER 2-neu, MDM2, MYC, PDGFR, Bcl-2, CCND1, p53, Rb, PTEN, LKB1, CDKN2A (p16/p14ARF), FHT, CAV1, APC, TUSC2, CDH 1, CDH 13, DAPK1, GSTP1, MGMT, RARβ, RASSF1A, SEMA3B, TIMP3, EML4-ALK fusion, etc. [9].

In LC as in other malignancies, oncogenesis is associated with growth promoting proteins activation such as, KRAS, EGFR, Braf, MEK-1, HER2, MET, ALK as well as inactivation of TSGs, such as P53, PTEN, LKB1 [3]. Activation of growth promoting oncogenes can occur by gene amplification or other genetic alterations including point mutations and structural rearrangements leading to uncontrolled signaling through oncogenic pathways [10].

A better understanding of molecular alterations and the multiple biochemical pathways involved in the molecular pathogenesis of LC at multiple levels, genetic, epigenetic, protein expression and their functional significance, are crucial to the development of treatment strategies that can target molecular aberrations and their downstream activated pathways [9], and could be contribute to LC diagnosis, treatment and prognosis. Moreover, cell survival depends on continued activation of the aberrant signaling [10] making them ideal candidates for targeted treatments. Identifying the genes and pathways involved, determining how they relate to the biological behavior of LC, and their utility as diagnostic and therapeutic targets are important basic and translational research issues. Consequently, current information on the crucial molecular stages in LC pathogenesis regarding the role of TSGs and their involvement in preneoplasia, primary cancer, and metastatic disease is the aim of the current review.

2. TUMOR SUPPRESSOR GENES

It is known that TSGs negatively regulate cell growth. Their function is crucial for carcinogenesis and requires inactivation of both gene alleles, according to Knudson’s two hit hypothesis. Mutation, epigenetic silencing or other aberrations, are responsible for the inactivation of the individual gene in one allele. The 2nd allele in some cases is targeted by deletion (homozygous deletions), methylation with consequent loss of expression, or mutation [11]. The final result is that the 2nd allele is inactivated due to LOH, leading to loss of a chromosome region by deletion, nonreciprocal translocation or mitotic recombination. The most frequently inactivated TSGs in LC cases are TP53, retinoblastoma (RB1), PTEN, RASSF1A, CDKN2A, Serine/threonine Kinase 11 (STK11/LKB1), and FHIT [9,12], and chromosomal regions that exhibit allelic loss in LC cases involve TSGs such as TP53 (17p13), PTEN (10q22), p16 (9p21), and RB1 (13q12) [12].(Fig. 1)

2.1. Pten Tumor Suppressor Gene

PTEN (phosphatase and tensin homolog) is a TSG which is mutated in many hereditary and sporadic human cancers, is located on 10q23 chromosome, encodes a protein that acts as a
dual lipid and protein phosphatase, and inhibits the PI3K/AKT/mTOR signaling pathway by the dephosphorylation of the PI(3,4,5)-triphosphate. PTEN inactivation causes unlimited activation of AKT/protein kinase B which is independent of ligand binding [13]. PTEN mutations have been identified in 5% of NSCLC cases [14] despite the fact that in NSCLC type has found reduced protein expression in about 75%. Those mutations are more frequent in SCC (10.2%) than LADC cases (1.7%) and are also associated with a smoking history [15]. The Nuclear Factor-kappa B (NF-kB) activates the transcription of Snail, which is a PTEN transcription suppressor, therefore PTEN is negatively regulated by NF-kappa B [16]. It has been demonstrated that NF-kappa B activation was necessary and sufficient for inhibition of PTEN expression in a subset of human LC cells [17].

3. FHIT TUMOR SUPPRESSOR GENE

The fragile histidine triad (FHIT) gene, a candidate TSG, was recently identified on chromosome 3p14.2. FHIT gene encompasses the common fragile site FRA3B on chromosome 3, where carcinogen-induced damage can lead to translocations and aberrant transcripts of it [18]. FHIT function has been investigated in several tumors by the upregulation of inducing cell cycle arrest, cell proliferation inhibition, and apoptosis by increasing its sensitivity to DNA damaging agents [19, 20].

