Molecular and Biological Basis of Lung Cancer-Part I

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ABSTRACT

Lung cancer (LC) consists the 5th leading cause of death worldwide, and an important cause of morbidity and mortality, nowadays, as the 5-year survival is extremely poor. LC molecular biology may lead to customized treatment based on targeting specific genes and signaling path-ways. The main signaling pathways in LC development concern growth promoting pathways such as EGFR/Ras/PI3K, growth inhibitory pathways such as p53/Rb/P14ARF, STK11, apoptotic pathways such as Bcl-2/Bax/Fas/FasL, and DNA repair and immortalization genes. Epigenetic alterations in LC are also responsible for cell transformation by modifying chromatin structures and the specific expression of genes and are involved in tumor suppressor genes silencing whereas enhancing oncogenes expression. In the present review, is presented the current state of knowledge regarding the cascade of events that are associated with LC development giving emphasis to oncogenes, tumor suppressor-genes and signaling pathways that are implicated in LC development. Those aberrations and abnormalities allow cells to escape the normal regulation of cell division, apoptosis and invasion. A better understanding of the molecular basis of LC and especially the identification of biologically significant genetic alterations in LC that lead to activation of oncogenes and inactivation of tumor suppressor genes could provide hypotheses for molecular early detection and targeted therapeutic strategies.

Keywords: *Cancer, Molecular Biology, Lung Cancer*

Introduction

In 2012 according to WHO report 1.8 million people were suffered from LC, whereas 1.6 million deaths were recorded worldwide (WHO, 2014a). Those observations lead to the conclusion that LC is the most common cause of cancer-related death in males and the second most common in females after breast cancer (WHO, 2014b). LC was the leading cause of cancer-related death in males and females in the United States, and was estimated 28 % of total cancer deaths in 2010 (Jemal *et al.*, 2010).

Cancer development is characterized by genetic alterations in DNA sequence and epigenetic ones that contribute to cancer initiation, promotion and progression as regulate gene expression and cellular

signaling pathways in the normal cells. Those alterations usually occur early in malignant transformation (Geutjes *et al.*, 2012). LC pathogenesis has not been fully elucidated, whereas LC molecular basis is complicated and heterogeneous. Previous investigations have shown that LC consists a multistage process in which genetic and epigenetic alterations are implicated, particularly activation of growth promoting pathways and inhibition of tumor suppressor pathways, that can lead to cause DNA damage, a procedure responsible for the transformation of lung epithelial cells into malignant (Sekido *et al.*, 1998). The susceptibility to that malignant transformation concerns possibly all lung cells, epithelial, or stem cells, whereas the tumor-initiating cell show specific mutations and acquire additional during the tumor development (Nowell, 1976).

According to histological features, clinical, and neuroendocrine characteristics, LC includes two main types, non-small cell LC (NSCLC) and small cell LC (SCLC), whereas their prevalence is 80%-85% and15%-20%, respectively. Those types have also differences in molecular basis. NSCLC is divided into the following histological subtypes, adenocarcinoma (ADC), squamous carcinoma (SQC), large-cell carcinoma (including l arge-cell neuroendocrine LC), bronchoalveolar LC, and mixed histologic types (e.g, adenosquamous carcinoma) (Gazdar, 2010).

Common molecular differences among NSCLC subtypes and between NSCLC and SCLC are associated with oncogenic mutations, gene amplification, increased protein expression, 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. Those genetic alterations implicate various cellular signaling pathways that are involved in cell functions such as cell growth, differentiation, proliferation, survival, programmed cell death, invasion, metastasis, etc., and include several oncogenes (ONCG) and tumor suppressor genes (TSG), such as BRAF, KRAS, MET, PIK3CA, EGFR, ErbB2/HER2-neu, MDM2, MYC, NKX2-1, PDGFRA, c-KIT, CD44, Bcl-2, CCND1, CRK, p53, PTEN, Rb, LKB1, CDKN2A (p16/p14ARF), FHIT, CAV1, APC, TUSC2 (FUS1), CDH 1, CDH 13, DAPK1, GSTP1, MGMT, RARβ, RASSF1A, SEMA3B, TIMP3, EML4-ALK fusion, etc. (Larsen and Minna, 2011) .

Other mechanisms that are implicated in LC development are epigenetic ones. Epigenetics is the alteration in gene expression without changing the nucleotide sequence. Activation and inactivation of cancer-associated genes which promote or prevent their development can occur by epigenetic mechanisms which concern gene regulation and conclude DNA methylation, histone deacetylation, chromatin remodeling, small non-coding RNA expression and gene imprinting. In recent years, epigenetic mechanisms have been investigated in a number of tumor types and epigenetic biomarkers

have been identified and are suitable for cancer detection, diagnosis, follow-up of treatment and screening high-risk populations. Epigenetic abnormalities consist targets for cancer therapy as regulators that are able to inhibit histone deacetylases and demethylate DNA, can lead to the reactivation of silenced genes (Banerjee and Verma, 2009).