FHIT gene is inactivated by LOH and methylation in cancer cells, whereas the occurrence of mutations is very rare [21]. FHIT promoter hypermethylation that leads to FHIT inactivation and FHIT protein expression lack, has been identified to play a crucial role in lung alveolar differentiation, regulation and epithelial tumorigenesis [22-24]. FHIT gene is inactivated in 50% to 70% of all LC cases [25].

Abnormal FHIT transcripts, including exons deletions, insertions between exons, and insertions that replace exons, have been identified in a high proportion of LC cases. Reduction or complete loss of FHIT expression have been found in about 30%-70% of NSCLC cases and in about 20% of bronchial biopsies from chronic smokers without evidence of LC, finding that supports the theory that FHIT gene is a molecular target of tobacco smoke carcinogens [26]. In a study by Fong et al. primary LCs, tumor cell lines, and preneoplastic bronchial lesions were examined for molecular genetic abnormalities in FHIT gene, which links the FRA3B fragile site on 3p14.2 region, and the outcomes showed that 3p14.2 allele loss was existed in 100% of SCLC and 88% of NSCLC cell lines and 45% of primary NSCLC cases and rare in LADC cases, with many break-points. Those findings suggest the involvement of several distinct regions in the FRA3B site. Homozygous deletions within the FHIT/FRA3B region were found in 4.4% of thoracic cancer cell lines [27].

4. APC TUMOR SUPPRESSOR GENE

Adenomatous polyposis coli (APC) is a protein that is encoded by the APC gene, a TSG and constitutes a negative regulator that controls beta-catenin concentrations as acts as an antagonist of the Wnt signaling pathway. It also interacts with E-cadherin, which is implicated in cell adhesion, cell migration, transcriptional activation, and apoptosis [28]. APC gene mutations may result in colorectal cancer [29], as cause familial adenomatous polyposis (FAP), finding that suggests its role as a potential predictor for cancer initiation or development.

It has been found that APC gene promoter methylation, inhibits its expression, and is mediated by changes of chromatin modulation and aberrant binding of CCAAT-box binding transcription factors [30]. Previous studies have shown that APC promoter hypermethylation in NSCLC cases has been reported as an effective biomarker for diagnosis [31, 32], as in general, the link between APC hypermethylation with cancers has been extensively assessed [33]. However, the results of those studies are controversial because of differences in epidemiological parameters examined and analyzed, detection methods, etc. LOH on chromosome 5q, the APC locus, is a frequent finding in LC cases, however previous studies have recorded no APC mutations. In a study by Ohgaki et al. [34], 114 human LC specimens were investigated for alterations in the mutation cluster region of the APC gene and revealed APC mutations in 5% of SCC cases, findings that suggest that APC mutations are infrequent, but may be involved in the pathogenesis of a small subset of LC cases.

5. STK11 (LKB1) TUMOR SUPPRESSOR GENE

Serine/threonine kinase 11 (STK11 or LKB1) gene encodes a serine/threonine kinase, is located on 19p13 chromosome, and functions as a TSG [35]. It acts as a mTOR inhibitor through mTOR path way inhibition via adenosine monophosphate-activated protein kinase (AMPK) [36] and regulates differentiation,
metabolism, cell polarity, motility and metastasis [35], whereas is also implicated in cell cycle regulation, and chromatin remodeling [36, 37]. mTOR signaling path way components deregulation, except for KRAS mutations, has been identified in 30% of LADC cases [38].STK11 gene inactivating mutations are responsible for Peutz-Jeghers syndrome [39], however somatic inactivation caused by point mutation and deletion on 19p13 are presented in 30% of LC cases, and constitutes the 3 rd most commonly mutated gene in LADC, caused by various somatic mutations or deletions that produce abnormal proteins, after p53 and RAS [38, 40-42]. STK11 gene inactivating mutations are often associated with KRAS activation and can lead to cell growth promotion [42], are more common in poorly differentiated LADC cases, whereas are rare in SCLC cases [40, 42, 43]. Moreover, STK11 gene inactivation is more frequent in LADC compared to SCC cases [40, 41]. An association has been recorded between STK11 gene mutations and smoking in males [41, 42, 44], and an association with KRAS mutations has also been found [41, 42].