Genomic Instability, Oncogenes and Tumor-Suppressor Genes

One of the main hallmarks of cancer is genomic instability that is obvious in LC (Sekido *et al.*, 1998), whereas the identification of gene amplification and deletions in cancer genome has led to the discovery of many ONCGs and TSGs (Larsen and Minna, 2011; Cooper *et al.*, 2013).

TSGs consist critical negative regulators of normal cell growth and the loss of a TSG function is responsible for cancer pathogenesis. According to Knudson's two hit hypothesis, carcinogenesis is associated with both gene alleles inactivation. Abnormalities such as mutation, epigenetic silencing or other aberrations lead to the inactivation of the gene in one allele, and the second one is often inactivated through loss of heterozygosity (LOH) in which is lost a chromosome region by genetic aberrations such as deletion, non-reciprocal translocation or mitotic recombination (Knudson, 1993a). A crucial step in lung tumorigenesis is the loss of TSGs function, and is usually caused by inactivation of both alleles. In that process LOH inactivates one allele through chromosomal translocation or deletion, and other aberrations such as point mutation, epigenetic, or transcriptional silencing inactivate the second allele (Knudson, 1989; Breuer *et al.*, 2005).

Commonly inactivated TSGs in LC concern TP53, RB1, STK11), CDKN2A, FHIT, RASSF1A and PTEN and the chromosomal locations with allelic loss in LC concern mainly TP53 (17p13), RB (13q12), CDKN2A (9p21), and PTEN genes (10q22) (Ding *et al.*, 2008; Larsen and Minna, 2011). Moreover, activation of ONCGs such as MYC, RAS, EGFR, B-RAF, ErbB2/HER2-neu, MET, NKX2-1, PDGFRA, CD44, BCL-2, PIK3CA, MDM2, c-KIT, SOX2, FGFR2, CRKL, CDK4, EML4-ALK fusion, and CCND1 due to gene amplification, overexpression, point mutation, or DNA rearrangements (Fong *et al.*, 1995a; Sekido *et al.*, 1998; Larsen and Minna, 2011; Cancer Genome Atlas Research Network, 2012) can lead to insistent upregulation of mitogenic growth signals that can induce cell growth as the cell depends on that abnormal oncogenic signaling for survival have also involved in LC development.

Genetic aberrations have been linked with LC development such as gain at 1q, 3q, 5p, and 17q, specific allelic loss at 3p, 4q, 5q, 9p, 11p, 17p, and18q (Fong *et al.*, 1994; Fong *et al.*, 1995a; Fong *et al.*, 1995b; Fong *et al.*, 1995c; Sanchez-Cespedes *et al.*, 2001; Sekido *et al.*1998; Sekido *et al.*, 2003; Weir *et al.*, 2007) (Table 1).

One of the most frequent and early genetic alterations in cancer is LOH or allele loss of one copy of 3p chromosome which has been observed in78% of lung preneoplastic lesions and in 96% of LC cases. On chromosome 3p are located various genes that function as TSGs, such as FHIT (3p14.2), RASSF1A, TUSC2/FUS1, semaphoring family members SEMA3B and SEMA3F (3p21.3), and RARb (3p24) (Fong *et al.*, 1999; Wistuba *et al.*, 2000). RASSF1A, FHIT, TUSC2, and SEMA3B TSGs are able to reduce malignant cell growth in case they will be introduced into LC cells (Siprashvili *et al.*, 1997), whereas RASSF1A, FHIT, SEMA3B, and RARb TSGs often show decreased expression in LC cells due to the promoter hypermethylation as an epigenetic mechanism (Ito *et al*,. 2005; Zochbauer-Muller *et al.*, 2007; Feng *et al.*, 2008).

Epigenetic Mechanisms

Epigenetics is the study of heritable phenotype changes that do not involve alterations in the DNA sequence. Epigenetic mechanisms are essential for normal development and maintenance of tissue specific gene expression standards in human. Epigenetic processes deregulation can lead to altered gene expression and function and thus in malignant cellular transformation and those alterations consist a hallmark of cancer. Cancer initiation and progression as a genetic disease implicates epigenetic abnormalities and genetic alterations. Recent researches have shown that the association between cancer and epigenetics concerns alterations such as DNA methylation (CpG island methylations), histone modifications (methylation, phosphorylation, acetylation, ubiquitylation, and sumoylation), chromatin remodeling, nucleosome positioning and non-coding RNAs, specifically microRNA expression. Epigenetic abnormalities have a reversible nature and a new field of epigenetic treatment is in progress in an effort to discover epigenetic drugs for cancer treatment (Sharma *et al.*, 2010) (Fig. 1).

DNA methylation can lead to gene silencing and post-translational modifications of histones, alterations that are implicated in gene expression abnormalities in cancer development and progression. It has been recorded that aberrant hypermethylation in 5′-CpG islands in the promoter regions is a mechanism for silencing TSGs or other cancer-associated genes in various cancers (Herman and Baylin, 2003; Cho *et al.*, 2010). The abnormal methylation of normally unmethylated CpG-rich areas of the promoter location can lead to the silencing of mRNA expression, and this epigenetic alteration is considered to be a significant mechanism for inactivation of those TSGs in LC development (Sulewska *et al.*, 2007). The loss of TSGs function can prevent inhibition of cancer cells growth, and can lead to malignant transcription and translation during DNA replication. Genes, such as the RASSF1A, cyclin-dependent kinase inhibitor (p16), and MGMT DNA repair gene, are methylated in LC (Kurakawa *et al.*, 2001; Gao *et al.*, 2012). In LC has been found abnormal promoter methylation in genes such as the TSGs

DAPK and RASSF1A. DAPK gene is located on chromosome 9q34.1 and encodes a pro-apoptotic protein which implicated in the apoptosis initiated by IFN-γ, Fas and TRAIL. Aggressiveness of malignancies have been associated with the methylation of the DAPK gene promoter location and the DAPK expression loss. The expression was partially restored after treatment with 5'-aza-2'-deo-xycitidine, which consists a demethylation agent, indicating a role for methylation in down regulation of DAPK (Tang *et al.*, 2000).

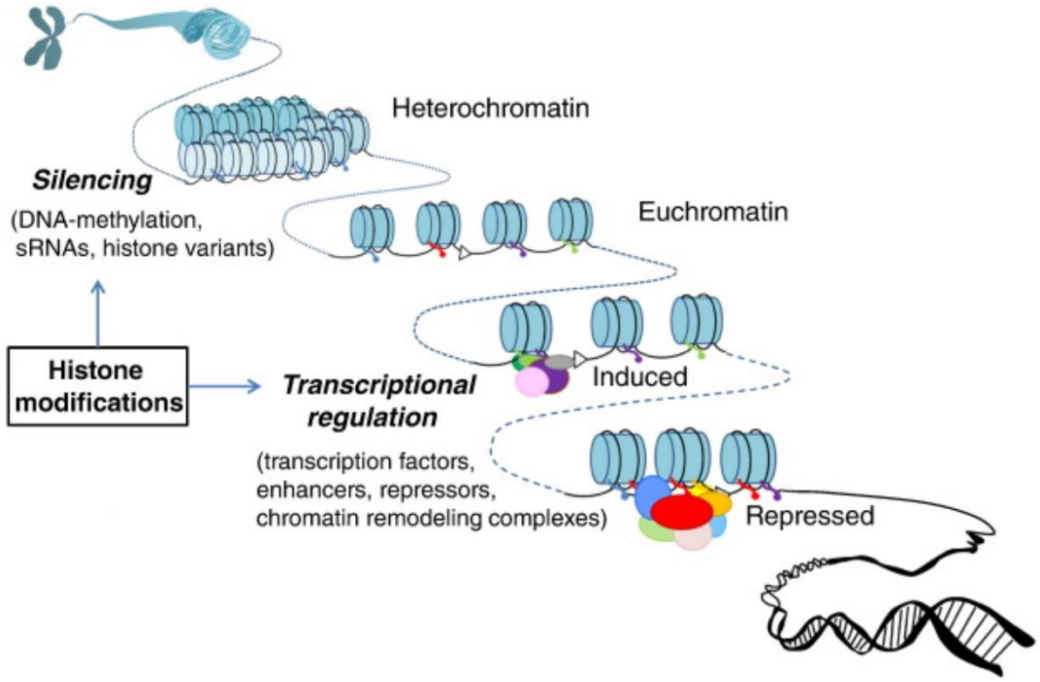

Figure 1: Epigenetic alterations

Oncogenes and LC

KRAS belongs to the proto-oncogenes RAS family which contains KRAS, NRAS and HRAS genes, and is implicated in RAS-RAF-MEK-MAPK/ERK transduction pathway which regulates cell survival, cell cycle progression, differentiation and cell motility (Downward, 2003). The RAS/RAF/MEK/MAPK pathway plays a critical role in many LC development with at least one mutation in the pathway (Ding *et al.*, 2008), however, the most common are mutations in KRAS. KRAS activating mutations are the commonest in LADC cases and have been observed in 25-40% of those cases (Ding *et al.*, 2008; Riely *et al.*, 2008; Schmid *et al.*, 2009), are very rare or absent in SQCLC and SCLC cases (Rekhtman *et al.*, 2012), whereas NRAS and HRAS activating mutations are rare in LADC cases (Cooper *et al.*, 2013). KRAS mutations concern G to T transversions in smokers, whereas never smokers are more likely to have G to A transitions (Riely *et al.*, 2008). In SCLC was identified only one KRAS mutation in codon 61 (Cancer

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Genome Atlas Research Network, 2012) and in LADC was found a single amino acid substitution in hotspots located mostly in codon12 but also more rarely in codons 13 and 61 (Downward, 2003).