6. TUSC2 TUMOR SUPPRESSOR GENE

Tumor suppressor candidate 2 gene (TUSC2), also known as FUS1, is considered as a candidate TSG. TUSC2 functions are still remain unclear. Previous researches showed that TUSC2 induces G1 cell cycle arrest and apoptosis [45, 46], regulates calcium signaling [47], modulates tyrosine kinases [48], and affects gene expression [49]. It is located on chromosome 3p21.3 which is homozygously deleted in lung and breast cancers. TUSC2 is considered as a TSG in LC cases as its mRNA expression loss has been recorded in 80% of the tumors, because of 3p21.3 deletion [50]. It has also recorded that 3p21.3 deletion was very rare in LC cases (1.1% TCGA), except for malignant mesothelioma (36%) [49], where as no evidence of methylation was identified in the TUSC2 gene promoter region in LC cases [46]. TUSC2 promoter region was partially methylated in oral tumors but unmethylated in normal mucosa [51]. TUSC2 somatic mutations have not been observed in any cancer specimens according to TCGA, although infrequent mutations have been identified in LC cell lines [46].

7. CDH1 TUMOR SUPPRESSOR GENE

CDH1/E-cadherin is a cell-cell adhesion transmembrane glycoprotein, and is encoded by the CDH1 gene which is located on 16q22.1 region [52]. CDH1 is a TSG and plays a critical role in maintaining cell adhesion and adherent junctions in normal tissues. Its expression is frequently absent in several epithelial tumors, and loss of normal intercellular junctions can promote cancer invasion and metastasis, whereas it is also associated with several types of cancers [53, 54]. CDH1 loss promotes β-catenin translocation into the nucleus, and regulates transcription of various targeted proteins [55]. It is also implicated in epithelial-mesenchymal transition (EMT) which is the crucial early step for cancer metastasis [56]. Therefore, CDH1 low expression levels associated with tumor invasiveness, metastasis, and poor prognosis. It has also found that decreased expression of CDH1 is responsible for the malignant phenotype of NSCLC [57], and CDH1 promoter methylation is associated with LC cases [58]. Y-box binding protein 1 (YBX1) is over-expressed in various tumors including LC cases and serves as a novel marker of LC progression [59]. In a study by Stella et al. [60] was found that down-regulated CDH1 strengthens EGFR transcription in a phospho-YBX1 dependent way and contributes to cell proliferation and metastasis in NSCLC cells. YBX1 plays a critical role in EGFR up-regulation, which consequently promotes cell proliferation and invasion ability in NSCLC cells. However, the mechanism that loss of CDH1 promotes NSCLC metastasis needs further investigation. Moreover, Xianfang et al. found down-regulation of CDH1 up-regulated EGFR transcription levels in NSCLC cells [61].

8. CDH 13 TUMOR SUPPRESSOR GENE

Cadherin 13, constitutes a member of the cadherin family, is coded by the CDH13 gene, which is located on chromosome 16q24.2 [62]. Cadherin proteins are implicated in the formation of intercellular junctions, such as N- and E-cadherin. In many epithelial cancers has been identified loss of cadherin expression and may play a critical role in malignant cell invasion and metastasis [63]. Recent researches have shown that CDH 13 functions as an anti-oncogene in lung [64], breast cancer [65] and in other organs, whereas downregulation of its expression could promote cancer progression. Toyooka et al. [66] were observed that CDH 13 expression is reduced in LC cases, and that its down regulation could be attributed to hypermethylation in the CDH13 promoter. In another study was found that single nucleotide polymorphisms (SNPs) in CDH13 gene could affect the methylation of CpG islands in CDH13.
gene [67]. Several studies have reported that SNPs in CDH13 gene were associated with cancer, such as colorectal and LC [62,64,66-69]. However, few studies have investigated the association between SNPs in CDH13 gene and NSCLC cases [70].

9. RASSF1A TUMOR SUPPRESSOR GENE

The RASSF1A gene constitutes a TSG which is located on chromosome 3p21, it acts at the point of G1/S phase cell cycle progression, inhibits the accumulation of Cyclin D1, and thus induce cell cycle arrest [71]. 3p21 location is epigenetically inactivated at high frequency in NSCLC cases. RASSF1A binds to the Ras-GTP binding protein Nore1, as a Rasoncoprotein negative effector [72]. In LC cells RASSF1A promoter is hyper methylated and the exogenous expression ofRASSF1A expresses tumorigenesis in nude mice [73]. Moreover, it has been identified that theRASSF1A gene is frequently inactivated in primary LC cases by the de novo methylation of CpG islands in the promoter location [74].