The MYC proto-oncogene consists one of the main downstream effectors of the RAS/RAF/MEK/MAPK signaling pathway. Under normal conditions this transcription factor is responsible for keeping tight control of cellular proliferation, however, its amplification or overexpression leads to aberrant expression and has been observed in LC cases (Richardson and Johnson, 1993). Gene amplification is the main mechanism for the MYC members activation and is observed frequently in NSCLC cases (Nau *et al.*1986), whereas MYCL and -N along with MYC, are usually activated in SCLC cases (Broers *et al.*1993; Fong *et al.*, 1999). Increased C-MYC expression has been shown to induce mitosis or apoptosis, depending on the availability of other critical growth stimuli. In the presence of such stimuli, C-MYC acts as a classic proto-oncogene stimulating mitosis, whereas in the absence of such stimuli, it initiates apoptosis (Kerr *et al.*, 1994).

BRAF is a gene that encodes a serine/threonine protein kinase, called B-RAF. The gene is also referred to as a proto-oncogene B-RAF and V-RAF murine sarcoma viral oncogene homolog B, while the protein is more formally known as serine/threonine-protein kinase B-RAF, that is the downstream KRAS effector protein and activates the MAPK pathway which is implicated in the regulation of cell survival and proliferation (Davies *et al.*, 2002). Following activation, B-RAF phosphorylates downstream mediators MEK1 and -2 which, in turn, activate ERK1 and ERK2, that is implicated in regulation of growth regulating factors, c-JUN and ELK1(Downward, 2003). B-RAF activating mutations increase kinase activity that display transforming activity *in vitro* (Davies *et al.*, 2002). Those mutations are rare in NSCLC cases (Brose *et al.*, 2002; Schmid *et al.*, 2009; Davies *et al.*, 2002; Paik *et al.*, 2011), however activating mutations in NSCLC cases are not the same with those in colorectal cancer and melanoma, showing a lower rate of V600E mutations that influence the protein kinase domain. In LADC cases, V600E mutations in exon 15 have been estimated to be 50% of B-RAF mutations followed by G469A in exon 11 and D594G in exon 15 (Marcheti *et al.*, 2011). In NSCLC cases some of the B-RAF mutations have been observed in the kinase domain, such as V600E, D594G and L596R, whereas others have been found in the activation domain G-loop of the B-RAF gene, such as G465V and G468 A (Davies *et al.*, 2002). B-RAF mutations have been observed mainly in LADC cases as already mentioned, whereas non-V600E B-RAF mutations have been l inked to current /former smokers and V600E mutations are more frequent in females never smokers (Marcheti *et al.*, 2011; Paik *et al.*, 2011).

MEK1 or MAPK1 is a serine-threonine kinase, functions as a downstream target of RAS activation, as activates MAPK2 and MAPK3 downstream of B-RAF (Downward, 2003). Few cases of somatic

mutations of MEK1 have been identified in LADC cases and carried an activating mutation in exon 2 that did not implicate the kinase domain (Marks *et al.*, 2008).

MET consists a proto-oncogene which is located on chromosome 7q21-q31 and encodes a membrane receptor tyrosine kinase (RTK), HGFR (hepatocyte growth factor receptor). Following binding of its ligand HGF, interactions have been observed in which are involved the RAS/RAF/MEK/MAPK, PI3K/AKT and c-SRC kinase downstream pathways (Sadiq and Salgia, 2013). In NSCLC cases, MET is changed by gene amplification (Cappuzzo *et al.*, 2009; Onozato *et al.*, 2009; Beau-Faller *et al.*, 2008), whereas increased MET copy number is mutually exclusive with KRAS mutations (Beau-Faller *et al.*, 2008; Onozato *et al.*, 2009) and are more common in SQCLC than in LADC cases. MET mutations have been found in 3-5% of LADC cases (Ding *et al.*, 2008; Onozato *et al.*, 2009).

ROS1 is a proto-oncogene, encodes a transmembrane RTK that has high homology with ALK in its protein kinase domain and is located on chromosome 6q22. Its activation implicates the RAS/MAPK/ERK, PI3K/AKT/m TOR, and STAT3 pathways (Chin *et al.*, 2012). ROS1 fusion was identified in a NSCLC cell line SLC 34A2-ROS1 and a patient sample CD 74-ROS1 (Rikova *et al.*, 2007). Another inframe fusion KDELR2-ROS1 was found, in a LADC sample from a non-smoker (Govindan *et al.*, 2012), whereas ROS1 rearrangements were identified in a lower rate in LADC cases (Bergethon *et al.*, 2012; Takeuchi *et al.*, 2012). In addition, ROS1 rearrangements were presented in younger ages, never smokers or in Asian population (Bergethon *et al.*, 2012) similar to ALK rearrangements (Shaw *et al.*, 2009).