10. SEMA FAMILY AS TUMOR SUPPRESSOR GENES

The semaphorin/collapsing family of molecules are implicated in neuronal development and has been shown that act as TSGs by inducing apoptosis. SEMA3 proteins are also involved invarious functions such as immune formation [75], organogenesis [76], neuronal apoptosis [77], and drug resistance [78]. Semaphorin3B (SEMA3B) is located on 3p21.3 chromosome, a location that is associated with increased allele loss and/or promoter methylation in the early pathogenesis of lung and breast cancer [78, 79], belongs to the class 3 semaphorins and constitutes a secreted protein [80].

SEMA3B gene encodes a protein with tumor suppressor activity for LC [81]. Previous studies showed that treatment by using exogenously SEMA3B or import of a plasmid encoding SEMA3B in H1299 NSCLC cells reinduced apoptosis and a significant reduction in colony formation [78,81], whereas tumor-acquired SEMA3B missense mutations do not show such functions. In another study was found that SEMA3B expression in a p53-negative glioblastoma cell line was increased after p53 reexpression, finding that suggest SEMA3B function as a mediator of p53 tumor-suppressor activity. However, because H1299 cells are p53 null, SEMA3B can induce tumor suppression even in the absence of p53 [82].

11. CAVI AS A TUMOR SUPPRESSOR GENE

Caveolin-1 (Cav-1), a major structural protein of caveolae, that are plasma membrane invaginations are implicated in cellular processes, such as lipid transport, cell adhesion, molecule transport, signal transduction, and tumor progression [83]. Recent researches suggests that Cav-1 has a positive regulatory effect on tumor growth and plays a central role in tumor invasion and metastasis, despite the fact that seems to function as a tumor suppressor protein at early stages of cancer progression [84,85]. Cav-1 regulates the activity of several pathways, including EGFR, Src family kinases, G-proteins, H-Ras, protein kinase C, endothelial nitric oxide synthase, andintegrins, which are potentially implicated in the development of cancer, by forming signaling complexes [83]. Therefore, Cav-1 could be a key molecule for growth-related signaling and cancer development.

It has been suggested that Cav-1 gene acts as both a TSG and an ONG. Its expression is down-regulated in lung, colon and other cancers, whereas cell oncogenic transformation has been associated with reduction of its expression, and antisense-mediated down-regulation of its expression could lead to oncogenic transformation in NIH 3T3 cells [86-88]. On the contrary, other reports have shown that Cav-1 expression was found to be up-regulated in several cancers such as esophagus and LC [89, 90]. Moreover, Cav-1 exogenous expression in malignancy transformed cells and cancer cell lines inhibited cell growth and tumorigenesis, observation that indicates its roleas a TSG [91, 92].

12. MGMT TUMOR SUPPRESSOR GENE

O-6-methylguanine-DNA methyltransferase (MGMT), is a specific DNA damage reversal repairprotein, which protects tissues against the carcinoogenic and toxic effects of methylating andchloro-ethylying agents by removing adducts from O 6 position of guanine and prevents mis-match and errors during DNA replication and transcription [93]. MGMT has been reported as a TSG in colorectal cancer [94], whereas its epigenetic silencing caused by its promoter methylation at specific CpG islands can lead to loss of its activity in several cancers, including LC [95,96]. The methylation status of the MGMT promoter has been observed in some cancers, such as NSCLC [97], glioblastoma

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[98], breast cancer [99], and others. In NSCLC cases has been identified a varying level of MGMT promoter methylation frequency [74,100], observation that could be attributed to various nature of the clinical samples, that were analyzed. Previous meta-analysis studies have shown that MGMT methylation is associated with NSCLC incidence [101-103], but those studies were based on a small amount of various samples and therefore could lead to unreliable outcomes, as they recorded quite different rates of MGMT hyper-methylation from different samples, whereas only the samples from tumor tissue showed higher methylation compared to control group[101,102]. Moreover, those studies have also not completely investigated the association between MGMT methylation and clinical characteristics of NSCLC, but only analyzed the risk between MGMT methylation and NSCLC[101-103].

13. TP53 TUMOR SUPPRESSOR GENE

TP53 gene is located on chromosome 17p13, encodes a nuclear phosphoprotein of 53 kDa that identifies and binds to regions of damaged DNA [104] and acts as a transcription factor controlling the expression of a large number of different genes. It is induced in case of DNA damage or carcinogenic or oxidative stress and leads to cell cycle arrest by inducing expression of Cyclin Dependent Kinase (CDK) inhibitors which regulate cell cycle checkpoint signals, causing the cell to undergo G1 arrest and allowing DNA repair or programmed cell death/apoptosis. As a transcription factor has downstream target genes involving cell cycle arrests G1 and G2, DNA repair or apoptosis, and upstream regulatory genes, including p14ARF and MDM2. MDM2 and p14ARF are implicated in cases of abnormal functions of p53 protein. p14ARF is a crucial TSG, is encoded in the 9p21 locus of p16INK4 from an alternative exon 1β, responding to both oncogenic stimuli (Ras, MYC, E2F1) and DNA damage and its activation induces G1 arrest and apoptosis, either dependently or independently of p53 protein [105,106]. TP53 TSG is the most frequently mutated gene in LC cases [107], whereas p14ARF protein expression loss, due to yet unknown mechanisms, occurs frequently in SCLC, LCNEC and in some LADC cases [108]. MDM2 amplification is rare, almost 6% of NSCLC cases, although over expression at the level of mRNA and protein is frequent, occurring in 30% of both SCLC and NSCLC cases [105].

TP53 gene’s inactivation is one of the most significant genetic abnormalities in LC cases with hemizygous deletion of 17p13, containing the locus of TP53, occurring in 90% of SCLC case sand about 65% of NSCLC cases [109]. Inactivating mutations of the TP53 gene which most of them are missense mutations within the DNA-binding domain, have been identified in 80-100% of SCLC cases, in 90% of LCNEC cases and in 50% of NSCLC, of which gain-of-function mutations prevent the p53 protein binding to MDM2 and subsequent p53 ubiquitin-dependent proteolysis [110-112]. Moreover, in a meta-analysis by Tamamemagi et al. [113] in over 4,000 NSCLC cases were recorded alterations by mutation or protein accumulation in only 46.8% of those cases, more commonly in SCLC than LADC cases and were associated with higher tumourgrade, stage and male gender. In a comprehensive genomic analysis according to The Cancer Genome Atlas (TCGA) project [114] mutations of TP53 gene were recorded in at least 81% of SCLC cases. In another research was recorded TP53 gene mutations in 85 of 188 LADC cases(45%) [38]. TP53 gene mutations in NSCLC cases, are associated with a positive history of smoking or exposure to environmen tal tobacco smoke [115,116]. The range of mutations of different types of TP53 gene mutations is different between smokers and non-smokers and it has been observed that smoking-related cancers show a significantly higher frequency of GC to TA transversions at CpG islands compared to G to C transversions, which were induced by PAHs into tobacco smoke, and G to A transitions at CpG dinucleotides more commonly found in neversmokers [116,117]. On the contrary, GC to TA(G-A) transition at non-CpG islands were associated with LC in never-smokers [111,112]. TP53 gene alterations and stabilization by mutation are frequent in proximal pre-invasive lesions of squamous dysplasia type and carcinoma in situ [111,112]. In a meta-analysis of 74 studies was recorded that aberrant TP53 gene detected by protein expression or mutational analysis was anadverse prognostic factor in NSCLC cases [118]. Some point mutations in TP53 gene accord again-of-function phenotype leading to increased aggressiveness of LC [119]. TP53 gene mutations can occur in association with KRAS and EGFR mutations [115], whereas genetic alterations of TP53 gene have also been
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associated with treatment resistance [104]. Ataxia telangiectasia mutated (ATM) gene is another gene upstream of the p53/p14ARF pathway, and mediates the response to DNA damage. It is known to be mutant in ataxia telangiectasia disease, characterized by a lack of DNA repair, but is not known as a mutant in LC cases. A recent DNA sequencing of 623 genes in 188 LADC cases showed ATM to be mutant in 14(7.4%), thus contributing the strong targeting of p53 pathway functions in LC cases [38]. The downstream p53 pathway includes target genes of TP53 transcription, which play key roles in the mitochondrial apoptotic pathway, as well as in the death receptor pathway Bcl-2 as antiapoptotic and Bax as pro-apoptotic, are up and down regulated by p53, respectively, Fas and the tumor necrosis factor receptor (TNFR)-like apoptosis inducing ligand (TRAIL) receptor DR5 (TRAIL-death receptor 5) belong to the TNFR family. Those four factors are strongly deregulated in LC cases, which results in strong resistance to both mitochondrial and death receptor-induced apoptosis [120].