Fibroblast growth factor receptor 1 (FGFR1), is a membrane RTK whose ligands are specific members of the fibroblast growth factor family and regulates cell proliferation through activation of the PI3K and MAPK pathways (Tran *et al.*, 2013). In SQCLC cases FGFR1 somatic gene amplifications have been identified (Turner and Grose, 2010), whereas FGFR1 amplifications show *in vitro* an oncogenic effect on NSCLC cell lines (Dutt *et al.*, 2011). Those abnormalities have not found in LADC cases (Cancer Genome Atlas Research Network, 2012; Dutt *et al.*, 2011). The PIK3CA ONCG is involved in p110 alpha (p110α) protein formation, which is one subunit of the enzyme PI3K. The p110α protein performs the action of PI3K. PI3K signaling is important for cell activities, such as cell growth, proliferation, migration, production of new proteins, transport of materials within cells, cell survival, whereas affects cancer and metabolic disorders (Engelman *et al.*, 2006; Yuan and Cantley, 2008; Courtney *et al.*, 2010). PIK3CA gene mutations have been observed in various human cancers and in NSCLC cases, affect the helical binding domain (exon 9, E545K or E542K) or the catalytic subunit (exon 20, H1047R or -7L) and are considered oncogenic (Samuels and Velculescu, 2004; Huang *et al.*, 2007; Engelman *et al.*, 2008;

Ding *et al.*, 2010). PIK3CA mutations in LADC cases have not been described to be mutually exclusive, finding that is not in accordance with classical oncogenic driver mutations like activating EGFR mutations, whereas are associated with EGFR, BRAF, ALK and, mainly, KRAS aberrations, thus it remains unclear if PIK3CA mutation alone is a sufficient event for NSCLC development (Yamamoto *et al.*, 2008; Chaft *et al.*, 2012). Similarly, the role of PIK3CA mutations in SQCLC cases is not obvious yet (Dearden *et al.*, 2013), despite the fact that an association between SQCLC and PIK3CA mutations in a frequency of 4.2% was recorded (Yuan and Cantley, 2008).

Platelet-derived growth factor receptors (PDGFRs), with the members PDGFRα and -β, consist a family of cell surface type III RTK (Shim *et al.*, 2010). Following binding of the ligands, the receptor complex is activated and interacts with several signaling molecules, such as PI3-K, GTP-ase activation protein (RAS-gap), growth factor binding protein (Grb2), and is implicated in the PI3-K, RASMAPK, and STAT3 pathways (Montmayeur *et al.*, 1997; Cancer Genome Atlas Research Network, 2012; Tian *et al.*, 2014). PDGFR signaling regulates biological functions, such as cellular growth, proliferation, differentiation, migration, invasion, angiogenesis and metastasis (Cooper *et al.*, 2013).

Deregulated PDGFR signaling has been implicated in the pathogenesis of several diseases and plays a crucial role in the development and progression of human malignancies such as breast, gastric, prostate, lung, colon, and other cancers (Coltrera *et al.*, 1995; Tejada *et al.*, 2006). PDGFRA somatic gene amplifications have also been found (Cancer Genome Atlas Research Network, 2012).

The RTKs of the ErbB family is consists of 4 members, EGFR, ErbB-2(HER2), ErbB-3, and -4, are able to form homodimers and heterodimers and also to bind different ligands which leads to the activation of the receptor (Normanno *et al.*, 2005). EGFR signal transduction activation is associated with RAS/RAF/MAPK/PI3K/AKT/m TOR, and JAK/STAT pathways (Scaglioti *et al.*, 2004; Sordella *et al.*, 2004). EGFR is implicated in the regulation of various oncogenic functions such as survival, cell proliferation, differentiation, invasion, metastasis, and neovascularization (Sordella *et al.*, 2004), whereas aberrations, such as activating mutations, associated with EGFR are implicated in the pathogenesis of several tumors, including NSCLC, as are able to lead to constitutive tyrosine kinase activation (Sordella *et al.*, 2004; Greulich *et al.*, 2005) and oncogenic transformation of lung epithetlial cells *in vitro* (Greulich *et al.*, 2005). Increased protein expression or increased gene copy number are additional mechanisms of increased EGFR signaling (Okabe *et al.*, 2007; Dahabreh *et al.*, 2010). In 50% to 90% of NSCLC cases has been observed EGFR overexpression or abnormal activation. Mutations in EGFRs, caused by exon19 deletion or exon21 L858R mutation, show an increased rate and duration of EGFR activation compared with wild-type receptors and prefer the activation of the PI3K/AKT and