14. RAR AS TUMOR SUPPRESSOR GENES

The retinoic acid receptor (RAR) is a type of nuclear receptor, act as a transcription factor [121] and is activated by both all-trans retinoic acid and 9-cis retinoic acid [122]. Two families of retinoic receptors have been identified, RARs and RXRs with three subtypes for each (α, β, γ) and several isoforms arising from promoter usage and alternate splicing and are encoded by the RARA, RARB, RARG genes, respectively. Moreover, RAR genes expression is under epigenetic regulation by promoter methylation [122,123]. The effects of retinoids are mainly mediated by the nuclear retinoid receptors, which are members of the steroid and thyroid hormone receptor super family [124]. The biological functions of the multiple isoforms of RARs are still remain unclear, however, those isoforms could explain the implication of RARs in various biological effects. It has been shown that the absence of two isoforms for RARα (α1 and α2) and for RAR γ(γ1 and γ2), and four isoforms for RARβ (β1-β4) and RARβ1 [125], could be responsible for retinoid resistance in lung carcinogenesis [126].

RARβ2 isoform is located on 3p24 region and its expression was reduced or even suppressed in LC cell lines, suggesting that its reexpression could suppress LC progression [127]. Loss or reduced RARβ2 expression has been identified in a high frequency in heavy smokers in NSCLCs and bronchial biopsy specimens [128,129], and in other solid tumors [130,131]. In a previous study was observed that RARs and RXRs were expressed in 89% of control normal bronchial tissue specimens in healthy individuals and that in distant normal bronchussspecimens in NSCLC patients RARα, RXRα and γ were expressed in more than 95% of the tumor-free specimens. However, RARβ, RARγ and RXRβ expression was reduced, and was observed in 76% of NSCLC specimens [128], finding that was in line with another research in which was recorded reduced or absent RARβ protein expression in 50% of resected NSCLC specimens [132]. In the mentioned studies was found normal or elevated RARα and RXRα expression in NSCLC specimens, whereas LOH at the region 3p24, in which is located RARβ gene was identified in a high frequency, and was also found in non-neoplastic lesions, suggesting that modified retinoid receptor expression may be involved in lung carcinogenesis.

The abnormal methylation of the genes promoter regions consists a mechanism of gene silencingin cancer [133]. As it has been mentioned RAR genes expression is under epigenetic regulation by promoter methylation [122,123]. RARβ gene hypermethylation frequently leads to loss of RARβ expression which has been recorded in 43% of primary resected NSCLC samples [134]. It is also implicated in the pathogenesis of SCLC, whereas it was found that LC cell lines treatment with the demethylation agent 5-aza-2&39;deoxycytidine (5-AZA-CdR) could restore RARβ expression. RARβ gene’s mRNA expression loss has been detected in many LC cell lines, observation that indicates its role as a TSG [135].

15. CDKN2A TUMOR SUPPRESSOR GENE

CDKN2A, also known as CDK inhibitor 2A, is a gene which in humans is located on chromosome 9 p21.3. The gene codes for two proteins, including the INK4 family member p16 (or p16INK4a) and p14 ARF. Both act as TSGs by regulating the cell cycle. p16 inhibits CDK4 and -6 and activates the RB family of proteins, which prevent the transition from G1 cell cycle phase to S-phase, p14ARF activates and interacts with the TP53 TSG [136]. Somatic mutations of CDKN2A are common in the majority of human cancers, and it has been assessed that CDKN2A gene is the 2nd most
commonly inactivated gene in cancer after TP 53. It has been shown that in LADC cases often exist genetic mutations in CDKN2A gene which leads to inactivation of p16 and is one of the most common genetic alterations in many forms of cancer including LADC [137-139].

Both alleles must be inactivated before its function is eliminated. In its inactivation are implicated mechanisms such as homozygous deletion (HD), hypermethylation in the promoter CpG island (methylation), and point mutation. p16 is frequently inactivated by HD or promoter hypermethylation, and rarely by point mutation in primary NSCLC cases [137,140]. It has also been observed that the frequency of p16 methylation is significantly higher in LADC with KRA Smutation, however, the associations between p16 inactivation mechanisms and other common genetic mutations in LADC such as EGFR and STK11 remain controversial or have never been investigated [141]. Associations between smoking and p16 methylation and between p16 HD and never smokers have been recorded in some researches, however those findings are thought to be inconsistent [137,140-142].