STAT3/-5 pathways rather than the RAS/RAF/MEK/MAPK pathway (Sordella *et al.*, 2004). In NSCLC cases, EGFR mutations have been identified in the first four exons of the intracellular tyrosine kinase domain, mainly in exon 19 in frame deletions, concerns over 20 variants, and the most common is the delE746-A750. Other EGFR mutations are missense, and mainly a single nucleotide point mutation in exon 21 that leads to a single amino acid change from leucine to arginine at codon 858, L858R (Yip *et al.*, 2013). However, less frequent mutations have been identified in exon 20, insertions or frame duplications (Tam *et al.*, 2006; Yamamoto *et al.*, 2006). The majority of EGFR mutations have been observed in LADC cases (Kosaka *et al.*, 2004; Shigematsu *et al.*, 2005) and few in adenosquamous (ADS) carcinomas and very rare in SQCLC cases (Ohtsuka *et al.*, 2007; Rekhtman *et al.*, 2012), however in SQCLC cases copy-number gains, protein overexpression and variant-III mutations which involve the extracellular domain of EGFR are common findings in those cases than in LADC (Heist *et al.*, 2012). EGFR mutations are strongly associated with the LADC histology and never smokers, (Fujino *et al.,* 1996; Franklin *et al.*, 2002; Shigematsu *et al.*, 2005), whereas the observed resistance to tyrosine kinase inhibitor therapy has been associated with KRAS mutation, or MET proto-oncogene amplification, or EGFR exon-20 insertions or a secondary T790M mutation (Pao *et al.*, 2005a; Pao *et al.*, 2005b; Engelman *et al.*, 2007; Gazdar, 2009) in which MET activates the PI3K pathway through phosphorylation of ERBB3, a process that is not associated with EGFR and ERBB2 (Engelman *et al.*, 2007). In LADC cases, activated mutant EGFR induces high levels of interleukin (IL)-6, and can lead to STAT3 activation (Gao *et al.*, 2007).

HER 2 activation leads to signaling through a sequence of signal transduction pathways such as PI3K, MAPK and JAK/STAT (Graus-Porta *et al.*, 1997) and its activation has been observed in few cases of LC with overexpression in 20% of cases, activating mutations in 1.6-4% and gene amplification in 2% (Heinmöller *et al.*, 2003) of NSCLC cases.

HER2 activating mutations concern exon 20 in frame insertions of 3 to12 base pairs in length (Shigematsu *et al.*, 2005). HER2 genetic aberrations have been identified mainly in LADC cases (Shigematsu *et al.*, 2005; Tomizawa *et al.*, 2011; Cancer Genome Atlas Research Network, 2012) and mutations in tumors which consist the wild-type for EGFR and KRAS (Shigematsu *et al.*, 2005; Cancer Genome Atlas Research Network, 2012) may be are associated with females and non-smokers (Shigematsu *et al.*, 2005; Tomizawa *et al.*, 2011).

Cyclin D1 (CCND1) is one of the more important human ONCG, involved in the pathogenesis of multiple tumor types. In normal cells, cyclin D1 forms complexes with cyclin-dependent kinases (CDK), activates those and regulates transcription process. Cyclin D1 protein is frequently overexpressed in

various cancers, as in a rate 5-20% of tumors occurs cyclin D1 gene amplification. In NSCLC cases CCND1is amplified and the protein is overexpressed in tumors and preinvasive bronchial lesions. Its deregulation is implicated in bronchial neoplasia and its overexpression is a critical event for malignant transformation in the lung and other tissues (Gautschi *et al.*, 2006). CCND1 overexpression through gene amplification or other gene abnormalities has been identified in about 40% of NSCLC cases. In NSCLC cases, the pathway is deactivated mainly because of the alterations of CCND1, CDK4 and the cyclin dependent kinase inhibitor p16 (CDKN2A) (Brambilla *et al.*, 1999).

B-cell-lymphoma-2(Bcl-2) is a mitochondrial apoptotic pathway regulator and promotes survival by inhibition of adapters which are necessary for the activation and cleavage of caspases. Bcl-2 protooncogene is encoded by a 230 kb gene and its major function appears to be to inhibit programmed cell death (apoptosis), to prolong cell survival by arresting cells in the cell cycle G0/G1 phase and is overexpressed in various human tumors including LC (Anagnostou *et al.*, 2010; Lawson *et al.*, 2010).

However, the role of the antiapoptotic protein Bcl-2 in LC remains controversial. In addition, transregulatory mechanisms appear to be responsible for the Bcl-2 protein high levels production that occur in many different solid tumors and LC (Pezzella *et al.*, 1993; Fontanini *et al.*, 1995). Bcl-2 protein is a known inhibitor of the p53-dependent and independent apoptosis pathway, at high levels protects cells apoptosis induced by C-MYC or wild-type p53 (McDonnell *et al.*, 1993; O'Neill *et al.*, 1996). However, the associations of those proteins, the correlation of their expression and the prognosis of LC are still controversial (Pezzella *et al.*, 1993; Gaffney *et al.*, 1994; Anton *et al.*, 1997). The RET proto-oncogene is located on chromosome 10q11.2 and encodes a RTK for members of the glial cell line-derived neurotrophic factor (GDNF) family of extra-cellular signaling molecules (Knowles *et al.*, 2006). RET gene gain of function mutations are associated with the development of various types of human cancer, including medullary thyroid carcinoma, multiple endocrine neoplasias (MEN) type 2A and 2B, pheochromocytoma and parathyroid hyperplasia (Wells and Santoro, 2009), whereas the activation of RET through chromosomal rearrangement has been found in a low rate of LC cases (Ju *et al.*, 2012; Kohno *et al.*, 2012; Lipson *et al.*, 2012). The functional RET kinase domain from exons 12-20 is fused to KIF5B (kinesin family 5B) and encodes a coiled-coil domain which is involved in organelle trafficking (Ju *et al.*, 2012; Kohno *et al.*, 2012). KIF5B-RET fusions have been found in 1-2% of LADC cases (Kohno *et al.*, 2012; Lipson *et al.*, 2012) and have also been found to be mutually exclusive of other driver mutations which involve EGFR, KRAS or ALK genes. RET rearrangements were identified in 6.3% of LADCs cases, in never or light smokers that are known to be wild type for other driver mutations (HER2, ALK, EGFR, KRAS, BRAF and ROS1) (Ju *et al.*, 2012; Kohno *et al.*, 2012; Lipson *et al.*, 2012).

DDR2 (discoidin domain receptor tyrosine kinase 2) is a gene that encodes a RTK known as the discoidin domain-containing receptor 2 protein. Binding of collagen to DDR2 leads to the activation of the downstream SRC and STAT pathways. Similar to integrin receptors, DDR2 may play a role in modulating cellular interactions with the extracellular matrix and is implicated in the regulation of survival and cell proliferation (Ikeda *et al.*, 2002). DDR2 mutations have been found in multiple tumor types, including LC, brain, breast, gynecologic, and prostate cancer (Valiathan *et al.*, 2012), whereas mutations in DDR2 were found in 3.8% of SQCLC cases (Hammerman *et al.*, 2011). The c-kit proto-oncogene encodes a transmembrane tyrosine kinase growth factor receptor which belongs to the PDGFR family (Yarden *et al.*, 1997). Its ligand SCF (kit-ligand or steel factor) is a hemopoietic growth factor that supports the proliferation of multiple hemopoietic cell lines (Ashman, 1999). Similar studies showed that SCLC cell lines and tumors express the mRNA for the ckit receptor and for SCF, suggesting that these gene products constitute an autocrine loop mediating tumor cell survival and growth. However, most of this knowledge is derived from cell culture experiments or animal models and is based on RNA or DNA analysis only. The clinical impact of ckit protein expression in SCLC patients remains unknown (Hibi *et al.*, 1991; Krystal *et al.*, 1996).

SOX2 is a stem cell transcription factor and plays a critical role in the embryonic development regulation as consists one of the genes (Oct4, SOX2, Nanog) that are able to reprogram human somatic cells to pluripotent stem cells. Overexpression of SOX2 has been observed in all types of LC tissues.

Amplification of the region 3q, the most common genomic aberration in SCLC, has been found in the evolution of pre-invasive SQCLC and involves SOX2 as a key target of this process. SOX2 is expressed in nearly 20% of LADC cases and is associated with poor prognosis (Karachaliou *et al.*, 2013).

After analyzing of SCLC cell lines, H446 and H720 was found that SOX2 is amplified in approximately 27% of cancers (Rudin *et al.*, 2012). In addition, it has been observed that SOX2 amplification and consequent SOX2 protein overexpression represent important mechanisms of tumor initiation and progression in a considerable subset of SQCLC cases (Maier *et al.*, 2011). Immunohistochemical analysis of SOX2 expression in various types of LC revealed that SCLC tissues showed a higher SOX2 expression level than in NSCLC tissues, whereas SOX2 was found to cooperate with crucial ONCGs like Wnt1, Wnt2, C-Myc and Notch to promote lung tumor occurrence, whereas downregulation of SOX2 inhibited proliferation and induced apoptosis in tumor cells (Chen *et al.*, 2012). SOX2 was found to be strongly and diffusely expressed in approximately 90% of SCLC and 20% of LADC cases (Sholl *et al.*, 2010a). As previously described, SOX2 gene amplification is more common in the SCLCs of smokers while the incidence of SOX2 amplification is in the early stage of tumorigenesis in

NSCLC. However, SOX2 is also activated in more advanced SQCL tumors (Sholl *et al.*, 2010b; Hussenet *et al.*, 2010). Therefore, the SOX2 gene is not only activated by amplification but is also affected by other regulators that promote its transcription, affecting its downstream genes. Somatic gene amplifications have been found in SCLC cases in a number of genes including SOX (Cancer Genome Atlas Research Network, 2012).