16. RB TUMOR SUPPRESSOR GENE

Retinoblastoma (RB) was the first TSG which discovered based on an association with a rare childhood tumor, retinoblastoma [143]. The Knudson hypothesis of a 2nd hit in retinal cells of children with germ line mutation led to the understanding the role of TSGs in cancer development [11]. A rate of 40% of retinoblastomas are hereditary and individuals with hereditary retinoblastoma are at high risk for malignancies such as breast cancer, melanoma, LC, osteosarcoma, bladder cancer and other epithelial cancers [144,145]. Somatic alterations in RB1 gene are common in various malignancies including LC, bladder, prostate, and breast cancer. RB1 gene is located on chromosome 13q14.1-q14.2 region, encodes the retinoblastoma pocket protein (RB) and is a downstream effector of p53-mediated G1 arrest through activation of the CDK inhibitor p21. CDK inhibitors, such as p16INK4A, p15INK4B, maintain RB in the unphosphorylated, active form. RB protein is implicated in the cell cycle regulation by binding to the unphosphorylated form of E2F transcription factors and suppresses their activity. After mitogenic stimulation, the CDKs, such as CDK4, CDK-6, Cyclin D and CDK2-cyclin E phosphorylate RB, which lead to release of the binding to E2F factors and progression through the cell cycle, and finally leads to G1-S transition [146]. RB, in addition to regulation of cell proliferation has also a crucial role in the regulation of EMT [147,148] and a possible role in immune response [149]. The absence of RB protein, is the most frequent mechanism of escape from G1 check-point in SCLC cases, whereas the RB hyperphosphorylation in NSCLC cases which is a common event, disarranges the G1 checkpoint control. Loss of RB protein occurs in more than 70% of highgrade LCNEC, in more than 90% of SCLC tumors, and in only 10-15% of NSCLC cases [150].

A wide analysis of DNA sequences in LADC also identified RB1 mutation in some cases [38] pointing to the persistent negative selection for RB functions in LC cases (loss, phosphorylation and mutation) in addition to LOH at 13q14 (allelic loss) which is common in NSCLC cases [151,152]. Inactivation of RB functions by phosphorylation is mainly caused by the loss of p16INK4A expression and/or overexpression of Cyclin D1 and Cyclin E, has been observed in NSCLC cases [120]. CDK4 is rarely over expressed but is amplified in a small subset of NSCLC cases. On the contrary, Cyclin D1 over expression and p16INK4A loss have been observed in 40–50% of NSCLC cases [153], whereas previous studies showed that overexpression of Cyclin D1 gene (CCD1) was observed in 35-50% of NSCLC cases and both CCD1 over expression and p16INK4A loss were early phenomena, and were present as soon as pre-invasive lesion appeared, with an increasing squamous dysplasia grade level [112,154]. Cyclin D1 is rarely amplified in LC, however, asmall percentage of amplification of CCD1 was recently identified in a wide genomic characterisation of the LADC. Moreover, Cyclin D1 (CCND1) and Cyclin E (CCNE2) genes were found among the top focal regions of amplification in LADC cases [151]. Loss of p16INK4A expression is attributed to its methylation in 40%, its homozygous deletion in 30% and its mutation in 10% in cases of expression losses [153]. Allelic loss in 9p21 region (p16INK4A loss of allele LOH) which contains the protein-coding genes CDKN2A and CDKN2B is a frequent genetic aberration and
leads to the p16 functions attenuation in addition to methylation [151].

A reverse relationship between Cyclin D1 over expression and RB loss shows that Cyclin D1 and p16INK4 are the only factors of RB phosphorylation that are not implicated in cases of RB lost, situation that is obvious in SCLC cases, in which RB is mostly lost, but Cyclin D1 or p16INK4 alterations are rare. In contrast to previous observation, Cyclin E may be over expressed in 30% of SCC cases, in the absence of RB, because of the Cyclin E response to DNA damage and genetic instability. Cyclin E over expression is an early event in LC bronchial preneoplasia [112].