NKX2-1, also known as TTF-1 (thyroid transcription factor-1), is a tissue-specific transcription factor of the thyroid, lung, and ventral forebrain. It has been shown to play a critical role in lung development, LC differentiation and morphogenesis (Li *et al.*, 2012), as encodes a lineage-specific transcription factor which is crucial for branching morphogenesis in lung development, as mentioned and the type II pneumocytes formation, the cells which cover the internal surface of lung alveoli (Ikeda *et al.*, 1995; Bingle, 1997). The oncogenic role of NKX2-1 in LADC cases has been extensively investigated (Tanaka *et al.*, 2007; Weir *et al.*, 2007; Kwei *et al.*, 2008) however, it was found the suppressive role of LADC progression by NKX2-1(Winslow *et al.*, 2011).

Mouse double minute2 homolog (MDM2) is an oncoprotein that is encoded by the MDM2 gene, consists an important negative regulator of the p53. MDM2 ligates the p53 protein via its E3 ubiquitin ligase, and the ubiquitinated p53 can be transferred to the cytoplasm and degraded by proteasomes. Therefore, MDM2 can maintain the stability of p53 pathway. MDM2 gene amplification and overexpression of its protein have been found in some malignancies, and can lead to tumorigenesis through inactivation of the p53 function, as shows a large effect on the antitumorigenic activity of the p53. Its amplification has been detected in LC, colon cancer and other malignancies (Higashiyama *et al.*, 1997).

CRK is an adapter molecule, also known as proto-oncogene c-Crk. CRK family of adaptor proteins are cellular homologues of v-Crk (Mayer *et al.*, 1998). Those adaptor proteins are widely expressed and are implicated in signal transduction from various receptors, signaling scaffold proteins, and oncoproteins and affect cellular proliferation, differentiation, and migration. Its role as mediated signal transduction protein contains ONCGs such as EGFR, Met, PDGF, BCR-ABL, Tel-Abl, VEGFR, erythropoietin receptor, and insulin receptor substrate (Feller, 2001; Birge *et al.*, 2009). CRK overexpression has been identified in NSCL, colon and brain cancers (Miller *et al.*, 2003; Takino *et al.*, 2003) and is responsible for cellular transformation process that has been found in the case of vCrk and CRK-I (Takino *et al.*, 2003). Thus, it is obvious that CRK is involved in the malignant process as an oncoprotein or through activation of down-stream effectors (Table 1).

Table 1: Genetic aberrations in NSCLC and SCLC

		NSCLC	
GENES	**SCLC**	**LADC**	**SQCLC**
ONCOGENIC ALTERATIONS			
MUTATIONS			
KRAS	**<1.0 %**	**15-35.0 %**	**<5.0 %**
B-RAF	**<1.0 %**	**1-5.0 %**	**<1.0 %**
EGFR	**<1.0 %**	**10-40.0 %**	**<1.0 %**
ErbB2 (HER2)	**<1.0 %**	**4.0 %**	**<1.0 %**
PIK3CA	**<1.0 %**	**<1.0 %**	**<5.0 %**
MET	**13.0 %**	**14.0 %**	**12.0 %**
GENE AMPLIFICATION			
MYC	**18-30.0 %**	-	-
EGFR	**<1.0 %**	**15.0 %**	**30.0 %**
ErbB2 (HER2)	**5-30.0 %**	**6.0 %**	**2.0 %**
MET	-	**20.0 %**	**21.0 %**
PIK3CA	**5.0 %**	**6.0 %**	**33-36.0 %**
MDM2	-	**14.0 %**	**22.0 %**
NKX2-1 (TITF1)	**<1.0 %**	**10-15.0 %**	**3-15.0 %**
INCREASED PROTEIN EXPRESSION			
MYC	**10-45.0 %**	-	-
EGFR	**<1.0 %**	**40-65.0 %**	**60-85.0 %**
ErbB2 (HER2)	**<10.0 %**	**16-38.0 %**	**6-16.0 %**
BCL-2	**75-95.0 %**	-	-
c-KIT	**46-91.0 %**	-	-
CCND1 (CYCLIN D1)	-	**35-55.0%**	**30-35.0 %**
CRK	-	**8-30.0%**	-
PDGFRA	**65.0 %**	**100.0 %**	**89.0 %**
TELOMERES			
TELOMERASE ACTIVITY	**75-100.0 %**	**65-85.0%**	**80-90.0 %**
CHROMOSOMAL ABERRATIONS			
GAIN	**3q, 5p, 8q, 18q**	**5p, 7p, 7q, 8q 11q, 19, 20q**	**2q, 3q, 5p, 8q, 7, 8q, 11q, 13q 19, 20q**
AMPLIFICATION	-	**1p34.3, 1p36.32, 1q21.2, 1q32.2, 2p24.3, 2q31.1, 2q11.2, 3q26.31, 5p14.3, 5p15.31, 5p15.33, 5q31.3, 6p21.1, 7p11.2, 8p12, 8q21.13, 8q24.21, 10q24.1, 10q26.3, 11q13.3, 12p12.1, 12q13.2, 12q14.1, 12q15, 14q32.13, 14q13.3, 16q22.2, 17q12, 18q12.1, 19q12, 19q13.33, 20q13.32, 22q11.21**	

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