17. DAPK TUMOR SUPPRESSOR GENE

Death-associated protein kinase (DAPK) is a pro-apoptotic Ca 2+ calmodulin-regulated serine/threonine kinase which is implicated in various cellular functions, promotes either apoptosis initiated by interferon (IFN)-γ, or autophagic cell death in response to various stimuli, including ONGs, transforming growth factor-β (TGFβ), activation of Fas/CD95 receptors detachment from extracellular matrix, and tumor necrosis factor-α (TNFα) [155,156].

DAPK-1 is a tumor suppressor protein and shows metastatic inhibition properties, whereas hypermethylation DAPK gene promoter has been identified in some types of cancers, leading to its functional loss rather than mutation [157,158]. DAPK dysfunction may be more crucial for the tumorigenesis of LC because of the high frequency of DAP-kinase promoter methylation on average in 40.5% of LC cases [159-163]. Less frequently, DAPK expression loss can be attributed to homozygous deletion DAP kinase CpG island [164].

18. GSTP1 ENZYMES AS TUMOR SUPPRESSORS

Human genes polymorphism that encodes enzymes which are implicated in metabolic activation and detoxification of lung carcinogens such as PAHs and aromatic amines has been revealed. Genetic differences among individual differences in their ability to activate and deactivate/detoxify these lung carcinogens are expected to affect the risk of LC developing [165]. Glutathione S-transferases (GSTs) are phase II transformation enzymes implicated in the detoxification of dangerous agents [166]. GST gene family encodes genes that are critical for detoxication and toxification mechanisms, as the main role of GSTs is to detoxify dangerous agents by catalyzing the nucleophilic attack by glutathione synthetase on electrophilic carbon, sulfur, ornitrogen atoms and transforms to non-polar compounds, preventing their interaction with crucialcellular proteins, nucleic acids, and other cellular components [167]. A review by Altinisik et al., which examined the role of GST genetic polymorphisms to LC in different populations and based on previous reports led to contradictory results [168]. Several classes of GST, including Alpha, Mu, P1, and Theta, were previously found in human tissues. GSTP1 is the most predominant GSTs in lung tissue also considered to be most important in determining risk for LC development [169]. GSTP1 gene is located on chromosome 11q13, four GSTP1 alleles have been recognized, GSTP1A-D, encodes a phase II metabolic enzyme that detoxifies reactive electrophilicintermediates, plays a crucial role in protecting cells from carcinogenic and cytotoxic agents and is expressed in normal tissues at variable levels in different cell types. Altered GSTP1 expression and activity have been identified in many tumors and could be mainly attributed to GSTP1 DNA hypermethylation of CpG island in the promoter-5′&39; [170]. SNPs in GSTP1 have been identified frequently and concern rs1695 and rs1138272 [171,172]. GSTP1 has the highest specific activity regarding the active benzo (a) pyrenoediol epoxide, which is cigarette metabolite, is almost exclusively active to the positive-enantiomer of anti-benzopyreneediol epoxide, which is considered to be the final mutagenic form of benzo (a) pyrene[173], whereas its accumulation is associated with LC development [174].

During cancer development, GSTP1 does not seem to act either as an ONG or as a TSG, since induced GSTP1 expression in cancer cell lines failed to suppress cell development. Instead, GSTP1 was proposed to act as a &quot;caretaker & quote; gene. In cases of GSTP1 inactivation, cells appear to become more unprotected to somatic alterations upon chronic exposure to genome-damaging stresses as oxidants and electrophiles that are contributed by environment and lifestyle [175].
The Role of Tumor Suppressor Genes in Molecular and Biological Basis of Lung Cancer

<table>
<thead>
<tr>
<th>GENES</th>
<th>SCLS</th>
<th>NON SMALL CELL LUNG CANCER (NSCLC)</th>
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<tbody>
<tr>
<td>TSGs ALTERATIONS</td>
<td></td>
<td>LADC</td>
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<tr>
<td>MUTATIONS</td>
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<tr>
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<tr>
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TSGs: Tumor Suppressor Genes  SCLS: Small Cell Lung Cancer  LADC: Lung Adenocarcinoma  SQCL: Squamous Cell Lung Cancer  LOH: Loss of Heterozygosity

**Figure 1:** Tumor suppressor gene alterations in Lung Cancer

**REFERENCES**


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Citation: Nikolaos Andreas Chrysanthakopoulos, The Role of Tumor Suppressor Genes in Molecular and Biological Basis of Lung Cancer. ARC Journal of Cancer Science. 2020; 6(1):29-45. DOI:doi.org/10.20431/2455-6009.0601005.

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